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Can we vaccinate against depression?

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Major depression is common in the context of autoimmune and inflammatory diseases and is frequently associated with persistently raised levels of proinflammatory cytokines and other markers of inflammation, even in the absence of another diagnosable immune pathology to account for these findings. Therefore immunoregulation-inducing vaccines or manipulations of the gut microbiota might prevent or treat depression. These strategies are already undergoing clinical trials for chronic inflammatory disorders, such as allergies, autoimmunity and inflammatory bowel disease. In this article, we summarize data suggesting that this approach might be effective in depression and encourage the initiation of clinical vaccination trials in this disorder.

The notion that it might be possible to vaccinate against some forms of depression is on the basis of the fact that (i) many individuals with depression who are otherwise healthy demonstrate a state of persistent inflammatory activation characterized by increased levels of circulating cytokines and other immune mediators and reductions in anti-inflammatory and/or immunomodulatory cytokines, and (ii) recent evidence supports a causal role for proinflammatory cytokines in depression. Thus, a vaccine to treat or prevent depression might be designed to enhance systemic immunoregulation, thereby correcting the imbalance between anti-inflammatory and proinflammatory cytokines frequently seen in this disorder.

Our route to this conclusion commenced with the hygiene hypothesis, which has recently been reformulated as the 'Old Friends' hypothesis. This hypothesis states that one reason for the dramatic increase in chronic inflammatory disorders (e.g. inflammatory bowel disease, allergic disorders, and autoimmunity) in the modern world is a depletion from the environment of microorganisms with which humans co-evolved, and which had come to have a crucial role in establishing immunoregulatory circuits in the human body (Box 1). These organisms, which were associated with feces, mud and animals have been lost from the modern concrete urban human environment leading to faulty immunoregulation and contributing to the emergence and rapid increase in the chronic inflammatory disorders listed above. As similar to these inflammatory disorders, major depression has also increased in prevalence in the modern world (see discussion below), consistent with the possibility that loss of contact with immunoregulatory microorganisms might contribute to depression by promoting the states of increased inflammation that are commonly observed in the disorder.

However the 'Old Friends' mechanism cannot be considered in isolation. One of the most important health-related scientific discoveries in recent years is the fact that manipulations of the immune system might act indirectly through changes in the gut flora, also known as the gut microbiota. For example, Wen and colleagues (2008) showed that specific-pathogen free (SPF) non-obese diabetic (NOD) mice, which spontaneously develop autoimmune type 1 diabetes, are protected from this autoimmune disease following knockout of the gene encoding MyD88 (an adaptor for multiple Toll-like receptors). However, this did not mean that MyD88 was directly involved in the autoimmune response to β-cells in the pancreas. Rather, it emerged that the modification of the immune system resulting from knocking out MyD88 caused profound changes in the interactions between the

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BOX 1

Identity and mechanisms of immunoregulatory 'Old Friends'.

The hygiene hypothesis, or as we prefer to call it, the 'Old Friends' hypothesis, suggests that one reason for the increasing incidences of chronic inflammatory disorders [49] in developed countries since the mid-19th Century is the depletion from the urban environment of organisms that accompanied mammalian evolution. Because it was necessary for these organisms to be tolerated, co-evolutionary forces ensured that they came to have essential roles in the optimal functioning of immunoregulatory pathways [27]. Overwhelming data show that a failure of immunoregulatory mechanisms reliably leads to simultaneous increases in the diverse types of immunopathology observed with increasing frequency in the modern world. For example, genetic defects of Foxp3 (a transcription factor important to immunoregulation) leads to the X-linked autoimmunity–allergic dysregulation syndrome (XLAAD), which includes aspects of allergy, autoimmunity and enteropathy [50].

The organisms involved are associated with feces (e.g. microbiota, helminths and fecal-oral transmission of infections/carrier states), animals (farm or pet) and mud [3,16,51–54]. Humans were continuously exposed to these elements and environments from early in evolution, right through the Neolithic age with the introduction of agriculture and husbandry, and were not deprived of them until the 2nd Epidemiological Transition (urbanization). Further details are reviewed elsewhere [27]. Table 1 lists some animal models of chronic inflammatory disorders that can be treated with immunoregulation-inducing 'Old Friends' such as helminths.

The microbiota constitute a subset of the 'Old Friends'. The intestinal flora of urban Europeans is totally different from that of African villagers living in a manner similar to the lifestyle of early man [3]. This difference is attributable to different diet, differential exposure to 'Old Friends', including helminths, (which modulate the bacterial flora), and antibiotic use in urban populations.

Germ-free mice have poorly developed lymphoid systems and are susceptible to inflammatory disorders. Colonization with certain organisms reverses these defects [55]. The precise pattern of restoration depends on the organisms used [56], but usually results in expansion of the Treg population [57,58], resulting in resistance to colitis and to systemic IgE responses [58]. The mechanisms have not all been worked out, but *Bacteroides fragilis* releases a polysaccharide that directly drives expansion of Treg and so restrains Th17 activity [54].

Intestinal helminths must also be regarded as part of the co-evolved microbiota. One intensely studied species in rodent models is *Heligmosomoide polygyrus*. This helminth exerts immunoregulatory effects via at least three pathways: (i) modulation of the bacterial microbiota [59], (ii) modulation of the maturation of intestinal dendritic cells (DC) [60], and 3) like *Bacteroides fragilis*, by directly driving proliferation of Treg [61].

immune system and the microbiota [1]. Consequent changes in the composition of the gut microbiota were responsible for the immunoregulatory effect that blocked the diabetogenic autoimmune process (Box 1).

