Chapter 15
Microbiota, Immunoregulatory Old Friends and Psychiatric Disorders

Graham A.W. Rook, Charles L. Raison, and Christopher A. Lowry

Abstract Regulation of the immune system is an important function of the gut microbiota. Increasing evidence suggests that modern living conditions cause the gut microbiota to deviate from the form it took during human evolution. Contributing factors include loss of helminth infections, encountering less microbial biodiversity, and modulation of the microbiota composition by diet and antibiotic use. Thus the gut microbiota is a major mediator of the hygiene hypothesis (or as we prefer, "Old Friends" mechanism), which describes the role of organisms with which we co-evolved, and that needed to be tolerated, as crucial inducers of immunoregulation. At least partly as a consequence of reduced exposure to immunoregulatory Old Friends, many but not all of which resided in the gut, high-income countries are undergoing large increases in a wide range of chronic inflammatory disorders including allergies, autoimmunity and inflammatory bowel diseases. Depression, anxiety and reduced stress resilience are comorbid with these conditions, or can occur in individuals with persistently raised circulating levels of biomarkers of inflammation in the absence of clinically apparent peripheral inflammatory disease. Moreover poorly regulated inflammation during pregnancy might contribute to brain developmental abnormalities that underlie some cases of autism spectrum disorders and schizophrenia. In this chapter we explain how the gut microbiota drives immunoregulation, how faulty immunoregulation and inflammation predispose to psychiatric disease, and how psychological stress drives further

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inflammation via pathways that involve the gut and microbiota. We also outline how this two-way relationship between the brain and inflammation implicates the microbiota, Old Friends and immunoregulation in the control of stress resilience.

Abbreviations

ASD	Autism spectrum disorders
BH4	Tetrahydrobiopterin
CD	Crohn's disease
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
dACC	Dorsal anterior cingulate cortex
DC	Dendritic cells
DCreg	Regulatory dendritic cells
fMRI	Functional magnetic resonance imaging
GABA	g-Aminobutyric acid
GCR	Glucocorticoid resistance
HPA	Hypothalamic-pituitary adrenal axis
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IDO	Indoleamine-2,3-dioxygenase
IFN-α	Interferon-alpha
IL	Interleukin
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MS	Multiple sclerosis
NO	Nitric oxide
Nod1	Nucleotide-binding oligomerization domain-containing protein-1
PBMCs	Peripheral blood monocyte cells
PET	Positron emission tomography
SCFA	Short chain fatty acids
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphisms
SNS	Sympathetic nervous system
SSRI	Selective serotonin reuptake inhibitors
T1D	Type 1 diabetes
TNF	Tumor necrosis factor
Treg	Regulatory T cells
UC	Ulcerative colitis
XLAAD	X-linked autoimmunity-allergic dysregulation syndrome

Introduction

This chapter concentrates on those gut-brain interactions that operate indirectly via the immune system, rather than via direct neural pathways. At least two sets of findings underlie this aspect of gut-brain interaction. First, we know that persistently raised levels of inflammatory mediators are associated with several psychiatric conditions. This will be discussed with particular reference to depression and to reduced stress resilience. The regulation of background levels of inflammation is dependent upon "learning" inputs to the immune system from appropriate microbial exposures during the prenatal and neonatal periods, and continuing diversity of input in later life. This concept, initially called the "hygiene hypothesis" is becoming renamed the "Old Friends" mechanism, which places it firmly within the field of Darwinian and evolutionary medicine. The various mammalian microbiotas, particularly the gut microbiota, are important components of the Old Friends mechanism, and have a continuing immunoregulatory role in the adult. Secondly, we know that inflammation during pregnancy can lead to abnormal development of the central nervous system (CNS). This will be illustrated by considering autism spectrum disorders (ASD) and schizophrenia, and aspects of epidemiology that suggest the importance of microbe-dependent immunoregulatory effects during pregnancy.

The expression "Hygiene hypothesis" was first published in 1989, following the observation that, when examined at 11 years old, children brought up in families with many older siblings were less likely to have developed allergic disorders. This concept was at first a narrow one, focusing on the notion that childhood infections somehow prevented subsequent allergies. In fact it had been known since the nineteenth century that the environment could modulate the likelihood of developing hay fever, which was increasing amongst wealthy townsfolk, while remaining rare amongst farmers [1]. This protective effect of the farming environment in allergic disorders has subsequently been rigorously confirmed and found to extend to juvenile-onset inflammatory bowel disease (IBD) as well [2]. Moreover to reap protection from these immune-mediated diseases it can be sufficient to expose the pregnant mother to the farming environment, rather than the infant itself [3]. The most recent observations indicate that the farm effect is mostly explained by exposure to increased microbial biodiversity, which was documented by analyzing the bacterial and fungal taxa present in the dust in children's bedrooms [4].

Meanwhile sporadic observations in other branches of medicine have confirmed that allergic disorders are not the only chronic inflammatory conditions that have been increasing in high-income countries, particularly in urban populations. Inflammatory bowel diseases and autoimmune diseases have increased at about the same rate, and in the same places [5, 6] as allergic conditions. Subsequently large epidemiological surveys have shown that childhood infections, originally implicated as the protective mechanism behind the hygiene hypothesis, do not protect against allergic disorders, and may in some cases, such as human rhinoviruses and respiratory syncytial viruses, actually trigger allergic responses. Considered

together, these observations led to a Darwinian reformulation of the hypothesis as the Old Friends mechanism, described in the next section.

Old Friends Mechanism

The Old Friends mechanism states that mammals co-evolved with various microbiotas and commensals (gut, skin, lung etc.), as well as with chronic infectious agents picked up at birth, helminths that persisted for life, and environmental organisms from animals, mud and untreated water with which we were in daily contact. Because all of these categories of organism needed to be tolerated, they took on a role of inducers of immunoregulatory circuits [7, 8]. For example, helminthic parasites need to be tolerated because, although not always harmless, once they are established in the host, efforts by the immune system to eliminate them are typically futile, and merely cause tissue damage [9].

Contact with the "Old Friends" rapidly diminished when industrialization occurred, and mankind started to inhabit a plastic and concrete environment, to consume washed food and chlorine-treated water, and to minimize our contact with mud, animals and feces. This withdrawal of the organisms that drive immunoregulatory circuits results in defective immunoregulation that, depending on the genetic background of any given individual, can manifest itself as a variety of chronic inflammatory disorders, including allergies, IBD and autoimmunity. We know that a failure of immunoregulatory mechanisms really can lead to simultaneous increases in diverse types of pathology. For example, defects in the gene encoding the immunoregulatory transcription factor Foxp3 lead to the X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) that includes aspects of allergy, autoimmunity and enteropathy [10].

The underlying Darwinian principle of the Old Friends mechanism is illustrated in Fig. 15.1. The immune system at birth is analogous to a computer with hardware, some software, but very little data. The minimal data that it does have comes from T lymphocyte selection in the thymus, and probably from transfer of at least some environmental and maternal antigenic material across the placenta. After birth the immune system requires the largest possible exposure to environmental microbial biodiversity in order to build a very broad repertoire of potential effector lymphocytes. Since all life forms are ultimately constructed with similar building blocks, such diversity of "education" can even provide the system with T cells that recognize, for example, some obscure viral pathogen that might be encountered in the future [11].

