Module V
Testing the Gut, Immune and Brain

Todd R. LePine, MD

Learning Objectives

- Describe the role of brain inflammation in conditions such as depression, chronic fatigue, MS, schizophrenia and dementia.
- Understand the connection to oral and gut dysbiosis in neuroinflammation
- Summarize how astroglial-like cells in the gut can act as signaling pathways for the gut to communicate to the brain the message of alarm which manifests as neuroinflammation
- Explain the role that tryptophan, quinolinic acid and NMDA receptor activation play in a variety of inflammatory neurological disorders.
- Discuss the key role restorative sleep plays in preventing the buildup of toxins and inflammation in the brain.

frontiers in
PHYSIOLOGY

REVIEW ARTICLE
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Brain–gut–microbe communication in health and disease

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Bidirectional signalling between the gastrointestinal tract and the brain is regulated at neural, hormonal, and immunological levels. This construct is known as the brain–gut axis and is vital for maintaining homeostasis. Bacterial colonization of the intestine plays a major role in the post-natal development and maturation of the immune and endocrine systems. These processes are key factors underpinning central nervous system (CNS) signaling. Recent research advances have seen a tremendous improvement in our understanding of the scale, diversity, and importance of the gut microbiome. This has been reflected in the form of a revised nomenclature to the more inclusive brain–gut–enteric microbiota axis and a sustained research effort to establish how communication along this axis contributes to both normal and pathological conditions. In this review, we will briefly discuss the critical components of this axis and the methodological challenges that have been presented in attempts to define what constitutes a normal microbiota and chart its temporal development. Emphasis is placed on the new research narrative that confirms the critical influence of the microbiota on mood and behavior. Mechanistic insights are provided with examples of both neural and humoral routes through which these effects can be mediated. The evidence supporting a role for the enteric flora in brain–gut axis disorders is explored with the spotlight on the clinical relevance for irritable bowel syndrome, a stress-related functional gastrointestinal disorder. We also critically evaluate the therapeutic opportunities arising from this research and consider in particular whether targeting the microbiome might represent a valid strategy for the management of CNS disorders and ponder the pitfalls inherent in such an approach. Despite the considerable challenges that lie ahead, this is an exciting area of research and one that is destined to remain the center of focus for some time to come.

Keywords: microbiota, central nervous system, enteric nervous system, irritable bowel syndrome, vagus nerve, inflammation, probiotic, dysbiosis

INTRODUCTION

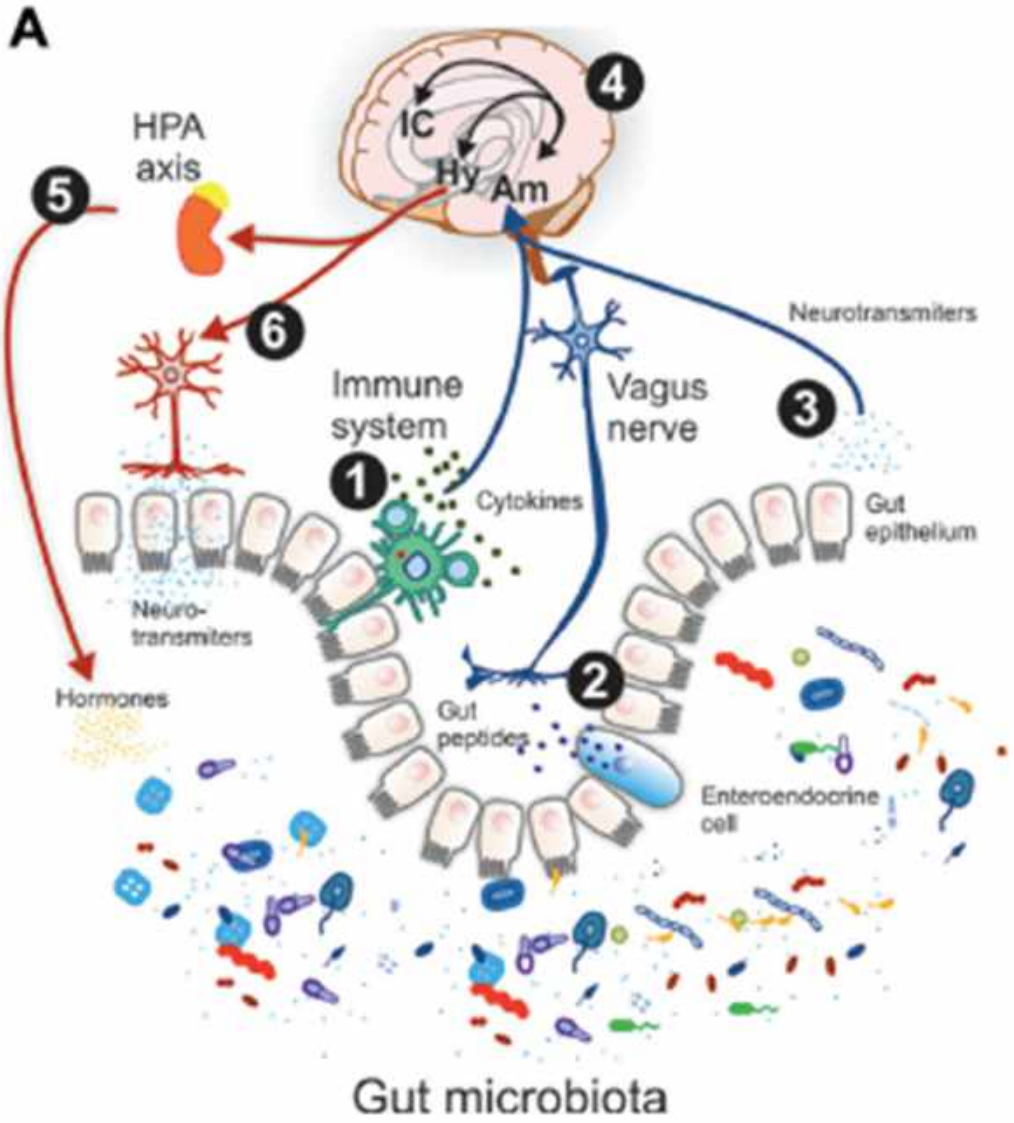
Scientific endeavor is increasingly characterized by a multidisciplinary approach to the study of both health and disease. Nowhere is this more evident than in the field of neurogastroenterology where the converging influence of experts across the diverse domains of gastroenterology, psychiatry, microbiology, pharmacology, immunology, and behavioral neuroscience, to name but a few, have helped shape emerging biological themes. Chief among these is the concept of the brain–gut axis, a term which describes the complex bidirectional communication system that exists between the central nervous system (CNS) and the gastrointestinal tract (GIT) and which is vital for maintaining homeostasis (Cryan and O'Mahony, 2011). Spurred in part by the discovery of *Helicobacter pylori* as a causative agent in ulcer diseases but also by other innovative research in the gastrointestinal sciences (Pandol, 2010; Shanahan, 2010b), there is a growing appreciation of the critical role played by the commensal microbiota, both in our general wellbeing and in the specific functioning of the brain–gut axis. This has been reflected in the form of a revised nomenclature to the more inclusive brain–gut–enteric microbiota axis and a sustained research effort to establish how communication along this axis contributes to both normal and pathological conditions (Rhee et al., 2009).

In this review, we will briefly discuss the critical components of this axis and the methodological challenges that have been presented in attempts to define what constitutes a normal microbiota and chart its temporal development. We examine the approaches that have been taken to elucidate the impact of the enteric microflora on this axis and vice-versa, with reference to the previously elucidated functions of the microbiota as well as an evaluation of exciting new data suggesting a role for the microbiota in the modulation of mood and behavior. Mechanistic insights are provided and the evidence supporting a role for the microbiota in disease states is discussed. The clinical implications are critically evaluated, therapeutic opportunities arising from these findings discussed and future perspectives are provided on this rapidly expanding area of research.

THE BRAIN–GUT–ENTERIC MICROBIOTA AXIS

The general scaffolding of the brain–gut–enteric microbiota axis includes the CNS, the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic

"It is now evident that the bidirectional signaling between the gastrointestinal tract and the brain, mainly through the vagus nerve, the so called **"microbiota–gut–vagus–brain axis,"** is vital for maintaining homeostasis and it may be also involved in the etiology of several metabolic and mental dysfunctions/disorders."


A
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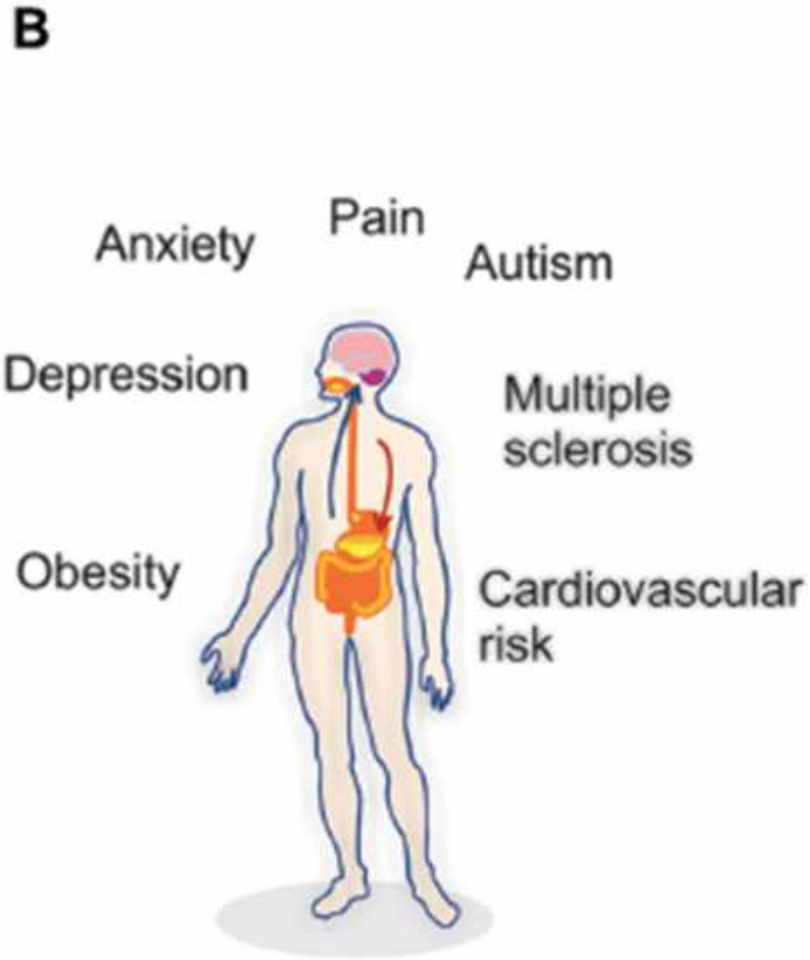
Gut microbiota


B
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Cardiovascular
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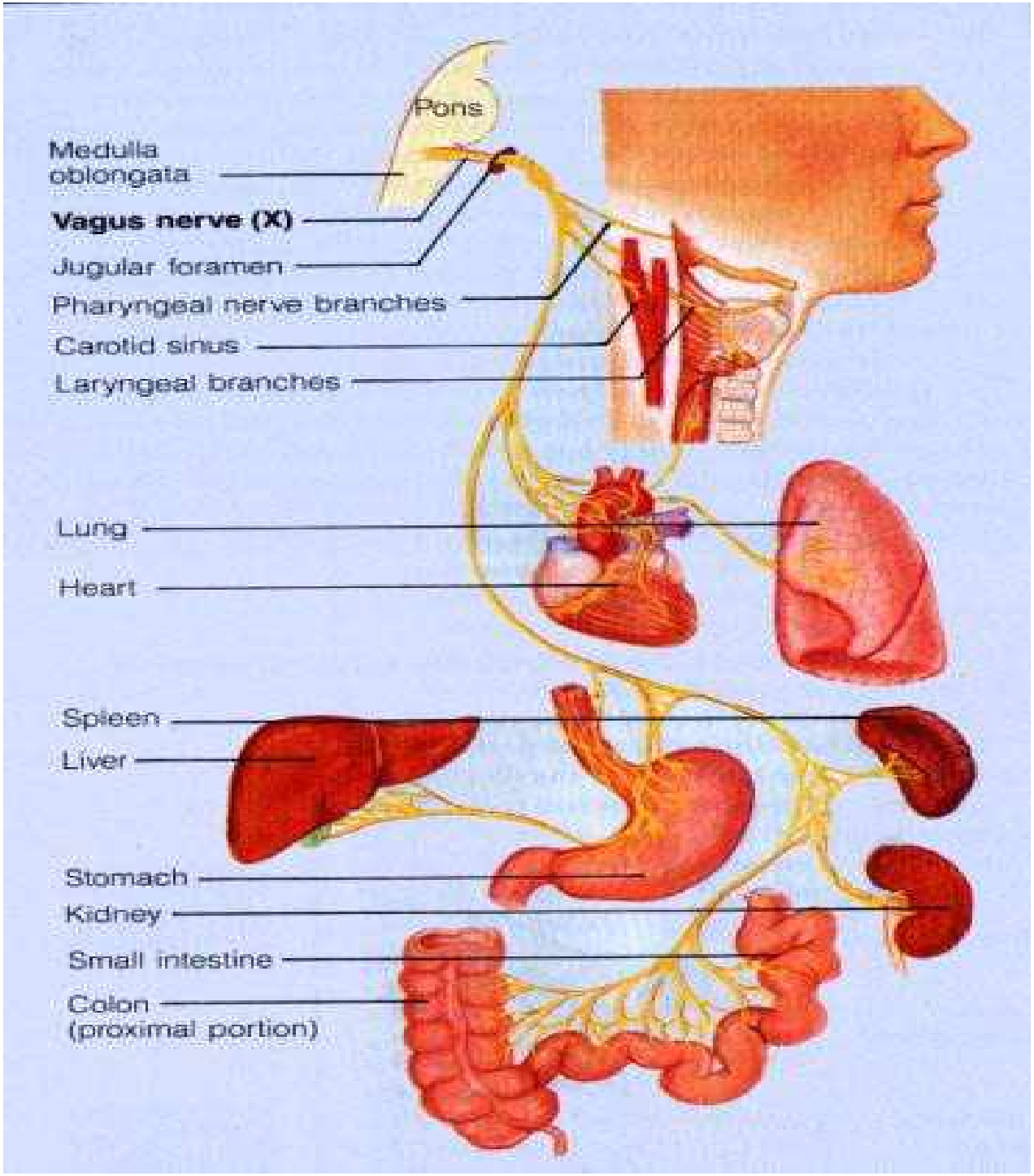
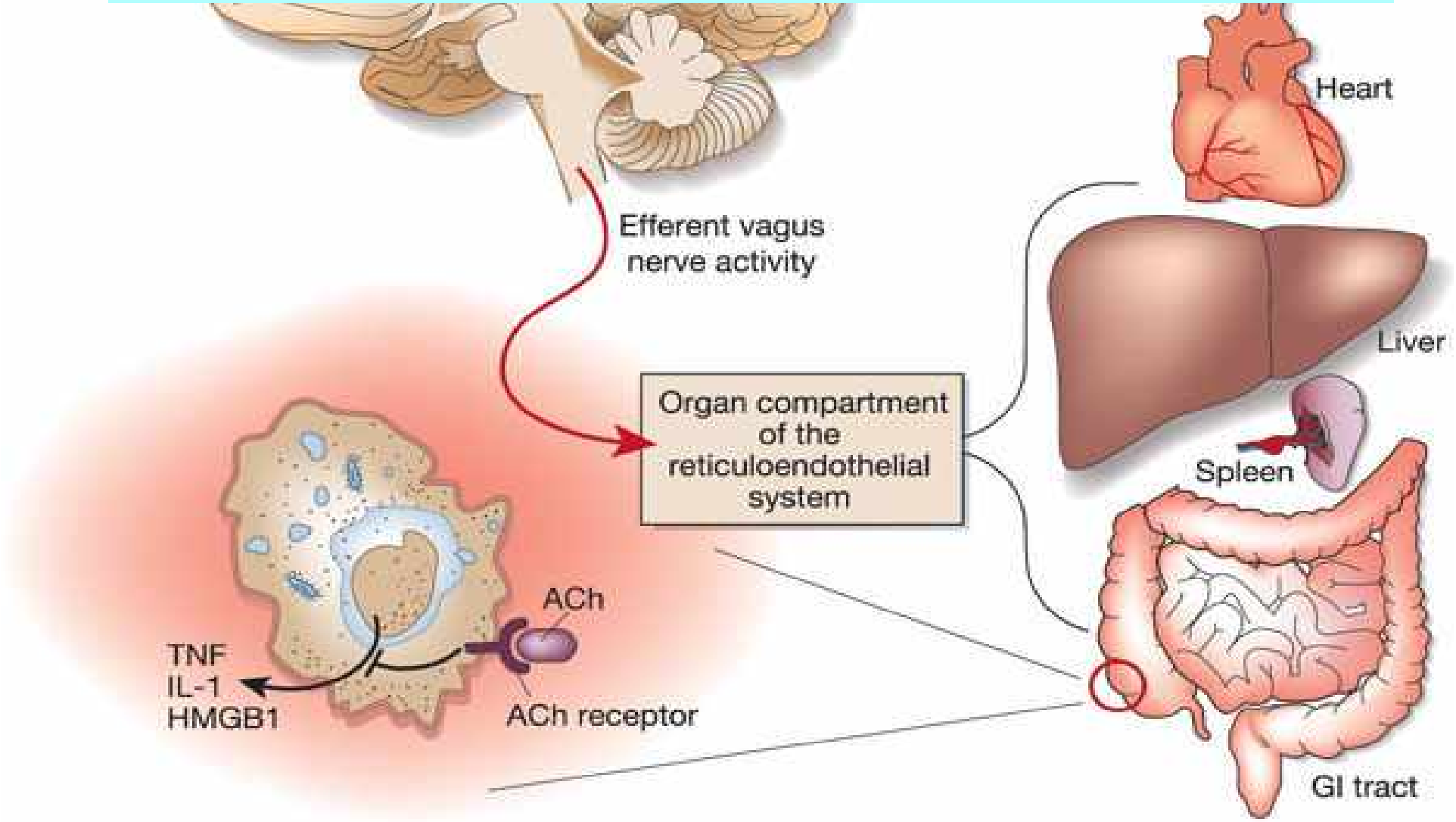

Pons
Medulla oblongata
Vagus nerve (X)
Jugular foramen
Pharyngeal nerve branches
Carotid sinus
Laryngeal branches
Lung
Heart
Spleen
Liver
Stomach
Kidney
Small intestine
Colon (proximal portion)

Macrophages in the spleen make tumor necrosis factor (TNF) a powerful inflammation-producing molecule. When the vagus nerve was stimulated, TNF production in the spleen decreased and survival increased in laboratory model.



Open

Molecular Psychiatry (2012), 1–23

www.nature.com/mp

HYPOTHESIS

The evolutionary significance of depression in Pathogen Host Defense (PATHOS-D)

CL Raison[1,2] and AH Miller[3]

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Given the manifold ways that depression impairs Darwinian fitness, the persistence in the human genome of risk alleles for the disorder remains a much debated mystery. Evolutionary theories that view depressive symptoms as adaptive fail to provide parsimonious explanations for why even mild depressive symptoms impair fitness-relevant social functioning, whereas theories that suggest that depression is maladaptive fail to account for the high prevalence of depression risk alleles in human populations. These limitations warrant novel explanations for the origin and persistence of depression risk alleles. Accordingly, studies on risk alleles for depression were identified using PubMed and Ovid MEDLINE to examine data supporting the hypothesis that risk alleles for depression originated and have been retained in the human genome because these alleles promote pathogen host defense, which includes an integrated suite of immunological and behavioral responses to infection. Depression risk alleles identified by both candidate gene and genome-wide association study (GWAS) methodologies were found to be regularly associated with immune responses to infection that were likely to enhance survival in the ancestral environment. Moreover, data support the role of specific depressive symptoms in pathogen host defense including hyperthermia, reduced bodily iron stores, conservation/withdrawal behavior, hypervigilance and anorexia. By shifting the adaptive context of depression risk alleles from relations with conspecifics to relations with the microbial world, the Pathogen Host Defense (PATHOS-D) hypothesis provides a novel explanation for how depression can be nonadaptive in the social realm, whereas its risk alleles are nonetheless represented at prevalence rates that bespeak an adaptive function.
Molecular Psychiatry advance online publication, 31 January 2012; doi:10.1038/mp.2012.2

Keywords: major depression; evolution; immune; inflammation; infection; genetic

Introduction

Major depression is so detrimental to survival and reproduction that it is hard to understand why allelic variants that promote the disorder have not been culled from the human genome, why in fact—far from being culled—genes that promote depression are so common and numerous and appear to have actually increased in prevalence during recent human evolution.[1] To address this issue, we have developed a novel theoretical framework positing that risk alleles for depression originated and have been largely retained in the human genome because these alleles encode for an integrated suite of immunological and behavioral responses that promote host defense against pathogens. This enhanced pathogen defense is accomplished primarily via heightened innate immune system activation, which results in reduced death from infectious causes,[2–5] especially in infancy when selection pressure from infection is strongest,[6] and the adaptive immune system is not yet fully operational.[6–8] A vast literature has associated depressive symptoms and/or major depressive disorder (MDD) with increased innate immune inflammatory responses,[10] with meta-analyses reporting the most consistent findings for increased plasma concentrations of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein and haptoglobin.[11–13] Recent longitudinal studies extend these cross-sectional observations by reporting that increased inflammatory markers in nondepressed individuals predict the later development of depression.[14–16] Because infection has been the primary cause of early mortality and hence reproductive failure across human evolution,[9,17–21] it would be expected that if depressive symptoms were an integral part of a heightened immunological response, allelic variants that support this response would have undergone strong positive selection pressure and thus would be both numerous and prevalent, as they appear to be. However, because

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"Depression risk alleles identified by both candidate gene and genome-wide association study (GWAS) methodologies were found to be regularly associated with immune responses to infection that were likely to enhance survival in the ancestral environment. **Moreover, data support the role of specific depressive symptoms in pathogen host defense including hyperthermia, reduced bodily iron stores, conservation/withdrawal behavior, hypervigilance and anorexia."**

CNS Drugs. 2012 May 1;26(5):391-401. doi: 10.2165/11632000-000000000-00000.

Minocycline: therapeutic potential in psychiatry.

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Abstract

Pharmacological interventions to treat psychiatric illness have previously focused on modifying dysfunctional neurotransmitter systems to improve symptoms. However, imperfect understanding of the aetiology of these heterogeneous syndromes has been associated with poor treatment outcomes for many individuals. Growing evidence suggests that oxidative stress, inflammation, changes in glutamatergic pathways and neurotrophins play important roles in many psychiatric illnesses including mood disorders, schizophrenia and addiction. These novel insights into pathophysiology allow new treatment targets to be explored. Minocycline is an antibiotic that can modulate glutamate-induced excitotoxicity, and has antioxidant, anti-inflammatory and neuroprotective effects. Given that these mechanisms overlap with the newly understood pathophysiological pathways, minocycline has potential as an adjunctive treatment in psychiatry. To date there have been promising clinical indications that minocycline may be a useful treatment in psychiatry, albeit from small trials most of which were not placebo controlled. Case reports of individuals with schizophrenia, psychotic symptoms and bipolar depression have shown serendipitous benefits of minocycline treatment on psychiatric symptoms. Minocycline has been trialled in open-label or small randomized controlled trials in psychiatry. Results vary, with findings supporting use in schizophrenia, but showing less benefit for nicotine dependence and obsessive-compulsive disorder. Given the limited data from rigorous clinical trials, further research is required. However, taken together, the current evidence suggests minocycline may be a promising novel therapy in psychiatry.

“Growing evidence suggests that oxidative stress, inflammation, changes in glutamatergic pathways and neurotrophins play important roles in many psychiatric illnesses including mood disorders, schizophrenia and addiction. These novel insights into pathophysiology allow new treatment targets to be explored. **Minocycline is an antibiotic that can modulate glutamate-induced excitotoxicity, and has antioxidant, anti-inflammatory and neuroprotective effects.”**

Maes et al. BMC Medicine 2012, 10:66
http://www.biomedcentral.com/1741-7015/10/66

BMC Medicine

REVIEW Open Access

Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways

Michael Maes[1*], Michael Berk[2,3,4,5], Lisa Goehler[6], Cai Song[7,8], George Anderson[9], Piotr Gałecki[10] and Brian Leonard[11]

Abstract

It is of considerable translational importance whether depression is a form or a consequence of sickness behavior. Sickness behavior is a behavioral complex induced by infections and immune trauma and mediated by pro-inflammatory cytokines. It is an adaptive response that enhances recovery by conserving energy to combat acute inflammation. There are considerable phenomenological similarities between sickness behavior and depression, for example, behavioral inhibition, anorexia and weight loss, and melancholic (anhedonia), physio-somatic (fatigue, hyperalgesia, malaise), anxiety and neurocognitive symptoms. In clinical depression, however, a transition occurs to sensitization of immuno-inflammatory pathways, progressive damage by oxidative and nitrosative stress to lipids, proteins, and DNA, and autoimmune responses directed against self-epitopes. The latter mechanisms are the substrate of a neuroprogressive process, whereby multiple depressive episodes cause neural tissue damage and consequent functional and cognitive sequelae. Thus, shared immuno-inflammatory pathways underpin the physiology of sickness behavior and the pathophysiology of clinical depression explaining their partially overlapping phenomenology. Inflammation may provoke a Janus-faced response with a good, acute side, generating protective inflammation through sickness behavior and a bad, chronic side, for example, clinical depression, a lifelong disorder with positive feedback loops between (neuro)inflammation and (neuro) degenerative processes following less well defined triggers.

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Keywords: depression, sickness behavior, inflammation, oxidative stress, cytokines

Introduction

The first inkling that there are phenomenological similarities between clinical depression and sickness behavior and that both conditions may share common pathways, that is, activation of the inflammatory responses system (IRS) was published in 1993 [1,2]. Sickness behavior is a behavioral complex that is typically induced by acute infections and tissue injury in many mammalian species. The characteristic behavioral pattern consists of malaise, hyperalgesia, pyrexia, listlessness and disinterest in social interactions with the environment, lethargy, behavioral inhibition, reduction of locomotor activity, exploration and grooming, reduction of reproductive performance, anhedonia, somnolence and sleepiness, anorexia and weight loss, failure to concentrate, and anxiety. There is evidence that sickness behavior is mediated through the effects of pro-inflammatory cytokines (PICs), such as IL-1, TNFα and IL-6 [3-10]. In this context, there is abundant evidence that clinical depression is an immuno-inflammatory disorder characterized by among other things increased levels of PICs and acute phase proteins, including C-reactive protein and haptoglobin [11-20].

Characteristic symptoms of major depression include anorexia, weight loss, fatigue, lethargy, sleep disorders, hyperalgesia, reduction of locomotor activity, and failure to concentrate (American Psychiatric Association). Moreover, 'vegetative symptoms' of depression, such as anorexia, weight loss, and psychomotor retardation, are significantly associated with inflammatory markers in clinical depression, such as increased levels of plasma haptoglobin, an acute phase protein, synthesis of which is induced by the three abovementioned PICs [1,2].

Thus, it may be concluded that there are striking behavioral and inflammatory similarities between both sickness behavior and clinical depression [1,2,11].

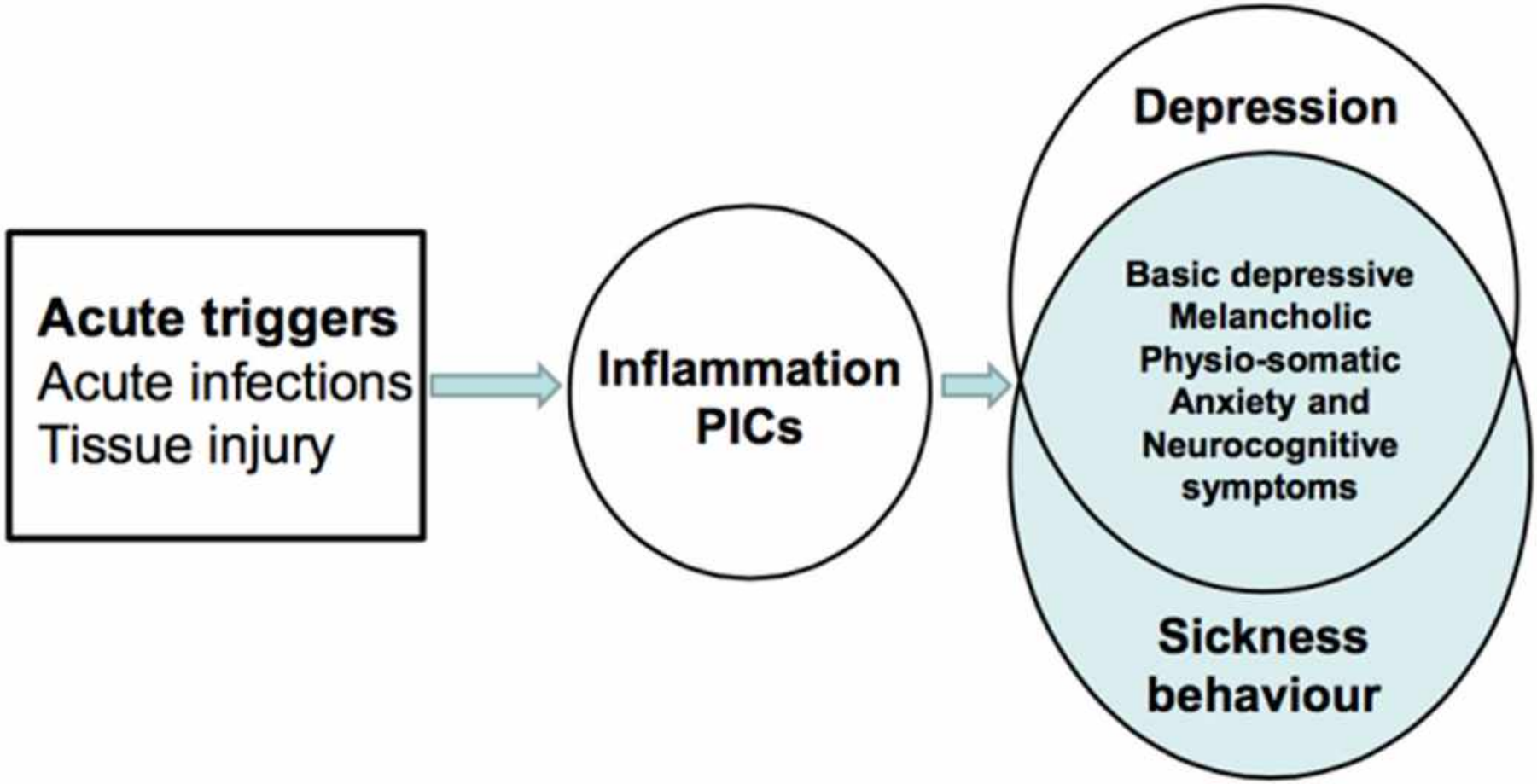

Figure 1 Inflammation causes sickness and depression. This Figure shows the theory that acute triggers cause inflammation and increased production of pro-inflammatory cytokines (PICs), which is associated with the onset of sickness behavior and clinical depression.

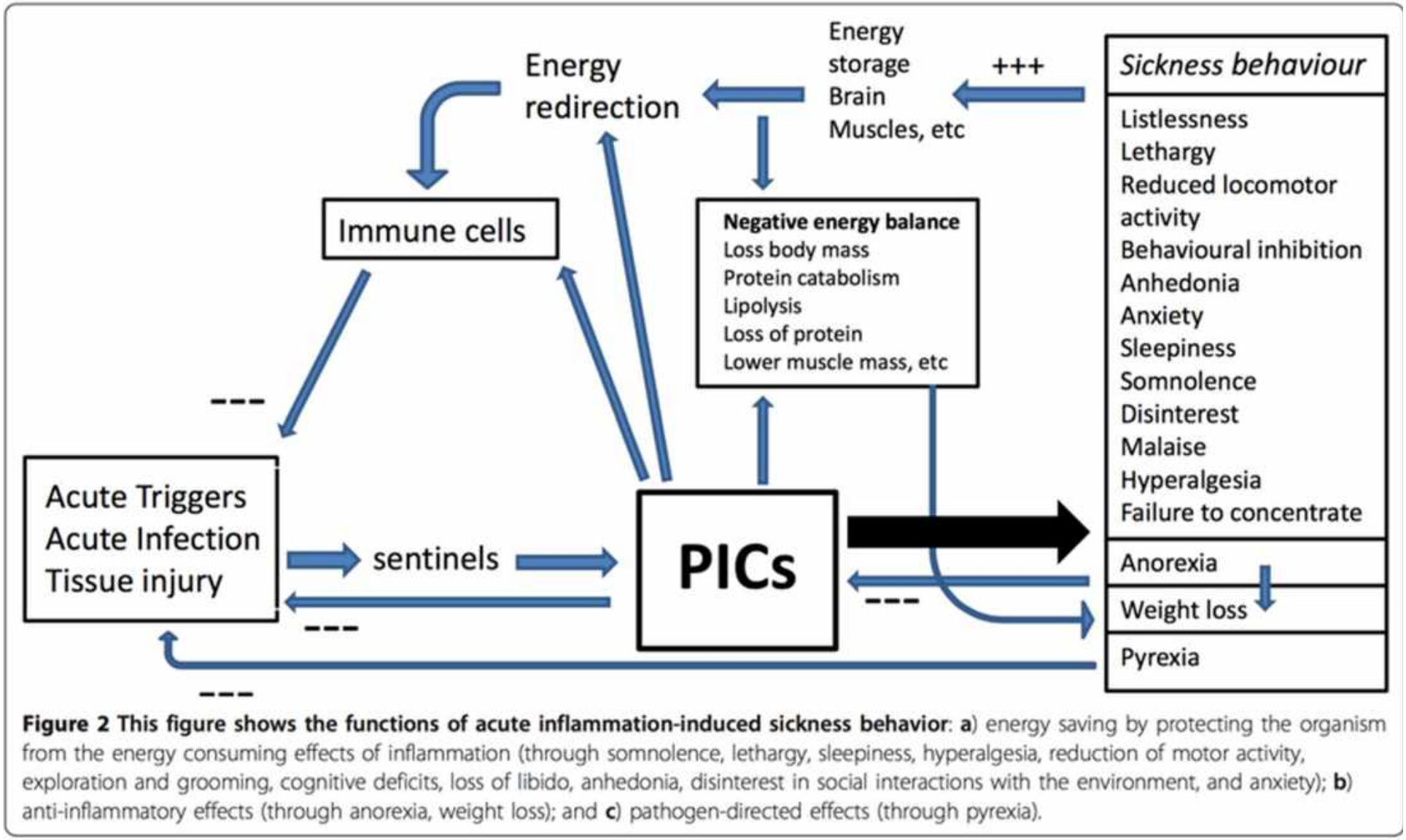

Figure 2 This figure shows the functions of acute inflammation-induced sickness behavior: **a**) energy saving by protecting the organism from the energy consuming effects of inflammation (through somnolence, lethargy, sleepiness, hyperalgesia, reduction of motor activity, exploration and grooming, cognitive deficits, loss of libido, anhedonia, disinterest in social interactions with the environment, and anxiety); **b**) anti-inflammatory effects (through anorexia, weight loss); and **c**) pathogen-directed effects (through pyrexia).

Review Article

GlycA: a new biomarker for systemic inflammation and cardiovascular disease (CVD) risk assessment

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: RA Ballout; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Alan T. Remaley, MD, PhD; Rami A. Ballout, MD. Lipoprotein Metabolism Section, Translational and Vascular Medicine Branch, National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health, Bethesda, MD, USA. Email: alan.remaley@nih.gov; rami-ballout@hotmail.com.

Abstract: The GlycA test is a recently developed proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy-based assay that has been gaining increased interest as a serum biomarker for systemic inflammation, and consequently, as a potential biomarker for cardiovascular disease (CVD) risk assessment. The test has undergone investigation in several large cohort studies, since its development, to assess its predictive value for incident CVD events, CVD-associated mortality, and all-cause mortality. Despite variation in the generated estimates by these studies, they have all consistently demonstrated moderate-strength positive correlations between baseline GlycA levels, and incident CVD event rates and associated mortality. These correlations withheld testing even after adjusting for several other established CVD risk factors, including notable inflammatory biomarkers such as high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Compared with hsCRP, which is a well-known inflammatory biomarker for CVD risk assessment, GlycA has a comparable predictive value for future CVD-related events. However, the indications to pursue GlycA testing, and its clinical utility in patient care management, are yet to be determined. In this review, we define the GlycA test and what it "measures", and provide a brief summary of the findings of studies showing its association with incident CVD rates, and CVD-related mortality, as well as its correlation with other inflammatory biomarkers, namely hsCRP. Finally, we highlight the analytical advantages of the GlycA test, compared with "traditional" inflammatory biomarkers, while also mentioning its current limitations.

Keywords: Atherosclerosis; coronary artery disease; GlycA; high-sensitivity C-reactive protein (hsCRP); N-glycans; acute phase reactants

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doi: 10.21037/jlpm.2020.03.03
View this article at: http://dx.doi.org/10.21037/jlpm.2020.03.03

Inflammation as a key driver of cardiovascular disease (CVD)

Although there was resistance when the concept was first proposed in the early 1990s, it is now widely accepted that inflammation plays a pivotal role in the pathogenesis of atherosclerosis (1-3). The actual sequence of events and initial triggers of the inflammatory cascade underlying atherosclerosis remain an active area of investigation. However, it is widely accepted that it starts with some type of endothelial injury, which allows for LDL and small remnant lipoproteins to across the endothelium, infiltrating into the intima. Within the intimal space, these lipoproteins are subject to various modifications,

"Compared with hsCRP, which is a well-known inflammatory biomarker for CVD risk assessment, GlycA has a comparable predictive value for future CVD-related events. However, the indications to pursue GlycA testing, and its clinical utility in patient care management, are yet to be determined."

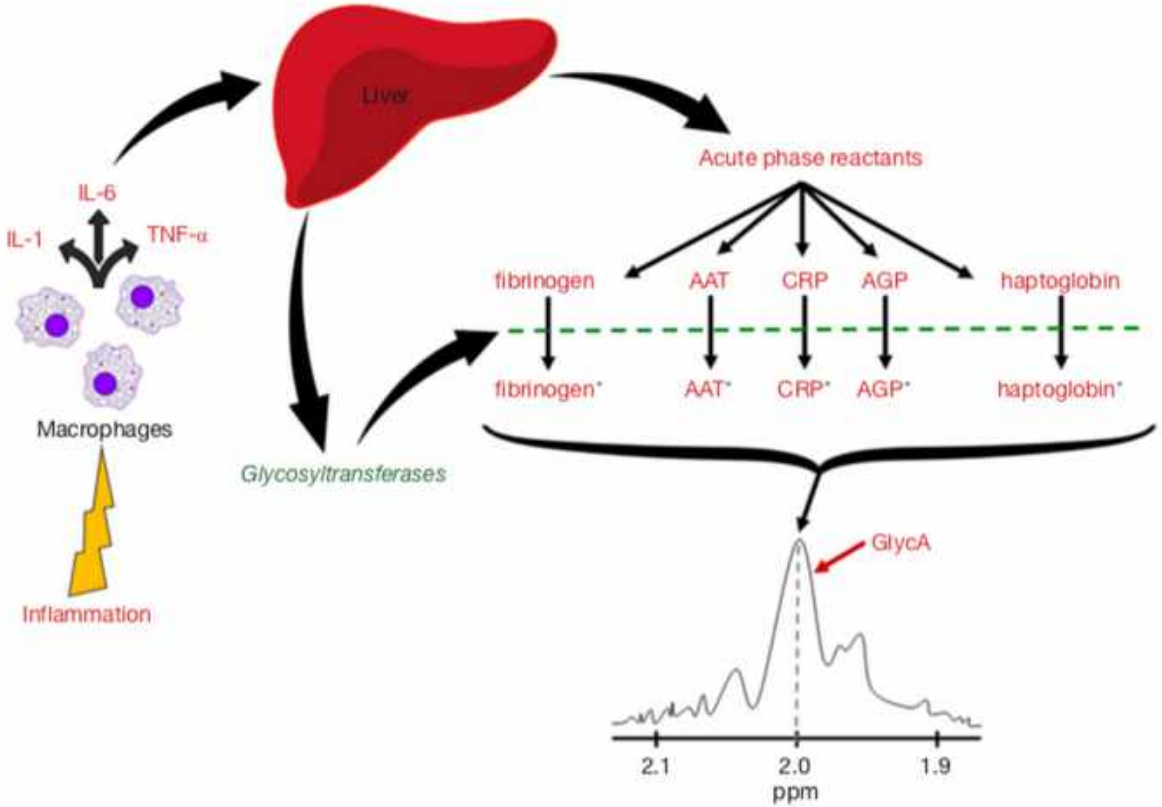

Figure 1 A simplified illustration showing how the GlycA 'peak' on ^{1}H-NMR relates to systemic inflammation. In the setting of inflammation, irrespective of the trigger, macrophages are recruited to the site of inflammation where they secrete a variety of cytokines, namely IL-1, IL-6, and TNF-alpha. These cytokines act locally to induce an inflammatory response aimed at removing the insulting trigger and promoting subsequent tissue recovery. However, some of these cytokines also enter the systemic circulation and reach the liver, where they induce an increased production and secretion of several so-called acute phase reactants, as well as various glycosylation-mediating enzymes, known as glycosyltransferases, which alter the glycosylation patterns of the latter acute phase reactants. The acute phase reactants themselves, and their glycosyltransferase-modified derivatives (denoted by * in the figure) contribute to the GlycA peak seen on the ^{1}H-NMR analyzer. AAT, alpha-1-antitrypsin; AGP, alpha-1-acid glycoprotein; CRP, C-reactive peptide.

Trends in Cognitive Sciences

CellPress REVIEWS

Review

Can't or Won't? Immunometabolic Constraints on Dopaminergic Drive

Michael T. Treadway [1,2,*] Jessica A. Cooper,[1] and Andrew H. Miller[2]

Inflammatory cytokines have been shown to have a direct effect on mesolimbic dopamine (DA) that is associated with a reduced willingness to expend effort for reward. To date, however, the broader implications of this communication between inflammation and mesolimbic DA have yet to be explored. Here, we suggest that the metabolic demands of chronic low-grade inflammation induce a reduction of striatal DA that in turn leads to a steeper effort-discounting curve because of reduced perceived ability (can't) versus preference (won't) for reward. This theoretical framework can inform how the mesolimbic DA system responds to increased immunometabolic demands during chronic inflammation, ultimately contributing to motivational impairments in psychiatric and other medical disorders.

Highlights

Converging evidence suggests that the mesolimbic dopamine (DA) system is directly affected by increases in inflammatory cytokines associated with chronic, low-grade inflammation.

The reasons for this immune–DA communication are unclear, but one novel hypothesis is that inflammatory cytokines signal immunometabolic shifts that impact the valuation of future actions as a function of available energy resources.

Future work on effort-based discounting models should incorporate variables related to inflammation and immunometabolic state.

Dopamine, Effort, and the Inflammatory Response

The efficient utilization of energy resources for goal-directed behaviors is believed to have been a driving force in the evolutionary development of the central nervous system (CNS) and its response to the environment [1]. Over the past several decades, our understanding of the neurobiological mechanisms that govern exploratory behavior and goal pursuit has expanded exponentially. This work has revealed a central role for mesolimbic signaling of the phylogenetically conserved neurotransmitter dopamine (DA) in shaping willingness to expend energy [2–5] or forage [6–8] and the drive to overcome obstacles [9,10] in pursuit of rewards. To date, however, the majority of studies have focused on the behavioral consequences of mesolimbic DA signaling within the striatal, limbic, and cortical areas that mediate various aspects of normal and abnormal reward-seeking behavior. By contrast, a smaller body of work has focused on inputs to the mesolimbic DAergic system from sources outside the CNS that communicate relevant bodily states to influence the responsivity of DAergic neurons and the calculus of effort-based decision-making.

One emerging source of this external regulation is inflammation. There is growing appreciation that many of the behavioral sequelae associated with infection and the related inflammatory response, including alterations in reward-seeking behavior (as occurs in so-called 'sickness behavior'), are a direct consequence of the impact of inflammatory cytokines on mesolimbic DA signaling [11,12]. To date, however, the broader implications of this mesolimbic DA–immune axis have yet to be fully explored. Herein, we propose that inflammatory signaling molecules play a critical role in communicating information relevant to shifts in immunometabolism that impact available energy resources in the body – a prerequisite for the mesolimbic DA system to generate accurate estimates of expected value of reward and guide effort allocation and energy expenditure. This function may have originated during evolution as a means of suppressing exploratory behavior and shifting energy resources to the immune system for fighting infection and healing wounds in an ancestral environment rife with pathogens and predators [13,14]. Nevertheless, we suggest that, in the modern world, communication between the immune

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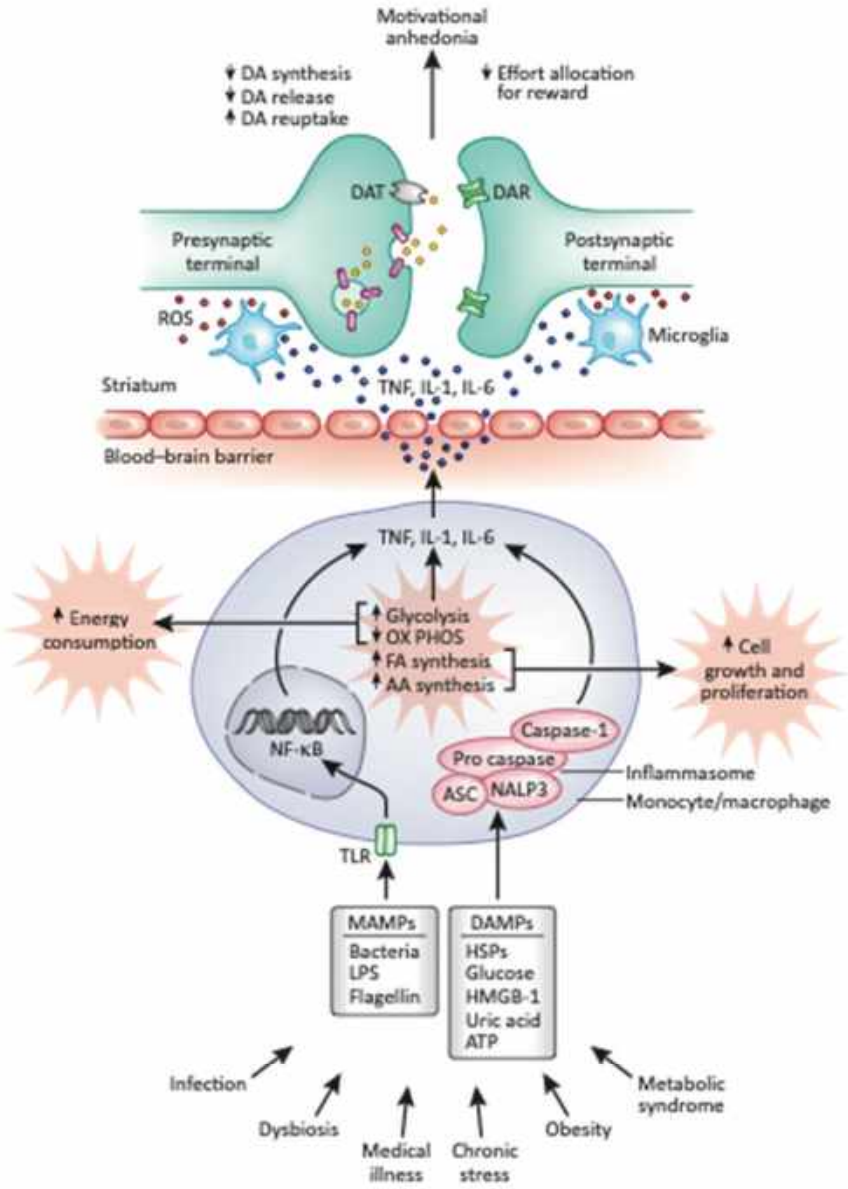
"Here, we suggest that the metabolic demands of chronic low-grade inflammation induce a reduction of striatal DA that in turn leads to a steeper effort-discounting curve because of reduced perceived ability (can't) versus preference (won't) for reward."



Trends in Cognitive Sciences

Figure 1. Causes and Consequences of the Immunometabolic Shift During Inflammation. Several well-established lifestyle and medical factors lead to the release of microbe-associated molecular patterns (MAMPs) and danger-associated molecular patterns (DAMPs), which bind to pattern recognition receptors on immune cells including Toll-like receptors (TLRs) that in turn activate nuclear factor kappa B (NF-κB) and the NACHT, LRR, and PYD domain-containing 3 (NLRP3) inflammasome. Activation of NF-κB and the protein components of the NLRP3 inflammasome including procaspase, caspase, and a caspase activation and recruitment domain (ASC) lead to immune cell activation and a metabolic shift from the more efficient oxidative phosphorylation (OX PHOS) to glycolysis to support rapid cell growth and proliferation, albeit at the cost of increased energy expenditure. Cellular activation also leads to the production and release of inflammatory cytokines that can access mesolimbic dopamine (DA) neurons and immune cells (microglia) in key brain regions including the striatum through stress- and potentially inflammation-induced disruptions in the blood–brain barrier, ultimately contributing to disruptions in DA synthesis, release, and reuptake. Activation of microglia can also lead to the release of reactive oxygen species (ROS), which can disrupt mitochondrial and ultimately neuronal function while also exacerbating inflammatory responses. These effects on mesolimbic DA lead to decreased effort allocation for reward and motivational anhedonia due to reductions in perceived ability (can't) but not preference (won't). Abbreviations: AA, amino acid; DAR, dopamine receptor; DAT, dopamine transporter; FA, fatty acid; HSP, heat shock proteins; HMGB-1, high-mobility group box-1; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor.

(Figure legend continued on the bottom of the next page.)

Comprehensive Neurotransmitter; urine

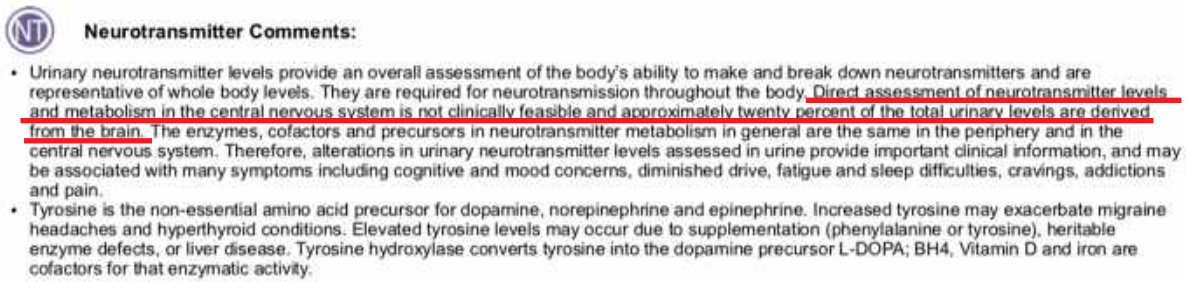
LABRIX by DOCTOR'S DATA

Order: SAMPLE REPORT
Client #: 12345
Doctor: Sample Doctor, MD
Doctors Data Inc.
3755 Illinois Ave.
St. Charles, IL 60174

Patient: Sample Report
Age: 33
Sex: Male
Body Mass Index (BMI): 24.4

Sample Collection	Date/Time
Date Collected	01/07/2019
Wake Up Time	0800
Collection Time	0805
Collection Period	2nd morning void
Date Received	01/08/2019
Date Reported	01/09/2019

Analyte	Result	Unit per Creatinine	WRI (L / H)	Reference Interval
Phenethylamine (PEA)	27	nmol/g		26 - 70
Tyrosine	112	µmol/g		28 - 75
Tyramine	1.9	µmol/g		1.6 - 3.2
Dopamine	211	µg/g		110 - 200
3,4-Dihydroxyphenylacetic acid (DOPAC)	331	µg/g		330 - 1000
3-Methoxytyramine (3-MT)	175	nmol/g		82 - 174
Norepinephrine	21	µg/g		18 - 42
Normetanephrine	133	µg/g		70 - 275
Epinephrine	4.3	µg/g		1.3 - 7.3
Metanephrine	55	µg/g		44 - 103
Norepinephrine / Epinephrine ratio	4.9			< 12
Tryptamine	0.3	µmol/g		0.10 - 0.75
Serotonin	83	µg/g		50 - 98
5-Hydroxyindolacetic acid (5-HIAA)	1450	µg/g		1600 - 6000
Glutamate	42	nmol/g		9.0 - 40.0
Gamma-aminobutyrate (GABA)	2.8	nmol/g		1.6 - 3.5
Glycine	2805	nmol/g		350 - 1500
Histamine	32	µg/g		12 - 30
Taurine	1111	µmol/g		240 - 900
Creatinine	125	mg/dL		35 - 240



Neurotransmitter Comments:

- Urinary neurotransmitter levels provide an overall assessment of the body's ability to make and break down neurotransmitters and are representative of whole body levels. They are required for neurotransmission throughout the body. Direct assessment of neurotransmitter levels and metabolism in the central nervous system is not clinically feasible and approximately twenty percent of the total urinary levels are derived from the brain. The enzymes, cofactors and precursors in neurotransmitter metabolism in general are the same in the periphery and in the central nervous system. Therefore, alterations in urinary neurotransmitter levels assessed in urine provide important clinical information, and may be associated with many symptoms including cognitive and mood concerns, diminished drive, fatigue and sleep difficulties, cravings, addictions and pain.
- Tyrosine is the non-essential amino acid precursor for dopamine, norepinephrine and epinephrine. Increased tyrosine may exacerbate migraine headaches and hyperthyroid conditions. Elevated tyrosine levels may occur due to supplementation (phenylalanine or tyrosine), heritable enzyme defects, or liver disease. Tyrosine hydroxylase converts tyrosine into the dopamine precursor L-DOPA; BH4, Vitamin D and iron are cofactors for that enzymatic activity.

Nat Rev Neurol. 2011 May 3;7(6):323-31. doi: 10.1038/nrneurol.2011.60.

Depression and risk of developing dementia.

Byers AL, Yaffe K.

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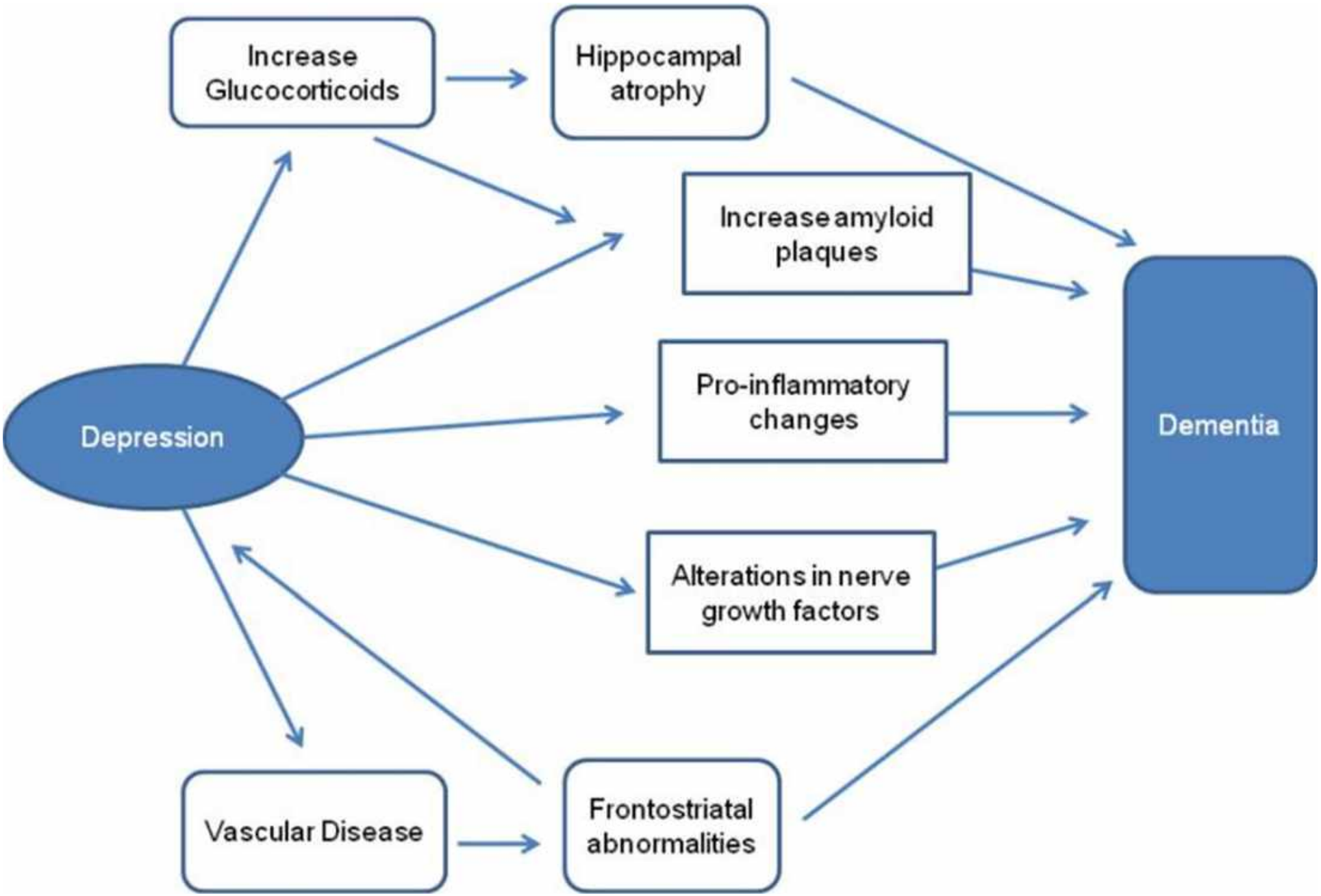
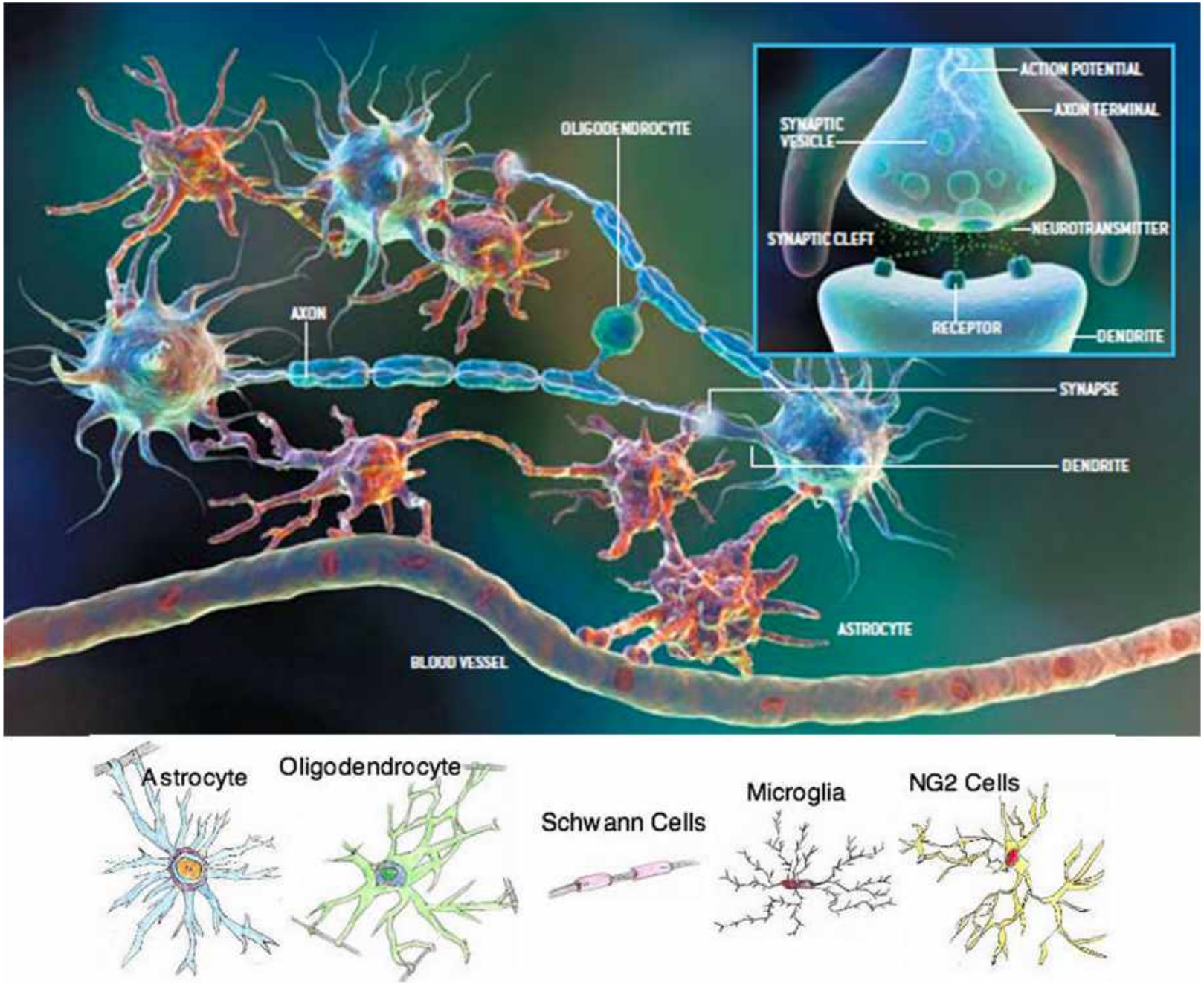
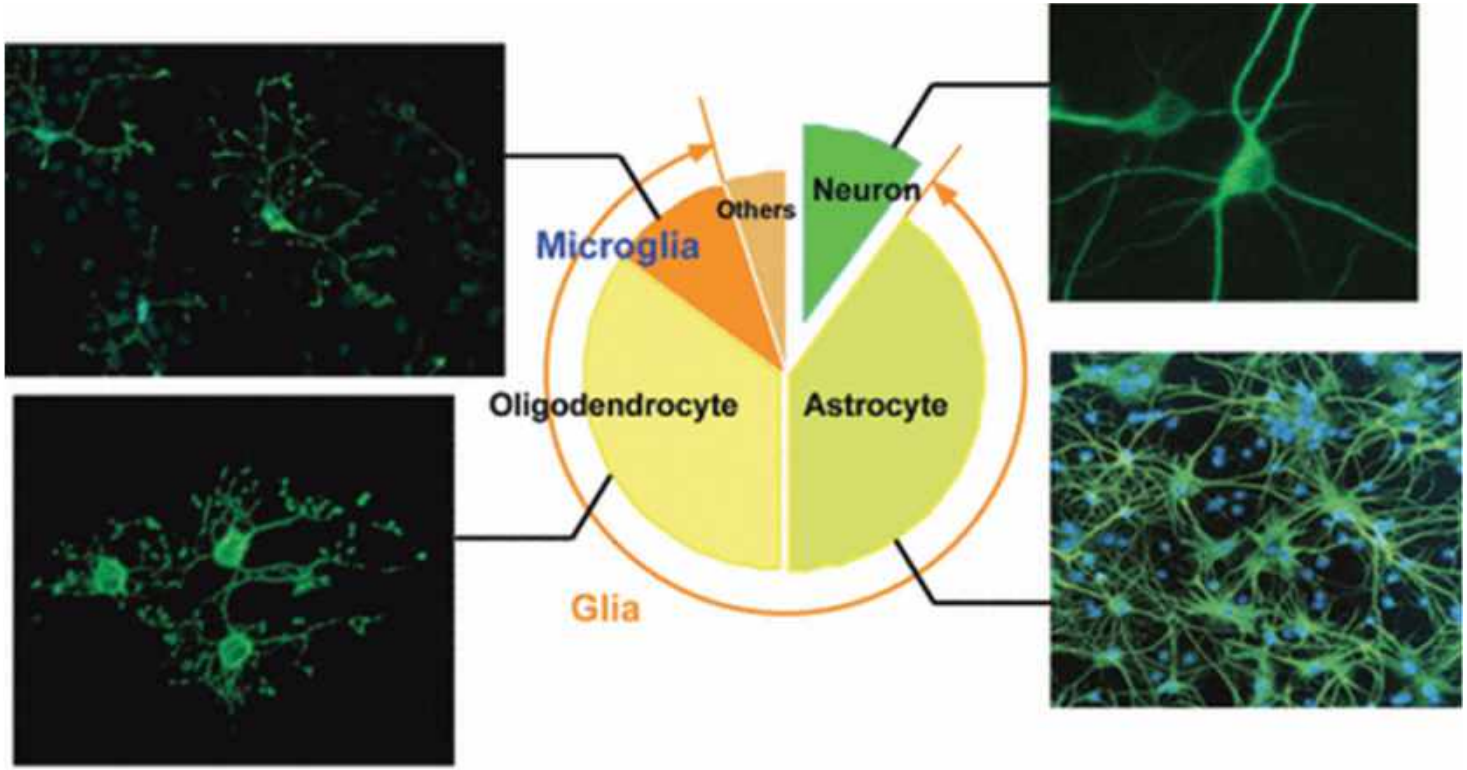
Abstract

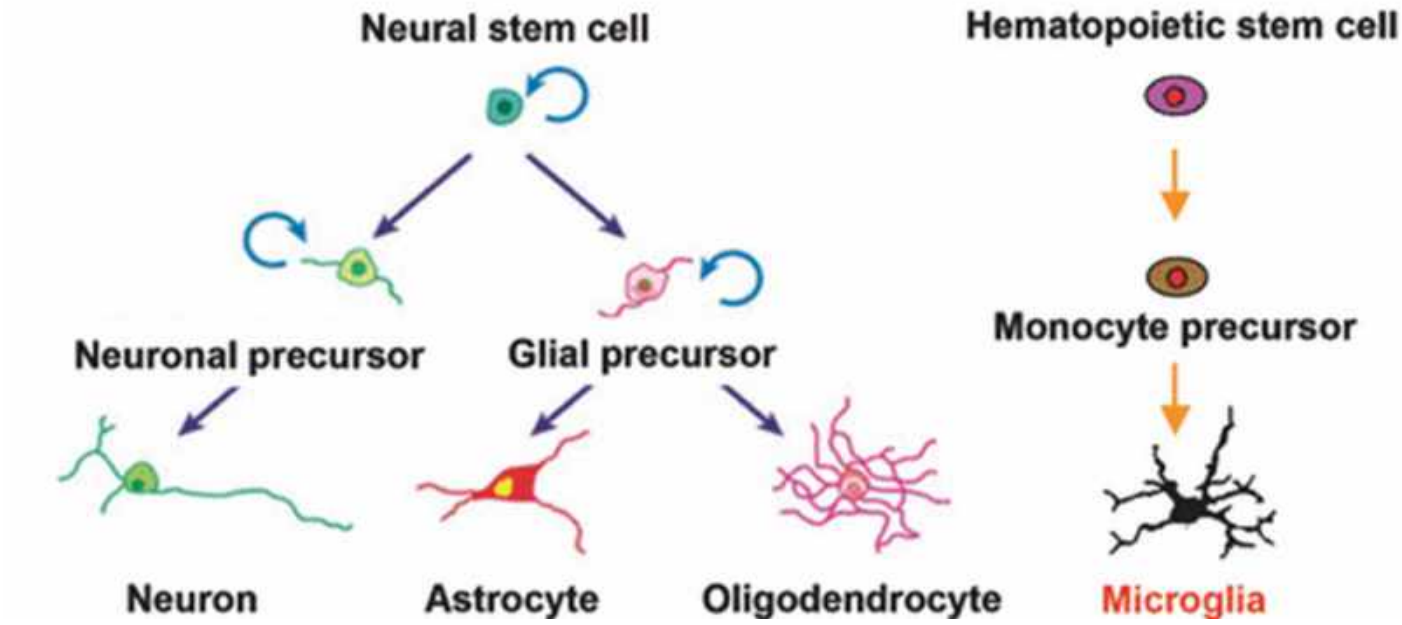
Depression is highly common throughout the life course and dementia is common in late life. Depression has been linked with dementia, and growing evidence implies that the timing of depression may be important in defining the nature of this association. In particular, earlier-life depression (or depressive symptoms) has consistently been associated with a more than twofold increase in dementia risk. By contrast, studies of late-life depression and dementia risk have been conflicting; most support an association, yet the nature of this association (for example, if depression is a prodrome or consequence of, or risk factor for dementia) remains unclear. The likely biological mechanisms linking depression to dementia include vascular disease, alterations in glucocorticoid steroid levels and hippocampal atrophy, increased deposition of amyloid-β plaques, inflammatory changes, and deficits of nerve growth factors. Treatment strategies for depression could interfere with these pathways and alter the risk of dementia. Given the projected increase in dementia incidence in the coming decades, understanding whether treatment for depression alone, or combined with other regimens, improves cognition is of critical importance. In this Review, we summarize and analyze current evidence linking late-life and earlier-life depression and dementia, and discuss the primary underlying mechanisms and implications for treatment.