Diminished exposure to intestinal helminths, fecal organisms of other humans and environmental fermenting species, such as Lactobacilli, among others, almost certainly contribute directly to the marked changes in gut flora seen in individuals in the modern world when compared with people living in more traditional societies. Alterations in the systemic load of 'Old Friend' microorganisms will change the composition of gut flora indirectly by changing immune system functioning more generally. Thus, changes in the microbiota observed in the modern world must be regarded as part of the 'Old Friends' hypothesis. Moreover, because the gut signals to the brain through multiple pathways (described later), modifications of the immune system that affect the gut microbiota will have consequences for the central nervous system (CNS), not only through immunoregulation, but also through the complex gut-brain axis.

The gut microbiota are changing for multiple reasons in addition to diminished exposure to the 'Old Friends'. For example, another major modulator of the microbiota is diet [2]. This factor was thought to be the major cause of the dramatic differences between the microbiota of Italians and individuals from a traditional village in Burkina Faso [3]. These relationships, which also include other contributory factors, such as diminished vitamin D3 levels and exposure to the T helper 17- (Th17-) driving dioxin pollutants, are indicated in Figs 1,2.

It is clearly beyond the remit of this article to explore all of these interacting factors in detail, but in the text that follows we do treat the 'Old Friends' mechanism and the immunoregulatory role of the gut microbiota as a continuum. Neither can be considered in isolation, and any vaccine that affects immunoregulation will also modulate the gut microbiota, and might indeed exert some of its systemic effects indirectly through the microbiota.

Depression is associated with inflammation

As outlined in the previous section and in Box 1, our changing lifestyle, which results in reduced contact with immunoregulatory organisms from animals, mud and feces, is one factor leading to alarming increases in chronic inflammatory disorders (allergies, autoimmunity, inflammatory bowel disease) in developed countries. All of these conditions are comorbid with depression. Why is this? And why is the prevalence of depression also increasing? There is overwhelming evidence that depression is associated with inflammation. There are many inflammation-inducing risk factors for depression, which include obesity (resulting in release of proinflammatory cytokines by adipose tissue and by macrophages aggregated around visceral fat stores), chronic inflammatory disorders (the incidence of depression correlates with the level of circulating proinflammatory cytokines), psychosocial stress, social isolation, sedentary life-style, smoking, female sex, and diminished sleep. Indeed depression is often accompanied by raised levels of proinflammatory cytokines, even when no other illness is detected. This has been reviewed in depth, and fully referenced [4]. Recent meta-analyses have confirmed that there is a dose–response relationship between depression and the inflammatory markers C-reactive protein (CRP), interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α [5,6]. By contrast, patients with major depressive disorder (MDD) have reduced circulating levels of the major regulatory mediators, IL-10 and transforming growth factor beta (TGF-β) [4].

Does this mean that the proinflammatory cytokines themselves, when chronically raised, drive symptoms of depression? (Note that brief exposure to raised cytokines can have the reverse effect, but this transient outcome is discussed later). There is strong evidence that prolonged elevations of proinflammatory cytokines do indeed drive depression. Administration of proinflammatory cytokines [IL-2, interferon-alpha (IFN-α), used as treatments for hepatitis or some

