The Intestinal Microbiome in Allergic Disease

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Abstract

Evidence from epidemiological studies demonstrates that the prevalence of allergic disease is increasing worldwide; an epidemic largely driven by environmental factors associated with the adoption of a modern, urbanized lifestyle. Changes in early-life intestinal colonization patterns caused by reduced exposure to traditional environmental microbes have been implicated in the failure of allergic individuals to develop appropriate immunoregulatory networks. Indeed, differences in intestinal microflora composition are observed between allergic and non-allergic persons, and colonization patterns measured within the first few months of life are especially predictive of allergic status. Several environmental factors have been associated with this microbiome imbalance, but there is conflicting evidence as to which exposures are truly driving disruption. Based on evidence linking microbiome perturbation to the pathogenesis of allergic disease, an obvious approach to prevention and treatment of allergy has been the use of probiotics and bacterial by-products (i.e. SCFAs). These interventions have seen some success in animal studies, but current attempts to therapeutically manipulate the microbiome in humans have been largely unable to impact systemic sensitization and alter host allergic status.

Glossary of Terms

Foxp3	Transcription factor important in the development of T regulatory cells
IPEX	Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome; a rare, lethal disorder caused by genetic defects in *FOXP3*
PAMP	Pathogen associated molecular pattern
PRR	Pattern recognition receptor
TLR	Toll-like receptor
OVA	Egg white protein commonly used in experimental models of allergy
Bet v 1	A common allergen from birch tree

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Allergic Disease: A Rising Epidemic

The prevalence of allergic disease is increasing globally and, with 30-40% of the world's population affected by 1 or more allergic condition(s), it now ranks among the most widespread chronic disorders.[1] The term "allergic disease" encompasses a broad range of conditions, ranging from eczema to asthma to life-threatening food allergy. Although an individual could theoretically develop an allergic hypersensitivity to nearly any material, most common allergens are derived from particular environmental, animal, food, and medication sources.[2]

The rapid rise in allergic disease excludes a primary genetic cause.[3] Rather, it is likely a result of changes in living conditions, as an upsurge in allergy prevalence has been observed as societies become more affluent and urbanized.[4] Studies of East and West German populations since the country's reunification in 1990 have shown a steep increase in allergy in East Germany corresponding to its adoption of a more Westernized lifestyle.[5-9] This observation has been supported by migration studies[10] and regional studies[11] from other areas of the world that report higher rates of allergic disease among members of families and ethnic groups who live in modern, industrialized areas, in comparison to rural-dwelling relatives. Increased personal cleanliness and reduced infection rates are hallmarks of an urbanized lifestyle. Indeed, lack of exposure to microbes, especially during crucial windows of growth and development, has been pinpointed as a likely cause of the aberrant immune responses driving allergic disease.

The Hygiene Hypothesis and its Evolution

The hygiene hypothesis was originally proposed over two decades ago by David Strachan,[12] who noticed an inverse correlation between family size and number of children in the household suffering from hay fever. This was the first suggestion that allergic disease may be caused by improvements in personal cleanliness and reduced exposure to childhood infections. The principal tenet of Strachan's idea remains largely accepted today, with an added emphasis on the role of intestinal commensal flora. Our current understanding of the hygiene hypothesis states that early gut microbiota establishment during critical developmental periods can influence an individual's risk of developing environmentally influenced diseases, and post-industrialization habits have depleted the ancestral microbiota we evolved to depend upon for optimal health. This is often referred to as the "disappearing gut microbiota hypothesis".[13]

The intestinal microbiota play an integral role in many physiologic, immunologic, and metabolic pathways. As such, aberrations in its diversity and composition have multisystem consequences that extend beyond the realm of the gut. Mi-

croflora imbalance has been implicated in the pathogenesis of several chronic inflammatory noncommunicable diseases, including diabetes, atherosclerosis, obesity, allergy, and asthma.[14] In allergy, a lack of exposure to appropriate microbes impairs proper induction of the immunoregulatory network, creating a predisposition towards Th2-driven immune overreaction.[15] For example, patients with food allergy exhibit a breakdown in oral tolerance and, most often, experience an immediate type I hypersensitivity reaction upon exposure to a trigger allergen. The resulting IgE/mast cell-mediated immune cascade produces symptoms characteristic of food-induced anaphylaxis: urticaria, angioedema, vomiting, diarrhea, respiratory distress, and hemodynamic collapse.[16]

A Brief Introduction to the Intestinal Microbiota

Though Theodor Escherich pioneered the study of early-life intestinal microbiota in the 1800s,[17] the gut microflora has since been classified as a "forgotten organ"; one which performs functions necessary for human life that we have not evolved to complete on our own.[18] In fact, some argue that the microbiota should be regarded as a contributor to genetic diversity between individuals.[19] The human intestinal tract is colonized with 10 x more microbial cells than there are human cells in the body and contains 150 x more bacterial genes than the human genome.[20] The intestinal microbiota act as a barrier to colonization and infection by pathogens and perform vital metabolic functions, such as dietary fibre fermentation and vitamin production.[21]

