CHAPTER

2

Childhood Microbial Experience, Immunoregulation, Inflammation, and Adult Susceptibility to Psychosocial Stressors and Depression

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INTRODUCTION

This book deals with the role of inflammation and immunity in depression. Our exposures to microorganisms in early life impact on this theme in many ways, and Fig. 1 attempts to provide a simplified summary of the text that follows. Microbes modulate susceptibility to depression via effects on the immune system, local regulation of gut neuroendocrine systems, afferent neuronal signaling, and microbial metabolites that enter the circulation and act on distant targets including the brain. First, microbes drive the development of the immune system by providing the data that the immune system requires before it can function correctly. Second, microbes, not only mostly from mother and other family members but also to a lesser extent from the environment, constitute the symbiotic microbiotas that colonize our body surfaces, notably the gut, airways, skin, and mucosal surfaces of the urinary and reproductive tracts. Vertebrates, which evolved about 500 million years ago, rapidly developed a very

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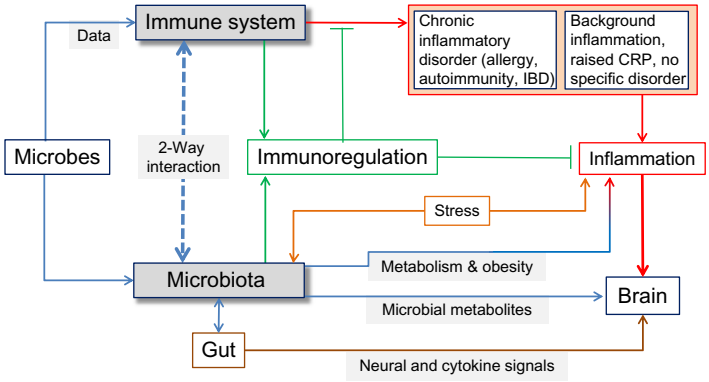

FIG. 1 Overview of the ways in which exposure to microorganisms and parasites can influence the brain via effects on the immune system and microbiota that modulate inflammation. Immunoregulatory microbial signals include metabolites and are illustrated in Fig. 2. Because the microbiota is critically involved in metabolism, obesity, and responses to psychosocial stressors, the role of these factors in depression is also modulated by microbial exposures. *Abbreviations*: CRP, C-reactive protein; IBD, inflammatory bowel disease.

complex and diverse gut microbiota that took on crucial roles in the development and subsequent function of essentially all organ systems, including the gut, immune system, and brain (McFall-Ngai et al., 2013). Some of the effects on the brain are mediated by microbial metabolites that are only beginning to be explored. Most evolutionary biologists now think that the adaptive immune system (which invertebrates do not have) evolved to enable the vertebrate immune system to control and regulate this very complex and physiologically essential microbiota (we might almost say it evolved to help "farm" the microbiota) while simultaneously excluding pathogens, which we can define as organisms that cause damage or disrupt the human-microbiota ecosystem (Pancer & Cooper, 2006). But communication between the microbiota and the immune system goes both ways, and in concert with the data from infections and environmental organisms, the microbiota plays a major role in setting up the regulatory mechanisms that limit and terminate inflammatory processes. Fig. 1 shows inflammation, highlighted in red, associated with chronic inflammatory disorders, such as allergies, autoimmune diseases, and inflammatory bowel diseases (IBDs), and also inflammation manifested as raised C-reactive protein or inflammatory cytokine levels in the absence of any of these diagnosable inflammatory disorders. These are all situations in which failing immunoregulation contributes to inflammation, and all are associated with increased risk of psychiatric problems. Moreover, inflammation can also be caused by poorly regulated metabolism and obesity, in which the microbiota again plays a major role. Finally, Fig. 1 highlights inflammation due to stress. But the inflammatory response to stress and the subsequent behavioral changes are again modulated by the microbiota and attenuated by the regulatory arms of the immune system. In conclusion, childhood microbial exposures, by supplying and modifying the microbiota, modifying the regulation of the immune system, and modifying the regulation of the microbiota *by* the immune system, have major effects on our susceptibility to some psychiatric disorders.

In much of what follows, we are forced to discuss the increases in disorders of immunoregulation indicated in Fig. 1 as surrogates for depression, because these are easily diagnosed, common, and intensively studied, though data on depression are used when available, particularly toward the end of the chapter. But it should be remembered that, as indicated in Fig. 1, depression is frequently comorbid with the chronic inflammatory disorders and associated with raised levels of blood biomarkers of inflammation (Hodes, Menard, & Russo, 2016; Maes, 1999; Raison, Lowry, & Rook, 2010). For example, prior hospitalization due to an autoimmune

disease has been associated with a 45% increased risk of subsequently developing mood disorder diagnosis (Benros et al., 2013). Migration, urbanization, modern medicine, and high-income lifestyle all lead to the loss of exposure to the organisms with which we coevolved ("old friends") and to increases in inflammatory and psychiatric disorders (reviewed and referenced in Rook, Raison, & Lowry, 2014). If we can understand these effects, we may be able to intervene and to counteract the trends toward higher incidences of inflammation and depression.

MICROBIAL EXPOSURES AND HUMAN EVOLUTION

Before analyzing these issues in greater depth, we need to identify the groups of organisms with which humans coevolved, and that might have become physiological necessities. The notion that modern life might deprive us of essential exposures initially emerged as "the hygiene hypothesis," following the observation that allergies are less frequent in children brought up with older siblings (Strachan, 1989). It was suggested that older siblings provided increased exposure to childhood infections that might somehow protect from allergic disorders. This was a valuable insight, but it implied a crucial role for the common infections of childhood and for hygiene, whereas neither implication is correct. The childhood infections are mostly "crowd" infections such as measles that either kill the host or induce solid sterilizing immunity, so they could not survive in isolated Paleolithic hunter-gatherer groups (Wolfe, Dunavan, & Diamond, 2007). They did not coevolve a necessary immunoregulatory role, and they do not protect from chronic inflammatory disorders and often actually trigger them (Bremner et al., 2008; Yoo, Tcheurekdjian, Lynch, Cabana, & Boushey, 2007). They are recent arrivals in human communities, endemic only since populations have increased (Wertheim & Kosakovsky Pond, 2011). Meanwhile, implicating hygiene was a reasonable guess, but as will be demonstrated later, hygiene is a minor factor in the contemporary reduction in microbial exposures. Therefore, we prefer terms such as the "biodiversity" or "old friends" hypothesis (Rook, 2010; von Hertzen, Hanski, & Haahtela, 2011), which place emphasis on our evolutionary heritage and are leading to the identification of relevant organisms and mechanisms.

Microbiota

Humans evolved in small hunter-gatherer groups. As outlined in the introduction, humans, like all vertebrates, were colonized internally and externally by a vast range of symbiotic species including viruses, archaea, bacteria, fungi, protozoa, and even multicellular mites found in hair follicles and sebaceous glands. These diverse organisms constitute the microbiotas of epithelial linings, including the skin, genitourinary system, airways, oropharynx, and gut. At least 50%, perhaps more, of the cells that make up our bodies are microbial (Sender, Fuchs, & Milo, 2016), and they contribute far more genes, DNA, and metabolic pathways than are encoded in our human genomes (O'Hara & Shanahan, 2006). Studies of human metabolomics reveal that much of "our" metabolism is in fact microbial (Wikoff et al., 2009).

Spores

The issue of spores has been neglected. Spores are remarkably resistant and can remain viable for thousands, possibly millions of years (reviewed in Nicholson, 2002). They are relevant in two contexts. First, about 1/3 of the organisms in the gut microbiota are spore-forming, and spores are readily demonstrable in human feces (Hong et al., 2009a). Human feces average up to 10^4 spores/g, while soil contains approximately 10^6 spores/g (Hong et al., 2009b). Wherever humans have lived, the natural environment is

inevitably seeded with human gut-adapted bacterial strains. A recent study revealed that the spore-forming strains within the human microbiota are more diverse than nonspore-forming bacteria and show a higher species turnover or a greater shift in relative abundance over the course of a year (Browne et al., 2016). Therefore, it is possible that when a gut organism becomes extinct as a result of dietary inadequacy or antibiotic misuse (Cox et al., 2014; Sonnenburg et al., 2016), it can be "reinstalled" via spores from the environment.