Increase Glucocorticoids
Hippocampal atrophy
Increase amyloid plaques
Pro-inflammatory changes
Alterations in nerve growth factors
Depression
Dementia
Vascular Disease
Frontostriatal abnormalities



SOURCE: **The Other half of the Brain** Scientific American 2004


Others
Neuron
Microglia
Oligodendrocyte
Astrocyte
Glia


Neural stem cell
Hematopoietic stem cell
Monocyte precursor
Neuronal precursor
Glial precursor
Neuron
Astrocyte
Oligodendrocyte
Microglia

Inflammation, Glutamate, and Glia in Depression: *A Literature Review*

By Leah McNally, BS, Zubin Bhagwagar, MD, PhD, and Jonas Hannestad, MD, PhD

ABSTRACT

Multiple lines of evidence suggest that inflammation and glutamate dysfunction contribute to the pathophysiology of depression. In this review we provide an overview of how these two systems may interact. Excess levels of inflammatory mediators occur in a subgroup of depressed patients. Studies of acute experimental activation of the immune system with endotoxin and of chronic activation during interferon-α treatment show that inflammation can cause depression. Peripheral inflammation leads to microglial activation which could interfere with excitatory amino acid metabolism leading to inappropriate glutamate receptor activation. Loss of astroglia, a feature of depression, upsets the balance of anti- and pro-inflammatory mediators and further impairs the removal of excitatory amino acids. Microglia activated by excess inflammation, astroglial loss, and inappropriate glutamate receptor activation

Needs Assessment

Depression is a serious disorder for which available treatments are inadequate and the pathogenesis of which is poorly understood. Recent research has highlighted the potential role of excess inflammation and dysregulated glutamate neurotransmission in depression. The convergence of these two fields may reveal novel treatment targets.

Learning Objectives

At the end of this activity, the participant should be able to:

- List inflammatory mediators commonly elevated in plasma in depression.
- Understand the potential role of the kynurenine pathway in depression.
- Enumerate two ways in which inflammatory mediators and glutamate interact.
- Describe the specific role and interactions between microglia and astroglia and how this may contribute to depression.

Target Audience: Neurologists and psychiatrists

CME Accreditation Statement

This activity has been planned and implemented in accordance with the Essentials and Standards of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the Mount Sinai School of Medicine and MBL Communications, Inc. The Mount Sinai School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation

The Mount Sinai School of Medicine designates this educational activity for a maximum of 3 *AMA PRA Category 1 Credit(s)™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

This activity has been peer-reviewed and approved by Eric Hollander, MD, chair at the Mount Sinai School of Medicine. Review date: April 28, 2008. Dr. Hollander does not have an affiliation with or financial interest in any organization that might pose a conflict of interest.

To Receive Credit for This Activity

Read this article and the two CME-designated accompanying articles, reflect on the information presented, and then complete the CME posttest and evaluation found on page 528. To obtain credits, you should score 70% or better. Early submission of this posttest is encouraged; please submit this posttest by June 1, 2010, to be eligible for credit. Release date: June 1, 2008. Termination date: June 30, 2010. The estimated time to complete all three articles and the posttest is 3 hours.

Ms. McNally is a fourth-year medical student at Yale University School of Medicine in New Haven, Connecticut. Dr. Bhagwagar is assistant professor and Dr. Hannestad is clinical instructor, both in the Department of Psychiatry at Yale University School of Medicine.

Faculty Disclosures: Ms. McNally and Dr. Hannestad do not have an affiliation with or financial interest in an organization that might pose a conflict of interest. Dr. Bhagwagar receives research/grant support from Bristol-Myers Squibb, and is on the speaker's bureaus of AstraZeneca, Bristol-Myers Squibb, and Janssen.

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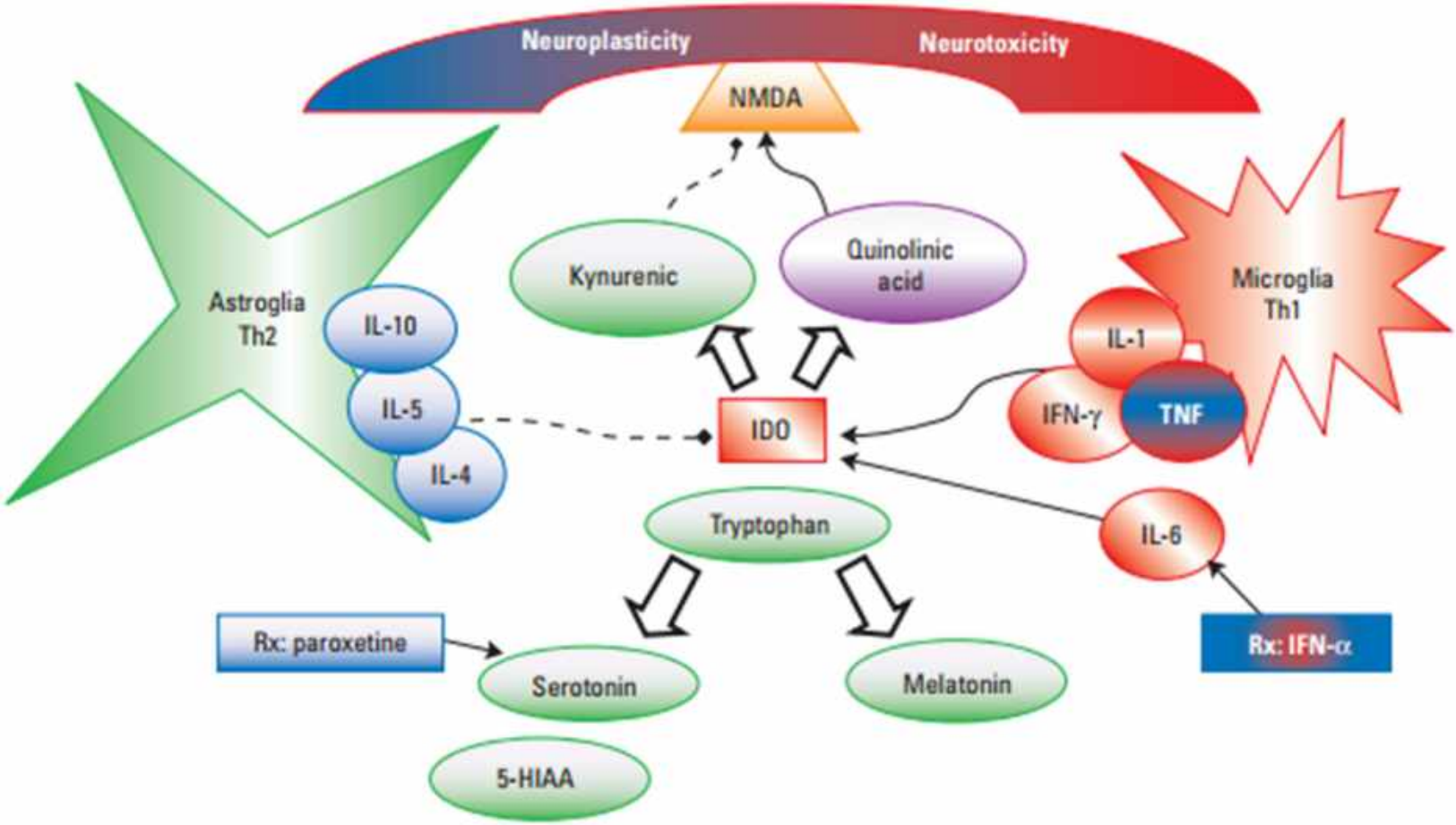
FIGURE 1.
Tryptophan, kynurenine, and quinolinic acid*



* IDO converts tryptophan to kynurenic acid, an NMDA receptor antagonist. This reduces tryptophan availability for serotonin synthesis. Microglia activated by inflammatory mediators can convert tryptophan to quinolinic acid, an NMDA agonist. Therefore, pro-inflammatory mediators favor the production of quinolinic acid, while anti-inflammatory mediators inhibit synthesis of quinolinic acid. Decreased serotonin availability and excessive glutamate receptor agonism have been implicated in depression. Depression associated with IFN-α treatment may occur because of interference with this pathway, and selective serotonin reuptake inhibitors, such as paroxetine, are, therefore, efficacious in treating depression caused by IFN-α. The word neurotoxicity denote consequences of excess excitatory amino acid levels, however, neurotoxicity has not been unequivocally demonstrated in depression.

NMDA=*N*-methyl-D-aspartate; Th=T helper cell; IL=interleukin; IDO=indoleamine 2,3-dioxygenase; IFN=interferon; TNF=tumor necrosis factor; Rx=prescription; 5-HIAA=5-hydroxyindoleacetic acid.

McNally L, Bhagwagar Z, Hannestad J. *CNS Spectr.* Vol 13, No 6. 2008.

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progress in Neurobiology

www.elsevier.com/locate/pneurobio

Kynurenines in the CNS: from endogenous obscurity to therapeutic importance

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Received 17 March 2000

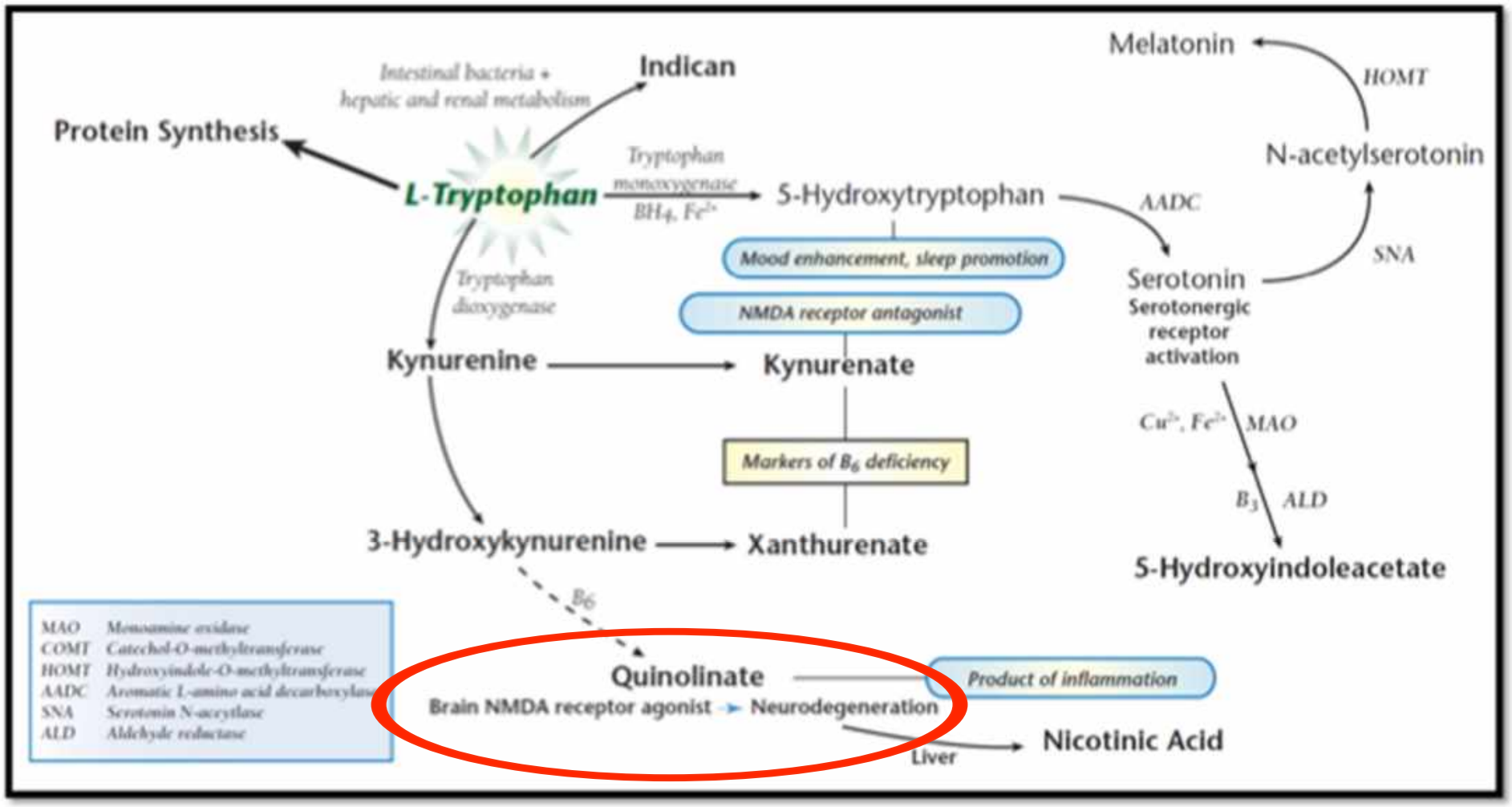
Abstract

In just under 20 years the kynurenine family of compounds has developed from a group of obscure metabolites of the essential amino acid tryptophan into a source of intensive research, with postulated roles for quinolinic acid in neurodegenerative disorders, most especially the AIDS-dementia complex and Huntington's disease. One of the kynurenines, kynurenic acid, has become a standard tool for use in the identification of glutamate-releasing synapses, and has been used as the parent for several groups of compounds now being developed as drugs for the treatment of epilepsy and stroke. The kynurenines represent a major success in translating a basic discovery into a source of clinical understanding and therapeutic application, with around 3000 papers published on quinolinic acid or kynurenic acid since the discovery of their effects in 1981 and 1982. This review concentrates on some of the recent work most directly relevant to the understanding and applications of kynurenines in medicine.

Keywords: Kynurenine; Kynurenic acid; Quinolinic acid; Tryptophan; Neurodegeneration; Neuroprotection; Excitotoxicity

Contents

" As an NMDA receptor agonist, quinolinic acid similarly proved able to cause neuronal death following direct intracerebral administration….. **There is strong evidence that the activation of NMDA receptors is critical in the production of brain damage in AIDS."**



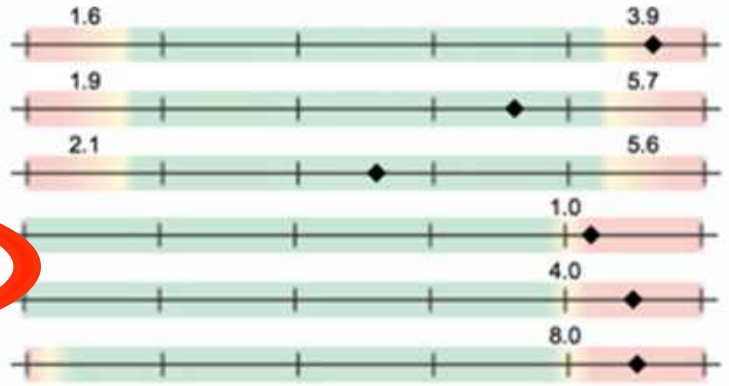
SOURCE: Laboratory Evaluations in Integrative and Functional Medicine
by Richard Lord, PhD & J. Alexander Bralley, PhD

Cell Regulation Markers

Neurotransmitter Metabolism Markers

(Tyrosine, Tryptophan, B6, antioxidants)

22	Vanilmandelate	4.3	H	1.2 - 5.3
23	Homovanillate	4.1		1.4 - 7.6
24	5-Hydroxyindoleacetate	3.2		1.6 - 9.8
25	Kynurenate	1.2	H	<= 1.5
26	Quinolinate	5.8	H	<= 5.8
27	Picolinate	9.6	H	2.8 - 13.5



Oxidative Damage and Antioxidant Markers

(Vitamin C and other antioxidants)

28	p-Hydroxyphenyllactate	0.44	H	<= 0.66
29	8-Hydroxy-2-deoxyguanosine *	4.7		<= 7.6

* Units for 8-Hydroxy-2-deoxyguanosine are ng/mg creatinine.

Toxicants and Detoxification

Detoxification Indicators

(Arg, NAC, Met, Mg and antioxidants)

30	2-Methylhippurate	0.014		<= 0.192
31	Orotate	<DL*		<= 1.01
32	Glucarate	11.1	H	<= 10.7
33	a-Hydroxybutyrate	1.2	H	<= 0.9
34	Pyroglutamate	35		28 - 88
35	Sulfate	2,066		690 - 2,988

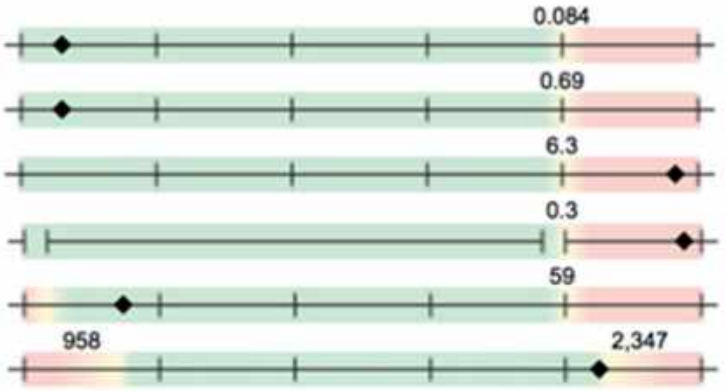
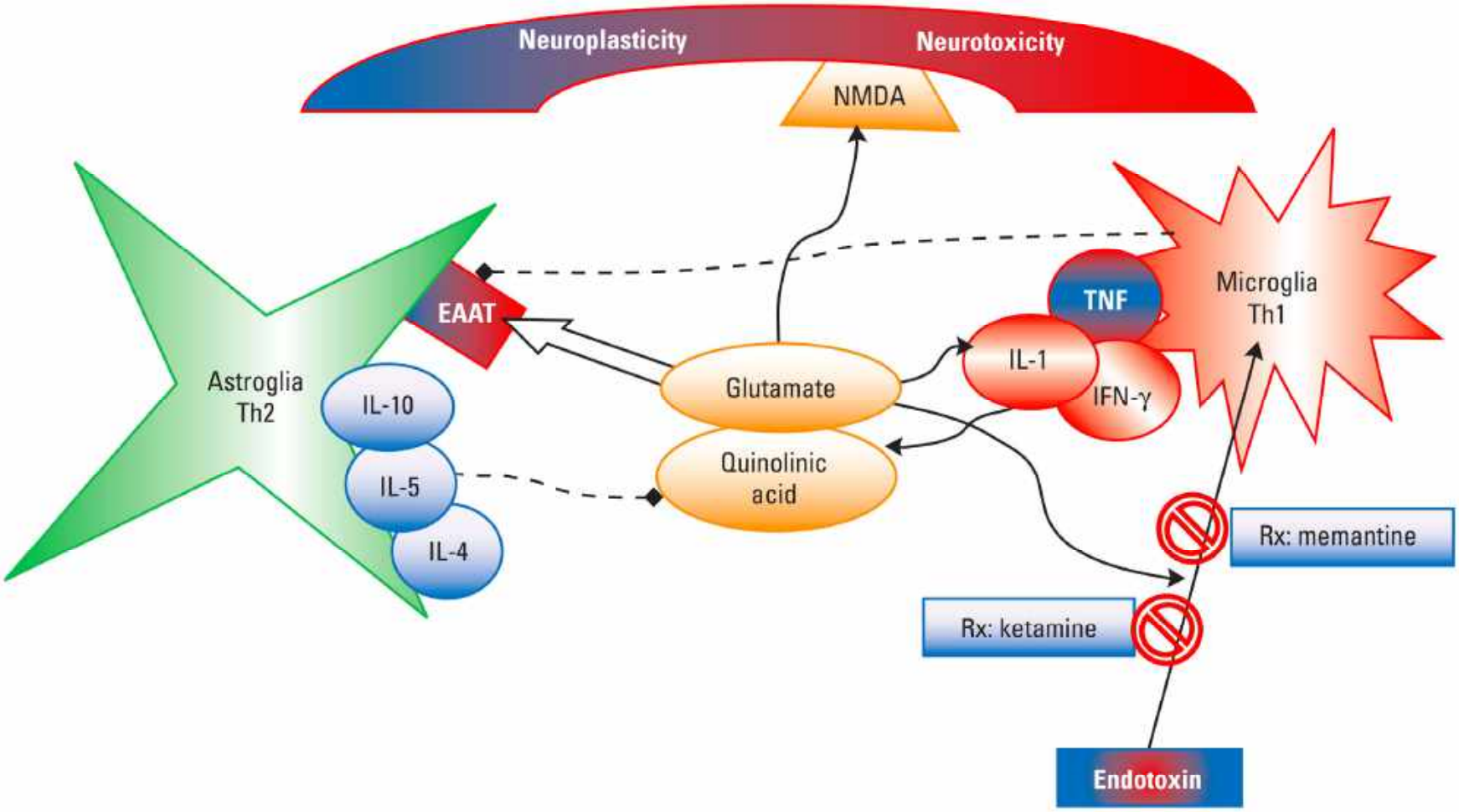


FIGURE 2.

Excitatory amino acid production and removal*



* Glutamate and quinolinic acid are excitatory amino acids that can have neurotoxic effects through NMDA receptor agonism. Excess glutamate is removed by astroglial EAAT. Microglia, activated by pro-inflammatory mediators, produce quinolinic acid and inhibit EAAT expression, potentially leading to excess NMDA agonism. NMDA antagonists such as ketamine and memantine can inhibit microglial release of pro-inflammatory mediators. How this occurs is not known.

NMDA=*N*-methyl-D-aspartate; Th=T helper cell; IL=interleukin; EAAT=excitatory amino acid transporter; TNF=tumor necrosis factor; IFN=interferon; Rx=prescription.

McNally L, Bhagwagar Z, Hannestad J. *CNS Spectr.* Vol 13, No 6. 2008.

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REVIEW ARTICLE
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NMDA receptor activity in neuropsychiatric disorders

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N-Methyl-d-aspartate (NMDA) receptors play a variety of physiologic roles and their proper signaling is essential for cellular homeostasis. Any disruption in this pathway, leading to either enhanced or decreased activity, may result in the manifestation of neuropsychiatric pathologies such as schizophrenia, mood disorders, substance induced psychosis, Huntington's disease, Alzheimer's disease, and neuropsychiatric systemic lupus erythematosus. Here, we explore the notion that the overlap in activity of at least one biochemical pathway, the NMDA receptor pathway, may be the link to understanding the overlap in psychotic symptoms between diseases. This review intends to present a broad overview of those neuropsychiatric disorders for which alternations in NMDA receptor activity is prominent thus suggesting that continued direction of pharmaceutical intervention to this pathway may present a viable option for managing symptoms.

Keywords: NMDA, psychiatry, schizophrenia, mood disorders, substance induced psychosis, Huntington's disease, Alzheimer's disease, neuropsychiatric systemic lupus erythematosus

INTRODUCTION

Diagnosis of psychiatric disorders is done clinically by focusing on observable symptoms and behaviors rather than on underlying psychodynamic processes or on the results of laboratory or imaging testing. Understanding the descriptive symptoms for mental disorders is vital in order to properly diagnose each psychiatric disease. The instruments most commonly used to diagnose and categorize mental illnesses, the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and the World Health Organization's International Statistical Classification of diseases and Related Health Problems (ICD-10), focus on objective observations, without offering any discussion on the etiologies for these diseases. From a molecular, biochemical, and ultimately therapeutic perspective, it is equally as essential to characterize the role of various receptors, ligands, and neurotransmitters that when modified alter the manifestation of these symptoms.

Psychotic symptoms can be present in primary psychiatric disorders (schizophrenia, schizoaffective disorder, mood disorders, substance intoxication) and in psychiatric disorders that occur due to a medical condition such as Huntington's disease (HD), Alzheimer's disease (AD), and systemic lupus erythematosus (SLE). Several neurotransmitters have been linked to the development of psychotic symptoms, with dopamine and serotonin being the most widely studied due to the treatment effect of blocking certain subtypes of these receptors with antipsychotics. Unfortunately, long-term treatment with typical or atypical antipsychotics is limited due to side effects profile and high rates of discontinuation (Lieberman et al., 2005). *N*-Methyl-D-aspartate (NMDA) receptors have also been implicated in the development of psychotic symptoms and are a potential target for the development of novel treatments in the future.

Abbreviations: AA, arachidonic acid; Aβ, amyloid beta; AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BPAD, bipolar affective disorder; CONSIST, Cognitive and Negative Symptoms in Schizophrenia Trial; DSM, Diagnostic and Statistical Manual of Mental Disorders; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, fourth-edition, text-revision; GABA, in γ-aminobutyric acid; HD, Huntington's disease; NMDA, *N*-Methyl-D-aspartate; SIP, substance induced psychosis; NPSLE, neuropsychiatric systemic lupus erythematosus; SLE, systemic lupus erythematosus.

NMDA RECEPTORS IN NEUROPSYCHIATRIC DISORDERS

N-Methyl-D-aspartate receptors are a class of glutamate receptor that when activated, mediate excitatory neurotransmission via passage of non-selective cations, including Ca^{2+}, through the channel. They are abundantly and ubiquitously located throughout the brain and are understood to play a key role in synaptic plasticity and memory function (Stephenson et al., 2008; Li and Tsien, 2009). They are activated by binding the co-agonists glutamate and glycine, in addition to exposure to a positive change in membrane potential across the cell. Functional NMDA receptor heterotetramers are generally formed through a "dimer of dimers" mechanism and are conventionally made up of two glycine binding NR1 subunits and two glutamate binding NR2 subunits (**Figure 1**) (Dongen, 2009). While the NR1 subunit is considered essential to the formation of the complex, data indicates that the NR2 subunits may be interchangeable with either one or two NR3 subunits (Schuler et al., 2008).

The NMDA receptor is known to play an integral role in the regulation of signal transduction in multiple regions of the brain. Accordingly, any homeostatic dysfunction of NMDA receptor activity has the potential to result in a variety of pathologies. Previous authors have found a particularly high concentration of post-synaptic NMDA receptors in limbic structures (Kretschmer, 1999; Tsapakis and Travis, 2002), which is of uttermost importance in the pathogenesis of many psychiatric disorders.

"Any disruption in this pathway, leading to either enhanced or decreased activity, may result in the manifestation of neuropsychiatric pathologies such as schizophrenia, mood disorders, substance induced psychosis, Huntington's disease, Alzheimer's disease, and neuropsychiatric systemic lupus erythematosus."

CASE REPORT

Anti-*N*-methyl-D-aspartate receptor encephalitis in a patient with a 7-year history of being diagnosed as schizophrenia: complexities in diagnosis and treatment

This article was published in the following Dove Press journal:
Neuropsychiatric Disease and Treatment
11 June 2015
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Abstract: Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is a form of autoimmune encephalitis associated with antibodies against the NR1 subunits of NMDARs. Although new-onset acute prominent psychotic syndromes in patients with NMDAR encephalitis have been well documented, there is a lack of case studies on differential diagnosis and treatment of anti-NMDAR encephalitis after a long-term diagnostic history of functional psychotic disorders. The present study reports an unusual case of anti-NMDAR encephalitis. The patient had been diagnosed with schizophrenia 7 years earlier, and was currently hospitalized for acute-onset psychiatric symptoms. The diagnosis became unclear when the initial psychosis was confounded with considerations of other neurotoxicities (such as neuroleptic malignant syndrome). Finally, identification of specific immunoglobulin G NR1 autoantibodies in the cerebrospinal fluid and greater effectiveness of immunotherapy over antipsychotics alone (which has been well documented in anti-NMDAR encephalitis) indicated the diagnosis of anti-NMDAR encephalitis in this case. Based on the available evidence, however, the relationship between the newly diagnosed anti-NMDAR encephalitis and the seemingly clear, long-term history of schizophrenia in the preceding 7 years is uncertain. This case report illustrates that psychiatrists should consider anti-NMDAR encephalitis and order tests for specific immunoglobulin G NR1 autoantibodies in patients presenting with disorientation, disturbance of consciousness, cognitive deficit, dyskinesia, autonomic disturbance, or rapid deterioration, even with a seemingly clear history of a psychiatric disorder and no specific findings on routine neuroimaging, electroencephalography, or cerebrospinal fluid tests in the early stage of the illness.

Keywords: anti-*N*-methyl-D-aspartate receptor encephalitis, schizophrenia, differential diagnosis, treatment

Introduction

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is a synaptic autoimmune disorder in which immunoglobulin (Ig)G autoantibodies recognize the GluN1 (also termed NR1 or NR1a) subunit of NMDARs.[1] According to available studies,[1–4] in most patients, development and progression of the disease occurs in well-defined clinical stages. The majority of patients (70%) will develop a viral prodrome with headache, fever, nausea, vomiting, diarrhea, and/or upper respiratory tract symptoms. Within 5 days to 2 weeks, patients develop prominent psychiatric symptoms, which may include delusions, hallucinations, mania, agitation, changes in speech, disorganization, and seizures. Most cases progress into an unresponsive phase with catatonic features, including severe neurological features like seizures, movement abnormalities,

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Anti-NMDA Receptor (NR1) IgG Antibodies

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune disease caused by antibodies against the brain protein, NMDA. Affected individuals display distinctive symptoms, including significant psychiatric disturbances, seizures, confusion, memory loss, and agitation. Woman are affected more often than men. Testing is used to confirm a diagnosis of NMDAR encephalitis and to monitor disease progression and treatment response.

Disease Overview

Incidence

Unknown

Age of Onset

Affects all age groups, with a low prevalence in individuals >50 years

Symptoms

- Prodromal symptoms similar to a nonspecific viral-like illness
  - Low-grade fever
  - Headache
- Rapid progression to other neurological symptoms (psychotic and catatonic phases)
  - Autonomic dysfunction (hypoventilation, tachycardia, hypertension, hyperthermia)
  - Cardiac dysrhythmias
  - Delusions, psychoses
  - Dyskinesia, movement disorders
  - Hallucinations
  - Memory loss
  - Paranoia
  - Seizures
  - Unresponsiveness
- Significant portion of patients are nonparaneoplastic
  - Ovarian teratoma is the most common tumor-related cause
  - Men, women, and children without tumors have also been diagnosed with anti-NMDAR encephalitis

Tests to Consider

N-methyl-D-Aspartate Receptor Antibody, IgG, Serum with Reflex to Titer 2004221

Method: Semi-Quantitative Indirect Fluorescent Antibody

- Confirm diagnosis of anti-NMDAR encephalitis
- May be used in monitoring treatment response in individuals who are antibody positive

N-methyl-D-Aspartate Receptor Antibody, IgG, CSF with Reflex to Titer 2005164

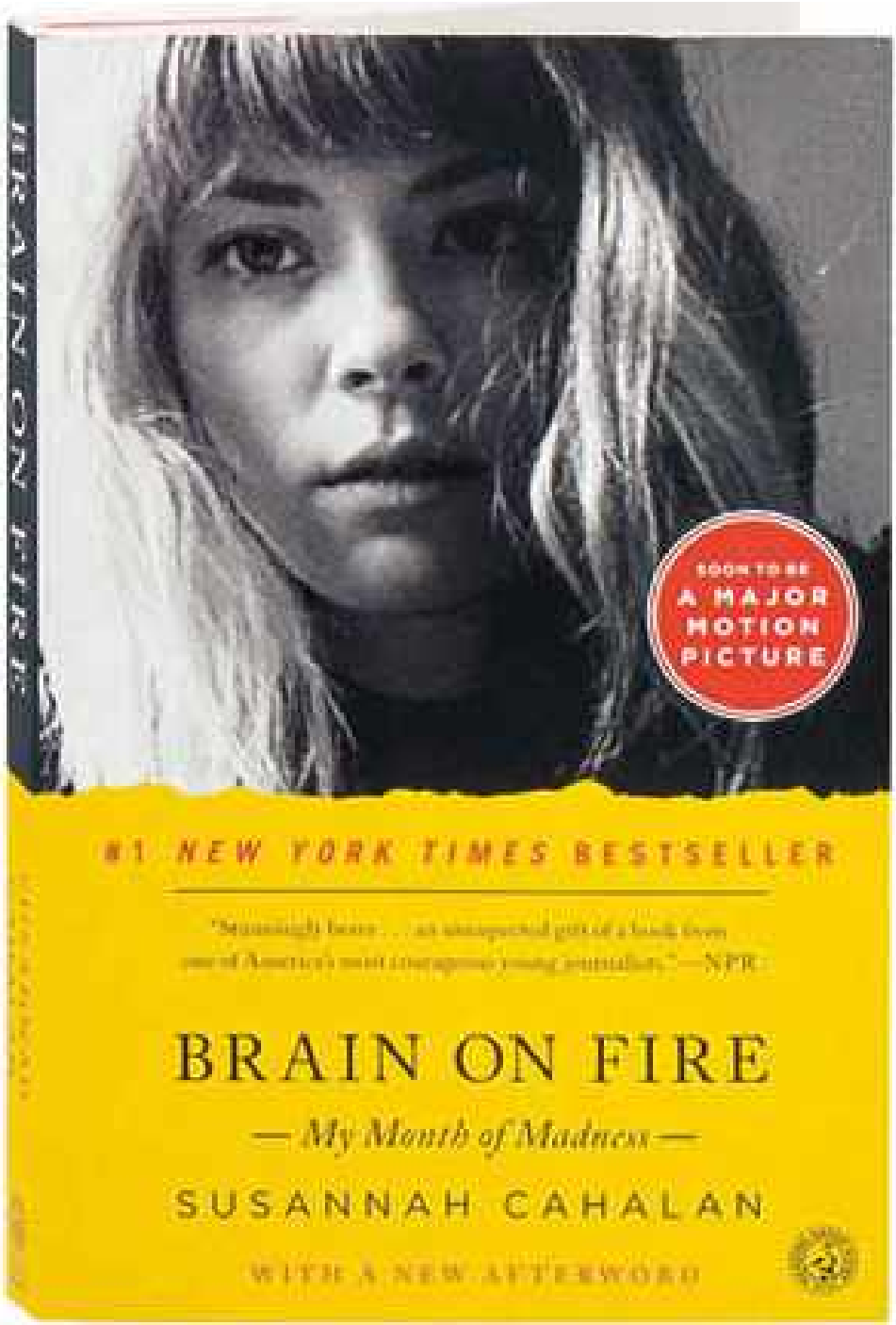
Method: Semi-Quantitative Indirect Fluorescent Antibody

- Confirm a diagnosis of anti-NMDAR encephalitis
- May be used in monitoring treatment response in individuals who are antibody positive

Autoimmune Encephalitis Reflexive Panel, Serum 2013601

Method: Semi-Quantitative Indirect Fluorescent Antibody/Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Quantitative Radioimmunoassay

- Differential evaluation of encephalitis of unknown origin with subacute onset of seizures, confusion, memory loss, and/or behavioral change
- Testing for LGI1 and CASPR2 antibodies always performed
- Panel includes NMDA receptor antibody, VGKC antibody, GAD65 antibody, AQP4 antibody.
- For adults and patients with suspicion of cancer, additional evaluation of paraneoplastic autoantibodies is recommended


BRAIN ON FIRE
SOON TO BE A MAJOR MOTION PICTURE
#1 NEW YORK TIMES BESTSELLER
BRAIN ON FIRE
—My Month of Madness—
SUSANNAH CAHALAN
WITH A NEW AFTERWORD

Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [^{11}C]PBR28 PET Brain Imaging Study

Peter S. Bloomfield, M.Sc., Sudhakar Selvaraj, M.D., Ph.D., Mattia Veronese, Ph.D., Gaia Rizzo, Ph.D., Alessandra Bertoldo, Ph.D., David R. Owen, M.D., Ph.D., Michael A.P. Bloomfield, M.D., Ilaria Bonoldi, M.D., Nicola Kalk, M.D., Federico Turkheimer, Ph.D., Philip McGuire, M.D., Ph.D., Vincenzo de Paola, Ph.D., Oliver D. Howes, M.D., Ph.D.

Objective: The purpose of this study was to determine whether microglial activity, measured using translocator-protein positron emission tomography (PET) imaging, is increased in unmedicated persons presenting with subclinical symptoms indicating that they are at ultra high risk of psychosis and to determine whether microglial activity is elevated in schizophrenia after controlling for a translocator-specific genetic polymorphism.

Method: The authors used the second-generation radioligand [^{11}C]PBR28 and PET to image microglial activity in the brains of participants at ultra high risk for psychosis. Participants were recruited from early intervention centers. The authors also imaged a cohort of patients with schizophrenia and matched healthy subjects for comparison. In total, 56 individuals completed the study. At screening, participants were genotyped to account for the rs6971 polymorphism in the gene encoding the 18Kd translocator protein. The main outcome measure was total gray matter [^{11}C]PBR28 binding ratio, representing microglial activity.

Results: [^{11}C]PBR28 binding ratio in gray matter was elevated in ultra-high-risk participants compared with matched comparison subjects (Cohen's d >1.2) and was positively correlated with symptom severity (r=0.730). Patients with schizophrenia also demonstrated elevated microglial activity relative to matched comparison subjects (Cohen's d >1.7).

Conclusions: Microglial activity is elevated in patients with schizophrenia and in persons with subclinical symptoms who are at ultra high risk of psychosis and is related to at-risk symptom severity. These findings suggest that neuroinflammation is linked to the risk of psychosis and related disorders, as well as the expression of subclinical symptoms.

AJP in Advance (doi: 10.1176/appi.ajp.2015.14101358)

Schizophrenia is a severe psychiatric disorder characterized by psychotic and cognitive symptoms, and it is a leading cause of global disease burden (1). It is generally preceded by a prodromal phase of attenuated psychotic symptoms and functional impairment (2). Individuals meeting standardized criteria for this phase have an ultra high risk for developing a psychotic disorder, in most cases schizophrenia (3). Approximately 35% of high-risk persons will develop a psychotic disorder within 24 months (4).

While the pathoetiology of schizophrenia is not fully understood, there is increasing evidence for the involvement of neuroinflammatory processes. Microglia are the resident immune cells of the CNS, and several lines of evidence indicate microglial involvement in the pathology of psychosis (5–7). In ultra-high-risk individuals, there are elevations in the levels of proinflammatory cytokines (8), which are also elevated in patients with schizophrenia (9). The levels of such peripheral markers have also been associated with reductions in gray matter volume in both ultra-high-risk individuals (10) and patients with schizophrenia (11). Postmortem investigation of brain tissue has found elevated microglial cell density (with a hypertrophic morphology) in persons with schizophrenia compared with control subjects (5), particularly in the frontal and temporal lobes (12), although some studies have found no differences (13). However, since microglial activity is dynamic, postmortem studies may miss alterations early in the development of the disease.

Elevations in microglial activity can be measured in vivo with positron emission tomography (PET) using radioligands specific for the 18kD translocator-protein (TSPO), which is expressed on microglia (14). Investigations using the first-generation radiotracer (R)-[^{11}C]PK11195 have revealed an increase in TSPO binding in medicated patients with schizophrenia when compared with healthy control subjects (6, 7). The first investigation of microglia using PET in schizophrenia, in a cohort of 10 patients, revealed a total gray

"**Microglial activity is elevated in patients with schizophrenia and in persons with subclinical symptoms who are at ultra high risk of psychosis and is related to at-risk symptom severity.** These findings suggest that neuro-inflammation is linked to the risk of psychosis and related disorders, as well as the expression of subclinical symptoms."

ARTICLE

doi:10.1038/nature13595

Biological insights from 108 schizophrenia-associated genetic loci

Schizophrenia Working Group of the Psychiatric Genomics Consortium*

Schizophrenia is a highly heritable disorder. Genetic risk is conferred by a large number of alleles, including common alleles of small effect that might be detected by genome-wide association studies. Here we report a multi-stage schizophrenia genome-wide association study of up to 36,989 cases and 113,075 controls. We identify 128 independent associations spanning 108 conservatively defined loci that meet genome-wide significance, 83 of which have not been previously reported. Associations were enriched among genes expressed in brain, providing biological plausibility for the findings. Many findings have the potential to provide entirely new insights into aetiology, but associations at *DRD2* and several genes involved in glutamatergic neurotransmission highlight molecules of known and potential therapeutic relevance to schizophrenia, and are consistent with leading pathophysiological hypotheses. Independent of genes expressed in brain, associations were enriched among genes expressed in tissues that have important roles in immunity, providing support for the speculated link between the immune system and schizophrenia.

Schizophrenia has a lifetime risk of around 1%, and is associated with substantial morbidity and mortality as well as personal and societal costs[1–3]. Although pharmacological treatments are available for schizophrenia, their efficacy is poor for many patients[4]. All available antipsychotic drugs are thought to exert their main therapeutic effects through blockade of the type 2 dopaminergic receptor[5,6] but, since the discovery of this mechanism over 60 years ago, no new antipsychotic drug of proven efficacy has been developed based on other target molecules. Therapeutic stasis is in large part a consequence of the fact that the pathophysiology of schizophrenia is unknown. Identifying the causes of schizophrenia is therefore a critical step towards improving treatments and outcomes for those with the disorder.

High heritability points to a major role for inherited genetic variants in the aetiology of schizophrenia[7,8]. Although risk variants range in frequency from common to extremely rare[9], estimates[10,11] suggest half to a third of the genetic risk of schizophrenia is indexed by common alleles genotyped by current genome-wide association study (GWAS) arrays. Thus, GWAS is potentially an important tool for understanding the biological underpinnings of schizophrenia.

To date, around 30 schizophrenia-associated loci[10–25] have been identified through GWAS. Postulating that sample size is one of the most important limiting factors in applying GWAS to schizophrenia, we created the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC). Our primary aim was to combine all available schizophrenia samples with published or unpublished GWAS genotypes into a single, systematic analysis[26]. Here we report the results of that analysis, including at least 108 independent genomic loci that exceed genome-wide significance. Some of the findings support leading pathophysiological hypotheses of schizophrenia or targets of therapeutic relevance, but most of the findings provide new insights.

108 independent associated loci

We obtained genome-wide genotype data from which we constructed 49 ancestry matched, non-overlapping case-control samples (46 of European and three of east Asian ancestry, 34,241 cases and 45,604 controls) and 3 family-based samples of European ancestry (1,235 parent affected-offspring trios) (Supplementary Table 1 and Supplementary Methods). These comprise the primary PGC GWAS data set. We processed the genotypes from all studies using unified quality control procedures followed by imputation of SNPs and insertion-deletions using the 1000 Genomes Project reference panel[27]. In each sample, association testing was conducted using imputed marker dosages and principal components (PCs) to control for population stratification. The results were combined using an inverse-variance weighted fixed effects model[28]. After quality control (imputation INFO score ≥ 0.6, MAF ≥ 0.01, and successfully imputed in ≥ 20 samples), we considered around 9.5 million variants. The results are summarized in Fig. 1. To enable acquisition of large samples, some groups ascertained cases via clinician diagnosis rather than a research-based assessment and provided evidence of the validity of this approach (Supplementary Information)[11,13]. Post hoc analyses revealed the pattern of effect sizes for associated loci was similar across different assessment methods and modes of ascertainment (Extended Data Fig. 1), supporting our *a priori* decision to include samples of this nature.

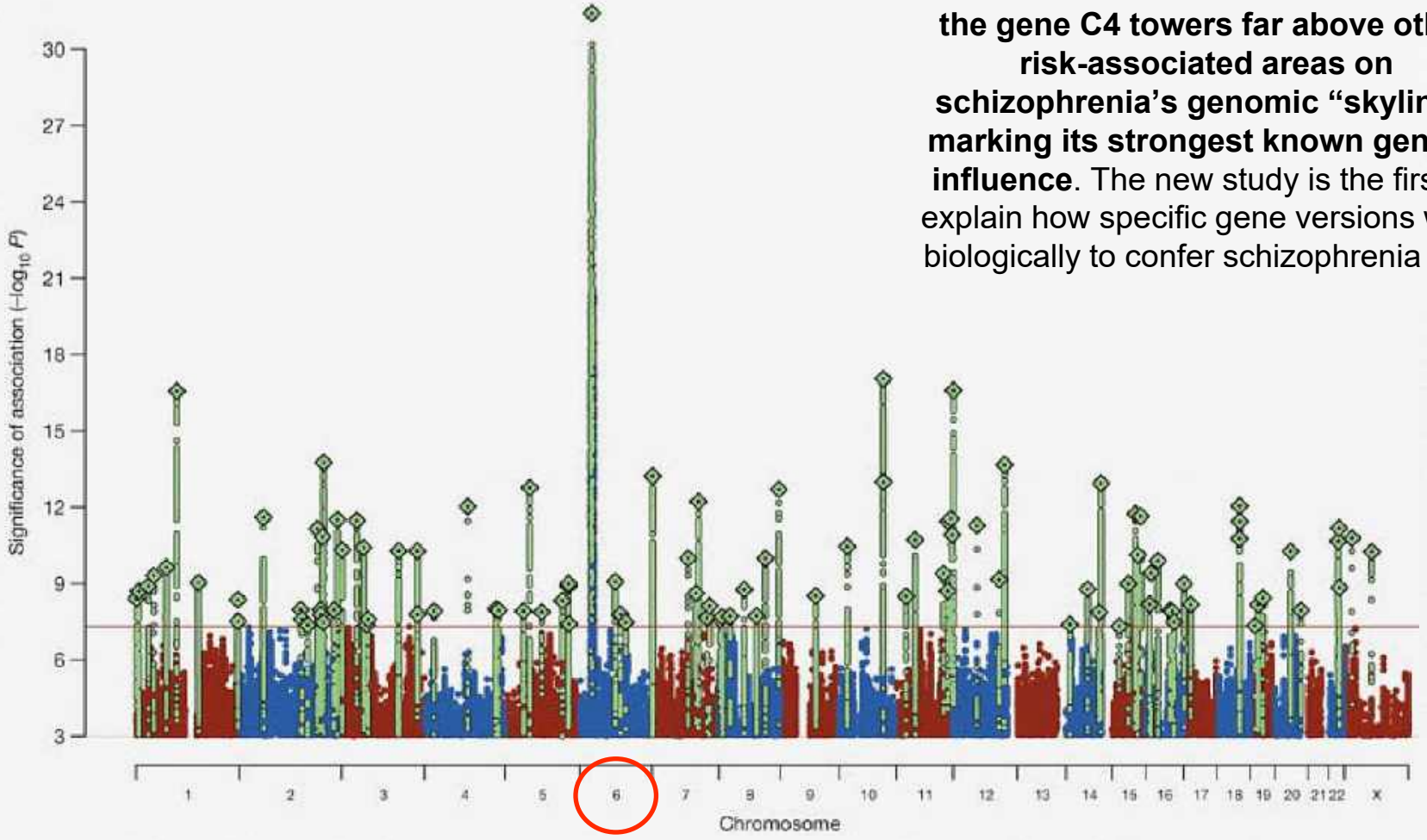
For the subset of linkage-disequilibrium-independent single nucleotide polymorphisms (SNPs) with $P < 1 \times 10^{-6}$ in the meta-analysis, we next obtained results from deCODE genetics (1,513 cases and 66,236 controls of European ancestry). We define linkage-disequilibrium-independent SNPs as those with low linkage disequilibrium ($r^2 < 0.1$) to a more significantly associated SNP within a 500-kb window. Given high linkage disequilibrium in the extended major histocompatibility complex (MHC) region spans ~8 Mb, we conservatively include only a single MHC SNP to represent this locus. The deCODE data were then combined with those from the primary GWAS to give a data set of 36,989 cases and 113,075 controls. In this final analysis, 128 linkage-disequilibrium-independent SNPs exceeded genome-wide significance ($P \leq 5 \times 10^{-8}$) (Supplementary Table 2).

As in meta-analyses of other complex traits which identified large numbers of common risk variants[17,29], the test statistic distribution from our GWAS deviates from the null (Extended Data Fig. 2). This is consistent with the previously documented polygenic contribution to schizophrenia[10,11]. The deviation in the test statistics from the null ($\lambda_{GC} = 1.47$, $\lambda_{1000} = 1.01$) is only slightly less than expected ($\lambda_{GC} = 1.56$) under a polygenic model given fully informative genotypes, the current sample size, and the lifetime risk and heritability of schizophrenia[29].

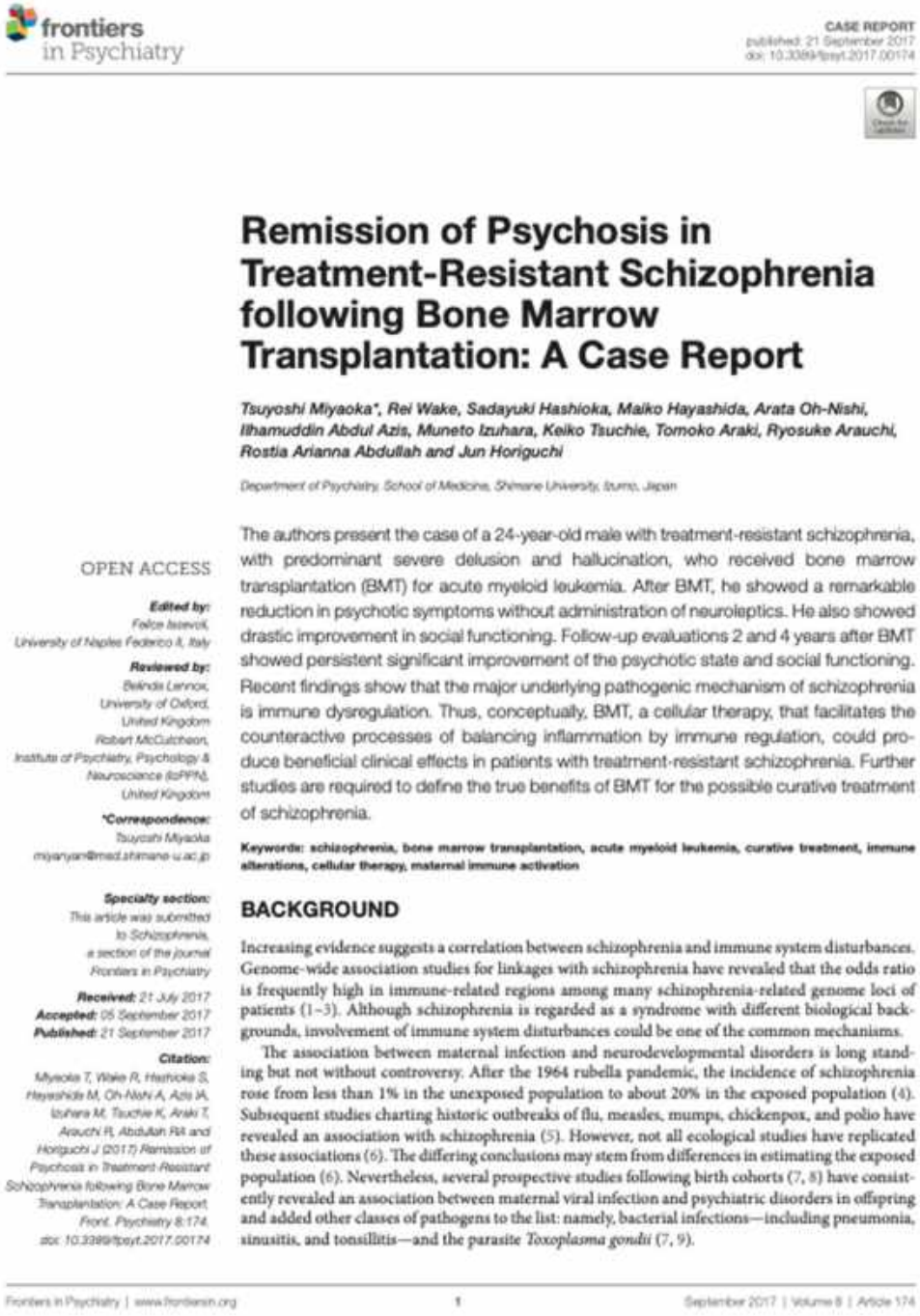
*A list of authors and affiliations appears at the end of the paper.

"Independent of genes expressed in brain, associations were enriched among genes expressed in tissues that have important roles in immunity, providing support for the speculated link between the immune system and schizophrenia."

The site in Chromosome 6 harboring the gene C4 towers far above other risk-associated areas on schizophrenia's genomic "skyline," marking its strongest known genetic influence. The new study is the first to explain how specific gene versions work biologically to confer schizophrenia risk.



Manhattan plot showing schizophrenia associations.



frontiers in Psychiatry

CASE REPORT
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Remission of Psychosis in Treatment-Resistant Schizophrenia following Bone Marrow Transplantation: A Case Report

Tsuyoshi Miyaoka, Rei Wake, Sadayuki Hashioka, Maiko Hayashida, Arata Oh-Nishi, Ilhamuddin Abdul Azis, Muneto Izuhara, Keiko Tsuchie, Tomoko Araki, Ryosuke Arauchi, Rostia Arianna Abdullah and Jun Horiguchi*

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The authors present the case of a 24-year-old male with treatment-resistant schizophrenia, with predominant severe delusion and hallucination, who received bone marrow transplantation (BMT) for acute myeloid leukemia. After BMT, he showed a remarkable reduction in psychotic symptoms without administration of neuroleptics. He also showed drastic improvement in social functioning. Follow-up evaluations 2 and 4 years after BMT showed persistent significant improvement of the psychotic state and social functioning. Recent findings show that the major underlying pathogenic mechanism of schizophrenia is immune dysregulation. Thus, conceptually, BMT, a cellular therapy, that facilitates the counteractive processes of balancing inflammation by immune regulation, could produce beneficial clinical effects in patients with treatment-resistant schizophrenia. Further studies are required to define the true benefits of BMT for the possible curative treatment of schizophrenia.

Keywords: schizophrenia, bone marrow transplantation, acute myeloid leukemia, curative treatment, immune alterations, cellular therapy, maternal immune activation

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BACKGROUND

Increasing evidence suggests a correlation between schizophrenia and immune system disturbances. Genome-wide association studies for linkages with schizophrenia have revealed that the odds ratio is frequently high in immune-related regions among many schizophrenia-related genome loci of patients (1–3). Although schizophrenia is regarded as a syndrome with different biological backgrounds, involvement of immune system disturbances could be one of the common mechanisms.

The association between maternal infection and neurodevelopmental disorders is long standing but not without controversy. After the 1964 rubella pandemic, the incidence of schizophrenia rose from less than 1% in the unexposed population to about 20% in the exposed population (4). Subsequent studies charting historic outbreaks of flu, measles, mumps, chickenpox, and polio have revealed an association with schizophrenia (5). However, not all ecological studies have replicated these associations (6). The differing conclusions may stem from differences in estimating the exposed population (6). Nevertheless, several prospective studies following birth cohorts (7, 8) have consistently revealed an association between maternal viral infection and psychiatric disorders in offspring and added other classes of pathogens to the list: namely, bacterial infections—including pneumonia, sinusitis, and tonsillitis—and the parasite *Toxoplasma gondii* (7, 9).

Recent findings show that the major underlying pathogenic mechanism of schizophrenia is immune dysregulation. Thus, conceptually, BMT, a cellular therapy, that facilitates the counteractive processes of balancing inflammation by immune regulation, could produce beneficial clinical effects in patients with treatment-resistant schizophrenia.

LETTER TO THE EDITOR

Severe chronic psychosis after allogeneic SCT from a schizophrenic sibling

Bone Marrow Transplantation (2015) **50,** 153–154; doi:10.1038/bmt.2014.221; published online 6 October 2014

Schizophrenia is a life-long disorder, usually starting at early adulthood and consisting of remitting or chronic psychosis and functional decline.

There is ample evidence that immune processes play a role in schizophrenia.[1–4] More than 20 different auto-antibodies are at elevated levels in patients with schizophrenia as compared to controls.[5] Autoimmune diseases (AID), such as thyrotoxicosis, celiac disease, acquired hemolytic anaemia, interstitial cystitis and Sjögren's syndrome, but also atopic diseases, have higher prevalence rates among patients with schizophrenia.[6,7] The strongest genetic association with schizophrenia is found in the MHC genes, including loci that influence immune responses.[8] Further evidence for an autoimmune component comes from the lower incidence of schizophrenia in men who used glucocorticosteroids for somatic diseases (odds ratio 0.52).[9] It is unclear if immune processes play a key role in all patients with schizophrenia, or only in ~30–40% of them.[10,11]

A common characteristic of AID[12] and severe allergies[13] is their favourable response to immune ablation and rescue with SCT. Accordingly, we (IES and DWvB) searched for transplant cases suffering from coincident schizophrenia.[14,15] No such cases had been discovered, but one of us (GT) retrieved the history of a patient who developed severe psychosis after receiving a BM transplant from his schizophrenic brother.

The patient (born 1940) had a blank psychiatric history. He was retired, married and had two adult children. At the age of 67 he developed fatigue and skin ecchymoses, and he was diagnosed with chronic lymphocytic leukemia (CLL) and bone marrow aplasia, requiring weekly blood transfusions. Chemotherapy (two courses of CY), and treatment with cyclosporin A, rituximab and prednisolone did not improve his CLL/aplastic anemia. In 2007 he received an allogeneic peripheral blood SCT from one of his brothers, who was the only HLA-matched family member. This brother (born 1952) had schizophrenia since early adulthood and he required treatment with multiple antipsychotic agents. No other first-degree relatives suffered from schizophrenia. The donor also had a history of Lyme disease 8 years before. Serology showed positive IgG and negative IgM for Lyme disease, and PCR was negative for *Borrelia burgdorferi* DNA, consistent with inactive prior infection. The donor used doxycycline 100 mg twice daily, starting 4 days before the stem cell collection, as a safety measure to prevent transmission. The patient was conditioned with fludarabine and CY, followed by an infusion of 5.0×10^6 $CD34^+$ cells/kg from the peripheral blood of his brother. Tacrolimus was administered to prevent GVHD. He never received steroids, and did not develop graft-vs-host reactions. He had complete hematologic recovery and reached full hematopoietic chimerism (>97% donor cells 4 weeks after SCT). We tapered off tacrolimus 4 months after the SCT, because of decreasing blood counts. A few weeks later, when off tacrolimus, he developed acute psychotic symptoms: frequent hallucinations (running commentary and threatening voices), bizarre and non-bizarre delusions, and thought broadcasting with clear consciousness. Insight and judgment were poor. He described his mood as 'angry', with a flat affect. When he developed suicidal and homicidal ideation, he was admitted to a psychiatric clinic. Neurological evaluation, magnetic resonance imaging and electroencephalography revealed no abnormalities. Extensive medical work-up did not reveal metabolic disorders, underlying infections and neoplasms. Results of lumbar puncture were normal, and screenings for viruses (including Herpes Simplex Virus and Human Herper Virus-6), bacteria, fungi and Lyme disease were all unremarkable. He was treated with risperidone 3 mg and citalopram 20 mg, unsuccessfully. Under the working diagnosis of delirium, all medications were discontinued and haloperidol 1 mg was administered, which was not helpful either.

