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IMPACT OF NUTRITION AND INTESTINAL MICROBIOTA ON DEVELOPMENT OF MUCOSAL IMMUNITY

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SUMMARY

It is now widely accepted that early life nutrition and the commensal gut microbiota play a key role in driving immune development, and maintaining immune homeostasis, but also in contributing to inflammatory and autoimmune diseases. Although much progress has been made on dissecting the various levels by which gut bacteria modulate gut barrier function and immunity, little is known about the host mechanisms and bacterial effector molecules and signals which drive these major physiological effects. Clearly microbial composition has a profound impact on gut health and disease. The quest to identify health-promoting gut bacteria and to unravel their mode of action continues to advance at a rapid rate. The commercial and clinical opportunities surrounding the exploitation of such research activities are significant, particularly in the development of functional foods and probiotics which promote, maintain, and restore gut health.

INTRODUCTION

During the last decade there has been a major surge in interest in the role of the gut microbiota in human health and disease. Since the publication by Eckburg and Relman (*Eckburg* et al., 2005), describing the diversity of the human gut microbiota, there have been significant advances in our understanding of how specific commensal gut bacteria influence mucosal immunity. The beneficial effects of commensal bacteria, particularly in promoting T regulatory cells (Tregs) and anti-inflammatory signalling pathways, have been scrutinised in an attempt to understand at a detailed mechanistic level how gut bacteria promote and maintain immune homeostasis.

Commensal microbes interact with the mucosal immune system in many ways and recent research has revealed that their beneficial effects can arise from specific interactions with epithelial cells which line the gut wall, mucosal dendritic cells, B cells and ultimately gut T cells within the human gut. New studies have also unveiled an important role for gut bacteria in regulating the enteric nervous system of the gut (*Rhee* et al., 2009). The accumulating data on host microbe interactions presents significant opportunities for exploiting the biological actions of live gut microbes and their component parts as mucosal adjuvants, anti-inflammatories and treatments for autoimmune, allergic and atopic diseases.

THE HUMAN GUT MICROBIOTA

The human gut is home to approximately 10^{14} gut bacteria, numbers of bacteria which outnumber human cells by a factor of 10 and human genes by a factor of 100 and whose collective genome is referred to as the gut microbiome. The diversity of the human gut microbiota, which results from strong host selection and co-evolution, was first comprehensively described by Eckburg and Relman (*Eckburg* et al., 2005). Using approximately 13,000 16S ribosomal RNA gene sequences they revealed that the human gut microbiota is dominated by three major bacterial phyla namely Bacteroidetes, Firmicutes and Actinobacteria all of which are highly diverse at both species and strain level. These authors also verified that mucosa-associated bacteria are distinct from those in the gut lumen and faeces, suggesting that mucosal-associated bacteria may in fact perform different host functions. A total of 395 phylotypes were identified of which 80% had never been cultivated. This astonishing finding serves to highlight the level of work required to fully appreciate the biological properties and function of individual members of the human gut microbiota.

Microbial diversity appears to vary between individuals and at different sites within the gastrointestinal tract (*Zoetendal* et al. 2008). However within an individual it is generally quite stable (*Ley* et al. 2008). The level of inter-individual variation probably reflects the functional redundancy among the constituent members of the human gut microbiota (*Turnbaugh* et al., 2007). This has been confirmed by additional studies which show that among family members, the human gut microbiome (microbial genes) is shared, but the gut microbial community within each individual varies in terms of the specific bacterial lineages. Different microbial diversities can in fact give rise to a core microbiome, supporting the concept of functional redundancy (*Turnbaugh* et al., 2009; *Qin* et al., 2010). Clearly diet, whether herbivore, omnivore, or carnivore has a highly significant impact on microbial diversity composition and tree-based clustering and appears to accounts for the diversity differences between unrelated host species (*Ley* et al., 2008). The gut microbiota of humans living a modern life-style appears typical of omnivorous primates (*Ley* et al., 2008).

