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Microreview

Intestinal microbiota, evolution of the immune system and the bad reputation of pro-inflammatory immunity

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Summary

The mammalian intestine provides a unique niche for a large community of bacterial symbionts that complements the host in digestive and anabolic pathways, as well as in protection from pathogens. Only a few bacterial phyla have adapted to this predominantly anaerobic environment, but hundreds of different species create an ecosystem that affects many facets of the host's physiology. Recent data show how particular symbionts are involved in the maturation of the immune system, in the intestine and beyond, and how dysbiosis, or alteration of that community, can deregulate immunity and lead to immunopathology. The extensive and dynamic interactions between the symbionts and the immune system are key to homeostasis and health, and require all the blends of so-called regulatory and pro-inflammatory immune reactions. Unfortunately, pro-inflammatory immunity leading to the generation of Th17 cells has been mainly associated with its role in immunopathology. Here we discuss the view that the immune system in general, and type 17 immunity in particular, develop to maintain the equilibrium of the host with its symbionts.

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Introduction: the intestinal metaorganism

We live in contact with countless microbes that colonize diverse niches in our house, in public places, in the animals and plants we touch, and in our friends and family members we meet. We also live with very large numbers ($> 10^{14}$) of microbes that have taken residence on and in ourselves, termed symbionts (from the Greek words *syn* and *biosis*, meaning 'with' and 'living'). Whether and how particular symbionts are mutualists that share a beneficial relationship with us, commensals that we simply ignore, or parasites and opportunistic pathogens that cheat on this relationship, is a matter of intense investigation (Backhed *et al*., 2005; Dethlefsen *et al*., 2007). A fast-growing literature shows the broad impact of symbionts on our physiology, modulating predisposition to obesity (Turnbaugh *et al*., 2006; Vijay-Kumar *et al*., 2010), diabetes (Wen *et al*., 2008), colitis (Garrett *et al*., 2007), cancer (Davis and Milner, 2009), skin and mucosal disorders and infection (Brandl *et al*., 2008; Lai *et al*., 2009), and altering behaviour (Gareau *et al*., 2011; Neufeld *et al*., 2011). The now common wisdom yields that understanding our interaction with symbionts will allow their manipulation to our own good, and designing pre- and probiotics that correct failing or pathogenic symbiotic communities (Round and Mazmanian, 2009).

The intestinal niche is of particular interest as it hosts the largest community of bacterial symbionts in mammals, and usual estimates suggest that they outnumber our own cells by a factor of 100 (Backhed *et al*., 2005; Dethlefsen *et al*., 2007). Their abundantly documented role in digestion and generation of metabolites has lead to their description as a 'metaorganism', providing the benefit, and potential danger, of their compound metagenome and metabolome to the host (Turnbaugh and Gordon, 2008). Together, the microbiota and the host form a higher-order entity termed the superorganism (Eberl, 2010), arguably the real target of natural selection, as the host does not exist germfree. Many sequencing-based projects worldwide aim at establishing the catalogue of bacterial species and the metagenome present in normal and sick individuals, and determining the influence of lineage and environment, including food, on these variables

(Turnbaugh and Gordon, 2009; Qin *et al*., 2010). Such studies are believed to lead to the identification of bacterial species, as well as fungal and viral species if included in the analysis, or more globally metaorganisms or metagenomes, that are associated with homeostasis and pathology.

Beyond such correlative studies, causal relationships between particular bacterial communities or species and phenotypes of the host are established by re-colonization of newborn or germfree mice. Using this experimental approach, it has been demonstrated that the microbiota isolated from obese ob/ob mice conferred a significant degree of obesity to lean mice (Turnbaugh *et al*., 2006). In another study, the microbiota from Myd88-deficient mice that lack a number of innate immune pathways, conferred protection to diabetes to genetically disease-prone NOD mice (Wen *et al*., 2008). At the species level, prominent cases include the induction of lymphoid tissue maturation, strong IgA responses and T-cell helper responses by *Candidatus arthromitus*, more commonly called segmented filamentous bacteria (SFB) (Talham *et al*., 1999; Gaboriau-Routhiau *et al*., 2009), and the induction of T regulatory cell responses by *Bacteroides fragilis* (Mazmanian *et al*., 2008) and *Helicobacter pylori* (Kao *et al*., 2010). One major caveat of the latter studies is the measure of the impact of bacterial symbionts in isolation, a situation that never occurs naturally and that may alter the bacteria's own expression programme (Toledo-Arana *et al*., 2009). Combinatorial approaches are eagerly awaited (Goodman *et al*., 2009), as are techniques to specifically ablate a species within complex communities (Mai *et al*., 2010). Microbial ecology is the scientific frame to explore the relationships between bacterial species within the niches defined by the host (Dethlefsen *et al*., 2007), and relies on 'metalevel' combinatorial analytical tools, including mathematics, which need to be urgently developed.