The patient's family decided for comfort care only and the patient was lost to follow-up. He died in 2010, of unknown cause.

The following differential diagnoses were considered and dismissed:

Delirium: Acute onset of psychotic symptoms in a 68-year-old man after somatic disease. However, the 4-month interval between transplantation and onset of psychosis is atypical. Furthermore, discontinuation of all medication did not improve his condition, nor could any somatic disorder be identified.

Endogenous schizophrenia: The patient was genetically predisposed to schizophrenia, given his brother had this disease. However, acute onset at the age of 68 is rare,[16] especially in males.[17] Age of onset is strongly correlated among affected siblings, and differences in onset of more than 10 years are very rare.[18]

Tick-borne infection transmitted through stem cells: The stem cell donor had Lyme disease 8 years earlier. Transfer of tick-borne pathogens might have caused psychotic symptoms. However, the donor was *Borrelia* IgM and PCR negative and received doxycycline before stem cell extraction.

Therefore, adoptive transfer of schizophrenia is the most appealing etiology, not only by exclusion, but also in view of the increasing evidence that some forms of schizophrenia have an autoimmune origin. Although the transplanted patient fulfills these criteria, a formal diagnosis was never made given his very unusual age of onset and potential relation to the SCT.

Adoptive transfer of AIDs seems to be rare, as we are aware of only 21 cases reported so far among ~200 000 long-term survivors of allogeneic SCTs. Stem cell donors are routinely subjected to complete medical examination; therefore chances are small that AID is overlooked. The 21 cases include thyroiditis (10 cases),[19] vitiligo (3),[20–22] psoriasis (2),[23,24] type I diabetes mellitus (2),[25] celiac disease (1),[26] thrombocytopenia (1),[27] polyglandular syndrome type II (1)[22] and systemic lupus erythematosus (1).[28] Moreover, not only AIDs may be transferred by SCT but also other immune diseases, such as allergies.[13] Adoptive transfer of AIDs and allergies is thought to be mediated by transfer of donor lymphocytes. This suggests that the subform of schizophrenia in the patient was mediated by lymphocytes.

Based on this single case report, we obviously cannot prove an immune pathogenesis of schizophrenia. However, the report supports the hypothesis of immunological involvement in schizophrenia pathogenesis and we suggest that physicians and patients involved in SCT take into consideration the possibility that schizophrenia may be transmitted by the transplant.

Based on this single case report, we obviously cannot prove an immune pathogenesis of schizophrenia. However, the report supports the hypothesis of immunological involvement in schizophrenia pathogenesis and we suggest that physicians and patients involved in SCT(Stem Cell Transplant) take into consideration the possibility that schizophrenia may be transmitted by the transplant.

NEUROSCIENCE

The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice

Peng Zheng[1,2,3]*, Benhua Zeng[4]*, Meiling Liu[5], Jianjun Chen[6], Junxi Pan[2,3,7], Yu Han[1,2,3], Yiyun Liu[1,2,3], Ke Cheng[1,2,3], Chanjuan Zhou[2,3], Haiyang Wang[1,2,3], Xinyu Zhou[1,2,3], Siwen Gui[1,2,3], Seth W. Perry[8], Ma-Li Wong[8], Julio Licinio[8†‡], Hong Wei[9†‡], Peng Xie[1,2,3†‡]

Schizophrenia (SCZ) is a devastating mental disorder with poorly defined underlying molecular mechanisms. The gut microbiome can modulate brain function and behaviors through the microbiota-gut-brain axis. Here, we found that unmedicated and medicated patients with SCZ had a decreased microbiome α-diversity index and marked disturbances of gut microbial composition versus healthy controls (HCs). Several unique bacterial taxa (e.g., Veillonellaceae and Lachnospiraceae) were associated with SCZ severity. A specific microbial panel (Aerococcaceae, Bifidobacteriaceae, Brucellaceae, Pasteurellaceae, and Rikenellaceae) enabled discriminating patients with SCZ from HCs with 0.769 area under the curve. Compared to HCs, germ-free mice receiving SCZ microbiome fecal transplants had lower glutamate and higher glutamine and GABA in the hippocampus and displayed SCZ-relevant behaviors similar to other mouse models of SCZ involving glutamatergic hypofunction. Together, our findings suggest that the SCZ microbiome itself can alter neurochemistry and neurologic function in ways that may be relevant to SCZ pathology.

INTRODUCTION

Schizophrenia (SCZ) is a devastating illness affecting approximately 0.5 to 1% of the general population worldwide (*1*). Previously, researchers have focused on analysis of the human genome to determine the pathogenesis of SCZ (*2*). Genome-wide association (GWAS) analysis of 36,000 patients identified 108 susceptibility loci for SCZ (*3*). However, the identified associations likely account for only about 4% of the variance in SCZ. Thus, we should also seek to identify the role of non-human genetic factors in the onset of SCZ.

The gastrointestinal (GI) tract is a complex ecosystem containing a large number of resident microorganisms (*4*). Recent evidence suggests that the gut microbiota could modulate brain function and behaviors via the "microbiota-gut-brain" (MGB) axis (*5*, *6*). For example, gut microbiota have been reported to be associated with alterations in anxiety (*7*), memory (*8*), cognition (*9*), and locomotor activity (*10*). Our groups recently showed that modulation of gut microbiota using the germ-free (GF) method or antibiotics could result in depressive-like behaviors (*11*, *12*). These findings highlight the novel possibility that disturbances of gut microbiota or the MGB axis may contribute to the onset of psychiatric disorders.

The relationships between the MGB axis and the SCZ are not yet fully understood. Emerging clinical and preclinical studies indicate potential associations between a disturbed gut microbiome and SCZ (*13*). Epidemiological studies have shown that prenatal microbial infection resulted in a 10- to 20-fold increased risk of developing SCZ (*14*). In addition, SCZ is frequently comorbid with GI disorders that are characterized by alterations of gut microbial communities (*15*). In animal studies, gut microbiota are crucial in postnatal development and maturation of neural, immune, and endocrine systems (*16*), and these behavioral and physiological processes are frequently impaired in patients with SCZ (*17*). These aforementioned studies suggest that disturbance of the MGB axis may be associated with development of SCZ.

To address this issue, a culture-independent, 16*S* ribosomal RNA (16*S* rRNA) gene sequence–based approach was used to compare the gut microbial communities of patients with SCZ and healthy controls (HCs) to evaluate whether microbiotal dysbiosis was linked with schizophrenic episodes or the severity of schizophrenic symptoms. Then, we transferred gut microbiota from patients with SCZ into GF mice to test whether SCZ-relevant behavioral phenotypes were transmissible via their gut microbiome. Last, to capture functional readout of microbial activity, we performed comparative metagenomic and metabolomic analyses of samples from the mice harboring "SCZ microbiota" versus "HC microbiota" to determine the potential mechanistic pathways by which the disturbed gut microbiota may modulate host physiology and behavior.

RESULTS

Human studies

Clinical characteristics of recruited participants

A total of 63 patients with SCZ and 69 HCs were recruited for this study. There were no significant differences in age, gender, or body mass index between the two groups. All patients with SCZ were undergoing some symptoms of this illness. The Positive and Negative Syndrome

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Here, we found that unmedicated and medicated patients with SCZ had a decreased microbiome diversity index and marked disturbances of gut microbial composition versus healthy controls (HCs)….**Together, our findings suggest that the SCZ microbiome itself can alter neurochemistry and neurologic function in ways that may be relevant to SCZ pathology.**

DOI: 10.1111/bdi.12652

ORIGINAL ARTICLE

WILEY BIPOLAR DISORDERS

Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: A randomized controlled trial

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Objective: Immunological abnormalities play a role in the pathophysiology of mania and have been associated with relapse. Probiotic organisms such as *Lactobacilli* and *Bifidobacteria* modulate inflammation in humans and animal models. The trial examined whether the administration of probiotic organisms prevents psychiatric rehospitalizations in patients recently discharged following hospitalization for mania.

Methods: Patients hospitalized for mania (N = 66) were randomized after discharge to receive 24 weeks of adjunctive probiotics (*Lactobacillus rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12) or adjunctive placebo in a parallel two-group design format. The effect of treatment group on the risk of rehospitalization was calculated using Cox regression models. The modulating effect of systemic inflammation was measured employing an inflammation score based on immunoglobulin levels directed at previously defined antigens.

Results: During the 24-week observation period there were a total of 24 rehospitalizations in the 33 individuals who received placebo and eight rehospitalizations in the 33 individuals who received the probiotics ($z = 2.63$, $P = .009$). Hazard functions indicated that the administration of the probiotics was associated with a significant advantage in time to all psychiatric rehospitalizations (hazard ratio [HR] = 0.26, 95% confidence interval [CI] 0.10, .69; $P = .007$). Probiotic treatment also resulted in fewer days rehospitalized (mean 8.3 vs 2.8 days for placebo and probiotic treatment, respectively; $\chi^2 = 5.17$, $P = .017$). The effect of the probiotic treatment on the prevention of rehospitalization was increased in individuals with elevated levels of systemic inflammation at baseline.

Conclusion: Probiotic supplementation is associated with a lower rate of rehospitalization in patients who have been recently discharged following hospitalization for mania.

KEYWORDS
immune, mania, probiotics, rehospitalization

Registered on clinicaltrials.gov # NCT01731171

In conclusion, *the adjunctive administration of a probiotic preparation containing defined levels of* ***Lactobacillus GG*** *and* ***Bifidobacterium lactis strain Bb12*** *resulted in a significant reduction in the rate of psychiatric rehospitalization in individuals recently discharged following hospitalization for mania.* The probiotic compound was well tolerated and had low levels of side effects.

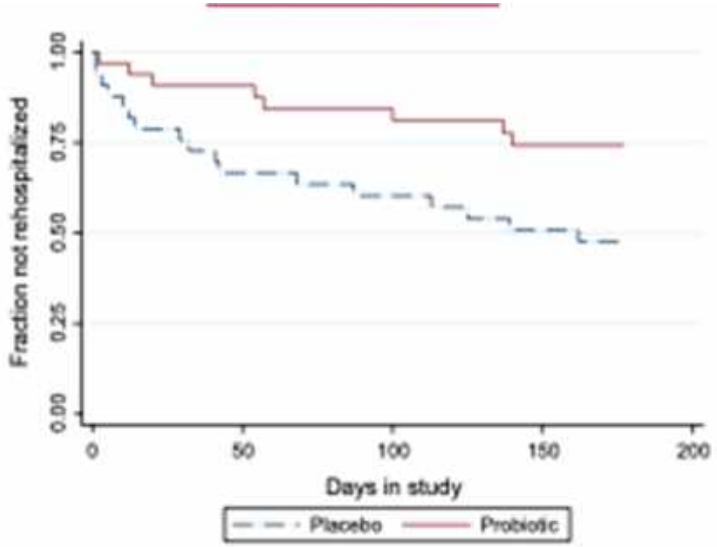

FIGURE 2 Time to first rehospitalization for the total study population by treatment group

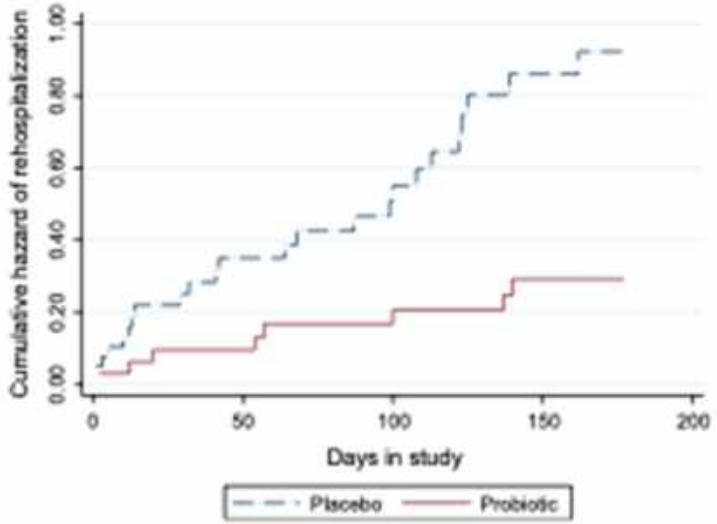

FIGURE 3 Cumulative hazard of rehospitalization for the total study population by treatment group

Probiotic treatment also resulted in fewer days rehospitalized (mean 8.3 vs 2.8 days for placebo and probiotic treatment, respectively; The effect of the probiotic treatment on the prevention of rehospitalization was increased in individuals with elevated levels of systemic inflammation at baseline.


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Review

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Neuroprotective Effects of Lithium: Implications for the Treatment of Alzheimer's Disease and Related Neurodegenerative Disorders

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ABSTRACT: Lithium is a well-established therapeutic option for the acute and long-term management of bipolar disorder and major depression. More recently, based on findings from translational research, lithium has also been regarded as a neuroprotective agent and a candidate drug for disease-modification in certain neurodegenerative disorders, namely, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and, more recently, Parkinson's disease (PD). The putative neuroprotective effects of lithium rely on the fact that it modulates several homeostatic mechanisms involved in neurotrophic response, autophagy, oxidative stress, inflammation, and mitochondrial function. Such a wide range of intracellular responses may be secondary to two key effects, that is, the inhibition of glycogen synthase kinase-3 beta (GSK-3β) and inositol monophosphatase (IMP) by lithium. In the present review, we revisit the neurobiological properties of lithium in light of the available evidence of its neurotrophic and neuroprotective properties, and discuss the rationale for its use in the treatment and prevention of neurodegenerative diseases.

KEYWORDS: *Lithium, neuroprotection, GSK-3β, autophagy, bipolar disorder, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis*

Lithium salts have long been used in psychiatry for the treatment of severe mental disorders.[1] Currently, the main medical indications of lithium are for the acute and long-term treatment of bipolar disorder (BD) and for the adjunctive treatment of major depression, given its well-established mood stabilizing properties.[2] More recently, there has been a growing body of evidence indicating that the neurobiological benefits of lithium may go beyond mood stabilization. In experimental and clinical models, lithium treatment has been associated with neuroprotection, due to its effects on several mechanisms of neuronal homeostasis involved in the activation of neurotrophic responses, modulation of oxidative stress, inflammatory cascades, up-regulation of mitochondrial function, and other specific biological effects implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS).[3] This article aims to review the mechanisms by which lithium may exert its neuroprotective effects, and how these mechanisms may help delay the progression of neurobiological changes in mood and neurocognitive disorders. Additionally, we address the potential of lithium as a disease-modifying agent for certain neurodegenerative and dementing conditions.

NEUROBIOLOGICAL PROPERTIES OF LITHIUM

The pharmacological mechanisms of lithium are not completely understood, but current evidence suggests the direct involvement of classic pharmacological targets affecting neurotransmission and signal transduction. These include the modulation of cell-surface receptors, the release of second-messengers and downstream signaling molecules, and the subsequent effect on the activity of important regulatory systems, with an impact on the release of transcription factors and gene expression.[4] Monovalent lithium (Li^+) competes with bivalent magnesium (Mg^{2+}) to the similar ionic radii of these cations (0.60 and 0.65 Å respectively), rendering the ability of lithium to bind to Mg^{2+} substrate sites. Therefore, lithium can inhibit a wide range of enzymes that depend on Mg^{2+} as a cofactor.[1,6] The competition between lithium and Mg^{2+} on these substrate sites has a significant influence on the activity of several enzymes and therefore the release of their metabolic products; in particular, glycogen synthase kinase-3 beta (GSK-3β), inositol monophosphatase (IMP), and Akt/β-arrestin2 (Akt) are important lithium targets. Therefore, the modification of these intracellular pathways through enzymatic inhibition is

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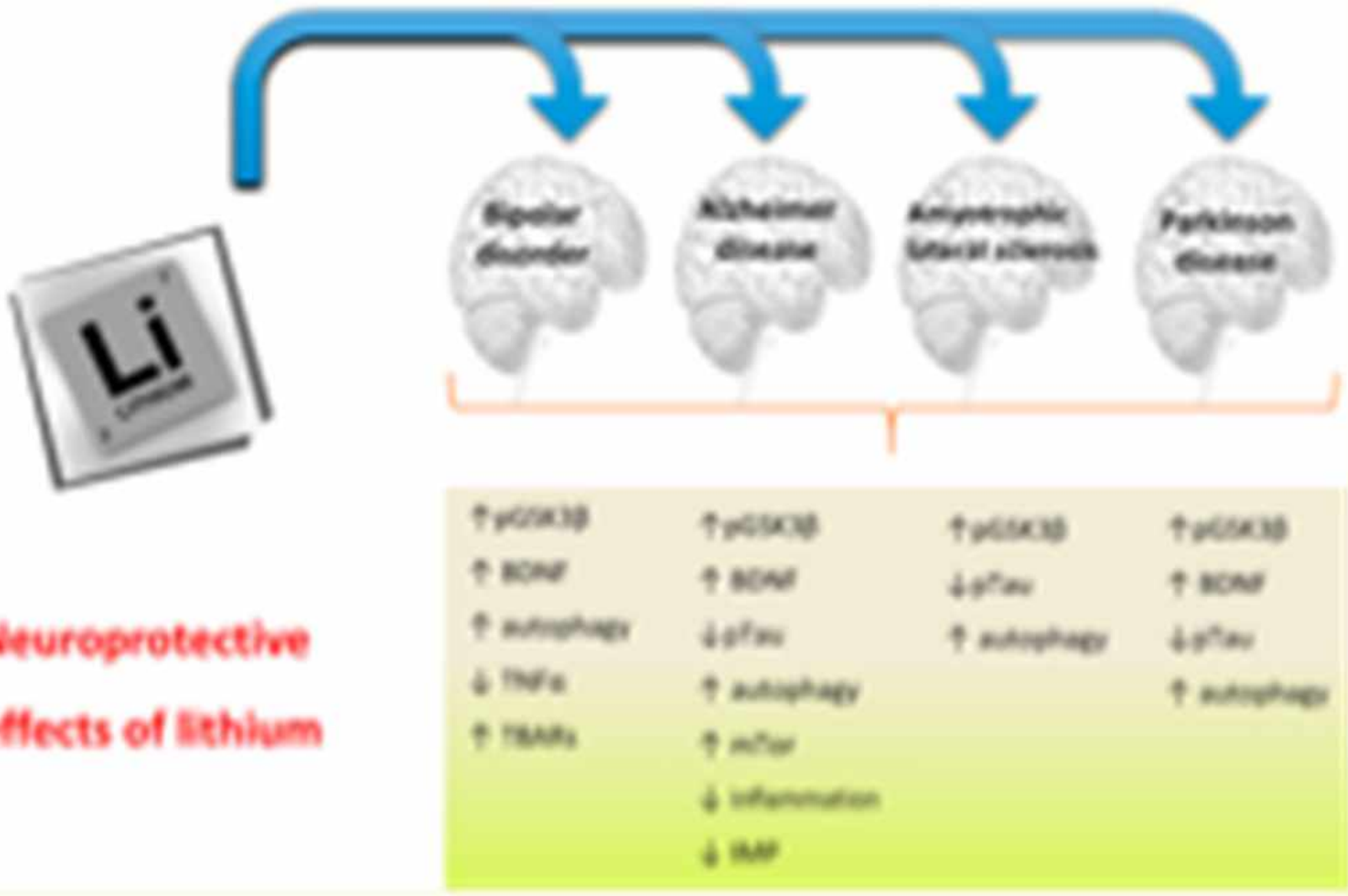
The putative neuroprotective effects of lithium rely on the fact that it modulates several homeostatic mechanisms involved in neurotrophic response, autophagy, oxidative stress, inflammation, and mitochondrial function. Such a wide range of intracellular responses may be secondary to two key effects, that is, the inhibition of glycogen synthase kinase-3 beta (GSK-3β) and inositol monophosphatase (IMP) by lithium.



Neuroprotective effects of lithium

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Review

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Lithium and Autophagy

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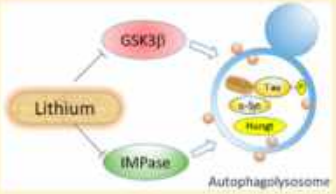
ABSTRACT: Lithium, a drug used to treat bipolar disorders, has a variety of neuroprotective mechanisms, including autophagy regulation, in various neuropsychiatric conditions. In neurodegenerative diseases, lithium enhances degradation of aggregate-prone proteins, including mutated huntingtin, phosphorylated tau, and α-synuclein, and causes damaged mitochondria to degrade, while in a mouse model of cerebral ischemia and Alzheimer's disease autophagy downregulation by lithium is observed. The signaling pathway of lithium as an autophagy enhancer might be associated with the mammalian target of rapamycin (mTOR)-independent pathway, which is involved in myo-inositol-1,4,5-trisphosphate (IP_3) in Huntington's disease and Parkinson's disease. However, the mTOR-dependent pathway might be involved in inhibiting glycogen synthase kinase-3β (GSK3β) in other diseases. Lithium's autophagy-enhancing property may contribute to the therapeutic benefit of patients with neuropsychiatric disorders.



KEYWORDS: *Lithium, autophagy, GSK3β, IMPase, Huntingtin, α-synuclein, tau, prion protein*

Lithium has been used clinically to treat bipolar disorders for over half a century, and various neuroprotective and neurotrophic properties have been described.[1] To the best of our knowledge, Sarkar et al. reported, for the first time, that lithium induced autophagy to enhance the degradation of mutant Huntingtin via the mTOR independent pathway in nonneuronal and neural precursor cells.[2] Since then, several papers have described lithium's autophagy regulation in various neuropsychiatric diseases such as Huntington's disease (HD), Alzheimer's disease (AD), Parkinson's disease (PD), prion disease, and amyotrophic lateral sclerosis (ALS). However, the signaling pathway to explain lithium's autophagy regulation has not been consistently described. Moreover, lithium did not always positively regulate autophagy in all pathological conditions. In a condition such as cerebral ischemia or AD, lithium has been shown to negatively regulate autophagy. In this review, we focus on lithium's autophagy-enhancing mechanism in various diseases.

I. THE BASICS OF AUTOPHAGY

I-1. Introducing Autophagy. Autophagy is the process of "self-eating." Under starvation conditions, bulk autophagy can be induced to catabolize cellular substrates to generate energy. There are three forms of autophagy: microautophagy, macroautophagy, and chaperone-mediated autophagy. The most common and well understood is macroautophagy, hereafter referred to simply as autophagy. For a more complete review of autophagy, see ref 3. Autophagy consists of four stages: initiation, elongation, maturation, and fusion (Figure 1). This process is initiated by formation of a cup-shaped membrane structure (the phagophore) in the cytoplasm. The phagophore accumulates additional proteins, which enables the membrane to elongate and form a double-membrane-bound structure called an autophagosome. A portion of the cytoplasm is enclosed in the autophagosome along with the cellular components to be degraded. Autophagosomes are then trafficked along microtubules to the perinuclear region of the cell (where lysosomes are clustered) to enhance the probability of autophagosome-lysosome fusion to form autophagolysosomes. After fusion with lysosomes, the protein and organelle contents of the autophagosome are degraded by acidic lysosomal hydrolases and recycled. Vacuolar H⁺ATPase (V-ATPases) are proton pumps that reside within the lysosomal membrane and enable acidification of the autolysosome contents. This acidification is essential for the activation of lysosomal enzymes, such as cathepsins or other acid hydrolases, which are responsible for proteolysis of the components in the autophagolysosome.[4]

Macroautophagy has physiological roles in both health and disease. Upon nutrient deprivation, autophagy catabolizes cytoplasmic components nonselectively into building blocks, such as amino acids. Autophagy also occurs constitutively at low levels even under nutrient-rich conditions and mediates global turnover of cytoplasmic materials. Constitutive autophagy acts as the quality-control machinery for cytoplasmic components, and it is crucial for homeostasis of various postmitotic cells, such as neurons. Although this quality control could be partially achieved by nonselective autophagy,

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"In neurodegenerative diseases, lithium enhances degradation of aggregate-prone proteins, including mutated Huntingtin, phosphorylated tau, and α-synuclein, and causes damaged mitochondria to degrade, while in a mouse model of cerebral ischemia and Alzheimer's disease autophagy downregulation by lithium is observed."

Toxic & Essential Elements; Hair

TOXIC METALS		RESULT µg/g	REFERENCE INTERVAL	PERCENTILE 68th 95th
Aluminum	(Al)	2.9	< 7.0	
Antimony	(Sb)	0.056	< 0.066	
Arsenic	(As)	0.035	< 0.080	
Barium	(Ba)	0.09	< 1.0	
Beryllium	(Be)	< 0.01	< 0.020	
Bismuth	(Bi)	0.030	< 2.0	
Cadmium	(Cd)	< 0.009	< 0.065	
Lead	(Pb)	0.08	< 0.80	
Mercury	(Hg)	0.89	< 0.80	
Platinum	(Pt)	< 0.003	< 0.005	
Thallium	(Tl)	< 0.001	< 0.002	
Thorium	(Th)	< 0.001	< 0.002	
Uranium	(U)	0.001	< 0.060	
Nickel	(Ni)	0.14	< 0.20	
Silver	(Ag)	0.05	< 0.08	
Tin	(Sn)	0.03	< 0.30	
Titanium	(Ti)	0.44	< 0.60	
Total Toxic Representation				

ESSENTIAL AND OTHER ELEMENTS		RESULT µg/g	REFERENCE INTERVAL	PERCENTILE 2.5th 16th 50th 84th 97.5th
Calcium	(Ca)	259	200- 750	
Magnesium	(Mg)	22	25- 75	
Sodium	(Na)	8	20- 180	
Potassium	(K)	< 3	9- 80	
Copper	(Cu)	11	11- 30	
Zinc	(Zn)	180	130- 200	
Manganese	(Mn)	0.10	0.08- 0.50	
Chromium	(Cr)	0.35	0.40- 0.70	
Vanadium	(V)	0.018	0.018- 0.065	
Molybdenum	(Mo)	0.032	0.025- 0.060	
Boron	(B)	0.14	0.40- 3.0	
Iodine	(I)	0.43	0.25- 1.8	
Lithium	(Li)	< 0.004	0.007- 0.020	
Phosphorus	(P)	198	150- 220	
Selenium	(Se)	0.85	0.70- 1.2	
Strontium	(Sr)	0.15	0.30- 3.5	
Sulfur	(S)	48400	44000- 50000	
Cobalt	(Co)	0.030	0.004- 0.020	
Iron	(Fe)	6.5	7.0- 16	
Germanium	(Ge)	0.031	0.030- 0.040	
Rubidium	(Rb)	0.005	0.011- 0.12	
Zirconium	(Zr)	0.27	0.020- 0.44	

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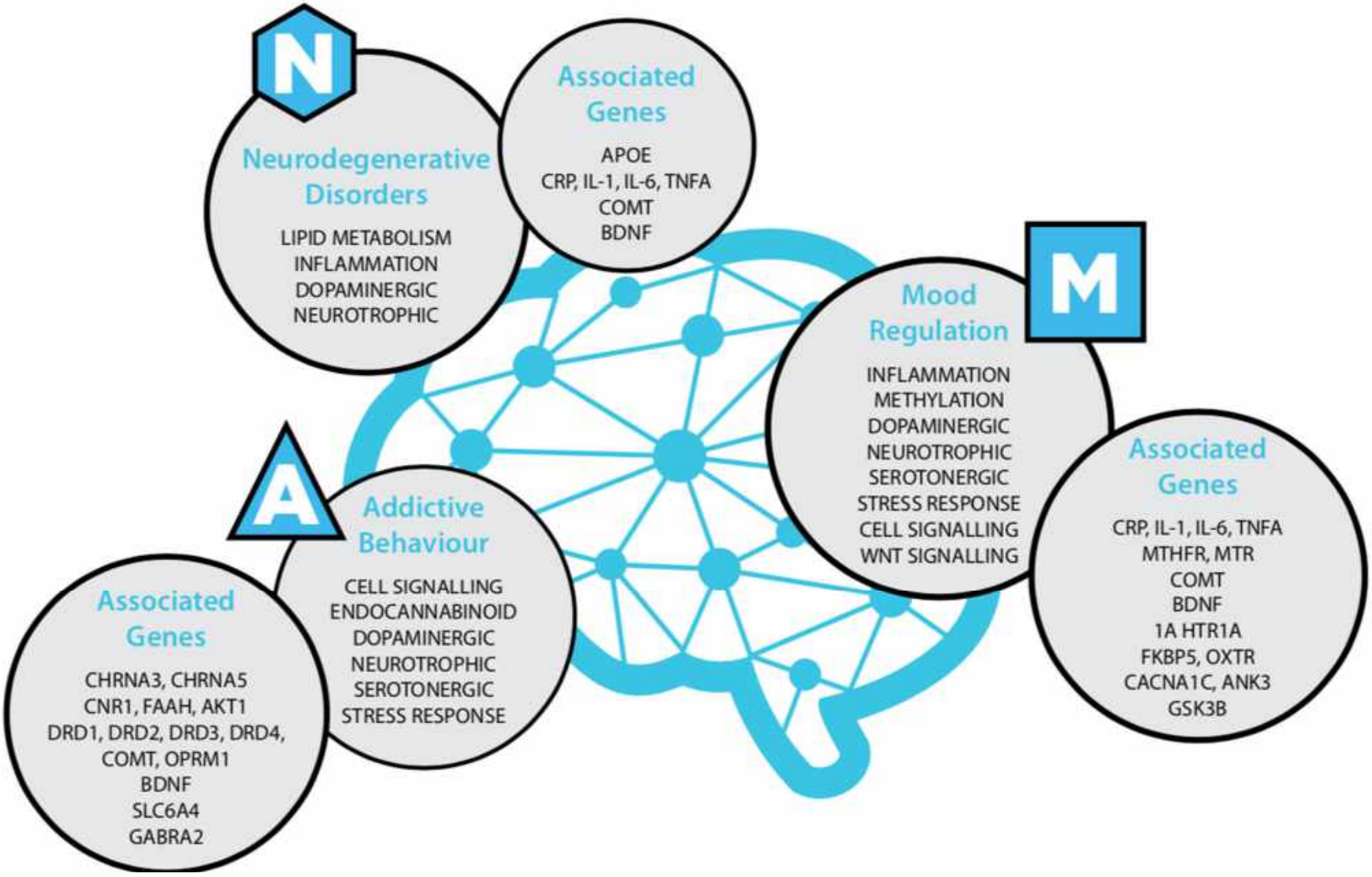
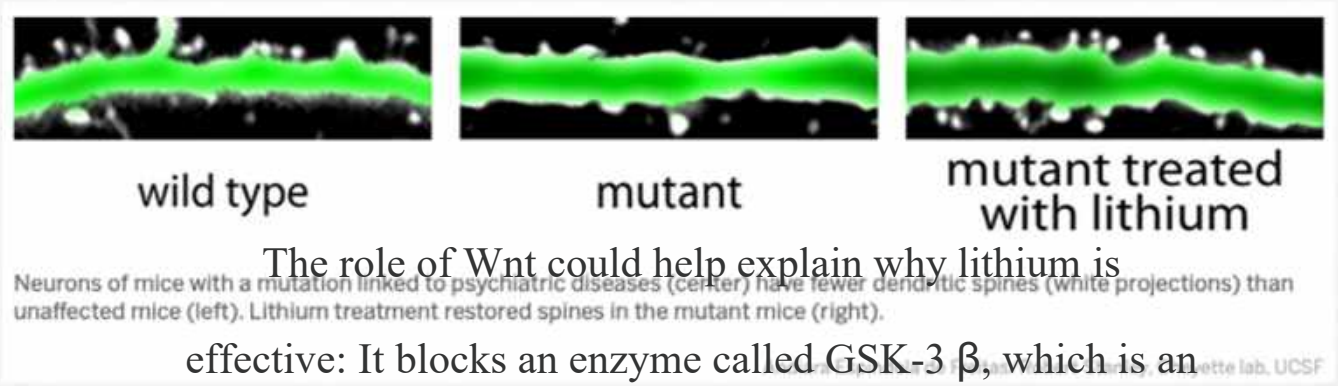


Summary table of results

Biological Area	Gene Name	Genetic Variation	Your Result	Impact N	Impact M	Impact A
Lipid metabolism	APOE	E2/E3/E4	E3/E4	●●●		
Inflammation	CRP	G>A	GA	●	●	
	IL1-A	4845 G>T	TG	●●	●●	
		-889 C>T	TC	●●	●●	
	IL1-B	3954 C>T	CT	●●	●●	
		-511 A>G	GG	●●●	●●●	
	IL1-RN	2108 C>T	CT	●	●	
	IL-6	-174 G>C	CC	●●●	●●●	
	TNFA	-308 G>A	GG	○	○	
Methylation	MTHFR	677 C>T	CC		○	
		1298 A>C	AA		○	
		[illegible]	AA			
Wnt Signalling	GSK3B	C>G	CG			
		A>C	CC		●●	
		G>A	GG			
Stress Response	FKBP5	[illegible]	[illegible]		●●●	
	OXTR	G>A	AG		●	
Cell Signalling	AKT1	T>C	CT			●
	ANK3	A>G	AA		○	
		C>T	CC		○	
	CACNA1	G>A	GG		○	
	CHRNA3	G>A	AG			●●
	CHRNA5	Asp398Asn	AG			●
Dopaminergic	COMT	Val158Met	GG	●●	○	●●
	DRD1	T>C	CC			○
		C>T	CC			○
	DRD2	Taq1A/2A	CC			○
	DRD3	Ser9Gly	TT			○
	DRD4	-521 C>T	CC			●
	OPRM1	Asn40Asp	AA			○
Endocannabinoid	CNR1	T>C	TT			○
	FAAH	385 C>A	CC			○
GABAergic	GABRA2	T>C	CT			○
Neurotrophic	BDNF	Val66Met	CC	○	○	○
Serotonergic	1A HTR1A	-1019 C>G	GG		●●●	
	SLC6A4	A>C	AC			○

Wnt signaling pathways are a group of signal transduction pathways

Lithium treatment restored healthy numbers of dendritic spines in mice engineered to carry a genetic mutation that is more common in people with autism, schizophrenia, and bipolar disorder than in unaffected people,



Neurons of mice with a mutation linked to psychiatric diseases (center) have fewer dendritic spines (white projections) than unaffected mice (left). Lithium treatment restored spines in the mutant mice (right).

The role of Wnt could help explain why lithium is effective: It blocks an enzyme called GSK-3 β, which is an inhibitor on the Wnt pathway. **By boosting Wnt signaling, lithium could produce a therapeutic effect in psychiatric diseases in which the Wnt pathway is underpowered.**

NOTE: Wnt signaling pathways are a group of signal transduction pathways

My New Scientist

Faecal transplant eases symptoms of Parkinson's

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Editorial: " Why we need to respect bacterial culture"

Diabetes and even obesity, as well as Parkinson's disease, might be cured just by replacing the bacteria in your gut

Faecal transplants: strange cures (Image: Artpartner-images/Getty)

1 more image

A FEW years ago, John Gillies had trouble picking up his grandchild. He would stand frozen, waiting for his Parkinson's disease to relinquish its hold and allow him to move. Then in May 2008, Gillies was given antibiotics to treat constipation, and astonishingly his Parkinson's symptoms abated. What on earth was going on?

Thomas Borody, a gastroenterologist at the Centre for Digestive Diseases in New South Wales, Australia, put Gillies on antibiotics because he had found that constipation can be caused by an infection of the colon. "He has now been seen by two neurologists, who cannot detect classic Parkinson's disease symptoms any more," says Borody.

Borody's observations, together with others, suggest that many conditions, from Parkinson's to metabolic disorders such as obesity, might be caused by undesirable changes in the microbes of the gut. If that is true, it might be possible to alleviate symptoms with antibiotics, or even faecal transplants using donor faeces to restore the bowel flora to a healthy state.

Borody has noticed that some of his patients also see improvements in symptoms of their other diseases, including Parkinson's, multiple sclerosis (MS), chronic fatigue syndrome (CFS) and rheumatoid arthritis.

Vagotomy and Subsequent Risk of Parkinson's Disease

Elisabeth Svensson PhD[1,*], Erzsébet Horváth-Puhó PhD[1], Reimar W. Thomsen PhD[1], Jens Christian Djurhuus DMSc[2], Lars Pedersen PhD[1], Per Borghammer DMSc[2,3] and Henrik Toft Sørensen DMSc[1]

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Objective

Parkinson's disease (PD) may be caused by an enteric neurotropic pathogen entering the brain through the vagal nerve, a process that may take over 20 years. We investigated the risk of PD in patients who underwent vagotomy and hypothesized that truncal vagotomy is associated with a protective effect, whereas superselective vagotomy has a minor effect.

Methods

We constructed cohorts of all patients in Denmark who underwent vagotomy during 1977–1995 and a matched general population cohort by linking Danish registries. We used Cox regression to compute hazard ratios (HRs) for PD and corresponding 95% confidence intervals (CIs), adjusting for potential confounders.

Results

Risk of PD was decreased in patients who underwent truncal (HR = 0.85; 95% CI = 0.56–1.27; follow-up of >20 years: HR = 0.58; 95% CI: 0.28–1.20) compared to superselective vagotomy. Risk of PD was also decreased after truncal vagotomy when compared to the general population cohort (overall adjusted HR = 0.85; 95% CI: 0.63–1.14; follow-up >20 years, adjusted HR = 0.53; 95% CI: 0.28–0.99). In patients who underwent superselective vagotomy, risk of PD was similar to the general population (HR = 1.09; 95% CI: 0.84–1.43; follow-up of >20 years: HR = 1.16; 95% CI: 0.80–1.70). Statistical precision of risk estimates was limited. Results were consistent after external adjustment for unmeasured confounding by smoking.

Interpretation

Full truncal vagotomy is associated with a decreased risk for subsequent PD, suggesting that the vagal nerve may be critically involved in the pathogenesis of PD. Ann

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Neuron-Glial Interactions in Blood-Brain Barrier Formation

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Abstract

The blood brain barrier (BBB) evolved to preserve the microenvironment of the highly excitable neuronal cells to allow for action potential generation and propagation. Intricate molecular interactions between two main cell types, the neurons and the glial cells, form the underlying basis of the critical functioning of the nervous system across species. In invertebrates, interactions between neurons and glial cells are central in establishing a functional BBB. However, in vertebrates, the BBB formation and function is coordinated by interactions between neurons, glial cells, and endothelial cells. Here we review the neuron-glial interaction–based blood barriers in invertebrates and vertebrates and provide an evolutionary perspective as to how a glial-barrier system in invertebrates evolved into an endothelial barrier system. We also summarize the clinical relevance of the BBB as this protective barrier becomes disadvantageous in the pharmacological treatment of various neurological disorders.

Keywords

septate junctions; tight junctions; endothelial cells; astrocytes; neurovascular unit; *Drosophila*

INTRODUCTION

Proper neuronal function necessitates a highly regulated extracellular environment, where the concentrations of sodium, potassium, and calcium ions need to be maintained within very narrow ranges. The central nervous system (CNS) is extremely sensitive to a wide range of substances that are otherwise readily metabolized without causing harm to the peripheral organ system. As a result, it is essential that the interface between the CNS and the peripheral circulatory system functions as a dynamic regulator of ion balance, a facilitator of nutrient transport, and a barrier to potentially harmful molecules (Hawkins & Davis 2005). Thus the structural aspects of the cerebral microcirculation, historically referred to as the blood-brain barrier (BBB), perform all these functions.

More than 100 years ago, Paul Ehrlich (1885) observed that certain pharmacologically active molecules and dyes injected into the bloodstream rapidly diffused into most organs with the exception of the CNS, composed of brain, spinal cord, and retina, because these were highly impermeable to most small molecules. Ehrlich proposed that the CNS environment possessed specialized properties that allow selective entry of only a small fraction of circulating factors. These and related findings formed the concept of a BBB that functions as a barricade to block blood-borne materials from entering the CNS microenvironment and also prevents permeability in the outward direction such that chemicals released from the nerve cells do not mix with blood (Goldmann 1913). Thus the concept of a vascular BBB, which also functions as a brain-blood barrier, was born (Bradbury 1979). Researchers now accept that several blood-

" Neurobiological diseases involving BBB breakdown and dysfunction include stroke, neoplasia, neurodegeneratve diseases (e.g., Parkinson's disease, Alzheimer's disease), epilepsy, infections, or inflammatory processes [meningitis, multiple sclerosis (MS), HIV], trauma etc"

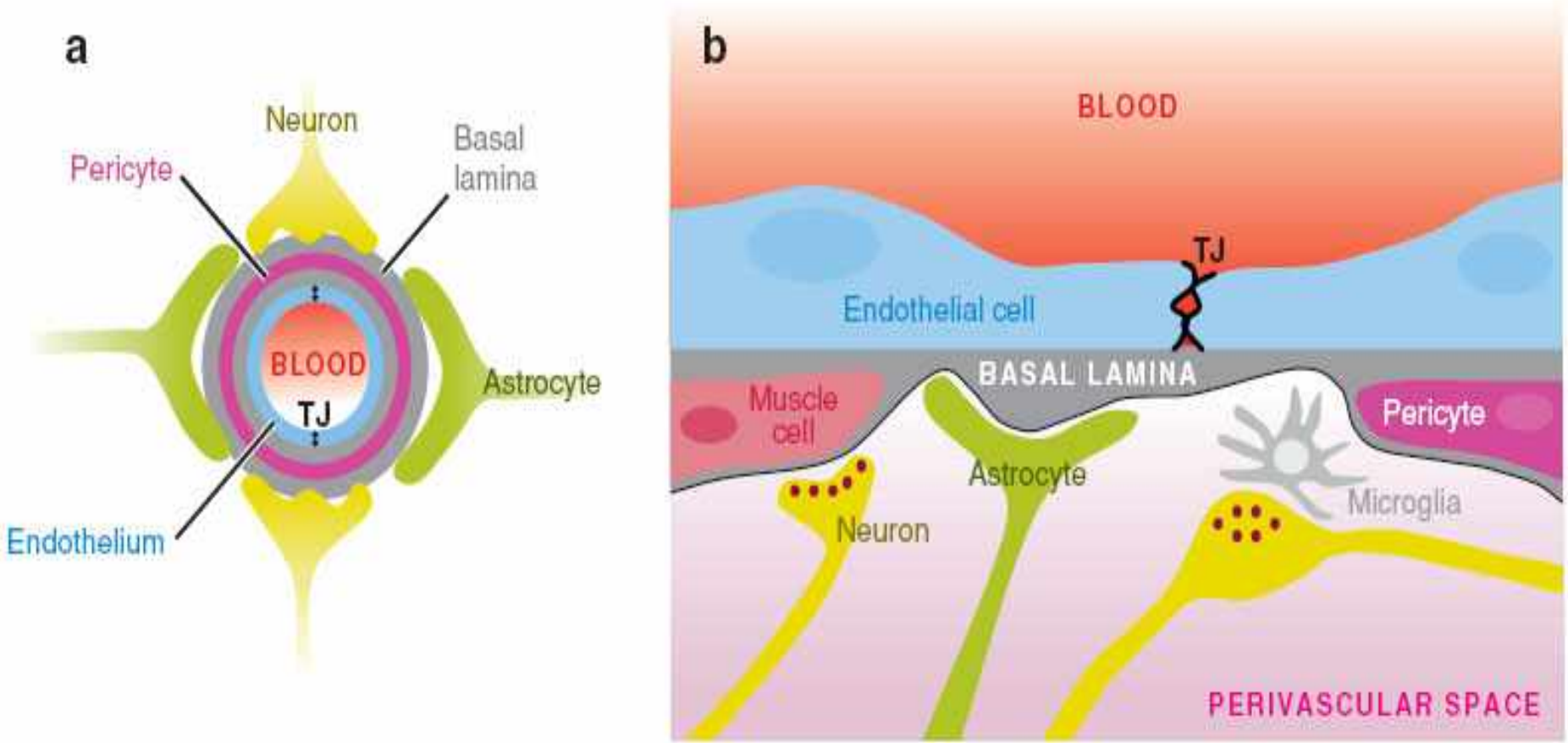

Figure 6

Schematic of the neurovascular unit. (*a*) A cross-section through a brain capillary shows adjacent endothelial cells connected by TJs that establish the BBB. The endothelial cell layer is surrounded by the basal lamina that separates the endothelium from the pericytes, astrocytes, and neurons. (*b*) A longitudinal section through a portion of a brain capillary reveals the presence of adjacent endothelial cells connected by TJs. Pericytes are present within the basal lamina in close proximity to the endothelial cells, whereas astrocytic endfeet are on the outer surface of the basal lamina. Microglia, nerve fibers, and neuromuscular synapses are found in the perivascular space. Panel *b* has been modified with permission from Abbott 2005; copyright 2005 by Springer Science and Business Media.

Starring roles for astroglia in barrier pathologies of gut and brain

Tor C Savidge[1], Michael V Sofroniew[2] and Michel Neunlist[3,4,5]

The gastrointestinal tract is a highly innervated organ and enteric neuropathy is emerging as a central feature of a wide range of gut diseases. Although most considerations of the enteric nervous system have focused on neuronal dysfunction, a large population of astrocyte-like glia populates gut muscle layers and the intestinal mucosa, and mounting new evidence points toward enteric glia as active participants in gut pathology. Similarly, in the central nervous system increasing evidence suggests that dysfunctions of astrocytes play central roles in disease mechanisms. On the basis of the premise that gut-brain disease paradigms may exist, we explore the possibility that enteric glia constitute a previously unrecognized disease target in pathologies associated with intestinal barrier dysfunction, notably inflammatory bowel disease, necrotizing enterocolitis, irritable bowel syndrome, diabetes, autoimmune disease and neurotrophic virus infection of the gut.

Laboratory Investigation (2007) **87,** 731–736; doi:10.1038/labinvest.3700600; published online 2 July 2007

KEYWORDS: blood–brain barrier; astrocyte; enteric glia; intestinal permeability; inflammatory bowel disease; S-nitrosothiol

THE ENTERIC NERVOUS SYSTEM AND ITS KNOWN DISEASES

Gastrointestinal tissues are innervated by a highly complex and extensive component of the peripheral nervous system known as the enteric nervous system (ENS).[1] Enteric neurons control several aspects of gut function, including motility, microvascular circulation, epithelial secretion of fluid, ions and bioactive peptides and intestinal barrier function. In addition to neurons, enteric glia represent an extensive component population of the ENS and show morphologic and functional similarities to CNS astrocytes.[2] Enteric glia have long been suggested to provide trophic and cytoprotective functions toward enteric neurons, and likely are involved in regulating neuronal activity as has been demonstrated for astrocytes.

An emerging concept in gastroenterology is that a wide range of diseases, for example motility disorders and inflammation, can be considered in part as enteric neuropathies. Although it often remains elusive to determine whether these neuropathies are the cause or effect of disease activity, ENS alterations may at least in part be symptomatic. Until recently, studies of enteric neuropathies have mainly focused on characterizing altered neuropeptide expression patterns and the involvement of enteric neurons[1] (Table 1). Scarce, but increasing data suggest that enteric glia are also major players in gut disease. Indeed, the main histopathologic observations made by the group of Bassotti *et al*[3] have demonstrated that motor disorders of the gut, such as slow transit constipation, diverticular disease and idiopathic megacolon, are associated with enteric glial abnormalities. Reinforcing these observations are *in vivo* animal experiments in which alterations of glial cell function result in reduced intestinal motility and a slowing of gastric emptying.[4,5]

Increasing evidence also suggests that enteric glia play a major role in gut pathologies associated with barrier dysfunction. Alterations in intestinal permeability are observed in a wide range of diseases ranging from high-grade inflammatory pathologies such as inflammatory bowel disease, celiac disease and enteric infection, to low-grade inflammatory diseases such as irritable bowel syndrome and diabetes. Indeed, all of these diseases present an increase in intestinal permeability that may be regarded as a contributing event in the onset of pathology. Therefore, regulation of intestinal barrier function by its microenvironment, and in

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"Increased barrier dysfunction is associated with a disruption of tight junction associated proteins expressed by BBB and IEC and is likely regulated by astroglial cell disruption, for example, by autoimmune reactions or neurotrophic virus infection."

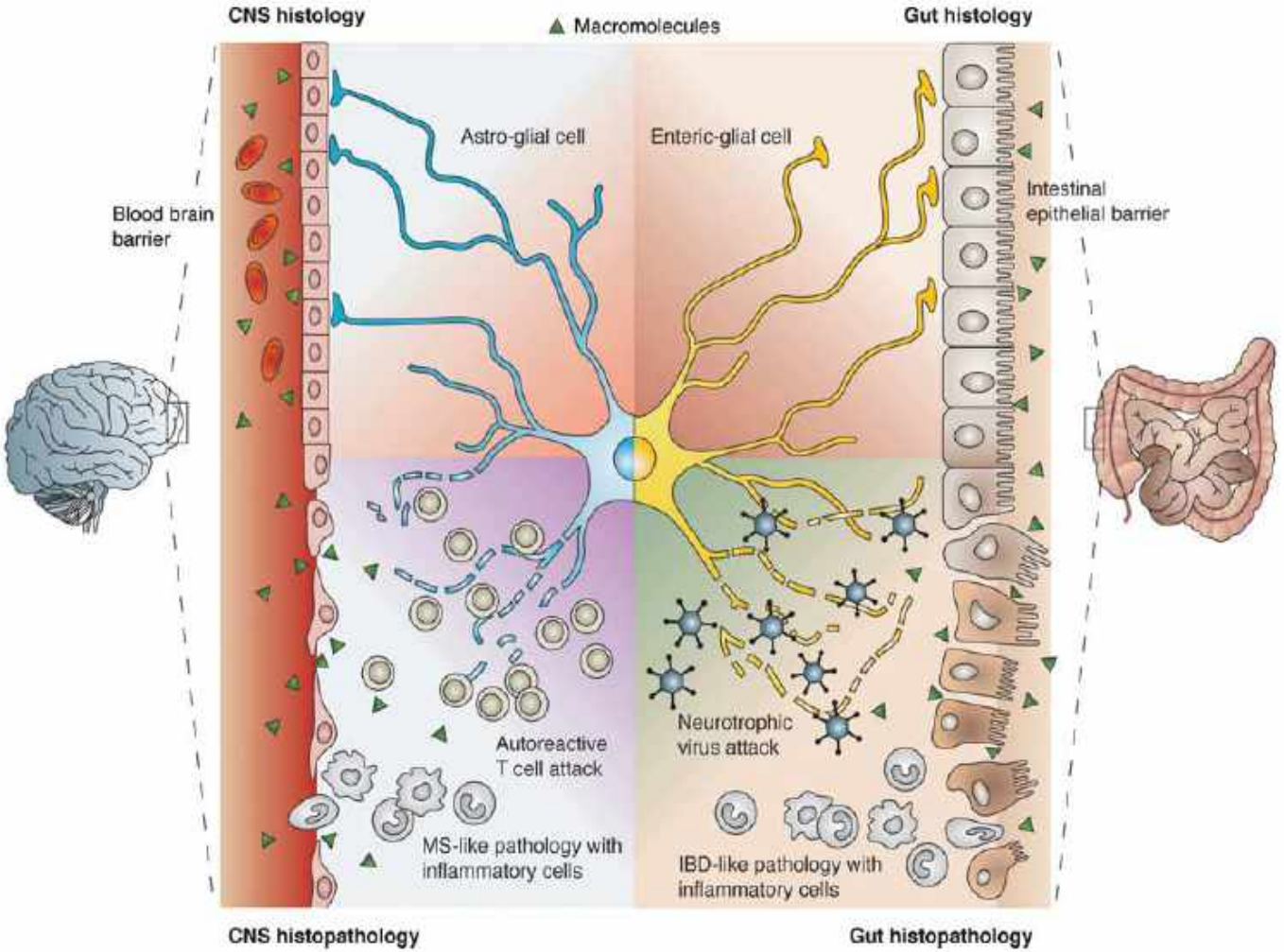

Figure 2 Gut-brain disease paradigm. Schematic illustration of astroglial regulation of barrier function in gut and CNS disease states. CNS morphology and pathology following disruption of blood–brain barrier (BBB) function are shown on the left. Gut morphology and pathology following disruption of intestinal epithelial cell (IEC) barrier function are shown on the right. Barrier dysfunction in both tissues is associated with an influx of polymorpholeukocytes, which contribute to disease activity. Increased barrier dysfunction is associated with a disruption of tight junction associated proteins expressed by BBB and IEC and is likely regulated by astroglial cell disruption, for example, by autoimmune reactions or neurotrophic virus infection.

“these findings provide evidence that astroglial-like cells in both brain and gut contribute interchangeably to barrier functions, suggesting a previously unrecognized paradigm whereby cellular interactions previously thought to be unique to the blood–brain barrier, also regulate gut epithelial permeability.”

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REVIEW ARTICLE - BRAIN INJURY

A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury

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Abstract
Background In order to improve injury assessment of brain injuries, protein markers of pathophysiological processes and tissue fate have been introduced in the clinic. The most studied protein "biomarker" of cerebral damage in traumatic brain injury (TBI) is the protein S100B. The aim of this narrative review is to thoroughly analyze the properties and capabilities of this biomarker with focus on clinical utility in the assessment of patients suffering from TBI.
Results S100B has successfully been implemented in the clinic regionally (1) to screen mild TBI patients evaluating the need to perform a head computerized tomography, (2) to predict outcome in moderate-to-severe TBI patients, (3) to detect secondary injury development in brain-injured patients and (4) to evaluate treatment efficacy. The potential opportunities and pitfalls of S100B in the different areas usually refer to its specificity and sensitivity to detect and assess intracranial injury.
Conclusion Given some shortcomings that should be realized, S100B can be used as a versatile screening, monitoring and prediction tool in the management of TBI patients.

Keywords S100B · Traumatic brain injury · Outcome · Monitoring · Screening · Biomarker · Serum · Humans

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Introduction

Traumatic brain injury (TBI) is a common cause of death and disability, primarily in the young but increasingly among the elderly [153]. The injury panorama stretches from the severely injured, unconscious patients in need of neuro-intensive care to the more common mildly injured patients, sometimes without any visual lesions. Many survivors, even from seemingly mild injuries, may suffer from permanent disabilities and be in need of long-term rehabilitation with high costs for society [56].

TBI is a complex disease and may change symptomatology over time [102]; it is heterogenic in nature and may contain a plethora of different hemorrhagic and non-hemorrhagic injures, both inside and outside the brain parenchyma. At admission to the hospital, the physicians often rely solely on a neurological examination and a computerized tomography (CT) scan, as other more advanced radiological options are unavailable in the acute care setting and monitoring tools are available only in specialized neuro-intensive care units (NICUs). Consequently, the assessment methods are often limited, and better surrogate markers of brain injury have been sought to help the treating clinician. In many fields of medicine, biological markers ("biomarkers") of injury have been introduced. A biomarker is defined as "A characteristic that is

The most studied protein biomarker of cerebral damage in traumatic brain injury (TBI) is the protein S100B…..**Conclusion:** Given some shortcomings that should be realized, *S100B can be used as a versatile screening, monitoring and prediction tool in the management of TBI patients.*

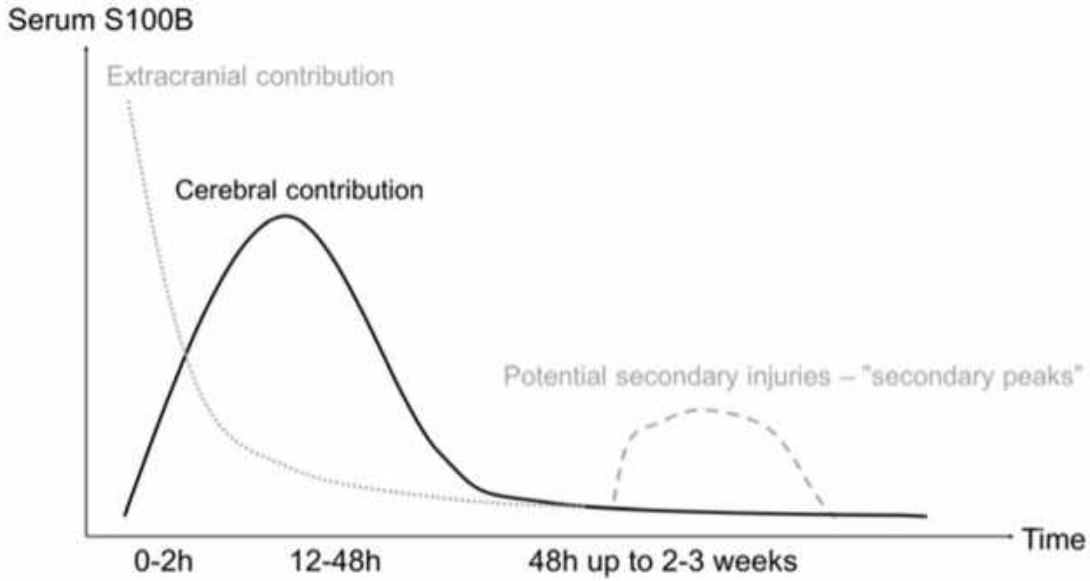

Fig. 1 Schematic overview of S100B release to serum. Schematic overview of the S100B release following severe TBI. Initially, there will be a great release of S100B from extracranial tissue to the serum (*dotted, gray line*), which will have a rapid wash-out the first hours after injury. While the cerebral release is more prolonged and shaped as a gamma function, as suggested by Ercole et al. 2016 (black line), it will initially be "masked" by these extracranial contributions. Our *black line* illustrates an "uneventful" release of S100B in a patient suffering from severe TBI; however, patients may suffer from subsequent injuries resulting in "secondary peaks" of S100B (*dashed, gray line*)

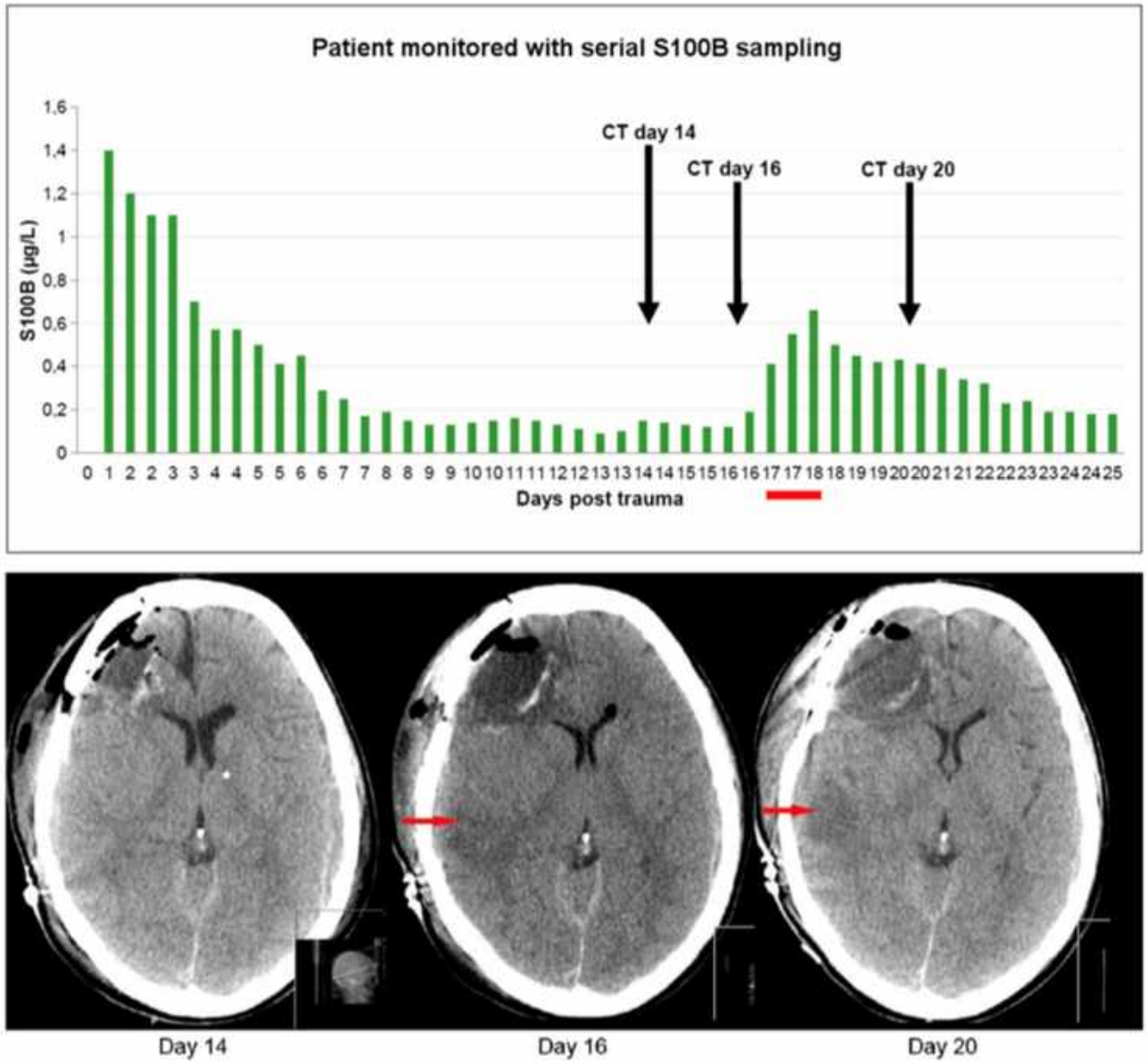

Fig. 2 S100B monitoring of a TBI patient. The patient was monitored with subsequent sampling of S100B (twice per day, y-axis µg/l of S100B and x-axis days after trauma) illustrating an initial decline the first days following trauma approaching baseline levels. However, at day 16–18 (*bar*), there is a secondary peak of S100B that correlates to the development of a right temporal infarction as seen on computerized tomography (*arrows*)

Clinical Interpretation Table

Array 7

ANTIGEN	ASSOCIATED WITH	TABLE REFERENCES
Myelin Basic Protein	• Demyelinating Diseases • Autism • PANDAS / OCD	Ponomarenko *et al. PNAS*, 2006; 103(2):281-286. Berger *et al. N Engl J Med*, 2003; 349:139-145. Vojdani *et al. J Int Med*, 2003; 254:363-374.
Asialoganglioside	• Chronic Inflammatory Demyelinating Polyneuropathy • Multiple Sclerosis • Guillain Barré Syndrome • PANDAS / ANDAS / OCD	Baba *et al. J Neuroimmunol.* 1989; 25:143-150. Bansal *et al. J Clin Pathol.* 1994; 14:300-302. Jacobs *et al. J Infect Disease.* 1997; 175:729-733. Vojdani A. *Latitudes.* 6(2):1-6.
α + β Tubulin	• Demyelinating Diseases • Early Onset Type 1 Diabetes • Thyroid Disorders	Kirvan *et al. J Immunol*, 2007; 178:7412-7421. Rousset *et al. Clin Exp Immunol*, 1983; 52:325-332. Rousset *et al. Diabetologia*, 1984; 27:427-432.
Cerebellar	• Celiac Disease • Gluten Ataxia Paraneoplastic Cerebellar Degeneration Syndrome	Vojdani *et al. Nutr Neurosci*, 2004; 7(3):151-161. Balegno *et al. Anticancer Res*, 2005; 25:3211-3214. Blaes *et al. Ann Neurol*, 2005; 58:313-317.
Synapsin	• Inhibited Neurotransmitter Release • Demyelinating Diseases	Gitlits *et al. J Invest Med*, 2001; 49(3):276-283. Bustos *et al. J Cell Sci*, 2001; 114:3695-3704. Bitsch *et al. J Neurol*, 2004; 251:1498-1501.