The gut is sterile at birth and microbial colonisation is influenced by such factors as mode of delivery, environment, diet and antibiotics. The major microorganisms colonising the newborn gut are derived from both maternal (vaginal and faecal) and environmental sources. Mode of delivery has a major effect on the composition of intestinal microbiota in early infancy, and it has been shown that infants born by Caesarean section have lower numbers of Bifidobacteria and Bacteroides compared with vaginally born infants (*Biasucci* et al., 2010). Furthermore, preterm infants have very different microbiotas, mainly characterised by a low diversity of culturable microorganisms (*Rouge* et al., 2010). Due to the accumulating evidence that the human gut microbiota in early life impacts on subsequent adult health, the issue of gestational age at birth and how it impacts on microbial composition and function in adult life is an important consideration worthy of further investigation. Furthermore, the ageing process is thought to affect the structure of the human gut microbiota, and disturbed immune homeostasis and age-related differences in gut microbiota composition may contribute to the

progression of disease and frailty in the elderly population (*Biagi* et al., 2010). As with early life, the composition and alteration of the gut microbiota in the elderly and the implications for immune status and health is also an important topic requiring more research.

In the first years of life the gut microbiota is highly dynamic and appears not to reach an adult phenotype until around 2 to 3 years of age. Immediately after birth the gut microbiota is characterised by high number of facultative anaerobes including lactobacteria, enterobacteria and streptococci. As oxygen levels begin to deplete within the gut other more obligate bacteria colonise and become established and these bacteria include clostridia and bacteroides (*Marques* et al., 2010). The factors which influence the specific gut bacteria which establish themselves as members of the gut microbiota are not fully understood. In addition to the influences of diet and environment other factors including host genotype and epithelial glycobiology are thought to be important. More specifically, the mucus gel layer overlying the intestinal epithelium has been proposed as a key contributor to the structural and functional stability of the microbiota and tolerance by the host (*Sonnenburg* et al., 2004). This notion gave rise to the view that microbial biofilms form within the gut and that these biofilms are stable communities composed of microorganisms able to utilised and degrade gut mucins as well as recognise mucin-associated glycan structures/carbohydrates as attachment structures (*Sonnenburg* et al., 2004).

COMMENSAL BACTERIAL GENOMES

The first microbial genome was published in 2003 by the Gordon lab (*Xu* et al., 2003). The decoding of the genetic make-up of *Bacteriodes thetaiotaomicron*, a Gram-negative bacterium which is a dominant member of the human gastrointestinal tract, provided the first opportunity to study the molecular mechanisms by which gut microbes shape human physiology. This bacterium containing a 4779-member proteome including an elaborate apparatus for hydrolyzing indigestible dietary polysaccharides and an associated environment sensing system presented the first opportunity to identify commensal-derived effector molecules that could regulate important functions of the host gut including its immune status. Since this significant advancement, the human microbiome project (HMP) has provided reference genomes for a large number of gut commensals, including multiple bacterial isolates belonging to the same species (*Turnbaugh* et al., 2009). Following on, other projects including MetaHIT (Metagenomics of the Human Intestinal Tract) and more recently the comprehensive publication of 3.3 million non-redundant human gut microbial genes (*Qin* et al., 2010) have provided the means to define microbial gene functionality in the context of host response and physiology. The rapid identification of bacterial gene products which influence host immunity, either by augmenting (adjuvants) or, attenuating (anti-inflammatories) specific mucosal immune responses is anticipated.

MUCOSAL IMMUNE DEVELOPMENT

Development of the mucosal immune system starts *in utero* and continues at a dynamic pace in early life, stabilises in adult life and then declines with advancing age through processes of senescence or cellular death (*Ogra*, 2010). The mucosal immune system is highly complex consisting of diverse populations of innate and adaptive immune cells as well as memory cells. These various cell populations are present in organised gut associated lymphoid structures including Peyer's patches, lamina propria, lymphoid aggregates and mesenteric lymph nodes. In addition to the structural complexity of the mucosal immune system, the functionality is also highly complex and involves processes of microbial and antigen recognition, presentation, and response. These processes critically differentiate between harmful (pathogenic) and harmless challenges; the mucosal immune system must be able to defend rigorously against infectious agents whilst maintaining oral tolerance to self-antigens as well as those derived from the diet and the commensal microbiota.

