Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

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Allergy

NEWS AND COMMENTARIES

Linking vitamin D, the microbiome and allergy

DOI:10.1111/all.13116

There is an ongoing interest in elucidating the causes of allergic diseases. Numerous genetic and environmental risk factors have been identified, including social class, early exposure to allergens, atmospheric pollution, month of birth, cesarean section, lack of breastfeeding, early and late introduction of solid food, and antibiotic use. Many of these are debatable because they could not be reproduced in different settings or do not provide a coherent picture. Obviously, there is no single risk factor responsible for the allergy epidemic. Indeed, allergy may be viewed more as a varying condition or a syndrome rather than a disease in the traditional sense because it involves multiple organs and unrelated pathology mechanisms.

Two extensively examined hypotheses are the hygiene hypothesis (lack of protective bacterial exposure which leads to subsequent allergy) and the vitamin D hypothesis (early vitamin D supplementation sensitizes newborns against allergens). During the past two decades, both concepts have undergone major revisions. The hygiene hypothesis has evolved into a cultural meme of modern civilization, ranging from the number of siblings and the use of coal heating to counting dung hills on farms while now describing a multitude of allegedly protective cowshed bacteria (1). The vitamin D hypothesis has also been expanded – from isolated supplementation effects to the impact of hypovitaminosis. While there is no lack of descriptive studies supporting both hypotheses, there are only a few randomized clinical trials testing either hygiene (2, 3) or vitamin D exposure (4). Both concepts agree on a vulnerable period during the newborn phase when the first allergen contact occurs. The interesting question is as follows: Are these concepts exclusive?

Already back in 2011, a synthesis of both divergent views was proposed (5), largely, however, at the cost of rejecting the hygiene hypothesis. But there is no doubt about many observations made in the context of the farming studies. The protection found in some farm children could of course be caused by infrequent doctor contact and therefore less vitamin D supplementation. In addition, raw milk consumers living on farms tend to avoid industrialized, supplemented milk (6). And likewise, due to the time spent outdoors, farmers have higher vitamin D levels than the general population, making them less susceptible to allergic sensitization.

Even if we are not satisfied with such a trivial explanation, any direct effect of vitamin D supplements on bacteria is unlikely because prokaryotic microorganisms do not have any nuclear or membrane receptor for vitamin D_3 (cholecalciferol) or vitamin D_2 (ergocalciferol). While other supplements like iron (7) influence the gut microbiome, this has been shown now also for vitamin D supplements. An interaction between vitamin supplementation, gut bacteria, and the immune system may occur at many levels – from endocrine signaling to direct sensing of immune cells – and may include both the innate and the adaptive immune system, for example, the early maturation of dendritic cells, the inhibition of Treg-cell development, and the promotion of the differentiation of TH1, TH2, and TH17 cells, among many other regulatory functions.

A first description of vitamin D regulating the gut microbiome was provided by Margherita Cantorna at Penn State (8). Back in 2013, she examined CYP27B1 knockout mice which cannot convert vitamin D into the active $1,25(OH)_2$-D_3 hormone, as well as their wild-type littermates. By means of RNA sequencing of stool samples, she found that CYP27B1 as well as vitamin D receptor (VDR) knockout mice have more bacteria from the Bacteroidetes and Proteobacteria phyla and fewer bacteria from the Firmicutes and Deferribacteres phyla compared to wild-type animals. In addition, there were more 'beneficial' bacteria, such as Lactobacillaceae and Lachnospiraceae. In a follow-up study, VDR knockout mice were resistant to colonization with *Citrobacter rodentium* (9), the rodent equivalent of the human enteropathogenic *Escherichia coli*. In 2015, another group (10), also examining VDR knockout mice, found fewer *C. rodentium*. Wu et al. (11) described a significant increase in Bacteroides. These changes do not relate to the gut only but also to the lung microbiome.

Unfortunately, results of mice studies cannot be directly extrapolated to the human host because mice are a notoriously poor model system in immune (12) and vitamin D research (13). The microbiome of mice raised under clean air conditions may be very different from the microbiome of the human host. While being coated and night-active animals, also the vitamin D metabolism is different in mice.

Human studies, published only this year, have therefore been long awaited. A clinical trial in pregnancy showed increased Lachnobacterium, but decreased *Lactococcus* in the newborn microbiome after vitamin D supplementation (14). Bashir et al. (15) reported changes in the human gut microbiome of adult volunteers given vitamin D_3 on a daily basis for 8 weeks. Vitamin D_3 supplementation mainly had effects in the upper gastrointestinal tract (gastric corpus, antrum, and duodenum), where a decreased relative abundance of Proteobacteria including *Pseudomonas* spp. and *Escherichia*/*Shigella* spp. was found together with increased *Actinomyces* species. Wang et al. (16) using data from colonic biopsies suggested an interplay between VDR and Parabacteroides and provided a genetic explanation for why we may have different microbiomes because the SNPs in the VDR were

associated with bacterial abundance. As an explanation, they point to secondary bile acids (bile acids transformed by the gut microbial metabolism) that serve as ligands for the VDR. Probably, equally important are direct antimicrobial effects of vitamin D_3 by stimulating the expression of cathelicidin antimicrobial peptide (CAMP, LL-37) or human ß-defensin 4 (DEFB4).

It would be interesting to compare these results with the variation found in the 'allergy' microbiome. Ismail et al. (17) described a lower microbial diversity in infants with eczema, while Sjögren et al. (18) reported children with allergy to have been less often colonized with Lactobacilli, *Bifidobacterium adolescentis*, and *Clostridium difficile* during their first 2 months. Chen et al. (19) showed more *Sphingomonas*, *Sutterella*, *Bifidobacterium*, *Collinsella*, *Clostridia*, *Enterococcus*, *Lactobacillus*, among others, in the food-sensitized group. *Bacteroides*, *Parabacteroides*, *Prevotella*, *Alistipes*, *Streptococcus*, and *Veillonella* were decreased. Azad et al. (20) found Enterobacteriaceae to be overrepresented and Bacteroidaceae to be underrepresented in the gut microbiota of food-sensitized infants. The picture here is not completely clear; it would be interesting to see not only the gut but also the lung and skin microbiome.

We now return to the initial question of whether there is a link between vitamin D supplementation, the microbiome, and allergy. There is some preliminary evidence that – like many other environmental factors – vitamin D may modify the human microbiome. Whether the pro-allergic effect of vitamin D is indeed mediated by the microbiome or by already known effects on dendritic cells, T-cell maturation, or antigen presentation remains an open research question. As always in biology, everything is connected to everything.

Conflicts of interest

None.

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