

Mechanisms of Disease: the hygiene hypothesis revisited

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SUMMARY

In industrialized countries the incidence of diseases caused by immune dysregulation has risen. Epidemiologic studies initially suggested this was connected to a reduction in the incidence of infectious diseases; however, an association with defects in immunoregulation is now being recognized. Effector T_H1 and T_H2 cells are controlled by specialized subsets of regulatory T cells. Some pathogens can induce regulatory cells to evade immune elimination, but regulatory pathways are homeostatic and mainly triggered by harmless microorganisms. Helminths, saprophytic mycobacteria, bifidobacteria and lactobacilli, which induce immunoregulatory mechanisms in the host, ameliorate aberrant immune responses in the setting of allergy and inflammatory bowel disease. These organisms cause little, if any, harm, and have been part of human microecology for millennia; however, they are now less frequent or even absent in the human environment of westernized societies. Deficient exposure to these 'old friends' might explain the increase in immunodysregulatory disorders. The use of probiotics, prebiotics, helminths or microbe-derived immunoregulatory vaccines might, therefore, become a valuable approach to disease prevention.

KEYWORDS microbiota, probiotics, prebiotics, regulatory T cells, tolerance

REVIEW CRITERIA

This review is based on a 2004 workshop sponsored by the International Scientific Association for Probiotics and Prebiotics. In writing this article, the authors critically reviewed original articles published in scientific journals and searchable in OVID, PubMed and the MEDLINE Plus Database up to April 2005. Owing to space limitations, the number of studies quoted is restricted to a critical minimum.

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INTRODUCTION

The incidence of allergic disorders in the US and Europe has increased since the late nineteenth century, and seems to have doubled in some decades, particularly during the 1960s and 1970s.¹ A link between the increasing incidence of allergies and the modern hygienic lifestyle was initially suggested, giving rise to the so-called hygiene hypothesis.² Allergic disorders primarily originate from responses from T-helper-2 cells, and a balance between T-helper-1 and T_H2 cells was considered pivotal for immune homeostasis. The original assumption, therefore, was that high standards of hygiene resulted in diminished exposure to microorganisms, which drive the activity of T_H1 cells, which should, in turn, cross-regulate the response of T_H2 cells. Such immunoregulatory imbalance, however, cannot explain the simultaneously increased incidence of several other immunologic disorders such as Crohn's disease, type 1 diabetes (insulin-dependent) and multiple sclerosis, which are all primarily driven by T_H1 effector cells. Notably, the incidence of allergic disorders (T_H2 -driven) and type 1 diabetes (T_H1 -driven) correlates closely both within and outside Europe.³

Epidemiologic data demonstrate that there has been a steady increase in the incidence of a number of immunoregulatory disorders in westernized countries during the past few decades.¹ For instance, asthma has become a common burden among children and young adults, and prevalence rates in Europe are very high (median 20.7%; from 8.5% in Pavia to 32.0% in Dublin).⁴ The incidence of type 1 diabetes has increased dramatically, from a very low prevalence of 0.04 per 1,000 at the turn of the nineteenth century;⁵ in Britain, the prevalence of type 1 diabetes among children under 11 years of age rose from 0.1 to 0.6 per 1,000 children between 1946 and 1958, and then to 1.3 per 1,000 children by 1970, as demonstrated in three national birth cohort studies.⁵ The incidence of multiple sclerosis has also shown a marked increase: in Lower Saxony, Germany, the incidence of multiple sclerosis

doubled over a period of 15 years.⁶ The incidence of inflammatory bowel disease (IBD) is also on the rise.^{7,8} The incidence of childhood IBD in the British Isles is now almost twice that in 1983.⁸ Only environmental factors can explain the rapid changes observed over the past few decades. A high level of hygiene has repeatedly been identified as a risk factor for the development of allergies,³ IBD,⁹ type 1 diabetes,¹⁰ and multiple sclerosis.¹¹

Amongst the major autoimmune diseases, rheumatoid arthritis seems to be an exception, in that no increase in incidence has been noted.¹² Interestingly, neither hygienic nor unhygienic living conditions are associated with the risk of developing juvenile rheumatoid arthritis.¹³