However in the context of this chapter, still more important than the diverse effector repertoire is the setting up of appropriate immunoregulation. Just as exposure early in life to a wide range of microbial and parasitic organisms trains the immune system regarding what to be on guard against, it also teaches immunity what to profitably ignore because the organisms in question either confer some benefit to the host, or confer no danger or despite posing some risk are not easily

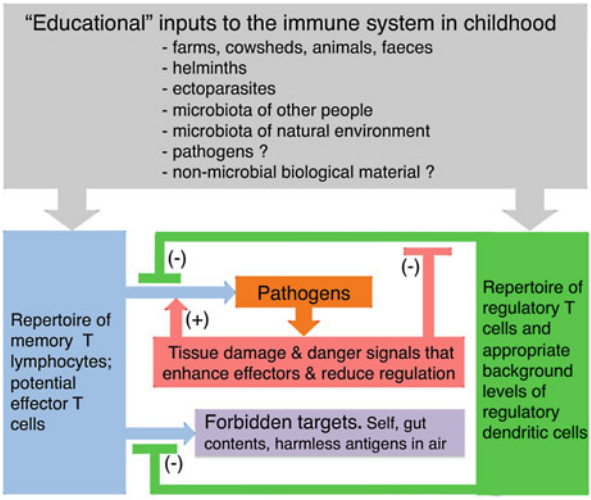

Fig. 15.1 The immune system requires "educational" input. The microbiota of others, tolerated organisms (such as helminths) with which we co-evolved and organisms from the natural environment are required to expand the effector and regulatory branches of the immune system. During subsequent encounters with pathogens, danger signals generated by tissue damage enhance effector mechanisms and attenuate regulatory pathways to permit an appropriate immune response. Adequate background levels of regulatory T cells and dendritic cells and other regulatory mechanisms are required to maintain suppression of responses to "forbidden targets" and to switch off inflammation completely when the danger is eliminated, so that proinflammatory mediators do not continue to circulate

eradicated by immune mechanisms once established. These immunoregulatory inputs benefit the host by teaching the immune system not to waste precious energy engaging in futile battles, by reducing the cost to the host of chronic inflammation and by reducing the risk of destruction of host tissues, either through bystander effects or via the induction of autoimmunity. Because humans in traditional environments were exposed to organisms that dampened, as well as stimulated, immune function, the Old Friends mechanism implies that inflammation should be better regulated in low-income than in high-income countries. At first sight this might seem paradoxical, because the high prevalence of infections in low-income countries might be expected to cause high levels of inflammation [12]. However recent work by McDade et al. [13, discussed in 14] has largely resolved this paradox. The results reveal that in a low-income country where there is still abundant exposure to the immunoregulation-inducing "Old Friends", immunoregulation is efficient, and the inflammatory response is vigorous during an infection, but is terminated when no longer needed, with the result that "resting" C-reactive protein (CRP) is close to zero. These longitudinal results illuminate a previous finding by McDade et al. [15] that *high* levels of microbial exposure in the perinatal period and in infancy correlated with *low* levels of "resting" CRP in adulthood. In contrast, in the USA and other high-income countries there is often constant low-grade inflammation

which tends to be stable across individuals, manifested as chronically raised CRP or interleukin (IL)-6, in the absence of any clinically apparent inflammatory stimulus. Such chronically elevated inflammation greatly increases the risk of subsequent inflammatory disease and cardiovascular problems and has been shown in some studies to predict the future development of depression [16].

Inflammation and Psychiatric Disorders

Inflammation is involved not only in chronic inflammatory disorders such as allergies, autoimmunity and IBD but also in many psychiatric disorders. We have reviewed this topic in detail elsewhere [17, 18]. Briefly, a large subset of depressed individuals has persistently raised levels of proinflammatory cytokines and other downstream inflammatory markers [19, 20], together with a relative deficit in anti-inflammatory mediators and regulatory T cells [fully referenced in 18, 21]. Interestingly, depressed individuals also show exaggerated release of inflammatory mediators in response to psychosocial stressors [22], implying altered immunoregulation (Fig. 15.2), and epidemiological studies in the United Kingdom (UK) showed that raised CRP and IL-6 predicts subsequent risk of depression assessed over a decade later [16].

The possibility that inflammatory mediators might play direct causal roles in depressive pathogenesis has been confirmed for interferon-alpha (IFN-α), interleukin 6 (IL-6), and tumor necrosis factor (TNF). When IFN-α is used therapeutically (to treat viral hepatitis or some cancers) it causes depression-like symptoms in a high percentage of patients. These symptoms have been repeatedly shown to respond to treatment with standard antidepressants, such as selective serotonin reuptake inhibitors (SSRI) [20, 23]. Similarly the cytokine antagonist infliximab, which blocks TNF actions, has been shown to have antidepressant properties, but only in depressed individuals with evidence of increased peripheral inflammation prior to treatment [24].

Finally, when IL-6 is administered to pregnant animals it causes abnormal brain development in the fetus, discussed later in relation to autism [25]. Similarly increased peripheral levels of IL-6 cause increased production of IL-6 in the CNS, and affect neurogenesis in the hippocampus [reviewed in 26]. That IL-6 is directly relevant to the changes seen is supported by the fact that these effects can be blocked by IL-6 receptor antagonists, and knockout mice with non-functional IL-6 genes have enhanced working memory compared to wild type mice [27] and are refractory to peripheral inflammation-induced impairments of spatial memory [28]. In humans raised levels of IL-6 are associated with diminished cognitive performance and reduced hippocampal gray matter [26, 29]. Mechanisms for these effects are discussed in a later section.

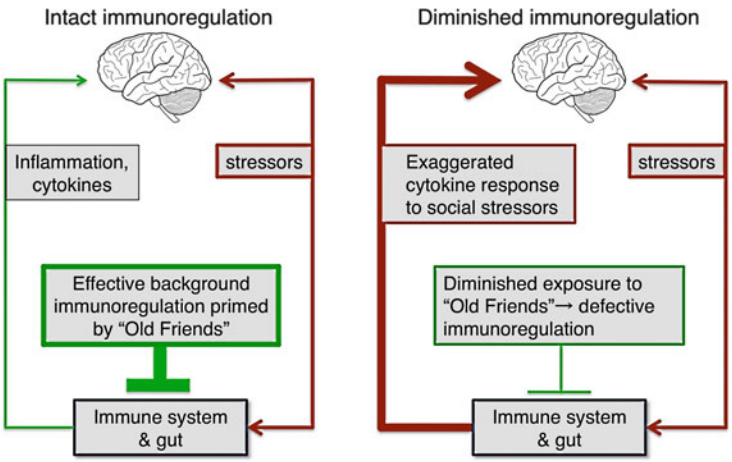

Fig. 15.2 Exaggerated and prolonged cytokine release in response to a psychosocial stressor in individuals with diminished immunoregulation. Populations that have poorly immunoregulatory gut microbiota and reduced exposure to immunoregulation-inducing "Old Friends" such as helminths are susceptible to excessive and prolonged cytokine release in response to psychosocial stressors, which may result in reduced stress resilience and inappropriate triggering of depressive episodes. Reprinted from Rook et al. (2013) *Evolution, Medicine and Pubic Health* (1): 46–64, doi: 10.1093/emph/eot004, by permission of Oxford University Press and the Foundation for Evolution, Medicine, and Public Health

Immunoregulation and Stress Resilience in Developing Countries

The vicious cycle described in Fig. 15.2, considered against the background of the Old Friends mechanism, suggests that in developing countries there will be less release of inflammatory mediators in response to psychosocial stressors, and less psychiatric consequences of such stressors. Recent data support this hypothesis. In experimental animals parental deprivation is a potent inducer of long-term changes to stress responses and immunoregulation [30]. Human studies suggest similar correlations [31]. However in a recent study performed in a developing country, parental absence in childhood was a significant predictor of raised CRP in adulthood, as it would be in a rich country, but *only in a subset* of the cohort raised in hygienic environments [32]. However, adults who had a high level of microbial exposure in infancy were resistant to the long-term proinflammatory effects of this severe childhood stressor [32]. The same was true of perceived stress during the previous month in young adults. CRP correlated with recent perceived stress in subjects with low microbial exposure in infancy, but not in those with high microbial exposure. Again, exposure to immunoregulation-inducing "Old Friends" seemed to provide resistance to the inflammation-inducing effects of psychosocial stressors [32].