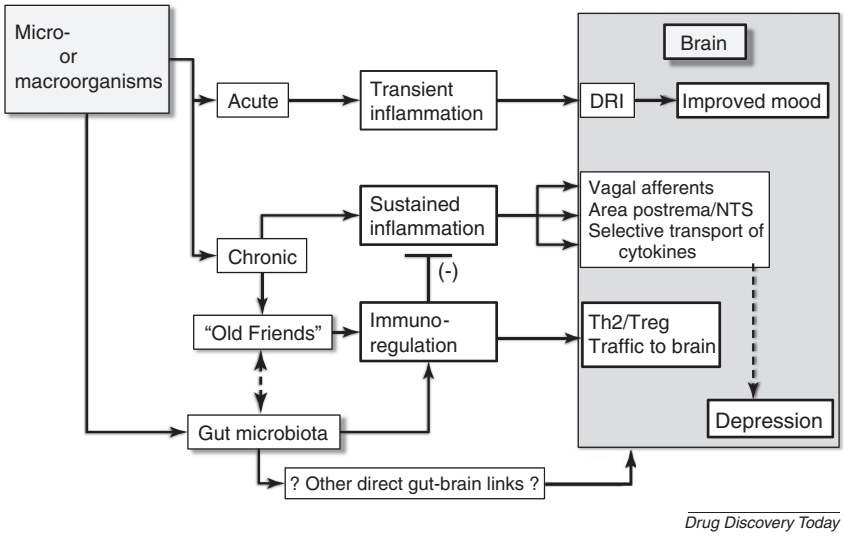

FIGURE 1

Pathways by which micro- or macroorganisms can modulate cognitive function and mood (excluding direct infection of the central nervous system (CNS)). Acute transient inflammation induced by infection with micro- or macroorganisms can activate serotonergic neurons in the interfascicular part of the dorsal raphe nucleus (DRI) through activation of spinal afferents. This results in an antidepressant-like behavioral effect demonstrable in standard laboratory models. By contrast, chronic inflammation, perhaps signaling through vagal sensory afferents, or following entry of cytokines into the CNS through the circumventricular organs where there is no blood–brain barrier (e.g. area postrema and/or nucleus of the solitary tract (NTS)), or through selective transport mechanisms, is associated with inhibition of DRI neuron activity and with depression. Chronic inflammation induced by infection with micro- or macroorganisms has multiple consequences. First, it alters the microbiota, both directly by local competition, symbiosis or colonization, or indirectly by modulating the immune system, and so altering the relationship between the gut immune system and the microbiota, leading to changes in the balance of strains and species, and to changes in microbiota-induced immunoregulation (in addition there are other poorly understood gut-brain links that also modulate CNS function; see main text). Finally the 'Old Friends' (a subset of the microbiota, and other organisms not associated with the gut, with which man has a long association) activate immunoregulatory pathways (regulatory dendritic cell (DCreg) and regulatory T cell (Treg)) leading to termination of inappropriate chronic inflammation and thus to diminished depressogenic cytokine signals. This figure illustrates multiple potential sites for therapeutic intervention that are discussed in the main text. *Abbreviations*: DRI: interfascicular part of the dorsal raphe nucleus; NTS: nucleus of the solitary tract; Treg: regulatory T cells; Th2: T helper 2 cells.
Figure adapted from Ref. [62].

cancers], induces states strikingly similar to naturally occurring depression that are treatable with antidepressant drugs, confirming the cause–effect relationship [7,8]. Presumably this also accounts for the common occurrence of mild transient depression after influenza, a virus that drives high levels of IFN-α.

Another type of evidence comes from monitoring the consequences of treating depression. Treatments that reduce depressive symptoms simultaneously lower levels of inflammatory mediators, or increase levels of regulatory ones (reviewed and referenced in [4]). By contrast, stressors, which promote depression, tend to downregulate immunoregulation, as evidenced by decreases in cluster of differentiation 25 $(CD25)^+$ T regulatory cells (Treg), IL-10, and forkhead box P3 (Foxp3), a master regulator in the development and function of Treg [9,10].

Cumulatively, these findings, together with the well-documented ability of proinflammatory cytokines to drive 'sickness behavior' in rodents [11] (an effect that can be opposed by IL-10), suggest that prolonged imbalance between proinflammatory and anti-inflammatory mediators can lead to depressive symptoms.

Rates of depression and the environment

While not universally accepted [12], significant evidence suggests that rates of MDD are increasing in developed countries, as would be expected if there is an association with chronic inflammatory disorders, stress and obesity (discussed and referenced in [4]). Moreover, moving from the developing world to the USA increases the risk for MDD. For example, Mexican immigrants to the USA have rates of depression similar to those seen in Mexico, whereas individuals of Mexican descent born in the USA have higher rates of MDD that are equivalent to the U.S. population at large, suggesting that it is American life itself, or an American upbringing, and not acculturation shock, that accounts for the increase [13]. Interestingly there is a significantly higher risk of mood and anxiety disorders in urban populations, compared to rural ones [14], which is compatible with a mechanism involving the 'Old Friends' hypothesis, as outlined in Box 1. A recent functional magnetic resonance imaging (fMRI) study compared the effects of social stress on individuals brought up in urban or rural environments. Urban versus rural upbringing correlated with significant differences in the activation of the perigenual anterior cingulate cortex, a region involved in control of negative affect and stress responses [15]. The authors attributed this to putatively different levels of social stressors in individuals with urban versus rural upbringing, but would social stressors in children differ significantly in the two environments in a wealthy European country (Germany)? It is equally probable that the findings were owing to the 'Old Friends' mechanism, leading to diminished regulation of proinflammatory mediators in those subjects who had an urban upbringing. Indeed the protective effects of the German farming environment against allergies and early onset inflammatory bowel disease require that the child be exposed to the farming environment during the first 2.5 years of life. . .a rural