The original hygiene hypothesis suggested that there was a link between decreased pathogen burden and the development of immunoregulatory disorders.¹ When the incidence of infections in the US was plotted for each year, the graphs suggested that the decrease in incidence of some infections occurred during the critical period (1960–1985) when the incidence of some inflammatory disorders was doubling every decade.¹ A more detailed analysis of European epidemiological data, however, revealed that, for the most part, reduced exposure to major pathogens took place long before that period.¹⁴ Moreover, data published in 2004 and 2005 suggested that childhood infections cause an increase, rather than a decrease, in the incidence of allergic disorders,¹⁵ including asthma.¹⁶ Infants with a recorded perinatal infection have been shown to have an increased risk for IBD.⁷

Altogether, neither reduced stimulation of T_H1 cells nor falling infection rates provide an adequate explanation for the rise of immune-mediated disorders in westernized countries. Since these disorders are at least partly attributable to aberrant immunoregulation, some other environmental factors of the modern lifestyle might limit the normal development of regulatory immune mechanisms.

PATHWAYS OF TOLERANCE

Numerous genes are involved in the regulation of innate and adaptive immunity, and various modifications have been introduced in the immune system over millions of years. In mucosal immunity, two layers of noninflammatory defense have evolved (Figure 1). First is immune exclusion, which is mediated by secretory antibodies

that modulate or inhibit surface colonization by microorganisms and reduce surface penetration by potentially dangerous soluble factors. Second are the suppressive mechanisms that allow local and peripheral hypersensitivity to innocuous antigens to be downregulated. Oral tolerance refers to the suppressive mechanisms induced via the gut, and explains the physiological absence of inflammation caused by food proteins or antigens from the commensal microbiota.

Immune exclusion

Immune exclusion is mediated mainly by secretory IgA and secretory IgM antibodies in cooperation with innate defense mechanisms.^{17,18} Intestinal lymphoid cells are located in three compartments: organized gut-associated lymphoid tissue (GALT); the lamina propria; and the surface epithelium. Induction and regulation of mucosal immunity occurs primarily in GALT and gut-draining mesenteric lymph nodes. Terminal differentiation of memory/effector B cells into plasma cells that produce dimeric IgA and pentameric IgM for the secretory antibody system takes place in the lamina propria, in which secondary signals are provided by antigen-sampling dendritic cells.¹⁹ The indigenous microbiota is important in this context, as shown by the fact that the intestinal IgA system of germ-free mice develops normally only after exposure to conventional microbes.²⁰

Oral tolerance

Mucosal homeostasis depends not only on the secretory immune system, but also on gut-induced suppressive mechanisms. There are several identifiable experimental variables for such oral tolerance: genetics; age, dose and timing of postnatal antigen feeding; antigen structure and composition; epithelial barrier integrity; and the degree of concurrent local immune activation, as reflected by microenvironmental cytokine profiles and the expression of costimulatory molecules on antigen-presenting cells (APCs).²¹ Studies in rodents suggest that the commensal microbiota is important both for induction of oral tolerance and for reconstitution of this function after experimentally induced abrogation.²²

In a healthy state, there is virtually no proinflammatory IgG response in the gut mucosa, and it contains very few hyperactivated T cells.²¹ Moreover, the systemic IgG response to dietary antigens tends to decrease with advancing age, and intradermal testing has shown that