The majority of intestinal flora can be assigned to five phyla: Actinobacteria (includes genera *Bifidobacterium*, *Colinsella*), Bacteroidetes (includes genera *Bacteroides, Prevotella*), Firmicutes (includes genera *Lactobacillus, Clostridium, Eubacterium, Ruminococcus, Enterococcus*), Proteobacteria (includes genera *Enterobacter*), and Verrucomicrobia (includes the major mucin- degrading species *Akkermansia muciniphila*).[22, 23] The flora of humans and mice have a very similar core, sharing to a large extent the same phyla and also a substantial fraction of common genera.[24] Murine studies have contributed greatly to our understanding of the mechanistic details governing interactions between commensal microbes and host.

When considering the development of the microbiota, it is important to note that the infant microbiome does not mature and gain adult-level diversity until 3 years of age.[19] The composition of intestinal microbiota evolves over the first 1 000 days of life, guided largely by changes in diet.[25] Pioneer species in neonates are mainly *Lactobacillus, Enterococcus, Enterobacter, Staphylococcus,* and *Streptococcus:* facultative anaerobic bacteria that create an environment for the obligate anaerobes that begin to predominate after 1-2 weeks of life. These are chiefly composed of *Bifidobacterium, Bacteroides, Clostridium,* and *Eubacterium.*[21] Significant changes are seen with the introduction of solid foods at 4-6 months: *Bifidobacteria* decrease and there is a gradual increase of more adult-type species, mainly *Bacteroides* and *Clostridium* clusters IV and XIV.[26] This maturation continues as the child's food and environmental exposures expand.

The Intestinal Microbiota: A Role in Educating the Immune System

It seems counterintuitive that the immune system would tolerate large populations of bacteria living within its host - the very crux of its function lies in its ability to recognize that which is non-self and destroy it. As a result, tandem evolution of commensal bacteria and host has created elegant molecular interactions that allow for such mutualism to exist. Symbiosis between the host and commensal flora is accomplished not through immunological ignorance,[27] but through specific interactions between flora and host immune system. These interactions not only prevent inflammatory responses directed towards commensals, but also play a vital role in the normal development of the immune system itself.[28] Indeed, the largest immune organ in the body, the gut-associated lymphoid tissue (GALT), fails to develop normally in germ-free mice and lacks maturation of ileal and colonic intestinal lymphoid follicles.[29]

There exist multiple mechanisms by which immune cells interact with, and are educated by, commensal flora. T cell tolerance is an essential component of healthy host immune function. Inappropriate inflammatory innate and adaptive immune responses are restrained by regulatory T cell populations consisting of three major $CD4^+$ T cell subsets: thymus-derived $Foxp3^+$ Treg cells, peripherally-induced $Foxp3^+$ iTreg cells, and peripherally-induced IL-10-producing $Foxp3^-$ type 1 regulatory (Tr1) cells.[30] T regulatory cells help control inflammation via a number of mechanisms, an essential one being the production of the immunoregulatory cytokine interleukin-10. In the context of allergy, IL-10 suppresses "allergic" IgE antibody production, generates protective IgG_4 blocking antibodies, suppresses allergen-specific Th2 cell proliferation, downregulates eosinophil function, and reduces pro-inflammatory cytokine release from mast cells.[31] Multiple cell types are capable of producing IL-10, but intestinal Tregs act as a central source, with 30-50% of them producing IL-10 under steady-state conditions.[32]

Most Tregs in the colon are $Foxp3^+$, and most Tregs in the small intestine are $Foxp3^-$ Tr1 cells. However, both regulatory T cell subsets are present in both intestinal compartments and produce IL-10.[30] Mice lacking Foxp3 develop fatal multiorgan inflammation,[33] mice lacking IL-10 succumb to wasting disease and colonic inflammation in response to normal commensal flora,[34] and human IPEX patients with germline loss of function mutations in *FOXP3* experience severe intestinal inflammation, food allergies, and widespread autoimmunity.[35]