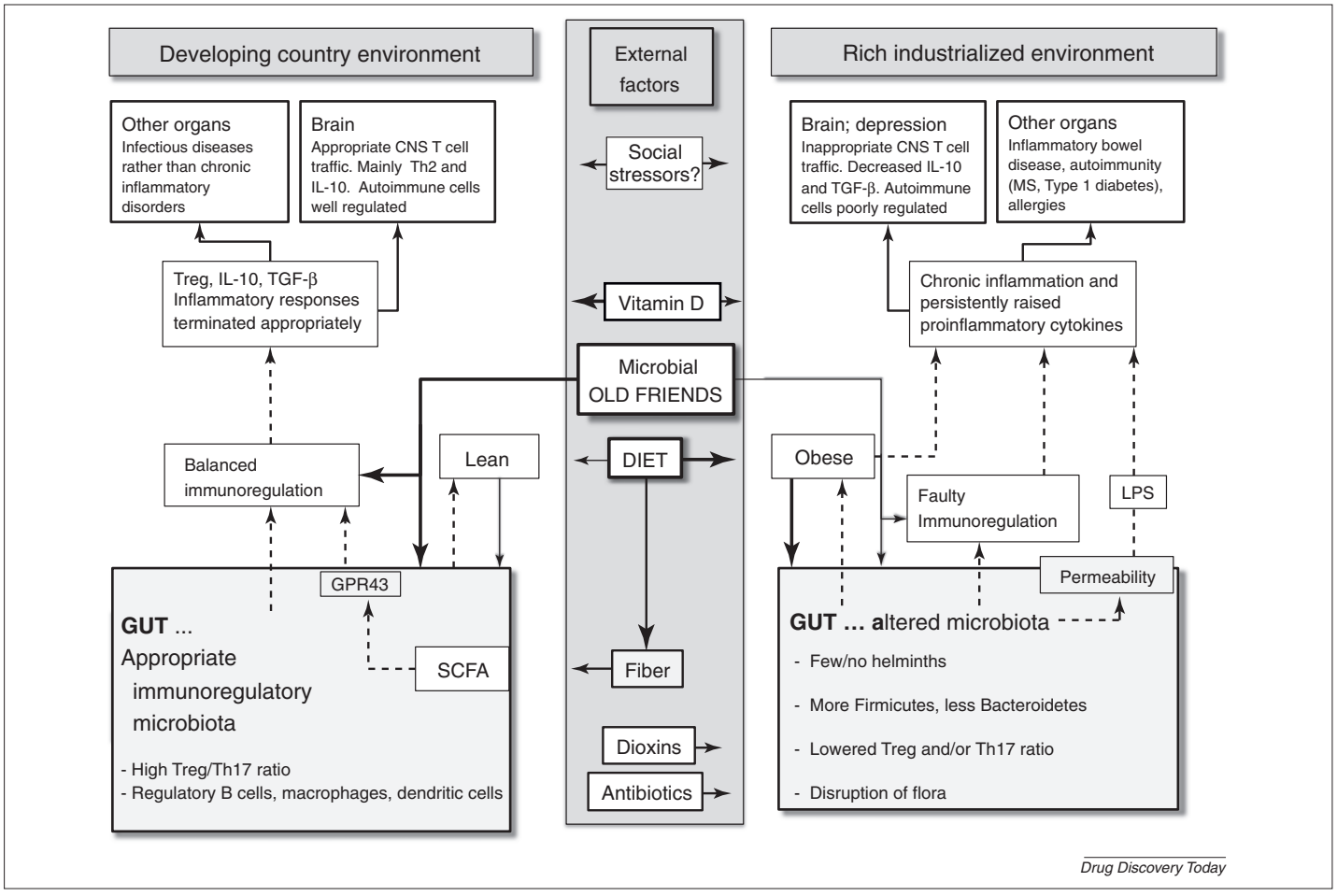

FIGURE 2

In the environments of developing countries contact with microbial 'Old Friends' primes immunoregulatory circuits, and, in concert with an appropriate diet, maintains an immunoregulatory microbiota. The diet is also rich in fiber that is fermented to short chain fatty acids (SCFA). These SCFA act through the G protein-coupled receptor 43 (GPR43) to exert further anti-inflammatory effects. Individuals tend to be lean, which limits release of proinflammatory mediators, and reduces absorption of lipopolysaccharide (LPS). Vitamin D, generated by regular exposure to sunlight, assists immunoregulation, whereas dioxins (which drive T helper 17 cell (Th17) cells via the aryl hydrocarbon receptor (AHR) or antibiotics (which disturb the microbiota) are avoided. Because immunoregulation is intact, chronic inflammatory diseases are uncommon, although infectious diseases can be widespread. The brain is not exposed to high ratios of proinflammatory to anti-inflammatory mediators, but rather to T helper 2 cell (Th2) cells and interleukin-10 (IL-10). By contrast, in affluent industrialized environments (right panel), the coevolved inducers of immunoregulatory circuits (the 'Old Friends') are diminished or absent, and the microbiota are profoundly altered, leading to faulty immunoregulation. This may be aggravated by deficient vitamin D, and exposure to dioxins. Inappropriate diet predisposes to obesity. Fat tissue releases proinflammatory mediators and fatty diets promote absorption of LPS, exacerbating the effect of disturbed bowel flora on gut permeability, and leading to further release of proinflammatory mediators. As outlined above, persistently raised inflammatory mediators can lead to depressive symptoms, and poor immunoregulation can lead to susceptibility to inflammatory bowel disease, autoimmunity and allergic disorders. *Abbreviations*: CNS: central nervous system; GPR43: G protein-coupled receptor 43; IL: interleukin; LPS: lipopolysaccharide; MS: multiple sclerosis; SCFA: short chain fatty acids; TGF-β: tumor growth factor beta; Th2: T helper 2 cell; Th17: T helper 17 cell; Treg: regulatory T cells.

upbringing [16]. The authors of the fMRI study did not measure the stress-induced levels of circulating proinflammatory cytokines in the two populations. The 'Old Friends' view of the data would postulate higher levels in the subjects who had urban upbringings.

'Old Friends', gut microbiota and signaling to brain

There is no space here to review all the data that definitively demonstrate the immunoregulatory effects of the 'Old Friends' and gut microbiota or the molecular mechanisms involved, although some of this work is described and referenced in Box 1. To provide examples, we show in Table 1 some of the many experimental models in which a variety of helminths have shown immunoregulatory efficacy. This was reviewed recently [17]. A close relationship between helminth load and Treg in multiple sclerosis has been reported [18,19]. A nice example of a human clinical trial using a helminth to treat multiple sclerosis has been published by Fleming and colleagues [20].

