REVIEW

The hygiene hypothesis: current perspectives and future therapies

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Abstract: Developed countries have experienced a steady increase in atopic disease and disorders of immune dysregulation since the 1980s. This increase parallels a decrease in infectious diseases within the same time period, while developing countries seem to exhibit the opposite effect, with less immune dysregulation and a higher prevalence of infectious disease. The "hygiene hypothesis", proposed by Strachan in 1989, aimed to explain this peculiar generational rise in immune dysregulation. However, research over the past 10 years provides evidence connecting the commensal and symbiotic microbes (intestinal microbiota) and parasitic helminths with immune development, expanding the hygiene hypothesis into the "microflora" and "old friends" hypotheses, respectively. There is evidence that parasitic helminths and commensal microbial organisms co-evolved with the human immune system and that these organisms are vital in promoting normal immune development. Current research supports the potential for manipulation of the bacterial intestinal microbiota to treat and even prevent immune dysregulation in the form of atopic disease and other immune-mediated disorders (namely inflammatory bowel disease and type 1 diabetes). Both human and animal model research are crucial in understanding the mechanistic links between these intestinal microbes and helminth parasites, and the human immune system. Pro-, pre-, and synbiotic, as well as treatment with live helminth and excretory/secretory helminth product therapies, are all potential therapeutic options for the treatment and prevention of these diseases. In the future, therapeutics aimed at decreasing the prevalence of inflammatory bowel disease, type 1 diabetes, and atopic disorders will likely involve personalized microbiota and/or helminth treatments used early in life.

Keywords: inflammatory bowel disease, microbiota, helminths, atopic disease, type 1 diabetes

Introduction

The Millennial generation (born 1980–1999) displays a marked increase in prevalence of atopic diseases (asthma, anaphylaxis, allergic rhinitis, food allergy, and atopic dermatitis [AD]) and immune-mediated disorders (including type 1 diabetes [T1D], and inflammatory bowel disease [IBD]), which have been steadily increasing in developed countries since the 1980s.[1–3] These disorders comprise a unique sector within immune dysregulation characterized by an irrational immune cell response to a foreign (or in the case of autoimmunity, a self) antigen which would, under normal circumstances, not occur. The short developmental timeframe of these diseases (from the 1980s onward, roughly within one generation) decreases the likelihood that a changing genetic component is significantly involved. Hence, researchers are assessing the potential effects of environmental factors, such as diet and antibiotic exposure.[4] Furthermore, the increase in immune disorders and

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atopic diseases parallels a decrease in prevalence of infectious diseases over the same time period, which can be attributed to increased vaccine and antibiotic treatments, and improved sanitation standards.[4] An in-depth look at the effects of these "hygienic" environmental factors suggests that lack of exposure to infectious agents may be the culprit for the increase in immune-mediated and atopic disease prevalence, a concept most commonly referred to today as the "hygiene hypothesis". This review aims to provide readers with the historical and current perspectives of the hygiene hypothesis and to elaborate on the modern scientific and medical applications of this theory. We also discuss the increasing evidence connecting the hygiene hypothesis to the development of atopic disease and immune-mediated disorders, in addition to discussing future therapies capitalizing on this knowledge.

A history of "hygiene" in immune modulation

One of the first observations relating infectious agents and immune dysregulation occurred in Western Nigeria, where Greenwood noted the low incidence of rheumatoid arthritis and deduced that this low incidence may be attributed to immunological disturbance resulting from frequent exposure to malaria (Figure 1).[5] Greenwood et al also observed suppressed spontaneous autoimmune disease, characterized by delayed Coombs test positivity and reticulocytosis in mice infected with *Plasmodium berghei* (a causative agent of rodent malaria).[6] In the late 1970s, a discrepancy between urbanized and rural environments emerged when Gerrard et al observed a lower prevalence of allergy in indigenous populations in Northern Canada compared to urban Caucasian populations.[7]

In 1989, Strachan proposed the hygiene hypothesis of allergic disease after observing that hay fever was less common among children with older siblings.[8] He reasoned that children growing up in larger families may experience increased exposure to microbes in early childhood due to inevitable unhygienic contact with older siblings or prenatal exposure from the mother infected by similar unhygienic contact.[8] Strachan proposed that this increased microbial exposure in early life could protect children from developing immune hypersensitivities later in life.[8] Strachan et al supported this theory by assessing family history, medical records, and allergy skin prick test results in a cohort of 11,765 children and found that household size was inversely correlated with the development of hay fever.[9] Additional epidemiological studies supporting the hygiene hypothesis associate a reduction in allergen sensitization with pet exposure, daycare attendance, and an increased number of siblings.[10,11] Early childhood infections have also been associated with decreased atopy in children. A retrospective case-control study showed that atopic patients exhibited a lower prevalence of *Toxoplasma gondii*, *Helicobacter pylori*, and hepatitis A when compared to nonatopic controls.[12] More recently, single-strand polymorphism analysis and culture techniques were used to identify microbial exposures among two cohort studies of European children.[13]
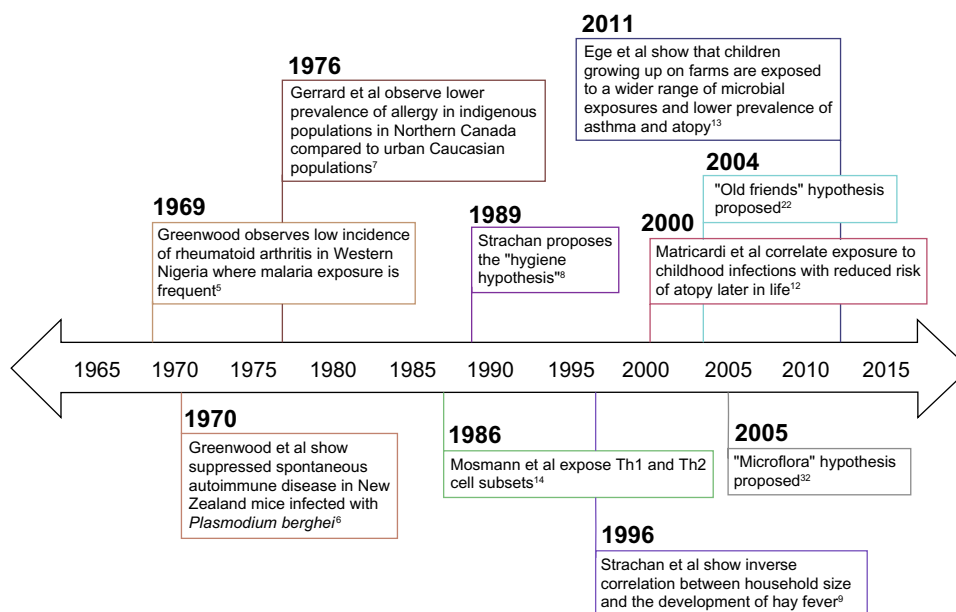