Interestingly, PAMP-PRR interactions play an important role in immune tolerance induction. Bacterial-derived ligands can influence T regulatory cell development by directly stimulating Treg-expressed PRRs, or stimulating neighbouring cells to create a cytokine milieu that drives T regulatory cell development. Microbial molecular patterns expressed by commensals that allow for paradoxical induction of tolerant host immune responses are termed "symbiont-associated molecular patterns" (SAMPs). The first example of these was found by Round et al who demonstrated that *Bacteroides fragilis* directly activates TLR2 on colonic $Foxp3^+CD4^+$ T cells

through production of the SAMP polysaccharide A (PSA), allowing for colonization of its niche and suppression of Th17 responses.[36] The importance of TLR activation in Treg development has been supported by subsequent studies. Jeon et al[37] demonstrated the induction of Foxp3$^-$ IL-10-producing Tr1 cells in the large intestine by *Bifidobacterium breve* is dependent upon its ability to stimulate CD103$^+$ dendritic cells to secrete IL-10 and IL-27 via TLR2. Likewise, mice deficient in the TLR adapter protein MyD88 manifest severe immune-mediated morbidity and mortality in response to even low doses of dextran sulfate sodium (a colonic epithelium toxin).[38] Thus, direct contact with microbes is essential for the development of a healthy immunoregulatory network and prevention of aberrant Th2 responses.

Some bacterial metabolites also facilitate interaction between the gut microbiota and the immune system and help generate peripheral Treg cells. Short chain fatty acids (SCFAs) are produced by fermentation of indigestible dietary fibre by colonic bacteria. The main intestinal SCFAs in mammals are acetic, butyric, and propionic acids; and alterations in gut microflora produce altered fecal SCFA profiles.[39] Bacteroides phylum produce high levels of acetic and propionic acids, and Firmicutes phylum produce high levels of butyric acids.[40]

SCFAs are rapidly absorbed in the gut and distributed systemically via the blood.[41] They act as an energy source for enterocytes[42] but also exert immune-modifying effects via two known mechanisms: enhancing histone H3 acetylation at the *FOXP3* locus and directly signaling through the G-protein coupled receptors GPR41, GPR43, GPR109A.[43] H3 acetylation permits *FOXP3* gene expression and, while the role of GPR signaling is less well defined, it may increase expression of *Aldh1a1* in gut antigen presenting cells, thereby increasing the production of retinoic acid, an important cofactor in Treg generation.[44] Administration of acetic, butyric, and propionic acids in drinking water increases Foxp3$^+$ Tregs in the colonic lamina propria of germ-free mice, with butyrate being the most potent Treg inducer.[45]

The Connection to Allergic Disease

IL-10-producing T regulatory cells are the dominant allergen-specific subset in non-allergic individuals. Conversely, allergic individuals generate IL-4-producing allergen-specific Th2 cells that produce pro-inflammatory cytokines and stimulate B cells to produce allergen-specific IgE antibodies.[31] As such, patients with allergy exhibit defective regulatory immune responses and mount pro-inflammatory reactions to innocuous antigens. Most commonly, these occur in the form of type I hypersensitivity reactions initiated when allergen crosslinks IgE bound to FcεRI receptors on the surface of mast cells, delivering a strong activation signal. Activated mast cells release multiple pro-inflammatory and vasoactive materials, including histamine, heparin, prostaglandins, leukotrienes, and Th2 cytokines (i.e. IL-5). Consequent vasodilation, vascular leakage, eosinophil recruitment, smooth muscle contraction, and mucus production give rise to typical allergy symptoms.[46]

Although the initiating triggers of allergic disease remain unknown, genetic predisposition, timing and dose of initial allergen exposure, and concurrent infections may all contribute. Additionally, perturbations in microbiome-induced immune tolerance are being increasingly recognized as important for the development of allergy. A Th2-skewed immunity is the primary driver of allergic disease, and newborns are born with a Th2-biased immune system that must mature to incorporate balancing Th1 and T regulatory elements.[47, 48] Evidence in the literature pinpoints interactions with commensal microflora as a major contributor to this maturation.

The composition of the ideal "healthy gut microbiome" that promotes immune tolerance has yet to be determined,[14] but many studies have uncovered differences in intestinal bacterial composition between allergic and non-allergic individuals. Kalliomäki et al[49] demonstrated that infants who were atopic at 12 months of age (defined as >=1 positive skin-prick test) had a reduced *Bifidobacteria* to *Clostridia* ratio in feces at 3 weeks of age. Interestingly, no differences were seen at 3 months of age, highlighting the importance of early immune programming. Björkstén et al[50] have reported that babies developing allergy in the first two years of life (defined as atopic dermatitis or >= 1 positive SPT) were less often colonized with *Bifidobacteria* and more often colonized with *Staphylococcus aureus*, and had higher counts of *Clostridia* and lower counts of *Bacteroides* in the first year of life. These differences were most pronounced in the first month of life. Colonization with *Clostridium difficile* at 1 month has been associated with wheeze and eczema through first 7 years of life and asthma at age 7.[51] In addition, altered fecal microbiota at both the phylum and genus levels have been measured in children diagnosed with food allergy.[18] Allergic children had reduced Bacteroides, Proteobacteria, Actinobacteria and highly enriched Firmicutes phyla. They also exhibited higher *Enterococcus* and *Staphylococcus* genera, levels of which correlated negatively with circulating IL-10 levels.