In addition to interacting with the 'Old Friends' to set up immunoregulatory circuits, the gut microbiota affect CNS function by several other routes. First, commensal organisms produce neuroactive molecules, such as serotonin, melatonin, gamma-aminobutyric acid, catecholamines, histamine and acetylcholine [21]. Restoring the gut microbiome of germ-free mice resulted in a 2.8-fold increase in plasma serotonin [22].

Experiments where microbiota were modified by antibiotics, or in which SPF gut microbiota was transferred into germ-free animals, demonstrated that the microbiota modulate brain-derived neurotrophic factor (BDNF) expression in the hippocampus, brain

TABLE 1

Experimental models in which helminths have shown beneficial, immunoregulatory effects

Type of animal model[a]	Helminth
Allergy	*Heligmosomoides polygyrus* *Schistosoma mansoni* *Strongyloides stercoralis*
Autoimmunity	
Type 1 diabetes	*Schistosoma mansoni* *Trichinella spiralis* *Heligmosomoides polygyrus*
Experimental autoimmune encephalomyelitis (EAE)	*Schistosoma mansoni* *Schistosoma japonicum* *Trichinella spiralis* *Fasciola hepatica*
Colitis	*Heligmosomoides polygyrus* *Schistosoma mansoni* *Hymenolepis diminuta*
Arthritis	*Schistosoma japonicum* *Schistosoma mansoni* *Hymenolepis diminuta*

[a] Reviewed and referenced in [17].

biochemistry and behavior [23]. This is consistent with a recent report of behavioral changes in mice in which the microbiota had been perturbed by dietary changes [24].

Interestingly, germ-free (GF) mice have not only defective immunoregulation [25], but also abnormally large hypothalamic-pituitary-adrenal (HPA) axis responses to restraint stress [26], and this can be corrected by mono-association with *Bifidobacterium infantis* before six weeks of age. This requirement for early reconstitution is perhaps reminiscent of the requirement for early exposure to rural upbringing or to the farming environment, as discussed earlier in relation to the hygiene hypothesis, susceptibility to allergic disorders, and changes in the CNS response to stress as detected by fMRI.

Can we use microorganisms to prevent or treat depression?

So there is evidence that MDD is increasing as a result of environmental factors, in parallel with chronic inflammatory diseases where, as in MDD itself, there is an underlying imbalance between anti-inflammatory and proinflammatory mechanisms [27]. So we speculate that vaccines or probiotics on the basis of 'Old Friends' might treat or protect against MDD, either by driving systemic immunoregulation, or by other subtle immune system-related changes to the microbiota and the gut-brain axis.

Bacterial probiotics

Chronic administration of *Bifidobacterium infantis* has been found to protect rats from the depression-like behavioral consequences of subjection to maternal separation stress [28]. Maternal separation also caused increased peripheral release of IL-6 and reduced levels of noradrenaline in the brain, but treatment with *Bifidobacterium infantis* normalized these parameters, again indicating an anti-inflammatory effect [28]. Rats given a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) for two weeks had significantly reduced anxiety-like behavior [29]. Interestingly, the same formulation was tested in humans. Volunteers who took it for 30 days showed significant improvement on scales that are routinely used to measure anxiety, hostility and depression [29]. Similarly, 39 patients suffering from chronic fatigue syndrome (CFS) were randomized to receive either $24x10^9$ colony forming units of *Lactobacillus casei* strain Shirota or a placebo daily. Consumption of the *Lactobacillus* was reported to result in a significant decrease in symptoms of anxiety [30]. While these results are intriguing, we suspect that to make further progress in this area it will be crucial to concentrate on those probiotic strains that promote immunoregulation, which is a property of only a small subset of these organisms.

Helminths

'Probiotic helminths' such as *Trichuris suis* or *Necator americanum* are being studied in several chronic inflammatory disorders. Preliminary results are encouraging [20,31], but regrettably, there has been no attempt to monitor any concomitant effects on mood. As pointed out above, helminths would be expected to directly drive immunoregulation, and also to modulate the microbiota towards a more immunoregulatory pattern. Helminth infection might not provide a final solution; in some individuals, or if the load of worms is too high, they can cause gastrointestinal symptoms. However if trials provide proof-of-principle, there will be greatly increased impetus behind efforts to discover the molecular mechanisms, and to devise vaccines that can replace the living organism.

Vaccines in humans

In contrast to the lack of evidence for mood-relevant effects of helminths, changes relevant to CNS effects have been evaluated in several clinical trials using heat-killed *Mycobacterium. vaccae*. This nonpathogenic environmental saprophyte (an 'Old Friend' from mud) induces Treg that downregulate chronic inflammatory states [32]. *M. vaccae* has undergone clinical trials for allergic disorders, psoriatic arthritis and some cancers. In several studies the patients who had received one or more intradermal injections of a heat-killed preparation of this organism showed unexpected improvements in quality of life scores [33–35]. *M. vaccae* activates human dendritic cells (DC) through the transcription factor cAMP response element-binding protein (CREB) rather than through nuclear factor-kappaB (NFκB) [36], which is interesting because CREB is increasingly implicated in the control of Treg [37]. Moreover CREB becomes phosphorylated (and hence activated) during successful treatment of MDD [38]. We postulate that the beneficial effect on quality of life (and related emotional constructs) seen in trials of *M. vaccae* was owing to correction of the balance of anti-inflammatory to proinflammatory mediators, resulting in cessation of the depressogenic stimulus. However, this is not the only potential antidepressant mechanism identified in studies of *M. vaccae*. Animal studies with *M. vaccae* have revealed a second quite different pathway by which *M. vaccae* might have antidepressant properties, discussed below.