Microbiota and immune system: partners in evolution and homeostasis

Margaret McFall-Ngai, dedicated to the study of the stunning though turbulent mutualistic relationship between the Gram-negative *Vibrio fischeri* and the Hawaiian squid *Euprymna scolopes* (McFall-Ngai *et al*., 2009), was the first to propose that microbial molecular patterns recognized by the innate immune system should be termed accordingly 'microbe-associated molecular patterns' (Koropatnick *et al*., 2004), instead of the more commonly adopted term 'pathogen-associated molecular patterns'. The latter reflects the perception that the immune system is primarily instructed to fight off pathogens, and to 'see' microbes, when it sees them, as pathogens. The inevitable correlate of this view is that microbes that cause disease, thus pathogens, are the prime force for the evolution of the immune system. However, the immune system sees microbes continuously; it reacts massively to intestinal symbionts through the largest routine production of immunoglobulins, delivered into the intestinal lumen as IgA (Macpherson and Uhr, 2004). And, even though notorious pathogens have profoundly marked human history, and continue to do so, we interact mostly with symbionts, on a daily basis, which do not cause significant pathology. McFall-Ngai has proposed, as a consequence, that the evolution of the immune system has been largely shaped by symbionts, and occasionally by pathogens (McFall-Ngai, 2007). As by definition symbionts live with their host, immunity directed against microbes may be therefore largely dedicated to establish a state of homeostasis between host and microbiota. In vertebrates, development of adaptive immunity allows for the recognition of a vast array of microbial antigens and thus, co-evolution of the host with a great diversity of symbionts. Accordingly, the intestine of vertebrates, in particular mammals, is populated with a large diversity of resident bacterial species (Dethlefsen *et al*., 2007), whereas the organs of invertebrates, such as *Drosophila* and *Euprymna*, harbour small number of unstable or 'tourist' symbionts (Ryu *et al*., 2008; McFall-Ngai *et al*., 2009). A notable exception is termites, which harbour a highly diverse intestinal microbiota (Hongoh, 2010), even though the stability of this community remains to be assessed.

Biomedical research is primarily driven by the quest to alleviate and prevent disease. Therefore, and rightly so, much emphasis is put on the mechanisms of pathogenesis when designing projects, experiments, and presenting data. Indeed, the identification of pathogenic cells, pathways and genes holds great promises to satisfy that quest. Nevertheless, and as a consequence, a biased view of the function of particular cells, pathways and genes occasionally develops that obscures the very reason why they have evolved in the first place. One such prominent example is Th17 cells. Even though IL-17 has been cloned nearly two decades ago (Rouvier *et al*., 1993) from activated T cells and shown early on to induce IL-6 and IL-8 production by human fibroblasts (Yao *et al*., 1995), it shot to prominence only 5 years ago once it was shown that IL-23, rather than IL-12, was the critical factor driving experimental autoimmune inflammation of the brain (Cua *et al*., 2003). It was subsequently demonstrated that IL-23 induces the generation of pathogenic IL-17-producing T helper cells (Harrington *et al*., 2005; Langrish *et al*., 2005; Park *et al*., 2005). Since then, many papers and reviews have rounded up the role of Th17 cells in pro-inflammatory pathology, and numerically dwarfed reports on their role in defence against microbial infections at mucosal surfaces (Khader *et al*., 2009). Furthermore, as Th17 cells are enriched in mucosal tissues,