Other spore-forming organisms from the environment might also be important despite not being definite components of the human microbiota. Spores of *Bacillus subtilis* can germinate in the small bowels of mice and rabbits (Casula & Cutting, 2002; Tam et al., 2006) and also humans (Hong, To, et al., 2009b). Moreover, after germination, they replicate in the small bowel and then resporulate as they enter the colon. This might be very relevant to the "old friends" mechanism, particularly to the clear importance of exposure to animals, agricultural land, and green spaces. After germinating in the small bowel, these organisms will provide data to the immune system in the ileum where dendritic cells sample gut contents and where recently ingested organisms can constitute a significant proportion of the microbes present (Schulz & Pabst, 2013).

Environmental Microorganisms

In addition to spores of gut-adapted organisms discussed above (Browne et al., 2016; Mulder et al., 2009), our ancestors were also exposed to many other microorganisms from the natural environment, many of which would have had significant immunologic impact, even when not able to establish themselves within the microbiotas. Large epidemiological studies demonstrate that living close to the natural rural or coastal environment, often denoted "green space or "blue space," respectively, reduces overall mortality, cardiovascular disease, and depressive symptoms and increases subjective feelings of well-being (Maas, Verheij, Groenewegen, de Vries, & Spreeuwenberg, 2006; Mitchell & Popham, 2008; Wheeler, White, Stahl-Timmins, & Depledge, 2012). The beneficial effects of exposure to green and blue space are particularly prominent in urban individuals of low socioeconomic status who tend to be most severely deprived of green space (Dadvand et al., 2012; Maas et al., 2006; Mitchell & Popham, 2008; Wheeler et al., 2012). It used to be assumed that these effects are explained by psychological mechanisms, but this view is untenable and supported only by experiments that lack relevant controls (Rook, 2013). While there undoubtedly are health benefits attributable to relaxation induced by exposure to the delights of nature and also benefits from accompanying exercise and sunlight, there is solid evidence for biological effects on the immune system mediated by exposures to environmental microorganisms.

Also, supporting the importance of natural environments in immunoregulation are studies demonstrating that exposure of pregnant mothers or infants to the farming environment protects the child against allergic disorders and juvenile forms of IBD (Radon et al., 2007; Riedler et al., 2001). This protection is attributable to airborne microbial biodiversity that can be assayed in children's bedrooms (Ege et al., 2011). Similarly, in a study performed in Finland, mere proximity to agricultural land rather than to urban agglomerations increased the biodiversity of skin microbiota; reduced atopic (allergic) sensitization; and increased release by blood cells of IL-10, an *anti*-inflammatory mediator (Hanski et al., 2012). It is important to note that in this study, hygiene was a constant, not a variable. The effect of the environment was seen in the presence of universally high levels of home hygiene. Most recently, studies of dust extracts obtained from Amish and Hutterite homes suggest that effects of

sustained microbial exposures on innate immune function can explain the low incidence of asthma and allergic sensitization in Amish children (Stein et al., 2016).

Some of the relevant microbiota come from animals. Contact with cows and pigs protects against allergic disorders (Riedler et al., 2001; Sozanska, Blaszczyk, Pearce, & Cullinan, 2013). Contact with dogs, with which humans have coevolved for many millennia (Axelsson et al., 2013; Thalmann et al., 2013), also protects from allergic disorders (Aichbhaumik et al., 2008; Ownby, Johnson, & Peterson, 2002). Dogs greatly increase the microbial biodiversity of the home (Dunn, Fierer, Henley, Leff, & Menninger, 2013; Fujimura et al., 2010). In a developing country, the presence of animal feces in the home correlated with better ability to control background inflammation in adulthood (McDade et al., 2012), and in Russian Karelia (where the prevalence of childhood atopy is four times lower and type 1 diabetes is six times lower than in Finnish Karelia), house dust contained a sevenfold higher number of clones of animal-associated species than was present in Finnish Karelian house dust (Pakarinen et al., 2008). A recent study investigated 10,201 participants aged 26–54 years from 14 countries and generated a "biodiversity score" based on reported childhood exposures to farms, rural versus urban environment, cats, dogs, day care, bedroom sharing, and older siblings. It emerged that a high biodiversity score correlated with reduced allergic sensitization and improved lung health (Campbell et al., 2017). The routes and mechanisms involved in these effects on immunoregulation are discussed later.

Old Infections

Finally, there are certain "old" infections that established lifelong carrier states or subclinical infections and so were able to survive within small hunter-gatherer groups. This term "old infections" was used by Jared Diamond and his colleagues in their classic paper in 2007 (Wolfe et al., 2007). In order to persist in small hunter-gatherer groups, the old infections had to avoid inducing sterilizing immunity or killing the host. And in order to maintain the health of the host, they had to be tolerated. Thus, they drive regulatory anti-inflammatory responses. (These old infections must not be confused with "old friends," a term used to include *all* the categories of organism with which humans coevolved.) The old infections include ancestral strains of *Mycobacterium tuberculosis*, *Helicobacter pylori*, gut helminths and blood nematodes, and hepatitis A virus. Analysis of their phylogenetic trees and comparison with the human phylogenetic tree reveal how the old infections coevolved and spread over the globe with human populations (Galagan, 2014; Linz et al., 2007; Wolfe et al., 2007). There are many examples of the immunoregulatory roles of these old infections. Deworming in pregnancy increases the risk of eczema and wheeze in the resulting infant (Mpairwe et al., 2011). Tuberculin-positive children are less likely to have allergic rhinitis or positive allergen skin-prick tests (Obihara et al., 2005), and individuals carrying *H. pylori* are also somewhat protected from allergic disorders (Hussain et al., 2016).

These three categories of organism—microbiota, environmental organisms, and old infections—were constantly present and had to be tolerated and so coevolved roles in setting up immunoregulatory pathways.

INNATE AND ADAPTIVE IMMUNE SYSTEMS REGULATE MICROBIOTA

How does the immune system regulate the microbiota, and what is the role of host genetics? The microbiomes of monozygotic (MZ) twins are more similar than those of dizygotic (DZ) twins. Using a large cohort of MZ and DZ twins, it was possible to identify many microbial taxa of which the abundance was influenced by host genetics, including one (*Christensenella minuta*)

that correlated with reduced obesity in the human subjects, and was shown to oppose obesity in an animal model (Goodrich et al., 2014). This important observation indicated that host genes can influence phenotype by controlling the organisms present in the microbiota (Goodrich et al., 2014, 2016). Much of this genetic effect is mediated via the immune system.

Innate Immune System and Microbiota

Some of this regulation of the microbiota is mediated via the innate immune system of which Toll-like receptors (TLR) and inflammasomes are essential components. If the gene encoding TLR5 is knocked out, mice develop the metabolic syndrome (hyperlipidemia, hypertension, insulin resistance, and adiposity) and altered microbiota. This altered microbiota will induce similar physiological changes when transferred to wild-type germ-free mice (Vijay-Kumar et al., 2010). Similar changes occur if components of inflammasomes are disabled. Inflammasomes are multiprotein oligomers of variable composition expressed in myeloid cells. They promote the maturation of the inflammatory cytokines IL-1β and IL-18 and play a major role in activating inflammatory responses. If various components of inflammasomes are knocked out, there are metabolic (Henao-Mejia et al., 2012) or inflammatory consequences (Elinav et al., 2011), and as in the TLR5 knockouts, these consequences are mediated by a changed microbiota. Several other genes have been shown to regulate the microbiota in a variety of animal models (Kostic, Howitt, & Garrett, 2013). So once again, it is evident that host genes within the immune system can influence phenotype by controlling the organisms present in the microbiota.