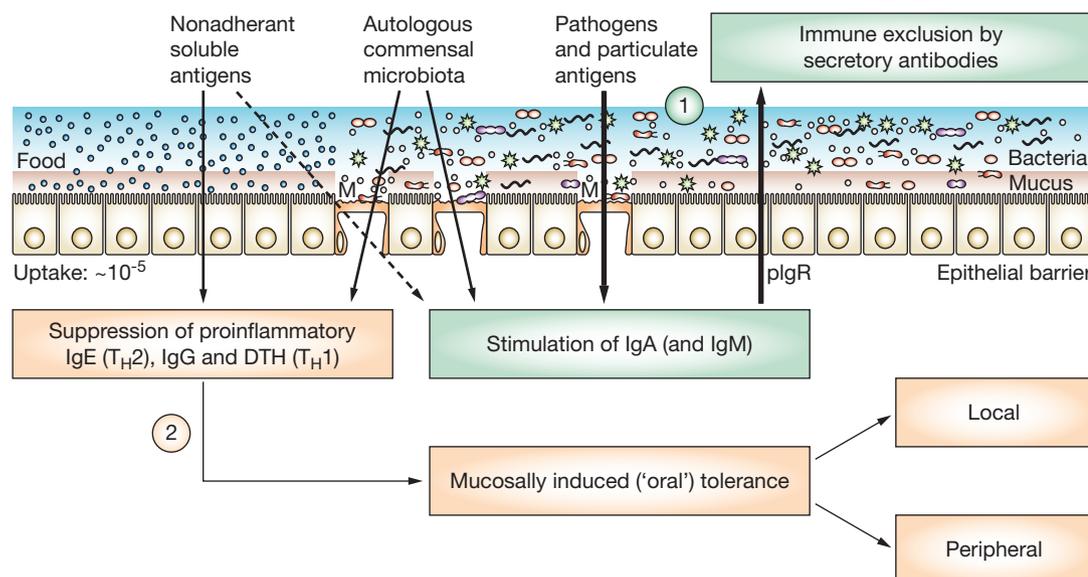


Figure 1 Schematic representation of two major adaptive immune mechanisms in the gut. Immune exclusion limits epithelial colonization by pathogens and inhibits penetration of harmful foreign material. Together with various nonspecific innate protective factors (not shown), this first line of defense is principally mediated by polymeric-immunoglobulin-receptor-dependent secretory antibodies of the IgA (and IgM) class (thick solid arrow upwards). Mucosal production of IgA (and IgM) is strongly stimulated by pathogens and other particulate antigens taken up through thin M cells (M) in the domes of gut-associated lymphoid tissue (thick solid arrow downwards) (①). Penetrating innocuous dietary antigens from food (magnitude of normal uptake indicated) and the commensal microbiota are less stimulatory for secretory immunity (broken and thin solid arrow, respectively) than pathogens, but induce suppression of proinflammatory humoral immune responses (IgG and T-helper-2 cytokine-dependent IgE antibodies) as well as suppression of T-helper-1 cytokine-dependent delayed-type hypersensitivity (②). Such homeostatic regulation of T-helper-1 and T-helper-2 responses in the gut is collectively termed 'oral tolerance', and exerts its downregulatory effects both locally and in the periphery. DTH, delayed-type hypersensitivity; pIgR, polymeric-immunoglobulin receptor; T_H1 , T-helper-1 cell; T_H2 , T-helper-2 cell

adults are hyporesponsive to bovine serum albumin.²³ This homeostatic state is abrogated in the setting of IBD.²⁴ The fact that resident APCs from normal human gut mucosa are quite inert in terms of their immune-productive stimulatory properties²⁵ supports the idea that they have a central role in oral tolerance.²¹

Decision making in the immune system

The decision to generate a productive systemic-type immune response, with the potential for tissue damage and inflammation, or a tolerogenic response seems to be largely determined by the microbial impact on APCs and T cells. Naive $CD4^+$ T_H cells are activated by APCs (chiefly dendritic cells) that provide appropriate costimulatory signals for differentiation into T_H1 or T_H2 cells with the resulting polarized cytokine secretion (Figure 2). Under what are, as yet, rather elusive circumstances, conditioned APCs

can also induce various subsets of regulatory T (T_{reg}) cells. By secreting cytokines interleukin (IL)-10 and transforming growth factor (TGF)- β , or by direct cellular interactions, T_{reg} cells can suppress both T_H1 and T_H2 responses, as well as innate immune activity. Such adequate balancing of the immune system seems to depend on appropriate 'crosstalk' between innate and adaptive immunity early in the neonatal period. APCs can subsequently also be conditioned to induce T_{reg} cells by environmental factors such as parasite cell-wall lipids,²⁶ or microbial heat-shock proteins that have an intrinsic capacity to trigger immunoregulatory events.²⁷

Regulatory cells in the control of immune responses

The identification of T_{reg} cells has prompted a paradigm shift in our understanding of immune regulation following infection. Several pathogens