Figure 1 Timeline displaying key findings leading up to the proposal of the "hygiene hypothesis", proposal of the "old friends" and "microflora" hypotheses, and key microbiological and immunological findings in support of these theories.
Abbreviation: Th, T-helper.

In both cohort studies, researchers found that children growing up on farms in Central Europe encountered a wider range of microbial exposures and had a lower prevalence of asthma and atopy than the reference group.[13] A closer look at the immunological mechanisms behind Strachan's hygiene hypothesis of allergic disease will enhance the connection between early life infectious exposures and the development of immune tolerance.

Immunological support for the hygiene hypothesis

In 1986, just prior to Strachan's proposal of the hygiene hypothesis, Mosmann et al described the T-helper (Th)1 and Th2 cell subtypes, providing an immunological basis for this otherwise observational theory.[14] They discovered that fully differentiated murine CD4⁺ T-cells secreted two separate cytokine profiles (Th1: IFN-γ; Th2: IL-4) and that the different cytokines produced two different inflammatory responses.[14] Th2 cells play a primary role in the allergen sensitization process.[15] Infection with viruses and intracellular bacteria generally stimulates Th1 immune responses, which suppress Th2 cytokine activity through the induction of IFN-γ.[16,17] Consequently, the concept of a Th1 versus Th2 balance arose whereby a Th1-dominated immune phenotype (brought on by early life microbial exposures) was thought to inhibit atopic immunopathology. Research related to helminth parasites stimulated the need for further explanation beyond this binary view, as these organisms paradoxically induce Th2 responses while suppressing allergic reactivity.[18] T-cell plasticity and additional T-cell phenotypes (eg, Th17, Th9, and T regulatory [Treg] cells) have more recently been implicated in the control of hypersensitivity disorders.[19,20] Additionally, many innate cytokines (eg, IL-25, IL-33, and thymic stromal lymphopoietin) and cell types (eg, eosinophils, basophils, mast cells, and epithelial cells) also play significant roles in hypersensitivity disease.[21] It is now understood that the process of allergen presentation and consequent initiation of the allergic response involves both the innate and adaptive branches of the immune system. Thus, the immunological foundation of the hygiene hypothesis has been modified to consider the balance between many adaptive and innate immune cell populations. Further, extending the hygiene hypothesis to account for the role of various parasites (ie, intestinal helminths) and microbiota compositional shifts provides insight into how early life environmental exposures shape the human immune system. These extensions are known as the "old friends" and "microflora" hypotheses, respectively.[22,23]

The old friends hypothesis: parasitic helminths

The old friends hypothesis, proposed by Rook et al, notes the co-evolution of microorganisms and macroorganisms, such as parasitic helminths, with the development of the human immune system.[22] Similar to the hygiene hypothesis, it suggests that these organisms are required for normal immune system development.[22,24] For example, a study in Gabon found that school children diagnosed with schistosomiasis, caused by infection with helminth parasites from the *Schistosoma* genus, exhibited lower levels of allergen reactivity than their uninfected classmates.[25] Since then, additional studies have highlighted this seemingly protective effect of helminths in many mouse models of allergic diseases.[26–28] A live *Heligmosomoides polygyrus* (*H. polygyrus*; a murine helminth parasite) infection reduces lung cellular influx, eosinophilia, allergen recall responses, bronchial hyperreactivity, and histopathology in ovalbumin (OVA)- and house dust mite (HDM)-driven mouse models of asthma.[26,27] Additionally, *Schistosoma mansoni* infection has been shown to be protective in an experimental mouse model of fatal anaphylaxis, probably due to the induction of a regulatory IL-10-producing B cell population.[28] There is also experimental animal model evidence suggesting the ability of helminths to ameliorate symptoms of T1D and colitis (Table 1). Non-obese diabetic (NOD) mice spontaneously develop T1D, which is significantly inhibited when they are infected with *H. polygyrus* or the filarial nematode *Litomosoides sigmodontis*.[29–32] Helminth infection has also been shown to reduce inflammation in murine models of colitis.[33] Studies such as these support live helminth infection as a potential therapy to combat hypersensitivity and other immune disorders; however, referring back to Strachan's original hygiene hypothesis, the question of whether live helminth infection in early life is an effective treatment to protect against the development of these disorders is still unclear. Future therapeutics to treat immune dysregulation may involve the excretory/secretory (ES) products of these parasites and/or the intestinal microbiota (Tables 1 and 2).