Adaptive Immune System and Microbiota

These experiments prove the role of the innate immune system, but other experiments make it clear that the adaptive immune system is just as crucial. For example, expression of major histocompatibility complex (MHC) class II on conventional dendritic cells (cDCs) is needed for effective control of the gut microbiota (Loschko et al., 2016). When MHC class II was not expressed on cDCs in a mouse model, there was chronic intestinal inflammation that could be reduced by antibiotic treatment. This inflammation did not occur in germ-free animals. Since the role of MHC class II is to present antigens to the T cells of the adaptive system, this is a formal proof of an essential role. Further evidence that the adaptive immune system is crucial comes from mice that lack both the transcription factor Tbet and the RAG2 gene. These animals cannot generate T cells or an adaptive receptor diversity. Such mice develop a severe colitis that can be treated with antibiotics or by infusion of regulatory T cells (Garrett & Glimcher, 2009). These observations confirm that both the innate and the adaptive immune systems are involved in maintaining and controlling the gut microbiota and so indirectly modulate the phenotype.

MECHANISMS OF IMMUNOREGULATION BY THE OLD FRIENDS

The previous section demonstrated that both arms of the immune system regulate the microbiota. The next question is how the microbial exposures that are the theme of this chapter, including the microbiota itself, influence the regulatory functions of the immune system. Conceptually, any input to the immune system might have direct immunoregulatory roles or might cause changes to the microbiota, leading secondarily to altered immunoregulation by the microbiota (Fig. 1). Some of the mechanisms of immunoregulation utilized by the three major categories of old friends are described here (Fig. 2).

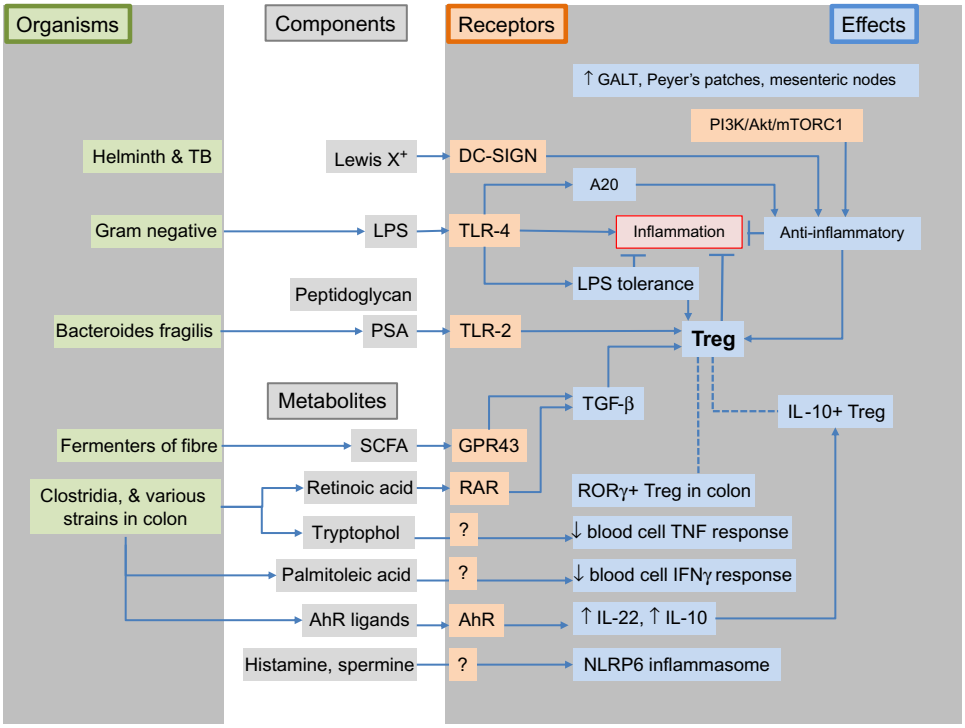

FIG. 2 Some mechanisms involved in microbial effects on immunoregulation and inflammation. Endotoxin (LPS) drives both inflammatory and anti-inflammatory pathways. The pathways shown are inevitably derived mostly from animal experiments, but human data are shown when available. References are provided in the main text. Pathways that *increase* inflammatory activity are not included on the figure. *Abbreviations*: GALT, gut-associated lymphoid tissue; PI3K, phosphatidylinositol-3 kinase; Akt, serine/threonine kinase Akt or protein kinase B (PKB); mTORC1, mammalian target of rapamycin complex 1; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin; TLR-2 and TLR-4, Toll-like receptors 2 and 4; GPR43, G-protein coupled receptor 43; RAR, retinoic acid receptor; AhR, aryl hydrocarbon receptor; ILC1, ILC2, and ILC3, intestinal innate lymphoid cells types 1, 2, and 3; NLRP6, NOD-like receptor pyrin domain-containing protein 6; RORγ, RAR-related orphan receptor γ; SCFA, short-chain fatty acids; PSA, polysaccharide A of *B. fragilis*.

Immunoregulation by the Old Infections

The mechanisms used by helminths to achieve immunoregulation include modification of the bacterial microbiota (including increased *Lactobacillus* levels), modification of the phenotype of DC in the gut so that they tend to drive regulatory pathways, and release of molecules that directly drive the expansion of Treg populations (Grainger et al., 2010). The soluble egg antigen (SEA) from *Schistosoma mansoni* drives Treg development (Zaccone et al., 2009), possibly because it imitates the LewisX trisaccharide motif and thus mimics lacto-N-fucopentaose III, an immunomodulatory glycan found in human milk. Such fucosylated glycans bind DC-SIGN and induce expansion of Th2 and Treg responses as shown in Fig. 2 (Lowry et al., 2016). *H. pylori* is also an inducer of Treg, both locally, in the stomach and duodenum (Lundgren et al., 2005; Robinson et al., 2008), and systemically (Arnold et al., 2011; Lundgren et al., 2005), and some *H. pylori* strains also express DC-SIGN-binding Lewis antigen.

Although modern strains of *M. tuberculosis* are virulent, the ancestral strains with which humans coevolved were not. Human-infecting organisms resembling *M. canettii* probably evolved in Africa from environmental soil mycobacteria as much as 2.8 million years ago, in which case they might have infected human ancestors as far back as *Homo habilis* (Galagan, 2014). The *M. tuberculosis* complex evolved from these strains in Africa, at least 70,000 years ago, and accompanied the out-of-Africa human migrations. These organisms express a range of immunoregulatory molecules, some once again acting via DC-SIGN, reviewed elsewhere (Lowry et al., 2016).

Immunoregulation by Organisms From the Natural Environment

The immunoregulatory effects of exposure to the natural environment will operate both via the airways and the gut (Fig. 2). The airways contain a number of cellular sensor systems that can monitor the content of inhaled air. One of these involves the PI3K/Akt/mTORC1 signaling system that plays a role in inflammatory pathways via NF-κB. Many natural products from bacteria, algae, fungi, and higher plants can inhibit the activities of these protein kinases, and the overall effect is thought to be anti-inflammatory (Moore, 2015). Microbial metabolites also exert anti-inflammatory effects via the aryl hydrocarbon receptor (AhR). Tryptophan can be metabolized to produce AhR ligands that drive the production of IL-22 by activated DC, T cells, and innate lymphoid cells (ILC) that are abundant at mucosal surfaces (Zelante et al., 2014). But this pathway also activates host indoleamine-2,3-dioxygenase 1. This enzyme generates further tryptophan-derived AhR agonists that drive the production of TGF-β (Bessede et al., 2014) and Treg (Quintana et al., 2008).

A major microbial component in inhaled air is endotoxin (LPS), and the phenomenon known as "endotoxin tolerance" is important in the gut and the airways (Fig. 2). In a mouse model, frequent low doses of LPS increase the production of anti-inflammatory A20 (encoded by *Tnfaip3*). A20 is an ubiquitin-modifying enzyme that attenuates NF-κB activation and therefore reduces influx and activation of DC in the airways (Schuijs et al., 2015). Thus, exposure to LPS or to farm dust blocked a mouse model of allergic asthma induced by house dust mite, and this effect was dependent upon the expression of A20 in lung epithelial cells (Schuijs et al., 2015).