Epithelial cells and dendritic cells (DCs) are the first cells involved in recognising and sampling commensal gut microbes. Epithelial cells undergo maturation in terms of their digestive and absorptive capabilities but also in aspects of their glycobiology, defence properties and synthesis and secretion of membrane bound and soluble mediators, all which affect their interactions with gut microbes and cells of the innate immune system. Important secreted epithelial factors include thymic stromal lymphopoietin (TSLP), IL-10 and TGFβ which promote the initiation and maintenance of oral tolerance (*Ziegler* and *Artis*, 2010). Gut bacteria are recognised by various classes of recognition receptors including Toll-like receptors (TLRs) expressed on the apical and basolateral surfaces of epithelial cells (*Abreu*, 2010). Following TLR recognition and ligation of specific bacterial structures referred to as microbial associated molecular patterns (MAMPS), a number of signalling cascades are activated which collectively influence host epithelial gene expression. These epithelial gene products operate in a paracrine and autocrine fashion to regulate the functional properties of both epithelial cells and neighbouring immune cells.

Gut DCs also respond to gut microbes through similar recognition events but as highlighted above they respond to factors secreted by intestinal epithelial cells such as TSLP and TGFβ which exert a profound effect on DC function within the gut (*Grainger* et al. 2010). Very significant progress has been made in recent years describing the role of intestinal DCs either in activating protective immune responses by engaging naive T cells or in promoting tolerance responses (*Rescigno* and *Di*, 2009). These divergent end points are managed by distinct DC subsets. An important DC subset has recently been defined within the gut which promotes the differentiation and expansion of Treg cells and responds to conditioning signals received from epithelial cells including TGFβ and TSLP (*Sun* et al. 2007; *Coombes* et al. 2007). Defective immunity in the neonatal gut has partly been explained by immaturity of antigen presenting cells including DCs. The precise developmental profiles of DC subsets within the neonatal gut, and the factors which influence their activation and maturation, are not currently known but clearly this information is essential to establishing the factors that influence tolerance and active immu-

nity and hence the susceptibility to allergic and infectious diseases.

In response to antigen presentation by DCs, T cells develop into a number of distinct subsets with associated effector functions depending on the specific cytokine milieu. Specifically T helper (Th)1 cells produce mostly interferon gamma (IFNγ), an inflammatory cytokine important in responses against microbial infections, while Th2 cells secrete interleukin (IL)-4 and IL-13, which participate in immunity against parasites but also play major roles in allergic reactions. Other T cells subsets include Th17 and T regulatory cell (Tregs), the former involved in driving inflammatory and autoimmune conditions and the latter functioning to suppress immune responses and induce tolerance.

Although neonates possess an immature immune system, as revealed by their under-developed lymphoid architecture, low numbers of T and B cells as well as DCs and memory cells, they are still able to mount immune responses. It has been suggested for some time that neonatal immunity is characterised by a dominance of Th2 responses, with a lower prevalence of Th1 responses thus contributing to the so called Th2 bias. Furthermore, although capable of mounting both Th1 and Th2 (mixed T cell responses) the overall response appears to default to a predominant Th2 response upon antigen re-challenge (*Zaghouani*, et al., 2009). Since many gut pathogens require robust Th1 immune responses for efficient clearance and immune protection, this may explain why the neonate is particularly susceptible to a number of important pathogens, resistant to the effects of certain vaccines as well as predisposed to allergic diseases.