When to Order Array 7X

- To capture the earliest stage and later stages of the autoimmune process
- IgM antibodies are the preferred biomarker for some neurological disorders:
  - multi-focal motor neuropathy
  - lower motor neuron syndromes
  - earlier development of relapses in multiple sclerosis and chronic inflammatory demyelinating polyneuropathy

The Central Role of BBB in Neuroautoimmunity

When the BBB is damaged it provides a gateway for environmental triggers to infiltrate the brain and nervous system. Due to the similarity between some of these triggers and neurological tissues, neuro-reactive antibodies can be formed. Neuronal autoantibodies contribute to the onset of neurological diseases. Known cross-reactions between neurological tissues and environmental triggers include:

- Asialoganglioside
  - Gliadin[21]
  - *Campylobactor jejuni* lipopolysaccharides[20]
  - Streptococcal proteins[22]
- Cerebellar
  - Gliadin[21 23]
  - Milk butyrophilin[23]
- Myelin Basic Protein
  - Gliadin[21]
  - *Chlamydia pneumoniae*[24]
  - Herpes-6[24]
  - Streptococcal protein[24 25]
- Myelin Oligodendrocyte Glycoprotein
  - Milk butyrophilin[23]
- Synapsin
  - Gliadin[21]
- Tubulin
  - Streptococcal protein[25]

TEST	RESULT			
Array 7X - Neurological Autoimmune Reactivity Screen - Expanded	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Myelin Basic Protein IgG + IgA	0.96			0.6-1.7
Myelin Basic Protein IgM	0.83			0.0-1.3
Asialoganglioside IgG+IgA	0.96			0.6-1.6
Asialoganglioside IgM	0.59			0.1-1.4
Alpha + Beta Tubulin IgG+IgA	0.77			0.0-2.7
Alpha + Beta Tubulin IgM	0.53			0.0-1.3
Cerebellar IgG+IgA	0.74			0.4-1.5
Cerebellar IgM	0.55			0.0-1.3
Synapsin IgG+IgA		2.10		0.0-2.1
Synapsin IgM		1.36		0.1-1.5

Cellular/Molecular

Enhancing Expression of Nrf2-Driven Genes Protects the Blood–Brain Barrier after Brain Injury

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The Vivian L. Smith Center for Neurologic Research and Departments of Neurobiology and Anatomy, and Neurosurgery, The University of Texas Medical School, Houston, Texas 77225

The integrity of the blood–brain barrier (BBB) is critical for normal brain function, and its compromise contributes to the pathophysiology of a number of CNS diseases and injuries. Using a rodent model of brain injury, the present study examines the pathophysiology of BBB disruption. Western blot and immunohistochemical analyses indicate that brain injury causes a loss of capillary endothelial cells and tight junction proteins, two critical components of the BBB. Activation of the transcription factor NF-E2-related factor-2 (Nrf2) by sulforaphane, a naturally occurring compound present in high levels in cruciferous vegetables, significantly increased the expression of endogenous cytoprotective genes in brain tissue and microvessels as indicated by real-time PCR analysis. Postinjury administration of sulforaphane reduced the loss of endothelial cell markers and tight junction proteins and preserved BBB function. These protective effects were dependent on the activity of Nrf2. Injured rats pretreated with decoy oligonucleotides containing the binding site of Nrf2, and mice lacking the *nrf2* gene, did not benefit from sulforaphane administration. These findings indicate a potential therapeutic usefulness for Nrf2-activating molecules to improve the function of the neurovascular unit after injury.

Key words: brain edema; endothelial cells; neurovascular unit; tight junction proteins; TBI; capillary

Introduction

The blood–brain barrier (BBB) is critical for the maintenance of brain homeostasis and neural functions. BBB compromise contributes to the pathological changes associated with a number of neurological diseases, brain tumors, and CNS injury (Petty and Lo, 2002; DeWitt and Prough, 2003; Oby and Janigro, 2006). Loss of BBB integrity not only disrupts the function of the neurovascular unit (an assembly of brain capillaries, extracellular matrix of the basal lamina, neurons, astrocytes, oligodendrocytes, and pericytes) but also initiates secondary pathological processes, including infiltration of inflammatory cells and circulating fluid. Cerebral edema, resulting in part from the accumulation of circulating fluid, causes increased intracranial pressure leading to decreased cerebral blood flow, tissue herniation, and poor outcome (Fishman, 1975).

A layer of brain capillary endothelial cells and the contiguous tight junctions between them are major components of the BBB. Ultrastructural studies have shown that brain endothelial cells have a paucity of fenestrae and endocytotic vesicles limiting the transcellular entry of circulating molecules into the brain. In addition, the interconnection of brain capillary endothelial cells via tight junction complexes severely restricts the paracellular entry of molecules. Although the mechanism(s) contributing to brain injury-induced BBB disruption has not been fully elucidated, it is likely that multiple processes, including endothelial cell death, tight junction breakdown, and decreased coupling between tight junction proteins and the cytoskeleton, could contribute to altered BBB permeability. Because multiple mechanisms may underlie BBB disruption after brain injury, combination treatments are likely to be more effective than single-target therapies to improve BBB function. As an alternate to combination treatments, a single agent that activates multiple cytoprotective mechanisms may also be suitable as a treatment option (Lyeth et al., 1993).

The transcription factor Nrf2 (nuclear factor E2-related factor 2) binds to the antioxidant/electrophilic response element (ARE/EpRE) and regulates the expression of multiple cytoprotective proteins, including antioxidant and glutathione generating enzymes (Thimmulappa et al., 2002; Lee et al., 2003; Hu et al., 2004, 2006). Nrf2 is a rapidly turned-over protein that is normally sequestered in the cytoplasm via an interaction with the actin-binding protein Keap1 (Kelch-like ECH associated protein 1). Recent studies have demonstrated that Keap1 acts as a substrate adaptor protein for Cul-3 (cullin-3)-dependent ubiquitination of Nrf2, which leads to rapid Nrf2 degradation by the proteosome system (McMahon et al., 2003; Zhang et al., 2004; Kobayashi and Yamamoto, 2006). Cellular stressors reduce the proteosomal degradation of Nrf2, resulting in its nuclear accumulation and increased expression of Nrf2-driven genes (Pi et al., 2003; Zhang and Hannink, 2003). In the present study, we demonstrate that systemic administration of sulforaphane, an isothiocyanate abundant in cruciferous vegetables (e.g., broccoli) but not en-

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 0270-6474/07/2710240-09$15.00/0

Activation of the transcription factor NF-E2-related factor-2 (Nrf2) by sulforaphane, a naturally occurring compound present in high levels in cruciferous vegetables, significantly increased the expression of endogenous cytoprotective genes in brain tissue and microvessels as indicated by real-time PCR analysis. **Post-injury administration of sulforaphane reduced the loss of endothelial cell markers and tight junction proteins and preserved BBB function.**


Optic chyasm
Pituitary gland
Vertebral artery

J Alzheimers Dis. 2010;20 Suppl 1:S127-41.

Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer's and Parkinson's diseases.

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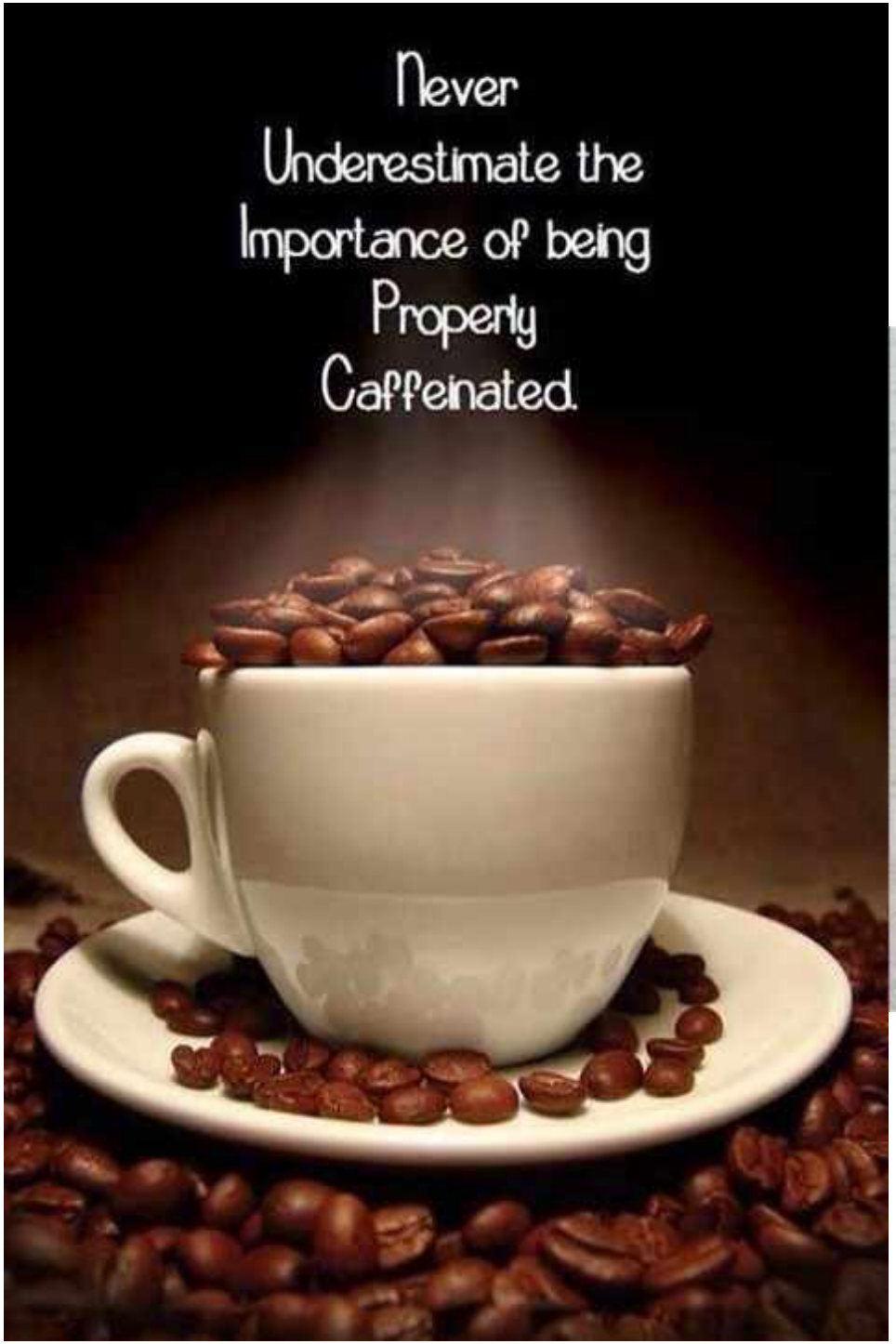
Department of Pharmacology, Physiology and Therapeutics, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND, USA.

Abstract

Sporadic Alzheimer's disease (AD) and Parkinson's disease (PD) are two of the most common neurodegenerative diseases and as such they represent major public health problems. Finding effective treatments for AD and PD represents an unmet and elusive goal largely because these diseases are chronic and progressive, and have a complicated and ill-understood pathogenesis. Although the underlying mechanisms are not fully understood, caffeine, the most commonly ingested psychoactive drug in the world, has been shown in human and animal studies to be protective against AD and PD. One mechanism implicated in the pathogenesis of AD and PD is blood-brain barrier (BBB) dysfunction and we reported recently that caffeine exerts protective effects against AD and PD at least in part by keeping the BBB intact. The present review focuses on the role of BBB dysfunction in the pathogenesis of AD and PD, caffeine's protective effects against AD and PD, and potential mechanisms whereby caffeine protects against BBB leakage.


Never
Underestimate the
Importance of being
Properly
Caffeinated.


+Dunken K Bliths

Bested et al. Gut Pathogens 2013, 5:5
http://www.gutpathogens.com/content/5/1/5

Gut Pathogens

REVIEW Open Access

Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part I – autointoxication revisited

Alison C Bested[1], Alan C Logan[2*] and Eva M Selhub[3]

Abstract

Mental health disorders, depression in particular, have been described as a global epidemic. Research suggests that a variety of lifestyle and environmental changes may be driving at least some portion of the increased prevalence. One area of flourishing research involves the relationship between the intestinal microbiota (as well as the related functional integrity of the gastrointestinal tract) and mental health. In order to appreciate the recent scientific gains in this area, and its potential future directions, it is critical to review the history of the topic. Probiotic administration (e.g. Lactobacillus) and fecal microbiota transfer for conditions associated with depression and anxiety is not a new concept. Here, in the first of a 3-part series, we begin by reviewing the origins of the contemporary research, providing a critical appraisal of what has become a revisionist history of the controversial term 'autointoxication'. We argue that legitimate interests in the gut-brain-microbiota connection were obscured for decades by its association with a narrow historical legacy. Historical perspectives provide a very meaningful context to the current state of the contemporary research as outlined in parts II and III.

Keywords: Intestinal microbiota, Autointoxication, Depression, Anxiety, Probiotics, Microbial ecology, Lipopolysaccharide endotoxin, Diet, Intestinal permeability

Series introduction

The global mental health crisis and prevalence of depression is increasingly being viewed, at least to some degree, as a consequence of modernization. There are numerous suspect candidates to explain what has been described as an epidemic increase in mental health disorders. These include, but are not limited to, socioeconomic changes, urbanicity, alterations in dietary habits, sedentary behavior, excessive screen-based information consumption, lack of adequate sunlight, erosion of real-world (off-line) social support, and an overall disconnect from nature [1-3]. Researchers are beginning to explore the ways in which these and other factors may combine to influence mental health in contemporary society.

One area of flourishing research involves the neuropsychological consequences of alterations to gut microbiota (formerly referred to as "flora" or "microflora") in conjunction with modern stressors, and an urbanized, Western lifestyle [4]. Almost a decade has passed since members of our group broke a 70-year-old scientific taboo by constructing a framework indicating that probiotics might play a beneficial role in conditions of human fatigue and depressive disorders [5,6]. Broadly speaking, ours was certainly not a new theory; it was, rather, a scientifically refined revival of select assertions that had been made a century prior. At our time of revival, in the early 2000s, the contention that the intestinal microbiota and the microbial-influenced integrity of the intestinal lining are of relevance to mental health disorders was, if it were to be suggested at all, a notion of nostalgia. Suggesting that intentional microbial manipulation could positively influence mental health, at least within scientific writing, was inevitably linked to the early 20th century, to a time when some within medicine had veered off a rational course in a relatively short-lived obsession with so-called 'autointoxication' and 'intestinal toxemia' [7-11]. During this period the colon was viewed as the central road to a limitless array

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Probiotic administration (e.g. Lactobacillus) and fecal microbiota transfer for conditions associated with depression and anxiety is not a new concept. Here, in the first of a 3-part series, we begin by reviewing the origins of the contemporary research, providing a critical appraisal of what has become a revisionist history of the controversial term ‘ autointoxication’ .

COLON IRRIGATION IN THE TREATMENT OF MENTAL DISEASE*

BY HAROLD K. MARSHALL, M.D.,† AND CHARLES E. THOMPSON, M.D.†

THE purpose of this article is to present a report of the result of the treatment of mental disease by the use of colon irrigation at the Gardner State Colony and to call attention to the possible wider value of this procedure in medical and surgical problems.

The first use of the enema dates back to the earliest days of our civilization, but the colon irrigation as practised today is of recent origin. The literature on this subject previous to an article by Mills and Bird[1] (1919) is scanty. These authors call attention to the beneficial use of colon irrigation in children with elevation of temperature, constipation, gas in the intestines, and in children needing mental sedation.

Bodkin[2] (1921) recommends this procedure and gives a technique which does not vary much from that used at the present time. Zobel[3] (1921) notes its efficacy in certain surgical cases, and advises caution in the technique. Schellberg[4] (1923) writes comprehensively of the technique of performing the procedure. Blackman[5] (1925) insists that the colon becomes engorged by neglect and emphasizes the necessity of keeping it clean by the use of irrigation. Shaine[6] (1926) concludes that many patients with chronic constipation are relieved by the use of colonic irrigation when medical treatment has failed. Snyder and Fineman[7] (1927) note beneficial results in arthritis, and publish a series of roentgenograms of colons illustrating their views. They credit Dr. Lockwood of New York with being the first physician to use this procedure. Rendall[8] (1929) notes that colon irri-

found it [illegible] 36 to 48 hours, [illegible] ating the circulation time of the colon in three groups of mental cases he reports his results as follows: dementia praecox 113 hours; manic depressive psychosis 154 hours; psychoneurosis 80 hours. After treating patients in each of these groups by colon irrigation he reports that of his dementia praecox cases 17% recovered; of his depressive psychosis cases 70% recovered; of his psychoneurosis cases 80% recovered. Of the total cases, all groups, 40% recovered, 33% improved, and 57% were released on visit. Jameson[12] (1930) states that 36 toxic substances; indol, skatol, phenol, botulin, triptophan, for example, exist [illegible] colon and that [illegible] reason that people can endure colon stasis is because of the detoxicating effect of the liver. He quotes Widal, Abrami and Ianeovesco[13] to prove that blood in the portal vein is not detoxicated and when the portal vein in animals is anastomosed into the inferior vena cava so that this blood enters the general circulation hemoclastic shock results every time following feeding. Jameson, therefore, believes that the liver is a buffer and that the detoxicating effect of this organ is sufficient to prevent the entrance of deleterious disintegrating products of colonic putrefaction into the general circulation. He concludes that colon irrigation is of value in mental and nervous states, certain forms of chronic nephritis and deforming diseases of the joints. Morse[14] (1930) writes of the benefits to be derived from this practise and of the technique and to Dr. Morse we are much indebted for his advice and assist-

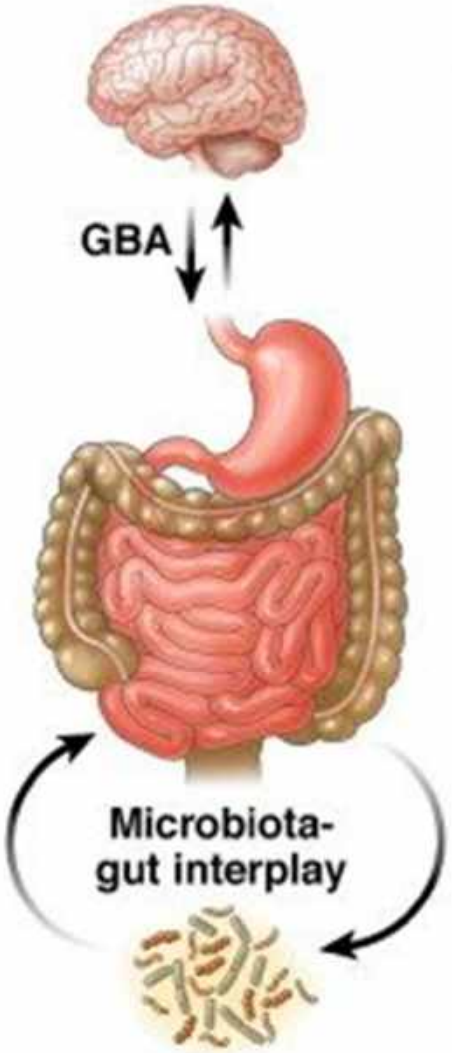
Integration of the microbiota into the gut-brain axis

The ability of the brain to influence the intestinal microbiota

Perturbation of normal habitat via stress-induced changes in gastrointestinal:

- Physiology
- Epithelial function
- Mucin production
- EE cell function
- Motility

Release of neurotransmitters



The ability of the microbiota to influence brain and behavior

Activation of neural afferent circuits to the brain

Activation of mucosal immune responses

Production of metabolites that directly influence the CNS

Giloteaux et al. Microbiome (2016) 4:30
DOI 10.1186/s40168-016-0171-4

Microbiome

RESEARCH Open Access

Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome

Ludovic Giloteaux[1], Julia K. Goodrich[1,2], William A. Walters[1,2], Susan M. Levine[3], Ruth E. Ley[1,2] and Maureen R. Hanson[1*]

Abstract

Background: Gastrointestinal disturbances are among symptoms commonly reported by individuals diagnosed with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). However, whether ME/CFS is associated with an altered microbiome has remained uncertain. Here, we profiled gut microbial diversity by sequencing 16S ribosomal ribonucleic acid (rRNA) genes from stool as well as inflammatory markers from serum for cases ($n = 48$) and controls ($n = 39$). We also examined a set of inflammatory markers in blood: C-reactive protein (CRP), intestinal fatty acid-binding protein (I-FABP), lipopolysaccharide (LPS), LPS-binding protein (LBP), and soluble CD14 (sCD14).

Results: We observed elevated levels of some blood markers for microbial translocation in ME/CFS patients; levels of LPS, LBP, and sCD14 were elevated in ME/CFS subjects. Levels of LBP correlated with LPS and sCD14 and LPS levels correlated with sCD14. Through deep sequencing of bacterial rRNA markers, we identified differences between the gut microbiomes of healthy individuals and patients with ME/CFS. We observed that bacterial diversity was decreased in the ME/CFS specimens compared to controls, in particular, a reduction in the relative abundance and diversity of members belonging to the Firmicutes phylum. In the patient cohort, we find less diversity as well as increases in specific species often reported to be pro-inflammatory species and reduction in species frequently described as anti-inflammatory. Using a machine learning approach trained on the data obtained from 16S rRNA and inflammatory markers, individuals were classified correctly as ME/CFS with a cross-validation accuracy of 82.93 %.

Conclusions: Our results indicate dysbiosis of the gut microbiota in this disease and further suggest an increased incidence of microbial translocation, which may play a role in inflammatory symptoms in ME/CFS.

Keywords: Myalgic encephalomyelitis, Chronic fatigue syndrome, Inflammation, Lipopolysaccharides, Microbiome, Microbial translocation, Beta-diversity

Background

Myalgic encephalomyelitis (ME), also known as chronic fatigue syndrome (CFS), or ME/CFS, is a debilitating illness of unknown etiology with no widely accepted therapy. Primary symptoms reported by patients are fatigue, muscle and/or joint paint, sore throat, headaches, unrefreshing sleep, and post-exertional malaise and have been the basis of the widely used Fukuda diagnostic criteria [1]. Many ME/CFS patients also report gastrointestinal (GI) symptoms, including but not limited to irritable bowel syndrome (IBS) [2–6]. Intestinal discomfort is also indicated in a survey of drug use by individuals with CFS compared to controls, which found significantly more use of antacids, H2 blockers, and proton pump inhibitors in the ME/CFS cohort [7].

The prevalence of bowel symptoms has led to attempts to treat the disease by probiotic oral or rectal supplements. Borody et al. [8] reported improvements in a majority of

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Conclusions: Our results indicate dysbiosis of the gut microbiota in this disease and further suggest an increased incidence of microbial translocation, which may play a role in inflammatory symptoms in ME/CFS.

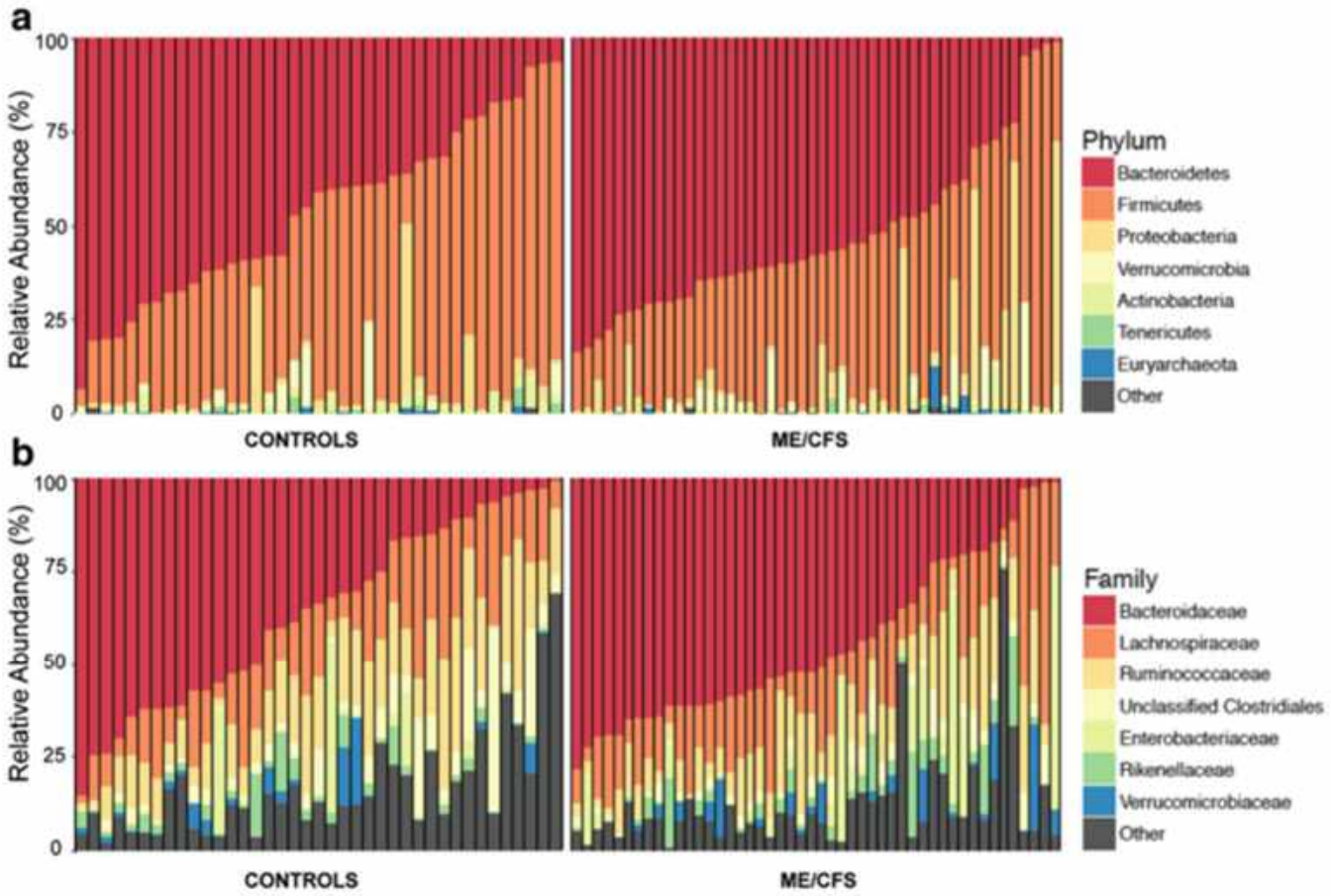

Fig. 4 Composition of the gut microbiome of healthy individuals and ME/CFS patients. Relative abundance of phylum-level (**a**) and family-level (**b**) gut microbial taxa

Neurobiol Aging. 2009 Sep;30(9):1350-60. Epub 2007 Dec 21.

APOE genotype-specific differences in the innate immune response.

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Abstract

Apolipoprotein-E protein is an endogenous immunomodulatory agent that affects both the innate and the adaptive immune responses. Since individuals with the APOE4 gene demonstrate worsened pathology and poorer outcomes in many neurological disorders, we examined isoform-specific differences in the response of microglia, the primary cellular component of the brain's innate immune response, in detail. Our data demonstrate that microglia derived from APOE4/4 targeted replacement mice demonstrate a pro-inflammatory phenotype that includes altered cell morphology, increased NO production associated with increased NOS2 mRNA levels, and higher pro-inflammatory cytokine production (TNFalpha, IL-6, IL12p40) compared to microglia derived from APOE3/3 targeted replacement mice. The effect is gene dose-dependent and increases with the number of APOE4 gene alleles. The APOE genotype-specific immune profile observed in the microglial immune response is also observed in the cortex of aged APOE3/3 and APOE4/4 mice treated with lipopolysacchride (LPS) and in peripheral (peritoneal) macrophages. To determine if APOE4's action resulted from an isoform-specific difference in effective levels of the apolipoproteins, we generated mice expressing only a single allele of APOE3. Immune-stimulated macrophages from APOE3/0 mice demonstrated an increased inflammatory response compared to APOE3/3 mice, but less than in APOE4/4 mice. These data suggest that inhibition of inflammation depends upon the dose of apoE3 protein available and that apoE4 protein may alter inflammation partly by dose effects and partly by being qualitatively different than apoE3. Overall, these data emphasize the important role of apolipoprotein E and of the APOE genotype on the immune responses that are evident in most, if not all, neurological disease.

“Our data demonstrate that microglia derived from APOE4/4 targeted replacement mice demonstrate a pro-inflammatory phenotype that includes altered cell morphology, increased NO production associated with increased NOS2 mRNA levels, and higher pro-inflammatory cytokine production (TNF alpha, IL-6, IL) compared to microglia derived from APOE3/3 targeted replacement mice.”

Expert Rev Mol Med. 2011 Sep 20;13:e30.

Emerging roles of pathogens in Alzheimer disease.

Miklossy J.

International Alzheimer Research Centre, Prevention Alzheimer Foundation, Martigny-Combe, Switzerland. judithmiklossy@bluewin.ch

Abstract

Chronic spirochetal infection can cause slowly progressive dementia, cortical atrophy and amyloid deposition in the atrophic form of general paresis. There is a significant association between Alzheimer disease (AD) and various types of spirochete (including the periodontal pathogen Treponemas and Borrelia burgdorferi), and other pathogens such as Chlamydophyla pneumoniae and herpes simplex virus type-1 (HSV-1). Exposure of mammalian neuronal and glial cells and organotypic cultures to spirochetes reproduces the biological and pathological hallmarks of AD. Senile-plaque-like beta amyloid (Aβ) deposits are also observed in mice following inhalation of C. pneumoniae in vivo, and Aβ accumulation and phosphorylation of tau is induced in neurons by HSV-1 in vitro and in vivo. Specific bacterial ligands, and bacterial and viral DNA and RNA all increase the expression of proinflammatory molecules, which activates the innate and adaptive immune systems. Evasion of pathogens from destruction by the host immune reactions leads to persistent infection, chronic inflammation, neuronal destruction and Aβ deposition. Aβ has been shown to be a pore-forming antimicrobial peptide, indicating that Aβ accumulation might be a response to infection. Global attention and action is needed to support this emerging field of research because dementia might be prevented by combined antibiotic, antiviral and anti-inflammatory therapy.

There is a significant association between Alzheimer disease (AD) and various types of spirochete (including the periodontal pathogen Treponemas and Borrelia burgdorferi), and other pathogens such as Chlamydophyla pneumoniae and herpes simplex virus type-1 (HSV-1)

Short-Chain Fatty Acids and Lipopolysaccharide as Mediators Between Gut Dysbiosis and Amyloid Pathology in Alzheimer's Disease

Article type: Research Article
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Abstract: Background:Metagenomic data support an association between certain bacterial strains and Alzheimer's disease (AD), but their functional dynamics remain elusive. Objective:To investigate the association between amyloid pathology, bacterial products such as lipopolysaccharide (LPS) and short chain fatty acids (SCFAs: acetate, valerate, butyrate), inflammatory mediators, and markers of endothelial dysfunction in AD. Methods:Eighty-nine older persons with cognitive performance from normal to dementia underwent florbetapir amyloid PET and blood collection. Brain amyloidosis was measured with standardized uptake value ratio versus cerebellum. Blood levels of LPS were measured by ELISA, SCFAs by mass spectrometry, cytokines by using real-time PCR, and biomarkers of endothelial dysfunction by flow cytometry. We investigated the association between the variables listed above with Spearman's rank test. Results:Amyloid SUVR uptake was positively associated with blood LPS ($rho \geq 0.32$, $p \leq 0.006$), acetate and valerate ($rho \geq 0.45$, $p < 0.001$), pro-inflammatory cytokines ($rho \geq 0.25$, $p \leq 0.012$), and biomarkers of endothelial dysfunction ($rho \geq 0.25$, $p \leq 0.042$). In contrast, it was negatively correlated with butyrate ($rho \leq -0.42$, $p \leq 0.020$) and the anti-inflammatory cytokine IL10 ($rho \leq -0.26$, $p \leq 0.009$). Endothelial dysfunction was positively associated with pro-inflammatory cytokines, acetate and valerate ($rho \geq 0.25$, $p \leq 0.045$) and negatively with butyrate and IL10 levels ($rho \leq -0.25$, $p \leq 0.038$). Conclusion:We report a novel association between gut microbiota-related products and systemic inflammation with brain amyloidosis via endothelial dysfunction, suggesting that SCFAs and LPS represent candidate pathophysiologic links between the gut microbiota and AD pathology.
Keywords: Brain amyloidosis, inflammation, lipopolysaccharide, microbiota, short chain fatty acids
DOI: 10.3233/JAD-200306
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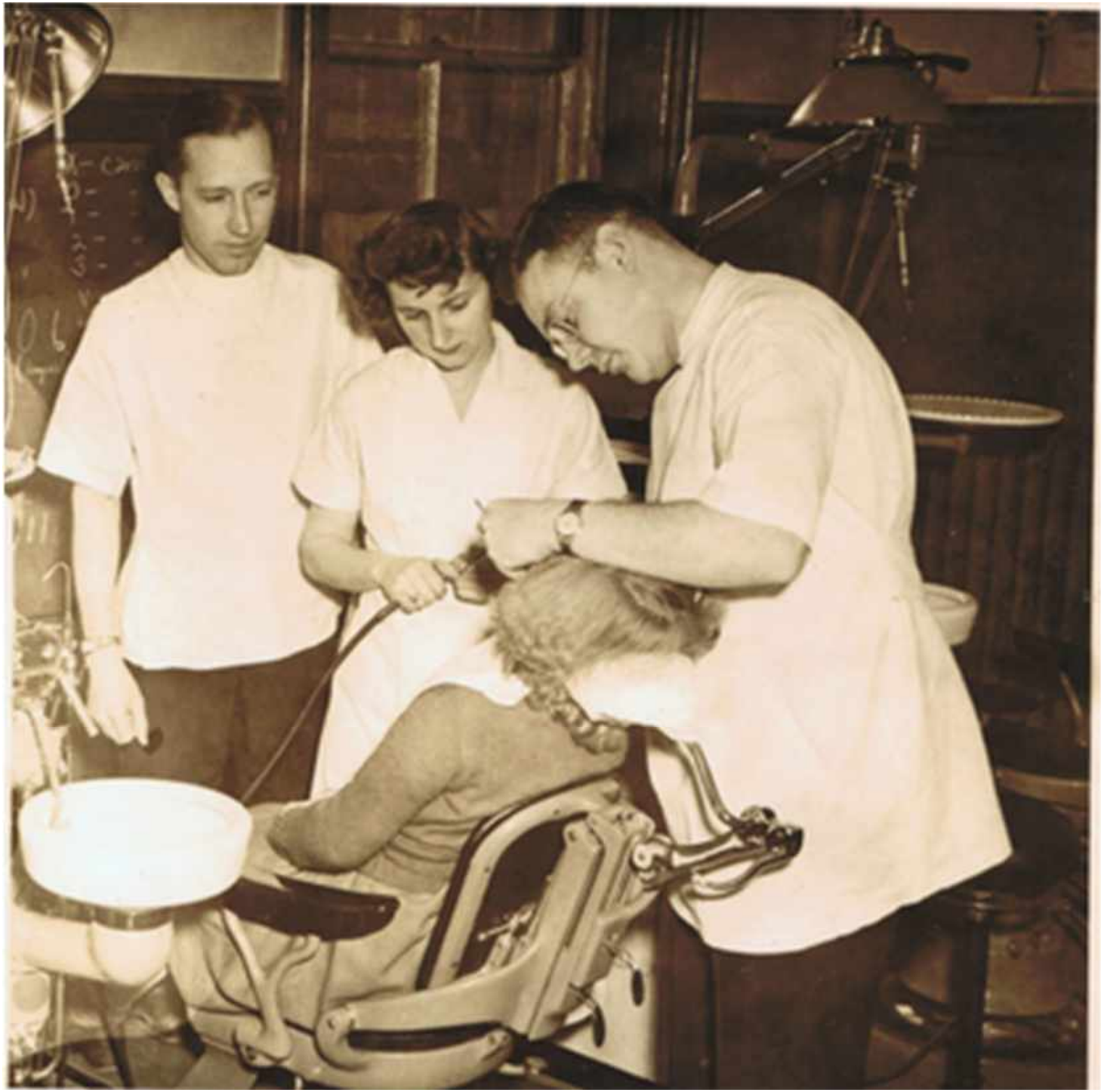
Accepted 28 August 2020

"We have already shown that the gut microbiota composition in patients with Alzheimer's disease was altered, compared to people who do not suffer from such disorders," he explains. "Their microbiota has indeed a reduced microbial diversity, with an over-representation of certain bacteria and a strong decrease in other microbes. **Furthermore, we have also discovered an association between an inflammatory phenomenon detected in the blood, certain intestinal bacteria and Alzheimer's disease; hence the hypothesis that we wanted to test here: could inflammation in the blood be a mediator between the microbiota and the brain?"**



Okamoto et al. Behavioral and Brain Functions 2010, 6:77
http://www.behavioralandbrainfunctions.com/content/6/1/77

BBF BEHAVIORAL AND BRAIN FUNCTIONS

RESEARCH Open Access

Relationship of tooth loss to mild memory impairment and cognitive impairment: findings from the fujiwara-kyo study

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Abstract

Background: This cross-sectional study investigated the relationship between the number of remaining teeth to mild memory impairment (MMI), which is a preclinical stage of dementia, and to cognitive impairment.

Methods: The subjects were aged 65 years or older and were grouped according to their score for the Mini-Mental State Examination (MMSE), the three-word delayed recall test in the MMSE, and the Geriatric Depression Scale into the control group (n = 3,696), the MMI group (n = 121), and the low MMSE score (23 or lower) group (n = 214). We collected data on the number of remaining teeth, the length of the edentulous period, health-related lifestyle, medical history, blood pressure, height, and body weight. Fasting venous blood samples were also obtained.

Results: Multiple logistic regression analysis, adjusted for depressive symptoms, age, sex, length of education, and other explanatory variables, revealed that the odds ratios of 0-10 remaining teeth to 22-32 remaining teeth were 1.679 (95% CI 1.073-2.627) for MMI and 2.177 (95% CI 1.510-3.140) for a low MMSE score. A significant relationship was also found between the length of the edentulous period and the risk of a low MMSE score (odds ratio 3.102, 95% CI 1.432-6.720) (15 years or more/less than 15 years).

Conclusions: Our findings suggest that tooth loss is associated with cognitive function.

Background

Tooth loss, one of the indicators of periodontal disease [1,2], has been reported to be associated with Alzheimer's disease (AD) and dementia [3,4]. Individuals with clinical dementia have an increased deterioration of their dental health [5], and tooth loss may induce nutritional deficits [6]. Reductions in the number of pyramidal cells [7] and acetylcholine levels [8] in the hippocampus due to the decrease of masticatory function caused by molar loss have been found in animal models. Furthermore, it has been hypothesized [9] that periodontal disease-derived inflammatory molecules, bacteria, and bacterial products enhance brain inflammation [10,11].

Among individuals with mild memory impairment (MMI), 21.2% progressed to illnesses with dementia, including AD (10.6%), vascular dementia (4.8%), or other types of dementia (5.8%), over a period of 5 years [12]; therefore, they represent a high-risk population for dementia. MMI was defined as [13]: (1) no impairment of the activities of daily living (ADL); (2) normal general cognitive function, as determined by a Mini-Mental State Examination (MMSE) score ≥ 24 [14]; (3) objective memory impairment, assessed by the MMSE three-word delayed recall test (Recall test) (low score: 1 or 0); and (4) absence of dementia or depression, diagnosed by geriatric neuropsychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, 3rd edn., revised (DSM-III R) criteria [15].

We hypothesized that tooth loss may also be associated with the preclinical stage of AD and dementia. To investigate our hypothesis in a community-based

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TNF-α and antibodies to periodontal bacteria discriminate between Alzheimer's disease patients and normal subjects

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Abstract

The associations of inflammation/immune responses with clinical presentations of Alzheimer's disease (AD) remain unclear. We hypothesized that TNF-α and elevated antibodies to periodontal bacteria would be greater in AD compared to normal controls (NL) and their combination would aid clinical diagnosis of AD. Plasma TNF-α and antibodies against periodontal bacteria were elevated in AD patients compared with NL and independently associated with AD. The number of positive IgG to periodontal bacteria incremented the TNF-α classification of clinical AD and NL. This study shows that TNF-α and elevated numbers of antibodies against periodontal bacteria associate with AD and contribute to the AD diagnosis.

Keywords

periodontal antibodies; Alzheimer's disease; TNF-α; inflammation; diagnosis; biomarkers

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease afflicting the elderly. In the United States, 4.5 million people have been diagnosed with AD and this number will undoubtedly increase as the population ages and the life-span increases. The specific factors involved in the etiology and pathogenesis of AD have not been completely characterized although

Consistent with these findings, **our study showed that AD subjects are more likely to have infections with periodontal bacteria than NL controls** suggesting that perhaps periodontal bacterial infection may be linked to the pathogenesis of AD (Kamer et al., 2008b).

BMC Neurology

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Research article

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Rapid improvement in verbal fluency and aphasia following perispinal etanercept in Alzheimer's disease

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Abstract

Background: Recent clinical studies point to rapid and sustained clinical, cognitive, and behavioral improvement in both Alzheimer's disease and primary progressive aphasia following weekly perispinal administration of etanercept, a TNF-alpha inhibitor that acts by blocking the binding of this cytokine to its receptors. This outcome is concordant with recent basic science studies suggesting that TNF-alpha functions *in vivo* as a gliotransmitter that regulates synaptic function in the brain. We hypothesized that perispinal etanercept had the potential to improve verbal function in Alzheimer's disease, so we included several standarized measures of verbal ability to evaluate language skills in a clinical trial of perispinal etanercept for Alzheimer's disease.

Methods: This was a prospective, single-center, open-label, pilot study, in which 12 patients with mild-to-severe Alzheimer's disease were administered etanercept, 25–50 mg, weekly by perispinal administration for six months. Two additional case studies are presented.

Results: Two-tailed, paired t-tests were conducted comparing baseline performance to 6-month performance on all neuropsychological measures. Test batteries included the California Verbal Learning Test-Second Edition, Adult Version; Logical Memory I and II(WMS-LM-II) from the Wechsler Memory Scale-Abbreviated; the Comprehensive Trail Making Test (TMT); Boston Naming Test; and letter(FAS) and category verbal fluency. All measures revealed a significant effect except for the Boston Naming Test and the TMT-4, with WMS-LM-II being marginally significant at $p = .05$. The FAS test for letter fluency was most highly significant with a $p < 0.0007$. In addition, rapid improvement in verbal fluency and aphasia in two patients with dementia, beginning minutes after perispinal etanercept administration, is documented.

Conclusion: In combination with the previously reported results of perispinal etanercept in Alzheimer's disease and primary progressive aphasia, these results further argue that larger scale studies of this therapeutic intervention, including Phase 3 trials, are warranted in dementias. In addition, these results may provide insight into the basic pathophysiologic mechanisms underlying Alzheimer's disease and related forms of dementia, and suggest the existence of novel, rapidly reversible, TNF-mediated pathophysiologic mechanisms in Alzheimer's disease which are worthy of further investigation.

"these results may provide insight into the basic pathophysiologic mechanisms underlying Alzheimer's disease and related forms of dementia, and ***suggest the existence of novel, rapidly reversible, TNF-mediated pathophysiologic mechanisms in Alzheimer's disease*** which are worthy of further investigation."

MYPERIOPROGRESS

COMPARISON OF RESULTS

ORALDNA LABS
Innovations in Salivary Diagnostics

Sample, Report
Date Of Birth: 09/20/1980 (37 yrs)
Gender: Female
Patient Id: 789
Patient Location: Test Location A

Previous Test
Specimen#: 5033032170
Accession#: 201807-12481
Specimen: Oral Rinse(P)
Collected: 05/17/2018

Current Test
Specimen#: 5110000014
Accession#: 201807-12514
Specimen: Oral Rinse(P)
Collected: 07/23/2018

COMPARISON OF TEST RESULTS

MyPerioPath - Previous Result

MyPerioPath - Current Result

Summary of Results

Total Bacterial Load

46%
Reduction

Since patient's last test on 05/17/2018:

- Congratulations, since the last test submitted 2 months 6 days ago, the clinical management of this patient has achieved a 46% reduction in periodontal pathogen (burden) load.
- The results show a reduction of the red (Aa, Tf, Td), orange (Fn, Pi, Pm) and green (Ec, Cs) complex pathogens.
- These current results are likely associated with a decrease in both oral and systemic inflammation. Consequences of high pathogenic bacteria present for years and decades add significantly to the risk of life threatening diseases beyond the mouth.
- For most treatment protocols, the maximal reduction in pathogen (burden) load is observed when follow-up testing is performed between 6-12 weeks. This sample was collected at 9 weeks 4 days from the previous test.

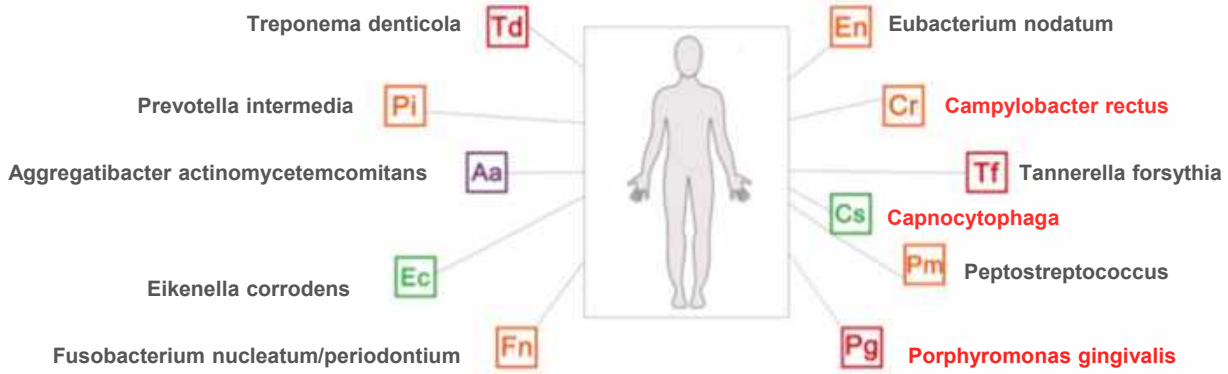
Clinical Comparison	Previous 05/17/2018	Current 07/23/2018
Total # Bacteria Present	8	7
Total # Bacteria Above Threshold	6	0
Deepest Pocket	5	5
Localized Infection	☐	☐
Generalized Infection	☑	☑
Inflammation/Redness	☑	☐
Bleeding on Probing	☐	☐
Bone Loss	☐	☐
Discharge	☐	☐
Halitosis/Malodor	☐	☐

A follow-up test is recommended to monitor the effectiveness of current treatments and to determine the type and frequency of future care.

Clinical Considerations

Reason for Testing	Clinical	Diagnostic	Medical History
☑ Not Provided	☑ Not Provided	☑ Periodontal Classification: Not Provided ☑ Tooth Numbers Pocket Depths(mm)	☑ Not Provided

Systemic Effects of Oral Pathogens



Cancer

Chronic gum disease, involving Aa, Pg, Td, Tf, & Fn is a risk factor for the development of certain cancers including ones involving the pancreas, esophagus, colon, lungs, and the head and neck. Additionally, untreated gum disease is a cause of ongoing inflammation, which may promote the advancing growth of tumors.

Cardiovascular Health

Select bacteria such as Aa, Td, Tf, Pg, Pi, & Fn can leak from blood vessels in the gums and travel to the heart, where cholesterol and other lipids deposit. These bacteria can incite inflammation in arteries, and if occluded, cause a heart attack. A goal of treatment is to minimize the levels of these bacteria as much and as long as possible.

Joint and Musculoskeletal Health

The periodontal bacteria Pg, Fn & Ec are a cause of arthritis. The oral inflammation caused by these bacteria also leads to total body inflammation which, combined with changes in a person's immunity, may result in chronic joint diseases like rheumatoid arthritis.

Dementia and Brain Health

Recent medical studies point to poor oral health, and high levels of the bacteria Pg, Cr, Cs in our gums, increasing the risk of developing dementias such as Alzheimer's.

Metabolic Health

Obesity, lack of exercise and chronic gum disease involving the bacteria Aa, Td, Tf, Pg, & Fn cause chronic inflammation. Inflammation can damage the pancreas where insulin is produced, possibly leading to diabetes. Also, diabetes worsens oral health by increasing the level of harmful bacteria in the gums.

Healthy Pregnancy

Bacteria associated with gum disease, especially Aa, Tf, Pg, Fn, and Ec, are known to put a pregnancy at risk for pre-term birth, decreased birth weight and even blood infection in the placenta or newborn. Every pregnant woman should be tested for these harmful bacteria.

Methodology: Genomic DNA is extracted from the submitted sample and tested for 10 species-specific bacteria [Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Tf: Tannerella forsythia, Td: Treponema denticola, En: Eubacterium nodatum, Fn: Fusobacterium nucleatum/periodontium, Pi: Prevotella intermedia, Cr: Campylobacter rectus, Pm: Peptostreptococcus (Micromonas) micros, Ec: Eikenella corrodens] and 1 genus of bacteria [Cs: Capnocytophaga species (gingavalis, ochracea, sputigena)] known to cause periodontal disease. The bacteria are assayed by real-time quantitative polymerase chain reaction (qPCR). Bacterial levels are reported in log 10 copies per mL of sample (e.g. 1x10^3 = 1000 bacteria copies per mL of collection). Cross-reactivity is possible with Leptotrichia buccalis, Fusobacterium hwasooki, Capnocytophaga granulosa and Capnocytophaga leadbetteri. This test was developed, and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

HEALTH AND MEDICINE

Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors

Stephen S. Dominy[1]*†, Casey Lynch[1]*, Florian Ermini[1], Malgorzata Benedyk[2,3], Agata Marczyk[2], Andrei Konradi[1], Mai Nguyen[1], Ursula Haditsch[1], Debasish Raha[1], Christina Griffin[1], Leslie J. Holsinger[1], Shirin Arastu-Kapur[1], Samer Kaba[1], Alexander Lee[1], Mark I. Ryder[4], Barbara Potempa[5], Piotr Mydel[2,6], Annelie Hellvard[3,6], Karina Adamowicz[2], Hatice Hasturk[7,8], Glenn D. Walker[9], Eric C. Reynolds[9], Richard L. M. Faull[10], Maurice A. Curtis[11,12], Mike Dragunow[11,13], Jan Potempa[2,5]*

Porphyromonas gingivalis, the keystone pathogen in chronic periodontitis, was identified in the brain of Alzheimer's disease patients. Toxic proteases from the bacterium called gingipains were also identified in the brain of Alzheimer's patients, and levels correlated with tau and ubiquitin pathology. Oral *P. gingivalis* infection in mice resulted in brain colonization and increased production of $A\beta_{1-42}$, a component of amyloid plaques. Further, gingipains were neurotoxic in vivo and in vitro, exerting detrimental effects on tau, a protein needed for normal neuronal function. To block this neurotoxicity, we designed and synthesized small-molecule inhibitors targeting gingipains. Gingipain inhibition reduced the bacterial load of an established *P. gingivalis* brain infection, blocked $A\beta_{1-42}$ production, reduced neuroinflammation, and rescued neurons in the hippocampus. These data suggest that gingipain inhibitors could be valuable for treating *P. gingivalis* brain colonization and neurodegeneration in Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) patients exhibit neuroinflammation consistent with infection, including microglial activation, inflammasome activation, complement activation, and altered cytokine profiles (*1*, *2*). Infectious agents have been found in the brain and postulated to be involved with AD, but robust evidence of causation has not been established (*3*). The recent characterization of amyloid-β (Aβ) as an antimicrobial peptide has renewed interest in identifying a possible infectious cause of AD (*4*–*6*).

Chronic periodontitis (CP) and infection with *Porphyromonas gingivalis*—a keystone pathogen in the development of CP (*7*)—have been identified as significant risk factors for developing Aβ plaques, dementia, and AD (*8*–*12*). A prospective observational study of AD patients with active CP reported a notable decline in cognition (Alzheimer's Disease Assessment Scale—Cognitive and Mini Mental State Examination scales) over a 6-month period compared to AD patients without active CP, raising questions about possible mechanisms underlying these findings (*13*). In *Apoe*$^{-/-}$ mice, oral infection with *P. gingivalis*, but not with two other oral bacteria, results in brain infection and activation of the complement pathway (*14*). In transgenic mice overexpressing mutated human amyloid precursor protein (hAPP-J20), oral infection with *P. gingivalis* impairs cognitive function, increases the deposition of AD-like plaques, and results in alveolar bone loss compared to control hAPP-J20 mice (*15*). *P. gingivalis* lipopolysaccharide has been detected in human AD brains (*16*), promoting the hypothesis that *P. gingivalis* infection of the brain plays a role in AD pathogenesis (*17*).

P. gingivalis is mainly found during gingival and periodontal infections; however, it can also be found at low levels in 25% of healthy individuals with no oral disease (*18*). Transient bacteremia of *P. gingivalis* can occur during common activities such as brushing, flossing, and chewing, as well as during dental procedures (*19*), resulting in documented translocation to a variety of tissues including coronary arteries (*20*), placenta (*21*), and liver (*22*). A recent study found that 100% of patients with cardiovascular disease had *P. gingivalis* arterial colonization (*23*).

P. gingivalis is an asaccharolytic Gram-negative anaerobic bacterium that produces major virulence factors known as gingipains, which are cysteine proteases consisting of lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). Gingipains are secreted, transported to outer bacterial membrane surfaces, and partially released into the extracellular milieu in soluble and outer membrane vesicle (OMV)–associated forms (*24*, *25*). Kgp and RgpA/B are essential for *P. gingivalis* survival and pathogenicity, playing critical roles in host colonization, inactivation of host defenses, iron and nutrient acquisition, and tissue destruction (*24*, *26*). Gingipains have been shown to mediate the toxicity of *P. gingivalis*

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"Porphyromonas gingivalis, the keystone pathogen in chronic periodontitis, was identified in the brain of Alzheimer's disease patients. Toxic proteases from the bacterium called gingipains were also identified in the brain of Alzheimer's patients, and levels correlated with tau and ubiquitin pathology."

Journal of Inflammation Research

Dovepress

REVIEW

Periodontal Disease and Periodontal Disease-Related Bacteria Involved in the Pathogenesis of Alzheimer's Disease

This article was published in the following Dove Press journal:
Journal of Inflammation Research

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Masae Yamada-Furukawa
Mie Kurosawa
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Abstract: Alzheimer's disease (AD) is the most common cause of dementia, and it exhibits pathological properties such as deposition of extracellular amyloid β (Aβ) and abnormally phosphorylated Tau in nerve cells and a decrease of synapses. Conventionally, drugs targeting Aβ and its related molecules have been developed on the basis of the amyloid cascade hypothesis, but sufficient effects on the disease have not been obtained in past clinical trials. On the other hand, it has been pointed out that chronic inflammation and microbial infection in the brain may be involved in the pathogenesis of AD. Recently, attention has been focused on the relationship between the periodontopathic bacterium *Porphyromonas gingivalis* and AD. *P. gingivalis* and its toxins have been detected in autopsy brain tissues from patients with AD. In addition, pathological conditions of AD are formed or exacerbated in mice infected with *P. gingivalis*. Compounds that target the toxins of *P. gingivalis* ameliorate the pathogenesis of AD triggered by *P. gingivalis* infection. These findings indicate that the pathological condition of AD may be regulated by controlling the bacteria in the oral cavity and the body. In the current aging society, the importance of oral and periodontal care for preventing the onset of AD will increase.

Keywords: *Porphyromonas gingivalis*, cognitive decline, amyloid β, blood-brain barrier, vascular inflammation

Introduction

Dementia is the most frequent neurological disease in the world and is recognized as a global public health priority by the World Health Organization. Although various methods for prevention and treatment of dementia have yet been studied, no effective method has been established. If risk factors for dementia and factors that suppress its onset and progression could be identified that information could be used effectively, it might be possible to prevent dementia and extend the healthy life span. Recently, the associations between dementia and systemic diseases have been focused on. The pathogenesis of Alzheimer's disease (AD), which accounts for the largest number of cases of dementia, and the relationships of the pathogenesis of AD with periodontitis and periodontitis-related bacteria are described in this review.

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Alzheimer's Disease

It is estimated that about 44 million people worldwide are currently suffering from dementia. Treatment costs exceed US $600 billion annually in the United States

Compounds that target the toxins of P. gingivalis ameliorate the pathogenesis of AD triggered by P. gingivalis infection. **These findings indicate that the pathological condition of AD may be regulated by controlling the bacteria in the oral cavity and the body. In the current aging society, the importance of oral and periodontal care for preventing the onset of AD will increase.**

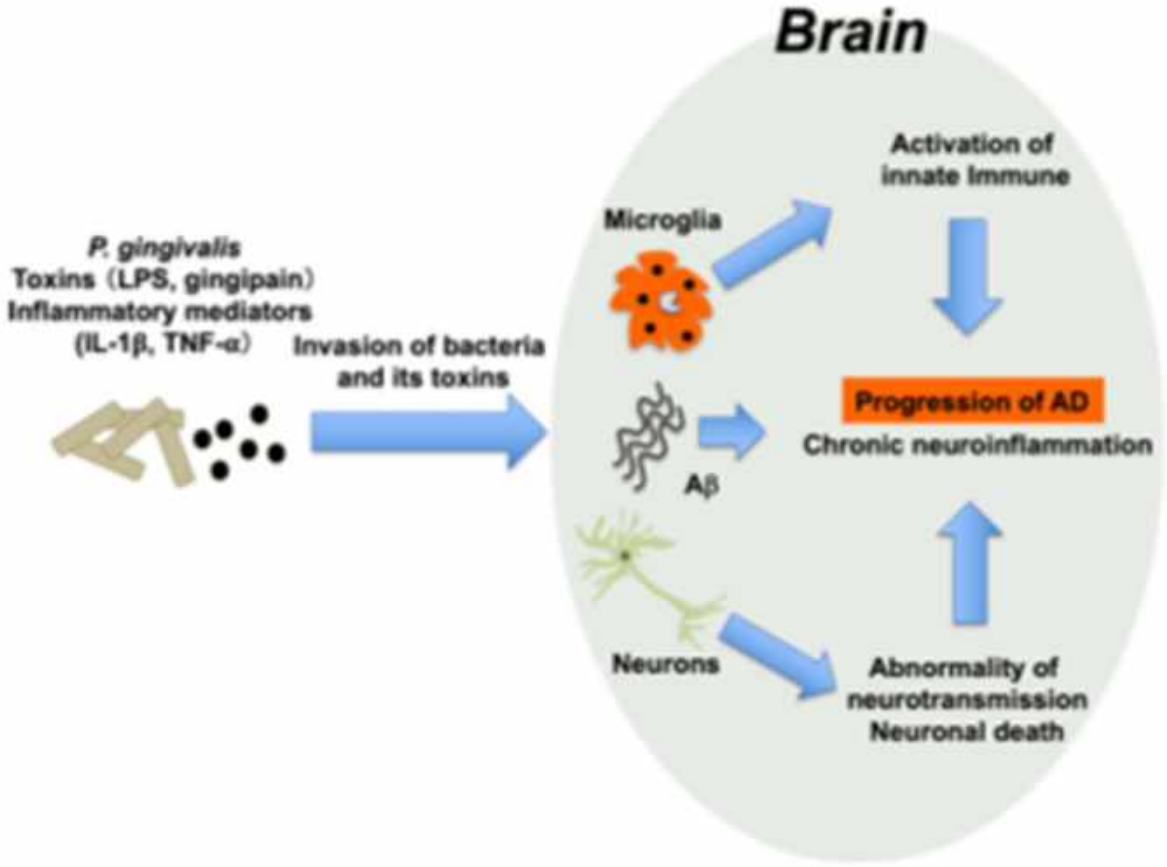


Figure 2 Possible mechanisms by which periodontal disease and *P. gingivalis* aggravate Alzheimer's disease.

70 yo female with fronto-temporal dementia

MYPERIOPATH MOLECULAR ANALYSIS OF PERIODONTAL AND SYSTEMIC PATHOGENS

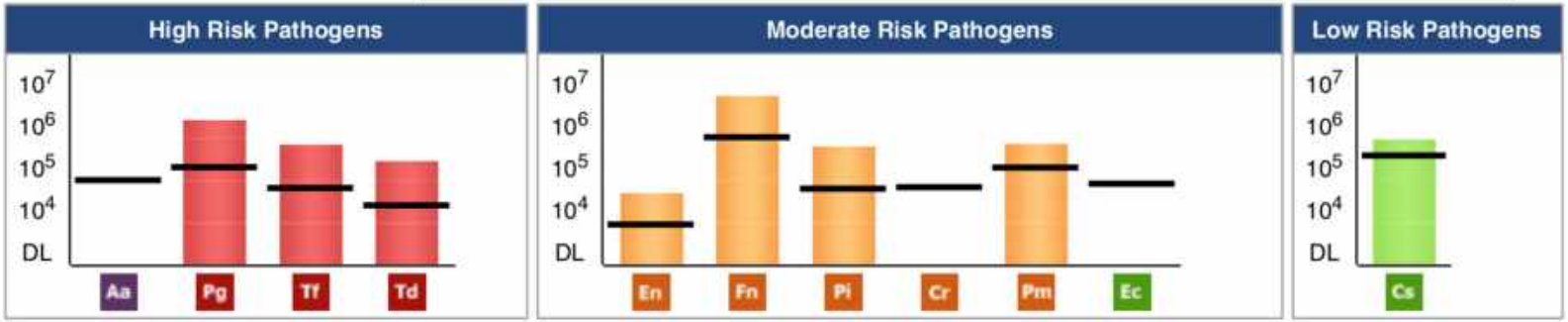
Result: PATHOGENIC BACTERIA DETECTED, 8 ABOVE THERAPEUTIC THRESHOLD

Pg Tf Td En Fn Pi Pm Cs

Bacterial Risk: HIGH - Very strong evidence of increased risk for attachment loss

Legend
▬ = Therapeutic Threshold*
DL = Detection Limit

Result Interpretation: Periodontal disease is caused by specific, or groups of specific bacteria. Threshold levels represent the concentration above which patients are generally at increased risk for attachment loss. Bacterial levels should be considered collectively and in context with clinical signs and other risk factors.