Animal studies have helped provide some insight into the immunological consequences of microbiome imbalance. Hill et al[52] reported higher levels of serum IgE and circulating basophils in germ-free mice. Sudo et al[53] found that oral tolerance protected against sensitization to OVA (determined by IgE, IgG1, and IL-4 production) in germ-free mice only if the intestine was first reconstituted with *Bifidobacterium infantis* during the neonatal period. Defects in TLR-signaling have been associated with the development of an allergic phenotype: MyD88-/- mice, and wild-type mice with adoptively transferred MyD88-/- B and T cells, have higher serum IgE concentrations, and higher frequencies of blood basophils.[52] TLR4 knockout mice are significantly more susceptible to peanut sensitization.[54] In addition, Grp43-/- mice exhibit exaggerated OVA-induced airway inflammation, as evidenced by increased inflammatory cell infiltrate and expression of eosinophil peroxide in lung tissue.[55] These studies provide insights into how bacterial interactions shape immune system development and ultimately impact host health.

Are there Specific "Microbiome Disruptors"?

Several studies have attempted to pinpoint the specific environmental factors responsible for microbiome disruption.

Fallani et al[56] reported that infants delivered by caesarean section displayed reduced diversity of intestinal flora and delayed colonization with *Bifidobacterium*. Penders et al[57] analyzed fecal samples at 1 month of age from a large Norweigan birth cohort of over 1 000 infants to investigate at the ability of 16 different influences to modify rate of colonization and bacterial counts of "beneficial" microbes (defined as *Bifidobacteria* species, *B. fragilis*) and "harmful" microbes (defined as *C. difficile* and *E. coli*). Intestinal microbiota composition was impacted negatively by C- section, formula feeding, prematurity and hospitalization, and antibiotic use in infancy. In contrast, farm residence, furry pets at home, maternal diet, maternal antibiotic use during pregnancy, and maternal probiotic use during pregnancy were found to have no impact. Feeding studies have identified higher *E. coli* and *C. difficile* counts in formula-fed infants, in comparison to breastfed infants.[39] Infants without older siblings have also been found to display increased proportions of *E. coli* and *Clostridia* in the gut,[58] while infants with older siblings have greater *Bifidobacteria* concentrations.[57]

One environmental exposure that has attracted particular attention is antibiotic use. Administration of antibiotics during infancy has been associated with drops in phylogenetic diversity and lower numbers of *Bifidobacteria* and *Bacteroides* in feces.[57] Alm et al[59] found that antibiotics in the first week of life conferred an increased risk for allergic rhinitis by 8 years of age. Metsälä et al[60] reported that maternal use of antibiotics, both before and during pregnancy, confers an increased risk for cow's milk allergy in offspring.

Mice treated with broad-spectrum antibiotics exhibit reduced and altered bacterial content in feces[54] and lower frequency of Foxp3 expression in the colonic lamina propria.[61] Hill et al[52] found that antibiotic-treated mice exhibited notably reduced levels of Firmicutes and Bacteroidetes phyla in addition to elevated serum IgE levels, increased circulating basophil populations, exaggerated Th2 cell responses (higher frequencies, increased IL-4 and IL-13 production), and exacerbated house dust mite-induced allergic lung inflammation. In a model of peanut allergy, mice treated with broad-spectrum antibiotics exhibited intestinal microbiome disruption and increased susceptibility to peanut, as evidenced by increased IgE levels, anaphylactic symptoms, and Th2-polarized cytokine production by splenocytes cultured with peanut.[54] The window of risk created by antibiotic use remains unknown, but it has been shown that their disruptive effects on the microbiome extend far beyond the treatment period.[39]

Microbiome-Altering Interventions: Attempts to Modify Allergy Risk

Based on evidence linking microbiome disruption to the pathogenesis of allergic disease, an intuitive approach to prevention and treatment of allergy has been the use of probiotics and bacterial-derived short chain fatty acids. These interventions have seen some success in animal studies, but human clinical trials have produced disappointing results.