This leads to an obvious question. If psychosocial stressors cause depression at least partly by triggering the release of proinflammatory mediators (Fig. 15.2), are inhabitants of developing countries resistant to psychosocial stress-induced depression? If so the prevalence of depression should be increasing in developed countries in parallel with the chronic inflammatory disorders [33], and lower in developing countries than in developed ones. Comparative studies are difficult to do, but this is indeed what data collected by the World Health Organization indicate [34]. Moreover one study failed to find a correlation between depression and raised CRP in a developing country whereas this association is routinely found in rich ones [35].

Urbanization and Immigration to High-Income Countries

If a dysregulated immune system resulting from diminished contact with immunoregulation-inducing "Old Friends" is partly to blame for the increasing prevalence not only of chronic inflammatory disorders such as allergies, autoimmunity and IBD, but also of those psychiatric disorders that can be triggered by inflammatory mediators, then it should be useful to examine urban-rural differences in disease prevalence, and the effect of migration from low-income to rich urban environments. In each case there will be loss of exposure to Old Friends.

Urban Versus Rural

A feature shared by most of the disorders discussed here is a higher prevalence in urban communities compared to rural ones. For example a meta-analysis of high quality studies performed in high-income countries since 1985 found that the prevalence of depression in urban areas was 39 % higher than in rural areas. Similarly, the prevalence of anxiety disorders was 21 % higher in urban than in rural areas [36], though a small minority of studies fails to find this urban-rural difference [37]. Peen et al. [36] also noted an increased urban prevalence of psychiatric disorders in general (38 % more in urban communities). This agrees well with another large meta-analysis that found a significantly raised prevalence of schizophrenia in urban communities [38]. Similarly, a study of all children born in Denmark between 1 January 1984 and 31 December 1998 found that the degree of urbanization of place of birth was very significantly correlated to risk of autism [39].

The urban > rural phenomenon is also well established for chronic inflammatory disorders, where the etiology is known to involve dysregulation of the immune system. Contact with the farming environment, whether early postnatal [40] or prenatal [3, 41] protects against allergic disorders, whereas the prevalence of these conditions increases with increasing urbanization [42]. The same is true for IBD

[43], and for autoimmune diseases such as multiple sclerosis (MS) [44, 45, discussed in 46].

Immigrants

Another striking parallel between chronic inflammatory diseases and psychiatric disorders concerns the effects that immigration has on these conditions. All the diseases discussed here, whether chronic inflammatory [43, 47–49] or psychiatric [50–52], tend to be more common in immigrants than in the birth population from which the immigrant was derived, at least when the migration is from a developing to a high-income country. Other relevant variables include the age of the individual at the time of immigration, and whether the prevalence increases in second generation immigrants, born in the adopted country. A study of these parameters provides some insight into whether the relevant influences, be they psychosocial or immunological, need to occur before birth, or in early childhood, or whether they can still exert their effects on adults.

Immigration and Psychiatric Disorders

Depression is particularly interesting in this respect [53, 54]. Mexicans, Cubans and African/Caribbean peoples were found to have a two to threefold increase in the prevalence of depression if immigration to the USA occurred when the individual was less than 13 years old, or was born in the USA, compared to the prevalence in those who migrated after the age of 13 [53]. But this is not likely due to psychosocial stress related to skin color, because white Eastern European immigrants show the same effect. In sharp contrast, the effect is not seen in immigrants from Western Europe, or from Puerto Rico, which is closely associated with the USA. (These last two populations already have a high prevalence of depression that is not increased by immigrating to, or being born in, the USA) [53]. These findings imply that influences important for depression occur perinatally, or in the early years of life.

The same is true for psychotic disorders [55]. A large Danish study noted that immigration into Denmark when less than 4 years old was associated with a strikingly increased risk for psychotic disorders, whereas the increased risk gradually decreased with older age at migration and disappeared in those immigrating when more than 29 years old [56]. Similarly a large meta-analysis confirmed that schizophrenia was increased amongst first generation immigrants, and further increased amongst second generation immigrants, particularly when the country of origin was a developing one [57]. Again, early events seem crucial.

Age at immigration is irrelevant to an early onset condition such as autism, but autism is strikingly (as much as tenfold) increased in second generation Caribbean or African immigrants born in the UK, compared to children of white UK-born

mothers [52]. *These findings implicate crucial early events in the perinatal period or early childhood as risk factors for depression, schizophrenia and autism.*

Immigration and Chronic Inflammatory Disorders

Migration has clear effects on the prevalence of MS, and the crucial events that confer increased risk for the disease occur very early in life, as is true for the psychiatric disorders [reviewed and referenced in 58, 59]. Iranians who migrate to Sweden have twice the prevalence of MS seen in their birth country [49]. Interestingly, if the second (or later) generation immigrants return to their developing country of origin, they retain their increased susceptibility to MS, which remains higher than in the local population that was not born abroad [60]. A similar phenomenon was seen when people born in the UK (a high MS country) migrated to South Africa (SA: a low MS country). Migration from the UK to SA was protective when the migrant was a child, whereas adult migrants retained their high UK prevalence of MS [61]. Analysis of this and other studies suggests that the environmental factors that protect from or predispose to MS act during the first two decades of life [58, 59]. The same is true for type 1 diabetes (T1D). Here the crucial factor is to have been *born* in the receiving developed country, again suggesting that relevant environmental factors act very early, or even in the prenatal period [48].

The role of migration in conferring risk for allergic disorders has been intensively examined. A study of children adopted into Sweden from developing countries showed that the prevalence of asthma, hay fever and eczema were highest in those adopted when less than 2 years old [62]. Similarly, for Mexican immigrants to the USA, the prevalence of asthma was highest for those born in the USA, while in those not born in the USA, the prevalence of asthma decreased as the age at immigration increased [63]. This effect of age at the time of childhood immigration was also seen in immigrants to Israel from the former Soviet Union or Ethiopia who were assessed when 17 years old [64]. These observations suggest the importance of early environmental influences for allergy/asthma risk, a conclusion that is powerfully supported by evidence that prenatal exposure (i.e. of the pregnant mother) to the farming environment protects the infant against some allergic manifestations [3, 41]. This is discussed later in another context.

Finally, a definitive study of all first- and second-generation immigrants in Sweden between January 1, 1964, and December 31, 2007 showed that some first generation immigrants remain partially protected from both ulcerative colitis (UC) and Crohn's disease (CD), presumably by environmental factors encountered in their countries of origin, but the diseases increased in prevalence in second generation immigrants, relative to first generation immigrants [65]. Similarly, the prevalence of UC in South Asian immigrants to Leicester in the UK was higher in second than in first generation immigrants [66]. This again implicates perinatal factors as potentially causative of this migration effect.