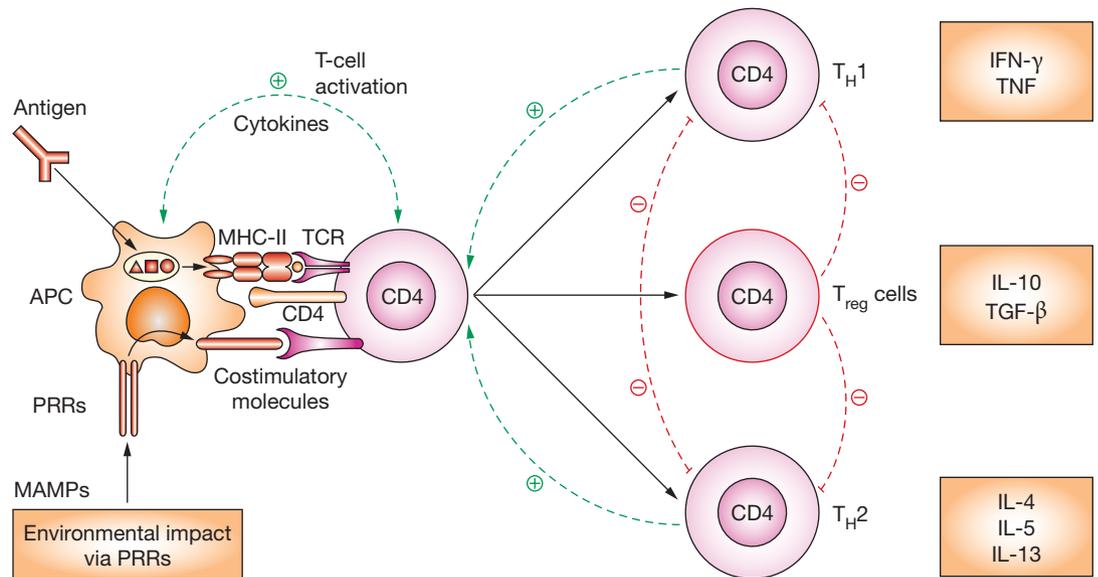


Figure 2 Decision-making in the adaptive immune system is modulated by innate costimulatory signals. Antigen-presenting cells take up a foreign antigen and degrade it to immunogenic peptides that are presented to the T-cell receptor. An immunological synapse is formed between the antigen-presenting cell and the T cell as indicated, resulting in cellular conditioning and various grades of activation. When naive CD4⁺ T-helper cells are activated by antigen-presenting cells that provide appropriate costimulatory signals (soluble factors and/or accessory ligating molecules), they differentiate into T-helper-1 or T-helper-2 cells with polarized cytokine secretion. Such skewing of the adaptive immune response depends on the presence of microenvironmental factors, including cytokines and microbe-associated molecular patterns. These are sensed by pattern-recognition receptors, from which intracellular signaling stimulates activation and functional maturation of antigen-presenting cells along different pathways, thereby dictating the provision of various costimulatory signals. Subsequent activation of T-helper-1 cells leads to predominant production of cytokines such as interferon- γ , tumor-necrosis factor and interleukin-2, while activated T-helper-2 cells are mainly capable of secreting interleukin-4, interleukin-5, and interleukin-13. In addition, under certain as yet unclear conditions, antigen-presenting cells can be conditioned to induce various subsets of regulatory T cells, which via their cytokines and transforming growth factor- β , or by direct cellular interactions, can suppress both T-helper-1 and T-helper-2 responses as well as innate immune activity. APC, antigen-presenting cell; IFN, interferon; IL, interleukin; MAMPs, microbe-associated molecular patterns; PPRs, pattern-recognition receptors; TCR, T-cell receptor; TGF- β , transforming growth factor- β ; T_{H1}, T-helper-1 cell; T_{H2}, T-helper-2 cell; TNF, tumor-necrosis factor; T_{reg} cells, regulatory T cells.

can induce both innate and adaptive immune cells to secrete high levels of immunosuppressive cytokines (e.g. IL-10, TGF- β). In certain cases, specific pathogen-derived immunoregulatory molecules have been identified that stimulate the release of IL-10 from macrophages and/or dendritic cells. For instance, antigen-specific T_{H1} immune responses in the lungs of mice infected with *Bordetella pertussis* are severely suppressed during the acute phase of infection.²⁸ The *B. pertussis* virulence factors filamentous hemagglutinin antigen and adenylate cyclase have been shown to inhibit production of the proinflammatory cytokine IL-12 and enhance production of IL-10 by macrophages and dendritic cells, and to selectively stimulate

the induction of IL-10-secreting Tr1 cells²⁹ (a subtype of T_{reg} cells). These findings suggest that certain pathogens have developed strategies to exploit T_{reg} cells, in order to counteract proinflammatory responses and thereby prolong their survival in the host.

