Review Article

THE TANGLED WEB OF THE IMMUNE SYSTEM: ALLERGY AND THE MICROBIOME

Eftyhia Vardas | BSc(Hons), MBBCh, DTM&H, DPH, MMed (Virology), FC Pathology (Clinical Virology)
Lancet Laboratories, Virology, Allergy and Autoimmunity, Johannesburg; and Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa
Email | Eftyxia.Vardas@lancet.co.za

INTRODUCTION

Allergic diseases are a major health problem especially in rapidly developing and high-income societies. They require chronic treatment and have a significant impact on quality of life (QoL) of the individuals, families and communities affected by them; in addition, they are a burden on limited health resources. What really causes allergic disease and why do some human beings develop them, whereas others do not? There are multiple layers to this immunological enigma that have been partially unravelled over the past few years. The development of atopy – or a genetic predisposition to allergic diseases and asthma – has been examined and, undoubtedly, allergic inflammation in an individual is a delicate interplay between their genetic milieu (which includes conventional chromosomal DNA and epigenetic DNA) and the environment.[1]

EPIDEMIOLOGY

Robust epidemiological evidence supports the increasing incidence of allergy[2] and asthma worldwide[3] over the past few decades. The hypothesis for these changes in global allergy epidemiology is simple; in its most basic form, it is postulated that a decrease in the frequency of infections leads to an increase in allergic conditions.[4] This idea developed further and became formalised as the 'hygiene hypothesis' in 2000. The hypothesis stated that the decrease in microbial exposure – specifically the loss of the symbiotic microbiological environment that evolves with the individual from early foetal life, birth and the crucial neonatal/early infancy period – resulted in the loss of protection from the development of allergic diseases.[5]

Animal models and some human-cohort data have demonstrated clear associations between decreased exposure to infectious organisms and 'clean' environments, on the one hand, and increased antibiotic use and caesarean section rates, on the other. Decreased exposure to environmental allergens and viral, bacterial, helminth loads can lead to significant differences in the rate of allergic-disease development.[6] But there is some confusion because this relationship is not clear cut, and certainly not linear, for not all infections are good. Indeed, some infections are detrimental and lead to disease. These include viral respiratory-tract infections in infants with respiratory syncytial virus or rhinovirus type C, both of which affect bronchial smooth musculature and result adversely in persistent wheeze and asthma in later life.[7]

ENVIRONMENT

The biome does not work in isolation: lifestyle changes and modern living conditions, with their associated reduction in allergen exposure, are fundamental to the loss of normal immunological tolerance to non-threatening environmental allergens.[9] Epidemiological evidence accumulated from many countries has shown that the rate and pattern of this increase in allergic diseases is skewed, with significantly higher prevalence and more severe disease occurring in higher-income countries.[3] The 'hygiene hypothesis' and the 'biome depletion' theories[5] are two sides of the same coin. Both refer to the impact that modern lifestyles have had on the normal microbiological flora (the human biome); indeed, many of the risk factors for allergic disease affect the microbiome of the placenta, skin, nasopharynx, lung and gut in the immunologically important early postnatal period (see Figure 1).

Recently, a well-designed study clearly demonstrated this concept in human beings and it has shown the protective effect of a traditional farming environment on the development of allergic sensitisation and asthma in two communities living in the United States: the Amish and the Hutterites. For religious reasons, these two communities have chosen to remain isolated from modern lifestyles and from marrying outside of their specific groups.[9] Because of their isolation, these communities afforded the opportunity for a natural human experiment which allowed the comparison of the effects of one critical factor – different farming practices – on children of similar genetic background and lifestyles and their development of allergy and asthma.

Both the Amish and the Hutterites are farming communities; however, the Amish use traditional methods whereas the Hutterites use industrialised methods. On the one hand, the Amish traditional farming environment was found to offer protection against the development of allergies; on the other hand, Hutterite children showed almost six times higher allergic sensitisation and four times higher asthma rates.[9] This study also analysed the microbial composition and endotoxin levels in household dust from these two communities. This showed that dust from Amish households had higher median endotoxin levels and significant differences in microbial composition when compared with the Hutterite examples.[9] Crucially, analysis indicated that the numbers and gene-expressing profiles of innate cells of periphery involved in the innate immune response