The microflora hypothesis

The microflora hypothesis is another modern extension of the hygiene hypothesis, which suggests that early life perturbations (driven by factors such as antibiotic use, infection, or diet) to the bacteria residing in the human intestine (the intestinal microbiota) disrupt the normal microbiota-mediated mechanisms promoting immunological tolerance and consequently bias the immune system toward

Table 1 Helminth-based therapeutic studies

Organism	Disease	Treatment	Description of effects	Reference
Mouse	OVA-alum and Der p1-alum (HDM allergen)-driven models of allergic airway inflammation	*H. polygyrus* larvae	*H. polygyrus*-infected mice had reduced airway cellular infiltrates (including reduced eosinophilia and neutrophilia), reduced lung type 2 cytokines, and reduced lung histopathology	26
Mouse	OVA-alum-driven model of allergic airway inflammation	*H. polygyrus* larvae	*H. polygyrus*-infected mice had reduced airway eosinophilia, reduced bronchial hyperreactivity, and reduced allergen-specific Th2 responses	27
Mouse	OVA-alum-driven model of allergic airway inflammation	HES	HES given at allergen sensitization or challenge reduced airway cellular infiltrates and lung eosinophilia	110
Mouse	*Alternaria alternata*-driven model of allergic airway inflammation	HES	HES blocked lung eosinophilia, IL-33 release, and innate lymphoid cell type 2 cytokine production	111
Mouse	TNBS-induced colitis	*Schistosoma mansoni*- or *Ancylostoma caninum*-soluble proteins	Intraperitoneal helminth protein administration reduced macroscopic inflammation scores and reduced proinflammatory cytokine release (IL-17 and IFN-γ)	112
Mouse	Systemic–fatal anaphylaxis	*S. mansoni* cercariae	*S. mansoni*-infected mice were protected from anaphylaxis	28
Mouse	T1D (spontaneous development in NOD mice)	*H. polygyrus* larvae	*H. polygyrus* infection delayed disease onset	29,30
Mouse	T1D (spontaneous development in NOD mice)	*Litomosoides sigmodontis* larvae	*L. sigmodontis* infection prevented disease onset	31,32
Mouse	OVA-alum-driven model of allergic airway inflammation and DSS-induced colitis	Recombinant cysteine protease inhibitor (cystatin) of *Acanthocheilonema viteae*	*A. viteae* cystatin treatment during OVA sensitization or prior to OVA challenge reduced airway BALF cell counts, airway eosinophilia, bronchial hyperreactivity, and lung histopathology. In the DSS–colitis model, intrarectal *A. viteae* cystatin resulted in significant reductions in colonic inflammatory index compared to control animals	114
Mouse	DSS-induced colitis	*Ancylostoma ceylanicum* crude extract or ES products	Helminth-product-treated mice had reduced clinical and colonic microscopic inflammation scores compared to control mice	113
Mouse	T1D (spontaneous development in NOD mice)	*S. mansoni* infection, or treatment with soluble worm or egg extracts	Exposure to worm or egg extract prevented disease onset if given before 4 weeks of age	116
Mouse	DSS-induced colitis	ES products from *A. caninum*	Exposure to helminth products reduced intestinal proinflammatory cytokine expression	115
Humans	CD and UC	Live *Trichuris suis* eggs	Three out of four CD patients entered remission; fourth patient had a reduction in symptoms. UC patients had a reduction in clinical colitis activity index	106
Humans	CD	Live *T. suis* ova	79.3% of patients had a reduction in CD activity index or remitted	103
Humans	CD	Live *T. suis* ova	All doses tested were well tolerated and did not result in treatment-related side effects. Efficacy of a reduction in disease severity not assessed	104
Humans	UC	Live *T. suis* ova	A reduction in disease activity was seen in helminth-infected patients compared to placebo group, although this did not reach statistical significance	108
Humans	CD	*Necator americanus* larvae	IBD questionnaire results were improved, and cumulative CD activity index scores were decreased	105
Humans	Allergic rhinoconjunctivitis	*N. americanus* larvae	Infection well tolerated; no significant differences in allergic symptoms between groups given placebo or *N. americanus* larvae	107

Abbreviations: OVA, ovalbumin; alum, potassium aluminum sulfate; ES, excretory/secretory; HES, *H. polygyrus* excretory/secretory product; HDM, house dust mite; BALF, bronchoalveolar lavage fluid; IL, interleukin; IFN, interferon; DSS, dextran sulfate sodium; TNBS, 2,4,6-trinitrobenzene sulfonic acid; T1D, type 1 diabetes; NOD, non-obese diabetic; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; Th, T-helper.

a state that promotes hypersensitivity disorders.[23] Current research focuses on the mechanisms by which the intestinal microbiota influences immune system development and homeostasis, and potentially confers protection against immune dysregulation.[35–50]

A mutualistic bond

The human intestine is a densely populated zone in the body harboring a diverse microbial community of 500–1,000 different bacterial species among other microbes such as archaea, eukarya, and viruses.[34] The most striking illustration