Although, in certain cases, induction of T_{reg} cells following infection promotes bacterial persistence, pathogen-specific T_{reg} cells mainly protect host-tissue integrity by controlling inflammation. This has been elegantly demonstrated by infecting mice defective for Toll-like receptor 4 with *B. pertussis*.³⁰ As innate and adaptive IL-10 production is defective in these mice, infection with *B. pertussis* is associated with enhanced inflammatory cytokine

production, cellular infiltration, and severe pathological changes in the lungs.³⁰ These findings suggest that certain conserved microbe-associated molecular patterns recognized by pattern-recognition receptors, promote the induction of T_{reg} cells, which prevent the host from adopting immunopathology as a strategy.

Many microbes, including aggressively pathogenic bacterial species, might be able to promote immunoregulatory responses. Hypothetically, a changed exposure rate to specific pathogens because of preventive vaccination could also have an impact on a defective development of immune tolerance.

Deficient immunoregulation and disease

Preliminary data from animal models of autoimmunity and from patients suggest that there is a link between defective T_{reg} cell activity and disease. In patients with multiple sclerosis, putative T_{reg} cells have been demonstrated in peripheral blood, but their capacity to suppress potentially disease-causing effector T cells is impaired.³¹ In healthy individuals, T cells directed against a disease-relevant islet cell auto-antigen were reported to show a regulatory phenotype; however, in patients with type 1 diabetes these T cells were proinflammatory.³² Individuals with an allergic predisposition have deficient T_{reg} cell activity.^{33,34} By contrast, studies in patients with rheumatoid arthritis have demonstrated that functionally active T_{reg} cells are present in peripheral blood and the synovial compartment during active disease.^{35,36} These data could be used to suggest that only those disorders that involve defective T_{reg} cell activity are increasing in incidence in westernized societies.

There are experimental data suggesting that induction of T_{reg} cell activity by certain microorganisms can prevent or alleviate inflammatory diseases. The suppressive effect of *Mycobacterium vaccae* on eosinophil-mediated airway inflammation in ovalbumin-primed mice was related to the induction of allergen-specific T_{reg} cells.³⁷ In the case of experimental IBD in IL-10^{-/-} mice, infection with the helminth *Heligmosomoides polygyrus* enhanced the T_{reg} cell marker Foxp3 in T cells and suppressed intestinal inflammation.³⁸ Furthermore, one study has demonstrated that parenteral administration of filamentous hemagglutinin antigen can dampen colitis in a mouse model.³⁹ The identification of

immunomodulatory molecules that promote the expansion of T_{reg} cells *in vivo* might lead to the development of novel immunotherapeutic drugs for the prevention and treatment of immunoregulatory disorders.

As mentioned earlier, the induction of regulatory pathways of mucosal immunity (T_{reg} cells) occurs primarily in GALT and the presence of commensal microorganisms is essential for the induction of immune tolerance.²² The gut, therefore, is where the events take place, and its dynamic microbial ecosystem seems to be crucial for the development of adequate individual–environment homeostasis.

THE GUT MICROBIOTA

The gut contains a large community of microorganisms, amounting to several hundred grams in an adult human being.⁴⁰ Gut bacteria include native species that are permanent colonizers, and a variable set of living microorganisms in temporary transit. Native bacteria are mainly acquired at birth and during the first year of life, whereas transient microorganisms are continuously being introduced from the external environment via ingestion.

The intestinal habitat contains at least 500–1,000 different species of microorganism.⁴¹ More than 50% of the bacteria observed in feces by microscopic examination cannot be grown in culture.⁴² The use of molecular methods to identify noncultivable bacteria is leading to the discovery of previously unknown genera and species.⁴³ Most of these techniques use the sequence diversity of the 16S ribosomal RNA gene to characterize species.^{42,44} Numerous DNA sequences (>200,000) from this gene are now available in databanks such as GenBank.⁴⁵

Molecular methods have highlighted the microbial diversity among individuals. Each subject harbors his or her own distinctive pattern of gut microbiota, which seems to be determined at least in part by host genotype. The similarity is much higher between twins than between genetically unrelated married couples sharing the same environment and dietary habits.⁴⁶ The composition of the fecal microbiota is stable over time in adults, but temporal fluctuations due to environmental variables can be detected. For instance, hospitalized individuals show reduced numbers and species diversity of some anaerobic genera (*Bacteroides*, *Bifidobacterium*, etc.) and an increase in enterobacteria.⁴⁷ Likewise,

environmental acquisition of bacteria has been demonstrated in preterm infants hospitalized in a neonatal intensive-care unit.⁴⁸