Pathogen	Result	Clinical Significance
Pg Porphyromonas gingivalis	High	Very strong association with PD: Transmittable, tissue invasive, and pathogenic at relatively low bacterial counts. Associated with aggressive forms of disease.
Tf Tannerella forsythia	High	Very strong association with PD: common pathogen associated with refractory periodontitis. Strongly related to increasing pocket depths.
Td Treponema denticola	High	Very strong association with PD: invasive in cooperation with other bacteria. Usually seen in combination with other bacteria.
En Eubacterium nodatum	High	Strong association with PD: specific role uncertain. Often seen in refractory disease.
Fn Fusobacterium nucleatum/periodonticum	High	Strong association with PD: adherence properties to several oral pathogens; often seen in refractory disease.
Pi Prevotella intermedia	High	Strong association with PD: virulent properties similar to Pg; often seen in refractory disease.
Pm Peptostreptococcus (Micromonas) micros	High	Moderate association with PD: detected in higher numbers at sites of active disease.
Cs Capnocytophaga species (gingavalis,ochracea,sputigena)	High	Some association with PD: Frequently found in gingivitis. Often found in association with other periodontal pathogens. May increase temporarily following active therapy.

Not Detected: (Aa) Aggregatibacter actinomycetemcomitans, (Cr) Campylobacter rectus, (Ec) Eikenella corrodens

Screenshot

Additional information is available from OralDNA.com

70 yo female with fronto-temporal dementia

TEST	RESULT			
Array 7 - Neurological Autoimmune Reactivity Screen **	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Myelin Basic Protein			1.58	0.1-1.4
Asialoganglioside			1.76	0.1-1.4
Alpha-Tubulin + Beta-Tubulin		1.13		0.4-1.4
Cerebellar		1.37		0.2-1.4
Synapsin			1.51	0.1-1.2

Lancet Neurol. 2005 Mar;4(3):195-202.

Infectious causes of multiple sclerosis.

Gilden DH.

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Erratum in
Lancet Neurol. 2005 May;4(5):269.

Abstract

Multiple sclerosis (MS) is a serious chronic neurological disorder in which demyelination and inflammation occur in the white matter of the CNS. The findings of many epidemiological studies and a discordance of MS in monozygotic twins suggest that the disorder is acquired. The most likely cause is a virus because more than 90% of patients with MS have high concentrations of IgG, manifest as oligoclonal bands, in the brain and CSF. Most chronic inflammatory CNS disorders are infectious. More indirect evidence that MS is caused by a virus is the association of several viruses with demyelinating encephalomyelitis in human beings, and the induction of demyelination in animals infected with viruses in research. Nevertheless, no virus has been isolated from the brains of patients who had MS. Molecular analysis of IgG gene specificity in the brain and CSF of those with MS has shown features of an antigen-driven response: clonal amplification and extensive somatic mutations. A viral antigen against which the IgG in MS brain and CSF is directed might be identified.

Review Article

Chlamydophila pneumoniae Infection and Its Role in Neurological Disorders

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Chlamydophila pneumoniae is an intracellular pathogen responsible for a number of different acute and chronic infections. The recent deepening of knowledge on the biology and the use of increasingly more sensitive and specific molecular techniques has allowed demonstration of *C. pneumoniae* in a large number of persons suffering from different diseases including cardiovascular (atherosclerosis and stroke) and central nervous system (CNS) disorders. Despite this, many important issues remain unanswered with regard to the role that *C. pneumoniae* may play in initiating atheroma or in the progression of the disease. A growing body of evidence concerns the involvement of this pathogen in chronic neurological disorders and particularly in Alzheimer's disease (AD) and Multiple Sclerosis (MS). Monocytes may traffic *C. pneumoniae* across the blood-brain-barrier, shed the organism in the CNS and induce neuroinflammation. The demonstration of *C. pneumoniae* by histopathological, molecular and culture techniques in the late-onset AD dementia has suggested a relationship between CNS infection with *C. pneumoniae* and the AD neuropathogenesis. In particular subsets of MS patients, *C. pneumoniae* could induce a chronic persistent brain infection acting as a cofactor in the development of the disease. The role of Chlamydia in the pathogenesis of mental or neurobehavioral disorders including schizophrenia and autism is uncertain and fragmentary and will require further confirmation.

1. Introduction and Background

Chlamydiae were taxonomically categorised into their own order *Chlamydiales*, with one family, *Chlamydiaceae*, and a single genus, *Chlamydia* which included four species: *C. trachomatis*, *C. pneumoniae*, *C. psittaci*, and *C. pecorum*. Two of the species, *C. trachomatis* and *C. pneumoniae*, are common pathogens in humans, but the routes of transmission, susceptible populations, and clinical presentations differ markedly. The other species occur mainly in animals although *C. psittaci* may be also implicated in human respiratory diseases. In 1999, a new taxonomic classification was proposed, renaming *Chlamydia pneumoniae* as *Chlamydophila pneumoniae* [1]. However, the proposal to change the taxonomic nomenclature for the *Chlamyadiaceae* family has not been universally accepted and both names are currently in use by different authors.

C. pneumoniae, a common cause of human respiratory disease, was first isolated from the conjunctiva of a child in Taiwan in 1965 but it was not until the early 1980s that it was scientifically identified as a distinct *Chlamydia* species and was established as a major respiratory pathogen in 1983 when it was isolated from the throat of a college student at the University of Washington. Most likely, *C. pneumoniae* is primarily transmitted from human to human by the respiratory tract without any animal reservoir [2, 3] and infection spreads slowly. The incubation period is several weeks, which is longer than that for many other

The demonstration of C. pneumoniae by histopathological, molecular and culture techniques in the late-onset AD dementia has suggested a relationship between CNS infection with C. pneumoniae and the AD neuropathogenesis***. In particular subsets of MS patients, C. pneumoniae could induce a chronic persistent brain infection acting as a cofactor in the development of the disease.***

J Pathol. 2009 Jan;217(1):131-8.

Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques.

Wozniak MA, Mee AP, Itzhaki RF.
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Abstract

The brains of Alzheimer's disease sufferers are characterized by amyloid plaques and neurofibrillary tangles. However, the cause(s) of these features and those of the disease are unknown, in sporadic cases. We previously showed that herpes simplex virus type 1 is a strong risk factor for Alzheimer's disease when in the brains of possessors of the type 4 allele of the apolipoprotein E gene (APOE-epsilon4), and that beta-amyloid, the main component of plaques, accumulates in herpes simplex virus type 1-infected cell cultures and mouse brain. The present study aimed to elucidate the relationship of the virus to plaques by determining their proximity in human brain sections. We used in situ polymerase chain reaction to detect herpes simplex virus type 1 DNA, and immunohistochemistry or thioflavin S staining to detect amyloid plaques. We discovered a striking localization of herpes simplex virus type 1 DNA within plaques: in Alzheimer's disease brains, 90% of the plaques contained the viral DNA and 72% of the DNA was associated with plaques; in aged normal brains, which contain amyloid plaques at a lower frequency, 80% of plaques contained herpes simplex virus type 1 DNA but only 24% of the viral DNA was plaque-associated ($p < 0.001$). We suggest that this is because in aged normal individuals, there is a lesser production and/or greater removal of beta-amyloid (Abeta), so that less of the viral DNA is seen to be associated with Abeta in the brain. Our present data, together with our finding of Abeta accumulation in herpes simplex virus type 1-infected cells and mouse brain, suggest that this virus is a major cause of amyloid plaques and hence probably a significant aetiological factor in Alzheimer's disease. They point to the usage of antiviral agents to treat the disease and possibly of vaccination to prevent it.

“Our present data, together with our finding of Abeta accumulation in herpes simplex virus type 1-infected cells and mouse brain, suggest that this virus is a major cause of amyloid plaques and hence probably a significant aetiological factor in Alzheimer's disease.”

Journal of Neuroinflammation

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Research

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Persisting atypical and cystic forms of *Borrelia burgdorferi* and local inflammation in Lyme neuroborreliosis

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Abstract

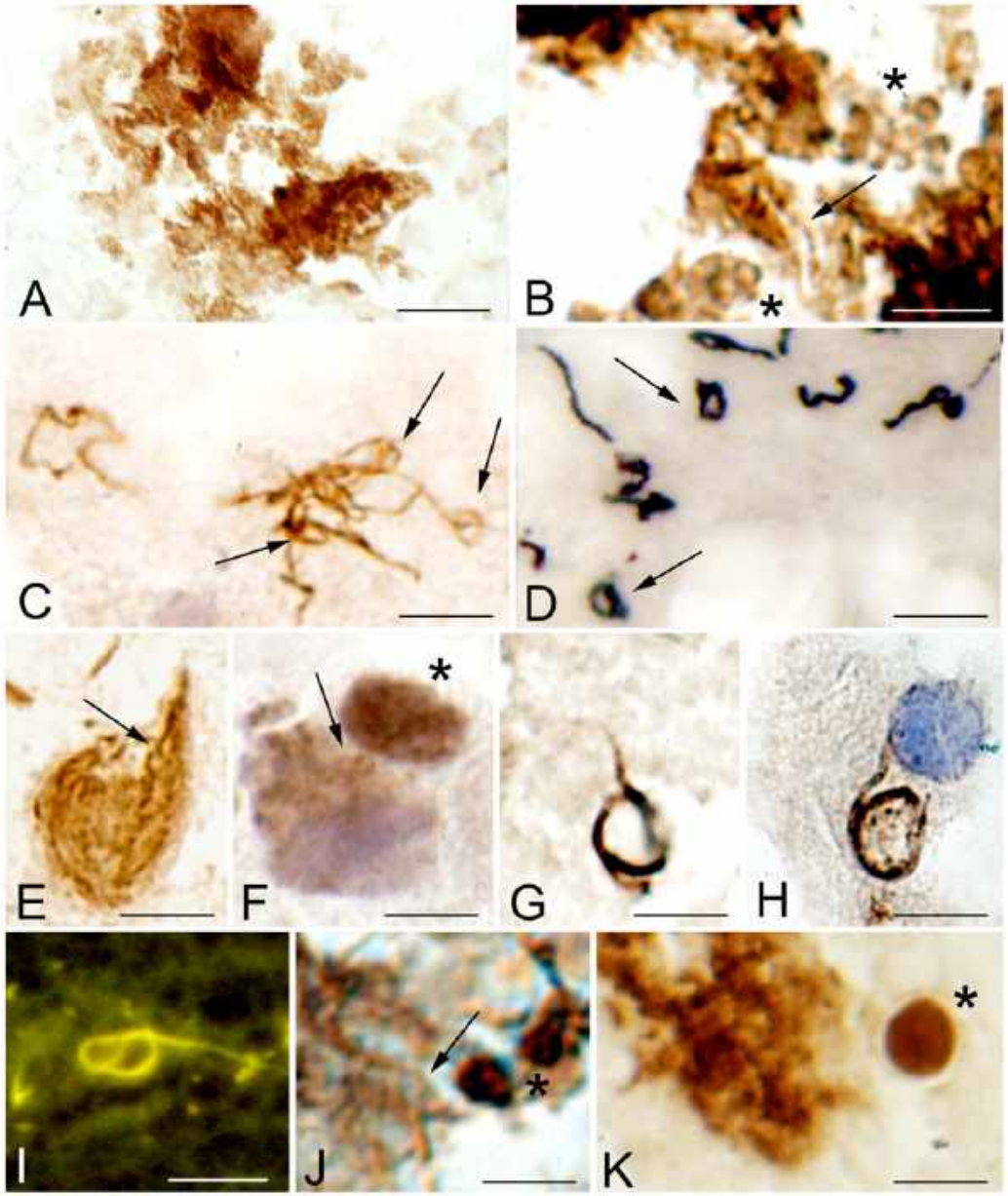
Background: The long latent stage seen in syphilis, followed by chronic central nervous system infection and inflammation, can be explained by the persistence of atypical cystic and granular forms of *Treponema pallidum*. We investigated whether a similar situation may occur in Lyme neuroborreliosis.

Method: Atypical forms of *Borrelia burgdorferi* spirochetes were induced exposing cultures of *Borrelia burgdorferi* (strains B31 and ADB1) to such unfavorable conditions as osmotic and heat shock, and exposure to the binding agents Thioflavin S and Congo red. We also analyzed whether these forms may be induced *in vitro*, following infection of primary chicken and rat neurons, as well as rat and human astrocytes. We further analyzed whether atypical forms similar to those induced *in vitro* may also occur *in vivo*, in brains of three patients with Lyme neuroborreliosis. We used immunohistochemical methods to detect evidence of neuroinflammation in the form of reactive microglia and astrocytes.

Results: Under these conditions we observed atypical cystic, rolled and granular forms of these spirochetes. We characterized these abnormal forms by histochemical, immunohistochemical, dark field and atomic force microscopy (AFM) methods. The atypical and cystic forms found in the brains of three patients with neuropathologically confirmed Lyme neuroborreliosis were identical to those induced *in vitro*. We also observed nuclear fragmentation of the infected astrocytes using the TUNEL method. Abundant HLA-DR positive microglia and GFAP positive reactive astrocytes were present in the cerebral cortex.

Conclusion: The results indicate that atypical extra- and intracellular pleomorphic and cystic forms of *Borrelia burgdorferi* and local neuroinflammation occur in the brain in chronic Lyme neuroborreliosis. The persistence of these more resistant spirochete forms, and their intracellular location in neurons and glial cells, may explain the long latent stage and persistence of Borrelia infection. The results also suggest that *Borrelia burgdorferi* may induce cellular dysfunction and apoptosis. The detection and recognition of atypical, cystic and granular forms in infected tissues is essential for the diagnosis and the treatment as they can occur in the absence of the typical spiral Borrelia form.

"In analogy to Treponema pallidum, Borrelia burgdorferi can persist in the brain in Lyme neuroborreliosis and may initiate and sustain chronic inflammation and tissue damage."



Extra- and intracellular atypical and cystic forms of spirochetes in the cerebral cortex of a patient with pathologically and serologically confirmed chronic Lyme neuroborreliosis where Borrelia burgdorferi sensu stricto was cultivated from the brain.

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DOI: 10.2353/ajpath.2010.091235

Immunopathology and Infectious Diseases

Bacterial Lipoproteins Can Disseminate from the Periphery to Inflame the Brain

Diana Londoño and Diego Cadavid

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The current view is that bacteria need to enter the brain to cause inflammation. However, in mice infected with the spirochete *Borrelia turicatae*, we observed widespread cerebral inflammation despite a paucity of spirochetes in the brain parenchyma at times of high bacteremia. Here we studied the possibility that bacterial lipoproteins may be capable of disseminating from the periphery across the blood-brain barrier to inflame the brain. For this we injected normal and infected mice intraperitoneally with lanthanide-labeled variable outer membrane lipoproteins of *B. turicatae* and measured their localization in blood, various peripheral organs, and whole and capillary-depleted brain protein extracts at various times. Lanthanide-labeled nonlipidated lipoproteins of *B. turicatae* and mouse albumin were used as controls. Brain inflammation was measured by TaqMan RT-PCR amplification of genes known to be up-regulated in response to borrelial infection. The results showed that the two lipoproteins we studied, LVsp1 and LVsp2, were capable of inflaming the brain after intraperitoneal injection to different degrees: LVsp1 was better than LVsp2 and Bt1 spirochetes at moving from blood to brain. The dissemination of LVsp1 from the periphery to the brain occurred under normal conditions and significantly increased with infection. In contrast, LVsp2 disseminated better to peripheral organs. We conclude that some bacterial lipoproteins can disseminate from the periphery to inflame the brain. (*Am J Pathol 2010, 176:2848–2857; DOI: 10.2353/ajpath.2010.091235*)

The traditional belief is that bacteria need to cross the blood brain barrier (BBB) to cause brain inflammation. However, during studies in mice persistently infected with the relapsing fever spirochete *Borrelia turicatae*, we noticed widespread cerebral microgliosis that could not be explained by the presence of spirochetes in the brain parenchyma.[1–4] Because cerebral microgliosis is much more pronounced during persistent than during intermittent bacteremia,[4,5] the possibility was raised that movement of pro-inflammatory bacterial products from blood to brain rather than the bacteria themselves could be responsible. Release of pro-inflammatory bacterial products occurs from spirochetes both spontaneously[6] and upon immune or antibiotic mediated lysis.[7–9] Furthermore, release of outer membrane lipoproteins from spirochetes into the extracellular environment can occur independently of blebs.[9]

However, little is known about whether bacterial products can be released *in vivo* and disseminate into tissues like the brain to cause inflammation.[10] Studies with lipopolysaccharide (LPS) from Gram-negative bacteria have shown that it does not cross the BBB but rather appears to cause brain inflammation by binding to its receptor, TLR4, on brain endothelial cells.[11] Although borrelia spirochetes lack LPS, they do contain an abundance of potent pro-inflammatory outer membrane lipoproteins that share a common palmitate 3 Cysteine modification with many other bacterial species.[12–15] Bacterial lipoproteins play prominent roles in disease pathogenesis.[12] Many, if not all, of their biological activities are dependent on the lipid modification.[15] The ability of eukaryotic cells to respond to lipoproteins correlates with expression of TLR2.[17]

Here we studied whether bacterial lipoproteins are capable of disseminating to the brain from the periphery. For this we injected mice intraperitoneally with lipidated outer membrane lipoproteins LVsp1 or LVsp2 of the relapsing fever

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This content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health.

D.C. is currently a full-time paid employee of Biogen Idec. His work on this article is not related to his employment in Biogen Idec.

Supplemental material for this article can be found on http://ajp.amjpathol.org.

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"We conclude that some bacterial lipoproteins can disseminate from the periphery to inflame the brain."

Am J Pathol 2010, 176:2848–2857

frontiers in Immunology

PERSPECTIVE
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Microbiome-Derived Lipopolysaccharide Enriched in the Perinuclear Region of Alzheimer's Disease Brain

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Abundant clinical, epidemiological, imaging, genetic, molecular, and pathophysiological data together indicate that there occur an unusual inflammatory reaction and a disruption of the innate-immune signaling system in Alzheimer's disease (AD) brain. Despite many years of intense study, the origin and molecular mechanics of these AD-relevant pathogenic signals are still not well understood. Here, we provide evidence that an intensely pro-inflammatory bacterial lipopolysaccharide (LPS), part of a complex mixture of pro-inflammatory neurotoxins arising from abundant Gram-negative bacilli of the human gastrointestinal (GI) tract, are abundant in AD-affected brain neocortex and hippocampus. For the first time, we provide evidence that LPS immunohistochemical signals appear to aggregate in clumps in the parenchyma in control brains, and in AD, about 75% of anti-LPS signals were clustered around the periphery of DAPI-stained nuclei. As LPS is an abundant secretory product of Gram-negative bacilli resident in the human GI-tract, these observations suggest (i) that a major source of pro-inflammatory signals in AD brain may originate from internally derived noxious exudates of the GI-tract microbiome; (ii) that due to aging, vascular deficits or degenerative disease these neurotoxic molecules may "leak" into the systemic circulation, cerebral vasculature, and on into the brain; and (iii) that this internal source of microbiome-derived neurotoxins may play a particularly strong role in shaping the human immune system and contributing to neural degeneration, particularly in the aging CNS. This "*Perspectives*" paper will further highlight some very recent developments that implicate GI-tract microbiome-derived LPS as an important contributor to inflammatory-neurodegeneration in the AD brain.

Keywords: Alzheimer's disease, inflammatory degeneration, lipopolysaccharide, microbiome, microRNA, small non-coding RNAs

"As LPS is an abundant secretory product of Gram-negative bacilli resident in the human GI-tract, these observations suggest (i) that a major source of pro-inflammatory signals in AD brain may originate from internally derived noxious exudates of the GI-tract microbiome; (ii) that due to aging, vascular deficits or degenerative disease these neurotoxic molecules may "leak" into the systemic circulation, cerebral vasculature, and on into the brain;"

frontiers in
AGING NEUROSCIENCE

OPINION ARTICLE
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Pathogenic microbes, the microbiome, and Alzheimer's disease (AD)

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Keywords: Alzheimer's disease, microbiome, virus replication, prion, miRNA

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of cognitive and behavioral impairment in industrialized societies. The cause of AD is unknown and the major risk factor for AD is age. About 5% of all AD cases have a genetic or familial cause however the vast majority of all AD cases (~95%) are of sporadic origin. Both the familial and the sporadic forms of AD share a common disease phenotype involving at least eight characteristic features including (i) evidence of uncontrolled oxidative stress; (ii) up-regulated pro-inflammatory signaling; (iii) changes in innate-immune signaling; (iv) the progressive accumulation of lesions including neurofibrillary tangles (NFT) and amyloid beta (Aβ)-containing senile plaques (SP); (v) significant synaptic signaling deficits; (vi) neurite and brain cell atrophy; (vii) progressively altered gene expression patterns that are different from healthy brain aging; and (viii) progressive cognitive impairment and dementia in the host. There is currently no cure or adequate clinical treatment for AD, and it remains unclear how AD originates and propagates throughout the brain and central nervous system (CNS). Results from recent genome-wide association studies (GWAS) indicate that a significant portion of AD-relevant gene signals are not located within gene coding regions suggesting the contribution of epigenetic or environmental factors to AD risk. The potential contribution of pathogenic microbes to aging and AD is becoming increasingly recognized (Miklossy, 2011; Cho and Blaser, 2012; Bhattacharjee and Lukiw, 2013; Poole et al., 2013; Heintz and Mair, 2014; Huang et al., 2014; Mancuso et al., 2014). Importantly, most of the changes seen in AD, such as inflammation, brain cell atrophy, immunological aberrations, amyloidogenesis, altered gene expression and cognitive deficits are also seen as a consequence of microbial infection (Cho and Blaser, 2012; Yatsunenko et al., 2012; Bhattacharjee and Lukiw, 2013; Foster and McVey Neufeld, 2013; Kim et al., 2013; Heintz and Mair, 2014; Mancuso et al., 2014). This brief communication will review some recent observations on the potential contribution of pathogens to neurological dysfunction, with specific reference to AD wherever possible.

Firstly, humans contain a complex and dynamic community of microbes called the microbiome that forms a "metaorganism" with symbiotic or commensal benefit to the host (Cho and Blaser, 2012; Bhattacharjee and Lukiw, 2013; Heintz and Mair, 2014). The microbiome of the human gastrointestinal (GI) tract contains the largest reservoir of microbes, containing about 10^{14} microbes from at least 1000 distinct microbial species, and outnumbering human host cells by about 100 to 1 (Whitman et al., 1998; Kim et al., 2013). The GI tract microbiome has been estimated to encode about 4×10^6 genes so the quantity of the microbiome genes outnumbers host genes by about 150 to 1 (Bhattacharjee and Lukiw, 2013). Over 99% of GI tract microbiota are anaerobic bacteria, with fungi, protozoa, archaebacteria and other microorganisms making up the remainder; interestingly only two bacterial divisions are prominent in GI tract microbiota, including *Firmicutes* (~51%) and *Bacteroidetes* (~48%), with the remaining 1% of phylotypes distributed amongst the *Cyanobacteria*, *Fusobacteria*, *Proteobacteria*, *Spirochaetes*, and *Verrucomicrobia*, with various species of fungi, protozoa, viruses and other microorganisms making up the remainder (http://www.genome.gov/pages/research/sequencing/seqproposals/hgmiseq.pdf). Of all human GI tract microbiota, bacterial densities of up to 10^{12} per ml are the highest recorded density in any known microbial ecosystem (Whitman et al., 1998; Bhattacharjee and Lukiw, 2013; Kim et al., 2013). Interestingly, the microorganisms making up the smallest 1% of the microbiome have a disproportionately large effect on disease, and it is a major function of the healthy GI tract microbiome to keep under control the proliferation of any potentially pathogenic microbes contained within (Hornig, 2013; Kim et al., 2013; Heintz and Mair, 2014; see below).

Recent interest in the role of the microbiome in human health and disease has rapidly expanded over the last several years with the advent of new sequencing and bioinformatics technologies for interrogating the genetics of complex microbial communities and microbial-host interactions. There is currently much interest in the ability of GI tract

"Here we list 10 recent, highly specific and illustrative insights into the potential contribution of pathogenic microbes, altered microbiome signaling and other disease-inducing agents to the development of AD"

(1) Fungal infection of the CNS
(2) HSV-1 is associated with AD
(3) Prion diseases
(4) Chlamydophila pneumoniae, other pathogenic bacteria
(5) HIV-1 and AD
(6) Toxoplasma and neurodegeneration
(7) Viroids, miRNAs and AD
(8) Hepatitis and AD
(9) Cytomegalovirus and AD
(10) GI tract and blood-brain barrier permeability

J Alzheimers Dis. 2014 Aug 13. [Epub ahead of print]

Direct Visualization of Fungal Infection in Brains from Patients with Alzheimer's Disease.

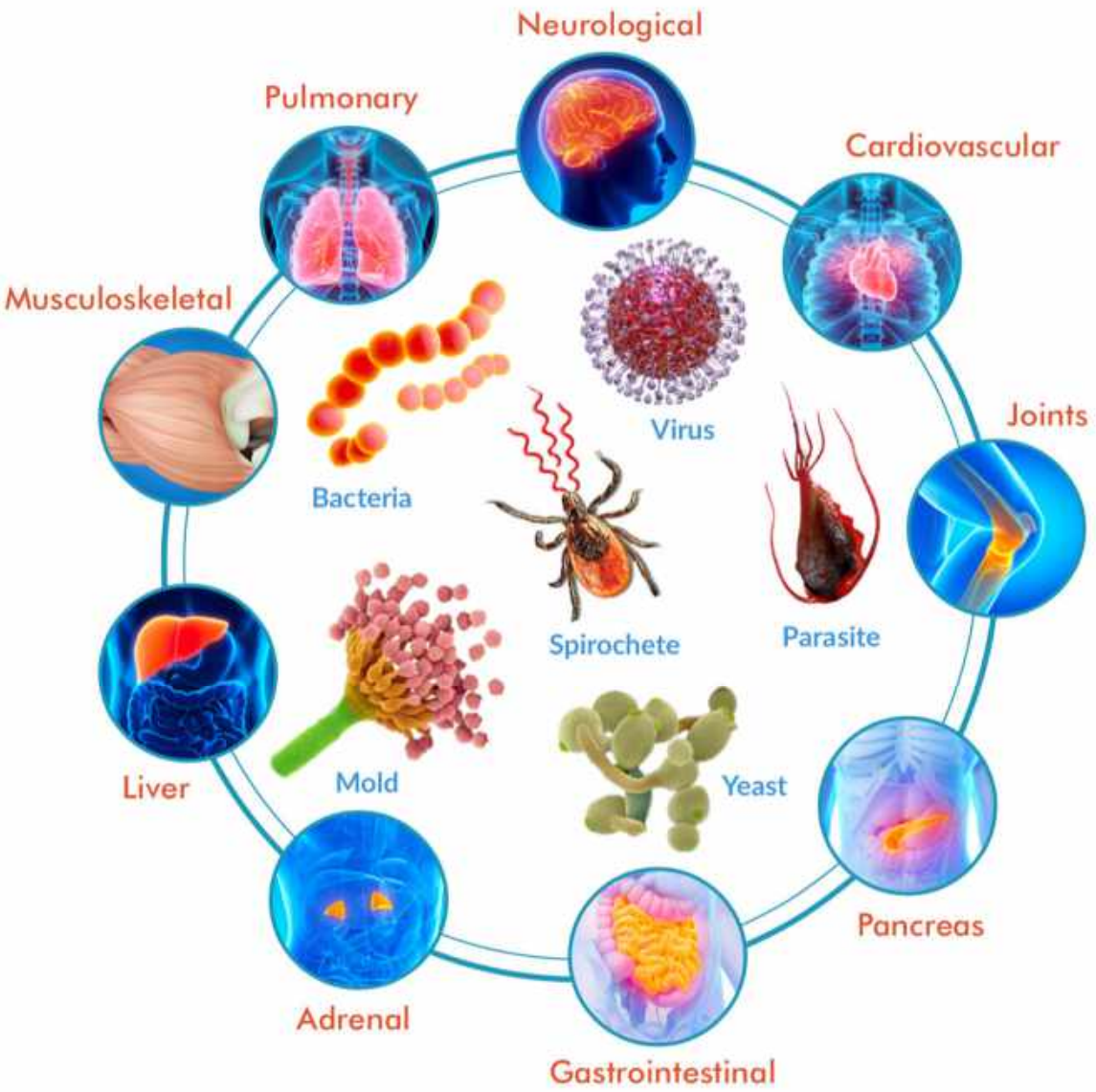
Pisa D[1], Alonso R[1], Juarranz A[2], Rábano A[3], Carrasco L[1].

Author information

Abstract

Recently, we have reported the presence of fungal infections in patients with Alzheimer's disease (AD). Accordingly, fungal proteins and DNA were found in brain samples, demonstrating the existence of infection in the central nervous system. In the present work, we raised antibodies to specific fungal species and performed immunohistochemistry to directly visualize fungal components inside neurons from AD patients. Mice infected with Candida glabrata were initially used to assess whether yeast can be internalized in mammalian tissues. Using polyclonal rabbit antibodies against C. glabrata, rounded immunopositive cells could be detected in the cytoplasm of cells from liver, spleen, and brain samples in infected, but not uninfected, mice. Immunohistochemical analyses of tissue from the frontal cortex of AD patients revealed the presence of fungal material in a small percentage (~10%) of cells, suggesting the presence of infection. Importantly, this immunopositive material was absent in control samples. Confocal microscopy indicated that this fungal material had an intracellular localization. The specific morphology of this material varied between patients; in some instances, disseminated material was localized to the cytoplasm, whereas small punctate bodies were detected in other patients. Interestingly, fungal material could be revealed using different anti-fungal antibodies, suggesting multiple infections. In summary, fungal infection can only be observed using specific anti-fungal antibodies and only a small percentage of cells contain fungi. Our findings provide an explanation for the hitherto elusive detection of fungi in AD brains, and are consistent with the idea that fungal cells are internalized inside neurons.

PATHOGEN-ASSOCIATED IMMUNE REACTIVITY SCREEN™



Pathogen-Associated Immune Reactivity Screen™

2 mL Serum

Antigens Tested (IgG)

- Porphyromonas gingivalis
- Streptococcus mutans
- Helicobacter pylori
- Campylobacter jejuni
- Yersinia enterocolitica
- Clostridium difficile
- Candida albicans
- Rotavirus
- Entamoeba histolytica
- Giardia lamblia
- Cryptosporidium
- Blastocystis hominis
- Human HSP-60 + Chlamydia HSP-60
- Chlamydias
- Streptozymes
- Streptococcal M Protein
- Mycoplasmas
- Acinetobacter
- Klebsiella
- Mycobacterium avium
- Aspergillus
- Penicillium
- Stachybotrys chartarum
- Citrullinated EBV
- Hepatitis C virus
- Cytomegalovirus
- Human Herpesvirus-6
- Borrelia burgdorferi
- Babesia+Ehrlichia +Bartonella

CLINICAL USE:

- Detect immune reaction to key pathogens that may lead to multiple autoimmune reactivities.
- Determine the role of pathogens in cases of 'unexplained' autoimmune reactivities.
- Monitor the effectiveness of clinical protocols for addressing pathogens associated with multiple autoimmunities.

RECOMMENDED FOR PATIENTS WHO:

- Present with chronic conditions such as gastrointestinal distress, fatigue, body aches or unexplained and general inflammation, including neuroinflammation.
- May have been exposed to bacteria, viruses, parasites, molds, and spirochetes associated with multiple autoimmunities.
- Have not fully responded to clinical interventions, such as detoxification and dietary modifications.

Detection of high levels of IgG antibody against the tested pathogens is not indicative of acute infection.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
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16S rRNA Next Generation Sequencing Analysis Shows Bacteria in Alzheimer's Post-Mortem Brain

*David C. Emery[1], Deborah K. Shoemark[2], Tom E. Batstone[3], Christy M. Waterfall[3], Jane A. Coghill[3], Tanya L. Cerajewska[4], Maria Davies[4], Nicola X. West[4] and Shelley J. Allen[1]**

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The neurological deterioration associated with Alzheimer's disease (AD), involving accumulation of amyloid-beta peptides and neurofibrillary tangles, is associated with evident neuroinflammation. This is now seen to be a significant contributor to pathology. Recently the tenet of the privileged status of the brain, regarding microbial compromise, has been questioned, particularly in terms of neurodegenerative diseases. It is now being considered that microbiological incursion into the central nervous system could be either an initiator or significant contributor to these. This is a novel study using 16S ribosomal gene-specific Next generation sequencing (NGS) of extracted brain tissue. A comparison was made of the bacterial species content of both frozen and formaldehyde fixed sections of a small cohort of Alzheimer-affected cases with those of cognitively unimpaired (normal). Our findings suggest an increase in bacterial populations in Alzheimer brain tissue compared with normal.

Keywords: Alzheimer's disease (AD), bacteria, human microbiome, 16S rRNA, next generation sequencing (NGS)

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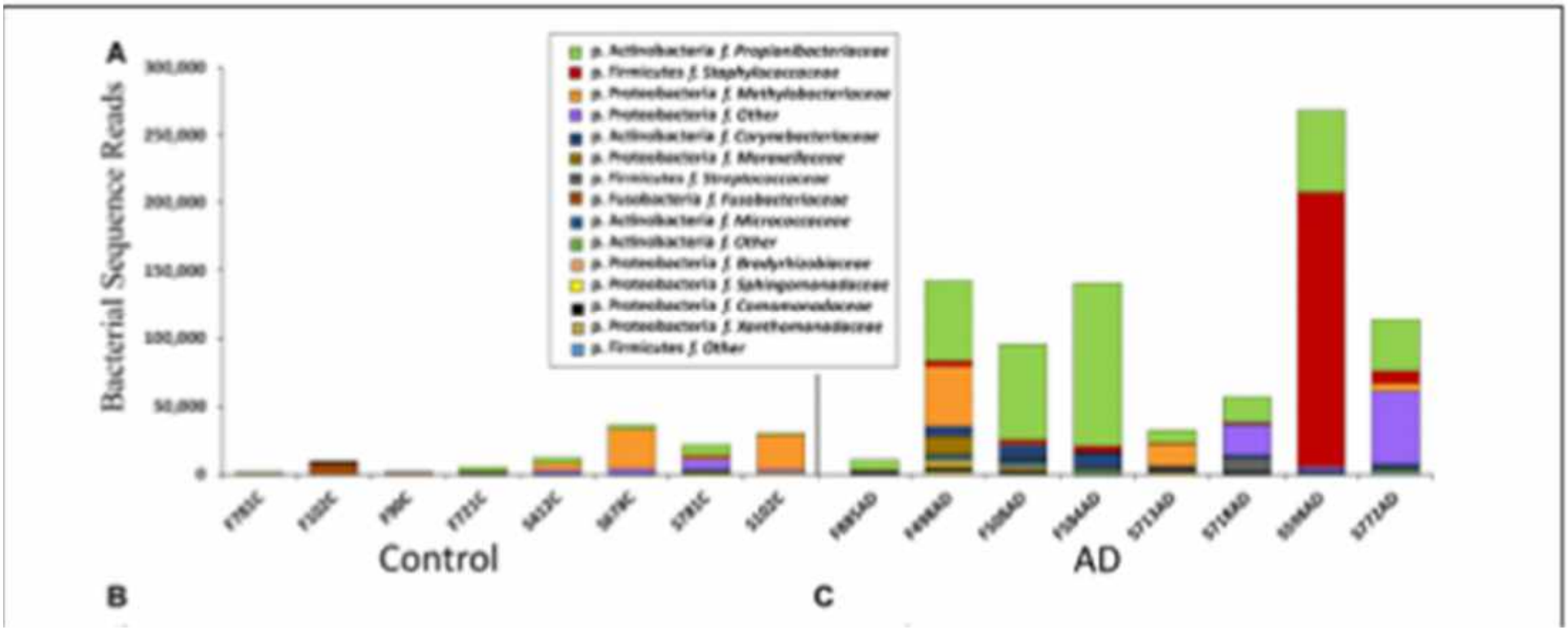
INTRODUCTION

Pathological triggers, culminating in the eventual loss of cognitive function in Alzheimer's disease (AD), are widely acknowledged to occur up to two decades before symptoms arise (Bateman et al., 2012). It is acknowledged that the increased level of amyloid Aβ42 in the brain parenchyma, due to either increased production of amyloid or its decreased removal, is likely to contribute substantially to this. However, understanding why the presence of excessive levels of Aβ do not necessarily result in cognitive impairment (Katzman et al., 1988; Hulette et al., 1998; Price and Morris, 1999; Aizenstein et al., 2008; Esparza et al., 2013) may be related to the known role of inflammation and the importance of the response of the innate immune system, which are also recognized as essential factors (Heneka et al., 2015b). The common sporadic form of AD arises from a large number of possible risk factors. The presence of the E4 polymorphism of apolipoprotein E4 (*APOE4*) has long been known to be the most potent risk factor for sporadic AD, second only to age. One reason for this is likely to be its importance in the clearance of Aβ, another may be its influence on inflammatory response and its adverse influence on the integrity of the blood-brain barrier (BBB; Bell, 2012), which is pertinent when discussing the level of privilege the brain retains (Yu et al., 2014). The E4 polymorphism is proinflammatory, unlike the more common E3 form, which facilitates suppression of inflammation (LaDu et al., 2001; Guo et al., 2004; Chen et al., 2005).

A comparison was made of the bacterial species content of both frozen and formaldehyde fixed sections of a small cohort of Alzheimer-affected cases with those of cognitively unimpaired (normal). **Our findings suggest an increase in bacterial populations in Alzheimer brain tissue compared with normal**.


A
Bacterial Sequence Reads
300,000
250,000
200,000
150,000
100,000
50,000
0
p. Actinobacteria f. Propionibacteriaceae
p. Firmicutes f. Staphylococcaceae
p. Proteobacteria f. Methylobacteriaceae
p. Proteobacteria f. Other
p. Actinobacteria f. Corynebacteriaceae
p. Proteobacteria f. Moraxellaceae
p. Firmicutes f. Streptococcaceae
p. Fusobacteria f. Fusobacteriaceae
p. Actinobacteria f. Micrococcaceae
p. Actinobacteria f. Other
p. Proteobacteria f. Brevundimonadaceae
p. Proteobacteria f. Sphingomonadaceae
p. Proteobacteria f. Comamonadaceae
p. Proteobacteria f. Xanthomonadaceae
p. Firmicutes f. Other
F781C
F502C
F90C
F721C
S412C
S879C
S781C
S102C
F895AD
F498AD
F508AD
F536AD
S711AD
S718AD
S598AD
S771AD
Control
AD
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OPINION ARTICLE
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Microbial-generated amyloids and Alzheimer's disease (AD)

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Keywords: 42 amino acid amyloid-beta peptide (Aβ42), Alzheimer's disease (AD), birefringence, central nervous system (CNS), Congo red, innate immunity, microbiome, toll receptor type 2 (TLR2)

INTRODUCTION

Atypical amyloid generation, folding, aggregation and impaired clearance are characteristic pathological features of human neurodegenerative disorders including Alzheimer's disease (AD). What is generally not appreciated is that a major secretory product of microbes is amyloid, and that the contribution of microbial amyloid to the pathophysiology of the human central nervous system (CNS) is potentially substantial. While earlier findings suggested that these amyloids may serve some immune-evasive strategy, it has recently become evident that humans have a tremendously heavy systemic burden of amyloid which may contribute to the pathology of progressive neurological diseases with an amyloidogenic component. This perspective will highlight some recent inroads made into our understanding of the enigmatic role that microbial amyloids may play in the homeostasis and pathology of the CNS with particular reference to AD wherever possible.

AMYLOID: MICROBIAL AND CNS SOURCES

"*Amyloid*" is a generic term for any aggregated, insoluble, lipoprotein-rich deposit exhibiting β-pleated sheet structures oriented perpendicular to the fibrillar axis (Steensma, 2001; Badtke et al., 2009; Blanco et al., 2012; Buxbaum and Linke, 2012). Amyloids are characterized by an apple-green birefringence (λ_{max} ~555 nm) when stained with the secondary diazo-dye Congo-red (λ_{max} ~498 nm) when viewed under polarized light (upon binding to amyloid, Congo Red displays bright fluorescence emission at λ_{max} ~614 nm after excitation at λ_{max} ~497 nm; Alexandrov et al., 2011; O'Brien and Wong, 2011). Amyloid fibrillation is initiated by self-aggregation of protein monomers-into-dimers, oligomers and fibrils, which accumulate over time, and this process is thought to result from the hydrophobic nature of the aromatic amino-acid peptides comprising the primary sequence of the amyloid (O'Brien and Wong, 2011; Lukiw, 2012). The Congo red dye-based intercalation of β-pleated sheets, induction of a positive anisotropy that is polarized and directionally dependent, and generation of a measureable wavelength shift and apple-green birefringence is the hallmark of all amyloids and is the "gold standard" in the diagnosis of amyloidogenic disease (Linke, 2006; Buxbaum and Linke, 2012). The polymerization of amyloidogenic proteins is cooperative, and can be accelerated by amyloid aggregates derived from the same protein in a selective "seeding" process. The identification of the "amylome," a classification of amino acid sequences within proteins with internal, self-complementary interfaces and high fiber-forming propensity has improved our understanding of the capability of different proteins to form amyloids that contribute to "dense-deposit" disease (Goldschmidt et al., 2010; O'Brien and Wong, 2011; Lukiw, 2012). The pathogenesis of diseases that accumulate amyloid, including AD, all involve a marked inflammatory response at sites of amyloid deposition, and this is mediated by microglial cells, the "roving macrophages" of the CNS. Microglia appear to utilize molecular sensors on their external surface, such as the Toll-like receptor 2, TLR2, to recognize abnormal forms of amyloid and initiate a phagocytic or "clearance" response (Zhao et al., 2013; Ferrera et al., 2014; Jones et al., 2014). Here we describe a relatively recent collection of stimulating research at the crossroads of microbial and AD amyloids highlighting 5 recent, specific and illustrative insights into the potential contribution of microbial-derived amyloids to CNS amyloidogenesis and AD.

MICROBIOME-DERIVED AMYLOIDS

The microbiome is the aggregate of all microorganisms that reside on and within the human body, forming a complex ecosystem that includes the skin, oral and nasal mucosa, the urogenital and gastrointestinal (GI) tracts. The microbiome of the GI tract is by far the largest reservoir of microbes in the human body, containing about 10^{14} microbes; over 99% of microbiota in the GI tract are anaerobic bacteria, with fungi, protozoa, archaebacteria and other microorganisms

"Microbes or their secretory or degradation products including their amyloids and LPSs are powerful inflammatory activators and inducers of cytokines and complement proteins, affecting vascular permeability and generating free-radicals that further support amyloidogenesis (Hill et al., 2014; Lin et al., 2014). These pathogenic signaling features are also highly characteristic of AD neuropathology."

OPEN ACCESS Freely available online

PLoS one

The Alzheimer's Disease-Associated Amyloid β-Protein Is an Antimicrobial Peptide

Stephanie J. Soscia[1,2], James E. Kirby[3], Kevin J. Washicosky[1], Stephanie M. Tucker[1], Martin Ingelsson[4], Bradley Hyman[1,5], Mark A. Burton[6,7], Lee E. Goldstein[6,7], Scott Duong[3], Rudolph E. Tanzi[1,5]*, Robert D. Moir[1,5]

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Abstract

Background: The amyloid β-protein (Aβ) is believed to be the key mediator of Alzheimer's disease (AD) pathology. Aβ is most often characterized as an incidental catabolic byproduct that lacks a normal physiological role. However, Aβ has been shown to be a specific ligand for a number of different receptors and other molecules, transported by complex trafficking pathways, modulated in response to a variety of environmental stressors, and able to induce pro-inflammatory activities.

Methodology/Principal Findings: Here, we provide data supporting an *in vivo* function for Aβ as an antimicrobial peptide (AMP). Experiments used established *in vitro* assays to compare antimicrobial activities of Aβ and LL-37, an archetypical human AMP. Findings reveal that Aβ exerts antimicrobial activity against eight common and clinically relevant microorganisms with a potency equivalent to, and in some cases greater than, LL-37. Furthermore, we show that AD whole brain homogenates have significantly higher antimicrobial activity than aged matched non-AD samples and that AMP action correlates with tissue Aβ levels. Consistent with Aβ-mediated activity, the increased antimicrobial action was ablated by immunodepletion of AD brain homogenates with anti-Aβ antibodies.

Conclusions/Significance: Our findings suggest Aβ is a hitherto unrecognized AMP that may normally function in the innate immune system. This finding stands in stark contrast to current models of Aβ-mediated pathology and has important implications for ongoing and future AD treatment strategies.

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Competing Interests: Dr Tanzi is a consultant to and holds stock options in Prana Biotechnology.

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Introduction

The past 25 years has witnessed the accrual of a large body of data concerning the physiochemistry and biological activities of the amyloid β-peptide (Aβ), the main component of β-amyloid deposits in the brains of Alzheimer's disease (AD) patients [1]. Aβ, which is generated in the brain and peripheral tissues, is widely believed an incidental catabolic byproduct of the amyloid β protein precursor (APP) with no normal physiological function. However, Aβ has been shown to be a ligand for a number of different receptors and other molecules [2,3,4], transported by complex trafficking pathways between tissues and across the blood brain barrier [1,5], modulated in response to a variety of environmental stressors, and able to induce pro-inflammatory activities [6,7]. Despite these clues, the normal physiological role of Aβ remains unknown. We have observed that many of the physiochemical and biological properties previously reported for Aβ are similar to those of a group of biomolecules collectively known as "antimicrobial peptides" (AMPs) which function in the innate immune system. AMPs (also called "host defense peptides") are potent, broad-spectrum antibiotics that target Gram-negative and Gram-positive bacteria, mycobacteria, enveloped viruses, fungi, protozoans and in some cases, transformed or cancerous host cells. AMPs are also potent immunomodulators that mediate cytokine release and adaptive immune responses (see review by Zasloff, 2007 [8]).

The three main families of mammalian AMPs are the defensins, the histatins, and the cathelicidins. Only one member of the cathelicidin family has been identified in humans, the LL-37 peptide [9]. The pleiotropic LL-37 peptide is a widely expressed archetypal AMP [10]. The rodent LL-37 homologue (CRAMP) has been shown to play a central role in combating bacterial infections in a range of tissues, including the CNS [11]. Patients that express low levels of LL-37 are at increased risk for serious

"Our findings suggest Ab is a hitherto unrecognized AMP that may normally function in the innate immune system. This finding stands in stark contrast to current models of Ab-mediated pathology and has important implications for ongoing and future AD treatment strategies."

ACS Chemical Neuroscience

Research Article

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Olive-Oil-Derived Oleocanthal Enhances β-Amyloid Clearance as a Potential Neuroprotective Mechanism against Alzheimer's Disease: In Vitro and in Vivo Studies

Alaa H. Abuznait, Hisham Qosa, Belnaser A. Busnena, Khalid A. El Sayed, and Amal Kaddoumi*

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ABSTRACT: Oleocanthal, a phenolic component of extra-virgin olive oil, has been recently linked to reduced risk of Alzheimer's disease (AD), a neurodegenerative disease that is characterized by accumulation of β-amyloid (Aβ) and tau proteins in the brain. However, the mechanism by which oleocanthal exerts its neuroprotective effect is still incompletely understood. Here, we provide in vitro and in vivo evidence for the potential of oleocanthal to enhance Aβ clearance from the brain via up-regulation of P-glycoprotein (P-gp) and LDL lipoprotein receptor related protein-1 (LRP1), major Aβ transport proteins, at the blood-brain barrier (BBB). Results from in vitro and in vivo studies demonstrated similar and consistent pattern of oleocanthal in controlling Aβ levels. In cultured mice brain endothelial cells, oleocanthal treatment increased P-gp and LRP1 expression and activity. Brain efflux index (BEI%) studies of ^{125}I-Aβ_{40} showed that administration of oleocanthal extracted from extra-virgin olive oil to CS7BL/6 wild-type mice enhanced ^{125}I-Aβ_{40} clearance from the brain and increased the BEI% from 62.0 ± 3.0% for control mice to 79.9 ± 1.6% for oleocanthal treated mice. Increased P-gp and LRP1 expression in the brain microvessels and inhibition studies confirmed the role of up-regulation of these proteins in enhancing ^{125}I-Aβ_{40} clearance after oleocanthal treatment. Furthermore, our results demonstrated significant increase in ^{125}I-Aβ_{40} degradation as a result of the up-regulation of Aβ degrading enzymes following oleocanthal treatment. In conclusion, these findings provide experimental support that potential reduced risk of AD associated with extra-virgin olive oil could be mediated by enhancement of Aβ clearance from the brain.

KEYWORDS: *Oleocanthal, β-amyloid clearance, β-amyloid degradation, P-glycoprotein, LRP1, BBB*

The Mediterranean diet is associated with beneficial health properties against Alzheimer's disease (AD), a neurodegenerative disease that affects about 30 million people worldwide.[1] Epidemiological studies indicate that the prevalence of AD and cognitive decline is low among the Mediterranean area populations compared to those of other geographical regions of the world.[2−4] One integral component of the Mediterranean dietary pattern is the consumption of extra-virgin olive oil (EVOO).[5] Typically, the intake of EVOO ranges from 25 to 50 mL per day in the Mediterranean diet.[6] Therefore, the apparent health benefits have been partially attributed to the dietary consumption of EVOO by Mediterranean populations.

Historically, the health promoting properties of EVOO were attributed to the high concentration of monounsaturated fatty acids, in particular oleic acid, contained in EVOO. However, other seed oils (i.e., sunflower, soybean, and rapeseed), which also contain high concentrations of oleic acid, do not exhibit the same health benefits as EVOO.[7−9] In addition to oleic acid, EVOO contains a minor, yet significant phenolic fraction that other seed oils lack and this fraction has generated much interest regarding its health promoting properties. Currently, 36 phenolic compounds have been identified in EVOO and in vitro and in vivo studies have demonstrated that olive oil phenolics have positive effects on certain physiological parameters such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity, and bone health.[10]

Among the phenolic olive oil constituents, (−)-oleocanthal, a naturally occurring phenolic secoiridoid isolated from EVOO, has shown an anti-inflammatory and antioxidant properties similar to the nonsteroidal anti-inflammatory drug ibuprofen.[11] (−)-Oleocanthal is the dialdehydic form of (−)-deacetoxyligstroside glycoside responsible for the bitter taste of EVOO, and its chemical structure is related to the secoiridoid glycosides ligstroside and oleuropein, which are also common in EVOO. Chemical structure of oleocanthal is shown in Figure 1.

Recently, oleocanthal has been demonstrated to have potential neuroprotective properties and contribute to preventing cognitive decline due to neurodegenerative diseases.[12−14] This has been supported by population-based studies indicating that Mediterranean diet, rich in olive oil and

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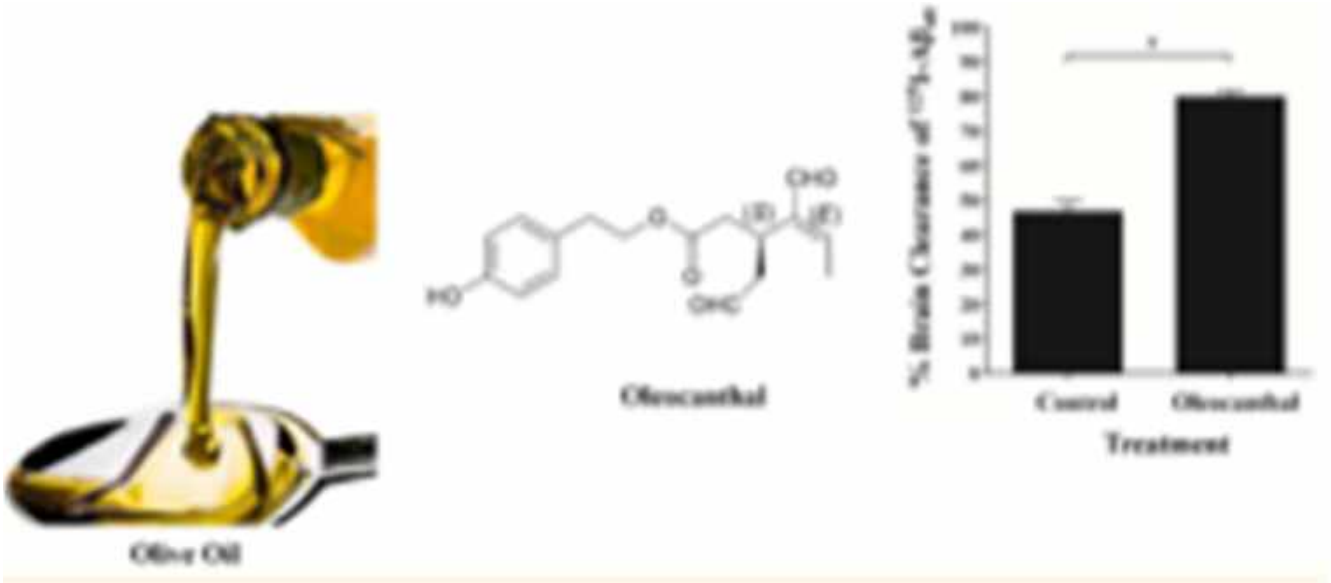
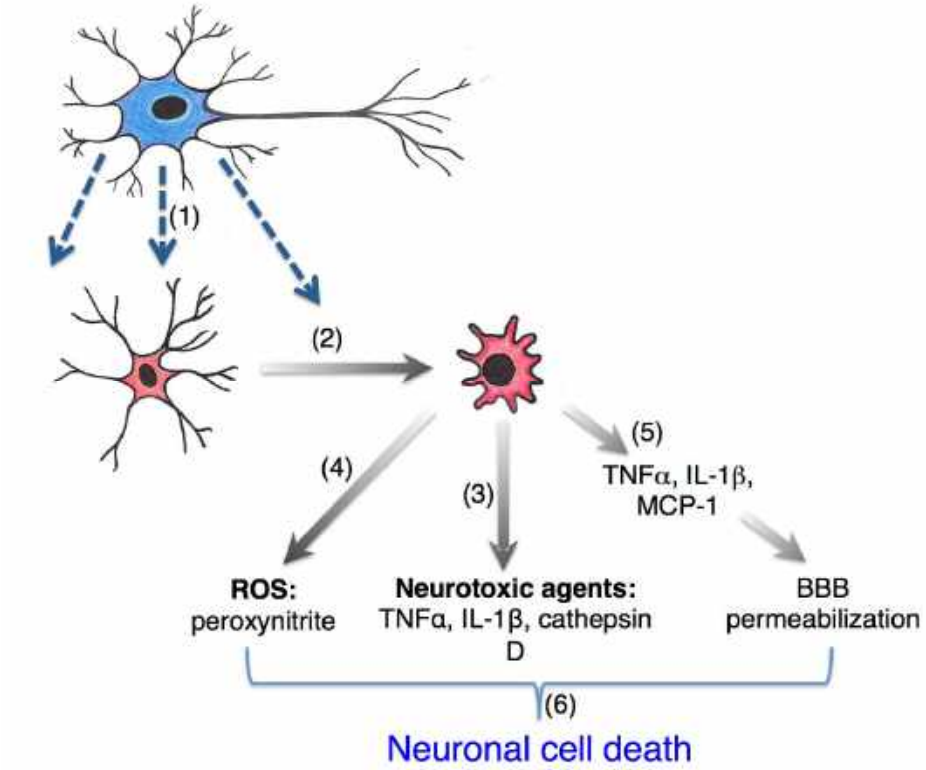
In conclusion, these findings provide experimental support that potential reduced risk of AD associated with extra-virgin olive oil could be mediated by enhancement of Aβ clearance from the brain.



In the present study, we aimed to demonstrate, using in vitro and in vivo studies, the role of oleocanthal in enhanced clearance of Aβ from the brain as an additional possible mechanism for its neuroprotective effect via its potential to up- regulate P-gp (P-glycoprotein) and LRP1 (LDL lipoprotein receptor related protein-1) at the BBB, and its ability to enhance Aβ degradation.

Brain on Fire
Glutamate Excitotoxicity Mediated NeuroInflammation



Immune Activators

- Lyme and Coinfections
- Viruses (e.g.HSV-1, HHV6, etc)
- Beta Amyloid
- Multiple Other Infectious sources e.g oral/gut bacteria
- Some Environmental Toxins hit immune triggers (e.g. mold toxins, heavy metals, etc)

npj | Aging and Mechanisms of Disease

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ARTICLE OPEN

Amyloid proteotoxicity initiates an inflammatory response blocked by cannabinoids

Antonio Currais[1], Oswald Quehenberger[2,3], Aaron M Armando[2], Daniel Daugherty[1], Pam Maher[1] and David Schubert[1]

The beta amyloid (Aβ) and other aggregating proteins in the brain increase with age and are frequently found within neurons. The mechanistic relationship between intracellular amyloid, aging and neurodegeneration is not, however, well understood. We use a proteotoxicity model based upon the inducible expression of Aβ in a human central nervous system nerve cell line to characterize a distinct form of nerve cell death caused by intracellular Aβ. It is shown that intracellular Aβ initiates a toxic inflammatory response leading to the cell's demise. Aβ induces the expression of multiple proinflammatory genes and an increase in both arachidonic acid and eicosanoids, including prostaglandins that are neuroprotective and leukotrienes that potentiate death. Cannabinoids such as tetrahydrocannabinol stimulate the removal of intraneuronal Aβ, block the inflammatory response, and are protective. Altogether these data show that there is a complex and likely autocatalytic inflammatory response within nerve cells caused by the accumulation of intracellular Aβ, and that this early form of proteotoxicity can be blocked by the activation of cannabinoid receptors.

npj Aging and Mechanisms of Disease (2016) **2**, 16012; doi:10.1038/npjamd.2016.12; published online 23 June 2016

INTRODUCTION

Nerve cell death from the accumulation of aggregated or amyloid-like proteins is a common theme in most age-dependent neurodegenerative diseases. However, there are no drugs that significantly inhibit cell death associated with Alzheimer's disease (AD), Parkinson's or Huntington's diseases. This could be because most interest has been in the late manifestations of the disease, not in the initial changes in cell metabolism that ultimately lead to nerve cell death.[1] In the context of life span, slowing down the removal of aggregated proteins in the brains of flies shortens life span, while expediting their rate of removal extends life span.[2] Therefore, it is likely that the accumulation of intracellular aggregated protein in the brain occurs throughout life, contributes to cognitive aging, and may also be involved in the initiation of many old age-associated diseases.

Although debated,[3,4] the accumulation of intracellular amyloid beta (Aβ) is an early event in AD. In both humans and rodents, intracellular Aβ accumulation is observed well before extracellular amyloid.[5–8] Similarly, both aggregated huntingtin and alpha synuclein are found in neurons before disease onset.[9,10]

As with the accumulation of intracellular proteins, central nervous system (CNS) inflammation is elevated with age and increases in disease.[11] As AD is associated with neuronal dysfunction, we hypothesized that proteotoxicity in nerve cells themselves may initiate an inflammatory response that can lead directly to their death and contribute to overall inflammation in the CNS. The following experiments identify the molecular basis of this inflammatory response using a human CNS nerve cell line that conditionally expresses Aβ.

RESULTS

MC65 cells are a human CNS nerve cell line that contains the C-99 fragment of the amyloid precursor protein under the control of a tetracycline (tet)-sensitive promoter.[12] The parent cell line is SK-N-MC from a human brain tumor, and it has an electrically excitable membrane typical of neurons.[13] When tet is withdrawn, cells express C-99 that is converted to Aβ by γ-secretase and the cells die within 4 days (Figure 1a,b). Aβ remains within the cell and forms aggregates.[12,14] In the presence of γ-secretase inhibitors (SI), cells accumulate C-99, but do not die, and C99 does not aggregate.

Intraneuronal Aβ induces the expression of proinflammatory molecules

Inflammation is associated with the elevated expression of cytokines and chemokines. To assay for the expression of these genes following the induction of Aβ in MC65 cells, the mRNA expression of 184 inflammation-associated genes was assayed sequentially for three days. Table 1 shows increases in the expression of 12 genes.

IL-8 expression is linked to late onset AD.[15] Importantly, IL-8 crosses the blood brain barrier and stimulates the recruitment of immune cells into the brain.[16] Aβ causes a 10-fold increase in IL-8 gene expression, and IL-8 is detected in the culture supernatant 2 days after the induction of Aβ (Table 1).

It was next asked whether the intracellular expression of Aβ leads to an increase in proinflammatory pathways. NFkB is a ubiquitous proinflammatory molecule whose activation includes phosphorylation. Phosphorylation of its p65 subunit is increased following the expression of Aβ in MC65 cells (Figure 1c,d). Inflammation is also associated with the activation of caspase 1. Figure 1c,d shows that following the expression of Aβ, caspase 1 is activated as defined by the appearance of activation-dependent cleavage products.

In cases of caspase-1 activation, cell death is ultimately caused by caspase-3.[17] Figure 1c also shows that caspase-3 is strongly

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"Cannabinoids such as tetrahydrocannabinol stimulate the removal of intraneuronal Aβ, block the inflammatory response, and are protective. Altogether these data show that there is a complex and likely autocatalytic inflammatory response within nerve cells caused by the accumulation of intracellular Aβ, and that this early form of proteotoxicity can be blocked by the activation of cannabinoid receptors."