More recently the subject of Th1 and Th2 balance has been investigated using a murine neonatal model of infection. Low doses of virulent *Yersinia enterocolitica* were found to induce strong inflammatory Th1 and Th17 cell responses with large quantities of IFNγ and IL-17 supporting the view that the neonate is perfectly capable of mounting a diverse range of T cell responses under certain circumstances (*Echeverry* et al., 2010). However, the enhanced susceptibility to infection in early life suggests that immune immaturity or defective Th1 immunity may be contributing factors. Recent data also suggests that low immune cell populations may not in fact fully account for the decreased immune responsiveness of neonates. An alternative mechanism may be due to an intrinsic "default" mechanism that neonatal $CD4^{(+)}$ T cells have to become Treg cells in response to T cell receptor (TCR) stimulations (*Wang* et al., 2010). This finding provides intriguing insights into Treg cell generation and the predisposition towards tolerance during early life. Equally it may explain the increased susceptibility of neonates to infectious diseases as well as the inadequate response to certain vaccines since neonatal Tregs could impair the specific T cell responses required for pathogenic clearance and account for the premature death of millions of human infants world-wide.

BREAST FEEDING AND IMMUNE DEVELOPMENT

It has long been suggested that breastfeeding confers protection against infections, diarrhoea, inflammatory and allergic diseases, but the mechanisms involved have remained elusive. Breast milk promotes strong anti-inflamma-

tory effects mediated by TGFβ, IL-10 and lactoferrin which serve to limit inflammation in the developing gut, and it also augments host defences through presentation of diverse antimicrobial factors (*Walker*, 2010). The protection against inflammatory and autoimmune diseases may also be related to the induction of oral tolerance mediated by milk antigen immunoglobulin immune complexes that promote antigen-specific FoxP3 regulatory T cells (*Mosconi* et al., 2010). As for B cell development and expansion within the gut, the recent identification of syntenin-1 which preferentially induces IgA production by B cells together with the biological effects of TGFβ may be significant (*Ogawa* et al., 2004; *Sira* et al. 2009)

LIVING ENVIRONMENT AND IMMUNE DEVELOPMENT

Environment during early life has for many years been considered to influence immune development and susceptibility to childhood allergies and asthma. This viewpoint was first postulated by Strachan in 1989 in the form of the hygiene hypothesis (*Strachan*, 1989) and was further endorsed with the notion that improved hygiene associated with decreased infectious agents in early life is a significant factor in the aetiology of atopic allergy disorders (*Sheikh* and *Strachan*, 2004). The latest version of this hypothesis suggests that exposure to farm animals, pets and non-pasteurized milk or fermented beverages may promote healthy development of the immune system (*Gern* et al., 2009). A recent study referred to as Urban Environment and Childhood Asthma (URECA) analysed 560 families from 4 urban areas who were at high risk of allergy along with 49 families without atopic diseases. This study revealed some associations between early life environment and subsequent risk of asthma but more studies are required (*Gern* et al., 2009). Experimental models mimicking the hygiene concept add additional support to the hypothesis that early life environment influences both microbial diversity and immune development and susceptibility to disease (*Mulder* et al., 2009).

COMMENSAL BACTERIA AND INNATE IMMUNITY

Barrier effects

Microbial colonisation is critical for the development and optimal functionality of the mucosal immune system. Beneficial effects on gut barrier function are one of the important biological actions of the colonising microbiota. These effects are thought to be induced down-stream of commensal-mediated TLR signalling through the production of interferon alpha which prevents intestinal epithelial apoptosis (*Mirpuri* et al., 2010).

Epithelial cells

Commensal bacteria also interact with epithelial cells to mediate and regulate NF-κB signalling (*Kelly* et al., 2004). Mice defective in NF-κB signalling developed gut barrier defects suggesting that NF-κB plays an important cytoprotective role in the gut in addition to its role in driving inflammatory responses (*Wullaert*, 2010). The ways in which commensal bacteria regulate host signalling are likely to be complex involving recognition recep-

tors such as TLRs and NODs but potentially other receptor systems that modulate NF-κB signalling responses in favour of immune homeostasis and cytoprotection.