Two other entirely different approaches to vaccination against depression have also been suggested. Both present greater uncertainties and difficulties than that described above, but we outline them below to provide a more complete picture.

Transient acute effects of inflammatory responses

The clinical effects of *M. vaccae* cited above led to an investigation of the CNS effects of peripheral administration of the bacterium in

a mouse model, and to the discovery that intratracheal or subcutaneous administration activated a specific subset of serotonergic neurons in the interfascicular part of the dorsal raphe nucleus (DRI) of mice [39]. This activation of DRI serotonergic neurons was associated with increases in serotonin metabolism within the medial prefrontal cortex (mPFC), an effect that is a common response to acute treatment with diverse classes of antidepressant drugs [40], and consistent with an effect of immune activation on mesolimbocortical serotonergic systems (heavily implicated in control of mood). These effects were temporally associated with reductions in immobility in the forced swim test, which is a standard test for antidepressant drug activity [39]. These neural, neurochemical, and behavioral changes were acute effects observed 6–12 hours after injection, which then waned rapidly. One of us (CAL) is actively investigating the pathway involved, and it might well be possible to exploit the underlying mechanism for more long-term effects by triggering relevant serotonergic effects in a different way, or with an intermittent stimulus.

We emphasize that this transient beneficial effect of peripheral inflammation is clearly distinct from the detrimental effects of sustained inflammatory stimuli, attributable to the long-term raising of the ratio of proinflammatory to anti-inflammatory mediators. We envision that activation of serotonergic neurons by peripheral immune activation, if unabated over a long period of time (weeks or months) would lead to dysregulation of serotonergic systems implicated in antidepressant pathways, and increased vulnerability to MDD. So in the context of established chronic inflammation the potential of *M. vaccae* is not as a transient inflammatory stimulus, but rather as an inducer of immunoregulation and anti-inflammatory mediators that might prevent dysregulation of serotonergic systems caused by chronic inflammatory conditions, as outlined in the previous section.

T cells with specificity for brain

In the previous sections we have discussed induction of immunoregulation in the periphery, and its potential consequences in the context of depression. A few workers have concentrated on the T cells that are normally present in certain compartments of the CNS (cerebrospinal fluid, meninges, subarachnoid space), and argued that T lymphocytes with specificity for brain antigens have a role in neuroprotection in response to tissue damage [41]. Initially Th1 cells were implicated, but more recent data show clearly that, in SPF mice, Th1 cells were protective only in the presence of other cell types, suggesting that the role of the Th1 cells was to attract regulatory cells to the relevant sites (analyzed in detail in [42]). Workers within the same institute also suggested that brain-recognizing lymphocytes could protect mice and rats from behavioral changes triggered by stressors [43,44], although no cell type was identified. More recently Kipnis and colleagues noted that performing cognitive tasks led to accumulation of IL-4-producing T cells in the meninges of rodents. These IL-4-secreting cells then led to alternative macrophage activation [45], and to regulatory events including increased IL-10 and decreased TNF-α. Moreover, cognitive defects were observed in IL-4-knockout mice, or following depletion of T cells from meningeal spaces by treatment with anti-very late antigen 4 (anti-VLA-4) [46].

Thus the issue might now be resolved, with agreement that protecting both structural and functional aspects of neuronal function requires immunoregulatory pathways, perhaps particularly IL-10 [47]. We are uneasy about the expression 'protective autoimmunity' that is often used for this effect, because to most immunologists this will imply proinflammatory effector cells, whereas the emphasis behind this idea as currently articulated is clearly on self-reactive immunoregulatory cells [42,48]. Perhaps 'protective autoimmunoregulation' would be less confusing, and would focus readers' attention on the potential of this process for targeting immunoregulation to the brain. Thus, it is suggested that we could attempt to increase self-reactive T cell traffic into the brain using vaccines that induce T cells (presumably, in view of the most recent work, IL-4$^+$ Th2-like or Treg cells) with specificity for brain antigens [41,43,44,48]. However the risks of inducing autoimmune encephalitis will make it difficult to initiate clinical trials of this type. It might be wise to first prove that there really is a deficit in meningeal Th2-like and/or Treg traffic in MDD and that the phenomena observed in clean SPF mice are not artifacts attributable to abnormally low cell traffic, or to immature IL-10-deficient Treg characteristic of these animals (discussed in detail in [42]).

Concluding remarks

In conclusion, there is overwhelming epidemiological, clinical and experimental evidence suggesting that prolonged increases in levels of proinflammatory mediators can cause symptoms of depression. This fact justifies ongoing clinical trials in depression of treatments such as infliximab, a neutralising antibody to TNF-α. However, we do not yet understand the cascade of events that links chronic inflammation with depression, so it is not clear which molecules should be targeted. Therefore other anti-inflammatory or immunoregulatory strategies including vaccines should be considered. Vaccines might alter fundamental immunoregulatory balances, and might do so in a long-lasting way, requiring minimal intermittent boosting. Since defective immunoregulation secondary to diminished exposure to immunoregulation-inducing 'Old Friends' is implicated in the increases in a range of chronic inflammatory diseases, new immunoregulation-inducing treatment strategies, such as helminths, are entering clinical trials. It would be helpful if such trials were to include simultaneous monitoring of affective and cognitive function. Moreover these 'Old Friends' should also be examined as treatments for MDD in otherwise medically healthy individuals.

