

Bacterial components plus vitamin D: The ultimate solution to the asthma (autoimmune disease) epidemic?

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In this issue of the *Journal*, 2 review articles (1 immunologic and 1 epidemiologic) draw attention to the human gut microbiome and its potential importance in human health and the development of allergic and autoimmune disease.^{1,2} This editorial will try to synthesize the central messages of these reviews and outline the research directions in this emerging field. I will first review the data on the human microbiome, discuss factors that might influence gut bacterial colonization in human subjects, review the evidence that the microbiome is involved in allergic disease, and finally consider the role of vitamin D in this process.

The gut microbiome is overwhelming in its size and its metabolic and antigenic complexity. There are 10^{14} bacteria in the gut, or 10 times more microbes in the human colon than there are cells in the human body. These bacteria belong to more than 1000 species and have 3.3 million genes, more than 150 times more genes than our own genome.³ Culture alone is inadequate to identify the mostly anaerobic organisms of the gut, and bacterial sequencing must be used, in conjunction with culture, to definitively speciate and hence identify all of these organisms.

These bacteria interact with the host in a variety of ways. First, they interact with the gut-associated lymphoid tissue, the largest immune organ in the human body, and by their sheer size, they represent the major source of antigenic load to the human immune system. Second, these bacteria function as a metabolically active force to digest and modify the human diet. Third, our own genome interacts with and helps determine which bacteria are important to us,⁴ thus providing a degree of complex gene-environment interaction effect. One of the most striking examples of this latter effect is that animal brain function and behavior can be influenced by the presence or absence of these commensal bacteria during development.⁵

The human gut flora is, quantitatively, the earliest, most diverse, and hence most important postnatal source of microbial stimulation of the immune system in most species, including the human, and hence the most likely determinant of normal tolerization.⁶ Experiments in rodents suggest that bacterial gut colonization is essential for the development of oral tolerance

and normal postnatal maturation of immune responses,⁷ including generation, expansion, or both of regulatory T (Treg) cells in peripheral tissues.⁸ Most of these “good bacteria” are anaerobes that are among the earliest life forms on the planet, having an evolutionary age of almost 6 billion years relative to our much more recent 150,000 years. Dobzhansky⁹ has said that “nothing in biology makes sense except in light of evolution,” and our coevolution with these anaerobic bacteria is critical to our understanding of how our immune system functions. Ultimately, how tolerization occurs and how the gut microbiome relates to the skin and other microbiomes, such as the oral cavity, is currently unknown. However, Noverr and Huffnagle⁶ have postulated that the gut microbiome actually is the sensor for immune tolerance in the lung. What does seem clear is that tolerization as an immune phenomenon is dominated by the gut flora because of the size of the gut-associated lymphoid tissue and the sheer magnitude of the gut microbiome’s antigenic mass. Initial colonizing bacteria can be a potent environmental stimulus to the host, inducing genes in the host’s gut epithelium and hence influencing the host’s immune response. Thus good bacteria can potentially control or prevent the growth of pathogenic bacteria in part through immune system development and metabolic processes and by mechanisms as yet not understood.¹⁰

The study of the natural history of microbial colonization of the human gut and its relevance to disease is in its infancy. Existing studies in this area are few because of the novelty of the technology, the high cost, and the technical difficulty of culturing anaerobes. Methodologic issues, such as small sample size, short duration of follow-up,¹¹ missing data on potentially relevant variables, and, most importantly, reliance on cultures alone, might miss relevant organisms, thus limiting what we know.¹² Multiple clinical and molecular factors are likely to determine gut colonization in early fetal life: bacterial characteristics, such as the number and diversity of cell-surface antigens; the presence or absence of pili; host mucosal cell properties, such as cell-surface receptors; and epidemiologic factors, such as gestational age, mode of delivery, exposure to maternal fecal flora, maternal diet, fetal antibiotic use, and type of infant feeding, including probiotics.^{10,12,13}