TLRs and commensals

The interactions between commensals and TLRs are not always positive in terms of immune homeostasis and health. For example, Type 1 diabetes (T1D) is a debilitating autoimmune disease and its incidence has increased significantly during the past several decades particularly in Westernised countries. In addition to T1D other autoimmune, inflammatory and atopic diseases are steadily increasing leading to a growing view that environment and in particular microbial exposure is playing a significant role in enhancing susceptibility to immune-mediated diseases. Scientific evidence strengthening this link reveals that the innate immune recognition of gut microbes can in fact promote T1D and elimination of MyD88, an important adapter molecule mediating bacterial TLR signalling protects against T1D. These findings indicate that interaction of the intestinal microbes with the innate immune system is a critical factor modifying T1D predisposition (*Wen* et al. 2008). Furthermore, a reduction in commensal microbiota by antibiotic treatment has been documented to impair the development of autoimmune encephalomyelitis (*Ochoa-Reparaz* et al., 2009). This protection was associated with reduced pro-inflammatory cytokines and increased IL-10 and IL-13. These cases of autoimmune disease clearly indicate that alterations in microbial composition can dramatically impact on disease susceptibility and outcome. They also highlight that not all interactions between the gut and the commensal microbiota are beneficial and that manipulation of microbial diversity profiles can have very significant impact on both intestinal and extra-intestinal diseases.

COMMENSAL MICROBIOTA AND ADAPTIVE IMMUNITY

Commensal Microbiota and T cells

Recent advances investigating the impact of the commensal microbiota in relation to T cell differentiation have revealed that certain bacteria belonging to the class Clostridia are potent inducers of Th17, Th1 and Treg responses (*Gaboriau-Routhiau* et al., 2009). The ability to influence mucosal T cells seems to be restricted to a relatively small group of gut colonising bacteria. Currently the features and biological actions of gut bacteria which can regulate T cell events are unknown but one bacterium shown to be effective, namely the Segmented Filamentous Bacteria, is firmly attached to the gut epithelium and may engage in signalling through a unique class of intestinal epithelial receptors. The identification of other bacteria that influence T cell differentiation is likely and insight into their mechanisms of action will be extremely helpful in developing strategies for manipulation of T cell responses during early life presenting obvious therapeutic benefits and opportunities.

Commensal Microbiota and B cells

The early gut microbiota is dominated by bifidobacteria and lactobacilli particularly if maternal milk is the main nutrient supply. It has recently been suggested that elevated Bifidobacterial diversity enhances the maturation of SIgA levels (*Sjögren* et al., 2009). As infants with higher levels of SIgA are less likely to develop allergic diseases, the presence of bifids in the early gut microbiota is thought to be beneficial.

EDUCATING THE IMMUNE SYSTEM THROUGH MICROBIAL SUPPLEMENTS

Many intestinal diseases are associated with dysregulated immune responses and include the inflammatory bowel diseases Crohn's Disease and Ulcerative Colitis. With both diseases, exaggerated immune responses directed against the commensal microbiota are a common feature and the normal function of cells of the innate (DCs) and adaptive immune (Th) systems are disrupted. Clearly, identification of bacteria which can restore the tolerogenic and regulatory functions of 'defective' DCs and Tregs in IBD patients would be an exciting outcome. Furthermore, the notion that immune protection can be induced in early life as a means of preventing or reducing the incidence of immune-mediated diseases in adult life is even more attractive.

As the beneficial effects of commensal bacteria and probiotics on health and disease prevention become increasingly more defined the application of live microbial supplements to promote and restore gut health will gain much more attention in many aspects of human health. Robust scientific evidence based on human studies is required for EFSA/FDA approved health claims. The future of probiotics as human health products lies with mechanistic studies which prove mode of action and efficacy in human subjects and consumers. One important outcome will be new food products designed to promote gut health at key life stages.