Thus the influence of immigration, acting via factors that occur perinatally or very early in life, is equally consistent and highly apparent for both psychiatric and chronic inflammatory disorders.

Mechanisms of Immunoregulation by Old Friends

Urbanization and immigration from low- to high-income countries cause diminished contact with Old Friends, and correlate with an increased incidence of chronic inflammatory disorders, all of which show evidence of failed immunoregulation [reviewed in 67]. Moreover adverse outcomes in animal models of all of these chronic inflammatory conditions can be prevented or treated with "Old Friends" such as helminths, certain gut commensals or probiotics that induce immunoregulation [68–70]. What are the mechanisms that enable the "Old Friends" to exert immunoregulatory effects? This is a vast topic, and here we outline some of its most studied aspects, particularly those that involve, or occur in, the gut.

Regulation of Innate Immunity

The gut microbiota has been shown to be necessary for priming of innate immunity, measured as the microbicidal activity of splenic macrophages [71]. This microbicidal activity was increased following a social disruption stressor. However, if the mice were germ-free no increase in microbicidal activity was seen [71]. Moreover depletion of microbiota with antibiotics attenuated the stressor-induced macrophage activation, and reduced stressor-induced increases in circulating bacterial cell wall peptidoglycan [71], and eliminated the increases in circulating IL-6 and monocyte chemoattractant protein-1 (MCP-1) usually seen in stressor-exposed mice [72]. These observations were in agreement with an earlier finding that systemic activation of the innate immune system by the gut microbiota involves recognition of *meso*-diaminopimelic acid (*meso*DAP)-containing peptidoglycan found predominantly in Gram-negative bacteria, by the pattern recognition receptor nucleotide-binding, oligomerization domain-containing protein-1 (Nod1) [73]. Similarly abdominal surgery causes systemic release of Nod2-binding bacterial components and consequent rises in several inflammatory biomarkers [74].

Regulatory Macrophages

Regulatory microorganisms can also operate via macrophages. During helminth infections there is expansion of the population of alternatively-activated macrophages, activated by Th2 rather than Th1 cytokines [75]. Such macrophages secrete

IL-10 and TGF-β rather than IL-12, are able to inhibit lymphocyte proliferation in a contact-dependent manner [76, 77], and may be responsible for preventing inflammation in mucosal surfaces such as the lung. However, some helminths drive types of regulatory macrophages that are distinct from alternatively activated macrophages [77]. Several species of filarial nematodes secrete cystatin, a cysteine protease inhibitor that induces macrophages to make IL-10 and IL-12 p40 through activation of intracellular signaling pathways. These can prevent allergic sensitization and airway hyperresponsiveness [78]. Similarly a colon-infiltrating macrophage population induced by Schistosoma infection was shown to prevent colitis in mice [79]. The protection was independent of T cells in general and regulatory T cells (Treg) in particular.

Regulatory B Cells

Helminths also induce regulatory B cells. *S. mansoni* infection prevented anaphylaxis in a mouse model, and this suppression of the effector phase of the allergic response was mediated by IL-10-secreting B cells [80]. These IL-10-secreting $CD1d^{hi}CD5+$ regulatory B cells can also suppress experimental autoimmune encephalomyelitis [81], and have recently been designated B10 cells [82]. They act in part by increasing the number of pulmonary CD4+CD25+Foxp3+ regulatory T cells in the lungs [83]. IL-10+ regulatory B cells are also found in humans [84]. Depletion of human B-cells using rituximab can occasionally exacerbate Th1-mediated conditions, suggesting that the rituximab removed a B-cell-mediated regulatory mechanism [84]. IL-10 production by B cells is increased in multiple sclerosis patients developing intestinal helminth infections [85], but not in patients infected by *Trypanosoma cruzi* [85]. The helminths also increase circulating Treg, as discussed below.

Regulatory Dendritic Cells (DC)

A particularly important immunoregulatory function is the generation of regulatory dendritic cells (DCreg) that tend to drive regulatory rather than inflammatory responses. This is crucial because such DCreg can process gut contents, autoantigens and allergens, and so downregulate responses to the target antigens of the major groups of chronic inflammatory disease. It is likely that health requires the presence of a certain background proportion of DCreg. This could be regarded as a "Treg adjuvant" function. A mixture of several putative probiotic organisms (VSL#3; four lactobacilli, three bifidobacteria, and one streptococcal strains) was found to ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and TGF-β+Treg [86]. In vitro this preparation caused human DC to release more IL-10 and inhibited their ability to drive Th1 cells [87]. Other probiotic strains [88, 89],

and a ubiquitous environmental saprophyte often present in untreated or muddy water [90] also modulate human DC function in vitro so as to induce T cell responses with a more regulatory bias. In the gut some of these DCreg express the integrin alpha chain CD103 and have unique immunoregulatory properties [91]. CD103 is involved in de novo conversion of Foxp3-CD4+ cells to Foxp3+ Treg cells [92]. Conversion of DC to this tolerogenic phenotype is driven locally by TGF-β and retinoic acid (RA). CD103+ DCs express *aldh1a2*, the gene encoding RALDH2. This enzyme is involved in conversion of dietary retinal to RA, which enhances development of FoxP3+ T cells rather than Th17 cells [reviewed in 93]. Some probiotic *Lactobacillus* strains, such as *L. plantarum* WCFS1 induce migration of these CD103+ DCreg as far as the spleen, and bias the response towards Treg [94].

An example of a Treg adjuvant effect that must at some stage involve DC is seen when MS patients become infected with helminths. The disease stops progressing, and circulating Treg appear in the peripheral blood [95, 96]. These Treg recognize the major epitope from myelin basic protein. Thus the immunoregulation caused by the helminths is not merely a bystander effect of IL-10 release, but rather a genuine Treg adjuvant effect that generates regulation specific for the autoantigen. Although the mechanism is not elucidated this is an exciting observation that has led to formal clinical trials [97].

Regulatory T Cells (Treg)

Ultimately many of these immunoregulatory mechanisms result in a relative increase in the numbers of Treg, whether secondary to changes in macrophages, B cells or DC. However some Old Friends release molecules that specifically expand Treg populations. The gut commensal *Bacteroides fragilis* releases a polysaccharide antigen that drives expansion of Treg via TLR-2 [98]. The helminth *Heligmosomoides polygyrus* drives Treg expansion via the TGF-β receptor [99]. Treatment with oral *Lactobacillus reuteri* for 9 days significantly increased the percentage and total number of CD4+CD25+Foxp3+ T cells in the spleens of experimental animals [70]. Colonization of mice by commensal *Clostridium* strains increased TGF−β levels and numbers of Foxp3+ Treg in the colon [100].

Gut Microbiota Diversity and Regulation of Inflammation

Interestingly the diversity of the gut microbiota appears to have consequences for immunoregulation, perhaps for the reasons discussed in relation to Fig. 15.1. From birth our microbiota are constituted by colonization with organisms from our mothers, from other social contacts [101, 102], and from the environment, and then further modified by factors such as diet and antibiotics [103–106]. Thus

lifestyle has major effects on an individual's microbiota and on its diversity. The gut microbiota of children from traditional villages in Burkina Faso is totally different from that of Europeans, and shows greater diversity [104]. There is abundant evidence that diversity of gut microbiota is associated with wellbeing. Mice exhibit at least two enterotypes (bacterial ecosystems in the gut microbiota), one of which has low biodiversity, and correlates with biomarkers of inflammation [107]. In humans reduced biodiversity of the gut microbiota has been associated with a range of inflammatory states, including allergic disorders [108], inflammatory bowel diseases [109–111] and obesity [112]. Loss of diversity in later life is associated with increases in circulating CRP and IL-6 levels [113]. This implies that diversity is associated with effective immunoregulation.