These observations are relevant to humans. A previously unrecognized genetic disorder has been described in families with an early onset systemic inflammatory disorder. The disease is caused by germ-line mutations in the gene that encodes A20 (Zhou et al., 2016). More evidence has come from a study of two culturally isolated farming communities in the United States. The Amish use traditional farming methods, while the Hutterites are industrialized. The Amish have much lower levels of asthma. Interestingly, peripheral blood cells from the Amish children express more *Tnfaip3* (Stein et al., 2016). Thus, it is suggested that chronic exposure of the airways to low-dose environmental microbiota sets up regulatory pathways within the airways. Endotoxin tolerance is likely to be an important part of this phenomenon.

Pulmonary neuroendocrine cells constitute another airway sensory system that is inevitably involved in the conditioning of the airway and its immune system. What is known is that stimulating the pulmonary neuroendocrine cells causes the release of neuropeptides that increase immune cell infiltrates (Branchfield et al., 2016).

Immunoregulation by the Microbiota

It used to be thought that the fetus was sterile before birth, but we now know that some transfer of maternal microbiota to the placenta and fetus starts in utero (Funkhouser & Bordenstein, 2013; Meropol & Edwards, 2015).

Animal experiments suggest that molecules derived from the maternal microbiota, some bound to maternal antibodies, cross the placenta and influence the development of the immune system (Gomez de Aguero et al., 2016). Some of these effects are mediated via the AhR and help to limit inflammatory responses to microbial molecules and translocation of intestinal microbes across the gut wall (Gomez de Aguero et al., 2016).

Germ-free animals show defects in the development of the immune system and of the gut itself (and also the brain, discussed later). This is particularly true of the gut-associated lymphoid tissue (GALT), Peyer's patches, and mesenteric lymph nodes (Round & Mazmanian, 2009). Bacterial strains that drive expansion of components of the immune system are beginning to be identified (Fig. 2). Segmented filamentous bacteria (SFB, provisionally known as *Candidatus savagella*) will expand Th17 cells, while in mouse models, certain members of the *Clostridia* (Atarashi et al., 2011) or *Bacteroides fragilis* (Round & Mazmanian, 2010) will drive Treg formation. There is a distinct subset of RORγ + Treg in the colon, and their formation is driven by a range of gut organisms from various different genera (Sefik et al., 2015). Interestingly, the RORγ transcription factor is also involved in driving Th17 cells in the small intestine, but this involves a different subset of gut organisms (Sefik et al., 2015). The microbiota also drives the development of intestinal innate lymphoid cells (ILC1, ILC2, and ILC3), and the transcriptomes of these cells are profoundly altered by antibiotic treatments (Gury-BenAri et al., 2016).

However, it is proving difficult to relate individual organisms to specific health problems, and the thinking is moving rapidly to the view that what really matters is the overall immunoregulatory potential and metabolome of the entire gut ecosystem and the resulting concentrations of certain critically important metabolites (Fig. 2). Progress is being made toward the identification of specific molecular signals from the microbiota to the immune system and brain (discussed later). Tryptophan metabolites such as indole-3-acetic acid have anti-inflammatory effects in the gut via AhR expressed on many cell types including DC (Lamas et al., 2016). Other microbial molecules also signal via AhR (Gomez de Aguero et al., 2016; Levy, Thaiss, & Elinav, 2016). AhR ligands influence the differentiation and function of Tregs by increasing the production of IL-10 and IL-22 (Goettel et al., 2016). The vitamin A metabolite retinoic acid enhances Treg and reduces Th17 via TGF-β and through the induction of histone acetylation at the FoxP3 promoter (Levy et al., 2016). Short-chain fatty acids (SCFA) help to drive Treg formation (Tan et al., 2016). Production of histamine from histidine and levels of spermine and taurine modulate the NLRP6 inflammasome (Levy et al., 2015). In the current context, it is of particular interest that the composition of the gut microbiota has strong influences on the release of cytokines by the donor's peripheral blood cells *in vitro* in the presence of bacterial and fungal stimuli (Schirmer et al., 2016). This shows that microbiota might influence background levels of inflammatory cytokines that are associated with depression. The strongest influences of the microbiota were on the production of IFNγ and TNFα, and the effects appeared to involve the tryptophan metabolite tryptophol, which has strong inhibitory effects on the TNF response, while the metabolism of palmitoleic acid was important for the IFNγ response (Schirmer et al., 2016).

Endotoxin Tolerance

Endotoxin tolerance, mentioned earlier in relation to the airways, is another mechanism that is important in the gut (Fig. 2). Animals can survive a potentially lethal dose of endotoxin if they have previously received one or more sublethal doses, and *in vitro* macrophages that have been exposed to endotoxin respond

differently when challenged again, with less release of TNF and reduced NF-κB translocation (Biswas & Lopez-Collazo, 2009). Repeated low-dose endotoxin administration *in vivo* leads eventually to increased Treg activity (Caramalho et al., 2011; Wang et al., 2015). A recent study has suggested that this pathway is relevant to the fact that the prevalence of childhood atopy is fourfold higher in Finland than in a bordering area of Russia with a genetically similar population, while the prevalence of type 1 diabetes is sixfold higher (Kondrashova et al., 2005; Pakarinen et al., 2008). The endotoxin in the guts of Russian infants was mostly derived from *Escherichia coli*, which can drive endotoxin tolerance, whereas the endotoxin in the guts of Finnish infants was overwhelmingly derived from a *Bacteroides* species that releases an endotoxin that acts as an *inhibitor* of the agonist effects of *E. coli* endotoxin. This *Bacteroides* endotoxin might therefore fail to modulate the potential for cytokine induction, fail to evoke endotoxin tolerance, and fail to enhance Treg induction in Finnish infants (Vatanen et al., 2016).

Microbial Exosomes

Membrane vesicles (MV) of various types are generated by essentially all life forms, including gram-negative and gram-positive bacteria and mycobacteria, archaea, fungi, protozoa, helminths, and mammalian cells (Coakley, Maizels, & Buck, 2015; Pathirana & Kaparakis-Liaskos, 2016). It is now evident that these have a major role in the regulation of the immune system (Coakley et al., 2015; de Candia, De Rosa, Casiraghi, & Matarese, 2016). Moreover, they are also important for the development and function of the brain and can cross the blood-brain barrier (Kramer-Albers & Hill, 2016). MV from prokaryotic microbiota and parasites can enter the host from the gut (Coakley et al., 2015; de Candia et al., 2016). Furthermore, it has long been known that materials can enter the brain via the nasal epithelium (Illum, 2004), and recent work indicates that in an animal model, synthetic MV can enter the brain by this route (Zhuang et al., 2011). It is too early to assess these findings, but if they are important, then some effects of changing exposures to microbes and parasites might operate in this way.

Other ways in which the immune system is regulated by the microbiota are considered below in relation to lifestyle changes that are causing our microbiota to differ from that with which humans evolved.

LIFESTYLE CHANGES THAT IMPAIR MICROBE-INDUCED IMMUNOREGULATION AND THAT MAY BE ASSOCIATED WITH DEPRESSION

A number of lifestyle factors, including a history of breast/formula feeding, vaginal/cesarean delivery, maternal obesity, and perinatal antibiotic exposure, modulate the development of the infant microbiota, yielding clear changes in microbiota composition during the first 1–3 years of life (Fig. 3). However, these effects on the infant microbiota do not necessarily cause detectable effects on the microbiota in adulthood (Falony et al., 2016). Nevertheless, persistent effects on the brain and on immune and metabolic systems are likely.

Perinatal and Early-Life Antibiotic Exposure

Antibiotics represent a particularly clear cause of dysbiosis, and work with antibiotics has also revealed why early dysbiosis, even when it does not persist into adulthood, is relevant to adult health.

Animal Experiments

In mice, periconceptual antibiotics also lead to weight gain later in life and to permanent changes in the immune system (Cox et al., 2014).