In addition, a further unexpected pathway has been discovered, involving specialized serotonergic neurons in the DRI. These can be acutely activated by transient inflammatory stimuli, and this appears to drive a short-term behavioral boost that will be replaced by depression if the inflammatory stimulus is prolonged and unremitting. Nevertheless this pathway might be exploitable when it is fully understood, if it can be triggered in an intermittent manner by an alternative non-inflammatory strategy.

Finally we have referred to studies suggesting that T cells with specificity for brain, which have privileged access to the CNS, might, at least in SPF mice, provide useful local immunoregulatory and anti-inflammatory functions. However, we hesitate to advocate trials of direct immunization with brain antigens or antigen-mimics at this stage, because we do not know enough about how to ensure a regulatory rather than a proinflammatory response. Nevertheless, induction of brain-recognizing regulatory T cells

in humans has been achieved by an indirect strategy. When patients with multiple sclerosis were allowed to become infected with helminth 'Old Friends' these worms exerted a 'Treg adjuvant' effect, resulting in myelin-specific Foxp3$^+$ Treg that secreted IL-10 and TGF-β in response to myelin peptide [18,19]. Therefore such a treatment, or other immunoregulatory vaccinations, might simultaneously induce (i) immunoregulation in the periphery that limits exposure of the brain to circulating proinflammatory mediators, and (ii) specific Treg that can damp down any inflammation in the brain itself.

There is, therefore, a spectrum of immunoregulation-inducing strategies that might need to be adopted to halt the inexorable rise of MDD towards the status of second most important scourge of mankind.