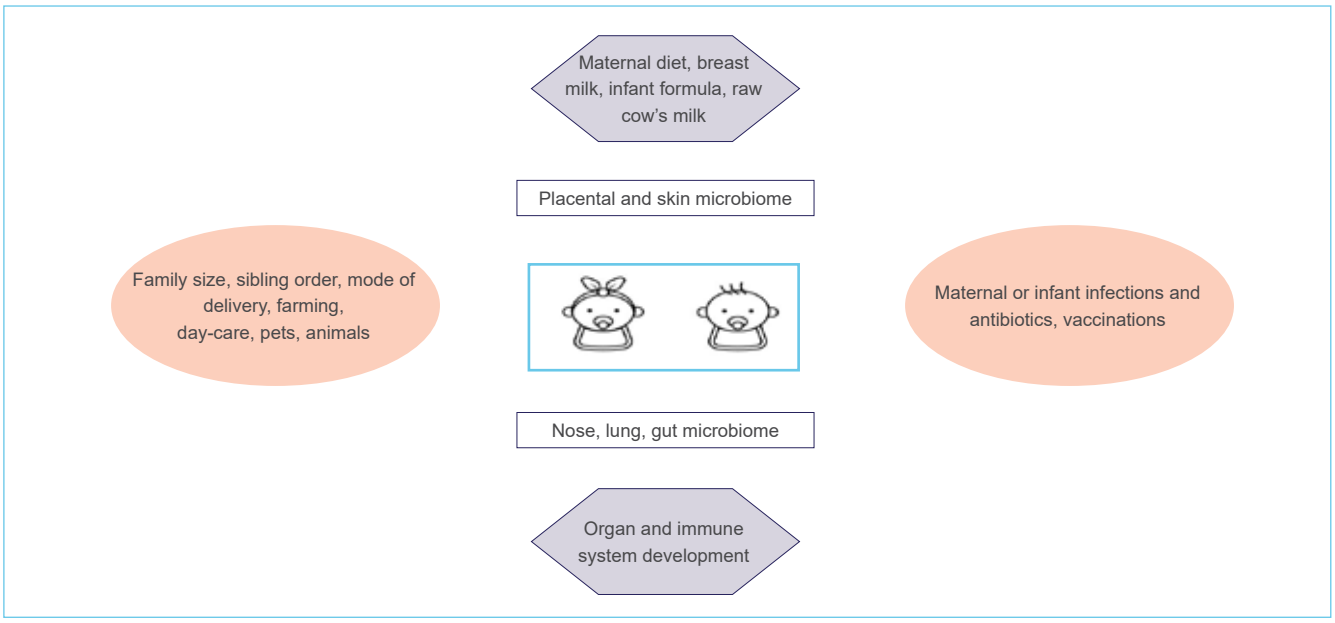

Figure 1: Factors that have an impact on the microbiome and increase the risk for the development of allergy

(monocytes, neutrophils and eosinophils) were different in the two groups of children.

The Amish children also displayed higher numbers of functional genes that limit the multiple inflammatory pathways essential in the innate immune responses against viruses. Sustained microbiological exposure leads to reduced expression of HLA-DR on monocytes and drives immature neutrophils from the bone marrow, both of which were confirmed in the Amish children.[9] These children also showed normal levels of T*regs* (regulatory T cells) and IL-10 that normally function to balance immune effects.

A study from South Africa of Xhosa adolescents has similarly demonstrated significant associations between polymorphisms in IL-10 and IL-4 genes, on the one hand, and susceptibility to allergy and asthma, on the other.[10] This study is a major step forward in describing allergy phenotypes in people of black ethnicity, although the definitions cannot be definitive because social and economic environments may confound ethnicity.

Stein et al,[9] in their study, then elegantly confirmed the findings observed in human beings by providing an immunological mechanism for the discrepancy in allergic disease in the children from these two communities. This was done by using a mouse model lacking *MyD88* and *Trif* adaptor proteins, which disables multiple pathways of the innate immune response and, more specifically, disables those pathways that mediate signalling by microbial products through toll-like receptors (TLRs). Dust samples from the Amish households suppressed the induction of airway inflammation in the mouse model of allergic asthma compared with the dust samples from Hutterite households.[9]

IMMUNOLOGY

The immunology of allergic disease is complex and the mechanistic details of the processes that lead to allergic disease are still being elucidated;[11] but the loss of innate, cellular and humoral immune-regulatory pathways is involved. The immune system functions by being able to receive and interpret signals internally from cells that make up the body, from environmental agents and from microorganisms, either pathogenic or commensal. Allergic reactions to usually innocuous environmental agents (aeroallergens and foods) interact with parts of the innate immune system that plays a pivotal role in shaping immune responses.