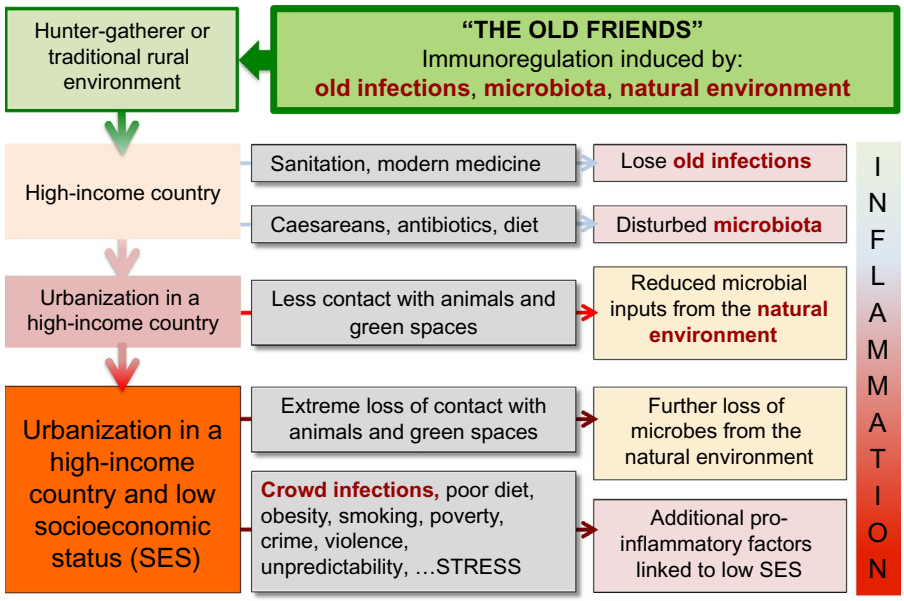

FIG. 3 The accumulation of factors that reduce microbial exposures as we progress from hunter-gatherer to urban life. The pro-inflammatory effects of reduced exposure to immunoregulation-inducing organisms are exacerbated by lifestyle problems in communities of low socioeconomic status, shown at the bottom of the figure. Abbreviations: SES, socioeconomic status. *Figure adapted, with permission from the publisher, from Rook, G. A., Raison, C.L., & Lowry, C. A. (2014). Microbial 'old friends', immunoregulation and socioeconomic status.* Clinical and Experimental Immunology, 177, *1–12, published by John Wiley & Sons Ltd on behalf of British Society for Immunology.*

The brain is also affected. Exposing pregnant rats to succinylsulfathiazole, a nonabsorbable antibiotic, from 1 month before breeding until gestational day 15 leads to behavioral abnormalities in the offspring (Degroote, Hunting, Baccarelli, & Takser, 2016). Even the adult mouse brain can be modified by antibiotics. Administering a broad-spectrum antibiotic mixture to adult mice reduced hippocampal neurogenesis and memory retention (Möhle et al., 2016). These defects, which appeared to involve a monocyte subset in the brain, could be treated by reconstituting a normal microbiota, particularly when supplemented with a commercially available probiotic mixture (VSL#3) consisting of eight bacterial strains (Fig. 4).

In these models, dysbiosis can be taken to an experimental extreme by studying germ-free animals, which are informative even if of uncertain relevance to humans. Germ-free mice have abnormal stress responses that can be corrected by early restoration of the microbiota, but cannot be corrected by normalization of the microbiota in adulthood (Diaz Heijtz et al., 2011; Sudo et al., 2004). The germ-free state is also associated with abnormal upregulation of genes involved in myelination in the prefrontal cortex (Fig. 4) (Hoban et al., 2016).

Human Data

The relevance of perinatal antibiotic use to human health is most obvious in allergic disorders, which, being very common, have received most attention (Fujimura et al., 2016). Administration of antibiotics during the second or third trimesters of pregnancy or during the first months after birth can increase the risk of allergies in the infant (Korpela et al., 2016;

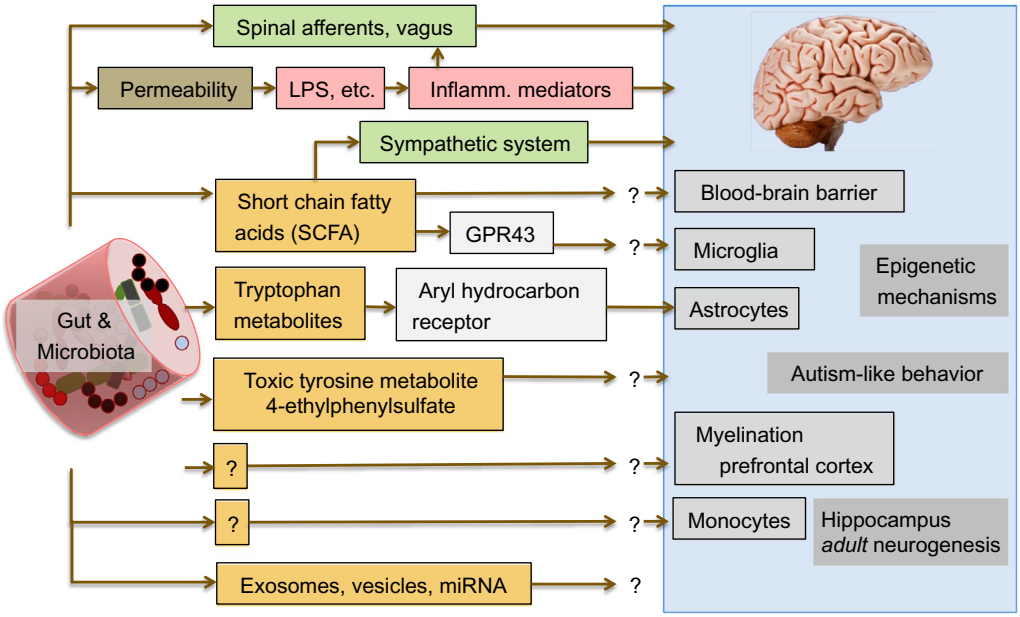

FIG. 4 Some mechanisms involved in the effects of the gut microbiota on the development and function of the brain. The pathways shown are inevitably derived from animal experiments. References and definitions are provided in the main text. Question marks imply that although the pathway has been demonstrated, the receptors or intermediary steps are unknown. References are provided in the main text. *Abbreviations*: GPR43, G-protein coupled receptor 43; LPS, lipopolysaccharide; SCFA, short-chain fatty acids.

Lapin et al., 2015; McKeever, Lewis, Smith, & Hubbard, 2002; Metsala et al., 2013). Similarly, just as in mice (Cox et al., 2014), epidemiological studies suggest that the perinatal antibiotics increase subsequent obesity in humans (Azad, Bridgman, Becker, & Kozyrskyj, 2014; Korpela et al., 2016; Trasande et al., 2013). As would be predicted by the overall adverse effects of antibiotics on the developing microbiota (as well as links between childhood antibiotic use and allergic/atopic disorders, obesity, and asthma—all associated with depression) (Kankaanranta, Kauppi, Tuomisto, & Ilmarinen, 2016; Maes, 1999; Raison et al., 2010), a large longitudinal cohort study in New Zealand found that children who received antibiotics in the first year of life had increased symptoms of depression at age 11, along with other behavioral disturbances, when compared with children not treated with antibiotics early in life (Slykerman et al., 2017). These findings reinforce the likelihood that a "window of opportunity" exists in infancy when an appropriate microbiota must be in place, for healthy development of the brain, immune system, and metabolic regulation.

Caesarean Delivery

Cesarean delivery results in delayed transfer of maternal microbiota and delayed maturation of the infant microbiota (Dominguez-Bello et al., 2016). This can be partially corrected by exposing the neonate to maternal vaginal fluids at birth (Dominguez-Bello et al., 2016). There is evidence from human studies that cesarean delivery increases the risk of obesity in later life (Blustein et al., 2013; Yuan et al., 2016). This effect, like that due to perinatal antibiotics or

the germ-free state, is probably due to early-life events, since a history of cesarean delivery did not cause detectable effects on the microbiota in adulthood (Falony et al., 2016). We know of no data demonstrating an increased risk for depression in individuals born by cesarean, but such an increase is a clear implication of the theoretical underpinnings informing this chapter.