In agreement with this, allergies are less common in children exposed to sources of microbial biodiversity such as farms [40], dogs [114], or the natural environment [115, 116]. Similarly, allergies are reduced where there is evidence of social interactions that promote exchange of microbiota. Indicators of such exchange include infection with orofecally transmitted organisms such as enteroviruses [117], *H. pylori*, *T. gondii*, and hepatitis A virus [118]. There is some evidence that these orofecally transmitted pathogens are themselves immunoregulatory. However it might be much more important that these organisms are markers of transfer of microbiota between individuals. Interestingly mothers who clean their baby's dummy/pacifier by sucking it rather than by sterilizing it have children with less allergic problems [119].

In sharp contrast, lifestyle events that are likely to restrict the diversity of gut microbiota are associated with increased risk of chronic inflammatory disorders. Birth by caesarian section may be a risk factor for allergic disorders [120, 121]. Similarly, excessive antibiotic use during pregnancy [122] or in early childhood is a risk factor for allergic disorders [123, 124] and IBD [125, 126]. And as discussed above, living in a high-income rather than in a low-income country is a risk factor for all of these disorders.

Organisms from the Natural Environment and Microbiota

Clearly microbiota from other people (and animals) can colonize our guts. But do organisms from the natural environment also colonize, or are these organisms "pseudocommensals" that impinge on the skin [116], airways and gut, and have independent immunoregulatory properties? Both mechanisms probably occur, though there are rather limited data on these issues. An interesting animal experiment compared piglets that were housed in a natural outdoor environment, with genetically similar piglets that had been reared in a very clean indoor facility. Firmicutes, in particular *Lactobacillus* strains were dominant in the gut microbiotas of the outdoor piglets, whereas the hygienic indoor piglets had reduced *Lactobacillus* and more potentially pathogenic phylotypes [127]. The indoor piglets also had less diverse gut microbiota, and a more inflammatory pattern of gene

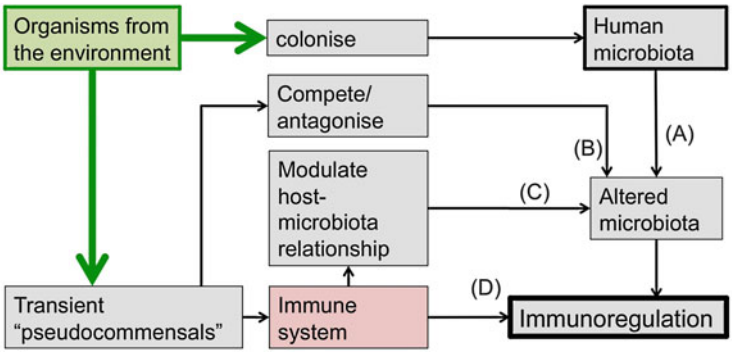

Fig. 15.3 Environmental organisms and immunoregulation. Microbial biodiversity from the environment can modulate immunoregulation by (*D*) directly interacting with the immune system, or (*A*, *B*, *C*) by leading secondarily to altered microbiota. The environmental organism may cause secondary changes to the microbiota by (*A*) colonizing, or (*B*) antagonizing or competing with established microbiota or (*C*) modulating the host immune system-microbiota relationship

expression in ileal biopsies [127]. For example they had increased Type 1 interferon activity, increased MHC Class 1, and upregulation of many chemokines [127], again confirming the correlation between reduced gut microbial biodiversity and poor control of inflammation discussed above.

Were these effects due to direct colonization by immunoregulation-inducing organisms from the outdoor environment [pathway (A) in Fig. 15.3], or did these organisms fail to colonize, but exert indirect effects on the immune system? The answer is unclear, but indirect effects certainly can occur in several ways. Some organisms compete with, or antagonize established organisms [pathway (B)] and so alter the microbiota [128]. Others alter the immune system directly [pathway (D)], or modulate the immune system in ways that lead secondarily to a change in the host-microbiota relationship, which in turn leads to changes in the microbiota [pathway (C) in Fig. 15.3].

The last mechanism is well established in experimental models. Genetic manipulations of the innate immune system that have profound effects on immune function (such as gene knockout) often operate indirectly by altering the gut microbiota. The phenotypic effects can then be transferred to wild-type mice that have not been genetically modified, by transferring the altered microbiota [129, 130]. It is the altered microbiota that is the proximate cause of the altered immunoregulation [129–133].

Is the Gut Still Involved in Immunoregulation by Organisms That Do Not Enter the Gut?

Does this mean that all immunoregulation by "Old Friends" operates indirectly by modulating the immune system, and so secondarily causing changes in the gut microbiota? It is likely that such indirect effects occur, but there could be direct effects too. For example the skin microbiota has at least some immunoregulatory role independent of the gut [134]. Indeed components of the skin microbiota extend into the subepidermal compartments, suggesting subtle and unexplored mechanisms [135]. Moreover psychological stressors cause increased bacterial translocation to lymphoid tissue from both the gut and the skin so the nature of the organisms present on skin is likely to be directly relevant to subsequent effects on immune function [136]. Interestingly, in the British field mouse (*Apodemus*), the burden of the louse *Polyplax serrata* correlated with the state of activation of the innate immune system in the spleen, implying that ectoparasites (fleas, lice, mites, ticks) might also have immunoregulatory roles [137, 138].

The blood nematodes are also of interest because these do not enter the gut at any phase of their life cycles, but they are powerfully immunoregulatory [9]. They cause impaired induction of T-bet and GATA-3 mRNA, Th1/Th2 deficiency and increased Foxp3, TGF-β, CTLA-4, PD-1, ICOS and IDO. Some blood nematodes secrete identifiable immunoregulatory molecules [139, 140].

Nevertheless, in view of the experiments listed in the previous section, it is still possible that in addition to direct effects on the immune system [pathway (D) in Fig. 15.3] these effects also operate indirectly via secondary modification of the gut microbiota [pathway (C) in Fig. 15.3].

Genetics and "Inflammatory Overshoot" in High-Income Countries

In parts of the world where there was a heavy load of organisms that drive potent immunoregulation (such as helminths) there has been selection for single nucleotide polymorphisms (SNP) or other variants to partially compensate for excessive immunoregulation, or to combat new infections such as malaria that spread from gorilla to man about 10,000 years ago [141, 142]. Such proinflammatory SNPs are seen for several proinflammatory cytokines [143], IgE [144] and STAT6, a transcription factor involved in Th2 responses [145]. There is also an increased frequency of the short allele of the serotonin transporter promoter that also has a marked proinflammatory effect [146]. However this results in a dangerous situation. As soon as the immunoregulation-inducing organisms are withdrawn by the modern lifestyle, or after immigration to a high-income country, these genetic variants lead to inflammatory overshoot. The proinflammatory variants become risk factors for chronic inflammatory disorders [143–146].

This is important because work that identifies proximate "causes" for diseases that were rare or nonexistent before the late nineteenth or early twentieth centuries, and that remain rare in low-income countries, may merely be unraveling a gene-environment interaction that would be irrelevant if the microbial status could be returned to that seen in the paleolithic age. For instance, the recent claim to have discovered that the "cause" of Crohn's disease is a genetically determined defect in the homing of neutrophils [147] is difficult to reconcile with the fact that 100 years ago the disease barely existed. But recent environmental changes could conceivably have caused this phenotype to become a risk factor.