Of the multiple factors noted above, maternal and fetal diet, prebiotics, and probiotics deserve some greater attention. At a CIBA Foundation symposium in 1996, I was the first to suggest that dietary changes could be driving the allergy and autoimmune disease epidemic.¹⁴ Food serves as an important substrate for bacteria digesting plant polysaccharides and hence producing short-chain fatty acids and other nutrients for the bacteria and hence amplifying their influence immunologically.¹⁵ This prebiotic function could potentially be exploited therapeutically if we knew which foods were promoting the good bacteria. Probiotics are cultures of normal gut flora organisms administered orally to influence immune function.¹⁶ Several studies, mostly with *Lactobacillus* species, have been performed,¹⁷ including 2 small

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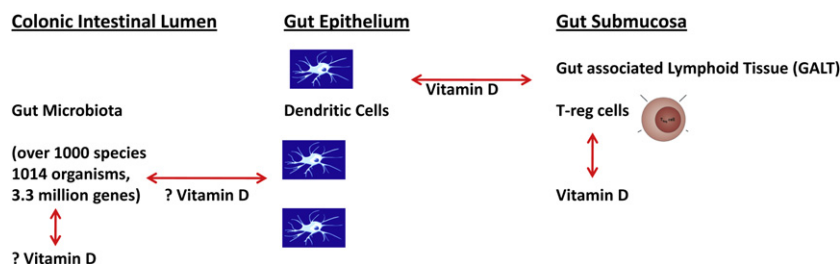


FIG 1. Vitamin D might determine how the gut flora interacts with the immune system.

clinical trials.¹⁸⁻²⁰ The problem is that these studies are premature because they beg a critical question: What are the relevant bacteria and their specific antigens that drive the putative immunoprotective effects? The evidence that lactobacilli are sufficient to do this is weak at best. *Bacteroides fragilis* would seem to be a better bet, but unlike the mouse, a single bacterium is unlikely to be sufficient to achieve normal immune tolerization in a human subject.

Existing clinical studies performed in the presequencing era suggest that the composition of the gut flora appears to differ between atopic and nonatopic infants, giving some credence to the hypothesis that the reduced number and diversity of gut bacteria early in life lead to faulty tolerization and the development of allergic and other autoimmune diseases.^{12,21-25} Ly et al² review much of this work in greater detail. In a small pilot study we found similar results to those of the studies cited above. We found that levels of IL-10, an anti-inflammatory Treg-controlling cytokine modulated in part by vitamin D, were increased in the cord blood of children whose mothers had a higher number of anaerobes in their guts. We went on to show that greater diversity of the infant's fecal flora was protective for atopic dermatitis occurrence at 6 months of age.²⁶ A recent review found that 14 of 17 studies in this area supported the findings that gut bacteria influenced the development of allergic disease.²⁷ These cited studies, including ours, are mostly of small sample size and, most importantly, lack robust, state-of-the-art gut microbiome assessment with both anaerobic culture and sequencing to maximally speciate the organisms. Finally, most studies do not adequately account for the confounding variables noted above. Suffice it to say that as the review by Ly et al² notes, we have no clear epidemiologic evidence as to the importance of specific bacterial species and whether their number, diversity, or both is the determining factor with regard to allergic diseases generally or asthma specifically. This is in contrast to Crohn disease, in which specific bacteria have been identified.²⁸

How is vitamin D related to this process? We have previously reviewed in these pages the potential role of vitamin D deficiency in the development of allergic and autoimmune diseases and specifically their dramatic increase in prevalence in the last 50 years.²⁹ As we noted, vitamin D has clear structural effects on lung and airway development *in utero*.²⁹ It is worth recapitulating the immune portion of the argument here. Vitamin D is essential in the development and function of both Treg cells^{30,31} and dendritic cells.^{32,33} It seems quite likely that vitamin D is an important modulator of the signaling traffic between gut bacterial surface antigens through its effects on dendritic and Treg cells not just in human subjects but also in organisms as evolutionarily well preserved as the fly.