The structure of the allergenic substance is the key initiating factor, with many allergenic substances being lipid-binding proteins (e.g. lipocalins of pets and lipid-transfer proteins of plants) and some being glycoproteins (e.g. peanut Ara h2). These proteins interact with pathogen-recognition receptors such as the TLRs on antigen-presenting cells, which push the immune system towards Th2 inflammation tissue injury, remodelling and chronicity.[12]

The first step in the complex interplay which leads to allergy is *sensitisation*. During sensitisation, allergen-specific IgE antibodies are produced and bind to the high-affinity FcεRI receptors on the surface of mast cells and basophils. During this phase, effector Th2 cells produce IL-4, IL-5 and IL-13. IL-4 and IL-13 induce class-switching to ε immunoglobulin heavy chains in B cells, IgE memory B-cell expansion and the production of allergen-specific IgE antibodies.[13] On re-encountering the allergen at a later time during the *effector phase* the stage has been set, the allergen causes cross-linking of the IgE-FcεRI receptor complexes on basophils and mast cells which release mediators (e.g. histamine, prostaglandins, IL-3, IL-4, IL-5, IL-13) that are responsible for the immediate hypersensitivity reactions. Inflammation in allergy is controlled by adaptive allergen-specific CD4+ helper T cells (T_H2 cells) and by group 2 innate lymphoid cells (ILCss) that drive IgE synthesis, eosinophilis, mucous

production and smooth-muscle contraction via interleukins (IL-4, IL-5, IL-13, IL25, IL33).[13]

The leading concept in trying to understand the immunological mechanisms of allergy has always depended on the concept of a T_H1–T_H2 balance. T_H1 cells are involved in infections and autoimmunity; T_H2 cells are involved in allergic disease; each set has reciprocal roles in regulating one another. It has been suggested that the loss of infectious pressure resulted in the loss of T_H1 cells and therefore in a rebound increase in T_H2 cells.[11] Unfortunately, this was way too simplistic and, what is more, the discovery of ILCs changed the perception of T cells as the effectors of immunity; accordingly, ILC2 cells took over the limelight and were shown in animal models to contribute significantly to TH2 inflammation.[13]

TOLERANCE AND REGULATORY T CELLS (T*regs*)

Allergic diseases occur because of a failure to develop tolerance towards a specific allergen, which results in allergen-specific T_H2 cells, IgE and cytokine production. T*reg* cells are the enforcers and regulators of tolerance to both self-antigens and innocuous environmental allergens. They are a distinct CD4+ T-cell subset identified by a marker required for their differentiation known as Forkhead Box 3 or FOXP3.[14] Some T*reg* cells are induced in the thymus and are involved chiefly in autoimmune diseases; a second subset, induced peripherally, are directed against microbial antigens or environmental allergens. These T*reg* subsets are characterised by non-overlapping T-cell receptors so there is a clear division of labour between cells that regulate responses to self-antigens and non-self-antigens.

T*regs* produce immune-regulatory cytokines IL-10 and TGF-β (transforming growth factor β). IL-10 is an anti-inflammatory cytokine that inhibits effector T-cell activation by suppressing stimulatory pathways; it also does so by suppressing the antigen-presenting capacity of dendritic cells (DCs) and eosinophil activation, by so doing interrupting both early and late allergic responses. TGF-β has a wide range of functions that include suppressing B- and T-cell proliferation and differentiation, control of airway inflammation and remodelling.[14]

In mice, alternations in the microbiome diversity with decreased fibre intake led to defective T*reg* cells.[11] In human beings, deep sequencing techniques show that allergen-specific FOXP3 T*reg* cells dominate in heathy individuals; however, in allergic individuals, T*reg* cells and memory/effector T_H2 cells exist together. The influence of the microbiome is demonstrated by the induction of T*regs* butyrate, a short-chain fatty acid produced by commensal bacteria (*Bacteriodes, Bifidobacterium, Fecalibacterium* and *Enterobacteria*). Further research is needed to determine how cleaner environments and modern lifestyles promote the loss of T*regs* and how microbes can promote allergen-specific T*regs* and dampen TH2 inflammation.

The importance of tolerance and these T*reg* mechanisms are illustrated further in establishing tolerance in allergy immunotherapy. This therapy requires, as a first step, basophil and mast-cell desensitisation to degranulation, the formation of allergen-specific T*reg* cells expressing multiple suppressor factors, and decreased eosinophil, mast-cell and basophil migration in the affected tissues.[8]

MECHANISMS OF INFLUENCE OF THE MICROBIOME

Multiple studies in both animal models and human beings have shown that disruption of the normal complex microbiological commensals on the skin, oral/nasal mucosa, gastrointestinal tract and lung leads to disease susceptibility. Associations with disease have been postulated as a function of the lack of or the change in diversity of the microbiome.[15]

Acquiring a functional microbiome during delivery, infancy and childhood is essential in maintaining homeostasis and preventing susceptibility to allergic disease. Experiments in mouse models of atopy show that dysbiosis, especially at the time of weaning, may have detrimental effects, leading to the development of atopy.[16] Increasing evidence also suggests that any effects on the microbiome may occur even earlier during gestation. Here, the use of maternal antibiotics and the significant impact of the mode of delivery show the significant differences in microbiological flora for vaginally delivered infants compared to those delivered by caesarian section.[7] During the postnatal period, a Th2-predominant immunological environment exists as a consequence of maternal–foetal tolerance. This is normal and assists in organ development; however, it allows easy sensitisation of the infant, given the immunological environment.