Breast Feeding

Breast milk contains oligosaccharides that cannot be metabolized by the infant and that serve as nutrients for bifidobacteria (Garrido, Barile, & Mills, 2012; Zivkovic, German, Lebrilla, & Mills, 2011). Milk is also a source of *Bifidobacterium* and *Lactobacillus* species that seem to be transferred from the maternal gut to the breast (Jost, Lacroix, Braegger, Rochat, & Chassard, 2014; Melnik, John, Carrera-Bastos, & Schmitz, 2016). Finally, milk contains exosomes that carry microRNAs and TGF-β. These constituents are thought to drive FoxP3 expression and long-lasting Treg differentiation (Melnik et al., 2016; Saarinen, Vaarala, Klemetti, & Savilahti, 1999). Nutritional factors in breast milk include omega-3 fatty acids, which are important for the development of the brain. Moreover, whether via nutritional or immunoregulatory pathways, via microbiota-induced effects, or via other mechanisms, breastfeeding is also important for the brain. Duration of breastfeeding is related to verbal and nonverbal intelligence later in life (Belfort et al., 2013), to better cognitive and motor development (Bernard et al., 2013), and to greater social mobility (Sacker, Kelly, Iacovou, Cable, & Bartley, 2013). Duration of breastfeeding is associated with reduced levels of circulating CRP in adulthood (Williams, Williams, & Poulton, 2006) and, as one would predict from this, has also been associated with a reduction in depression in adults who were breastfed as infants (Peus et al., 2012). Again, this is likely to be due to an early-life effect, since a history of formula feeding did not cause detectable effects on the microbiota in adulthood (Falony et al., 2016).

Diet

The high-income urban diet, rich in fat and processed sugars, leads to a gut microbiome that differs markedly from that seen in communities living as traditional subsistence farmers or hunter-gatherers, and that also has a much reduced diversity (De Filippo et al., 2010; Gomez et al., 2016; Obregon-Tito et al., 2015; Rampelli et al., 2015; Schnorr et al., 2014). Diversity seems to be crucial, and there is now strong evidence that important dietary factors for the maintenance of diversity are fiber (polysaccharides that are fermented by gut microorganisms to yield short-chain fatty acids; SCFA) (Sonnenburg et al., 2016) and plant polyphenols (phenolic acids, flavonoids, stilbenoids, resveratrol, proanthocyanidins, curcuminoids, tannins, and lignans) (Vanamala, Knight, & Spector, 2015). Moreover, SCFA help to increase Treg (Tan et al., 2016). Thus, mothers failing to consume a diet rich in fiber and polyphenols might pass on an inappropriate microbiota to their infants. Excessive consumption of fat causes further problems. In a rat model, administering a high-fat diet during pregnancy caused dysbiosis in the offspring, with striking depletion of certain strains accompanied by behavioral social deficits. One of the depleted strains was *Lactobacillus reuteri*, and administering this organism could correct the behavioral deficits, as could cohousing with control mice (Buffington et al., 2016).

The nature of the fat consumed is also relevant. It is estimated that during human evolution, we consumed roughly equal quantities of omega-6 and omega-3 fatty acids, but thanks to recent dietary changes, we now consume 10–50 times more omega-6 than omega-3 (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011; Kaliannan, Wang, Li, Kim, & Kang, 2015).

In a mouse model, feeding a diet high in omega-6 fatty acids resulted in high levels of metabolic endotoxemia (excessive absorption of LPS from the gut) and low-grade systemic inflammation that could be blocked by antibiotic treatment, implying that the effect was secondary to changed microbiota (Kaliannan et al., 2015). To our knowledge, it is not known whether maternal diet during pregnancy impacts the later development of depression in offspring, but an unhealthy diet during pregnancy independently increases the risk for offspring behavioral/emotional dysregulation at age 7 (Pina-Camacho, Jensen, Gaysina, & Barker, 2015). Overwhelming data now demonstrate that pro-inflammatory diets in adulthood are a risk factor for subsequent depression, and a recent randomized trial found that a dietary intervention designed to enhance healthy nutrition outperformed a social support protocol for reducing depressive symptoms in adults with MDD (Jacka et al., 2017).

Maternal Obesity

The fact that pro-inflammatory diets are associated with obesity provides a further link between diet and depression. It is extremely probable that the transfer of a dysfunctional microbiota from an obese mother to her offspring explains the fact that maternal obesity is a major risk factor for obesity in the child (Galley, Bailey, Kamp Dush, Schoppe-Sullivan, & Christian, 2014; Soderborg, Borengasser, Barbour, & Friedman, 2016). This, just like obesity due to perinatal antibiotic exposure discussed above, is likely to lead to increased levels of inflammatory mediators later in life. It is difficult to establish links between human maternal obesity and later depression in offspring, but the link with autism, another inflammation-associated disorder, is clear, and a recent review concluded that a link with depression was also probable (Edlow, 2017).

Immigration

Depression in immigrants also points to the importance of early-life events (Breslau, Borges, Hagar, Tancredi, & Gilman, 2009; Vega, Sribney, Aguilar-Gaxiola, & Kolody, 2004). Mexicans, Cubans, and African/Caribbean people have a two- to threefold increase in the prevalence of depression if immigration to the United States occurred when the individual was less than 13 years old or was born in the United States, compared with the prevalence in those who migrated after the age of 13 (Breslau et al., 2009; Vega et al., 2004). This implies that there is a protective effect of early childhood environmental influences, as has been shown for autoimmune disorders and IBD (Rook et al., 2014) and obesity (Trasande et al., 2013).

Stress

Perinatal stress causes changes in the regulation of the immune system and of the HPA axis and altered development of the brain, notably the hippocampus and amygdala. We reviewed these issues elsewhere (Rook, Lowry, & Raison, 2015). However, some aspects of these effects of stress are relevant to this chapter, because they are mediated via stress-induced changes in the microbiota. Stress alters the microbiota of experimental animals (Bailey et al., 2011; Kiliaan et al., 1998), and the same is true of the microbiota of severely stressed critically ill humans, where the changes are rapid and prolonged (Hayakawa et al., 2011). *Prenatal* stressors have been shown to alter the microbiome in rhesus monkeys by reducing the overall numbers of *Bifidobacteria* and *Lactobacilli* during adulthood (Bailey, Lubach, & Coe, 2004). In a rat model, the stress of maternal separation in the neonatal period had long-term effects on the diversity of the microbiota that was still apparent when the pups became adults (O'Mahony et al., 2009). Recently, it has been shown that when human mothers are stressed during pregnancy, the

infants carry higher relative abundances of *Proteobacteria* some of which might be pathogens and lower relative abundances of lactic acid bacteria and *Bifidobacteria* (Zijlmans, Korpela, Riksen-Walraven, de Vos, & de Weerth, 2015). These findings, together with the effects on brain development and function of perinatal antibiotics and the germ-free state discussed earlier, make it very probable that part of the effect of perinatal stress on rates of depression is mediated via the microbiota.

DEPRESSION

A number of predictions can be made if a failure of immunoregulation, arising from deficient exposure to immunoregulatory microbial inputs during early life, is a risk factor for depression in adulthood. A first prediction is that lifestyle and environmental factors that promote immune dysregulation (and disorders of immune dysregulation) should also increase the risk for depression. As we've touched upon in the preceding sections, this appears to be widely true. Here, we also consider other predictions that stem from a link between immunodysregulation/disturbed microbial interactions and depression, including that (1) depression should be associated with chronic low-grade inflammation and exaggerated pro-inflammatory cytokine responses to psychosocial stressors during adulthood; (2) treatment with pro-inflammatory cytokines should induce depressive symptoms or clinical depression; (3) depressed patients should have a higher incidence and prevalence of autoimmune disorders, allergies, and other conditions associated with reduced FoxP3$^+$ Treg; (4) anti-inflammatory or immunoregulatory therapies should be effective in reducing symptoms of depression; and (5) depression should be associated with changes in the composition and function of the gut microbiota. Evidence supporting each of these predictions is described in the following paragraphs.