How Does Stress Cause Inflammation?

Two major issues were avoided in the discussion of the role of immunoregulation in determining stress resilience (Fig. 15.2). First we did not discuss why stress causes release of inflammatory mediators, and secondly we did not discuss why such mediators trigger depression. Stress leads to activation of the hypothalamic-pituitary adrenal axis (HPA) and the sympathetic nervous system (SNS), and to changes in the microbiota and gut permeability. How do these mechanisms combine to result in raised proinflammatory cytokine levels?

GC Resistance

Depression is commonly associated with hypercortisolaemia and glucocorticoid resistance (GCR) [148]. A recent analysis has revealed that persistently raised levels of inflammatory cytokines cause GCR by impairing the function of glucocorticoid receptors [148], leading to further loss of control of inflammation. This was the suggested mechanism in individuals with recent exposure to severe psychosocial stressors who developed GCR and subsequently released more proinflammatory cytokines in response to an inflammatory stimulus (virus challenge to airways) [149].

Sympathetic Nervous System (SNS)

There is an increase in plasma concentrations of norepinephrine following exposure to a standardized laboratory stressor, the Trier Social Stress Test. This is accompanied by activation of the master regulator of inflammation, nuclear factor-kappa beta (NF-κB), in peripheral blood monocyte cells (PBMCs) [150]. Blocking the effects of activation of the SNS with the β-adrenergic receptor antagonist

propranolol blocked the stressor-induced increases in proinflammatory cytokines, and reduced the development of GCR [151].

Corticotropin-Releasing Hormone (CRH)

Increased expression of corticotropin-releasing hormone (CRH) is found in CSF and in the limbic brain regions in depression [152, 153], but CRH is also involved in the control of gut permeability [154–156]. For example, chronic administration of CRH via minipumps caused colonic barrier dysfunction in rats [155]. Moreover when released in the periphery by T cells, CRH is not only a regulator of intestinal permeability [154, 155], but also a potent pro-inflammatory cytokine [157]. In many cell types CRH activates NF-κB, and stimulates expression of IL-1β, IL-6, and TNF mRNAs [158], so some of the effects of CRH on permeability are secondary to the release of proinflammatory cytokines. Paracellular permeability is controlled by tight junctions, intermediate junctions and desmosomes, which constitute a size- and cation-selective filter for small molecules. However, TNF, IL-17, IFN-γ and nitric oxide (NO) increase permeability. IFN-γ disrupts the tight junctions, and can modify para-epithelial traffic of inflammatory cells. By contrast, the immunoregulatory cytokine TGF-β decreases permeability [154, 155].

The composition of the microbiota, particularly *Lactobacillus* strains and helminth "Old Friends" also modulate permeability. When idiopathic chronic diarrhea in rhesus monkeys was treated with the whipworm *Trichuris trichiura*, clinical improvement was accompanied by striking changes in the microbiota attached to the mucosa [159]. Similarly in a mouse model of IBD, infection with *H. polygyrus* caused an increase in lactobacilli.

Indirect Effects of Psychosocial Stress via the Microbiota

Stress induces changes in the composition of the microbiota of rodents [72], and induces bacterial translocation from gut and skin [136]. The same is true in humans. When sampled within hours of admission to the emergency room fecal bacterial counts were decreased 1,000-fold compared to control subjects, and obligate anaerobes and *Lactobacillus* species were significantly decreased [160]. Similarly, a large change in the microbiota after allogeneic bone marrow transfer was identified as a risk factor for subsequent inflammation and graft-versus host disease [161], implying that stress alters immunoregulation at least partly by altering the microbiota.

The gut microbiota is involved in the activation of the HPA axis by stress, and in the systemic release of cytokines. A social disruption stressor caused increases in circulating IL-6 and MCP-1 that correlated with changes in the composition of the microbiota. But this response was greatly attenuated by pre-treatment with an

antibiotic cocktail to deplete the microbiota [72]. Therefore much of the systemic cytokine response to stress might be secondary to uptake of LPS and other proinflammatory microbial components. Uptake of LPS was measured in another study. Animals subjected to restraint stress show increased portal blood LPS, together with HPA axis activation manifested as increased plasma ACTH and corticosterone, increased hypothalamic CRF and increased IL-1β, IL-6 and TNF. All of these manifestations were blocked by treatment with *Lactobacillus farciminis,* which blocks leakiness due to HPA axis activation [162].

Stress Resilience and the Microbiota

The microbiota might also be involved in the observation that poor stress resilience and an exaggerated cytokine response to environmental [31] or laboratory [22] stressors is characteristic of people who have suffered increased early life stress. We know that early life stress can modulate the microbiota [30, 163]. This interpretation is in agreement with the observation that in a low-income country population the adults who had a high level of microbial exposure in infancy were resistant to the long-term proinflammatory effects of a very severe childhood stressor [32]. In addition to the role in immunoregulation, the microbiota in the first weeks of life also modulates the development of the HPA axis and stress response [164], and the development of the brain [165].

How Does Inflammation Alter Behavior and Cognition?

The mechanisms that cause inflammatory responses to alter behavior and cognition have been extensively reviewed recently [166] and are summarized briefly here. From an evolutionary point of view this link between immunity and psychiatry is explained by the fact that during an acute infection, withdrawal (to conserve resources, fight infection and heal wounds) and hypervigilance (to detect danger) are adaptive responses [167]. Withdrawal and hypervigilance probably result from inflammation-mediated signals to distinct parts of the brain. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been used to identify brain regions affected by inflammatory cytokines, and the list includes the basal ganglia, the dorsal anterior cingulate cortex (dACC), amygdala, hippocampus, insula, dorsolateral prefrontal cortex, and subgenual ACC [reviewed in 166]. It is suggested that involvement of the basal ganglia is important for withdrawal, while the effects on the dACC are important for hypervigilance. However these states are not sustainable and if prolonged, withdrawal becomes depression and hypervigilance becomes anxiety. This relationship between prolonged inflammatory stimuli and behavioral changes that resemble depression

and anxiety was postulated long ago following observations of "sickness behavior" in mice [168].

Inflammation-associated depression and anxiety are more likely in situations where there is poor control of inflammation [169]. This is seen in high-income countries where persistently high CRP is common [170], as discussed earlier in the context of the Old Friends mechanism. Similarly, depression and anxiety often accompany the chronic inflammatory disorders that are increasing in high-income countries. They are also seen in obesity where fat tissue contains cytokine-secreting activated macrophages, and in people who underwent traumatic childhoods, perhaps because of developmental changes in the HPA axis, brain, and microbiota [31, discussed in 169]. Genetic factors also play a role. For example individuals who have the short allele of the serotonin transporter-linked polymorphic region were more likely to get depression after IFN-α treatment [171].

Cytokines and Cellular Infiltration

Recent studies of patients receiving IFN-α have confirmed in humans many of the mechanisms previously reported in animal studies. For example, recipients of IFN-α develop raised CSF levels of IL-6 and MCP-1, proving that the inflammatory signal is transmitted to the brain [172]. Signals from the inflamed periphery enter the brain via several pathways. First, cytokines can enter the brain in areas such as the circumventricular organs where there is no blood-brain barrier. Perhaps these areas should be regarded as sensory organs of which one role is the detection of inflammation. Signals also pass via activation of endothelial cells within the cerebral vasculature, leading secondarily to release of prostaglandins and NO in the CNS. Furthermore brain endothelium expresses specific cytokine transporters. Cytokines in the periphery can also signal via afferent fibres within the vagus and other sensory nerves. This has been called the "facsimile" mechanism, because the cytokine stimulating the peripheral nerve terminals may subsequently be synthesized de novo and released within the brain.