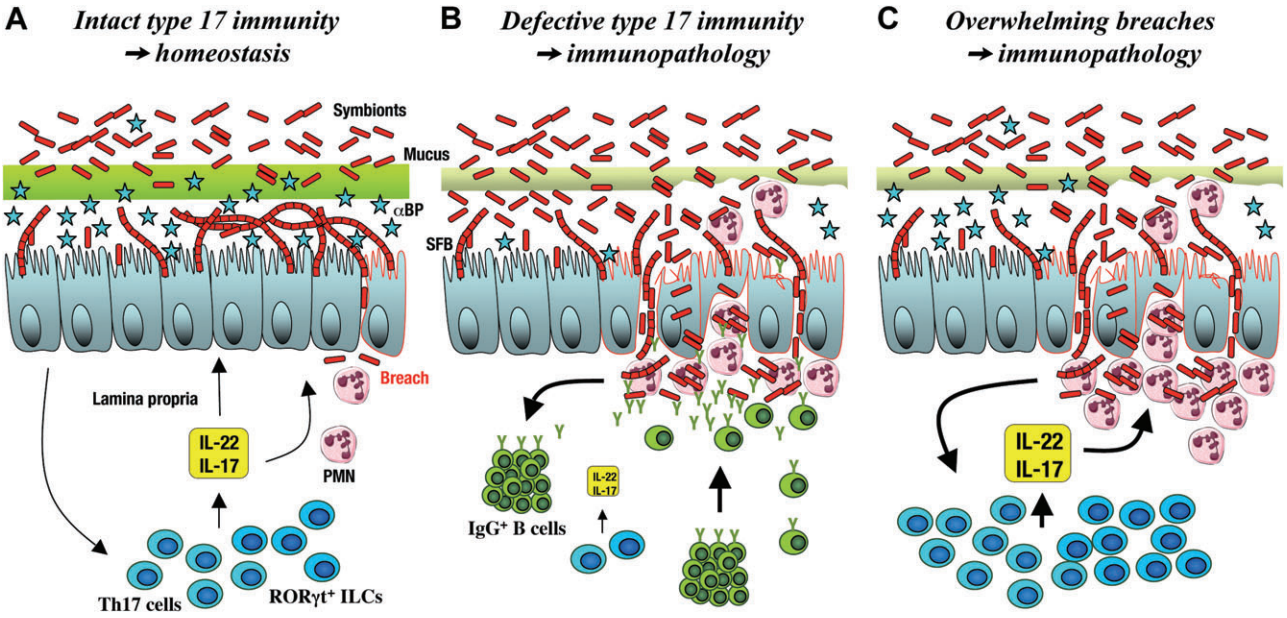

Fig. 1. Type 17 pro-inflammatory immunity, intestinal homeostasis, and immunopathology.
A. An effective barrier separates the intestinal lamina propria from the large community of symbionts. A single layer of epithelial cells creates not only a physical wall, but also produces anti-bacterial peptides (αBP), a function that is enhanced by IL-17 and IL-22 produced by Th17 cells, Tγδ cells and RORγt$^+$ innate lymphoid cells (ILCs). Goblet cells, a specialized type of epithelial cells, secrete mucus, which keeps most of the bacteria at bay from direct contact with the epithelium. Segmented filamentous bacteria (SFB) are symbionts that uniquely locate underneath the mucus and attach directly to the epithelial cells of the small intestine. Occasional breaches may occur in the barrier that leads to low-key bacterial penetration, which are cleared by type 17 immunity and the consequent recruitment of polymorphonuclear neutrophils (PMN). Homeostasis is maintained.
B. In the case of a defective type 17 immunity caused by mutation or immunosuppression, the homeostatic pathway driven by Th17 cells and RORγt$^+$ cells is broken and occasional breaches are dealt with by other means, such as IgG$^+$ B cells. However, the capacity of this alternative pathway to maintain homeostasis is limited and overwhelmed in the case of larger breaches, leading to immunopathology.
C. Intact type 17 immunity promotes a feed-forward loop when it first failed to prevent the amplification of breaches. Large breaches induced by chemicals or pathogens, or a deregluated mucosal immunity, may lead to this immunopathological scenario.

in particular in the intestine, the tempting hypothesis emerged that a specific type of symbiont induces the generation of Th17, and thus, favours pro-inflammatory pathology. The time was ripe for the hunt of this hypothetical pro-inflammatory symbiont, and culminated in the identification of SFB as the prime Th17 inducer (Ivanov *et al*., 2009), as well as the driver of autoimmune arthritis in re-colonized germfree K/BxN mice (Wu *et al*., 2010) and of experimental autoimmune encephalomyelitis in re-colonized and immunized germfree mice (Lee *et al*., 2010). However, even though SFB is an efficient inducer of Th17 cells, it also efficiently induces other types of T helper cells (Gaboriau-Routhiau *et al*., 2009) and the production of IgA (Talham *et al*., 1999). Furthermore, other types of bacteria can induce Th17 cells (Gaboriau-Routhiau *et al*., 2009; Lochner *et al*., 2011a), and the impact of an SFB-less complex microbiota on autoimmune disease progression remains to be assessed.