Chronic Stress-Induced Inflammation in Depression

A massive database, captured in several meta-analyses, confirms that depressed patients as a group have increased levels of circulating pro-inflammatory cytokines and downstream inflammatory markers (i.e., CRP), with the cytokine data being strongest for IL-6, CRP, and TNF. Depressed individuals have also been reported to present with a relative deficit in anti-inflammatory mediators and Treg (Chen et al., 2011; fully referenced in Raison et al., 2010). These peripheral changes are almost certainly reflected in immune functioning in the CNS, given data from noninvasive positron-emission tomography (PET) scanning that reveals the presence of inflammation in the brains of depressed individuals (Setiawan et al., 2015). In addition to these cross-sectional associations, studies now document that increased CRP or IL-6 *predicts* later depression in children (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014) and adults in the United Kingdom (Gimeno et al., 2009) and also *predicts* later susceptibility to posttraumatic stress disorder (PTSD) in marines (Eraly et al., 2014). Depressed individuals (and those at high risk for depression via early-life adversity) have been shown in several studies to respond to laboratory-based psychosocial stressors with increased production of IL-6 and induction of the inflammatory signaling molecule NF-κB (Carpenter et al., 2010; Pace et al., 2006), and increases in inflammation in response to a laboratory stressor have been shown to predict increased depressive symptoms up to a year later (Aschbacher et al., 2012).

Proinflammatory Cytokines Induce Depression

Mercifully, for patients with hepatitis C, treatment with the cytokine interferon IFN-alpha has been supplanted by more effective and tolerable

treatment options. But during the years of its clinical hegemony, IFN-alpha provided a unique model system for understanding behavioral and biological responses to chronic inflammation relevant to depression. Results from many studies have been quite consistent in demonstrating that IFN-alpha exposure produces a widespread increase in depressive and anxious symptoms, with a sizable minority of patients meeting full criteria for major depressive disorder (MDD) within a month of commencing treatment. As reviewed in Miller and Raison (2016), IFN-alpha treatment also produces all known biological changes associated with MDD more generally, including increased circulating pro-inflammatory cytokines, disruption of the diurnal cortisol rhythm and induction of glucocorticoid resistance, altered sleep physiology, and changes in monoamine metabolism, with many of these changes associating with increased depression during treatment (Miller & Raison, 2016). Supporting these findings are studies showing that even a single exposure to inflammatory stimuli (e.g., lipopolysaccharide or typhoid vaccine) induces depressive symptoms and depressive-style social cognitions in healthy volunteers, with these effects being strongest in women (Harrison et al., 2009; Moieni et al., 2015).

Depression is Associated With Autoimmune, Allergic and Inflammatory Bowel Disorders

A prospective population-based study in Denmark that included approximately 1.1 million people identified 145,217 individuals with depression and found that depression was associated with a higher incidence and prevalence of a wide range of autoimmune disorders, allergic disorders, IBDs, and enteropathies (Andersson et al., 2015). The association with bowel disorders is consistent in other studies of celiac disease and IBDs (Jackson, Eaton, Cascella, Fasano, & Kelly, 2012; Regueiro, Greer, & Szigethy, 2017). Similarly, when a large birth cohort was followed prospectively for 31 years, it was found that in females, the presence of atopy, confirmed by skin-prick tests, increased the risk of depression up to 4.7-fold compared with nonatopic females from the same cohort (Timonen et al., 2003). The effect is large enough to explain why spring peaks in aeroallergens coincide with peaks in suicide (Postolache, Komarow, & Tonelli, 2008). A Finnish sample of 1337 monozygotic and 2506 dizygotic twin pairs suggested a shared genetic risk for atopy and depression (Wamboldt et al., 2000). Interestingly, depression is associated with reduced circulating $FoxP3^+$ Treg even in those patients who do not have manifest inflammatory disorders (Grosse et al., 2016).

Antidepressant Effects of Anti-Inflammatory and Immunoregulatory Treatments

Studies on the antidepressant effects of anti-inflammatory or immunoregulatory agents have done much to reinforce many years' evidence that only a subset of depressed patients evince increased inflammation and that making this distinction has very significant treatment implications. While meta-analyses suggest that non-specific anti-inflammatory agents (i.e., those with biological effects relevant to depression other than inflammation, such as selective serotonin reuptake inhibitors) may produce small effect-size improvements in MDD generally, studies using specific cytokine antagonists (which have no off-target effects) paint a more precise and interesting picture. In particular, Raison et al. showed in a study of treatment-resistant MDD that treatment with the TNF antagonist infliximab had no overall effect on depressive symptoms when compared with placebo. On the other hand, infliximab performed as well as typical antidepressants in depressed subjects with baseline CRP concentrations >5 mg/L, suggesting that peripheral inflammatory processes were driving depressive

symptomatology in these subjects (Raison et al., 2013). However, in depressed subjects with lower levels of CRP at baseline, placebo actually far outperformed infliximab—highlighting the dangers in making too easy assumptions about depression being "an inflammatory condition." Rather, it appears that a subset of depressed patients may come to their illness, at least in part, via chronic increased inflammation, while in other depressed patients, the immune system is either less relevant or actually abnormal in ways not subsumed under the moniker of "increased inflammation." Consistent with this possibility are recent findings that healthy patients with MDD and increased inflammation (as indexed by peripheral CRP) show differential glutamate activity in anterior striatum and different patterns of prefrontal-anterior striatum connectivity when compared with depressed patients with low levels of inflammation (Felger et al., 2016; Haroon et al., 2016). Further supporting the possibility that anti-inflammatory treatments may have antidepressant effects but only in those with elevated inflammation is a study by Rappaport et al. that found results identical to the infliximab study: depressed patients with elevated baseline CRP showed an antidepressant response to treatment with omega-3 fatty acids, whereas depressed subjects with low levels of inflammation obtained more benefit from placebo treatment (Rapaport et al., 2016).

Microbiota in Human Depression

So, is there any evidence of changes to the microbiota in human depression? There are few studies, and they are not conclusive. Patients suffering from MDD had reduced levels of *Faecalibacterium*, which showed a negative correlation with the severity of depressive symptoms (Jiang et al., 2015). This finding is of note as *F. prausnitzii*, the sole known species of the *Faecalibacterium* genus, is an abundant commensal that synthesizes butyrate and other SCFA implicated in driving immunoregulation (Khan et al., 2012; Qiu, Zhang, Yang, Hong, & Yu, 2013; Tan et al., 2016). Consistent with the thesis of this paper, low relative abundances of *F. prausnitzii* have also been associated with Crohn's disease, obesity, and psoriasis (Newton et al., 2015; Qiu et al., 2013; Sokol et al., 2008). Patients also had increased levels of *Enterobacteriaceae* and *Alistipes* (Jiang et al., 2015). A second study also found some increases in members of the *Alistipes* group in the fecal microbiota of depressed individuals (Naseribafrouei et al., 2014). There is one report that transfer of fecal microbiota from depressed patients to germ-free mice results in depression-like behavioral changes in the latter (Zheng et al., 2016). Moreover, microbiota from patients with irritable bowel syndrome (IBS) will induce similar symptoms after transfer to rodents, including anxiety-like behavior when this symptom was present in the human donor (reviewed in Collins, 2016). The future may lie in seeking changes in levels of critical metabolites, rather than changes in the microbial composition.

However, there is good evidence that human behavior can be changed by modulation of the microbiota. A probiotic-rich fermented milk product or a matching placebo was given to women for 4 weeks. Both before and after this regimen, the women were exposed to emotive images of faces while undergoing functional magnetic resonance imaging (fMRI) of their brains. Consumption of the probiotic product was shown to have affected the activity of brain regions involved in central processing of emotion and sensation (Tillisch et al., 2013). Another randomized and blinded study used 20 healthy participants without current mood disorder who received a complex probiotic food supplement or placebo for 4 weeks. Assessment before and after the intervention indicated that the active preparation reduced negative thoughts associated with sad mood (Steenbergen, Sellaro, van Hemert, Bosch, & Colzato, 2015).

Emerging data suggest that microbe-based immunoregulatory interventions may also hold

promise for the treatment of depression. A recent meta-analysis of five randomized trials of various probiotic formulations versus placebo found an overall effect size of 0.30 favoring probiotics in patients with MDD and an effect size for improving depressive symptoms of 0.25 in nonclinically depressed individuals, although effects were not significant for patients over 65 years of age for unknown reasons (Huang, Wang, & Hu, 2016). Less is known about the antidepressant effects of environmentally based microorganisms although both animal and human data suggest that a heat-killed preparation from the saprophytic microorganism, *M. vaccae*, may hold promise for the reduction of depressive and anxious symptoms (Lowry et al., 2007; O'Brien et al., 2004; Reber et al., 2016).