Probiotics are cultures of potentially beneficial bacteria.[62] Many probiotic varieties include *Lactobacilli* and *Bifidobacteria*, mainly because of early observations that these microbes were reduced in allergic individuals.[14] Administration of *Bifidobacterium longum* during pregnancy can suppress Bet v 1 sensitization in germ-free mice and increase serum IL-10 and TGF-β levels.[63] Conrad et al[64] exposed mice to intranasal *Acinetobacter lwoffii* during pregnancy and were able to prevent the development of OVA-induced allergic asthma pathology in adult offspring. This benefit was dependent on intact TLR- signaling and was abrogated in TLR2/3/4/7/9-/- animals. Barletta et al[65] reported that probiotics administered to peanut-sensitized mice were capable of reducing anaphylactic symptoms and serum histamine levels while increasing IL-10 production from $CD103^+$ DCs in mesenteric lymph nodes, and Foxp3 expression in jejunum mucosa. Here, TGF-β was also upregulated in the jejunum and proved necessary for the protective effect. A probiotic-supplemented diet fed to lactating dams and weaned pups was able to boost numbers of naturally occurring T regulatory cells in the spleens of mice sensitized with peanut and cholera toxin.[66] Therapeutic effects of probiotics have also been shown at a cellular level: DCs treated with *Lactobacillus rhamnosus* can protect against OVA-mediated allergic disease when transplanted intravenously.[67]

In humans, much of the clinical benefit of probiotics in allergic disease has been demonstrated in the context of eczema.[68-70] A recent meta-analysis of probiotic use concluded that probiotics show some efficacy in reducing the incidence of eczema (~31%), but are unable to impact systemic sensitization (IgE levels and SPT results) and thus cannot alter overall host allergic status.[71] However, interest in using probiotics to ameliorate asthma and food allergy remains strong and clinical studies in this area continue.

One Finnish study gave 1 223 mothers with infants at high risk for developing allergy a probiotic mixture during their final month of pregnancy, and then gave a prebiotic supplement to their infants from birth until 6 months of age. This regimen was unable to protect the children from developing IgE-associated allergic diseases (eczema, food allergy, rhinitis, and asthma) in the first 5 years of life.[72] This pattern of failed protection has been replicated in several other studies of probiotics in infancy.[73-75] However, in one study by Cao et al,[76] the addition of *L. rhamnosus* to extensively hydrolyzed casein formula was seen to hasten acquisition of oral tolerance to cow's milk in children previously diagnosed with cow's milk allergy. Interestingly, this effect was later attributed to an expansion of butyrate-producing *Clostridia* species, whose colonization was aided by the *L. rhamnosus*. Nevertheless, due to lack of evidence, consumption of probiotics and/or prebiotics is not currently recommended for prevention or treatment of allergy.

Recently, therapeutic use of bacterial products, mainly SCFAs, has been investigated, though this early research has been limited to animal studies. Mice fed propionate were protected against HDM-induced airway inflammation. This was mediated not through increased $Foxp3^+$ Treg cells but through alterations in DC hematopoiesis: lungs were populated with DCs with high phagocytic capacity but impaired ability to induce Th2 cells.[77]

The inconsistency in results from probiotic human trials may have a number of causes, including species selection, timing, dose, and duration of treatment.[78] Genotype hetero-

geneity coupled with highly varied environmental exposures make it difficult to develop a universal probiotic or prebiotic formula for allergy prevention and treatment.

However, bacterial isolation and sequencing techniques continue to improve, and our understanding of the human microbiome in health and disease is expanding rapidly. Further research in this area may reveal the key to a healthy gut and the immunological benefits it confers.

Conclusions

Epidemiological studies have linked increasing allergy prevalence to the adoption of a modern, urbanized lifestyle. A large body of evidence suggests that improper colonization with particular commensal flora compromises proper induction of the immunoregulatory network and creates a predisposition to Th2-driven immune overreaction. Although current probiotic formulas have been unable to successfully prevent or treat allergic diseases, newly emerging sequencing technologies are expanding our knowledge of the human microbiome. This research may pave the way for more effective allergy treatment and prevention strategies in the future.