The interaction between gut bacteria and host is a mutually beneficial symbiotic relationship. The host provides a nutrient-rich habitat and the bacteria can confer important benefits on the host.⁴⁴ Gut bacteria have an essential role in the development of the immune system. Germ-free animals have only a low number of intestinal T and B cells, GALT follicles are small with no germinal centre, and serum Ig levels are low.²⁰ Shortly after microbial exposure, all such immune parameters are normalized.^{20,22,49} Multiple and diverse interactions between microbes, gut epithelium and GALT are constantly reshaping mucosal and systemic immunity.

Gut microbiota in disease

It has been demonstrated many times that the fecal microbiota of patients with IBD and that of healthy controls differs.⁵⁰ Approaches derived from molecular methods have shown that a substantial proportion of the dominant microbiota in patients with active Crohn's disease (around 30% of the species) belongs to uncommon phylogenetic groups, suggesting that there are major differences between Crohn's disease and a healthy state.⁵¹ The fecal microbial community in individuals with Crohn's disease was found to be unstable in structure over time.⁵¹ Instability might reflect increased susceptibility to environmental microorganisms.

Other studies have focused on the mucosa-associated microbiota. High counts of mucosa-associated bacteria were found in patients with IBD using both conventional culture and molecular methods.⁵² Fluorescent *in situ* hybridization with molecular probes has revealed mucosal bacterial invasion in most colonic specimens (83%) of ulcerative colitis, and in ileal (56%) and colonic (25%) specimens of Crohn's disease, but not in normal control mucosa.⁵³ In addition, bifidobacteria and lactobacilli were absent from, or present in low numbers in, the mucosa of IBD patients.⁵³

Several studies have reported a relationship between the composition of the microbiota and allergy. Compared with healthy children, allergic children in Estonia and Sweden were less often colonized with lactobacilli.⁵⁴ By contrast, allergic children harbored higher numbers of aerobic microorganisms, particularly coliforms

and *Staphylococcus aureus*.⁵⁴ Babies who developed allergies harbored enterococci and bifidobacteria colonies less often, and had higher counts of clostridia compared with healthy infants.⁵⁵ Fluorescent *in situ* hybridization has also demonstrated more clostridia and fewer bifidobacteria in children with atopic dermatitis than in healthy children, resulting in a reduced bifidobacteria : clostridia ratio in stools.⁵⁶

The available data, therefore, demonstrate that the composition of the gut microbiota in patients with allergy or IBD differs from that of healthy individuals. Theoretically, the absence of specific protective commensals might impair immune homeostasis. Results from interventional studies with live microorganisms, including helminths⁵⁷ and probiotic bacteria,^{58,59} support this idea.

Commensals and probiotics

A dynamic mutualism between the human host and its commensal microbiota is likely to have important implications for health.⁴⁴ The vast number of bacteria that colonize mucosal surfaces and the skin interact directly with the host, and have, over millions of years, developed mechanisms for coexistence. For instance, multiple bacterial genes are induced during growth in the mammalian gastrointestinal tract, while the same genes are not expressed during routine laboratory culture.⁶⁰

There is ample experimental evidence showing that commensal bacteria can regulate mucosal immune responses. Various bacteria directly modulate cytokine networks and innate immunity by multiple mechanisms. Messenger molecules that are important for communication among bacteria (e.g. quorum sensing) can affect the intestinal physiology of the host.⁶¹ The quorum-sensing molecule 3-oxododecanoyl-L-homoserine lactone, produced by *Pseudomonas aeruginosa*, is known to suppress the production of IL-12 and tumor-necrosis factor by lipopolysaccharide-stimulated macrophages.⁶¹ Some other bacteria can diminish proinflammatory cytokine production by inhibiting NF- κ B activation. Avirulent strains of *Salmonella* inhibit the ubiquitination of the NF- κ B inhibitory subunit, I κ B- α ,⁶² and *Bacteroides thetaiotaomicron* modulates the NF- κ B signaling pathway by facilitating the nuclear export of RelA (an NF- κ B subunit), via peroxisome proliferative activated receptor gamma.⁶³