Table 2 Microbiota-based therapeutic studies

Organism	Disease	Treatment	Description of effects	Reference
Rat	HLA-B27 transgenic rats (colitis model)	Inulin and FOS	Decreased severity of intestinal inflammation (FOS treatment resulted in less disease severity than inulin)	135
Rat	T1D (BB-DP rat model)	*Lactobacillus johnsonii*	Administration of *L. johnsonii* isolated from BB-diabetes-resistant rats resulted in decreased incidence of T1D and reduced levels of IFNγ and inducible nitric oxide synthase in BB-diabetes-prone rats	92
Mouse	T1D (spontaneous development in NOD mice)	VSL#3 (probiotic compound: containing *Bifidobacteria*, *Lactobacilli*, and *Streptococci* species).	Reduced insulitis and decreased beta cell destruction	124
Mouse	HDM-driven model of allergic airway inflammation	Diet supplemented with 30% pectin	Increased concentrations of SCFAs and decreased allergic inflammation in the lungs of murine HDM model of airway inflammation	46
Mouse	OVA-alum-driven model of airway inflammation	scGOS/lcFOS, and scGOS/lcFOS + pAOS	Suppressed airway inflammation and hyperresponsiveness	134
Mouse	CMA model	scGOS/lcFOS + *Bifidobacterium breve*	Increased serum galectin-9 and galectin-9 expression by intestinal epithelial cells. Also, reduced acute allergic skin reaction and mast cell degranulation	137
Rat	T1D (STZ model)	*Lactobacillus gasseri* engineered to secrete GLP-1(1-37)	GLP-1(1-37) secreted by *L. gasseri* stimulated rat intestinal epithelial cells to become glucose-responsive insulin-secreting cells. Resulted in increased insulin levels and glucose tolerance in diabetic rats	125
Mouse	IBD (IL-10-deficient colitis model)	*Lactobacillus plantarum*	Prior to SPF flora exposure, treatment of GF IL-10 deficient mice with *L. plantarum* and continued *L. plantarum* therapy attenuated colitis	122
Mouse	IBD (DSS-induced colitis model)	*Lactobacillus rhamnosus, L. plantarum, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium lactis, Bifidobacterium bifidum, Bifidobacterium adolescentis, Bifidobacterium infantis*	Mice receiving the probiotic mixture for 7 days prior to DSS induction of colitis showed reduced mucosal inflammation and damage compared to controls that did not receive the therapy	123
Mouse	IBD (IL-10-deficient and DSS-induced colitis models)	*Lactobacillus salivarius*	Oral treatment with *L. salivarius* did not attenuate colitis symptoms in IL-10-deficient or DSS-treated mice	132
Mouse	OVA-alum-driven model of airway inflammation	*Bifidobacterium longum*	Protected against airway inflammation in OVA-sensitized mice and blocked induction of OVA-specific IgE	66
Mouse	OVA-alum-driven model of airway inflammation	*Lactobacillus reuteri, L. salivarius*	*L. reuteri* decreased airway hyperresponsiveness. *L. salivarius* had no effect	64
Human	Eczema	*Lactobacillus rhamnosus* and *L. reuteri*	After 6 weeks of probiotic therapy, 56% of children (aged 1–13 years) experienced improved eczema, while only 15% of placebo controls reported improved symptoms	127
Human	UC	Enema solution containing *L. reuteri*	Improved mucosal inflammation and decreased inflammatory cytokines in children with UC	128
Human	AR	*L. johnsonii* + levocetirizine	Compared with patients receiving levocetirizine only, *L. johnsonii* + levocetirizine improved AR symptoms including increased IFNγ and IL-10 and decreased IL-4 concentrations, and improved FVC and FEV1 spirometry measurements in a 24-week, two-phase crossover treatment program	141
Human	Pollen allergy	*B. longum*	Reduced ocular symptom scores during exposure to Japanese cedar pollen	142
Human	Peanut allergy	*L. rhamnosus* + peanut oral immunotherapy	Subjects (82.1%) receiving combination peanut oral immunotherapy + *L. rhamnosus* achieved possible sustained unresponsiveness to peanut 2–5 weeks after discontinuation of treatment compared to only 3.6% receiving placebo	126
Human	AD, recurrent wheeze, allergic urticaria	scGOS + lcFOS	Prebiotic group had significantly lower incidences of allergic manifestations	56
Human	AD	scGOS/lcFOS + *B. breve* (Immunofortis®)	Increased galectin-9 expression and reduced AD in infants with IgE-mediated eczema 12 weeks posttreatment	137
Human	Asthma	scGOS/lcFOS + *B. breve* (Immunofortis)	Decreased prevalence of frequent wheezing and usage of asthma medications in children with AD after 1 year follow-up evaluation	139

(*Continued*)

Table 2 (*Continued*)

	Disease	Treatment	Description of effects	Reference
Human	Asthma, eczema, allergic rhinoconjunctivitis	*L. reuteri*	Oral supplementation with *L reuteri* ATCC 55730 in the last month of gestation through the first year of life is not associated with lower prevalence of allergic disease at 7 years of age	130
Human	UC	*Escherichia coli* Nissle (Mutaflor®)	Mutaflor® is as effective at preventing relapses as the established mesalazine therapy in patients with UC. Patients (36.4%) receiving Mutaflor for 12 months experienced relapses compared to 33.9% in the mesalazine group	129
Human	UC	Inulin-oligofructose (Synergy® 1) + *B. longum*	Reduced chronic inflammatory markers of UC (TNFα and IL-1α)	140

Abbreviations: HLA, human leukocyte antigen; FOS, fructooligosaccharide; T1D, type 1 diabetes; BB-DP, bio-breeding diabetes-prone; NOD, non-obese diabetic; IFN, interferon; IL, interleukin; OVA, ovalbumin; alum, potassium aluminum sulfate; SCFA, short-chain fatty acid; DSS, dextran sulfate sodium; scGOS, short-chain galactooligosaccharide; lcFOS, long-chain fructooligosaccharide; pAOS, pectin-derived acidic oligosaccharide; CMA, cow's milk allergy; GLP, glucagon-like peptide; UC, ulcerative colitis; STZ, streptozotocin; SPF, specific pathogen-free; GF, germ-free; AD, atopic dermatitis; AR, allergic rhinitis; HDM, house dust mite; IBD, inflammatory bowel disease; FVC, forced expiratory vital capacity; FEV1, forced expiratory volume in 1 second; ATCC, American Type Culture Collection; Ig, immunoglobulin.

of the importance of the intestinal microbiota for mammalian immune development comes from studies conducted in germ-free (GF) mice, in which the lack of a microbiota results in reduced Peyer's patches, smaller germinal centers and fewer plasma cells, and increased susceptibility to pathogen invasion when compared to conventionally raised mice.[35–38] Although GF murine models are valuable in mechanistic studies, they do have many caveats.[39] To fully elucidate the underlying mechanisms driving the relationship of the gut microbiota with atopic disease development, many different murine models, including GF, gnotobiotic, and antibiotic-treated models, along with models supplemented with specific bacterial species, should be used. In addition, murine systems with a reconstituted human immune system would be even more valuable.