Thus, even though Th17 cells are associated with pro-inflammatory pathology, we believe that this 'function' is associated with a particular context or genetic susceptibility of the host. As Th17 cells are enriched even in the apparent absence of pathogens in specific pathogen-free mice that harbour a 'normal' complex microbiota (Lochner *et al*., 2008), we have proposed that pro-inflammatory immunity is a necessary component of the immune system to maintain homeostasis of the host with its intestinal metaorganism (Eberl, 2010) (Fig. 1). We are now discussing recent data supporting this hypothesis, and suggesting that inadequate tempering with type 17 immunity may have pathological consequence in the intestine, and possibly beyond.

Fatal consequences of a loss in pro-inflammatory immunity

The immune system shows an extraordinary level of adaptation even when one of its arms is disabled. In the intestine, such adaptive potential is best illustrated in mice partially deficient in innate immunity, which, however, still manage to contain the intestinal microbiota. In the absence of the adaptor molecules Myd88 and TRIF, mice resort to systemic production of IgG targeting the symbionts (Slack *et al*., 2009). Such a response is not normally observed, as bacteria-loaded dendritic cells trafficking from the intestine do not wander beyond the

mesenteric lymph node barrier (Macpherson and Uhr, 2004). However, this compensatory solution is not optimal and has not been selected by evolution to be normally triggered by symbiotic microbiota. There is a price to pay and risks to incur for the host. Indeed, such mice showed a marked increase in susceptibility to colitis and wasting disease when challenged with dextran sodium sulfate (DSS), which is toxic to intestinal epithelial cells (Brandl *et al*., 2010) and models some aspects of human inflammatory bowel disease (IBD). In another example, mice that produce only germline-encoded IgM compensate the lack of IgA in the intestine by massive production of IgM (Fagarasan *et al*., 2002). The supernumerary B cells required for such an increased IgM production are generated in hyperplastic isolated lymphoid follicles (ILFs) of the intestinal lamina propria. Although the microbiota is significantly altered in both qualitative and quantitative terms, these mice do not apparently suffer from bacterial penetration and intestinal pathology. However, the price to pay and risks to incur are increased susceptibility to infection by intestinal pathogens and autoimmunity (Agarwal and Mayer, 2009).

In mice that lack Th17 cells, it is nevertheless expected that the loss of this arm of pro-inflammatory immunity rather protects from IBD. In accordance with this view, the IL-17/IL-23 signalling pathway is involved in a number of chronic inflammatory pathologies, including colitis (Kastelein *et al*., 2007). Most convincingly, a gain of function mutation in the IL-23 receptor predisposes patients to the development of IBD (Duerr *et al*., 2006). Furthermore, Th17 cells have been shown to be required for disease development in an adoptive transfer model of colitis (Leppkes *et al*., 2009), and IL-17R-deficient mice are resistant to trinitrobenzenesulfonic acid-induced colitis, even in the presence of increased levels of IL-12 and IFNγ (Zhang *et al*., 2006). It is therefore suggested that antagonists of IL-17R or of the nuclear hormone receptor RORγt, which is required for the generation of Th17 cells (Ivanov *et al*., 2006), prevent or at least modulate IBD. RORγt is involved in the generation of a number of pro-inflammatory cell types that produce IL-17, such as TCRαβ$^+$ Th17 cells, subsets of TCRγδ$^+$ cells and invariant NKT cells (Michel *et al*., 2008), as well as innate lymphoid cells (ILCs) (Eberl *et al*., 2004). RORγt$^+$ ILCs include lymphoid tissue inducer LTi cells, required for the development of lymph nodes, Peyer's patches and ILFs (Bouskra *et al*., 2008), as well as colitis-inducing Thy-1$^+$ cells (Buonocore *et al*., 2010). Thus, RORγt-deficient mice that lack IL-17$^+$ cells, as well as secondary lymphoid tissues and ILFs, should only poorly sustain inflammatory pathology.