CBD, Neuroprotection, and NeuroInflammation

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0007–1188/07 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Cannabidiol *in vivo* blunts β-amyloid induced neuroinflammation by suppressing IL-1β and iNOS expression

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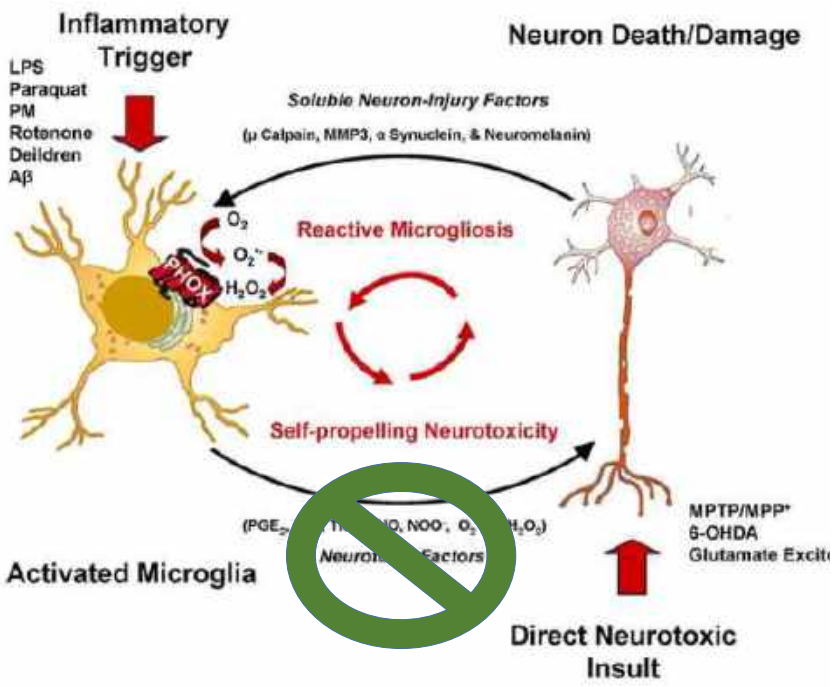
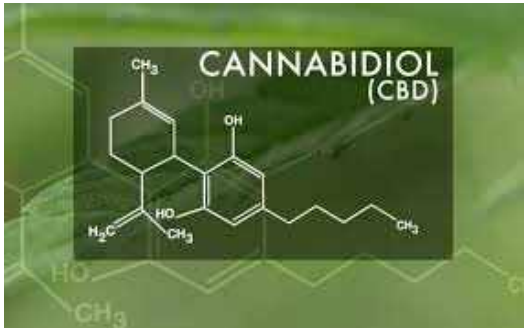
Background and purpose: Pharmacological inhibition of beta-amyloid (Aβ) induced reactive gliosis may represent a novel rationale to develop drugs able to blunt neuronal damage and slow the course of Alzheimer's disease (AD). Cannabidiol (CBD), the main non-psychotropic natural cannabinoid, exerts *in vitro* a combination of neuroprotective effects in different models of Aβ neurotoxicity. The present study, performed in a mouse model of AD-related neuroinflammation, was aimed at confirming *in vivo* the previously reported antiinflammatory properties of CBD.
Experimental approach: Mice were inoculated with human Aβ (1–42) peptide into the right dorsal hippocampus, and treated daily with vehicle or CBD (2.5 or 10 mg kg^{-1}, i.p.) for 7 days. mRNA for glial fibrillary acidic protein (GFAP) was assessed by *in situ* hybridization. Protein expression of GFAP, inducible nitric oxide synthase (iNOS) and IL-1β was determined by immunofluorescence analysis. In addition, ELISA assay of IL-1β level and the measurement of NO were performed in dissected and homogenized ipsilateral hippocampi, derived from vehicle and Aβ inoculated mice, in the absence or presence of CBD.
Key results: In contrast to vehicle, CBD dose-dependently and significantly inhibited GFAP mRNA and protein expression in Aβ injected animals. Moreover, under the same experimental conditions, CBD impaired iNOS and IL-1β protein expression, and the related NO and IL-1β release.
Conclusion and implications: The results of the present study confirm *in vivo* anti-inflammatory actions of CBD, emphasizing the importance of this compound as a novel promising pharmacological tool capable of attenuating Aβ evoked neuroinflammatory responses.
British Journal of Pharmacology (2007) **151**, 1272–1279; doi:10.1038/sj.bjp.0707337; published online 25 June 2007





Studies Show How Vitamin D3 Helps Clear Amyloid in AD

Megan Brooks

March 15, 2012 — A team of researchers has uncovered the intracellular mechanisms regulated by vitamin D3 that may help clear amyloid-beta from the brain, the hallmark of Alzheimer's disease (AD).

"This new study helped clarify the key mechanisms involved, which will help us better understand the usefulness of vitamin D3 and curcumin as possible therapies for Alzheimer's disease," Milan Fiala, MD, of the David Geffen School of Medicine at University of California Los Angeles, notes in a written statement.

The study also supports mounting evidence that adequate levels of vitamin D "may be a key factor in AD prevention," the researchers say. Their work was published March 6 in the *Journal of Alzheimer's Disease*.

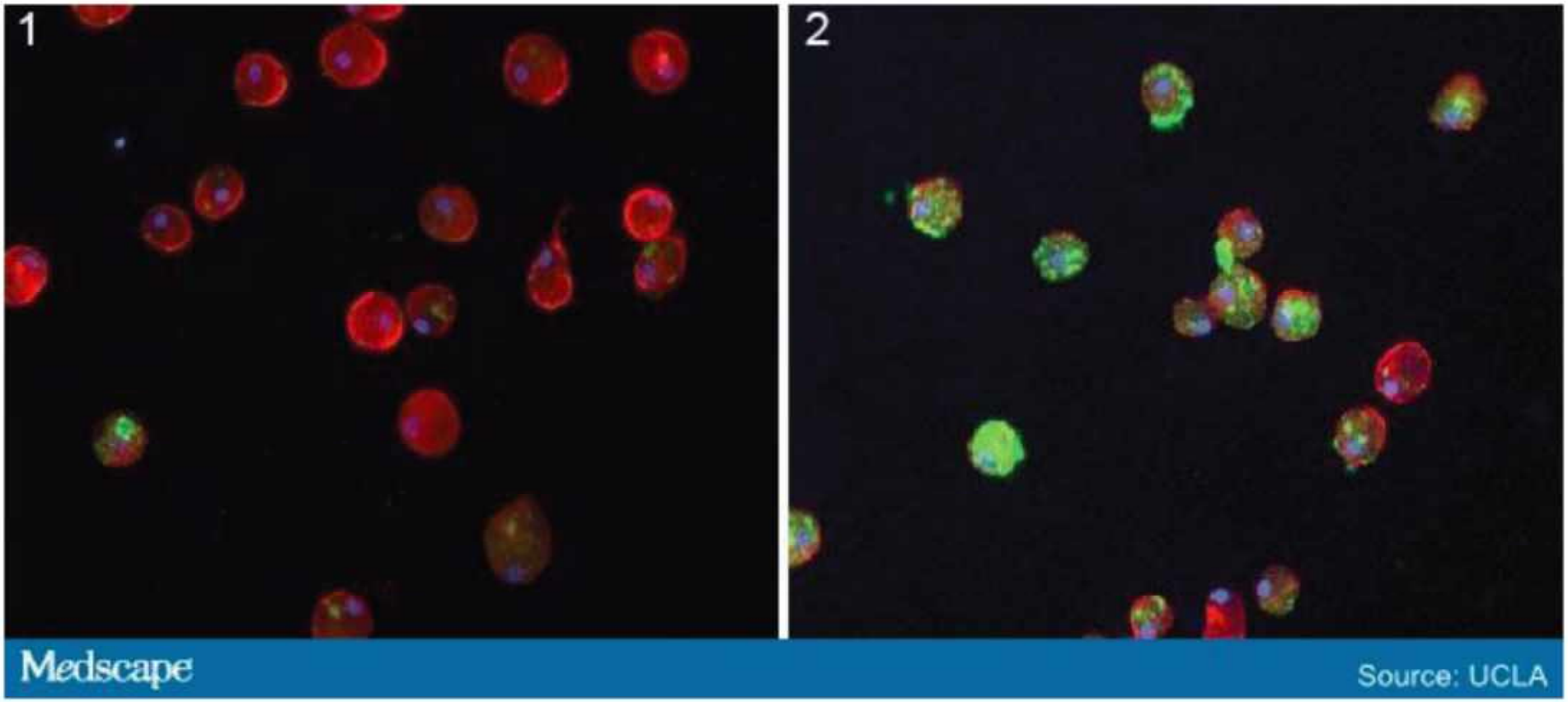
"The clinical implications," Dr. Fiala told *Medscape Medical News*,"are that vitamin D3 protects the brain through the immune system and that recommended blood levels of the 25-hydroxy vitamin D3 should be maintained in all seasons including winter when the sun is not helping to produce vitamin D in the skin."

She said clinical trials assessing the therapeutic potential of vitamin D3 are "ongoing or planned."

Retuning AD Macrophages

Brain clearance of amyloid-beta 1-42 (Aβ-42) by innate immune macrophages is required for maintenance of normal brain function. This process of phagocytosis is defective in patients with AD.

In earlier laboratory studies, Dr. Fiala and colleagues identified 2 types of macrophages in patients with AD, type I and type II macrophages. They found that the function of type I macrophages can be improved by adding vitamin D3 and curcuminoids, a synthetic form of curcumin, a chemical found in turmeric spice. Type II macrophages, on the other hand, are improved only by adding vitamin D3. However, the exact mechanism of these effects remained unclear, until now.



Macrophages from AD patients without (Figure 1) and with (Figure 2) vitamin D3. Macrophages in Figure

"Our findings demonstrate that active forms of vitamin D3 may be an important regulator of immune activities of macrophages in helping to clear amyloid plaques by directly regulating the expression of genes, as well as the structural physical workings of the cells," said study author Mizwicki,

frontiers in PSYCHIATRY

REVIEW ARTICLE
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Treating depression and depression-like behavior with physical activity: an immune perspective

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The increasing burden of major depressive disorder makes the search for an extended understanding of etiology, and for the development of additional treatments highly significant. Biological factors may be useful biomarkers for treatment with physical activity (PA), and neurobiological effects of PA may herald new therapeutic development in the future. This paper provides a thorough and up-to-date review of studies examining the neuroimmunomodulatory effects of PA on the brain in depression and depression-like behaviors. From a neuroimmune perspective, evidence suggests PA does enhance the beneficial and reduce the detrimental effects of the neuroimmune system. PA appears to increase the following factors: interleukin (IL)-10, IL-6 (acutely), macrophage migration inhibitory factor, central nervous system-specific autoreactive CD4+ T cells, M2 microglia, quiescent astrocytes, CX3CL1, and insulin-like growth factor-1. On the other hand, PA appears to reduce detrimental neuroimmune factors such as: Th1/Th2 balance, pro-inflammatory cytokines, C-reactive protein, M1 microglia, and reactive astrocytes. The effect of other mechanisms is unknown, such as: CD4+CD25+ T regulatory cells (T regs), CD200, chemokines, miRNA, M2-type blood-derived macrophages, and tumor necrosis factor (TNF)-α [via receptor 2 (R2)]. The beneficial effects of PA are likely to occur centrally and peripherally (e.g., in visceral fat reduction). The investigation of the neuroimmune effects of PA on depression and depression-like behavior is a rapidly developing and important field.

Keywords: physical activity, exercise, depression, psychiatry, immune, neurobiology

The increasing burden of major depressive disorder (MDD; WHO, 2008) makes the search for an extended understanding of etiology, and for the development of additional treatments highly significant. The global "pandemic" of physical inactivity (Lee et al., 2012) – a significant etiological factor for many non-communicable diseases, including depression (Garber et al., 2011; Kohl et al., 2012; Lee et al., 2012) – as well as the growing evidence supporting the clinical utility of physical activity (PA) in many psychiatric disorders, make the biological effects of PA highly relevant (Knochel et al., 2012; Lautenschlager et al., 2012; Rimer et al., 2012). Biological factors may be useful biomarkers for treatment with PA, and neurobiological effects of PA may herald new therapeutic developments in the future.

The neuroimmune system is important in the pathogenesis and pathophysiology of depression-like behaviors (Eyre and Baune, 2012c). Elevations in pro-inflammatory cytokines (PICs), causing neuroinflammation, are well known to be involved in the development of depression-like behaviors – e.g., sickness-like behavior, cognitive dysfunction, and anhedonia – in pre-clinical and clinical populations (Dantzer et al., 2008; McAfoose and Baune, 2009; Miller et al., 2009). The involvement of PICs in the development of depression-like behavior is often referred to as the cytokine model of depression (Dantzer et al., 2008; McAfoose and Baune, 2009; Miller et al., 2009).The neuroinflammatory state is associated with neurotransmitter dysfunction [e.g., reductions in serotonin (5-HT), as well as neurotoxic levels of glutamate (GLU) and tryptophan catabolites], reduced hippocampal (HC) neuroplasticity [e.g., neurogenesis, synaptic plasticity, and long-term potentiation (LTP)], oxidative stress, and glucocorticoid insensitivity (Dantzer et al., 2008; Miller et al., 2009; Eyre and Baune, 2012c; Leonard and Maes, 2012; Moylan et al., 2012).

A variety of novel neuroimmune mechanisms may also be involved in the development of depression-like behaviors (Eyre and Baune, 2012c; Littrell, 2012). Cellular immune factors include various T cells [e.g., CD4+CD25+ T regulatory cells (T regs), CNS-specific autoreactive CD4+ T cells] and macrophages (e.g., M2-type blood-derived macrophages) involved in the model of protective immunosurveillance (Schwartz and Shechter, 2010a,b; Martino et al., 2011; Ron-Harel et al., 2011). These neuroprotective immune cells – found to release neurotrophic factors and anti-inflammatory cytokines (AICs; Schwartz and Shechter, 2010a,b; Martino et al., 2011; Ron-Harel et al., 2011) – may be dysfunctional in the disease state (Schwartz and Shechter, 2010b). Moreover, the function of immunomodulatory proteins such as CX3CL1 (aka fractalkine; Rogers et al., 2011; Corona et al., 2012; Ginnti et al., 2012), insulin-like growth factor-1 (IGF-1; Park et al., 2011a), and CD 200 (Lyons et al., 2007; Ojo et al., 2012) may be reduced.

In clinical studies, PA has shown efficacy in the treatment of MDD (Rimer et al., 2012), schizophrenia (SCZ; Knochel et al., 2012), anxiety-based disorders (Asmundson et al., 2013), and in

"PA appears to increase the following factors: interleukin (IL)-10, IL-6 (acutely), macrophage migration inhibitory factor, central nervous system-specific autoreactive CD4+ T cells, M2 microglia, quiescent astrocytes, CX3CL1, and insulin-like growth factor-1. **On the other hand, PA appears to reduce detrimental neuroimmune factors such as: Th1/Th2 balance, pro-inflammatory cytokines, C-reactive protein, M1 microglia, and reactive astrocytes."**

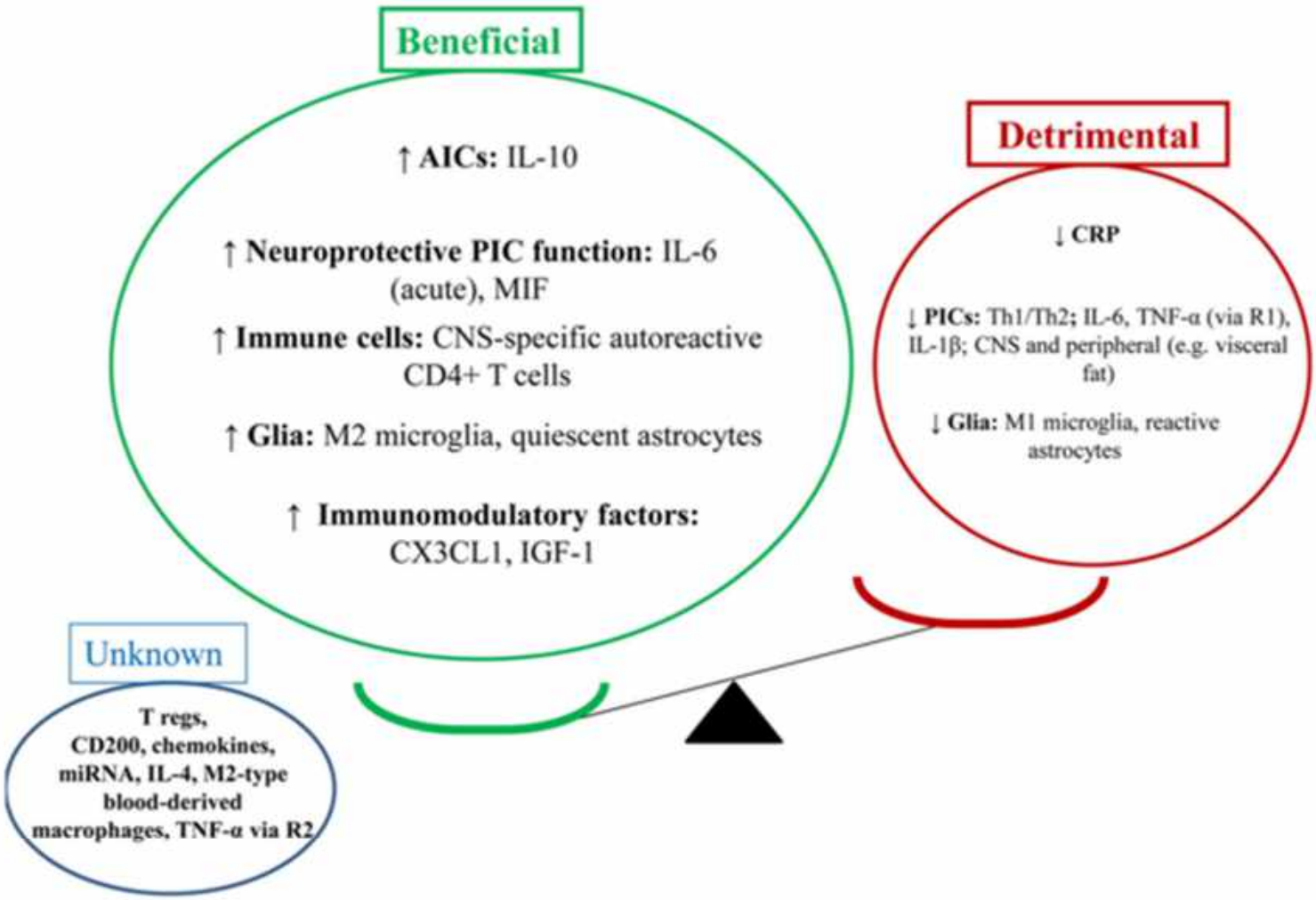
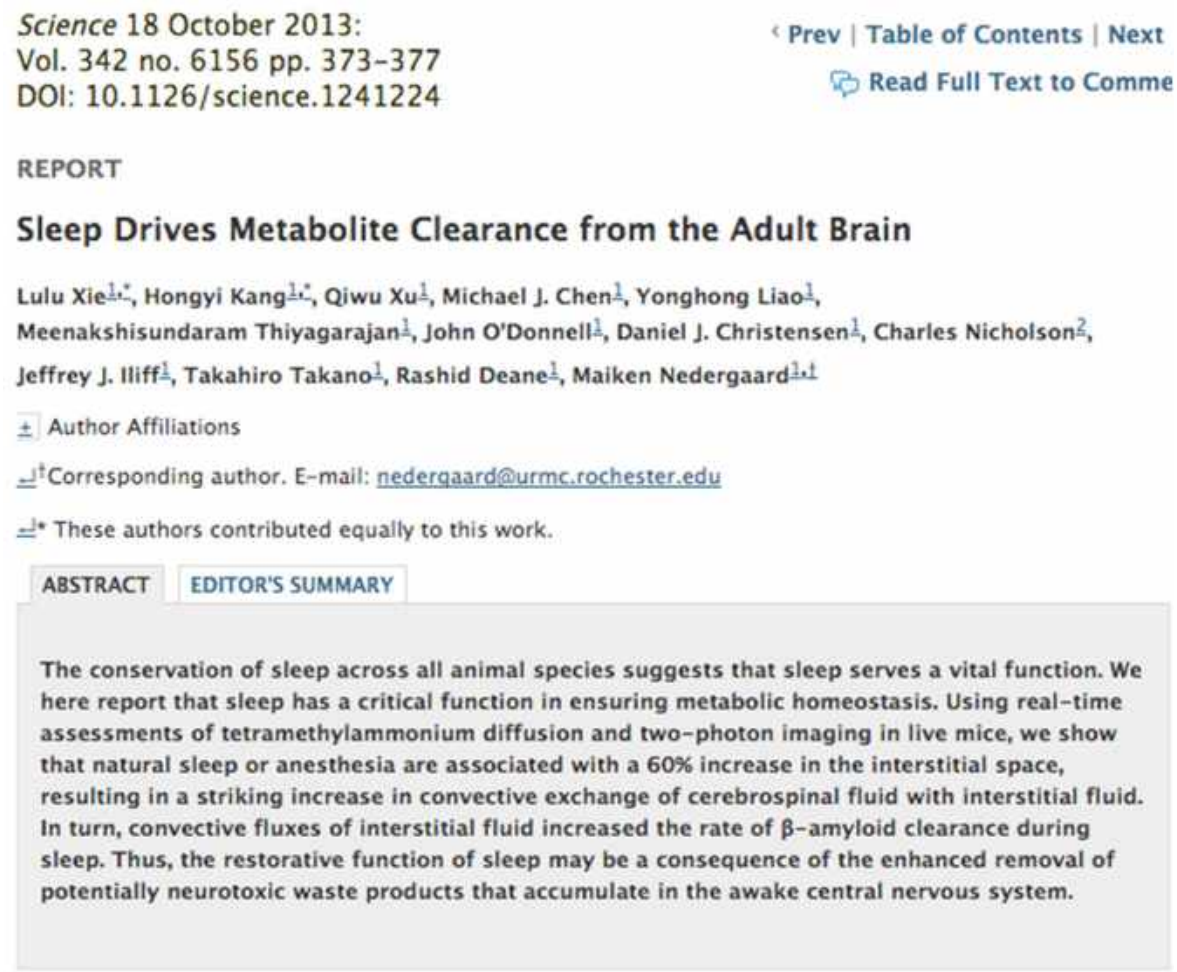


FIGURE 2 | Physical activity in depression: antidepressant via enhancing the beneficial effects of the neuroimmune system. This figure illustrates the effects of PA on the brain as per the balance between beneficial and detrimental effects of neuroimmune factors. PA appears to enhance the beneficial effects of the neuroimmune system and reduce the detrimental effects. From a behavioral perspective, this may lead to reduced depression-like behaviors. From a clinical perspective, this may lead to reduced depressive symptoms, depressive episode resolution, and reduced relapse rates (disease prevention).



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REPORT

Sleep Drives Metabolite Clearance from the Adult Brain

Lulu Xie[1,*], Hongyi Kang[1,*], Qiwu Xu[1], Michael J. Chen[1], Yonghong Liao[1], Meenakshisundaram Thiyagarajan[1], John O'Donnell[1], Daniel J. Christensen[1], Charles Nicholson[2], Jeffrey J. Iliff[1], Takahiro Takano[1], Rashid Deane[1], Maiken Nedergaard[1,†]

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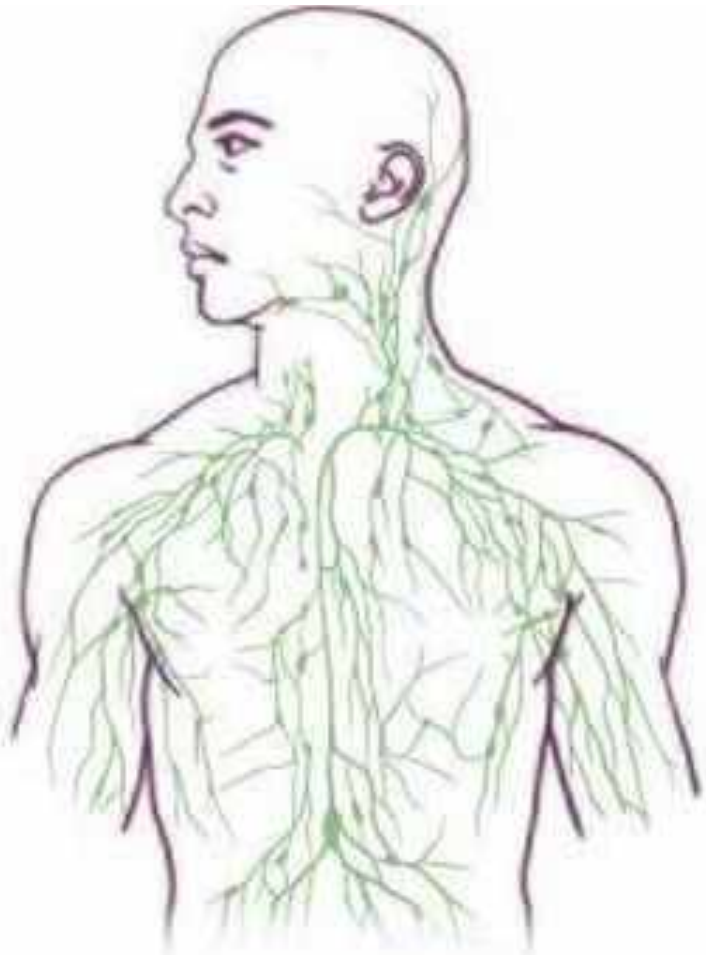
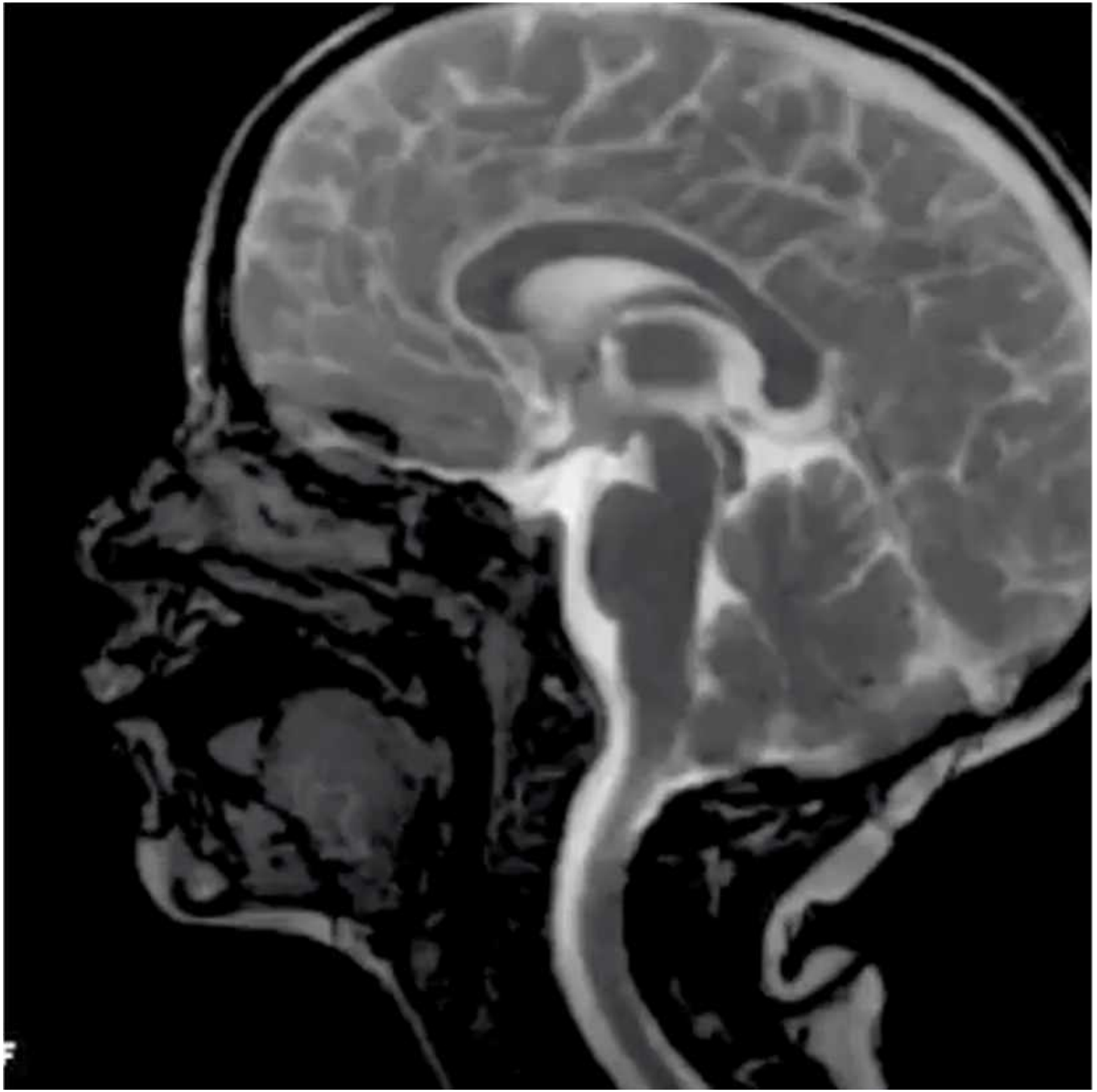
* These authors contributed equally to this work.

ABSTRACT | EDITOR'S SUMMARY

The conservation of sleep across all animal species suggests that sleep serves a vital function. We here report that sleep has a critical function in ensuring metabolic homeostasis. Using real-time assessments of tetramethylammonium diffusion and two-photon imaging in live mice, we show that natural sleep or anesthesia are associated with a 60% increase in the interstitial space, resulting in a striking increase in convective exchange of cerebrospinal fluid with interstitial fluid. In turn, convective fluxes of interstitial fluid increased the rate of β-amyloid clearance during sleep. Thus, the restorative function of sleep may be a consequence of the enhanced removal of potentially neurotoxic waste products that accumulate in the awake central nervous system.

"natural sleep or anesthesia are associated with a 60% increase in the interstitial space, resulting in a striking increase in convective exchange of cerebrospinal fluid with interstitial fluid.....Thus, ***the restorative function of sleep may be a consequence of the enhanced removal of potentially neurotoxic waste products that accumulate in the awake central nervous system."***





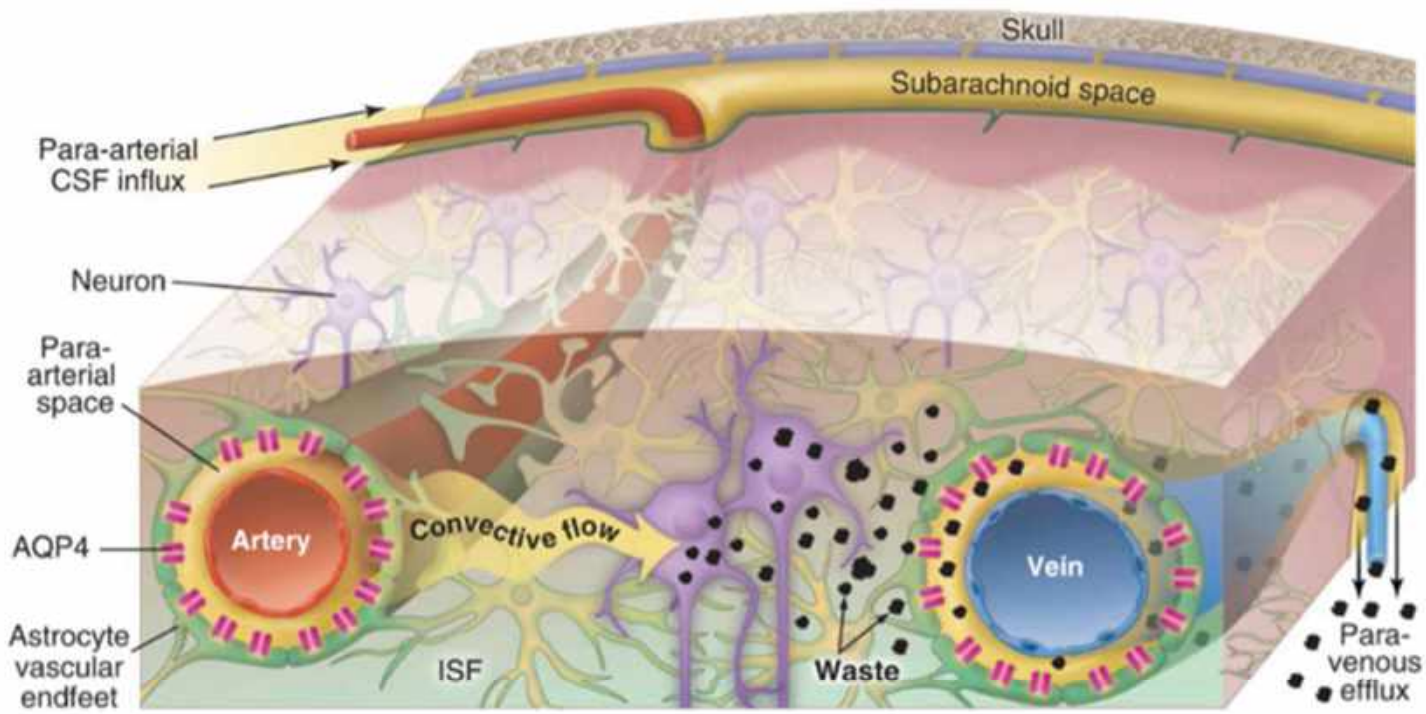


Figure 1. Go with the flow

Convective glymphatic fluxes of CSF and ISF propel the waste products of neuron metabolism into the paravenous space, from which they are directed into lymphatic vessels and ultimately return to the general circulation for clearance by the kidney and liver.

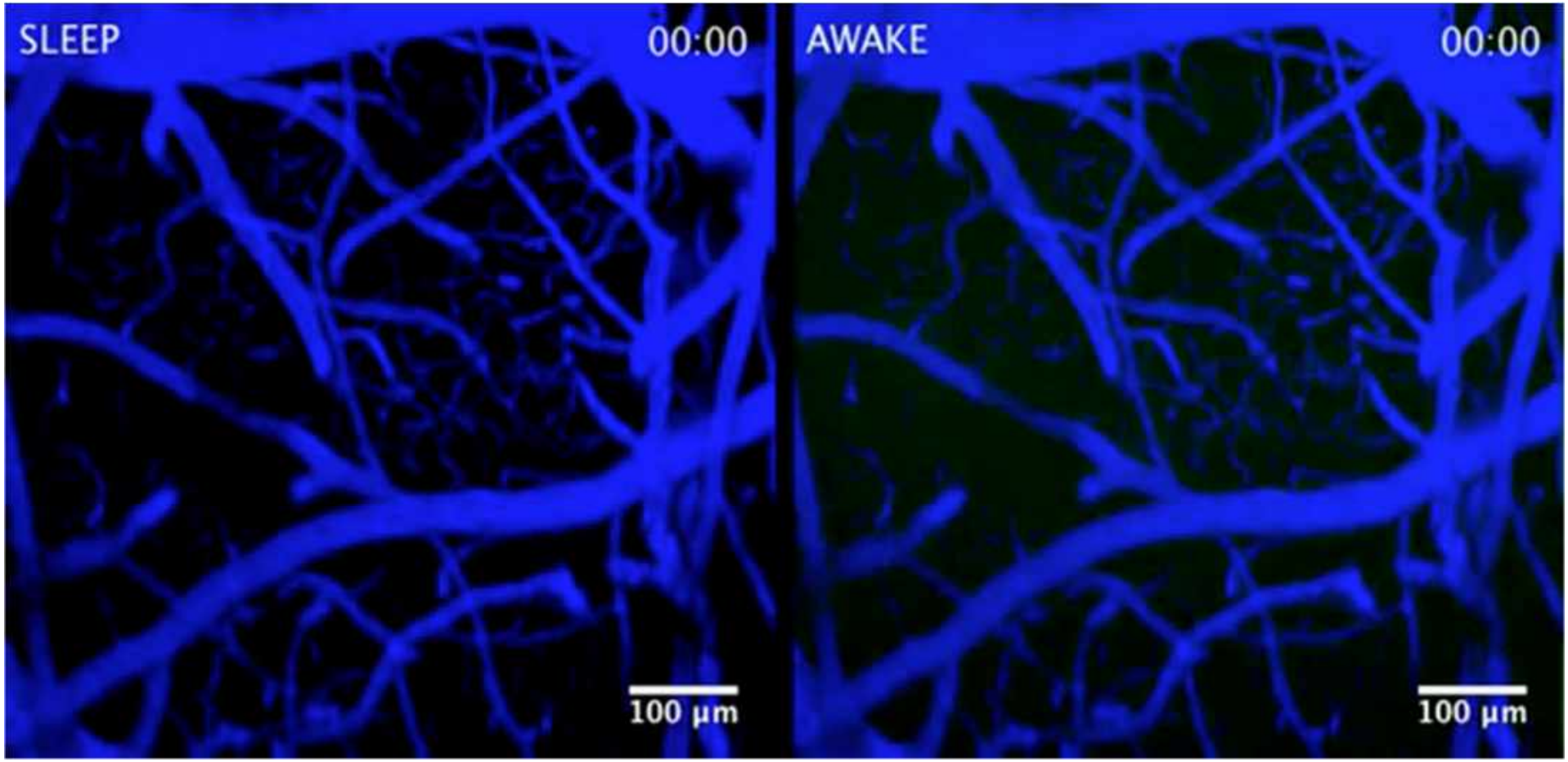
Garbage Truck of the Brain

Maiken Nedergaard

Science. 2013 June 28; 340(6140): 1529–1530.

doi:10.1126/science.1240514.

SLEEP AWAKE



Free Radic Biol Med. 2012 Nov 8. pii: S0891-5849(12)01800-X. doi: 10.1016/j.freeradbiomed.2012.10.558. [Epub ahead of print]

Metallostasis in Alzheimer's disease.

Ayton S, Lei P, Bush AI.

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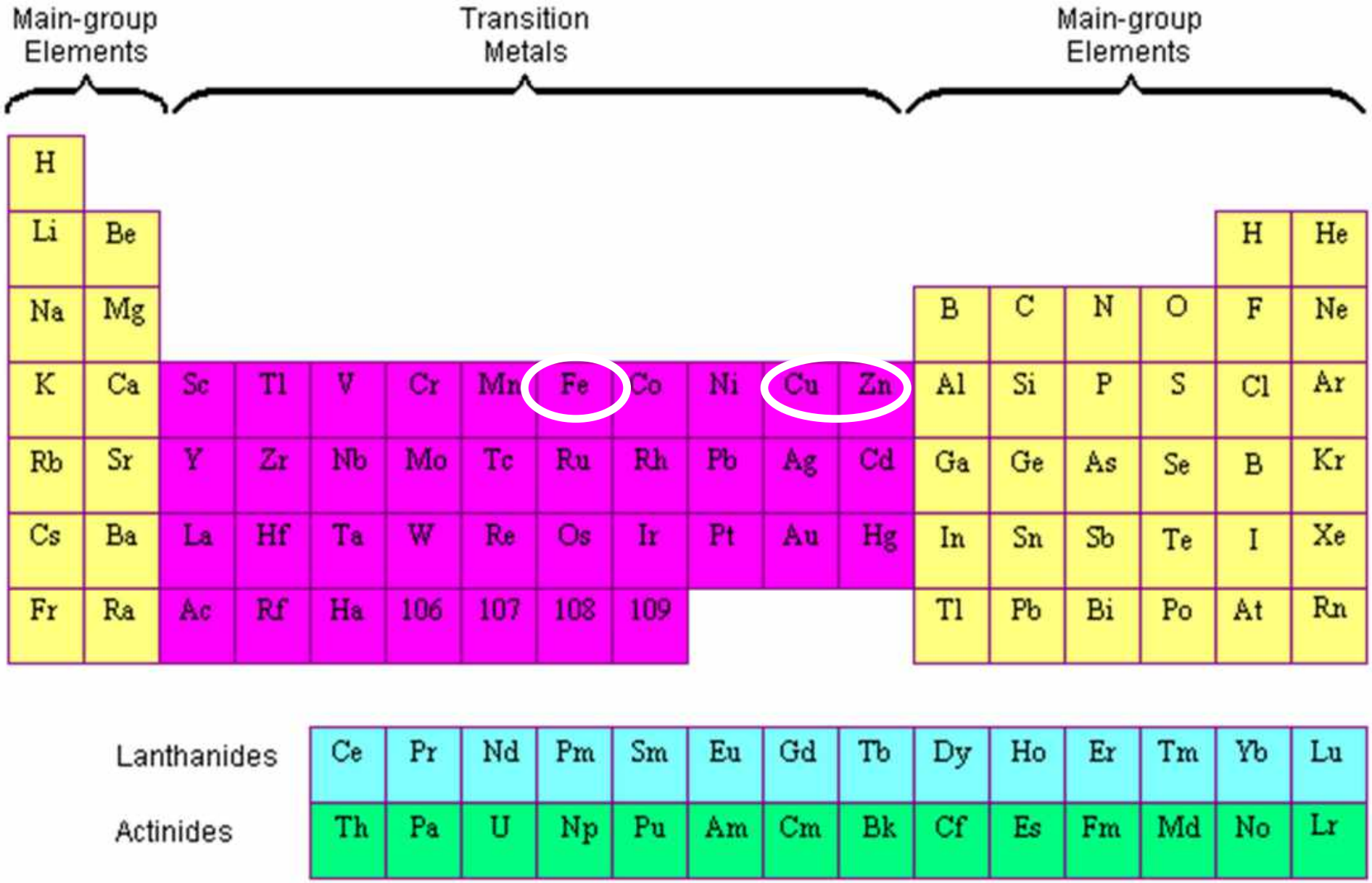
Abstract

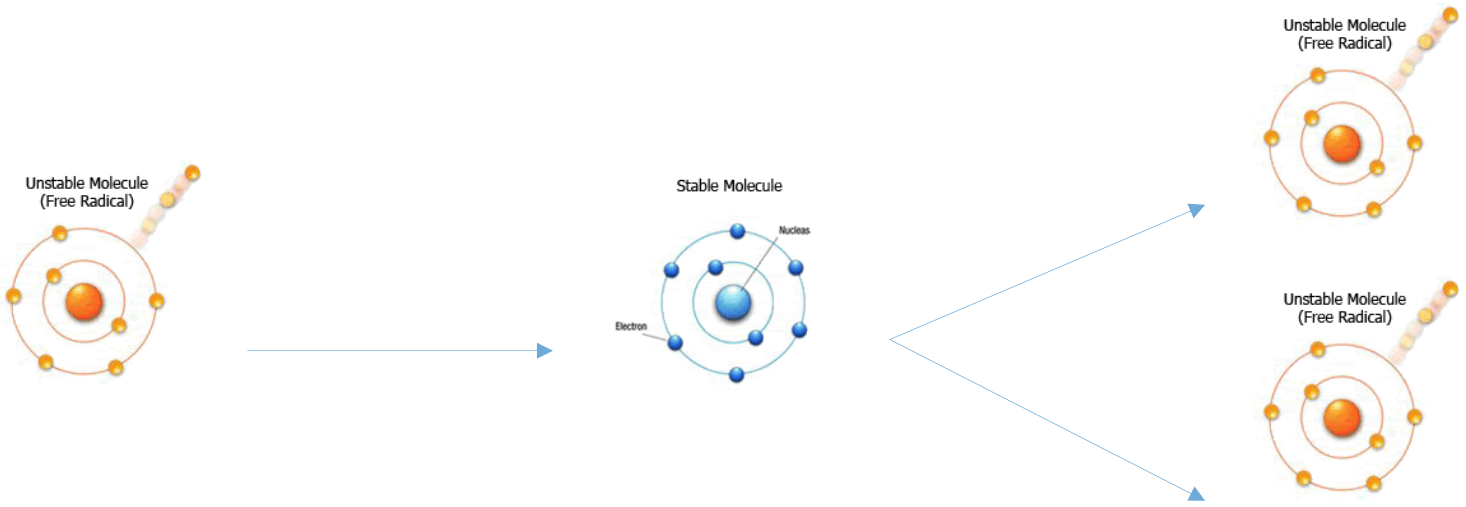
2012 has been another year where multiple large scale clinical trials for Alzheimer's disease (AD) have failed to meet their clinical endpoints. With the social and financial burden of this disease increasing every year, the onus is now on the field of AD researchers to investigate alternative ideas in order to deliver outcomes for patients. While several major clinical trials targeting Aβ have failed, three smaller clinical trials targeting metal interactions with Aβ have all shown benefit for patients. Here we review the genetic, pathological, biochemical and pharmacological evidence that underlie the metal hypothesis of AD. The AD-affected brain suffers from metallostasis, or, fatigue of metal trafficking resulting in redistribution of metals into inappropriate compartments. The metal hypothesis is built upon the triad of transition elements: iron, copper, and zinc. The hypothesis has matured from early investigations showing amyloidogenic and oxidative stress consequences of these metals; recently, disease related proteins: APP, tau and presenilin, have been shown to have major roles in metal regulation, which provides insight into the pathway of neurodegeneration in AD and illuminates potential new therapeutic avenues.

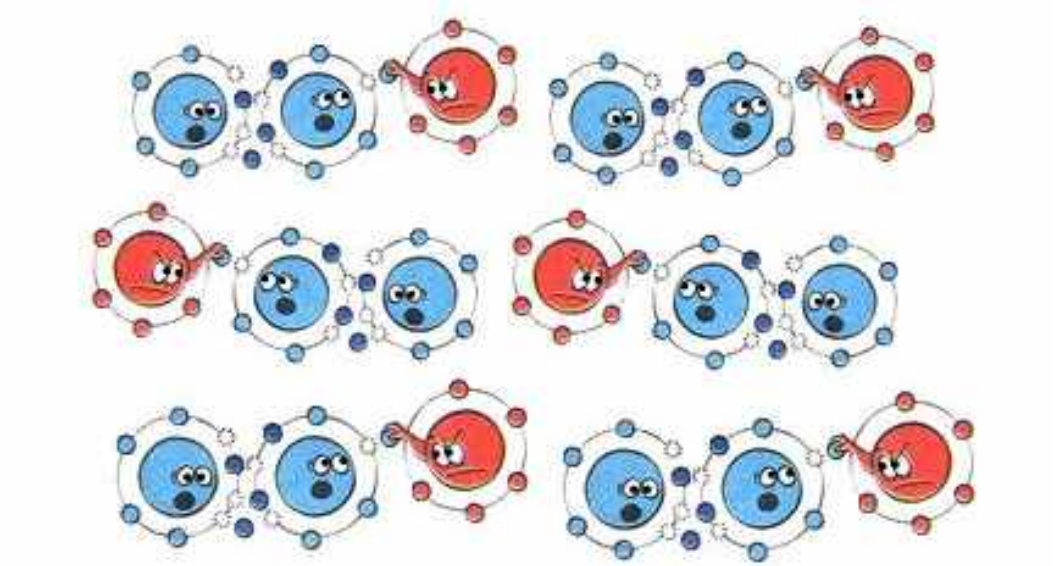

Main-group Elements
Transition Metals
Main-group Elements
H
Li Be
Na Mg
K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn
Rb Sr Y Zr Nb Mo Tc Ru Rh Pb Ag Cd
Cs Ba La Hf Ta W Re Os Ir Pt Au Hg
Fr Ra Ac Rf Ha 106 107 108 109
H He
B C N O F Ne
Al Si P S Cl Ar
Ga Ge As Se B Kr
In Sn Sb Te I Xe
Tl Pb Bi Po At Rn
Lanthanides Ce Pr Nd Pm Sm Eu Gd Tb Dy Ho Er Tm Yb Lu
Actinides Th Pa U Np Pu Am Cm Bk Cf Es Fm Md No Lr

Free Radicals

Free Radicals are highly reactive/unstable molecules containing unpaired electrons

- *A Reactive Oxygen Species (ROS)* is a free radical containing an oxygen molecule
  - To improve stability, the free radical remove/steal electrons from stable molecules
  - Propagation steps perpetuate the formation of free radicals
  - Free radical excess can affect tissues, lipids, proteins and DNA



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Acc Chem Res. 2014 Aug 19;47(8):2252-9. doi: 10.1021/ar400293h. Epub 2014 May 29.

Metal Ions and Intrinsically Disordered Proteins and Peptides: From Cu/Zn Amyloid-β to General Principles.

Faller P[1], Hureau C, La Penna G.

Author information

Abstract

Conspectus The interaction of d-block metal ions (Cu, Zn, Fe, etc.) with intrinsically disordered proteins (IDPs) has gained interest, partly due to their proposed roles in several diseases, mainly neurodegenerative. A prominent member of IDPs is the peptide amyloid-β (Aβ) that aggregates into metal-enriched amyloid plaques, a hallmark of Alzheimer's disease, in which Cu and Zn are bound to Aβ. IDPs are a class of proteins and peptides that lack a unique 3D structure when the protein is isolated. This disordered structure impacts their interaction with metal ions compared with structured metalloproteins. Metalloproteins either have a preorganized metal binding site or fold upon metal binding, resulting in defined 3D structure with a well-defined metal site. In contrast, for Aβ and likely most of the other IDPs, the affinity for Cu(I/II) and Zn(II) is weaker and the interaction is flexible with different coordination sites present. Coordination of Cu(I/II) with Aβ is very dynamic including fast Cu-exchange reactions (milliseconds or less) that are intrapeptidic between different sites as well as interpeptidic. This highly dynamic metal-IDP interaction has a strong impact on reactivity and potential biological role: (i) Due to the low affinity compared with classical metalloproteins, IDPs likely bind metals only at special places or under special conditions. For Aβ, this is likely in the neurons that expel Zn or Cu into the synapse and upon metal dysregulation occurring in Alzheimer's disease. (ii) Amino acid substitutions (mutations) on noncoordinating residues can change drastically the coordination sphere. (iii) Considering the Cu/Zn-Aβ aberrant interaction, therapeutic strategies can be based on removal of Cu/Zn or precluding their binding to the peptide. The latter is very difficult due to the multitude of metal-binding sites, but the fast koff facilitates removal. (iv) The high flexibility of the Cu-Aβ complex results in different conformations with different redox activity. Only some conformations are able to produce reactive oxygen species. (v) Other, more specific catalysis (like enzymes) is very unlikely for Cu/Zn-Aβ. (vi) The Cu/Zn exchange reactions with Aβ are faster than the aggregation process and can hence have a strong impact on this process. In conclusion, the coordination chemistry is fundamentally different for most of IDPs compared with the classical, structured metalloproteins or with (bio)-inorganic complexes. The dynamics is a key parameter to understand this interaction and its potential biological impact.

BMC Medical Genomics

BioMed Central

Review

Open Access

Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases

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Abstract

Background: The production of peroxide and superoxide is an inevitable consequence of aerobic metabolism, and while these particular 'reactive oxygen species' (ROSs) can exhibit a number of biological effects, they are not of themselves excessively reactive and thus they are not especially damaging at physiological concentrations. However, their reactions with poorly liganded iron species can lead to the catalytic production of the very reactive and dangerous hydroxyl radical, which is exceptionally damaging, and a major cause of chronic inflammation.

Review: We review the considerable and wide-ranging evidence for the involvement of this combination of (su)peroxide and poorly liganded iron in a large number of physiological and indeed pathological processes and inflammatory disorders, especially those involving the progressive degradation of cellular and organismal performance. These diseases share a great many similarities and thus might be considered to have a common cause (i.e. iron-catalysed free radical and especially hydroxyl radical generation).

The studies reviewed include those focused on a series of cardiovascular, metabolic and neurological diseases, where iron can be found at the sites of plaques and lesions, as well as studies showing the significance of iron to aging and longevity. The effective chelation of iron by natural or synthetic ligands is thus of major physiological (and potentially therapeutic) importance. As systems properties, we need to recognise that physiological observables have multiple molecular causes, and studying them in isolation leads to inconsistent patterns of apparent causality when it is the simultaneous combination of multiple factors that is responsible.

This explains, for instance, the decidedly mixed effects of antioxidants that have been observed, since in some circumstances (especially the presence of poorly liganded iron) molecules that are nominally antioxidants can actually act as pro-oxidants. The reduction of redox stress thus requires suitable levels of both antioxidants and effective iron chelators. Some polyphenolic antioxidants may serve both roles.

Understanding the exact speciation and liganding of iron in all its states is thus crucial to separating its various pro- and anti-inflammatory activities. Redox stress, innate immunity and pro- (and some anti-)inflammatory cytokines are linked in particular via signalling pathways involving NF-kappaB and p38, with the oxidative roles of iron here seemingly involved upstream of the IkappaB kinase (IKK) reaction. In a number of cases it is possible to identify mechanisms by which ROSs and poorly

Conclusion: Overall we argue, by synthesizing a widely dispersed literature, that the role of poorly liganded iron has been rather under-appreciated in the past, and that in combination with peroxide and superoxide its activity underpins the behavior of a great many physiological processes that degrade over time.

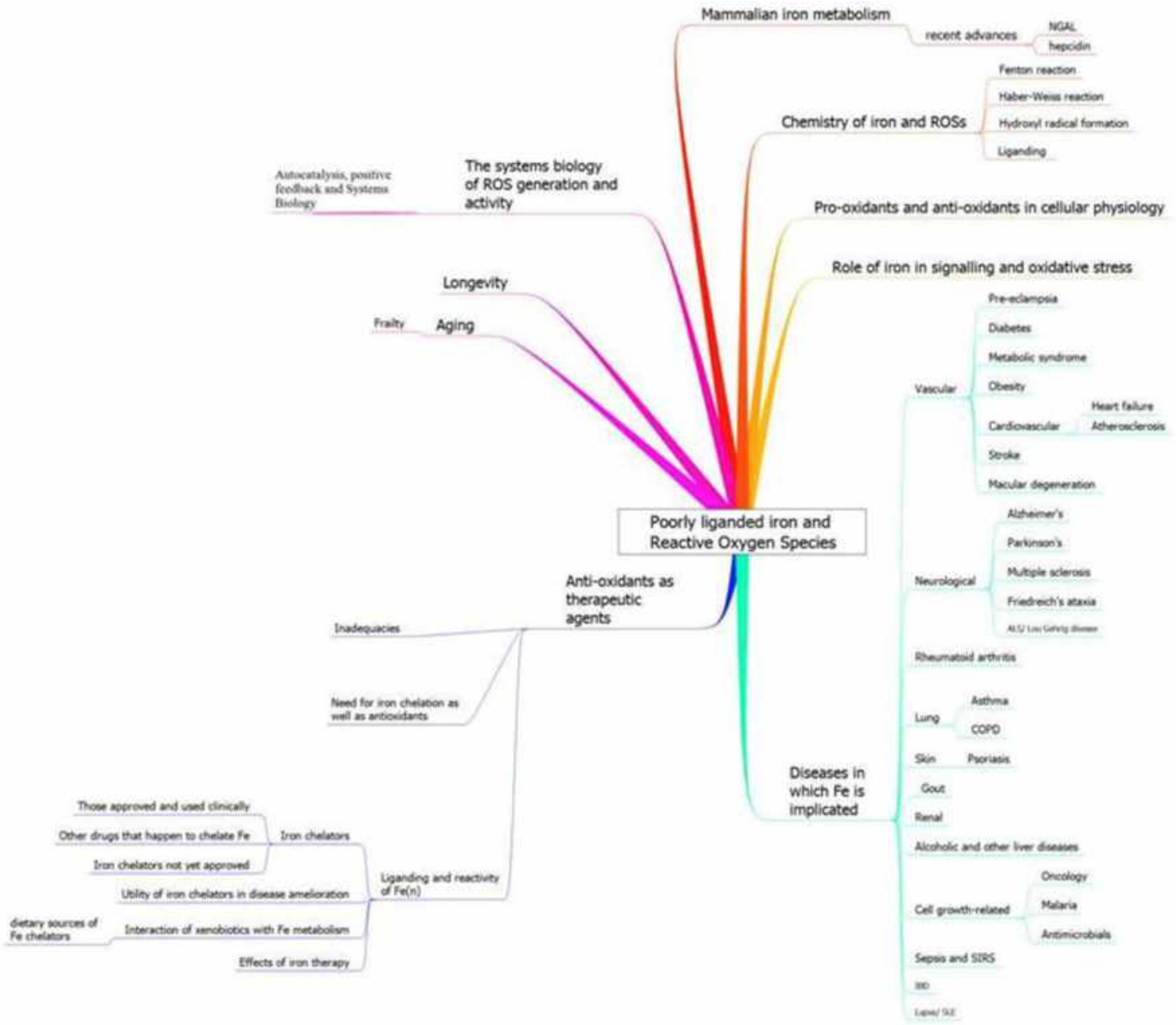
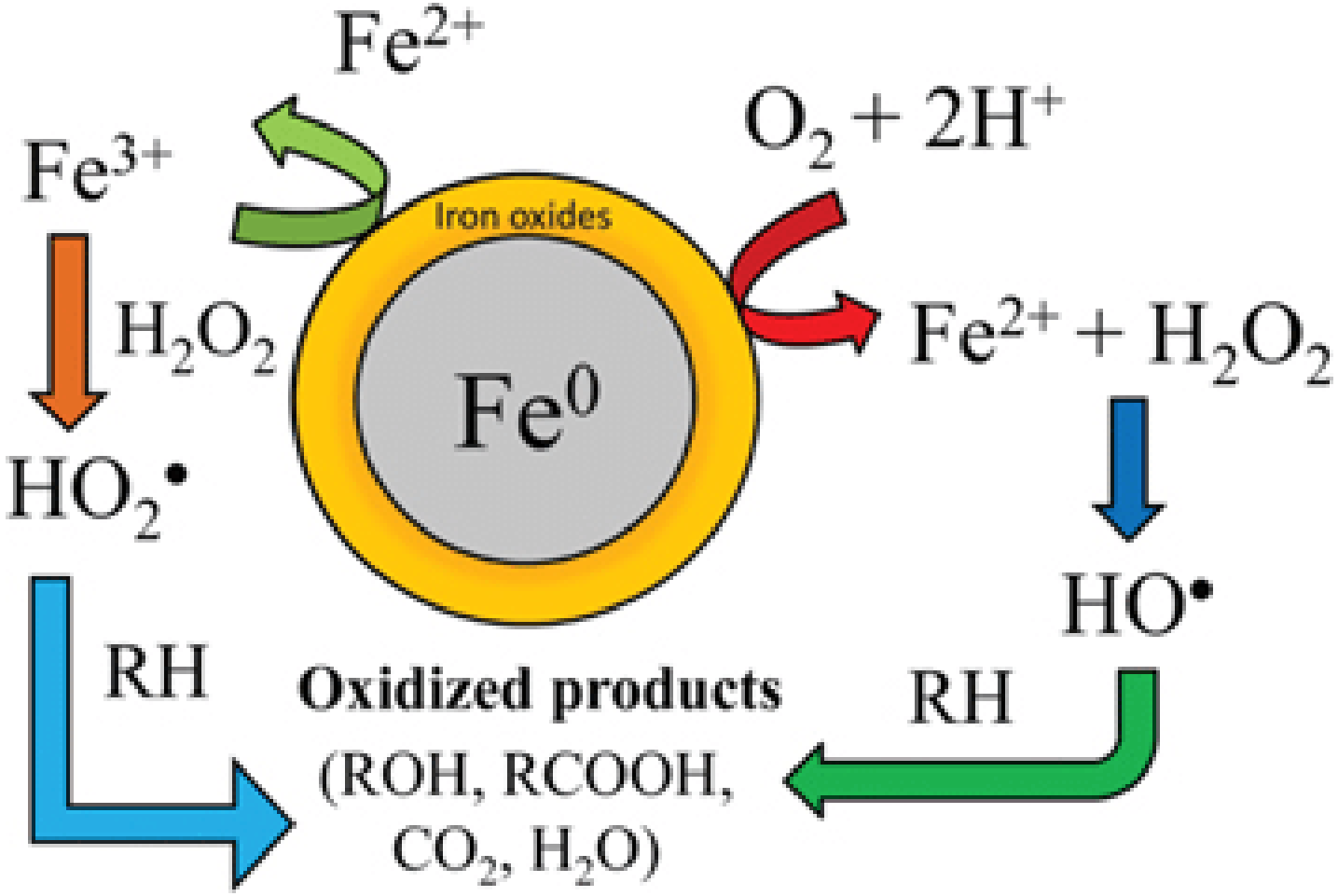


Figure 1
An overview of this article, set out in the form of a 'mind map' [64].

Fenton Reaction



Insights into antiamyloidogenic properties of the green tea extract (−)-epigallocatechin-3-gallate toward metal-associated amyloid-β species

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[a]Department of Chemistry, [b]Biophysics, and [c]Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109

Edited by Harry B. Gray, California Institute of Technology, Pasadena, CA, and approved January 25, 2013 (received for review November 22, 2012)

Despite the significance of Alzheimer's disease, the link between metal-associated amyloid-β (metal–Aβ) and disease etiology remains unclear. To elucidate this relationship, chemical tools capable of specifically targeting and modulating metal–Aβ species are necessary, along with a fundamental understanding of their mechanism at the molecular level. Herein, we investigated and compared the interactions and reactivities of the green tea extract, (−)-epigallocatechin-3-gallate [(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate; EGCG], with metal [Cu(II) and Zn(II)]–Aβ and metal-free Aβ species. We found that EGCG interacted with metal–Aβ species and formed small, unstructured Aβ aggregates more noticeably than in metal-free conditions in vitro. In addition, upon incubation with EGCG, the toxicity presented by metal-free Aβ and metal–Aβ was mitigated in living cells. To understand this reactivity at the molecular level, structural insights were obtained by ion mobility-mass spectrometry (IM-MS), 2D NMR spectroscopy, and computational methods. These studies indicated that (*i*) EGCG was bound to Aβ monomers and dimers, generating more compact peptide conformations than those from EGCG-untreated Aβ species; and (*ii*) ternary EGCG–metal–Aβ complexes were produced. Thus, we demonstrate the distinct antiamyloidogenic reactivity of EGCG toward metal-Aβ species with a structure-based mechanism.

amyloid-β peptide | metal ions | natural products | amyloidogenesis

The brain of individuals with Alzheimer's disease (AD) has protein aggregates composed of misfolded amyloid-β (Aβ) peptides (1–4). The Aβ peptides are produced endogenously through enzymatic cleavage of amyloid precursor protein. Aβ monomers can misfold and oligomerize into various intermediates before the formation and elongation of fibrils that exhibit a characteristic cross-β-sheet structure (1–4). The accumulation of aggregated Aβ species has been a key feature of the amyloid cascade hypothesis, which cites that these aggregates are possible causative agents in AD. In addition, transition metals, such as Cu and Zn, whose misregulation leads to aberrant neuronal function, have a suggested link to AD pathology (1, 3–8). In vitro and in vivo studies have provided evidence for the direct interactions of metal ions with Aβ and their presence within Aβ plaques, indicating the formation of metal-associated Aβ (metal–Aβ) species. These metal–Aβ species have been implicated in processes that could lead to neurotoxicity (e.g., metal-induced Aβ aggregation and metal–Aβ-mediated reactive oxygen species generation) (1, 3–8). The involvement of metal–Aβ species in AD pathogenesis, however, has not been clearly elucidated. To advance our understanding of the potential neurotoxicity of metal–Aβ species, efforts to develop chemical tools capable of interacting directly with metal–Aβ species and modulating their reactivity in vitro and in biological systems are under way (1, 8–18). In particular, novel bifunctional compounds that contain elements for metal chelation and Aβ interaction have recently been prepared or identified via rational structure-based design strategies or systematic selection of natural products.

Naturally occurring flavonoids have been shown to interact with amyloidogenic peptides and arrest or redirect aggregation pathways (19–26). These studies have mainly been conducted under metal-free conditions. For example, the green tea extract, (−)-epigallocatechin-3-gallate [(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate; EGCG; Fig. 1*A*], is known as an antioxidative and anti-inflammatory agent for numerous human diseases (27) and has exhibited antiamyloidogenic reactivity with various disease-related peptides (e.g., Aβ, α-synuclein, islet amyloid polypeptide, semen-derived enhancer of virus infection) (19–21, 24, 25, 28–31). Nontoxic amorphous species were observed upon incubation of α-synuclein or Aβ with EGCG in the absence of metal ions, presumably through the direct peptide–EGCG interactions that were proposed to alter the peptide assembly from the expected fibrillar structures in favor of an off-pathway intermediate (20, 21). EGCG has also been shown to restructure preformed metal-free Aβ aggregates into unstructured, stable, and nontoxic conformations (21). These observations suggest a broad ability for EGCG to disrupt early-stage and late-stage aggregation processes. Although EGCG is also able to chelate metal ions (32, 33), its influence on metal-bound Aβ structure and reactivity has not been fully elucidated (34).