Commensal bacteria can produce immunoregulatory factors, known as immunomodulins,

which suppress cytokine production. Different *Lactobacillus* species have been shown to inhibit proinflammatory cytokine production in bone-marrow-derived dendritic cells⁶⁴ and cultured mucosal explants from patients with Crohn's disease.⁶⁵ Some bacteria are known to secrete anti-inflammatory metabolites (<10 kDa) that inhibit human tumor-necrosis factor production by human peripheral blood mononuclear cells.⁶⁶

In addition to the effects on innate immunity, some bacteria can induce adaptive immune responses of the regulatory type. Oral administration of *Lactobacillus casei* to mice has been shown to reduce antigen-specific skin inflammation mediated by interferon- γ -producing CD8⁺ T effectors, and this effect requires T_{reg} cells.⁶⁷ Another *Lactobacillus* strain stimulates the proliferation of T cells that produce regulatory cytokines such as IL-10 and TGF- β .⁶⁸ One proposed model is that commensal bacteria stimulate production of TGF- β by intestinal epithelial cells, and TGF- β induces expression of IL-10 in dendritic cells and macrophages. These cytokines favor the differentiation of T_{reg} cells at the mucosal inductive sites (Figure 2).

Strong evidence suggests that some probiotic preparations that are therapeutically active in chronic inflammatory disorders can induce T_{reg} cells.⁶⁹ In mice with colitis induced by trinitrobenzene sulfonic acid, probiotic administration was associated with changes in the lamina propria mononuclear-cell population, consisting of an increased number of T_{reg} cells bearing surface TGF- β . Moreover, such lamina propria mononuclear cells were able to protect against colitis in a cell-transfer system.⁶⁹ Their effects in the recipients depended on TGF- β and IL-10, and could be blocked by appropriate neutralizing antibodies.

Helminths and immunoregulation

Helminths are multicellular worm parasites that infect more than 2 billion people around the world,⁷⁰ but are being eliminated in westernized societies where helminth carriage has steadily declined.⁷¹ Most helminths are well tolerated and many of the carriers are clinically asymptomatic.⁷⁰ A special host-parasite relationship has developed over time, so that some species have the ability to live for decades in the human host.⁷⁰

There is increasing evidence to indicate that helminth carriage might protect the host from

immunological disorders. An inverse relationship between helminthiasis and allergy has been clearly established: children with chronic helminth infestation are less prone to allergy⁷² and have a reduced allergic reaction to the house dust mite.⁷³ Moreover, one study found that antihelminthic treatment of infested children resulted in increased atopic reactivity.⁷⁴ Experimental data in animal models also support the theory that helminths protect from immunological disorders such as allergic airway inflammation,⁷⁵ autoimmune encephalomyelitis,⁷⁶ type 1 diabetes⁷⁷ and intestinal inflammation.^{78,79} The positive outcome of clinical trials using helminths to treat IBD further supports the concept.^{57,80}

Most helminths stimulate the production of T_{H2} cytokines (IL-4, IL-5, IL-9, IL-13). This mechanism would explain the protective role of helminths in T_{H1}-driven disorders such as intestinal inflammation. Indeed, induction of T_{H2} pathways (IL-4 and/or IL-13 and their Stat-6 signaling pathway) has been proven in animal models of IBD.^{77,78} However, helminthiasis also prevents T_{H2}-driven allergic reactions. Thus, the key mechanism seems to be the ability of some helminths to induce regulatory cytokines, including IL-10 and TGF- β , via stimulation of both T_{reg} cells and regulatory-type non-T cells.^{75,81} Generation of T_{reg} cells suppresses both T_{H1} and T_{H2} arms of immunity. The helminths seem to stimulate certain T_{reg} cell subsets (CD4CD25 Foxp3 T cells) through interactions with dendritic cells and/or other types of APCs.^{74,81}

CONCLUSIONS

Because of mankind's long evolutionary association with a number of relatively harmless organisms, including saprophytic mycobacteria, bifidobacteria, lactobacilli, and helminths, they are recognized by the human innate immune system as innocuous. Some of these organisms have been shown to induce an unusual maturation pattern of dendritic cells,^{26,82} facilitating their ability to stimulate a T_{reg} cell response (Figure 3). Rather than eliciting a productive and potentially aggressive immunity, these microorganisms therefore skew immune responses towards regulatory modulation.