These various inflammatory signals may secondarily activate local CNS cell populations. The microglia are derived from a subset of CD45+ monocytic cells and enter the CNS during embryogenesis in utero and during early post-natal life. These stable long-lived cells form a network with surveillance functions within the brain parenchyma, and under normal conditions are in a non-activated, non-terminally differentiated state, with low expression of major histocompatibility complex class II [173]. However inflammation propagated to the brain by the pathways listed above can cause these cells to express inflammatory cytokines and to release reactive oxygen and nitrogen species [166]. Activated microglia may also be a source of the MCP-1 mentioned earlier, which recruits monocytes into the brain [174].

Recruited leukocytes can enter the CNS by several routes. In healthy individuals there is background traffic via the choroid plexus or through postcapillary venules

located in the subarachnoid space [175, 176]. However in inflammatory states cells can cross the endothelium of parenchymal post-capillary venules, and so enter the perivascular space. The relevant adhesion molecules are not expressed on these endothelial cells under resting conditions but they are induced by peripheral inflammatory signals such as LPS or TNF, facilitating the MCP-1-driven recruitment [176].

Indoleamine-2,3-Dioxygenase (IDO)

Inflammatory cytokines also activate the enzyme, indoleamine-2,3-dioxygenase (IDO), converting tryptophan to kynurenine. It has been suggested that this can deplete tryptophan sufficiently to cause depression. Recent mouse work showed that inflammation induced by peripheral administration of lipopolysaccharide (LPS) activated IDO and caused mice to display a depression-like behavioral syndrome that could be inhibited by blocking IDO [reviewed in 177]. However, the syndrome could be reproduced by administering kynurenine, which is taken up into the brain by the large amino acid transporter, where it can be further metabolized in microglia, astrocytes and macrophages to quinolinic acid, kynurenic acid and other metabolites [178]. This suggested that depletion of tryptophan was not the primary mechanism of the depression-like syndrome [179] (though this cannot be ruled out as an additional factor because there is evidence that kynurenine can compete with tryptophan for transport into the brain [180]). These mouse findings have been confirmed in patients with hepatitis C who were being treated with IFN-α, in whom there were depressive symptoms, but no reduction in CSF concentrations of tryptophan [181]. The treatment did however cause increased CSF levels of kynurenine, quinolinic acid and kynurenic acid, and also of IFN-α, soluble tumor necrosis factor-α receptor 2 and MCP-1 [181]. This suggested that as in the mouse, the depressogenic effect might involve transport of kynurenine into the brain followed by local generation of further active metabolites, rather than depletion of tryptophan [181]. There is increasing evidence that these neuro-active tryptophan metabolites are important in depression and in schizophrenia, and this topic has been reviewed in detail recently [177].

Transporters and Reuptake

Inflammatory cytokines also increase the expression of the transporters responsible for reuptake of dopamine, norepinephrine and serotonin. For example, in mice, IL-1, TNF and LPS all cause increased expression of the serotonin transporter paralleled by depression-like behavior [182]. In humans administration of IFN-α increases the reuptake and decreases the release of radiolabeled L-DOPA, the precursor of dopamine [183].

Tetrahydrobiopterin (BH4)

Inflammation also disturbs the availability of tetrahydrobiopterin (BH4), which is an essential cofactor for tryptophan hydroxylase and tyrosine hydroxylase. These are the rate-limiting enzymes for the synthesis of serotonin, dopamine and norepinephrine [166]. It is possible that the BH4 gets used up when cytokines drive NO synthase to generate NO, but this pathway is less active in man than in mouse. However BH4 can also be degraded by oxygen radicals and nitrogen radicals. Evidence for depletion of BH4 in the human brain in the presence of inflammatory mediators has recently been obtained in patients receiving IFN-α [184]. In the CSF of treated patients levels of the inactive oxidized form BH2 were increased, while levels of BH4 were inversely correlated with increased IL-6 [184].

Anti-Inflammatory Neurotransmitter Pathways

Inflammation in the CNS may also interfere with neurotransmitter pathways that have anti-inflammatory roles, and so weaken negative feedback on the inflammatory response. For example neural signals transmitted to the periphery via the vagus nerve inhibit cytokine release through a mechanism that requires the α7-subunit-containing nicotinic acetylcholine receptor [185]. This cholinergic anti-inflammatory pathway can be inhibited centrally by mediators such as IL-1 that increase neuronal acetylcholinesterase activity [185]. Similarly IL-1β might reduce signaling by γ-aminobutyric acid (GABA), and so enhance local inflammation [186]. This is due to the fact that GABA-ergic tone is anti-inflammatory because it inhibits the NF-κB and p38 MAPK pathways, and so reduces the response of microglia to LPS or IFN-γ [186].

Abnormal Brain Development

In addition to the postnatal mechanisms described above, inflammation can also act in utero to cause developmental defects in the foetal CNS that lead later to psychiatric problems. The likelihood of this phenomenon occurring will be influenced by the efficiency of immunoregulation in mother and child during pregnancy (Fig. 15.4).

The Old Friends mechanism will be one factor that determines this immunoregulation, though as discussed below, there are also genetic factors. Interestingly autism spectrum disorders (ASD) are increased in towns [39] and in second generation immigrants [52]. These findings parallel the simultaneous increases in chronic inflammatory disorders, and depression in which the Old Friends mechanism likely plays a role [169].

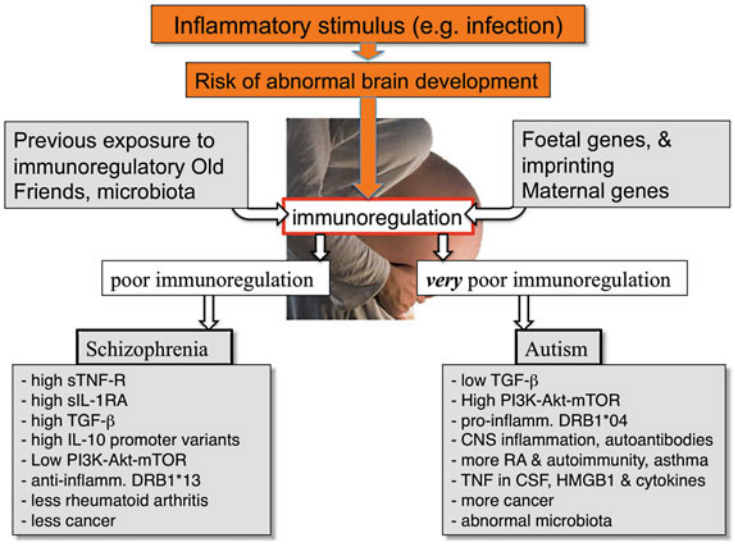

Fig. 15.4 Reduced efficiency of immunoregulation during pregnancy could predispose to inflammatory episodes in utero that lead to neurodevelopmental abnormalities. Such abnormalities are seen in ASD and schizophrenia, and both disorders are accompanied by evidence of failing immunoregulation that is most striking in ASD and in family members. The immunological points listed in the boxes at lower left and right are taken from, and fully explained within, the references in the main text

Maternal Infection, Immunoregulation, Fetal Inflammation and ASD

There has been a significant and genuine increase in the prevalence of autism spectrum disorders (ASD) that cannot be explained only by increased awareness [187]. There is debate about the relative contribution of genetics and environment. A very recent study suggested that "Susceptibility to ASD has moderate genetic heritability and a substantial shared twin environmental component" [188]. Rather than worrying about the relative importance of genes and environment it is important to note that known autism susceptibility genes include a neuronal module and a module enriched for immune genes and glial markers [189]. Another study of interactome networks associated with highly expressed ASD-candidate genes found that immune signaling through NF-κB, TNF, and Jnk were strongly represented and that these interactomes involved glia in addition to neurons [190]. Thus the genetics point to inflammation and the immune system, which could modulate susceptibility to the types of environmental influence discussed in this chapter. A recent study suggests that there might be an underlying immune phenotype (whether genetic or environmental): the immune systems of autistic children and their healthy siblings were found to have similar immune

dysregulation, when compared to the immune systems of matched healthy children [191].