Specific bacterial species within the microbiota have been shown to induce expression of antimicrobial peptides (eg, *Bacteroides thetaiotaomicron* induction of regenerating islet-derived 3γ expression by Paneth cells) and mucin production, which ultimately confers protection against pathogen invasion, and combined with regular stimulation of pattern recognition receptors, contributes to intestinal homeostasis.[40–42] The presence of the microbiota can stimulate CD4+ T-cell proliferation, Th17 cell differentiation through the induction of IL-1β, and accumulation of colonic Tregs.[43–45] The intestinal microbiota also metabolizes food components that are indigestible by mammalian enzymes, such as human milk oligosaccharides (HMOs) and dietary fiber.[46,47] This produces short-chain fatty acids (SCFAs), which are essential energy sources for many host tissues and prominent immune modulators.[48–50] There are many factors that likely contribute to the development of immune dysregulation: perturbations to the composition of

the intestinal microbiota, caused by environmental factors such as antibiotic exposure, birth mode, or diet, are one potential explanation linking early life hygiene with the development of atopic and immune-mediated disorders (Figure 2).

The intestinal microbiota in atopic disease: human studies

A longitudinal study comparing the early life intestinal microbiota compositions of school-age asthmatic and non-asthmatic children showed that significant decreases in overall gut microbial diversity at 1 week and 1 month of age were correlated with asthma development at school age.[51] Additionally, a recent characterization of the gut microbiota of 166 Canadian infants revealed an increased Enterobacteriaceae/Bacteroidaceae ratio in children sensitized to food allergens at 3 months and 1 year of age compared to non-sensitized children.[52] Also, lower gut microbial richness was observed at 3 months of age only.[52] Studies such as these suggest that therapeutic microbial intervention early in human life may be favorable, and highlight the need for animal studies in which experimentation to confirm causality is possible.

Many human studies lend support for the hygiene and microflora hypotheses by assessing the impact of early life environmental factors known to disturb the intestinal microbiota on atopic disease development later in life. For example, antibiotic usage in the first 2 years of life has been associated with the development of asthma at 7.5 years of age in a dose-dependent manner.[53] Additionally, antibiotic usage was reported to precede the manifestation of wheeze in the first 2 years of life in a questionnaire-based analysis of the KOALA (acronym in Dutch for "Child, parents and health: lifestyle and genetic constitution") Birth Cohort
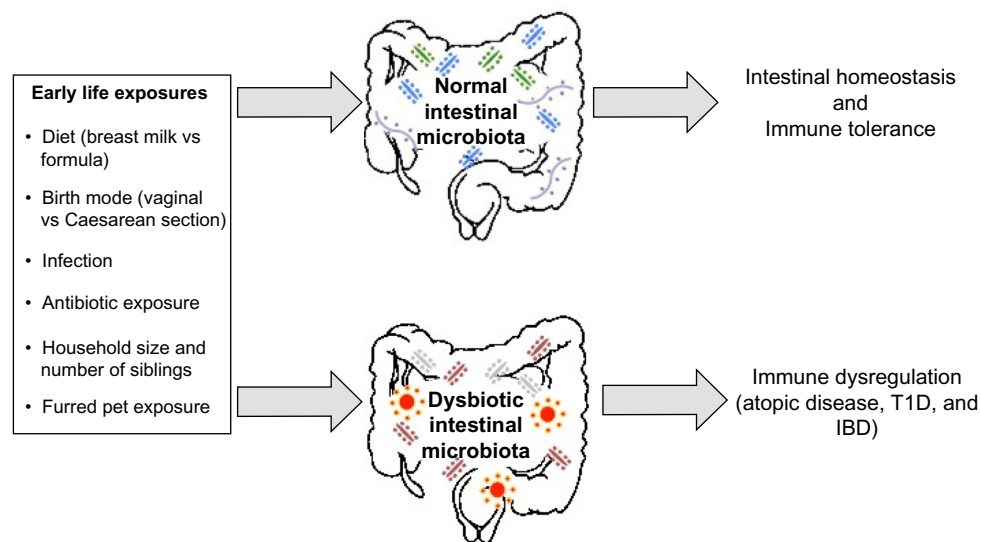
Figure 2 A depiction of the early life environmental exposures differentially associated with promoting a healthy intestinal microbiota, which results in intestinal homeostasis and immune tolerance, and a dysbiotic (unhealthy) intestinal microbiota, which may induce the development of immune dysregulation.
Abbreviations: vs, versus; T1D, type 1 diabetes; IBD, inflammatory bowel disease.