Experimental evidence suggests that the biologically active form of vitamin D (1,25[OH]₂ vitamin D₃) influences the process

by which immune cells acquire signaling molecules that enable them to migrate to normal extralymphoid tissue sites, as well as sites of inflammation. The gastrointestinal mucosa, as the main body surface exposed to environmental antigens (specifically gut bacteria), has well-described tissue-specific adhesion and chemoattractant homing receptors.³⁴ Vitamin D can block or down-regulate gut homing of both normal immune and inflammatory cells. For example, vitamin D receptor gene expression is required for dendritic cells, T cells, and other immune cells to control inflammation in the *il10* knockout mouse model.³⁵ By upregulating the normal immune function of Treg cells, specifically through *IL10* and *TGFB1*, vitamin D can promote normal tolerization. Conversely, reduced vitamin D levels, a reduced number and diversity of gut bacteria, or both will promote inflammation that might disrupt the mucosal barrier and promote food and other allergen sensitization or abnormal tolerization. Thus pathogenic and commensal gut bacteria can play opposing roles in allergy development, and these roles might be modulated by the vitamin D receptor gene, other host genes, and vitamin D itself. It is also possible that independent of its immune effects, vitamin D has a direct effect on gut bacterial flora to increase or decrease the number of specific species of bacteria or species diversity. These complex effects are synthesized in Fig 1. Given the current Institute of Medicine report on vitamin D dosage (<http://www.IOM.edu/reports/2010/dietary-reference-intakes-for-calcium-and-vitamin-D.aspx>), it is worth considering what level of vitamin D would provide immunoprotection for the development of allergic and autoimmune disease. Although this is currently unknown, it would seem that higher doses than those currently recommended by the Institute of Medicine report for bone health will almost certainly be necessary. Further research is needed in this area.

It is worth noting that the microbiome/vitamin D hypothesis to explain the origins of the autoimmune disease epidemic fits the facts far better than the hygiene hypothesis.²⁹ The hygiene hypothesis never could explain the increase in T_H1 autoimmune disease in addition to asthma and allergy and the high rates of asthma and allergy among the urban poor in the United States. Both phenomena are easily explained by vitamin D deficiency. The strong link between the microbiome and vitamin D closes the loop by providing a plausible immunologic substrate for vitamin D's effects on normal tolerization. We have clear evidence for dramatic changes in vitamin D levels over the 50-year period that has seen the dramatic increase in autoimmune disease; what we do not know is what has happened to the gut microbiome during that period. Current clinical trials in the United States and Denmark will test whether giving vitamin D to pregnant women will prevent asthma in their offspring (Vitamin D Antenatal Asthma Reduction Trial, VDAART, U01 HL091528, ClinicalTrials.gov: NCT00856947), although this is unlikely to be the whole story because this trial will not test the

effect of postnatal sufficiency on asthma risk. A funded ancillary study will allow an exploration of the relative importance of vitamin D and the gut flora on disease risk.

Identification of the good bacteria in the human gut microbiome is of primary scientific importance in understanding allergic diseases. This will not be a trivial exercise given the size and diversity of the gut microbiome. If this task can be accomplished, then it is conceivable that in the not-too-distant future we will prevent asthma, food allergies, atopic dermatitis, allergic rhinitis, and other autoimmune diseases, such as Crohn disease, type 1 diabetes mellitus, and multiple sclerosis, with a cocktail of anaerobic bacterial antigens, dietary constituents, or the bacteria themselves (prebiotics and probiotics) in combination with vitamin D administered orally to both pregnant women and their offspring. First we need to find the protective bacterial species, identify their antigens, understand more clearly how vitamin D modulates the signaling from gut bacteria to dendritic cells and Treg cells, and determine what dose of vitamin D is necessary. This is an exciting time for asthma research and for autoimmune disease research generally, and the gut microbiome will be an important extension of genomics to the environment. Elucidating its influence on the development of allergic disease is going to certainly contribute to this excitement.

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