References

1. Pawanker R, Canonica G, Holgate S, Lockey R, editors. World Allergy Organization (WAO) white book on allergy. Milwaukee (WI): World Allergy Organization; 2011.
2. Leung PSC, Shu S-A, Chang C. The changing geoepidemiology of food allergies. Clin Rev Allergy Immunol [Internet]. 2014 Jun [cited 2015 Jan 2];46(3):169–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24535418.
3. Spergel JM. From atopic dermatitis to asthma: the atopic march. Ann Allergy Asthma Immunol [Internet]. 2010 Aug [cited 2014 Jun 18];105(2):99–106; quiz 107–9, 117. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20674819.
4. Björkstén B. The environmental influence on childhood asthma. Allergy [Internet]. 1999 Jan [cited 2014 Jun 10];54 Suppl 4:17–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10422743.
5. Von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med [Internet]. 1994 Feb [cited 2014 Jun 11];149(2 Pt 1):358–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8306030.
6. Nicolai T, Bellach B, Mutius E V, Thefeld W, Hoffmeister H. Increased prevalence of sensitization against aeroallergens in adults in West compared with East Germany. Clin Exp Allergy [Internet]. 1997 Aug [cited 2014 Jun 11];27(8):886–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9291284.
7. Duhme H, Weiland SK, Rudolph P, Wienke A, Kramer A, Keil U. Asthma and allergies among children in West and East Germany: a comparison between Münster and Greifswald using the ISAAC phase I protocol. International Study of Asthma and Allergies in Childhood. Eur Respir J [Internet]. 1998 Apr [cited 2014 Jun 11];11(4):840–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9623686.
8. Wichmann HE. Possible explanation for the different trends of asthma and allergy in East and West Germany. Clin Exp Allergy [Internet]. 1996 Jun [cited 2014 Jun 11];26(6):621–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8809418.
9. Krämer U, Oppermann H, Ranft U, Schäfer T, Ring J, Behrendt H. Differences in allergy trends between East and West Germany and possible explanations. Clin Exp Allergy [Internet]. 2010 Feb [cited 2014 Jun 10];40(2):289-98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20210807.
10. Smith JM. Skin tests and atopic allergy in children. Clin Allergy [Internet]. 1973 Sep [cited 2014 Jun 10];3(3):269–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4778074.
11. Van Niekerk CH, Weinberg EG, Shore SC, Heese H V, Van Schalkwyk J. Prevalence of asthma: a comparative study of urban and rural Xhosa children. Clin Allergy [Internet]. 1979 Jul [cited 2014 Jun 10];9(4):319–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/476905.
12. Strachan DP. Hay fever, hygiene, and household size. BMJ [Internet]. 1989 Nov 18 [cited 2014 Jun 9];299(6710):1259–60. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1838109&tool=pmcentrez&rendertype=abstract.
13. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy [Internet]. 1998 Jan [cited 2014 Jun 20];53(46 Suppl):20–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9825991.
14. West CE, Jenmalm MC, Prescott SL. The gut microbiota and its role in the development of allergic disease: a wider perspective. Clin Exp Allergy [Internet]. 2014 Apr 29 [cited 2014 May 24]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24773202.
15. West CE, Renz H, Jenmalm MC, Kozyrskyj AL, Allen KJ, Vuillermin P, et al. The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies. J Allergy Clin Immunol [Internet]. 2015 Jan [cited 2015 Jan 6];135(1):3–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2556703.
16. Longo G, Berti I, Burks AW, Krauss B, Barbi E. IgE-mediated food allergy in children. Lancet [Internet]. 2013 Nov 16 [cited 2014 May 24];382(9905):1656–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23845860.
17. Escherich T. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung [Internet]. F. Enke; 1886 [cited 2014 Jun 18]. Available from: http://books.google.com/books?hl=en&lr=&id=LCsLAQAAIAAJ&pgis=1.
18. Ling Z, Li Z, Liu X, Cheng Y, Luo Y, Tong X, et al. Altered fecal microbiota composition associated with food allergy in infants. Appl Environ Microbiol [Internet]. 2014 Apr [cited 2014 Jun 4];80(8):2546–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24532064.
19. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature [Internet]. 2012 Jun 14 [cited 2014 May 23];486(7402):222–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3376388&tool=pmcentrez&rendertype=abstract.
20. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature [Internet]. 2010 Mar 4 [cited 2014 May 23];464(7285):59–65. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3779803&tool=pmcentrez&rendertype=abstract.
21. Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. Pediatr Allergy Immunol [Internet]. 2014 Jun 5 [cited 2014 Jun 10]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24899389.
22. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol [Internet]. 2007 Dec [cited 2014 May 23];73(23):7767–70. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2168041&tool=pmcentrez&rendertype=abstract.
23. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science [Internet]. 2005 Jun 10 [cited 2014 May 25];308(5728):1635–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1395357&tool=pmcentrez&rendertype=abstract.
24. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. PLoS One [Internet]. 2013 Jan [cited 2014 May 28];8(5):e62578. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3641060&tool=pmcentrez&rendertype=abstract.
25. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A [Internet]. 2011 Mar 15 [cited 2014 May 27];108 Suppl :4578–85. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3063592&tool=pmcentrez&rendertype=abstract.
26. Roger LC, McCartney AL. Longitudinal investigation of the faecal microbiota of healthy full-term infants using fluorescence in situ hybridization and denaturing gradient gel electrophoresis. Microbiology [Internet]. 2010 Nov [cited 2014 Jun 17];156(Pt 11):3317–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20829292.
27. Hooper L V. Do symbiotic bacteria subvert host immunity? Nat Rev Microbiol [Internet]. 2009 May [cited 2014 Jun 18];7(5):367–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19369952.
28. Eberl G. A new vision of immunity: homeostasis of the superorganism. Mucosal Immunol [Internet]. 2010 Sep [cited 2014 May 23];3(5):450–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20445502.
29. Kamada N, Seo S-U, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol [Internet]. 2013 May [cited 2014 May 23];13(5):321–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23618829.