Study in the Netherlands.[54] Birth by Caesarean section was associated with lower total microbial diversity, delayed colonization with Bacteroidetes, and decreased Th1 responses in the first 2 years of life.[55] Breastfeeding promotes colonization with commensal microbes such as *Bifidobacteria* spp. and provides the intestinal microbiota with necessary nutrients in the form of HMOs.[46,56] Specific HMOs, short-chain galactooligosaccharides (GOSs) and long-chain fructooligosaccharides (FOSs), administered in the first 6 months of life have been shown to reduce the cumulative incidences of AD, recurrent wheezing, and allergic urticaria.[57] In line with Strachan's original proposal, one study found that an increased number of older siblings was associated with decreased colonization with *Clostridium difficile* and *Clostridium* cluster 1, and a decreased risk of developing AD.[58] Correlative human studies such as these shed light on the environmental factors that may be associated with atopic disease through manipulation of the intestinal microbiota; however, research regarding factors such as antibiotic exposure, breastfeeding, and birth mode remains controversial, and there are studies that suggest these factors have little or no effect on atopic disease development.[59–64] Additional longitudinal human studies are necessary to determine which early life factors are most influential in promoting the intestinal dysbiosis associated with the development of immune hypersensitivities, and animal model research is a crucial complementary approach to elucidate the mechanisms behind these associations.

The intestinal microbiota in atopic disease: mouse models

Murine model studies mechanistically support a link between the intestinal microbiota and atopic disorders through the experimental manipulation of microbiota compositions. In an OVA-driven model of asthma, Forsythe et al show that oral supplementation with live *Lactobacillus reuteri* reduced airway hyperresponsiveness as well as levels of TNFalpha, monocyte chemotactic protein 1, IL-5, and IL-13 in the bronchoalveolar lavage fluid (BALF), while treatment with *Lactobacillus salivarius* had no effect.[65] Intranasal supplementation of mice, polysensitized to birch and grass pollen allergens, with *Bifidobacterium longum* and *Lactobacillus paracasei* at the time of sensitization resulted in reduced IgE-dependent basophil degranulation in response to allergen challenge.[66] Only *B. longum* displayed protective effects when mice were supplemented prior to allergen sensitization.[66] Additionally, oral supplementation of mice with *B. longum* protected against airway inflammation, increased Peyer's patch and splenic Tregs, and blocked serum IgE induction in OVA-sensitized animals.[67]

More recent research focuses on the earliest time point at which gut microbial intervention must occur to prevent the onset of hypersensitivity disease. In an OVA-driven model of allergic inflammation, neonatal (but not adult) exposure of previously GF mice to a conventional microbiota reduced the severity of allergic inflammation characterized by decreased accumulation of invariant natural killer (NK) T-cells to the lung and reduced serum IgE levels and eosinophil frequencies

in the BALF.[68] Arnold et al show in OVA- and HDM-driven mouse models of allergic inflammation that oral infection of neonatal mice with *H. pylori* prior to OVA or HDM challenge resulted in the significant reduction of eosinophils in the BALF, and a decrease in IL-5 and IL-13 cytokine levels when compared to uninfected mice and infected adult mice.[69] Russell et al found that perinatal vancomycin treatment of OVA-challenged mice alters gut microbial composition and exacerbates asthma-related immune responses, which may be driven by increased serum IgE levels and reduced Treg populations.[70] Interestingly, perinatal treatment with streptomycin did not result in exacerbated disease after OVA challenge, but perinatally streptomycin-treated mice showed exaggerated lung inflammation when compared to untreated or vancomycin-treated mice in a Th1/Th17-driven model of hypersensitivity pneumonitis.[70,71] This highlights the ability of altered microbiota compositions to differentially control disease severity depending on the immunological basis of the disease.[71] Additional studies including human subjects and supporting mechanistic animal models are necessary to provide a holistic view of the role of the intestinal microbiota in atopic disease. Currently, there is also increasing evidence supporting a role of the intestinal microbiota and early life environmental exposures in other immune-mediated disorders.[72] For the purpose of this review, we focus on IBD and T1D.

The hygiene and microflora hypotheses and immune-mediated disorders
IBD

IBD is an inflammatory disorder of the gastrointestinal (GI) tract encompassing Crohn's disease (CD) and ulcerative colitis (UC), both of which are highest in prevalence in North America and Europe.[73] The presence of intestinal bacteria appears to be required for the development of experimental colitis, while the composition influences the severity of IBD. GF IL-10-deficient mice show no evidence of experimental colitis, while IL-10-deficient mice housed under specific pathogen-free (SPF) conditions spontaneously develop the disease.[74] Additionally, antibiotics have been shown to attenuate the symptoms of experimental colitis.[75–77] Exposure of SPF IL-10-deficient mice to antibiotics displays differential and localized roles of specific bacteria in mediating experimental colitis.[77] For example, treatment of SPF IL-10-deficient mice with vancomycin–imipenem and metronidazole eliminated anaerobic bacteria and reduced colonic injury,

while ciprofloxacin and vancomycin–imipenem decreased cecal inflammation and reduced the prevalence of *Escherichia coli* and *Enterococcus faecalis*.[77]

Some human studies suggest that early life antibiotic exposure is associated with IBD.[78–80] This discrepancy is likely because antibiotics in murine IBD experiments are typically given as treatment after disease onset, whereas human studies are often retrospective and assess the effects of antibiotic exposure prior to disease onset. In a nested case-control study, children diagnosed with IBD at approximately 8 years of age were 2.9 times more likely to have received antibiotics in the first year of life.[80] Additionally, antibiotic exposure in the first 3 months of life was associated with childhood CD.[79] Conversely, antibiotic combination therapy has been shown to be effective in treating UC in humans.[81] Thus, effects after antibiotic exposure in humans are likely disease specific and/or dependent on when antibiotics are administered (ie, before or after disease onset).