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LITERATURE

Abreu, M.T. Toll-like receptor signalling in the intestinal epithelium: How bacterial recognition shapes intestinal function. Nat. Rev. Immunol. 10, 131-144 (2010).

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkïla, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P., and de Vos, W.: Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS One 5, e10667 (2010).

Biasucci, G., Rubini, M., Riboni, S., Morelli, L., Bessi, E., and Retetangos, C.: Mode of delivery affects the bacterial community in the newborn gut. Early Hum Dev. Suppl. 1, 13-15 (2010).

Coombes, J.L., Siddiqui, K.R., Arancibia-Cárcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., and Powrie, F.: 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. 204, 1757-1764 (2007).

Echeverry, A., Saijo, S., Schesser, K., and Adkins, B.: Yersinia enterocolitica promotes robust mucosal inflammatory T cell immunity in murine neonates. Infect. Immun. 78, 3595-3608 (2010).

Eckburg, P.B., Bik, E.M., Beernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A.: Diversity of the human intestinal microbial flora. Science 308, 1635-1638 (2005).

Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi,

G., Eberl, G., Snel, J., Kelly, D., and Cerf-Bensussan, N.: The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31, 677-689 (2009).

Gern, J.E., Visness, C.M., Gergen, P.J., Wood, R.A., Bloomberg, G.R., O'Connor, G.T., Kattan, M., Sampson, H.A., Witter, F.R., Sandel, M.T., Shreffler, W.G., Wright, R.J., Arbes, S.J. Jr., and Busse, W.W.: The Urban Environment and Childhood Asthma (URECA) birth cohort study: Design, methods, and study population. BMC Pulm. Med. 9, 17 (2009).

Grainger, J.R., Hall, J.A., Bouladoux, N., Oldenhove, G., and Belkaid, Y.: Microbe-dendritic cell dialog controls regulatory T-cell fate. Immunol. Rev. 234, 305-316 (2010).

Kelly, D., Campbell, J.L., King, T.P., Grant, G., Jansson, E.A., Coutts, A.G., Pettersson, S., and Conway, S.: Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat. Immunol. 5, 104-112 (2004).

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., and Gordon, J.I.: Evolution of mammals and their gut microbes. Science 320, 1647-1651 (2008).

Marques, T.M., Wall, R., Ross, R.P., Fitzgerald, G.F., Ryan, C.A., and Stanton, C.: Programming infant gut microbiota: Influence of dietary and environmental factors. Curr. Opin. Biotechnol. 21, 149-156 (2010).

Mirpuri, J., Brazil, J.C., Berardinelli, A.J., Nasr, T.R., Cooper, K., Schnoor, M., Lin, P.W., Parkos, C.A., and Louis, N.A.: Commensal Escherichia coli reduces epithelial apoptosis through IFN-alphaA-mediated induction of guanylate binding protein-1 in human and murine models of developing intestine. J. Immunol. 184, 7186-7195 (2010).

Mosconi, E., Rekima, A., Seitz-Polski, B., Kanda, A., Fleury, S., Tissandie, E., Monteiro, R., Dombrowicz, D.D., Julia, V., Glaichenhaus, N., and Verhasselt, V.: Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. Mucosal. Immunol. 3, 461-474 (2010).

Mulder, I.E., Schmidt, B., Stokes, C.R., Lewis, M., Balley, M., Aminov, R.L., Prosser, J.L., Gill, B.P., Pluske, J.R., Mayer, C.D., Musk, C.C., and Kelly, D.: Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol. 7, 79 (2009).

Ochoa-Reparaz, J., Mielcarz, D.W., Ditrio, L.E., Burroughs, A.R., Foureau, D.M., Haque-Begum, S., and Kasper, L.H.: Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J. Immunol. 183, 6041-6050 (2009).