Here, we present the ability of EGCG to modulate metal [Cu(II) or Zn(II)]-induced Aβ aggregation to produce small, unstructured peptide aggregates to a different extent than metal-free Aβ aggregation, which may translate to reduced metal–Aβ toxicity in living cells. To rationalize the antiamyloidogenic reactivity of EGCG at the molecular level, Aβ interaction properties in the absence and presence of metal ions were investigated by ion mobility-mass spectrometry (IM-MS) (35, 36) and 2D NMR spectroscopy. Our IM-MS and 2D NMR results were also supported by molecular dynamics (MD) simulations to create a comprehensive molecular-level mechanism of EGCG action and reactivity. The interactions of EGCG with metal-free Aβ monomers and dimers induced structurally compact peptide conformations that likely led to the generation of amorphous Aβ

Author contributions: S.-J.H., A.S.D., J.R.B., A.R., B.T.R., and M.H.L. designed research; S.-J.H., A.S.D., J.R.B., S.L., S.V., A.K., and J.-S.C. performed research; S.-J.H., A.S.D., J.R.B., S.L., S.V., A.K., A.R., B.T.R., and M.H.L. analyzed data; and S.-J.H., A.S.D., J.R.B., A.R., B.T.R., and M.H.L. wrote the paper.

The authors declare no conflict of interest.

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"Thus, we demonstrate the distinct anti-amyloidogenic reactivity of EGCG toward metal–Aβ species with a structure based mechanism."

PNAS March 5, 2013 vol. 110 no. 10.pg. 3743–3748


EGCG
Alzheimer's Disease
Cu(II)
Zn(II)
Amyloid-β

OPEN ACCESS Freely available online

PLoS one

Curcumin Enhances Neurogenesis and Cognition in Aged Rats: Implications for Transcriptional Interactions Related to Growth and Synaptic Plasticity

Suzhen Dong[1,2☯], Qingwen Zeng[1☯], E. Siobhan Mitchell[3], Jin Xiu[4], Yale Duan[1☯], Chunxia Li[1☯], Jyoti K. Tiwari[5], Yinghe Hu[1,2], Xiaohua Cao[1*], Zheng Zhao[1*]

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Abstract

Background: Curcumin has been demonstrated to have many neuroprotective properties, including improvement of cognition in humans and neurogenesis in animals, yet the mechanism of such effects remains unclear.

Methodology: We assessed behavioural performance and hippocampal cell proliferation in aged rats after 6- and 12-week curcumin-fortified diets. Curcumin enhanced non-spatial and spatial memory, as well as dentate gyrate cell proliferation as compared to control diet rats. We also investigated underlying mechanistic pathways that might link curcumin treatment to increased cognition and neurogenesis via exon array analysis of cortical and hippocampal mRNA transcription. The results revealed a transcriptional network interaction of genes involved in neurotransmission, neuronal development, signal transduction, and metabolism in response to the curcumin treatment.

Conclusions: The results suggest a neurogenesis- and cognition-enhancing potential of prolonged curcumin treatment in aged rats, which may be due to its diverse effects on genes related to growth and plasticity.

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Competing Interests: ESM, JX and JKT are employees of Unilever R&D, the research arm of a food company which does sell products with very small amounts of curcumin. There are no patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

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☯ These authors contributed equally to this work.

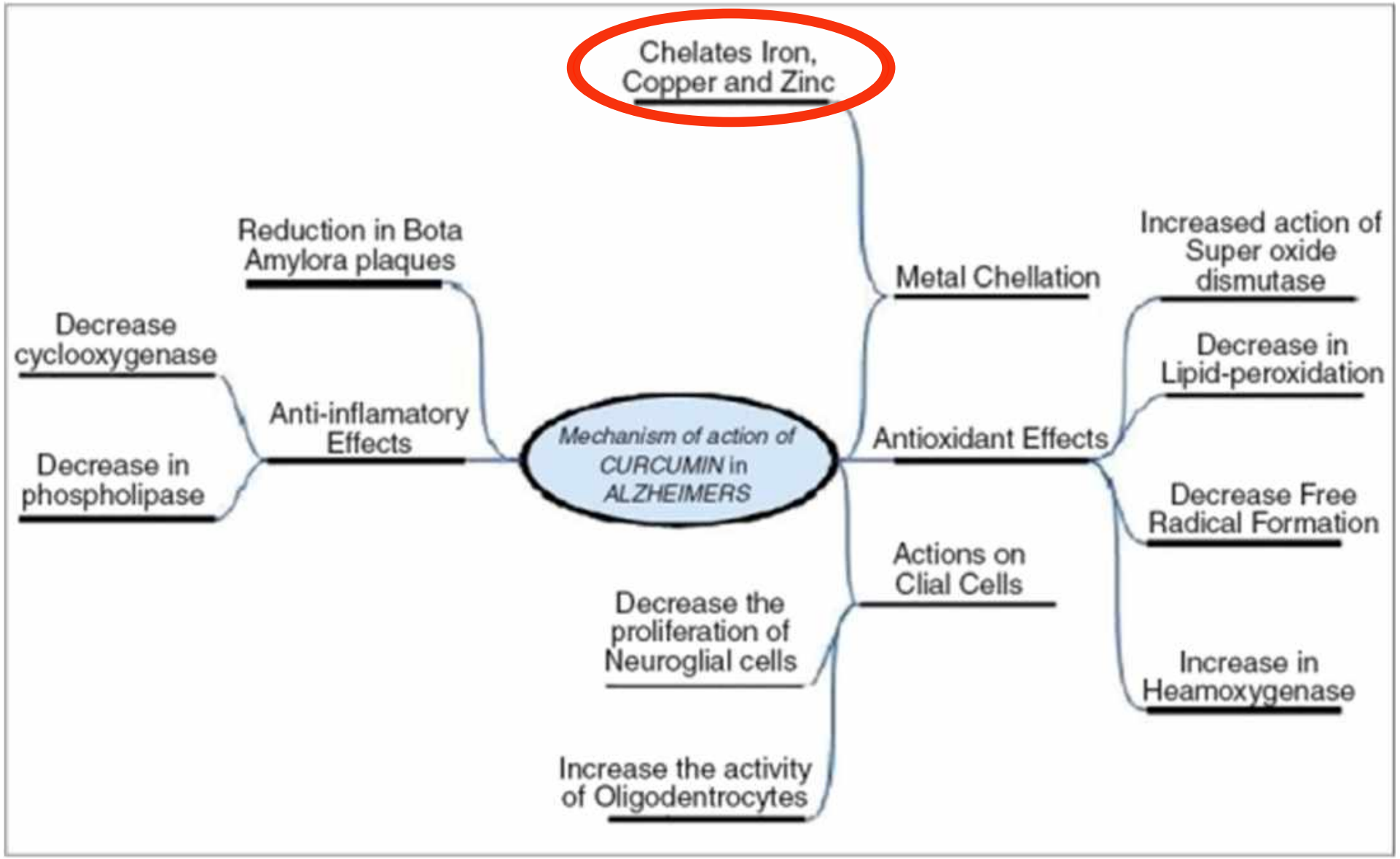
Introduction

The polyphenol compound curcumin is the main component of turmeric curcuminoids derived from tumeric spice, which exhibits many therapeutic properties. Numerous studies have shown that curcumin possesses not only anti-inflammation, anti-oxidative stress, and tumor reduction properties [1], but also neuroprotection against a wide spectrum of neurodegenerative conditions in animal models [2]. Due to its therapeutic potential, curcumin is currently undergoing human clinical trials for treating inflammatory-linked diseases and several types of cancer [1].

In recent years the focus has been shifted to neuroprotective effects of curcumin on cognition. Epidemiologic data has shown that regular curcumin intake may be related to better cognitive function in healthy elderly [3], while in rat models curcumin appears to reverse various forms of cognitive impairment [4,5,6,7,8,9]. For example, chronic administration of curcumin can ameliorate age-related spatial memory deficits [10]. These effects may be due to curcumin's activity on oxidative stress [4,5,7], BDNF and ERK/P38 kinase signalling pathways [6], degradation of PKCδ [10] or inhibition of histone acetyltransferase [11], as well as several other activities. Curcumin also may protect against Alzheimer's disease (AD) pathology. Both *in vitro* and *in vivo* studies have shown that curcumin prevents amyloid-beta build-up, one of pathological hallmarks of AD [12,13,14]. Despite the above observations, understanding of curcumin's diverse neuroprotective activities is still limited, especially how curcumin influences neuronal proliferation.

Adult neurogenesis has been suggested to be an important event for cognitive function [15,16]. Two recent publications revealed that curcumin enhanced neurogenesis in adult rodents. Xu et al found that oral administration of curcumin increased the proliferation of hippocampal progenitor cells in chronically stressed rats [17], while Kim et al. showed that curcumin could stimulate proliferations of hippocampal neural progenitor cells both at embryonic stage and adult in mice [18]. These studies, however, used relatively young animals (only several weeks old) that have relatively high rates of neurogenesis. Furthermore, neither study investigated behavior, which could have shed light on the functional implications of curcumin-induced neurogenesis. Thus, it is still not clear if neurogenesis is responsible for treatment effects on learning and memory.

Conclusions: "The results suggest a neurogenesis- and cognition-enhancing potential of prolonged curcumin treatment in aged rats, which may be due to its diverse effects on genes related to growth and plasticity."


Chelates Iron, Copper and Zinc
Reduction in Bota Amylora plaques
Decrease cyclooxygenase
Anti-inflamatory Effects
Decrease in phospholipase
Mechanism of action of CURCUMIN in ALZHEIMERS
Metal Chellation
Increased action of Super oxide dismutase
Decrease in Lipid-peroxidation
Antioxidant Effects
Decrease Free Radical Formation
Increase in Heamoxygenase
Actions on Clial Cells
Decrease the proliferation of Neuroglial cells
Increase the activity of Oligodentrocytes

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Review article

Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health?

Megan W. Bourassa[a,b], Ishraq Alim[a,b], Scott J. Bultman[c], Rajiv R. Ratan[a,b,*]

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HIGHLIGHTS

- Interest in how diet influences brain function via the gut microbiome is growing.
- Butyrate can protect the brain and enhance plasticity in neurological disease models.
- Gut microbiota produce butyrate by fermenting carbohydrates in a high fiber diet.
- Hypothesis: A high fiber diet can elevate butyrate to prevent/treat brain disorders.

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ABSTRACT

As interest in the gut microbiome has grown in recent years, attention has turned to the impact of our diet on our brain. The benefits of a high fiber diet in the colon have been well documented in epidemiological studies, but its potential impact on the brain has largely been understudied. Here, we will review evidence that butyrate, a short-chain fatty acid (SCFA) produced by bacterial fermentation of fiber in the colon, can improve brain health. Butyrate has been extensively studied as a histone deacetylase (HDAC) inhibitor but also functions as a ligand for a subset of G protein-coupled receptors and as an energy metabolite. These diverse modes of action make it well suited for solving the wide array of imbalances frequently encountered in neurological disorders. In this review, we will integrate evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of a high fiber diet in the gut can alter gene expression in the brain to prevent neurodegeneration and promote regeneration.

Contents

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"Butyrate has been extensively studied as a histone deacetylase (HDAC) inhibitor but also functions as a ligand for a subset of G protein-coupled receptors and as an energy metabolite....**In this review, we will integrate evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of a high fiber diet in the gut can alter gene expression in the brain to prevent neurodegeneration and promote regeneration. "**

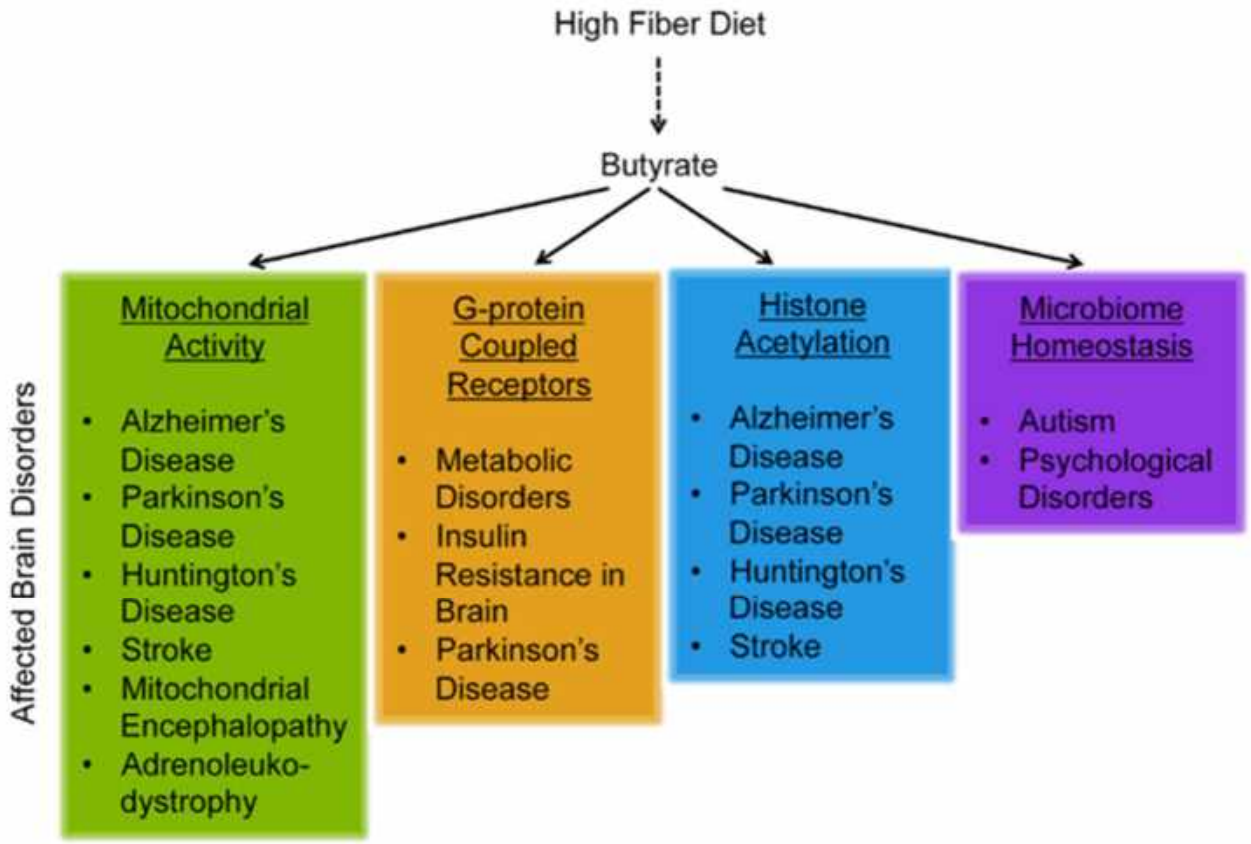

Fig. 2. The proposed mechanisms for the neuroprotective effects of butyrate and the diseases which may benefit from butyrate treatment or a high fiber diet.

frontiers in Immunology

ORIGINAL RESEARCH
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Butyrate and Dietary Soluble Fiber Improve Neuroinflammation Associated With Aging in Mice

*Stephanie M. Matt[1,2], Jacob M. Allen[3], Marcus A. Lawson[1], Lucy J. Mailing[4], Jeffrey A. Woods[3,4] and Rodney W. Johnson[1,2,4,5]**

[1]Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [2]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [3]Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [4]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [5]Integrative Immunology and Behavior Program, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Aging results in chronic systemic inflammation that can alter neuroinflammation of the brain. Specifically, microglia shift to a pro-inflammatory phenotype predisposing them to hyperactivation upon stimulation by peripheral immune signals. It is proposed that certain nutrients can delay brain aging by preventing or reversing microglial hyperactivation. Butyrate, a short-chain fatty acid (SCFA) produced primarily by bacterial fermentation of fiber in the colon, has been extensively studied pharmacologically as a histone deacetylase inhibitor and serves as an attractive therapeutic candidate, as butyrate has also been shown to be anti-inflammatory and improve memory in animal models. In this study, we demonstrate that butyrate can attenuate pro-inflammatory cytokine expression in microglia in aged mice. It is still not fully understood, however, if an increase in butyrate-producing bacteria in the gut as a consequence of a diet high in soluble fiber could affect microglial activation during aging. Adult and aged mice were fed either a 1% cellulose (low fiber) or 5% inulin (high fiber) diet for 4 weeks. Findings indicate that mice fed inulin had an altered gut microbiome and increased butyrate, acetate, and total SCFA production. In addition, histological scoring of the distal colon demonstrated that aged animals on the low fiber diet had increased inflammatory infiltrate that was significantly reduced in animals consuming the high fiber diet. Furthermore, gene expression of inflammatory markers, epigenetic regulators, and the microglial sensory apparatus (i.e., the sensome) were altered by both diet and age, with aged animals exhibiting a more anti-inflammatory microglial profile on the high fiber diet. Taken together, high fiber supplementation in aging is a non-invasive strategy to increase butyrate levels, and these data suggest that an increase in butyrate through added soluble fiber such as inulin could counterbalance the age-related microbiota dysbiosis, potentially leading to neurological benefits.

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Keywords: microglia, butyrate, aging, neuroinflammation, epigenetics, microbiome, fiber diet

INTRODUCTION

During healthy aging, there is a disruption in the communication and balance between the brain and immune system. Microglia shift to a pro-inflammatory phenotype that makes them hypersensitive to signals from the peripheral immune system (1, 2). The precise mechanisms during aging that are responsible for this detrimental transition is not known, but overproduction of the

"Taken together, high fiber supplementation in aging is a non-invasive strategy to increase butyrate levels, and these data suggest that an increase in butyrate through added soluble fiber such as inulin could counterbalance the age-related microbiota dysbiosis, potentially leading to neurological benefits."

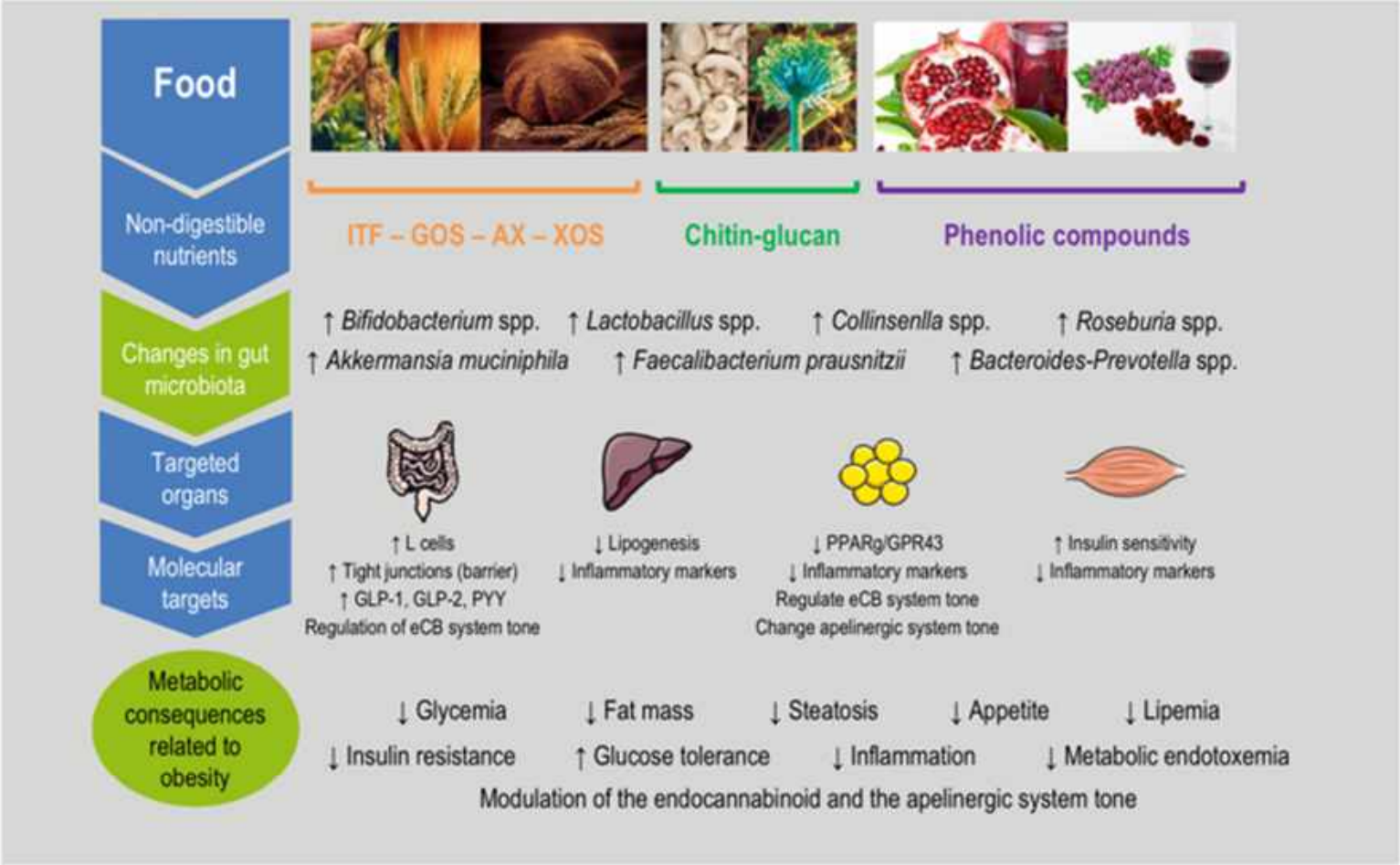
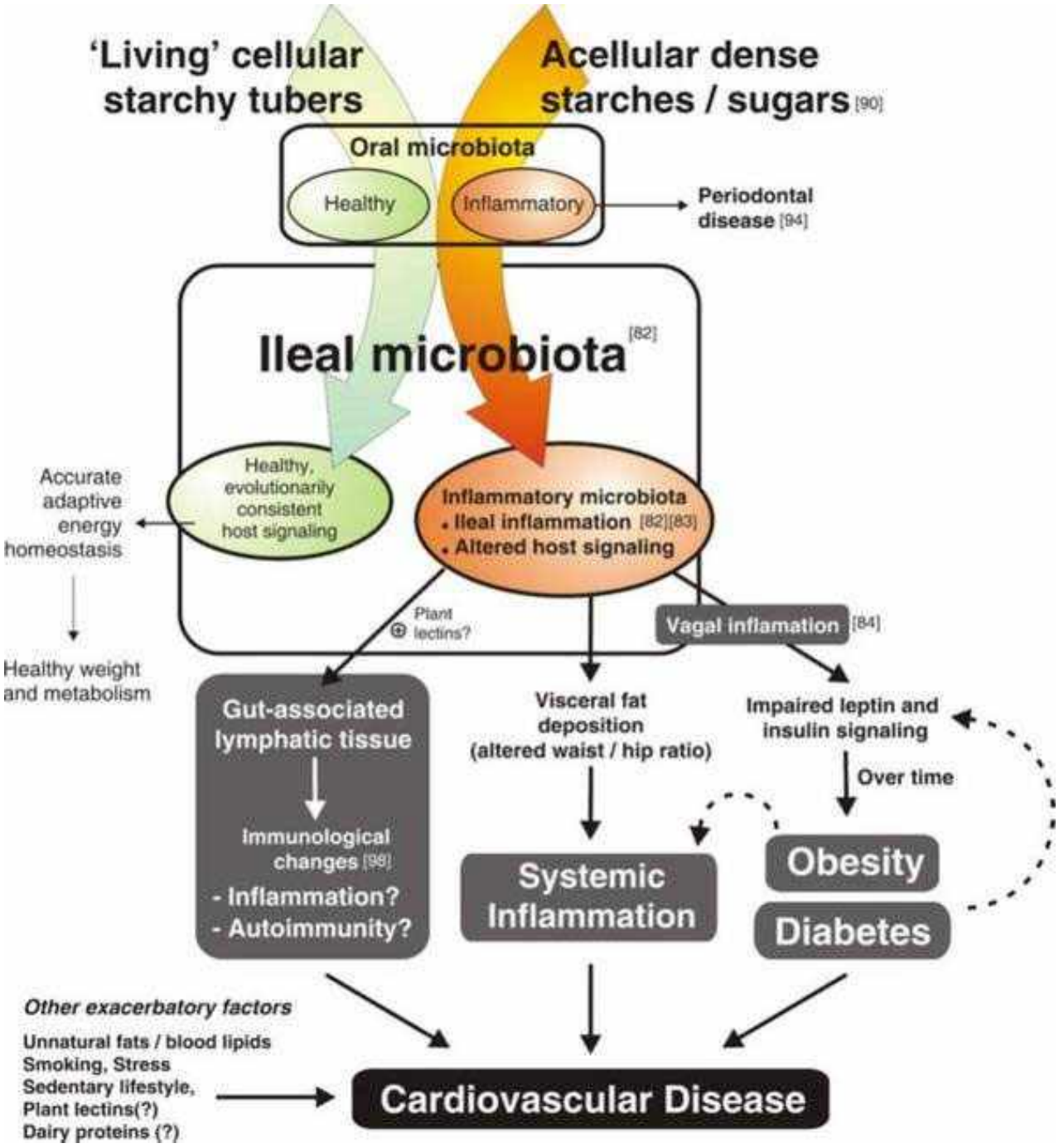


Figure 1. Effect of non-digestible nutrients with prebiotic properties on host pathophysiology related to obesity. In intervention studies in animals and humans, non-digestible nutrients with prebiotic properties, such as inulin-type fructans, galacto-oligosaccharides, arabinoxylan and arabinoxylan oligosaccharides derived from wheat, fungal chitin-glucan and several phenolic compounds present in pomegranate or grapes, have been shown to change the gut microbiota composition by favouring bacteria that confer health benefits to the host. Prebiotics reinforce the gut barrier and promote gut hormones that control appetite, glucose homeostasis and systemic inflammation. The prebiotic approach also counteracts hepatic steatosis (lipogenesis), hepatic insulin resistance, and adiposity by modifying gene expression at the tissue level. LPS = lipopolysaccharide, APJ = apelin receptor, eCB = endocannabinoid.

Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics

Beneficial Microbes, March 2014; 5(1): 3-17


'Living' cellular starchy tubers
Acellular dense starches / sugars [90]
Oral microbiota
Healthy
Inflammatory
Periodontal disease [94]
Ileal microbiota [82]
Healthy, evolutionarily consistent host signaling
Inflammatory microbiota
• Ileal inflammation [82][83]
• Altered host signaling
Accurate adaptive energy homeostasis
Healthy weight and metabolism
Plant lectins?
Vagal inflamation [84]
Gut-associated lymphatic tissue
Immunological changes [98]
- Inflammation?
- Autoimmunity?
Visceral fat deposition (altered waist / hip ratio)
Impaired leptin and insulin signaling
Over time
Systemic Inflammation
Obesity
Diabetes
Other exacerbatory factors
Unnatural fats / blood lipids
Smoking, Stress
Sedentary lifestyle,
Plant lectins(?)
Dairy proteins (?)
Cardiovascular Disease

Review Paper

N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action

Olivia Dean, BSc, PhD; Frank Giorlando, MBBS, BMedSc;
Michael Berk, MBBCh, MMed(Psych), PhD

Dean, Berk — Mental Health Research Institute, Parkville; Dean, Giorlando, Berk — Department of Clinical and Biomedical Sciences, Barwon Health, University of Melbourne, Geelong; Berk — Youth Health Orygen Research Centre, Parkville, and the School of Medicine, Faculty of Health, Medicine, Nursing and Behavioural Sciences, Deakin University, Geelong, Victoria, Australia

There is an expanding field of research investigating the benefits of alternatives to current pharmacological therapies in psychiatry. *N*-acetylcysteine (NAC) is emerging as a useful agent in the treatment of psychiatric disorders. Like many therapies, the clinical origins of NAC are far removed from its current use in psychiatry. Whereas the mechanisms of NAC are only beginning to be understood, it is likely that NAC is exerting benefits beyond being a precursor to the antioxidant, glutathione, modulating glutamatergic, neurotropic and inflammatory pathways. This review outlines the current literature regarding the use of NAC in disorders including addiction, compulsive and grooming disorders, schizophrenia and bipolar disorder. *N*-acetylcysteine has shown promising results in populations with these disorders, including those in whom treatment efficacy has previously been limited. The therapeutic potential of this acetylated amino acid is beginning to emerge in the field of psychiatric research.

Historical use of *N*-acetylcysteine

N-acetylcysteine (NAC) has been used as an antioxidant precursor to glutathione (γ-glutamylcysteinylglycine; GSH) in the treatment of paracetamol overdose for more than 30 years.[1] As more is understood about the actions of NAC, the clinical applications have also broadened. *N*-acetylcysteine is now widely used as a mucolytic and in the treatment of HIV, and it has reported efficacy in chronic obstructive pulmonary disease and contrast-induced nephropathy.[2] Specific to brain disorders, NAC has been trialled with some efficacy in patients with Alzheimer disease.[3] The present review will explore the role of NAC in the treatment of psychiatric conditions and the possible mechanisms of benefit for these disorders.

Role in oxidative homeostasis

The use of NAC in restoring GSH levels is well established (Fig. 1). Glutathione is the primary endogenous antioxidant. Glutathione neutralizes reactive oxygen and nitrogen species from the cell through both direct and indirect scavenging. As the most abundant and ubiquitous antioxidant, it is responsible for maintaining the oxidative balance in the cell. This occurs through both direct removal of reactive species through the formation and breakdown of adducts and is also catalyzed by glutathione peroxidase (GPx) in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reaction. The resulting oxidized glutathione is then reduced by glutathione reductase to begin the cycle again.[4] Glial cells contain much higher levels of GSH than neuronal cells and support neuronal GSH production. Astrocytes release GSH into the extracellular space and γ-glutamyltranspeptidase breaks down GSH to a cysteine–glycine dipeptide and glutamate. The dipeptide is hydrolyzed to glycine and cysteine, and all 3 amino acids are then available for neuronal GSH synthesis. Neuronal GSH production is believed to be primarily mediated by astrocytic GSH release, and astrocytic GSH production is rate-limited by cysteine and the enzyme glutamate-cysteine ligase.[4,5]

In addition to providing cysteine for GSH production, NAC has been shown to scavenge oxidants directly, particularly the reduction of the hydroxyl radical, -OH and hypochlorous acid.[6]

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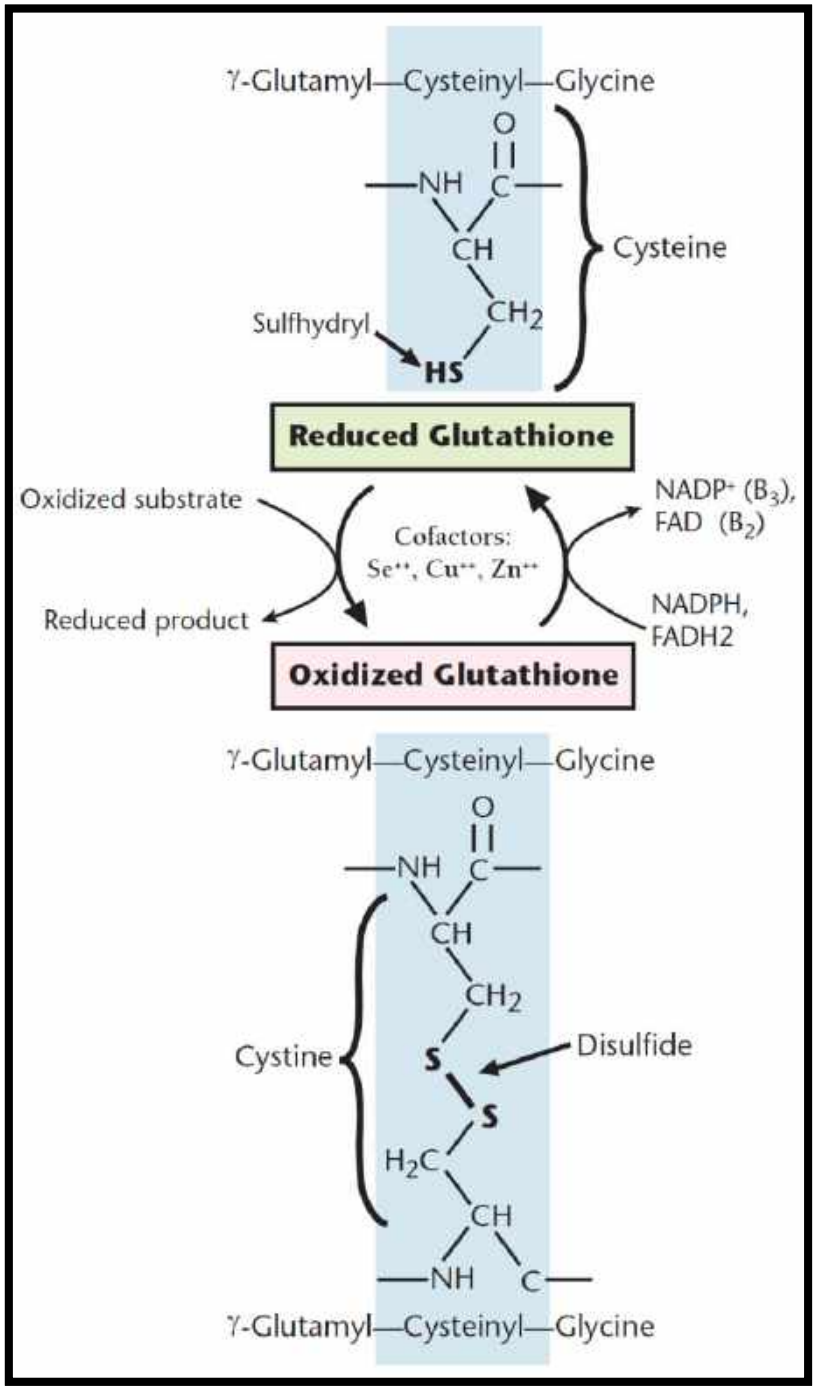
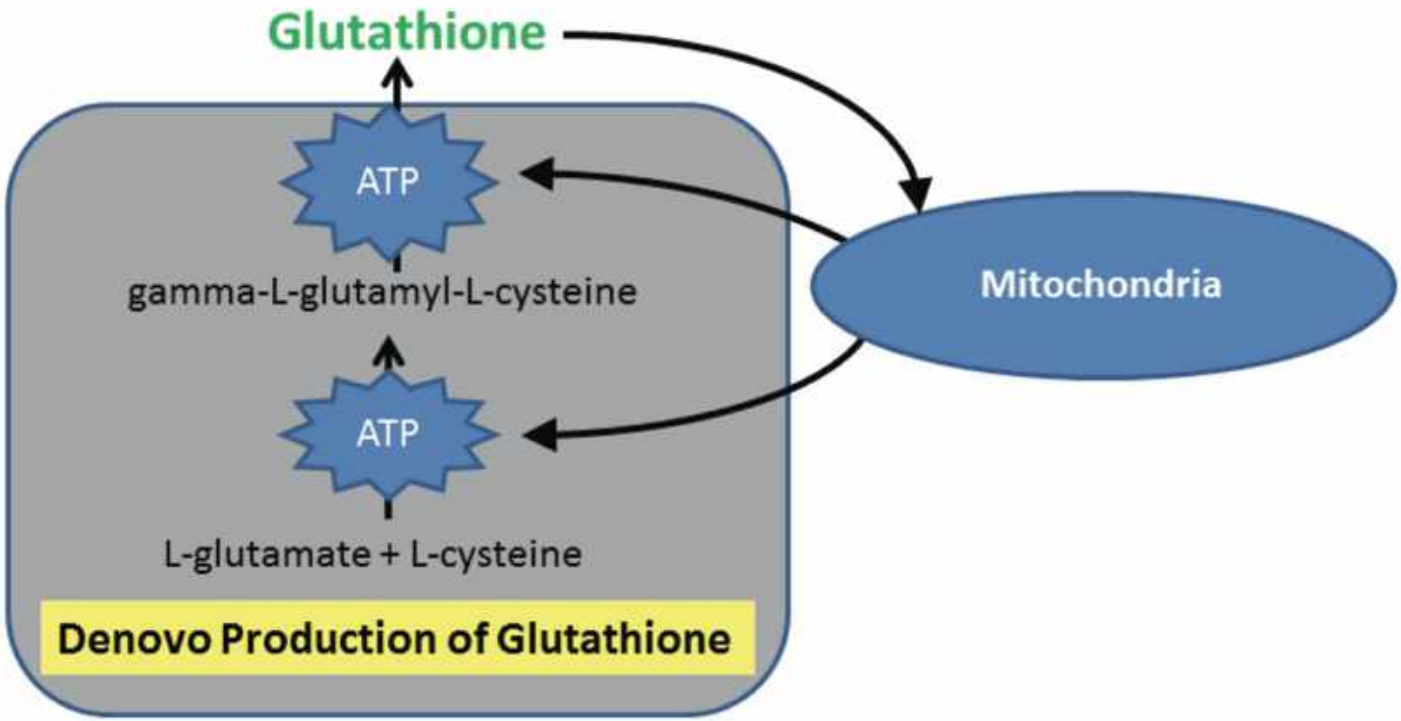
J Psychiatry Neurosci 2011;36(2):78-86.

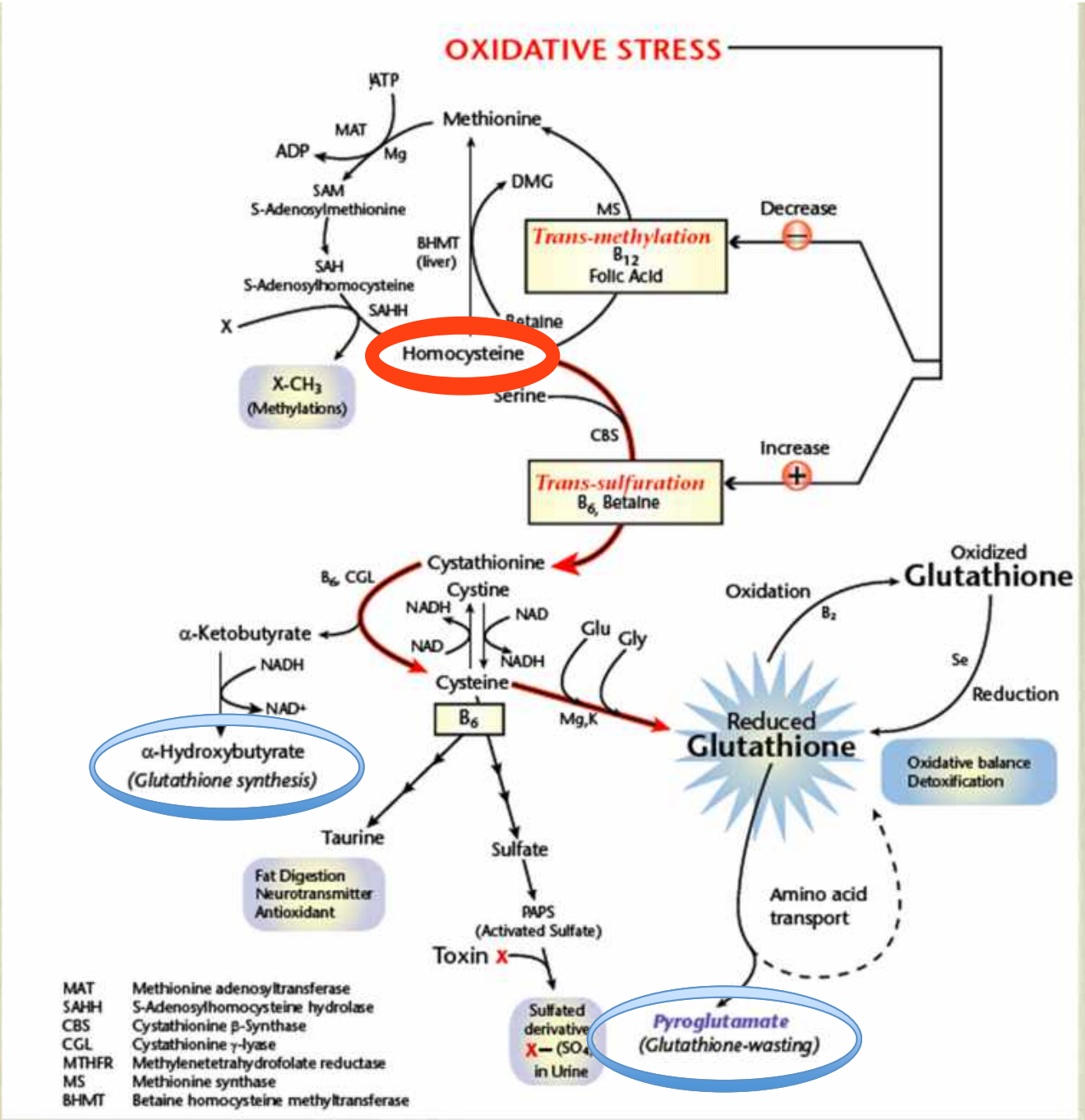
Whereas the mechanisms of NAC are only beginning to be understood, it is likely that NAC is exerting benefits beyond being a precursor to the antioxidant, glutathione, modulating glutamatergic, neurotropic and inflammatory pathways. This review outlines the current literature regarding the use of NAC in disorders including addiction, compulsive and grooming disorders, schizophrenia and bipolar disorder

Reduced and Oxidized Glutathione

The ratio of GSSH/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell, that is, **it is critical that the cell maintains high levels of the reduced glutathione and a low level of the oxidized Glutathione disulfide**




Glutathione
ATP
gamma-L-glutamyl-L-cysteine
ATP
L-glutamate + L-cysteine
Denovo Production of Glutathione
Mitochondria

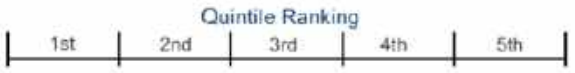


Laboratory Evaluations in Functional and Integrative Medicine textbook pg. 223

Organix™ Comprehensive - Urine

Methodology: LC/Tandem Mass Spectroscopy, Colorimetric

Ranges: Ages 13 and over.



95% Reference Interval

Cell Regulation Markers

Neurotransmitter Metabolism Markers

(Tyrosine, Tryptophan, B6, antioxidants)

				95% Reference Interval
22	Vanilmandelate	2.3		1.3 - 4.9
23	Homovanillate	2.7		1.6 - 10.9
24	5-Hydroxyindoleacetate	2.8		1.6 - 9.8
25	Kynurenate	1.6		<= 2.7
26	Quinolinate	1.9		<= 5.8
27	Picolinate	3.6		2.8 - 13.5

Oxidative Damage and Antioxidant Markers

(Vitamin C and other antioxidants)

				95% Reference Interval
28	p-Hydroxyphenyllactate	0.09		<= 1.45
29	8-Hydroxy-2-deoxyguanosine *	2.6		<= 7.6

* Units for 8-Hydroxy-2-deoxyguanosine are ng/mg creatinine.

Toxicants and Detoxification

Detoxification Indicators

(Arg, NAC, Met, Mg and antioxidants)

				95% Reference Interval
30	2-Methylhippurate	0.066		<= 0.192
31	Orotate	0.22		<= 1.01
32	Glucarate	4.0		<= 10.7
33	a-Hydroxybutyrate	0.7	H	<= 0.9
34	Pyroglutamate	62	H	28 - 88
35	Sulfate	1,413		690 - 2,988

Free Radic Biol Med. 1997;22(1-2):359-78.

Neuroprotection by the metabolic antioxidant alpha-lipoic acid.

Packer L, Tritschler HJ, Wessel K.

Author information

Abstract

Reactive oxygen species are thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant alpha-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6, 8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood-brain barrier. alpha-Lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence, protection is afforded to both intracellular and extracellular environments. Both alpha-lipoate and especially dihydrolipoate have been shown to be potent antioxidants, to regenerate through redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutathione levels. Thus, it would seem an ideal substance in the treatment of oxidative brain and neural disorders involving free radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. We propose that the various metabolic antioxidant properties of alpha-lipoate relate to its possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas alpha-lipoic acid can. In vitro, animal, and preliminary human studies indicate that alpha-lipoate may be effective in numerous neurodegenerative disorders.

ereas alpha-lipoic acid can. In vitro, animal, and preliminary human studies

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Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease ☆

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that destroys patient memory and cognition, communication ability with the social environment and the ability to carry out daily activities. Despite extensive research into the pathogenesis of AD, a neuroprotective treatment – particularly for the early stages of disease – remains unavailable for clinical use. In this review, we advance the suggestion that lipoic acid (LA) may fulfil this therapeutic need. A naturally occurring cofactor for the mitochondrial enzymes pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, LA has been shown to have a variety of properties which can interfere with the pathogenesis or progression of AD. For example, LA increases acetylcholine (ACh) production by activation of choline acetyltransferase and increases glucose uptake, thus supplying more acetyl-CoA for the production of ACh. LA chelates redox-active transition metals, thus inhibiting the formation of hydroxyl radicals and also scavenges reactive oxygen species (ROS), thereby increasing the levels of reduced glutathione. In addition, LA down-regulates the expression of redox-sensitive pro-inflammatory proteins including TNF and inducible nitric oxide synthase. Furthermore, LA can scavenge lipid peroxidation products such as hydroxynonenal and acrolein. In human plasma, LA exists in an equilibrium of free and plasma protein bound form. Up to 150 μM, it is bound completely, most likely binding to high affinity fatty acid sites on human serum albumin, suggesting that one large dose rather than continuous low doses (as provided by "slow release" LA) will be beneficial for delivery of LA to the brain. Evidence for a clinical benefit for LA in dementia is yet limited. There are only two published studies, in which 600 mg LA was given daily to 43 patients with AD (receiving a standard treatment with choline-esterase inhibitors) in an open-label study over an observation period of up to 48 months. Whereas the improvement in patients with moderate dementia was not significant, the disease progressed extremely slowly (change in ADAScog: 1.2 points•year, MMSE: −0.6 points•year) in patients with mild dementia (ADAScog < 15). Data from cell culture and animal models suggest that LA could be combined with nutraceuticals such as curcumin, (−)-epigallocatechin gallate (from green tea) and docosahexaenoic acid (from fish oil) to synergistically decrease oxidative stress, inflammation, Aβ levels and Aβ plaque load and thus provide a combined benefit in the treatment of AD.

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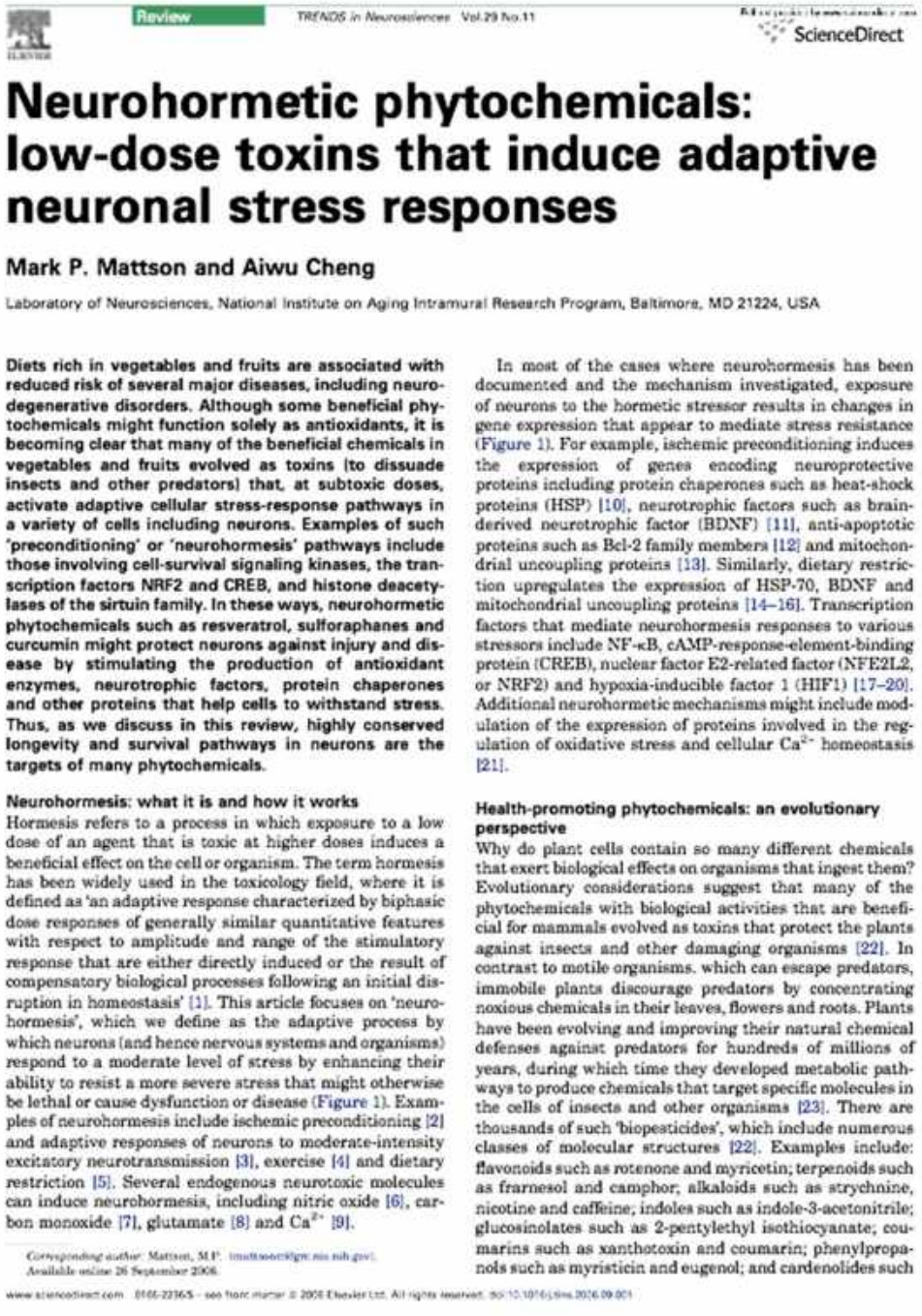

Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses

Mark P. Mattson and Aiwu Cheng

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Diets rich in vegetables and fruits are associated with reduced risk of several major diseases, including neurodegenerative disorders. Although some beneficial phytochemicals might function solely as antioxidants, it is becoming clear that many of the beneficial chemicals in vegetables and fruits evolved as toxins (to dissuade insects and other predators) that, at subtoxic doses, activate adaptive cellular stress-response pathways in a variety of cells including neurons. Examples of such 'preconditioning' or 'neurohormesis' pathways include those involving cell-survival signaling kinases, the transcription factors NRF2 and CREB, and histone deacetylases of the sirtuin family. In these ways, neurohormetic phytochemicals such as resveratrol, sulforaphanes and curcumin might protect neurons against injury and disease by stimulating the production of antioxidant enzymes, neurotrophic factors, protein chaperones and other proteins that help cells to withstand stress. Thus, as we discuss in this review, highly conserved longevity and survival pathways in neurons are the targets of many phytochemicals.

Neurohormesis: what it is and how it works

Hormesis refers to a process in which exposure to a low dose of an agent that is toxic at higher doses induces a beneficial effect on the cell or organism. The term hormesis has been widely used in the toxicology field, where it is defined as 'an adaptive response characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range of the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis' [1]. This article focuses on 'neurohormesis', which we define as the adaptive process by which neurons (and hence nervous systems and organisms) respond to a moderate level of stress by enhancing their ability to resist a more severe stress that might otherwise be lethal or cause dysfunction or disease (Figure 1). Examples of neurohormesis include ischemic preconditioning [2] and adaptive responses of neurons to moderate-intensity excitatory neurotransmission [3], exercise [4] and dietary restriction [5]. Several endogenous neurotoxic molecules can induce neurohormesis, including nitric oxide [6], carbon monoxide [7], glutamate [8] and Ca^{2+} [9].

In most of the cases where neurohormesis has been documented and the mechanism investigated, exposure of neurons to the hormetic stressor results in changes in gene expression that appear to mediate stress resistance (Figure 1). For example, ischemic preconditioning induces the expression of genes encoding neuroprotective proteins including protein chaperones such as heat-shock proteins (HSP) [10], neurotrophic factors such as brain-derived neurotrophic factor (BDNF) [11], anti-apoptotic proteins such as Bcl-2 family members [12] and mitochondrial uncoupling proteins [13]. Similarly, dietary restriction upregulates the expression of HSP-70, BDNF and mitochondrial uncoupling proteins [14–16]. Transcription factors that mediate neurohormesis responses to various stressors include NF-κB, cAMP-response-element-binding protein (CREB), nuclear factor E2-related factor (NFE2L2, or NRF2) and hypoxia-inducible factor 1 (HIF1) [17–20]. Additional neurohormetic mechanisms might include modulation of the expression of proteins involved in the regulation of oxidative stress and cellular Ca^{2+} homeostasis [21].

Health-promoting phytochemicals: an evolutionary perspective

Why do plant cells contain so many different chemicals that exert biological effects on organisms that ingest them? Evolutionary considerations suggest that many of the phytochemicals with biological activities that are beneficial for mammals evolved as toxins that protect the plants against insects and other damaging organisms [22]. In contrast to motile organisms, which can escape predators, immobile plants discourage predators by concentrating noxious chemicals in their leaves, flowers and roots. Plants have been evolving and improving their natural chemical defenses against predators for hundreds of millions of years, during which time they developed metabolic pathways to produce chemicals that target specific molecules in the cells of insects and other organisms [23]. There are thousands of such 'biopesticides', which include numerous classes of molecular structures [22]. Examples include: flavonoids such as rotenone and myricetin; terpenoids such as farnesol and camphor; alkaloids such as strychnine, nicotine and caffeine; indoles such as indole-3-acetonitrile; glucosinolates such as 2-pentylethyl isothiocyanate; coumarins such as xanthotoxin and coumarin; phenylpropanols such as myristicin and eugenol; and cardenolides such

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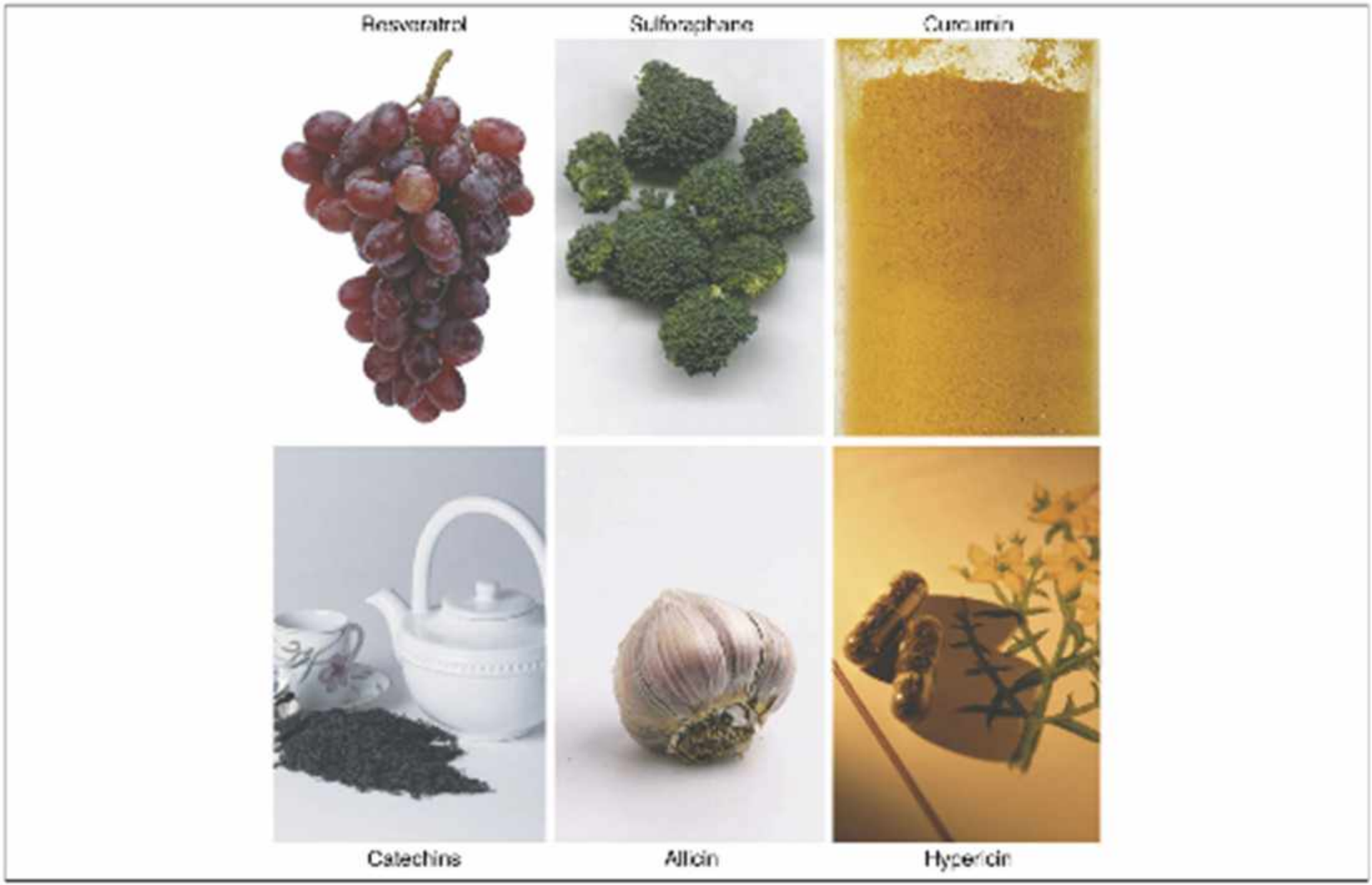
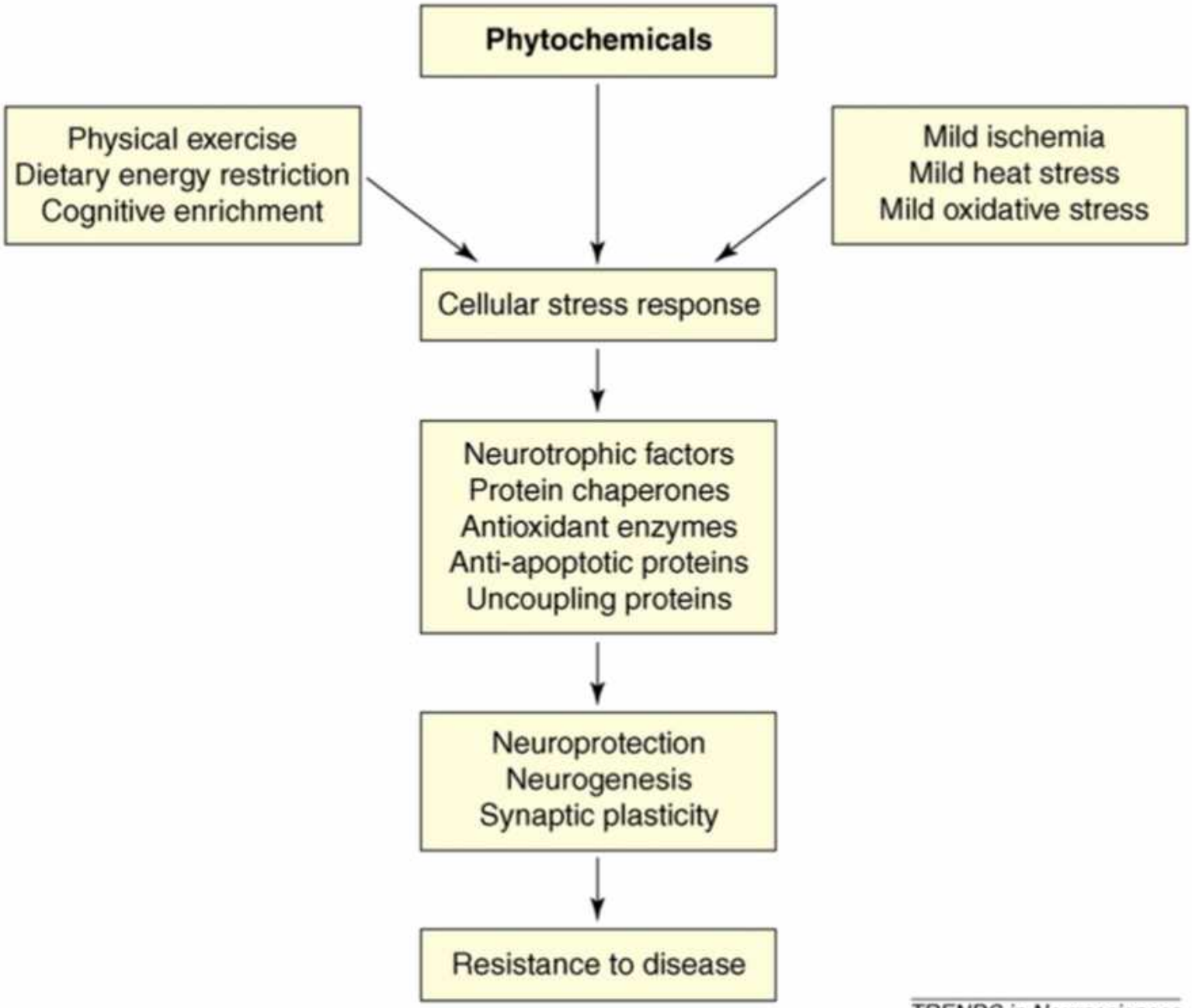


Figure 2. Neurohormetic phytochemicals include compounds from a range of botanical sources and chemical classes. Resveratrol is a polyphenolic compound present in high amounts in red grapes and wine, and in peanuts and soy. Broccoli and other cruciferous vegetables contain high amounts of the isothiocyanate sulforaphanes. Curcumin is the yellow pigment in the roots of turmeric. Green tea contains high amounts of catechins. Garlic is rich in allicin, allium and other organosulfur compounds. St John's wort contains the phenanthroperylene quinone hypericin.