Diet may also play an important role in IBD. Maternal secretory IgA (a component of breast milk) has been shown to alter the intestinal microbiota composition and the expression of genes associated with intestinal inflammation.[82] Additionally, a systematic review negatively correlated breast milk exposure with the development of early onset IBD in humans, suggesting a protective effect of breastfeeding on IBD development.[83]

Altogether, these results suggest that IBD is driven by the composition of the intestinal microbiota, which is strongly influenced by early life environmental factors. Early life diet (breastfeeding) is likely protective against IBD development, while effects of antibiotic exposure are more complicated. If antibiotics are given in early life, they may result in an intestinal microbiota that promotes IBD development.[79,80] However, after disease onset, antibiotics alleviate disease severity by shifting the prevalence of specific microbes that may be promoting the disease.[75–77,79,81] Regardless, factors related to early life hygiene are involved in IBD development, and there is also evidence that the hygiene and microflora hypotheses are applicable to immune-mediated disorders not associated with the GI tract, such as T1D.[84–96]

T1D

The prevalence of childhood T1D, an autoimmune disorder resulting from T-cell mediated destruction of beta cells in the pancreas, is steadily increasing worldwide, and developed countries such as Canada and the UK exhibit the highest incidences of the disease.[84,85] Epidemiological evidence supports a link between environmental factors associated with

the hygiene hypothesis and the onset of T1D. Having older siblings is negatively correlated with childhood onset T1D, suggesting a protective effect.[86] Furred pet exposure seems to also play a role, as one study found in a birth cohort of 3,000 children: children exposed to an indoor dog were less likely to develop T1D than unexposed children.[87] Breast-feeding has been associated with protection from T1D, and children born by Caesarean section exhibit a higher risk of T1D than children born vaginally.[88–90]

Lending support for the microflora hypothesis, a recent study compared the gut microbial compositions of children with T1D and healthy children and concluded that children with T1D showed a significant increase in *Bacteroides* spp., which was later reduced to that of controls after insulin treatment for 2 years.[91] Oral administration of *Lactobacillus johnsonii* isolated from bio-breeding (BB) diabetes-resistant rats was shown to delay the onset of T1D in BB-diabetes prone rats.[92] Additionally, MyD88-deficient NOD mice are protected from disease onset in SPF environments, and segmented filamentous bacteria have been reported to protect female NOD mice from disease development.[93,94] Additionally, antibiotic therapy in mice has been shown to protect against virus-induced T1D through the alteration of intestinal microbiota composition.[95] However, in humans the contribution of antibiotics to T1D development is currently unclear, as a population-based human cohort study found no association between T1D and antibiotic exposure in the first 8 years of life.[96] Thus, similar to atopic disease and IBD, early life factors common to industrialized countries such as birth mode, diet, and antibiotic exposure seem to play a role in T1D development. However, additional mechanistic research is needed before significant conclusions regarding the gut microbial composition and immunological consequences can be made. The use of appropriate animal models will be critical in continuing to determine whether the relationship between microbiota composition and immune dysregulation is causal, or an effect of a dysregulated immune environment. Regardless, research related to the hygiene, old friends, and microflora hypotheses supports early life intervention as the primary therapeutic component for averting immune dysregulation in the form of atopic and immune-mediated disorders.

Future therapeutics

Future therapeutic options to prevent the development of immune dysregulation will likely involve the millions of micro- and macroorganisms living commensally or symbiotically (microbiota), or even parasitically (helminths) in the human body. In this section, we discuss potential helminth-based (Table 1) and microbiota-based therapies (Table 2) in the prevention of these disorders.

Helminth-based therapies

Clinical trials to date have focused on the use of live helminth infection as an ameliorative, rather than preventative, strategy due to the potential for diminished vaccine responsiveness in mice and humans infected with helminths early in life.[97–101] The majority of early phase clinical trials to determine the safety and efficacy of live helminth infection have been conducted in CD and UC patients.[102] Initial clinical trials using ova from the porcine whipworm, *Trichuris suis*, or larvae from the human hookworm, *Necator americanus*, have not yet found any cause for major safety concerns in IBD or asthma patients.[103–107] *T. suis* ova administration seemed to reduce intestinal inflammation in a small number of CD and UC patients, and administration of *N. americanus* larvae to CD patients resulted in a nonsignificant improvement in intestinal inflammation scores.[104–108] These initial clinical trials were promising, although follow-up studies with the inclusion of placebo control groups show mixed results.[102,107,108]

Live helminth parasites release a suite of ES immunomodulatory products that likely mediate many of their suppressive effects in models of allergic disease and experimental colitis.[109] In mice exposed to both OVA- and *Alternaria alternata*-driven asthma models, administration of ES material from the murine intestinal nematode, *H. polygyrus* (HES), was sufficient to suppress lung eosinophilia and histopathology in response to antigen challenge.[110,111] HES appears to suppress lung inflammation when given at the point of antigen sensitization and antigen challenge, making it a promising therapeutic candidate.[110] Soluble products from several different helminth parasites have also been shown to reduce measures of disease severity in murine models of trinitrobenzene sulfonic acid-induced and dextran sulfate sodium-induced colitis and T1D.[112–116] Administration of helminth ES products rather than live helminths has not yet begun in human patients, but evidence from murine models suggests that this is a promising approach for future clinical trials.

Researchers are beginning to elucidate the mechanisms that mediate the potent immunoregulatory effects of these helminth products. ES products from *N. americanus* mediate the rapid proteolysis of eotaxin, an eosinophil chemoattractant, and HES can stimulate the induction of Tregs through a TGF-β-dependent pathway.[117,118] Whether the administration of helminth products modifies the composition of the intestinal

microbiota is not yet reported. However, infection of mice with live helminth parasites results in a marked disruption of intestinal microbiota composition, suggesting that the immunosuppressive effects following helminth infection could be due to an indirect modulation of the microbiota.[119–121] The relative contribution of the microbiota or helminth-secreted products in ameliorating immune dysregulation remains to be determined. If microbiota compositional shifts following helminth infection are shown to have a direct role in disease modulation, future probiotic administration to drive the microbiota composition toward that seen during helminth infection may be a novel therapeutic approach.