References

1 Wen, L. *et al.* (2008) Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature* 455, 1109–1113
2 Cani, P.D. and Delzenne, N.M. (2011) The gut microbiome as therapeutic target. *Pharmacol. Ther.* 130, 202–212
3 De Filippo, C. *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. U. S. A.* 107, 14691–14696
4 Raison, C.L. *et al.* (2010) Inflammation, sanitation and consternation: loss of contact with co-evolved, tolerogenic micro-organisms and the pathophysiology and treatment of major depression. *Arch. Gen. Psychiatry* 67, 1211–1224
5 Howren, M.B. *et al.* (2009) Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom. Med.* 71, 171–186
6 Dowlati, Y. *et al.* (2010) A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457
7 Musselman, D.L. *et al.* (2001) Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N. Engl. J. Med.* 344, 961–966
8 Capuron, L. *et al.* (2009) Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? *J. Affect. Disord.* 119, 181–185
9 Buske-Kirschbaum, A. *et al.* (2007) Altered distribution of leukocyte subsets and cytokine production in response to acute psychosocial stress in patients with psoriasis vulgaris. *Brain Behav. Immun.* 21, 92–99
10 Freier, E. *et al.* (2010) Decrease of CD4(+)FOXP3(+) T regulatory cells in the peripheral blood of human subjects undergoing a mental stressor. *Psychoneuroendocrinology* 35, 663–673
11 Dantzer, R. *et al.* (2008) Identification and treatment of symptoms associated with inflammation in medically ill patients. *Psychoneuroendocrinology* 33, 18–29
12 Simon, G.E. *et al.* (1995) Is the lifetime risk of depression actually increasing? *J. Clin. Epidemiol.* 48, 1109–1118
13 Vega, W.A. *et al.* (2004) 12-month prevalence of DSM-III-R psychiatric disorders among Mexican Americans: nativity, social assimilation, and age determinants. *J. Nerv. Ment. Dis.* 192, 532–541
14 Peen, J. *et al.* (2010) The current status of urban–rural differences in psychiatric disorders. *Acta Psychiatr. Scand.* 121, 84–93
15 Lederbogen, F. *et al.* (2011) City living and urban upbringing affect neural social stress processing in humans. *Nature* 474, 498–501
16 Riedler, J. *et al.* (2001) Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 358, 1129–1133
17 Osada, Y. and Kanazawa, T. (2010) Parasitic helminths: new weapons against immunological disorders. *J. Biomed. Biotechnol.* 2010, 743–758
18 Correale, J. and Farez, M. (2007) Association between parasite infection and immune responses in multiple sclerosis. *Ann. Neurol.* 61, 97–108
19 Correale, J. and Farez, M.F. (2011) The impact of parasite infections on the course of multiple sclerosis. *J. Neuroimmunol.* 233, 6–11
20 Fleming, J. *et al.* (2011) Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult. Scler.* 17, 743–754
21 Iyer, L.M. *et al.* (2004) Evolution of cell–cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* 20, 292–299
22 Wikoff, W.R. *et al.* (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl Acad. Sci. U. S. A.* 106, 3698–3703
23 Bercik, P. *et al.* (2011) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* 141, 599–609
24 Li, W. *et al.* (2009) Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. *Physiol. Behav.* 96, 557–567
25 Sudo, N. *et al.* (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J. Immunol.* 159, 1739–1754
26 Sudo, N. *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J. Physiol.* 558, 263–275
27 Rook, G.A.W. (2010) 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. *Clin. Exp. Immunol.* 160, 70–79
28 Desbonnet, L. *et al.* (2010) Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* 170, 1179–1188
29 Messaoudi, M. *et al.* (2011) Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br. J. Nutr.* 105, 755–764
30 Rao, A.V. *et al.* (2009) A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathog.* 1, 6
31 Summers, R.W. *et al.* (2005) *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology* 128, 825–832
32 Zuany-Amorim, C. *et al.* (2002) Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat. Med.* 8, 625–629
33 Dalbeth, N. *et al.* (2004) A randomised placebo controlled trial of delipidated, deglycolipidated *Mycobacterium vaccae* as immunotherapy for psoriatic arthritis. *Ann. Rheum. Dis.* 63, 718–722
34 O'Brien, M.E. *et al.* (2004) SRL172 (killed *Mycobacterium vaccae*) in addition to standard chemotherapy improves quality of life without affecting survival, in patients with advanced non-small-cell lung cancer: phase III results. *Ann. Oncol.* 15, 906–914
35 O'Brien, M.E. *et al.* (2000) A randomized phase II study of SRL172 (*Mycobacterium vaccae*) combined with chemotherapy in patients with advanced inoperable non-small-cell lung cancer and mesothelioma. *Br. J. Cancer* 83, 853–857
36 Le Bert, N. *et al.* (2011) DC priming by *M. vaccae* inhibits Th2 responses in contrast to specific TLR2 priming and is associated with selective activation of the CREB pathway. *PLoS ONE* 6, E18346
37 Wen, A.Y. *et al.* (2010) The role of the transcription factor CREB in immune function. *J. Immunol.* 185, 6413–6419
38 Gass, P. and Riva, M.A. (2007) CREB, neurogenesis and depression. *Bioessays* 29, 957–961
39 Lowry, C.A. *et al.* (2007) Identification of an immune-responsive mesolimbocortical serotonergic system: potential role in regulation of emotional behavior. *Neuroscience* 146, 756–772
40 Lowry, C.A. *et al.* (2009) That warm fuzzy feeling: brain serotonergic neurons and the regulation of emotion. *J. Psychopharmacol.* 23, 392–400
41 Kipnis, J. *et al.* (2002) Myelin specific Th1 cells are necessary for post-traumatic protective autoimmunity. *J. Neuroimmunol.* 130, 78–85
42 Rook, G.A. *et al.* (2011) Lymphocytes in neuroprotection, cognition and emotion: is intolerance really the answer? *Brain Behav. Immun.* 25, 591–601
43 Lewitus, G.M. *et al.* (2008) Reducing post-traumatic anxiety by immunization. *Brain Behav. Immun.* 22, 1108–1114
44 Lewitus, G.M. *et al.* (2009) Vaccination as a novel approach for treating depressive behavior. *Biol. Psychiatry* 65, 283–288
45 Derecki, N.C. *et al.* (2011) Alternatively activated myeloid (M2) cells enhance cognitive function in immune compromised mice. *Brain Behav. Immun.* 25, 379–385
46 Derecki, N.C. *et al.* (2010) Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J. Exp. Med.* 207, 1067–1080
47 Xin, J. *et al.* (2011) IL-10 within the CNS is necessary for CD4(+) T cells to mediate neuroprotection. *Brain Behav. Immun.* 25, 820–829
48 Schwartz, M. and Shechter, R. (2010) Protective autoimmunity functions by intracranial immunosurveillance to support the mind: the missing link between health and disease. *Mol. Psychiatry* 15, 342–354
49 Bach, J.F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347, 911–920
50 Wildin, R.S. *et al.* (2002) Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J. Med. Genet.* 39, 537–545

51 McDade, T.W. *et al.* (2010) Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. *Proc. Biol. Sci.* 277, 1129–1137
52 Strachan, D.P. (1989) Hay fever, hygiene, and household size. *Br. Med. J.* 299, 1259–1260
53 Aichbhaumik, N. *et al.* (2008) Prenatal exposure to household pets influences fetal immunoglobulin E production. *Clin. Exp. Allergy* 38, 1787–1794
54 Round, J.L. *et al.* (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332, 974–977
55 Kohashi, O. *et al.* (1985) Reverse effect of gram-positive bacteria vs. gram-negative bacteria on adjuvant-induced arthritis in germfree rats. *Microbiol. Immunol.* 29, 487–497
56 Umesaki, Y. *et al.* (1999) Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. *Infect. Immun.* 67, 3504–3511
57 Geuking, M.B. *et al.* (2011) Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 34, 794–806
58 Atarashi, K. *et al.* (2011) Induction of colonic regulatory T cells by indigenous clostridium species. *Science* 331, 337–341
59 Walk, S.T. *et al.* (2010) Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm. Bowel Dis.* 16, 1841–1849
60 Hang, L. *et al.* (2010) *Heligmosomoides polygyrus* infection can inhibit colitis through direct interaction with innate immunity. *J. Immunol.* 185, 3184–3189
61 Grainger, J.R. *et al.* (2010) Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. *J. Exp. Med.* 207, 2331–2341
62 Rook, G.A.W. *et al.* (2011) Microbes and mood: a new approach to the therapy of depression? *Microbiologist* 12, 32–36