Commensal microbiological flora in the gut work through different immune cells of either the innate or the adaptive immune systems to influence allergic responses. Microbiological flora promote IgA production by T*reg*-cell-dependent MyD88-dependent mechanisms that allows T*regs* in the gut to differentiate into follicular T-helper cells (T_{FH}). Th2 cells predominate in situations where antibiotics deplete commensal bacteria. These, however, are usually held in check by the MyD88 microbial-dependent suppression of IgE.[16]

Bacterial metabolites produced by the bacterial fermentation of dietary fibre such as acetate propionate and butyrate (short-chain fatty acid) increase the production of T*reg* cells via a specific receptor FFAR2 (G protein-coupled receptor, GPR43) in the case of acetate proprionate. In the case of butyrate, which is a histone deactylase (HDAC) inhibitor, this increases FOXp3 protein acetylation, which in turn increases the stability and suppressive function of intestinal T*regs*.[5] IL-22, which decreases gut permeability and therefore oral allergen uptake, is promoted by the presence of gut *Clostridia* species that allow the development of oral tolerance by the production of IL-10 and control systemic IgE production by decreasing IL-4 production from CD4+ T cells.[7] Evidence suggests that microbiota promote the expansion of the ROR-γt T*reg* cell and the regulation of DC activations; a deficiency of these cells therefore leads to a Th2 environment.

CONCLUSION

More than sufficient and convincing evidence confirms the significant impact on human health and disease of the

environment and, in particular, the commensal microbiological environment that constitutes the microbiome. The challenge now is that the pathways and complex interactive immunological mechanisms that have been teased out need to be distilled into a common understanding of the fine balance that exists between them. This information can then be used with confidence in providing directed and novel interventions for the primary prevention of allergic diseases through the use of probiotics and microbiome supplementation. It could also be used to direct new therapies towards these diseases of immune disruption.

DECLARATION OF CONFLICT OF INTEREST
The author declares no conflict of interest

This article has been peer reviewed.

REFERENCES

1. Meyers DA, Bleecker ER, Holloway JW, Holgate ST. Asthma genetics and personalised medicine. Lancet Respir Med 2014;2:405–415.
2. Platts-Mills TAE. The allergy epidemics 1870–2010. J Allergy Clin Immunol 2015;136(1):3–13.
3. Asher MI. The Asthma epidemic – global and time trends of asthma in children. Chapter 3a. In Global Atlas of Asthma. 2013. EAACI. Editors Akdis CA, Agache I.
4. Strachan DP. Hay-fever, hygiene and household size. BMJ 1989;299:1259–1260.
5. Lynch SV, Bouchey HA. The microbiome and the development of allergic disease. Curr Opin Allergy Clin Immunol 2016;16(2):165–171.
6. Smits HH, Hiemstra PS, Prazeres da Costa C, Ege M, et al. Microbes and asthma: opportunities for intervention. J Allergy Clin Immunol 2016;137(3):690–697.
7. Bendiks M, Kopp MV. The relationship between advances in understanding the microbiome and the maturing hygiene hypothesis. Curr Allergy Asthma Rep 2013; 13:487–494.
8. Linneberg A. Are we getting enough allergens. Int Arch Allergy Immunol 2008;147:93–100.
9. Stein MM, Hrush CL, Gozdz J, Igartua C, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. NEJM 2016;375(5):411–421.
10. Laurence C, Van der Merwe L, Zhang G, Le Souef P, Levin M. Association between pro-inflammatory alleles and allergic phenotypes in Xhosa adolescents. Pediatric Allergy Immunology 2018;29:311–317.
11. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. Nat Immunol 2017;18(10):1077–1082.
12. Breiteneder H. Mechanisms of allergenicity of allergens. Chapter 3b. In Global atlas Allergy. 2014;9–10. EACCI. Editors Akdis CA, Agache I.
13. Akdis CA. The underlying mechanisms in allergy of allergens. Chapter 3b. In Global atlas Allergy. 2014;39–41. EACCI. Editors Akdis CA, Agache I.
14. Rivas MN, Chatila TA. Regulatory T cells in allergic disease. J Allergy Clin Immunol 2016;138(3):639–652.
15. McCoy KD, Koller Y. New developments providing mechanistic insight into the impact of the microbiota on allergic disease. Clin Immunol 2015;159:170–176.
16. Wesemann DR, Nagler CR. Commensal bacteria, timing and barrier function in the context of allergic disease. Immunity 2016;19(44)728–738.