30. Barnes MJ, Powrie F. Regulatory T cells reinforce intestinal homeostasis. Immunity [Internet]. 2009 Sep 18 [cited 2014 Jun 8];31(3):401–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19766083.
31. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol [Internet]. 2014 Mar [cited 2014 Sep 8];133(3):621–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24581429.
32. Bollrath J, Powrie FM. Controlling the frontier: regulatory T-cells and intestinal homeostasis. Semin Immunol [Internet]. 2013 Nov 30 [cited 2014 Jun 20];25(5):352–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24184013.
33. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol [Internet]. 2003 Apr [cited 2014 May 23];4(4):330–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12612578.
34. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity [Internet]. 2008 Apr [cited 2014 Jun 5];28(4):546–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18387831.
35. Verbsky JW, Chatila TA. Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) and IPEX-Related Disorders: an Evolving Web of Heritable Autoimmune Diseases. Curr Opin Pediatr. 2013;25(6):708-14.
36. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science [Internet]. 2011 May 20 [cited 2014 May 23];332(6032):974–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3164325&tool=pmcentrez&rendertype=abstract.
37. Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, et al. Probiotic Bifidobacterium breve induces IL-10-producing Tr1 cells in the colon. PLoS Pathog [Internet]. 2012 Jan [cited 2014 Jun 6];8(5):e1002714. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3364948&tool=pmcentrez&rendertype=abstract.
38. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell [Internet]. 2004 Jul 23 [cited 2014 Jun 2];118(2):229-41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15260992.
39. Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. Gut Microbes [Internet]. [cited 2014 Jun 9];3(3):203–20. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3427213&tool=pmcentrez&rendertype=abstract.
40. Mcfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc. 2003;62(1):67–72.
41. Pomare EW, Branch WJ, Cummings JH. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. J Clin Invest [Internet]. 1985 May [cited 2014 Jun 18];75(5):1448–54. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=425482&tool=pmcentrez&rendertype=abstract.
42. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature [Internet]. 1997 Sep 25 [cited 2014 May 29];389(6649):349–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9311776.
43. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Adv Immunol [Internet]. 2014 Jan [cited 2014 May 26];121:91–119. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24388214.
44. Coombes JL, Siddiqui KRR, Arancibia-Cárcamo C V, Hall J, Sun C-M, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med [Internet]. 2007 Aug 6 [cited 2014 May 23];204(8):1757–64. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2118683&tool=pmcentrez&rendertype=abstract.
45. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science [Internet]. 2013 Aug 2 [cited 2014 Jun 1];341(6145):569–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3807819&tool=pmcentrez&rendertype=abstract.
46. Kumar S, Verma AK, Das M, Dwivedi PD. Molecular mechanisms of IgE mediated food allergy. Int Immunopharmacol [Internet]. 2012 Aug [cited 2015 Jan 12];13(4):432-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22668720.
47. Tulic MK, Andrews D, Crook ML, Charles A, Tourigny MR, Moqbel R, et al. Changes in thymic regulatory T-cell maturation from birth to puberty: differences in atopic children. J Allergy Clin Immunol [Internet]. 2012 Jan [cited 2014 Jun 20];129(1):199–206.e1-4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22104606.
48. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol [Internet]. 1998 May 15 [cited 2014 Jun 20];160(10):4730-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9590218.
49. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol [Internet]. 2001 Jan [cited 2014 May 29];107(1):129-34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11150002.
50. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol [Internet]. 2001 Oct [cited 2014 Jun 10];108(4):516-20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11590374.
51. Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol [Internet]. 2011 Nov [cited 2014 May 26];128(5):948-55.e1-3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21872915.
52. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. Nat Med [Internet]. 2012 Apr [cited 2014 Jun 10];18(4):538-46. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3321082&tool=pmcentrez&rendertype=abstract.
53. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol [Internet]. 1997 Aug 15 [cited 2014 Jun 8];159(4):1739-45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9257835.
54. Bashir MEH, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol [Internet]. 2004 Jun 1 [cited 2014 Jun 19];172(11):6978-87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15153518.
55. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature [Internet]. 2009 Oct 29 [cited 2014 May 27];461(7268):1282-6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3256734&tool=pmcentrez&rendertype=abstract.
56. Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, et al. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. J Pediatr Gastroenterol Nutr [Internet]. 2010 Jul [cited 2014 May 27];51(1):77-84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20479681.
57. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics [Internet]. 2006 Aug [cited 2014 May 30];118(2):511-21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16882802.
58. Adlerberth I, Strachan DP, Matricardi PM, Ahrné S, Orfei L, Aberg N, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol [Internet]. 2007 Aug [cited 2014 Jun 11];120(2):343-50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17604093.
59. Alm B, Goksör E, Pettersson R, Möllborg P, Erdes L, Loid P, et al. Antibiotics in the first week of life is a risk factor for allergic rhinitis at school age. Pediatr Allergy Immunol [Internet]. 2014 Jun 9 [cited 2014 Jun 16]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24912441.
60. Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. Epidemiology [Internet]. 2013 Mar [cited 2014 Jun 20];24(2):303-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23348066.
61. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science [Internet]. 2011 Jan 21 [cited 2014 May 23];331(6015):337-41. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3969237&tool=pmcentrez&rendertype=abstract.
62. Salminen S, Bouley C, Boutron-Ruault MC, Cummings JH, Franck A, Gibson GR, et al. Functional food science and gastrointestinal physiology and function. Br J Nutr [Internet]. 1998 Aug [cited 2014 Jun 11];80 Suppl 1:S147-71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9849357.
63. Schwarzer M, Srutkova D, Schabussova I, Hudcovic T, Akgün J, Wiedermann U, et al. Neonatal colonization of germ-free mice with Bifidobacterium longum prevents allergic sensitization to major birch pollen allergen Bet v 1. Vaccine [Internet]. 2013 Nov 4 [cited 2014 Jun 16];31(46):5405-12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24055352.
64. Conrad ML, Ferstl R, Teich R, Brand S, Blümer N, Yildirim AO, et al. Maternal TLR signaling is required for prenatal asthma protection