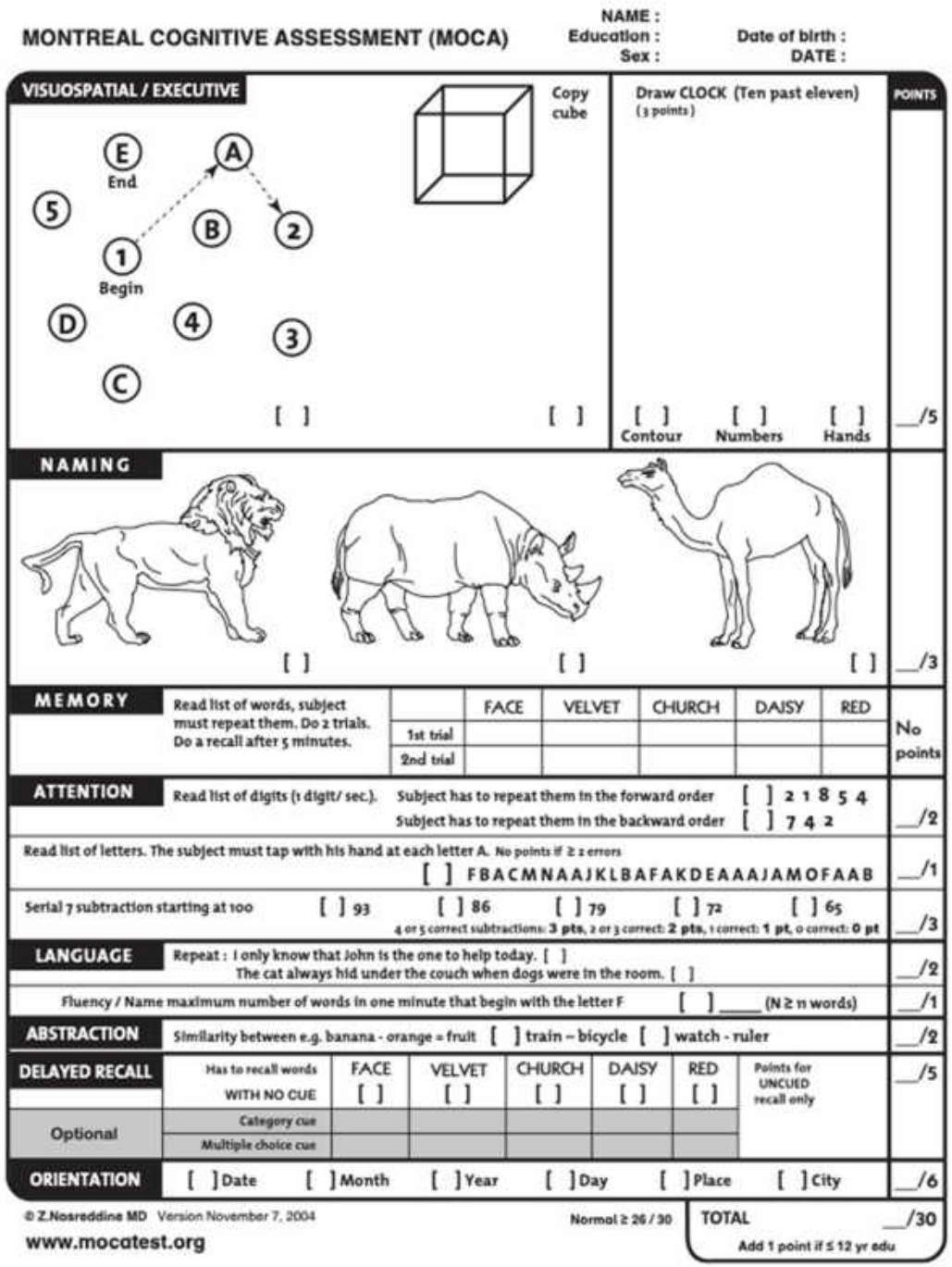

Phytochemicals
Physical exercise
Dietary energy restriction
Cognitive enrichment
Mild ischemia
Mild heat stress
Mild oxidative stress
Cellular stress response
Neurotrophic factors
Protein chaperones
Antioxidant enzymes
Anti-apoptotic proteins
Uncoupling proteins
Neuroprotection
Neurogenesis
Synaptic plasticity
Resistance to disease
TRENDS in Neurosciences

Case Study of 71 yo female with LOAD

71yo presents with a diagnosis of “Alzheimer's disease”, speech impairment and difficulty problem solving. Husband first noted forgetfulness 8 yrs ago. Current symptoms started about a year and a half ago when she began having difficulty organizing and expressing her thoughts. On trip to France was out of her element and was very confused.

All blood tests and brain MRI were normal
No history of head trauma, worked as hospital administrator & had excellent verbal skills.
Eats a mediterranean diet.....no junk food
Longstanding constipation.....does not drink much water.
Eats canned tuna
Used Dristan every night 10 yrs ago and lost her sense of smell.....prone towards constipation
Can go up to 2-3 days without a BM

Retired 9 yrs ago and in retirement drank champagne & wine daily
Took Ativan 9 yrs ago for 2 yrs at night but stopped as she got AM confusion
Began HRT at 55 yo.



MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :
Sex :
Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE

Copy cube

Draw CLOCK (Ten past eleven) (3 points)

POINTS

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ]

[ ] Contour [ ] Numbers [ ] Hands

/5

NAMING

[ ] [ ] [ ]

/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt

/3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words)

/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

/6

© Z.Nasreddine MD Version November 7, 2004

www.mocatest.org

Normal ≥ 26 / 30

TOTAL /30

Add 1 point if ≤ 12 yr edu

MOCA
24 out of 30

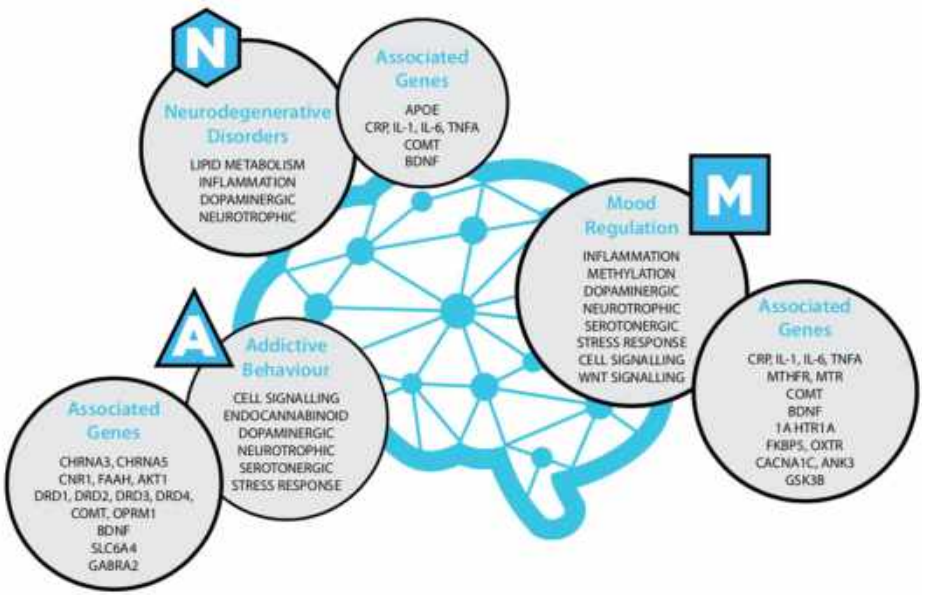
Triggers

Drivers

DNA Mind Genetic Profile

Summary table of results

Biological Area	Gene Name	Genetic Variation	Your Result	Impact N	Impact M	Impact A
Lipid metabolism	APOE	E2/E3/E4	E4/E4	●●●		
Inflammation	CRP	G>A	AA	○	○	
	IL1-A	4845 G>T	TG	●●	●●	
		-889 C>T	TC	●●	●●	
	IL1-B	3954 C>T	CT	●●	●●	
		-511 A>G	GG	●●●	●●●	
	IL1-RN	2108 C>T	TT	●●	●●	
	IL-6	-174 G>C	CC	●●●	●●●	
	TNFA	-308 G>A	GG	○	○	
Methylation	MTHFR	677 C>T	TT		●●●	
		1298 A>C	AA		○	
	MTR	2756 A>G	AA		○	
Wnt Signalling	GSK3B	C>G	CG			
		A>C	CC		●●	
		G>A	GG			
Stress Response	FKBPS	C>T	CC		○	
	OXTR	G>A	AG		●	
Cell Signalling	AKT1	T>C	TT			○
	ANK3	A>G	AA		○	
		C>T	CC		○	
	CACNA1	G>A	AG		●	
	CHRNA3	G>A	GG			○
	CHRNA5	Asp398Asn	GG			○
Dopaminergic	COMT	Val158Met	AA	○	●●	○
	DRD1	T>C	TT			●●
		C>T	CC			○
	DRD2	Taq1A/2A	CC			○
	DRD3	Ser9Gly	TT			○
	DRD4	-521 C>T	CC			●
	OPRM1	Asn40Asp	AA			○
Endocannabinoid	CNR1	T>C	TT			○
	FAAH	385 C>A	AC			●
GABAergic	GABRA2	T>C	TT			○
Neurotrophic	BDNF	Val66Met	CC	○	○	○
Serotonergic	1A HTR1A	-1019 C>G	GG		●●●	
	SLC6A4	A>C	AA			●●



APOE E2/E3/E4

APOE encodes Apoliprotein E, a lipid-transporting protein functioning in both the periphery and the central nervous system. It is involved in multiple biological processes related to AD development and progression. Two SNPs on APOE results in three possible isoforms. The isoform affects the structure and function of apoE including binding to lipids, receptors and Aβ.

YOUR RESULT: **E4/E4**

The APOE E4 allele leads to an altered apolipoprotein function associated with Aβ clearance in the brain, which may be a consequence of reduced ApoE protein quantity and reduced affinity of ApoE for Aβ binding, resulting in impaired ApoE-mediated efflux and transport of Aβ across the blood-brain barrier. Carriers of the ε4 allele have been associated with greater brain atrophy, decreased cerebral glucose metabolism, impaired synaptic function, as well as defective hippocampal neurogenesis.

There is a significantly increased risk associated with development of LOAD in individuals with the APOE E4 allele. Furthermore, being a carrier of the APOE E4/E4 genotype shifts the age of onset of LOAD an average of 5 to 10 years earlier.
In E4 carriers, it is important to reduce total saturated, and increase polyunsaturated fat intake, specifically DHA, and increase antioxidant-rich foods. Strongly encourage regular moderate intensity exercise and weight management. Cessation of smoking and alcohol intake should be advised.

IL-1: IL-1A, IL1-B & IL-1RN

IL-1 has been increasingly implicated as an important leverage point in the inflammatory cascade, and IL-1 expression is therefore key in the pathogenesis of several chronic diseases. The biological activity of IL-1 involves the two agonists – IL-1alpha (IL-1A) and IL-1beta (IL-1B), specific IL-1 receptors, and an IL-1 receptor antagonist (IL-1RN), which is a negative regulator of the pro-inflammatory response. Certain genetic variations in IL-1A, IL-B and IL-1 RN lead to a more active

IL-1: IL-1A, IL1-B & IL-1RN

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YOUR RESULT: **Positive**

Individuals carrying variations in IL-1A, IL-1B or IL-1RN have a more active inflammatory response and can be considered to have increased IL-1 activity. This has been linked to increased risk for chronic, low-grade inflammation and predisposition with a number of mental health disorders.

Individuals with increased IL-1 activity are at increased risk for neuro-inflammatory disorders, including cognitive decline and mood disorders specifically depressive disorder. The association is modulated by the presence of an environmental trigger such as psycho-social stress. Increase intake of nutrients known to inhibit secretion of pro-inflammatory markers. These include omega 3 fatty acids, curcumin, ginger, and phytonutrient rich foods including certain berries that contain compounds such as resveratrol, anthocyanins and dehydro-ascorbate.

IL-6 -174 G>C

Interleukin 6 is a pro-inflammatory cytokine that plays a crucial role in inflammation and regulates expression of CRP.

YOUR RESULT: **CC**

The C allele of this functional SNP has been associated with raised IL-6 and CRP concentrations and has been associated with increased risk for chronic, low-grade inflammation.

The IL-6 C allele is associated with increased risk for cognitive decline as well as mood disorders, especially when exposed to psychosocial stressors and a pro-inflammatory environment. The risk associated with the C allele is further pronounced in smokers.
Individuals with the C allele should follow a diet to reduce inflammation that includes increasing n-3 fatty acids, decreasing saturated fatty acids, and increasing dietary antioxidants. A healthy weight and avoidance of all smoking is also imperative in managing inflammation.

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
HSV 1 and 2-Spec Ab, IgG w/Rfx					
HSV 1 IgG, Type Spec	**32.60**	**High**	index	0.00 - 0.90	01
			Negative	**<0.91**	
			Equivocal	**0.91 - 1.09**	
			Positive	**>1.09**	
Note: Negative indicates no antibodies detected to HSV-1. Equivocal may suggest early infection. If clinically appropriate, retest at later date. Positive indicates antibodies detected to HSV-1.					
HSV 2 IgG, Type Spec	<0.91		index	0.00 - 0.90	01
			Negative	<0.91	
			Equivocal	0.91 - 1.09	
			Positive	>1.09	
Note: Negative indicates no antibodies detected to HSV-2. Equivocal may suggest early infection. If clinically appropriate, retest at later date. Positive indicates antibodies detected to HSV-2.					
Trans. Growth Fact. beta 1*	1986		pg/mL	867 - 6662	04
The result is reported in pg/mL. The assay range is approximately 98 to 400,000. The reference range for a healthy population is 867-6662. However it should be noted that these ranges are obtained from a limited population of apparently healthy adults and are not diagnostic thresholds. *This test was developed and its performance characteristics determined by Viracor Eurofins. It has not been cleared or approved by the U.S. Food and Drug Administration.					
Reverse T3, Serum	**33.8**	**High**	ng/dL	9.2 - 24.1	02
C-Reactive Protein, Cardiac	0.18		mg/L	0.00 - 3.00	01
Relative Risk for Future Cardiovascular Event					
Low				<1.00	
Average				1.00 - 3.00	
High				>3.00	
Interleukin-6, Plasma	1.1		pg/mL	0.0 - 12.2	02
Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.					
HSV, IgM I/II Combination	**0.96**	**High**	Ratio	0.00 - 0.90	01
			Negative	**<0.91**	
			Equivocal	**0.91 - 1.09**	
			Positive	**>1.09**	
Triiodothyronine (T3)	101		ng/dL	71 - 180	01

Vitamin D 19

Glucose X3+Hb A1c+Insulin X3

Glucose, Fasting	93	mg/dL	65 - 99	01
RECEIVED ANOTHER TUBE LABELED 30 MINUTES GLUCOSE = 142 mg/dL				
Glucose, 2 hour (120 min)	120	mg/dL	65 - 139	01

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
Hemoglobin A1c	5.4		%	4.8 - 5.6	01
Please Note:					01
Prediabetes: 5.7 - 6.4					
Diabetes: >6.4					
Glycemic control for adults with diabetes: <7.0					
Insulin, Fasting	6.1		uIU/mL	2.6 - 24.9	01
RECEIVED ANOTHER TUBE LABELED 30 MINUTES Insulin = 28.2 uIU/ml					
Insulin, 2 hour (120 min)	34.3		uIU/mL	0.0 - 145.4	01

HNK1 (CD57) Panel

% CD8-/CD57+ Lymphs	2.6		%	2.0 - 17.0	01
This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration.					
Abs.CD8-CD57+ Lymphs	**36**	**Low**	/uL	60 - 360	
This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration.					

Toxic Metals; Urine

TOXIC METALS		RESULT µg/g creat	REFERENCE INTERVAL	WITHIN REFERENCE / OUTSIDE REFERENCE
Aluminum	(Al)	3	< 35	
Antimony	(Sb)	< dl	< 0.2	
Arsenic	(As)	39	< 80	
Barium	(Ba)	3.4	< 7	
Beryllium	(Be)	< dl	< 1	
Bismuth	(Bi)	< dl	< 4	
Cadmium	(Cd)	0.3	< 1	
Cesium	(Cs)	8	< 10	
Gadolinium	(Gd)	< dl	< 0.8	
Lead	(Pb)	1.8	< 2	
Mercury	(Hg)	1.5	< 4	
Nickel	(Ni)	3.9	< 10	
Palladium	(Pd)	< dl	< 0.3	
Platinum	(Pt)	< dl	< 0.1	
Tellurium	(Te)	< dl	< 0.5	
Thallium	(Tl)	0.4	< 0.5	
Thorium	(Th)	< dl	< 0.03	
Tin	(Sn)	0.6	< 5	
Tungsten	(W)	< dl	< 0.4	
Uranium	(U)	< dl	< 0.04	

URINE CREATININE	RESULT mg/dL	REFERENCE INTERVAL	-2SD -1SD MEAN +1SD +2SD
Creatinine	52.3	30- 225	

Baseline

Toxic Metals; Urine

TOXIC METALS		RESULT µg/g creat	REFERENCE INTERVAL	WITHIN REFERENCE / OUTSIDE REFERENCE
Aluminum	(Al)	79	< 35	
Antimony	(Sb)	0.7	< 0.2	
Arsenic	(As)	45	< 80	
Barium	(Ba)	6.9	< 7	
Beryllium	(Be)	< dl	< 1	
Bismuth	(Bi)	0.2	< 4	
Cadmium	(Cd)	0.8	< 1	
Cesium	(Cs)	7.3	< 10	
Gadolinium	(Gd)	< dl	< 0.8	
Lead	(Pb)	7.9	< 2	
Mercury	(Hg)	44	< 4	
Nickel	(Ni)	3.9	< 10	
Palladium	(Pd)	< dl	< 0.3	
Platinum	(Pt)	< dl	< 0.1	
Tellurium	(Te)	< dl	< 0.5	
Thallium	(Tl)	0.5	< 0.5	
Thorium	(Th)	< dl	< 0.03	
Tin	(Sn)	4.8	< 5	
Tungsten	(W)	0.2	< 0.4	
Uranium	(U)	< dl	< 0.04	

URINE CREATININE	RESULT mg/dL	REFERENCE INTERVAL	-2SD -1SD MEAN +1SD +2SD
Creatinine	45.3	30- 225	

SPECIMEN DATA

Comments: Result Checked.

Date Collected: 12/13/2018
Date Received: 12/15/2018
Date Completed: 12/21/2018
Method: ICP-MS

pH upon receipt: Acceptable
<dl: less than detection limit
Provoking Agent: DMPS 500
Creatinine by Jaffe Method

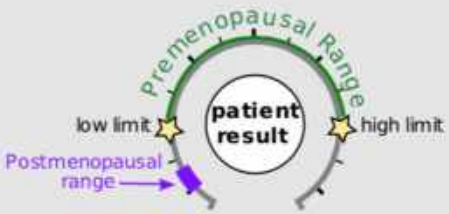
Collection Period: timed: 6 hours
Volume:
Provocation: POST PROVOCATIVE

Results are creatinine corrected to account for urine dilution variations. **Reference intervals and corresponding graphs are representative of a healthy population under non-provoked conditions.** Chelation (provocation) agents can increase urinary excretion of metals/elements. V13

Post Provocation with DMPS 500mg

Hormone Testing Summary

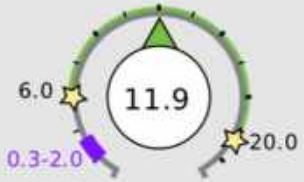
Key (how to read the results):



Sex Hormones

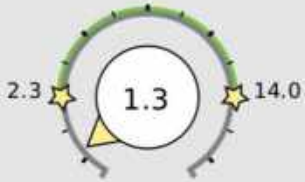
See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites

Estradiol(E2)



Progesterone
(Serum Equivalent, ng/mL)

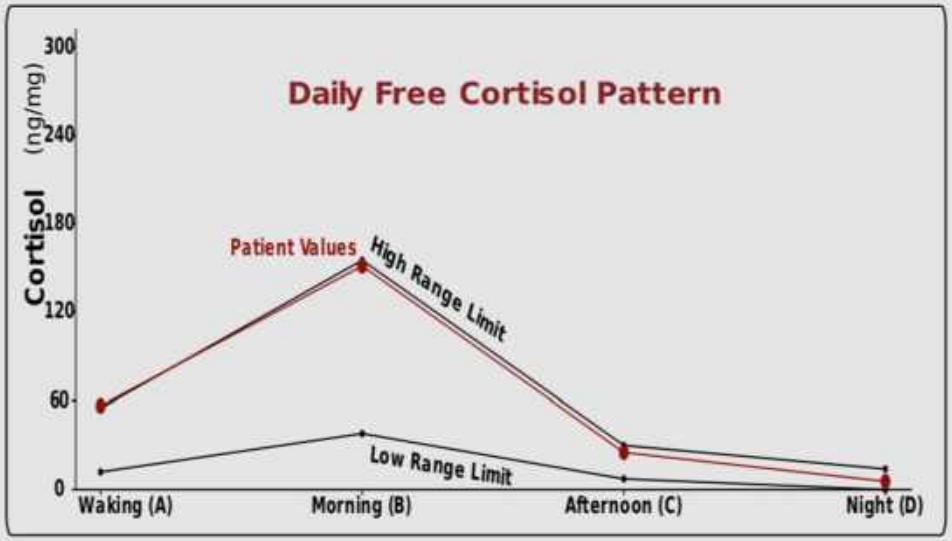
Progesterone Serum Equivalent is a calculated value based on urine pregnanediol.



Testosterone

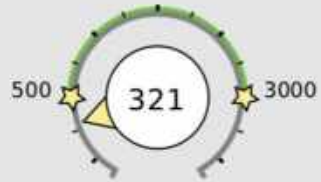
Adrenal Hormones

See pages 4 and 5 for a more complete breakdown of adrenal hormones



Total DHEA Production

Age	Range
20-39	1300-3000
40-60	750-2000
>60	500-1200



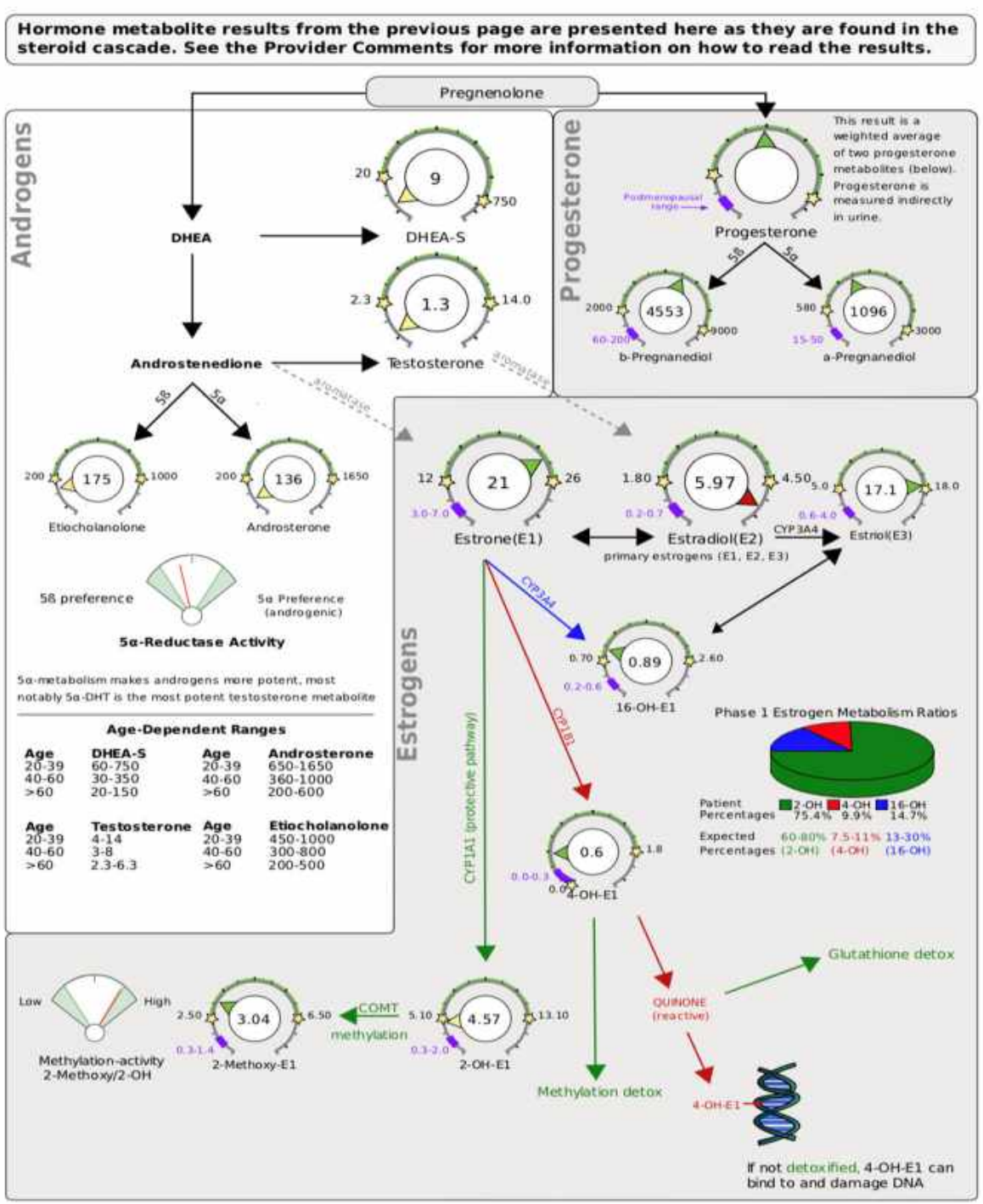
Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)

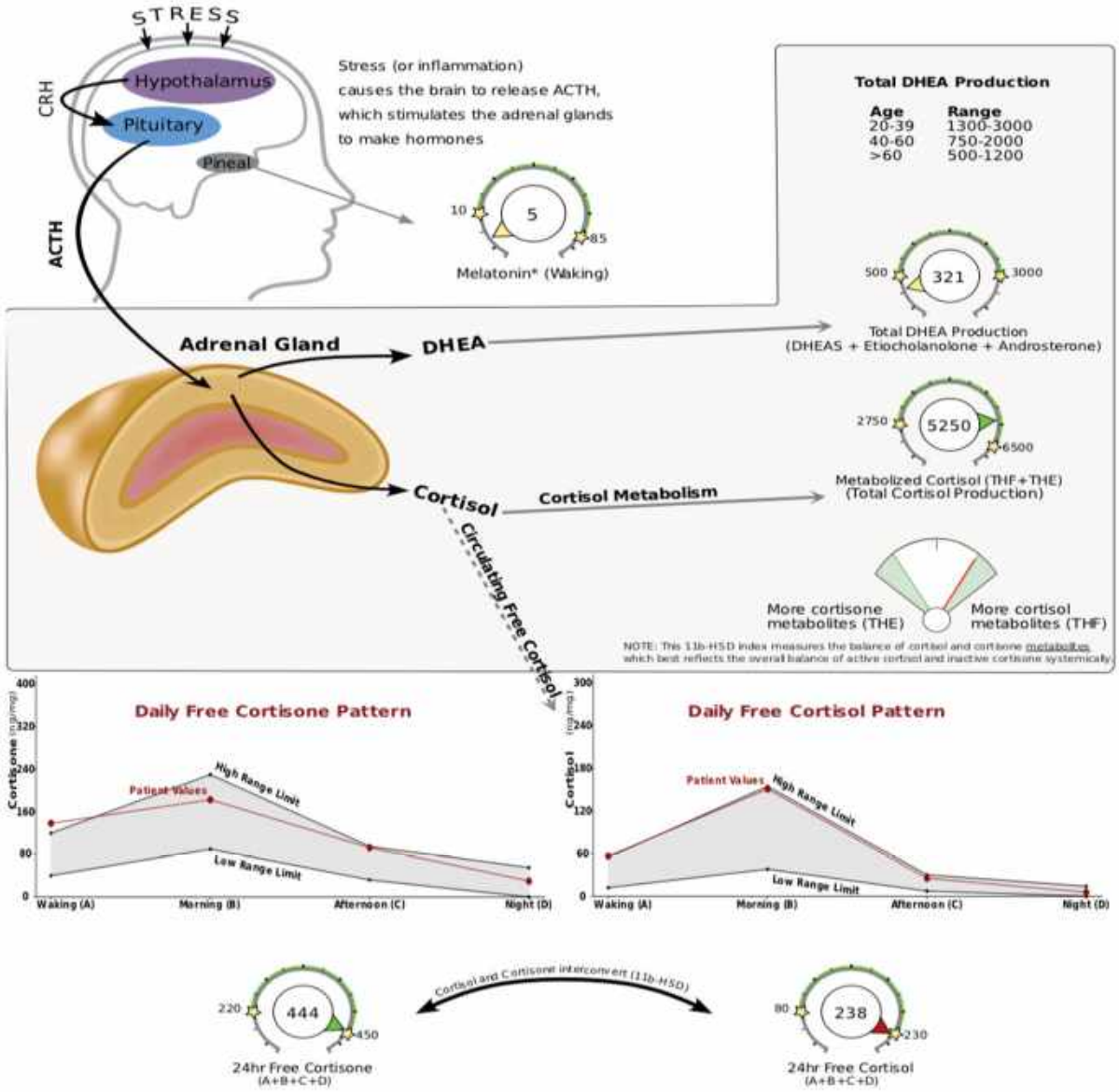
24hr Free Cortisol
(A+B+C+D)

cortisol metabolism

Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.


Hormone metabolite results from the previous page are presented here as they are found in the steroid cascade. See the Provider Comments for more information on how to read the results.
Pregnenolone
Androgens
DHEA
DHEA-S
20
9
750
Androstenedione
Testosterone
2.3
1.3
14.0
aromatase
5β
5α
Etiocholanolone
200
175
1000
Androsterone
200
136
1650
5β preference
5α Preference (androgenic)
5α-Reductase Activity
5α-metabolism makes androgens more potent, most notably 5α-DHT is the most potent testosterone metabolite
Age-Dependent Ranges
Age DHEA-S
20-39 60-750
40-60 30-350
>60 20-150
Age Androsterone
20-39 650-1650
40-60 360-1000
>60 200-600
Age Testosterone
20-39 4-14
40-60 3-8
>60 2.3-6.3
Age Etiocholanolone
20-39 450-1000
40-60 300-800
>60 200-500
Progesterone
This result is a weighted average of two progesterone metabolites (below). Progesterone is measured indirectly in urine.
Postmenopausal range
Progesterone
5β
5α
2000
4553
9000
60-200
b-Pregnanediol
580
1096
3000
15-50
a-Pregnanediol
Estrogens
12
21
26
3.0-7.0
Estrone(E1)
1.80
5.97
4.50
0.2-0.7
Estradiol(E2)
5.0
17.1
18.0
0.6-4.0
Estriol(E3)
CYP3A4
primary estrogens (E1, E2, E3)
CYP3A4
0.70
0.89
2.60
0.2-0.6
16-OH-E1
CYP1B1
CYP1A1 (protective pathway)
Phase 1 Estrogen Metabolism Ratios
Patient Percentages
2-OH 75.4%
4-OH 9.9%
16-OH 14.7%
Expected Percentages
60-80% (2-OH)
7.5-11% (4-OH)
13-30% (16-OH)
0.6
1.8
0.0-0.3
0.0
4-OH-E1
Glutathione detox
QUINONE (reactive)
Low
High
Methylation-activity
2-Methoxy/2-OH
2.50
3.04
6.50
0.3-1.4
2-Methoxy-E1
COMT
methylation
5.10
4.57
13.10
0.3-2.0
2-OH-E1
Methylation detox
4-OH-E1
If not detoxified, 4-OH-E1 can bind to and damage DNA



The first value reported (Waking "A") for cortisol is intended to represent the "overnight" period. When patients sleep through the night, they collect just one sample. In this case, the patient woke during the night and collected (see the top of the report for the times collected). We call this value "A1" and the value from the sample collected at waking "A2." These values are used to create a "time-weighted average" to create the "A" value. The individual values are listed here for your use:

The middle-of-the-night "A1" sample registered a cortisol value of 58.3ng/mg.
The waking "A2" sample registered a cortisol value of 53.0ng/mg.
These two values are averaged together, taking into account the amount of time each one represents, to create the "A" value of approximately 56.5ng/mg that you will see on the report.

MYPERIOPATH®

FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

[redacted]

Ordering Provider
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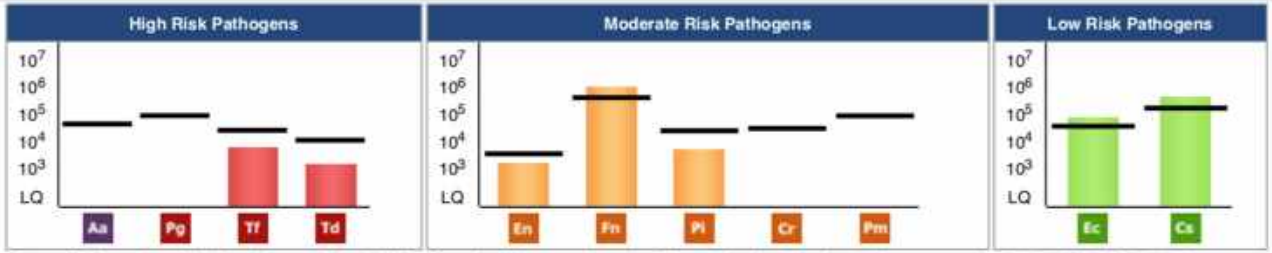
[redacted]

MYPERIOPATH MOLECULAR ANALYSIS OF PERIODONTAL AND SYSTEMIC PATHOGENS

Results



Legend: The result graphic (above) shows the bacterial level for each of the assayed species. The vertical axis displays bacterial genome copies/milliliter in log10. The limit of quantification (LQ) is the lowest bacteria level that can be repeatedly measured. The black lines across each colored bar are the Therapeutic Threshold.

Interpretation of Results

- This result shows a combination of 2 high risk (Tf, Td) below, and 1 moderate risk (Fn) pathogens above, the therapeutic threshold. High levels of Ec, Cs are frequently part of this complex bacterial profile.
- The bacterial species Tf and/or Td are strongly associated with chronic periodontitis, are transmissible and tissue invasive even at low amounts of these organisms. Moreover, Td is present in 20-40% of cases of periodontitis where because it possesses proteins needed for adherence and invasion of host cells, it can cause destruction of periodontal tissue.
- The detected pathogens are also risk factors for various systemic diseases, including atherosclerosis, type 2 diabetes, arthritis, dementia and several types of cancer. Periodontal infections involving Ec have been associated with widespread and tissue invasive diseases of the lung, pancreas, heart, and bone. The spread of Ec infections is direct and often associated with abscess formation.

Treatment Considerations: to be determined by the healthcare professional

- **Mechanical/Debridement:** Scaling and root planing (SRP) is a mainstay of therapy to disrupt biofilm, remove plaque and debride compromised tissue. This patient harbors a series of pathogens (Tf, Pi) that may be refractory to this treatment.
- **Local Antibiotics and Chemical Hygiene:** As an adjunct to SRP, sub-antimicrobial doses of doxycycline hyclate lower collagenase activity and reduce periodontal pocket depth. Alternatively, locally delivered antimicrobial agents (LDA) including minocycline microspheres, doxycycline hyclate in an absorbable polymer, or chlorhexidine in a gelatin matrix have been shown to decrease pocket depth modestly.
- **Pocket or Field Decontamination:** Laser decontamination as an adjunct therapy to SRP may be beneficial in reducing probing depth and bacterial loads. The consideration of using lasers as an adjunct to SRP is dependent on type of laser used and the particular protocol.
- **Chemical and Gaseous antiseptics:** Chlorhexidine or Povidine iodine rinses can reduce periodontal pocket depth. Prescription tray application of peroxide gel, as an adjunct to frequent periodontal maintenance appointments for refractory patients, demonstrated significant reductions in bleeding on probing. Ozone is a volatile antiseptic that can disrupt microbial membranes.
- **Probiotics and Prebiotics:** Probiotics are live, beneficial bacteria, typically administered as a food or dietary supplement. Prebiotics are non-digestible ingredients that promote growth of commensal bacteria. Research shows that prebiotics and probiotics control the growth of pathogens and reverse tissue destruction caused by periodontitis.
- **Periodontal Surgery:** For severe and/or refractory periodontitis - surgical approaches such as gum flap repairs, procedures to reduce pocket depth, or other restorative procedures may be indicated.

Follow up Recommendations

- ☑ Good periodontal health depends on compliance of a home care regimen as detailed by your healthcare provider. Daily brushing, flossing, as well as attention to nutrition, proper rest and cessation of smoking are essential.
- ☑ Follow-up testing between 6-12 weeks with MyPerioPath is recommended. Persistence of bleeding on probing is often indicative of unresolved infection. Retesting will identify residual or refractory bacteria. Currently there is not a cure for periodontal disease, only periods of remission.
- ☑ Assessment of a patient's level of inflammation with Celsus One is valuable in deciding the frequency of patient recall and treatment.

MYPERIOPATH®

FINAL REPORT

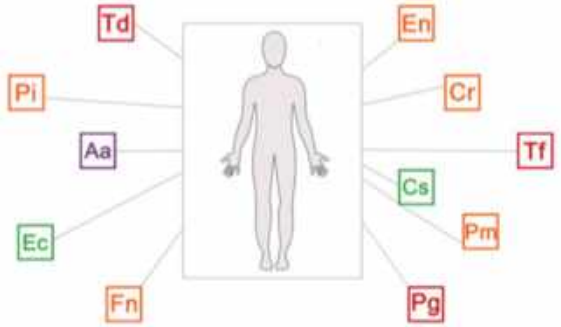
[redacted]

ORALDNA LABS
Innovations in Salivary Diagnostics

Clinical Considerations

Reason for Testing	Clinical	Diagnostic	Medical History
☑ Not Provided	☑ Not Provided	☑ Periodontal Classification: Not Provided ☑ Tooth Numbers Pocket Depths(mm)	☑ Not Provided

Systemic Effects of Oral Pathogens



Cancer

Chronic gum disease, involving Aa, Pg, Td, Tf, & Fn is a risk factor for the development of certain cancers including ones involving the pancreas, esophagus, colon, lungs, and the head and neck. Additionally, untreated gum disease is a cause of ongoing inflammation, which may promote the advancing growth of tumors.

Cardiovascular Health

Select bacteria such as Aa, Td, Tf, Pg, Pi, & Fn can leak from blood vessels in the gums and travel to the heart, where cholesterol and other lipids deposit. These bacteria can incite inflammation in arteries, and if occluded, cause a heart attack. A goal of treatment is to minimize the levels of these bacteria as much and as long as possible.

Joint and Musculoskeleta Health

The periodontal bacteria Pg, Fn & Ec are a cause of arthritis. The oral inflammation caused by these bacteria also leads to total body inflammation which, combined with changes in a person's immunity, may result in chronic joint diseases like rheumatoid arthritis.

Dementia and Brain Health

Recent medical studies point to poor oral health, and high levels of the bacteria Pg, Cr, Cs in our gums, increasing the risk of developing dementias such as Alzheimer's.

Metabolic Health

Obesity, lack of exercise and chronic gum disease involving the bacteria Aa, Td, Tf, Pg, & Fn cause chronic inflammation. Inflammation can damage the pancreas where insulin is produced, possibly leading to diabetes. Also, diabetes worsens oral health by increasing the level of harmful bacteria in the gums.

Healthy Pregnancy

Bacteria associated with gum disease, especially Aa, Tf, Pg, Pm, and Ec, are known to put a pregnancy at risk for pre-term birth, decreased birth weight and even blood infection in the placenta or newborn. Every pregnant woman should be tested for these harmful bacteria.

Methodology: Genomic DNA is extracted from the submitted sample and tested for 10 species-specific bacteria [Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Tf: Tannerella forsythia, Td: Treponema denticola, En: Eubacterium nodatum, Fn: Fusobacterium nucleatum/periodontium, Pi: Prevotella intermedia, Cr: Campylobacter rectus, Pm: Peptostreptococcus (Micromonas) micros, Ec: Eikenella corrodens] and 1 genus of bacteria [Cs: Capnocytophaga species (gingavalis, ochracea, sputigena)] known to cause periodontal disease. The bacteria are assayed by real-time quantitative polymerase chain reaction (qPCR). Bacterial levels are reported in log 10 copies per mL of sample (e.g. 1x10^3 = 1000 bacteria copies per mL of collection). Cross-reactivity is possible with Leptotrichia buccalis, Fusobacterium hwasooki, Capnocytophaga granulosa and Capnocytophaga leadbetteri. This test was developed, and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

Toxic & Essential Elements; Hair

TOXIC METALS		RESULT µg/g	REFERENCE INTERVAL	PERCENTILE 68th 95th
Aluminum	(Al)	1.6	< 7.0	
Antimony	(Sb)	< 0.01	< 0.050	
Arsenic	(As)	< 0.01	< 0.060	
Barium	(Ba)	0.82	< 2.0	
Beryllium	(Be)	< 0.01	< 0.020	
Bismuth	(Bi)	0.88	< 2.0	
Cadmium	(Cd)	< 0.009	< 0.050	
Lead	(Pb)	0.18	< 0.60	
Mercury	(Hg)	1.2	< 0.80	
Platinum	(Pt)	< 0.003	< 0.005	
Thallium	(Tl)	< 0.001	< 0.002	
Thorium	(Th)	< 0.001	< 0.002	
Uranium	(U)	0.001	< 0.060	
Nickel	(Ni)	0.21	< 0.30	
Silver	(Ag)	0.10	< 0.15	
Tin	(Sn)	0.08	< 0.30	
Titanium	(Ti)	0.37	< 0.70	
Total Toxic Representation				

ESSENTIAL AND OTHER ELEMENTS		RESULT µg/g	REFERENCE INTERVAL	PERCENTILE 2.5th 16th 50th 84th 97.5th
Calcium	(Ca)	3660	300- 1200	
Magnesium	(Mg)	470	35- 120	
Sodium	(Na)	150	20- 250	
Potassium	(K)	17	8- 75	
Copper	(Cu)	26	11- 37	
Zinc	(Zn)	160	140- 220	
Manganese	(Mn)	1.0	0.08- 0.60	
Chromium	(Cr)	0.34	0.40- 0.65	
Vanadium	(V)	0.010	0.018- 0.065	
Molybdenum	(Mo)	< 0.01	0.020- 0.050	
Boron	(B)	0.19	0.25- 1.5	
Iodine	(I)	0.34	0.25- 1.8	
Lithium	(Li)	0.009	0.007- 0.020	
Phosphorus	(P)	147	150- 220	
Selenium	(Se)	0.32	0.55- 1.1	
Strontium	(Sr)	21	0.50- 7.6	
Sulfur	(S)	47700	44000- 50000	
Cobalt	(Co)	0.045	0.005- 0.040	
Iron	(Fe)	12	7.0- 16	
Germanium	(Ge)	0.035	0.030- 0.040	
Rubidium	(Rb)	0.013	0.007- 0.096	
Zirconium	(Zr)	0.12	0.020- 0.42	

GI Map Stool quantitative PCR, stool test

Ordered by: Todd LePine, MD

Pathogens		
Bacterial Pathogens	Result	Normal
Campylobacter	**<dl**	<1.00e3
C. difficile, Toxin A	**<dl**	<1.00e3
C. difficile, Toxin B	**<dl**	<1.00e3
Enterohemorrhagic E. coli	**<dl**	<1.00e3
E. coli O157	**<dl**	<1.00e3
Enteroinvasive E. coli/Shigella	**<dl**	<1.00e2
Enterotoxigenic E. coli LT/ST	**<dl**	<1.00e3
Shiga-like Toxin *E. coli* stx1	**<dl**	<1.00e3
Shiga-like Toxin *E. coli* stx2	**<dl**	<1.00e3
Salmonella	**<dl**	<1.00e4
Vibrio cholerae	**<dl**	<1.00e5
Yersinia enterocolitica	**<dl**	<1.00e5
Parasitic Pathogens	Result	Normal
Cryptosporidium	**<dl**	<1.00e6
Entamoeba histolytica	**<dl**	<1.00e4
Giardia	**3.05e2**	<5.00e3
Viral Pathogens	Result	Normal
Adenovirus 40/41	**<dl**	<1.00e10
Norovirus GI/II	**<dl**	<1.00e7

H. pylori			
	Result		Normal
Helicobacter pylori	**2.8e4**	**High**	<1.0e3
Virulence Factor, babA	**Negative**		Negative
Virulence Factor, cagA	**Negative**		Negative
Virulence Factor, dupA	**Negative**		Negative
Virulence Factor, iceA	**Positive**		Negative
Virulence Factor, oipA	**Negative**		Negative
Virulence Factor, vacA	**Negative**		Negative
Virulence Factor, virB	**Negative**		Negative
Virulence Factor, virD	**Negative**		Negative

Normal Bacterial Flora			
	Result		Normal
Bacteroides fragilis	**8.0e9**		1.60e9 - 2.50e11
Bifidobacterium spp.	**2.1e9**		>6.70e7
Enterococcus spp.	**4.3e5**		1.9e5 - 2.00e8
Escherichia spp.	**1.8e8**		3.70e6 - 3.80e9
Lactobacillus spp.	**1.9e6**		8.6e5 - 6.20e8
Clostridium spp.	**2.52e5**		1.20e3 - 1.00e6
Enterobacter spp.	**4.63e6**		1.00e6 - 5.00e7

Phyla Microbiota	Result		Normal
Bacteroidetes	**2.89e12**		8.61e11 - 3.31e12
Firmicutes	**3.95e11**	**High**	5.70e10 - 3.04e11
Firmicutes:Bacteroidetes Ratio	**0.14**		<1.00

Opportunistic Bacteria			
Additional Dysbiotic/Overgrowth Bacteria	Result		Normal
Bacillus spp.	**6.85e3**		<1.50e5
Enterococcus faecalis	**1.75e3**		<1.00e4
Enterococcus faecium	**<dl**		<1.00e4
Morganella spp.	**<dl**		<1.00e3
Pseudomonas spp.	**<dl**		<1.00e4
Pseudomonas aeruginosa	**<dl**		<5.00e2
Staphylococcus spp.	**4.26e5**	**High**	<1.00e4
Staphylococcus aureus	**2.86e0**		<5.00e2
Streptococcus spp.	**4.64e2**		<1.00e3
Potential Autoimmune Triggers	Result		Normal
Citrobacter spp.	**<dl**		<5.00e6
Citrobacter freundii	**<dl**		<5.00e5
Klebsiella spp.	**<dl**		<5.00e3
Klebsiella pneumoniae	**1.13e4**		<5.00e4
M. avium subsp. paratuberculosis	**<dl**		<5.00e3
Prevotella copri	**<dl**		<1.00e7
Proteus spp.	**<dl**		<5.00e4
Proteus mirabilis	**<dl**		<1.00e3
Fungi/Yeast			
	Result		Normal
Candida spp.	**<dl**		<5.00e3
Candida albicans	**<dl**		<5.00e2
Geotrichum spp.	**<dl**		<3.00e2
Microsporidium spp.	**<dl**		<5.00e3
Rodotorula spp.	**<dl**		<1.00e3
Viruses			
	Result		Normal
Cytomegalovirus	**<dl**		<1.00e5
Epstein Barr Virus	**<dl**		<1.00e7

Parasites			
Protozoa	Result		Normal
Blastocystis hominis	**<dl**		<2.00e3
Chilomastix mesnili	**<dl**		<1.00e5
Cyclospora spp.	**<dl**		<5.00e4
Dientamoeba fragilis	**1.19e3**		<1.00e5
Endolimax nana	**<dl**		<1.00e4
Entamoeba coli	**<dl**		<5.00e6
Pentatrichomonas hominis	**<dl**		<1.00e2
Worms	Result		Normal
Ancylostoma duodenale	**Not Detected**		Not Detected
Ascaris lumbricoides	**Not Detected**		Not Detected
Necator americanus	**Not Detected**		Not Detected
Trichuris trichiura	**Not Detected**		Not Detected
Taenia spp.	**Not Detected**		Not Detected
Intestinal Health			
Digestion	Result		Normal
Elastase-1	**730**		>200 ug/g
Steatocrit	**8**		<15 %
GI Markers	Result		Normal
b-Glucuronidase	**3168**	**High**	<2486 U/mL
Occult Blood - FIT	**0**		<10 ug/g
Immune Response	Result		Normal
Secretory IgA	**5453**	**High**	510 - 2010 ug/g
Anti-gliadin IgA	**132**		0 - 157 U/L
Inflammation	Result		Normal
Calprotectin	**232**	**High**	<173 ug/g

TEST	RESULT			
Array 3X - Wheat/Gluten Proteome Reactivity & Autoimmunity	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Wheat IgG	0.95			0.3-1.5
Wheat IgA	0.80			0.1-1.2
Wheat Germ Agglutinin IgG	0.45			0.4-1.3
Wheat Germ Agglutinin IgA	0.44			0.2-1.1
Non-Gluten Proteins A IgG	0.20			0.2-2.1
Non-Gluten Proteins A IgA	0.35			0.2-2.1
Non-Gluten Proteins B IgG	0.29			0.2-1.9
Non-Gluten Proteins B IgA	0.42			0.2-2.1
Gliadin Toxic Peptides IgG	0.57			0.2-1.9
Gliadin Toxic Peptides IgA	0.81			0.2-1.8
Native & Deamidated Gliadin 33 IgG	0.34			0.2-1.2
Native & Deamidated Gliadin 33 IgA	0.70			0.1-1.1
Alpha Gliadin 17-mer IgG	0.21			0.1-1.5
Alpha Gliadin 17-mer IgA	0.57			0.1-1.1
Gamma Gliadin 15-mer IgG	<0.50			0.5-1.5
Gamma Gliadin 15-mer IgA	0.42			0.1-1.0
Omega Gliadin 17-mer IgG	0.63			0.3-1.2
Omega Gliadin 17-mer IgA	0.24			0.1-1.2
Glutenin 21-mer IgG	0.31			0.1-1.5
Glutenin 21-mer IgA	0.56			0.1-1.3
Gluteomorphin + Prodynorphin IgG	0.30			0.3-1.2
Gluteomorphin + Prodynorphin IgA	0.43			0.1-1.2
Gliadin-Transglutaminase Complex IgG	0.60			0.3-1.4
Gliadin-Transglutaminase Complex IgA	1.06			0.2-1.5
Microbial Transglutaminase IgG	0.63			0.2-1.8
Microbial Transglutaminase IgA	0.82			0.2-2.3
Transglutaminase-2 IgG	<0.30			0.3-1.6
Transglutaminase-2 IgA	0.70			0.1-1.6
Transglutaminase-3 IgG	0.72			0.2-1.6
Transglutaminase-3 IgA	0.48			0.1-1.5
Transglutaminase-6 IgG	0.50			0.2-1.5
Transglutaminase-6 IgA	0.80			0.1-1.5

TEST	RESULT			
Array 2 – Intestinal Antigenic Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Actomyosin IgA **	9.47			0.0-20
Occludin/Zonulin IgG	0.73			0.2-1.5
Occludin/Zonulin IgA	0.80			0.1-1.8
Occludin/Zonulin IgM	0.75			0.1-2.1
Lipopolysaccharides (LPS) IgG			1.63	0.1-1.6
Lipopolysaccharides (LPS) IgA		1.56		0.1-1.8
Lipopolysaccharides (LPS) IgM	0.95			0.1-2.0

Analysis		Result	Units	Reference Range		Chart
Haematology *						
5 Blood count						
5 Leucocytes		4,62	Tsd./ul	4,00 - 10,40	[	*.....]
5 Erythrocytes		4,92	Mill./ul	3,96 - 5,16	[	.....*.]
5 Hemoglobin		14,6	g/dl	11,6 - 15,5	[	.....*.]
5 Hematocrit	**+**	**49,20**	**%**	**35,00 - 45,00**	**[**	**..... *>**
5 MCV	**+**	**100,00**	**fl**	**80,00 - 96,00**	**[**	**..... *>**
5 MCH		29,70	pg	26,00 - 33,00	[	....*...]
5 MCHC	**-**	**29,70**	**g/dl**	**32,00 - 36,00**	**<***	**.....]**
5 Thrombocytes		217,00	Tsd./ul	176,00 - 391,00	[	.*.....]
5 Differential Blood count						
5 Neutroph. Granulocytes		55,40	%	40,00 - 75,00	[	...*....]
5 Lymphocytes		33,10	%	17,00 - 47,00	[	....*...]
5 Monocytes		6,10	%	4,00 - 12,00	[	..*.....]
5 Eosin. Granulocytes		1,30	%	< 7,00	[	.*.....]
5 Basoph. Granulocytes	**+**	**4,10**	**%**	**< 2,00**	**[**	**..... *>**
Borrelia EliSpot *						
1 Borrelia b. Full Antigen		1	SI			
0-1 = negative						
2-3 = weak positive						
> 3 = positive						
1 Borrelia b. OSP-Mix		1	SI			
0-1 = negative						
2-3 = weak positive						
> 3 = positive						
1 Borrelia burgdorferi LFA-1		1	SI			
0-1 = negative						
2-3 = weak positive						
> 3 = positive						

The results of the EliSpot tests indicate no current cellular activities against Borrelia burgdorferi.

Explanation of antigens:
Borrelia-burgdorferi Full Antigen: Borrelia burgdorferi B31 reference strain (Borrelia burgdroferi sensu stricto)
Borrelia-burgdorferi Peptide-Mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant.
Borrelia-burgdorferi LFA-1 (Lymphocyte Function Antigen 1) Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). LFA1 can be associated with autoimmune diseases: collagenosis, Rheumatoid Arthritis, vasculitis. If positive or borderline positive look at: ANA, CCP-antibodies, ANCA.

CD3-/CD57+ Cells

5	CD3-/CD56+ Flow Cytometry					
5	T cells CD3+ (%)	71,00 %	62,00 - 80,00	[	....*...	]
5	T cells CD3+ (absolute)	1086 /ul	900 - 1900	[	.*.....	]
5	NK cells CD56+ CD3- (%)	18,98 %	6,00 - 29,00	[	....*...	]
5	NK cells CD56+ CD3- (absolute)	290 /ul	60 - 700	[	..*.....	]
5	CD57+ NK-cells (%)	10,56 %	2,00 - 77,00	[	*.....	]
5	**CD57+ NK-cells (absolute) -**	**31 /ul**	**100 - 360**	**<***	**.....**	**]**

The result of the CD57-cell count indicates chronic immune-suppression, which can be caused by Borrelia burgdorferi or other bacteria like Chlamydia pneumoniae or Mycoplasma pneumoniae.

Six Week Follow up Appointment

Patient has been following protocol

increased her water intake, drinking green tea, eating more dietary fiber

avoiding all gluten, dairy, sugar, tuna,

6 months ago was fragile, problem solving diff.

Doing better now, about 20-30 % better.

going to gym and interacting with others better.

Treatment Regimen

1. Start MCT oil 1 tbsp twice a day
2. Start Lithium Orotate 5mg/day (can go up to 25mg/day)
3. Start Memory Pro 2 caps twice a day
4. Start Melatonin 3mg QHS
5. Start PhytoMulti 2/day with active folate

1. Start L-Lysine 500mg 3 caps twice a day
2. Start Turkey Tail Mushroom 2 caps BID
3. Start Buffered Vitamin C 2-4 grams QD
4. Start Therbiotic Complete 2 caps/day (all for HSV-1 and Low CD57)

Treatment Regimen

1. Start GastroMend HP 3 caps TID for 2 weeks
2. Start Oncoplex 1 cap BID for 2 weeks
3. Start PeptoBismol 2 tabs TID for 2 weeks (all for H. Pylori)

1. Start L-glutamine 3 grams BID
2. Start Glutagenics 1 heaping tsp BID
3. Start Quercitin Ascorbate 1/4 tsp BID (all for 1 month for leaky gut)

Treatment Regimen

1. Start Vitamin D Supreme 2 caps a day for 1 month then 1/day
2. CoQ10 200mg QD
3. Start Oral Irrigator, Electric Toothbrush and Dentalcidin toothpaste

1. Decrease Estro-Gel 0.75mg/1 gram to 1 pump/day
2. Continue Progesterone 100mg/day
3. Start DHEA 10mg a day

Four Month Follow up Appointment

MSQ 13 was 40

speech is mildly impaired but communicating better

Physical health and disposition is good

doing painting now....will be starting yoga

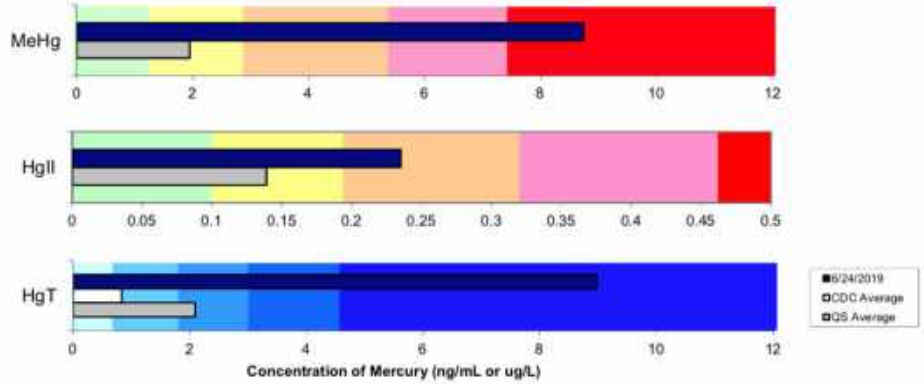
brain fog is gone!

QUICKSILVER SCIENTIFIC

		Dates	Taken	Arrived	Analyzed
Physician Name	Todd LePine		6/24/2019	6/27/2019	7/2/2019
Date Of Birth	9/17/1947		NA	NA	NA

Blood Results

Blood Mercury Comparison

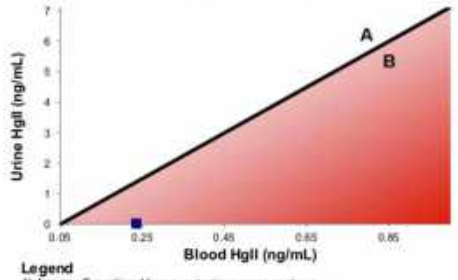


	[redacted] Results (ng/mL)			Reference Ranges QS n=1011; CDC n=1928			Percentile			
	6/24/2019	NA	% Change	Source	Range	Average	50th	75th	90th	95th
Methylmercury— MeHg	8.74	NA	NA	QS	<0.003 to 23.3	1.95	1.2	2.9	5.4	7.4
Inorganic Mercury— HgII	0.235	NA	NA	QS	<0.007 to 1.75	0.139	0.10	0.19	0.32	0.46
Sum— HgT	8.98	NA	NA	CDC	0.038 to 9.96	0.833	0.7	1.7	3	4.6

Blood Reference Values: Quicksilver Scientific (QS) Data represents 1011 males and females that have utilized our testing. CDC data represents 1928 females, ages 16 to 49. QS blood Hg concentrations are higher that CDC because QS analyzes blood a population that already suspects mercury toxicity.
Data and Analysis Information: Mercury speciation was performed at Quicksilver Scientific, and all values are in concentrations of ng Hg per mL of blood

Urine Results

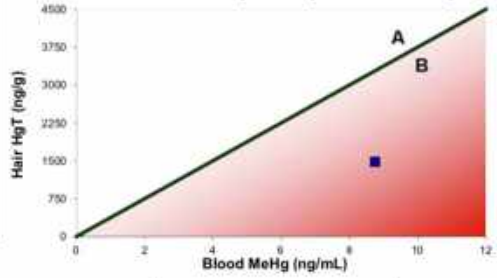
Indication of Inorganic Mercury Excretion Ability



Legend
A) Average Excretion: Mercury output is average or above average when at a ratio of at least 375:1 HgT in hair to MeHg in blood and 6.9:1 HgT in urine to HgII in blood.
B) Below Average Excretion: Mercury output is below average when the tissue Hg comparisons are below ratios mentioned above (red area)

Hair Results

Indication of Methylmercury Excretion Ability



	[redacted] Urine Results (ng/mL)			Hair (ng/g)
	6/24/2019	NA	%Change	6/24/2019
Methylmercury— MeHg	<0.005	NA	NA	NA
Inorganic Mercury— HgII	0.019	NA	NA	NA
Sum— HgT	0.019	NA	NA	1477

reenshot

Treatment Regimen

1. Start HepatoThera Forte 1 cap/day
2. Start Liposomal Glutathione 2 pumps BID
3. Start DMSA 250 mg, 2 capsules three times a day for 3 days every other week, 3 months then stop.

MYPERIOPROGRESS

COMPARISON OF RESULTS

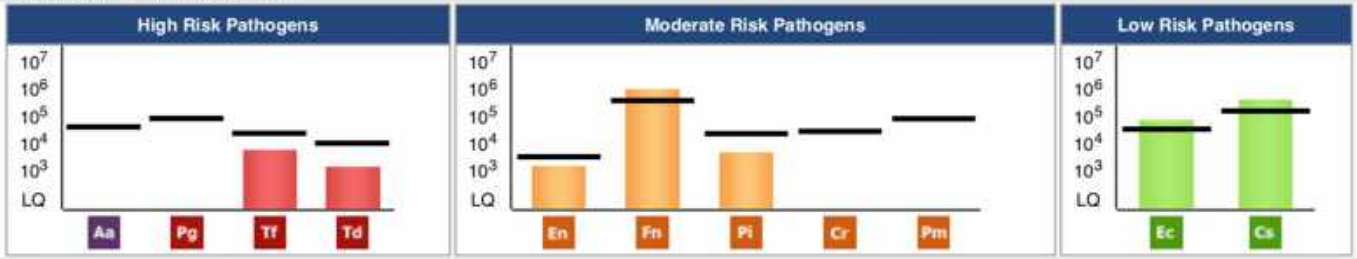
ORALDNA LABS
Innovations in Salivary Diagnostics

[redacted]

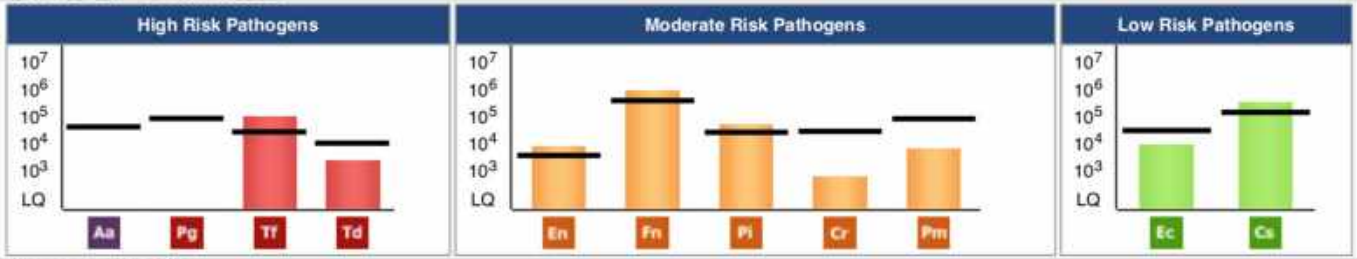
[redacted]

COMPARISON OF TEST RESULTS

MyPerioPath - Previous Result



MyPerioPath - Current Result



Summary of Results

Total Bacterial Load

34%
Increase

Since patient's last test on 12/11/2018:

- Of concern, since the last test submitted 7 months 6 days ago, the clinical management of this patient has resulted in 34% increase in periodontal pathogen (burden) load.
- The results show an increase in the level of the red (Tf, Td) , orange (En, Fn, Cr, Pi, Pm) complex pathogens. This increase in the bacterial level may be due to a prolonged time since the last testing, the failure of treatment, or changes in the patients general state of health.
- These results would likely not be associated with a reduction in either oral or systemic inflammation. Consequences of high pathogenic bacteria present for years and decades add significantly to the risk of life threatening diseases beyond the mouth.
- For most treatment protocols, the maximal reduction in pathogen (burden) load is observed when follow-up testing is performed between 6-12 weeks. This sample was collected at 31 weeks 1 day from the previous test.

Clinical Comparison	Previous 12/11/2018	Current 07/17/2019
Total # Bacteria Present	7	9
Total # Bacteria Above Threshold	3	5
Deepest Pocket	--	--
Localized Infection	☐	☐
Generalized Infection	☐	☐
Inflammation/Redness	☐	☐
Bleeding on Probing	☐	☐
Bone Loss	☐	☐
Discharge	☐	☐
Halitosis/Malodor	☐	☐
Not Provided	☑	☑

A follow-up test is recommended to monitor the effectiveness of current treatments and to determine the type and frequency of future care.

Seven Month Follow up Appointment

Patient is doing well....has some good days and bad days, now has sparkle in her eyes…husband says: *"I have my wife back."* She can walk up and down the mountain where they live, problem solving and planning is still difficult

Now able to drive 2 hrs on her own, she is able to take care of her own medications/supplements.

Rec. F/U with integrative dentist to address periodontal disease more aggressively

Take Home Pearls

Probiotics are useless without dietary prebiotics

Investigate Stealth Infections as a cause of Neuroinflammation

Caffeine in coffee and sulforophane in Broccoli both help with blood brain barrier function.

NAC (600mg bid), Lipoic acid 300-600mg/day and Lithium orotate (5 to 30 mg/day) are powerful neuroprotective agents