An obvious link between immunoregulation and ASD is provided by evidence that maternal infection during pregnancy increases the risk of ASD in the infant. In one study 13 % of infants developed ASD following exposure to congenital rubella [192]. However it seems that any infection increases the risk, particularly if severe enough to require hospitalization during pregnancy [193]. A study of 1.2 million births in Finland showed that raised maternal CRP early in gestation was associated with increased risk, whatever the cause [194]. Animal work proves that maternal inflammation during pregnancy is transmitted to the foetus. TNF-α and IL-1β expression was upregulated in a dose dependent manner in the fetuses of pregnant rats exposed to LPS [discussed in 195]. Similarly I^{125}-labelled IL-6 administered i.v. to pregnant rats was found in the fetal compartment [196].

Experimental animals exposed to maternal immune activation in utero also display developmental changes measured by MRI, and behavioral changes suggestive of ASD [197]. In pregnant mouse models these effects are dependent upon IL-6, and can be mimicked by administering IL-6 itself rather than an indirect inflammatory stimulus [25]. Interestingly these abnormalities are accompanied by a systemic deficit in $CD4^+$ $TCR\beta^+$ $Foxp3^+$ $CD25^+$ T regulatory cells, and by increased IL-6 and IL-17 production by $CD4^+$ T cells [198]. The behavioural abnormalities can be attenuated by transplanting normal bone-marrow, implicating the immune system both in the development of the syndrome, and in its subsequent maintenance [198].

Inflammation and Faulty Immunoregulation in ASD

The role of inflammation in utero in the development of the CNS abnormalities that accompany at least some cases of human ASD is not in doubt. But there is increasing evidence for an ongoing immunoregulatory deficit in human ASD [199, 200], as in the mouse model mentioned above [198]. ASD patients have increased circulating levels of proinflammatory cytokines, and reduced levels of TGF-β [200], and there is an increased prevalence of asthma and autoimmunity in family members, reinforcing the view that there is an hereditary, or at least familial, immunoregulatory deficit [191, 199, 200]. This tendency to autoimmunity is manifested as brain autoantibodies in plasma from children with ASD, and from their mothers [200].

Studies of autistic brains reveal activation of microglia and astroglia and increased expression of a range of proinflammatory mediators such as TNF, IFN-γ, IL-8 and IL-6 [201–203]. IL-6 is normally expressed at very low levels in the brain, but it is able to cross the placenta [196] and induce an ASD-like state in the offspring when administered to pregnant mice [25]. Overexpression of IL-6 in transgenic mice causes neuroanatomical and neurophysiological alterations

associated with neurological disease [204]. Immunohistochemistry studies confirmed that IL-6 is raised in the cerebellum of autistic brain [205].

Autoantibodies to Brain in ASD

There is an alternative way of interpreting some of the data. There is no doubt that autoantibodies present during fetal life [206], or during inflammatory episodes when blood-brain barrier function is compromised [176], can alter CNS function [207]. Such antibodies have been shown in autism [206] and, as suggested by a murine model, might explain the high frequency of learning disorders in the offspring of mothers suffering from systemic lupus erythematosus (SLE) [208]. The association between autoimmune disease and psychiatric disease has been confirmed in a massive recent study [209]. Patients with MDD have not only a higher frequency of brain-reactive antibodies, but also an increased circulating Th17/Treg ratio [21]. In short, the relationship between immunodysregulation and psychiatric disease might involve additional autoantibody-mediated damage when autoimmunity is one of the forms of chronic inflammatory disorder present in a given individual, even if subclinical. The evidence for this in some cases of ASD is strong [206].

GI Symptoms and Immunoregulation in ASD

Gastrointestinal symptoms are common in ASD. These include diarrhea, constipation, vomiting/reflux, abdominal pain/discomfort, gaseousness, and unusually foul-smelling stools [210] and are similar to the symptoms of irritable bowel syndrome (IBS), which affects 10–20 % of the US population [discussed in 211]. While symptoms are not in doubt, there is controversy about whether these correlate with detectable inflammation in the gut mucosa [discussed in 211]. The observation that low-grade endotoxemia occurs in patients with severe autism tends to suggest that some gut inflammation might be present [212]. Meanwhile the use of modern culture-independent methods has revealed that the gut microbiota of autistics is abnormal [213–215]. Some authors argue that this abnormality might lead to excessive production and absorption of short chain fatty acids (SCFA; for example propionic acid) that in experimental animals induce autism-like states [216].

Brain Development and Schizophrenia

The etiology of schizophrenia is not known but the predominant view is that, like at least some cases of ASD, it can involve abnormalities in brain development occurring during fetal/neonatal life (Fig. 15.4) long before manifestation of the illness in adolescence or early adulthood [173, 217]. There is correlational evidence for this. Raised maternal levels of IL-8 and TNF (but not of IL-6 and IL-1β) in mid gestation were associated with psychosis in the children [173, 218, 219]. As in ASD there is evidence for immunoregulatory problems in adults with schizophrenia, though these are less extreme than in ASD [199]. Interestingly a relationship between ASD and schizophrenia has been postulated that attributes the differing disease manifestations to relative expression of maternal or paternal copies of imprinted genes [220]. It might be possible to reconcile this hypothesis with the differing degrees of immunodysregulation characteristic of the two conditions [199]. It is of particular interest that some genes that predispose to ASD do not need to be expressed in the fetus, involve the immune system and probably act during pregnancy [221].

Conclusions

The study of the gut microbiota by modern molecular methods, and the modulation of the microbiota by modern lifestyle and dietary habits, have led to a vast expansion of our knowledge, and to a tendency for the study of the microbiota to be seen as an independent medical discipline. Meanwhile, the original hygiene hypothesis was seen as a narrow concept dealing mostly with factors affecting allergic disorders in childhood, while an offshoot of the hygiene hypothesis, sometimes known as the "helminth hypothesis" has been applied mostly to the increases in IBD and MS. In this chapter we suggest that these concepts need to be studied together, or even unified under one heading such as the "Old Friends", or "biodiversity" mechanism. They all deal with the issue of the education and regulation of the immune system by microbial contact. The gut microbiota is certainly a major component of this system, but it acts in concert with other environmental inputs that regulate the immune system, including organisms that never enter the gut. By seeing the whole picture we may be able to determine whether immunodysregulation due to divergence of our microbial exposure from that with which we evolved is able to explain the worrying and parallel increases in chronic inflammatory disorders and inflammation-linked psychiatric disorders in high-income countries.

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