Animal Data

These human findings are supported by a mass of animal data, and two of these studies are of particular relevance. First, spleen cells from individual mice were tested *in vitro* for IL-6 output in the presence of endotoxin. Individual animals could then be classified as high or low IL-6 releasers. When the spleen cell donors were subsequently subjected to stress, only the high IL-6 releasers showed depression-like behavioral changes (Hodes et al., 2014). This is analogous to the human study mentioned in the previous paragraph (Pace et al., 2006). A still more relevant study exploited a model in which exposure to stress induces changes in the gut microbiota accompanied by an inflammatory colitis, exaggerated release of pro-inflammatory cytokines from mesenteric lymph node cells stimulated *ex vivo*, and anxiety-like behavioral changes (Reber et al., 2016). In this model, injections of a heat-killed environmental mycobacterium were able to block the colitis, the stress-induced exaggeration of inflammation, and the behavioral changes, and all these effects were shown to be attributable to the induction of Treg (Reber et al., 2016). This simultaneous modulation of a peripheral inflammatory disorder (colitis) and a behavioral disorder by microbe-induced Treg is a striking finding (Fig. 5).

Microbial Metabolites and the Brain

Not all the effects of the microbiota are mediated indirectly via altered immunoregulation. Several neurotransmitters are derived from amino acids, and humans generate them using genes obtained by horizontal gene transfer from bacteria (Iyer, Aravind, Coon, Klein, & Koonin, 2004). It is therefore not surprising that organisms within the microbiota synthesize these mediators and also physiologically active variants (Fig. 4). Tyrosine (the precursor of dopamine, noradrenaline, and adrenaline) and tryptophan (the precursor of serotonin, neuroactive kynurenine metabolites, and melatonin) are two examples. When pregnant mice were exposed to poly(I:C) to mimic inflammation induced by virus infections, the offspring exhibited behavioral impairments (Hsiao et al., 2013). This was accompanied by dysbiosis and changes in blood levels of 4-ethylphenyl sulfate (a tyrosine metabolite) and indolepyruvate (a tryptophan metabolite). Administering 4-ethylphenyl sulfate to normal mice was able to elicit similar behavioral changes. Moreover, a probiotic preparation (*B. fragilis*) was able to normalize the blood levels of these metabolites.

Other tryptophan metabolites are agonists for the AhR, and several of these (indole, indoxyl-3-sulfate, indole-3-propionic acid, and indole-3-aldehyde) were found to exert anti-inflammatory effects in the CNS via AhR expressed on astrocytes (Rothhammer et al., 2016). Similarly, SCFA derived by the fermentation of fiber by organisms in the colon have been shown to regulate the development of microglia (Erny et al., 2015) and to be necessary for the development of an intact blood-brain barrier

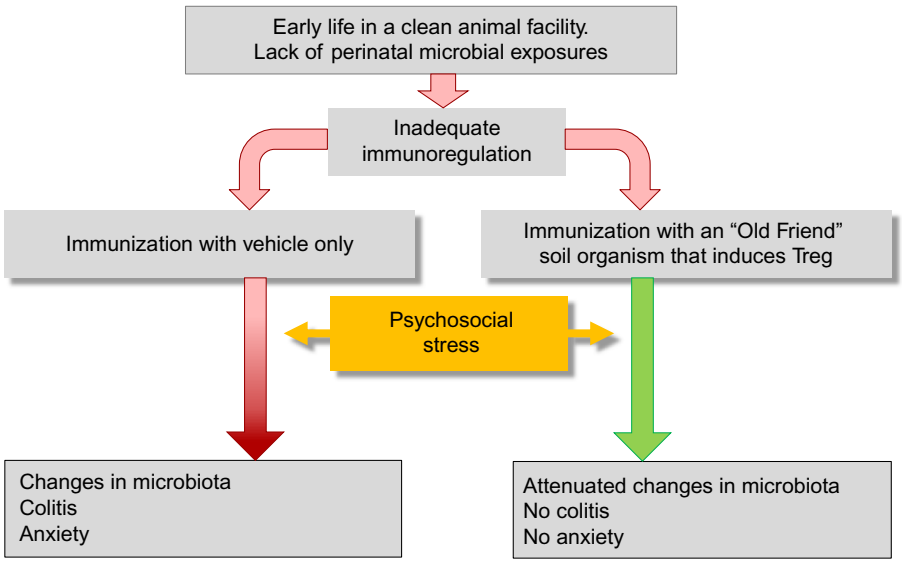

FIG. 5 An experimental model where stress leads to simultaneous development of altered microbiota, colitis, changes in brain chemistry, and behavioral changes resembling posttraumatic stress disorder (PTSD). The striking thing about this model is that the induction of regulatory T cells (Treg) by injecting an environmental organism blocks both the colitis and the PTSD-like state.

(Braniste et al., 2014). Thus, these microbial metabolites, already discussed above in relation to their indirect effects on the brain via the regulation of the immune system, are also relevant to brain function via direct effects on brain development (Fig. 4).

CONCLUSIONS

This chapter has followed the rather narrow brief of describing how susceptibility to depression may be modified by childhood exposures to microbes acting via effects on the regulation of the immune system that help to limit inflammation and via effects on the composition of the microbiota and gut-brain axis function. We hope to encourage greater awareness of the fact that environmental changes, particularly those associated with the 21st century urban lifestyle, can affect our brains via physical and biochemical pathways as well as via psychosocial ones. Consequently, there are many dietary and lifestyle factors with the potential to increase the immunoregulatory influence of microbial exposure and reduce the risk of depression. These include (1) increased consumption of fresh plants and fermented foods, which may increase alpha diversity of the microbiome and its immunoregulatory potential (Selhub, Logan, & Bested, 2014; Sonnenburg et al., 2016; Tillisch et al., 2013); (2) increased consumption of prebiotics, which have been shown to selectively increase the abundance of bifidobacteria (Davis, Martinez, Walter, Goin, & Hutkins, 2011) and to attenuate negative outcomes of stress (Thompson et al., 2016); (3) minimized use of antibiotics, especially in early life (Slykerman et al., 2017); (4) increased exercise, which increases alpha diversity of the gut microbiome (O'Sullivan et al., 2015); (5) increased sleep (Benedict et al., 2016; Poroyko et al., 2016); and (6) increased exposure to the outdoor environment (Rook, 2013). It should be possible

to modify indoor and urban environments so that their microbiotas resemble that of the natural environment with which we evolved (Hoisington, Brenner, Kinney, Postolache, & Lowry, 2015; Logan, 2015; Lowry et al., 2016; Stamper et al., 2016). This will require a much deeper understanding of the different roles of environmental organisms, followed by collaborations with town planners, architects, and designers of air-conditioning systems, building materials, and water supplies.

It is possible that not all the effects of microbial exposures on susceptibility to depression act via modulation of immunoregulation and inflammation. For example, Fig. 4 indicates the effects of the microbiota operating via microglia, astrocytes, myelination in the prefrontal cortex, neurogenesis in the hippocampus, and modification of the blood-brain barrier. It is not clear that all of these effects are dependent only on the balance of inflammation and immunoregulation, so it is entirely possible that we will learn to modulate the gut microbiota in ways that generate other signals that influence the development or function of the brain.

Anti-inflammatory treatments for depression are already showing efficacy (Kappelmann, Lewis, Dantzer, Jones, & Khandaker, 2018), and the time has come to test immunoregulation-inducing microorganisms. Ultimately, we anticipate the development of immunoregulatory immunizations and probiotics that will exert long-term therapeutic effects on those patients whose disorders are associated with inflammation or with inappropriate microbiota. Such immunizations or probiotics may also hold great promise when administered in early life during key developmental windows for protecting against the development of stress-related psychiatric disorders—including major depression—in adulthood.

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