Our 'old friends'⁸³ are microorganisms associated with farms, untreated water supplies, cowsheds, pets, fermented foods, and the like, and have been part of the human

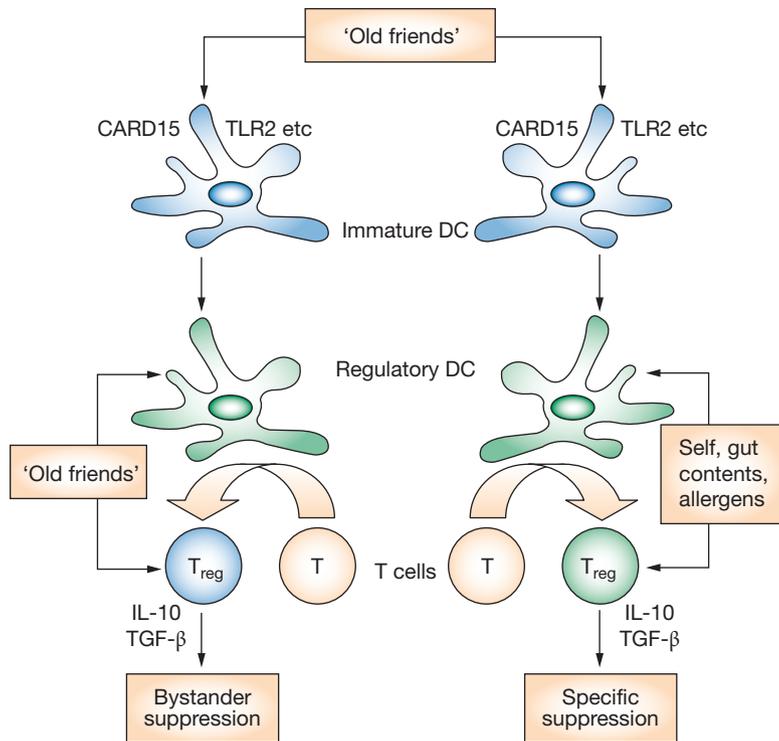


Figure 3 The 'old friends' hypothesis. Organisms recognized as harmless by the innate immune system (through pattern-recognition receptors, such as CARD15 and Toll-like receptor 2) cause dendritic cells to mature into regulatory dendritic cells that drive regulatory-T-cell polarization. Some of these cells will recognize the 'old friends' themselves, and so provide a continuous background bystander regulation. Regulatory dendritic cells, however, might also process and present epitopes from self, allergens and gut content (antigens, bacterial DNA motifs, microbial heat-shock proteins) and so drive specific immunoregulation.

DC, dendritic cells; IL, interleukin; TGF-β, transforming growth factor-β; TLR2, Toll-like receptor 2; T_{reg}, regulatory T cell.

microbiological environment for millennia. Such 'domestic' commensals are, however, mostly depleted in the westernized environment. The reduced numbers of our old friends is probably associated with defects in the development of immunoregulatory pathways and, set against a background of genetic susceptibility, it might explain the increased prevalence of immunological disorders in societies with high hygienic standards.

Autoimmune diseases and asthma are a major cause of human morbidity worldwide. Treatment of these disorders generally relies on immunosuppressive drugs, which lack specificity of action and can have serious side effects. A novel approach for the treatment of such diseases might be to exploit the immunomodulatory function of certain microorganisms,

or their active components (Figure 3). Most importantly, prevention of the epidemic of immune-mediated disorders associated with a hygienic lifestyle should be grounded in the rational use of probiotics, prebiotics, helminths, or immunoregulatory vaccines to induce and maintain immune homeostasis. Further research is required to establish a solid scientific foundation for this approach.

KEY POINTS

- The recent identification of regulatory T cells has prompted a paradigm shift in our understanding of immune regulation
- Deficient activity of regulatory T cells has been demonstrated in individuals with immune-mediated diseases
- Induction and regulation of mucosal immunity occurs primarily in gut-associated lymphoid tissues and the gut-draining mesenteric lymph nodes
- Helminths, saprophytic mycobacteria and lactobacilli have all been shown to stimulate regulatory-T-cell responses
- Reduced exposure to relatively harmless microorganisms in modern society could be associated with defects in the development of immunoregulatory pathways

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Competing interests

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