by the nonpathogenic microbe Acinetobacter lwoffii F78. J Exp Med. 2009;206:2869-77.
65. Barletta B, Rossi G, Schiavi E, Butteroni C, Corinti S, Boirivant M, et al. Probiotic VSL#3-induced TGF-α ameliorates food allergy inflammation in a mouse model of peanut sensitization through the induction of regulatory T cells in the gut mucosa. Mol Nutr Food Res [Internet]. 2013 Dec [cited 2014 May 23];57(12):2233-44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23943347.
66. Toomer OT, Ferguson M, Pereira M, Do A, Bigley E, Gaines D, et al. Maternal and postnatal dietary probiotic supplementation enhances splenic regulatory T helper cell population and reduces peanut allergen-induced hypersensitivity responses in mice. Immunobiology [Internet]. 2014 May 14 [cited 2014 Jun 5]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24882556.
67. Kim H-J, Kim Y-J, Lee S-H, Kang M-J, Yu H-S, Jung Y-H, et al. Effects of Lactobacillus rhamnosus on asthma with an adoptive transfer of dendritic cells in mice. J Appl Microbiol [Internet]. 2013 Sep [cited 2014 Jun 12];115(3):872-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23725296.
68. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet [Internet]. 2001 Apr 7 [cited 2014 May 28];357(9262):1076-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11297958.
69. Rautava S. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. J Allergy Clin Immunol [Internet]. 2012 [cited 2014 Jun 11];130(6):1355-60. Available from: http://resolver.scholarsportal.info/resolve/00916749/v130i0006/1355_mpsdparoeiti.xml.
70. Dotterud CK. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. Br J Dermatol [Internet]. 2010 [cited 2014 Jun 11];163(3):616-23. Available from: http://resolver.scholarsportal.info/resolve/00070963/v163i0003/616_pipwtpadardt.xml.
71. Dang D, Zhou W, Lun ZJ, Mu X, Wang DX, Wu H. Meta-analysis of probiotics and/or prebiotics for the prevention of eczema. J Int Med Res [Internet]. 2013 Oct [cited 2014 May 23];41(5):1426–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23908398.
72. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. J Allergy Clin Immunol [Internet]. 2009 Feb [cited 2015 Feb 2];123(2):335-41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19135235.
73. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. J Allergy Clin Immunol [Internet]. 2007 Jan [cited 2015 Feb 2];119(1):184-91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17208600.
74. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. Pediatrics [Internet]. 2008 Apr [cited 2015 Feb 2];121(4):e850-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18332075.
75. West CE, Hammarström M-L, Hernell O. Probiotics in primary prevention of allergic disease–follow-up at 8-9 years of age. Allergy [Internet]. 2013 Aug [cited 2015 Jan 22];68(8):1015-20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23895631.
76. Cao S, Feehley TJ, Nagler CR. The role of commensal bacteria in the regulation of sensitization to food allergens. FEBS Lett [Internet]. 2014 May 1 [cited 2014 May 24]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/24791655.
77. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med [Internet]. 2014 Feb [cited 2014 May 25];20(2):159-66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24390308.
78. West CE. Gut microbiota and allergic disease: new findings. Curr Opin Clin Nutr Metab Care [Internet]. 2014 May [cited 2014 Jun 9];17(3):261-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24535216.