Microbiota-based therapies

Probiotics are live bacteria which, when administered, are beneficial to host health. Animal model research using probiotics shows their ability to ameliorate symptoms in atopic disease, IBD, and T1D.[66,92,119,122–125] Additionally, probiotic administration in humans has been shown to protect against allergic rhinitis, peanut allergy, AD, and UC.[124,126–129] However, research thus far reveals many gaps in probiotic therapy, likely due to individualized disease phenotypes that may or may not be linked to the specific microbial species tested.[130–132] Consequently, prebiotic and synbiotic therapeutics are also being explored.

Prebiotics are chemicals or food components (eg, inulin, pectin, GOSs, and FOSs), which are indigestible by pancreatic and intestinal enzymes, but are important in the growth and proliferation of intestinal microbiota.[133] Prebiotic substances can induce the production of SCFAs by intestinal microbes, which have been shown to promote effector (Th1 and Th17) and anti-inflammatory IL-10-producing FoxP3$^+$ and non-FoxP3$^+$ T-cell differentiation.[48,49] As such, they continue to be a promising microbe-based therapeutic option to modulate intestinal immune responses. Supplementation of mice with a mixture of short-chain GOS, long-chain GOS, and pectin-derived acidic oligosaccharides prior to OVA challenge suppressed airway inflammation and hyperresponsiveness compared to controls.[134] Additionally, Trompette et al show that a high-fiber diet (diet supplemented with 30% pectin) metabolized by the gut microbiota increases the concentrations of circulating SCFAs and decreases allergic inflammation in the lungs of an HDM-driven model of allergic inflammation.[47] In humans, prebiotic oligosaccharide formula supplementation in the first 6 months of life has been associated with decreased incidences of allergic manifestations until 2 years of age, supporting early life intervention in humans.[57] Additionally, prebiotics have been implicated

in protection from IBD development. Human leukocyte antigen-B27 transgenic (HLA-B27, TG) rats supplemented with FOS and inulin prior to disease onset showed decreased intestinal inflammation compared to untreated rats; however, FOS-treated rats compared to inulin-treated rats showed less intestinal inflammation, suggesting FOS as a more effective prebiotic treatment for spontaneous colitis.[135] Conversely, FOS was not an effective treatment for CD, as patients receiving the treatment after 4 weeks exhibited higher GI symptoms compared to the placebo group, despite the reduced IL-6 and increased IL-10 production from lamina propria dendritic cells.[136]

Synbiotic therapies involve supplementation with both pre- and probiotics. In a murine model for cow's milk allergy, mice fed the synbiotic mixture (GOS, FOS, and *Bifidobacterium breve* M-16V) showed increased galectin-9 expression by intestinal epithelial cells, which correlated with reduced acute skin reaction and mast cell degranulation.[137] Similar results were measured in humans fed the synbiotic mixture, suggesting a mechanism by which this therapy may be effective in protecting against AD in humans.[137] Conversely, a clinical trial using a similar synbiotic mixture, Immunofortis®, found no difference in AD severity in the synbiotic group versus the placebo group.[138] However, this research group did later find in infants with AD that supplementation with this mixture for 12 weeks correlated with decreased prevalence of wheezing and asthma medication usage after 1 year.[139] Synbiotics are also potential therapeutics for IBD. In a controlled pilot trial involving 18 patients with active UC, short-term synbiotic therapy combining *B. longum* and inulin-oligofructose significantly reduced chronic inflammatory biomarkers of the disease, including decreased TNFα and IL-1α levels.[140]

The effects of early life factors such as diet and antibiotic exposure discussed throughout this review suggest that the application of live helmiths/helminth ES products, and pro-, pre-, and synbiotics prior to disease onset may be key in averting disease development, because interventions occurring later in life or after disease onset may be ineffective after the neonatal immune developmental window has closed. The timing of this developmental window could be driven by epigenetic alterations to specific, microbially regulated factors, such as the *CXCL16* gene described by Olszak et al.[68] In previously GF mice colonized neonatally with a conventional microbiota, the presence of a conventional microbiota decreased hypermethylation of *CXCL16*, which consequently decreased accumulation of invariant NK T-cells in the colon (this did not occur in previously GF mice colonized until

they reached adulthood). This suggests that microbe-based therapeutics aimed at protecting against hyperinflammatory diseases are age-sensitive.[67] Additionally, the incongruity of current research highlights the need for future microbiota-based treatments that are constructed as individualized therapeutics specific to the disease phenotype and microbiota of the affected patient.

Conclusion

The progression of research since Strachan's 1989 proposal of the hygiene hypothesis exemplifies the scientific method in health research, progressing from observational theory to experimental therapy. The hygiene hypothesis has been expanded today to include commensal and symbiotic intestinal microbes, which are profoundly involved in human immune development, and parasitic helminths, which are also strong therapeutic candidates to protect against immune dysregulation. More research addressing the early life "critical window" for microbiota intervention, currently being assessed in mice for hypersensitivity diseases, is needed if researchers hope to use these therapeutics to prevent immune dysregulation in humans.[68–71] Children undergo large shifts in their intestinal microbiota compositions throughout the first few months of life; thus, it may be possible in the near future to shift the gut microbial composition using pro-, pre-, and synbiotics toward a microbiota that promotes immune tolerance.[72]

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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