Ogawa, J., Sasahara, A., Yoshida, T., Sira, M.M., Futatani, T., Kanegane, H., and Myawaki, T.: Role of transforming growth factor-beta in breast milk for initiation of IgA production in newborn infants. Early Hum. Dev. 77, 67-75 (2004).

Ogra, P.L.: Ageing and its possible impact on mucosal immune responses. Ageing Res. Rev. 9, 101-106 (2010).

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J.; MetaHIT Consortium, Bork, P., Ehrlich, S.D., and Wang, J.: A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65 (2010).

Rescigno, M. and Di Sabatino A.: Dendritic cells in intestinal homeostasis and disease.

J. Clin. Invest. 119, 2441-2450 (2009).

Rhee, S.H., Pothoulakis, C., and Mayer, E.A.: Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat. Rev. Gastroenterol. Hepatol. 6, 306-314 (2009).

Rougé, C., Goldenberg, O., Ferraris, L., Berger, B., Rochat, F., Legrand, A., Göbel, U.B., Vodovar, M., Voyer, M., Rozé, J.C., Darmaun, D., Piloquet, H., Butel, M.J., de La Cochetière, M.F.: Investigation of the intestinal microbiota in preterm infants using different methods. Anaerobe 16, 362-370 (2010).

Sheikh, A. and Strachan, D.P.: The hygiene theory: Fact or fiction? Curr. Opin. Otolaryngol. Head Neck Surg. 12, 232-236 (2004).

Sira, M.M., Yoshida, T., Takeuchi, M., Kashiwayama, Y., Futatani, T., Kanegane, H., Sasahara, A., Ito, Y., Mizuguchi, M., Imanaka, T., and Miyawaki, T.: A novel immunoregulatory protein in human colostrum, syntenin-1, for promoting the development of IgA-producing cells from cord blood B cells. Int. Immunol. 21, 1013-1023 (2009).

Sjögren, Y.M., Tomicic, S., Lundberg, A., Böttcher, M.F., Björkstén, B., Scerremark-Ekström, E., and Jenmalm, M.C.: Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin. Exp. Allergy 39, 1842-1851 (2009).

Sonnenburg, J.L., Angenent, L.T., and Gordon, J.I.: Getting a grip on things: How do communities of bacterial symbionts become established in our intestine? Nat. Immunol. 5, 569-573 (2004).

Strachan, D.P.: Hay fever, hygiene, and household size. BMJ 299, 1259-1260 (1989).

Sun, C.M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., and Belkaid, Y.: Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J. Exp. Med. 204, 1775-1785 (2007).

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R., and Gordon, J.I.: A core gut microbiome in obese and lean twins. Nature 457, 480-484 (2009).

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I.: The human microbiome project. Nature 449, 804-810 (2007).

Walker, A.: Breast milk as the gold standard for protective nutrients. J. Pediatr. 156 (Suppl.), S3-S7 (2010).

Wang, G., Miyahara, Y., Guyo, Z., Khattar, M., Stepkowski, S.M., and Chen, W.: "Default" Generation of Neonatal Regulatory T Cells. J. Immunol. 185, 71-78 (2010).

Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.CV., Hu, C., Wing, F.S., Szot, G.L., Bluestone, J.A., Gordon, J.I., and Chervonsky, A.V.: Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455, 1109-1113 (2008).

Wullaert, A.: Role of NF-kappaB activation in intestinal immune homeostasis. Int. J. Med. Microbiol. 300, 49-56 (2010).

Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V., and Gordon, J.I.: A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science 299, 2074-2076 (2003).

Zaghouani, H., Hoeman, C.M., and Adkins, B.: Neonatal immunity: Faulty T-helpers and the shortcomings of dendritic cells. Trends Immunol. 30, 585-591 (2009).

Ziegler, S.F. and Artis, D.: Sensing the outside world: TSLP regulates barrier immunity. Nat. Immunol. 11, 289-293 (2010).

Zoetendal, E.G., Rajilic-Stojanovic, M., and de Vos, W.M.: High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 57, 1605-1615 (2008).