We directly tested this prediction (Lochner *et al*., 2011b). In RORγt-deficient mice, numerous tertiary (ectopic) lymphoid tissues were induced in the colon by the microbiota, and microbiota-specific IgG were detected in the serum. In the absence of type 17 immunity, IFNγ production by Th1 cells was markedly enhanced. Thus, similar to mice deficient in Myd88 and TRIF, RORγt-deficient mice compensated the loss of IL-17 and efficiently contained the intestinal microbiota. What was the price to pay and the risks incurred for this shift in the response to microbiota? Importantly, was the loss of RORγt-controlled type 17 immunity matched by a gain in resistance to inflammatory immunopathology? No. When mice were challenged with DSS, hell was unleashed in the intestine. RORγt-deficient mice suffered from severe colonic tissue destruction and wasting disease. Th1-type of immunity was not involved in the pathology, but the now very large numbers of tertiary lymphoid tissues present in the colon expressed markedly elevated levels of activation-induced deaminase required for the maturation of immunoglobulin genes (Muramatsu *et al*., 2000), and IgG$^+$ B cells were abnormally recruited to the lamina propria. When the generation of these lymphoid tissues was blocked by treatment with soluble decoy receptor for lymphotoxin β (Rennert *et al*., 1998), the pathology was averted. The severity of disease was also markedly reduced by treatment with intravenous immunoglobulin, which saturates Fc receptors (Nimmerjahn and Ravetch, 2008), indicating a central role for B cells and IgG in the inflammatory immunopathology (Brandtzaeg *et al*., 2006) caused by DSS in the absence of type 17 immunity. Accordingly, the severity of disease was also reduced when wild-type cells including Th17 cells were transferred back into RORγt-deficient mice.

The prime cause of the severe pathology induced in RORγt-deficient mice by DSS was penetration by the microbiota: development of disease was completely blocked by treatment with antibiotics (Lochner *et al*., 2011b). Thus, even though during steady state, these mice could compensate their loss of pro-inflammatory immunity through the production of IgG against symbionts, among several mechanisms probably, this strategy was fatal when bacterial penetration was augmented. So, we suggest that type 17 pro-inflammatory immunity is an essential part of the forces that have been selected during evolution to maintain homeostasis with the intestinal metaorganism, best illustrated when symbionts breach the intestinal barrier (Fig. 1). Engagement of type 17 immunity primarily prevents intestinal inflammatory pathology, thus is anti-inflammatory, by restraining breaches by bacteria. Only when the extent of breaches has overcome this barrier does type 17 immunity show its immunopathological effects by inducing an efficient feed-forward loop in an attempt to reverse invasion. As a correlate, therapeutic strategies aimed at modulating inflammatory pathology by suppressing type 17 immunity must be extremely well timed, or may expose the host to

the risks of a weakened mucosal barrier. Thus, these risks should be carefully evaluated and minimized through for example the concomitant use of antibiotics.

Post-scriptum: recent evidence shows that RORγt$^+$ ILCs include a large fraction of the cells producing IL-22 in the intestine (Satoh-Takayama *et al*., 2008; Cella *et al*., 2009; Luci *et al*., 2009; Sanos *et al*., 2009). IL-22, also produced by Th17 cells, plays an important role in epithelial immunity by inducing, like IL-17, the production of anti-bacterial peptides (Liang *et al*., 2006; Aujla *et al*., 2008; Zheng *et al*., 2008) and the expression of anti-apoptotic Bcl-2 family members (Pan *et al*., 2004; Zenewicz *et al*., 2007; 2008). In the absence of IL-22, the susceptibility to DSS-mediated colitis and intestinal pathogens is markedly increased. We also find that RORγt$^+$ ILCs, in contrast to Th17 cells (Ivanov *et al*., 2008), are programmed to develop (Sawa *et al*., 2010) and constitutively express IL-17 and IL-22 (S. Sawa, unpubl. obs.). We suggest that RORγt$^+$ ILCs develop to pre-empt bacterial colonization of the intestine, and therefore play a fundamental role in intestinal homeostasis (Sawa *et al*., 2010).

Conclusion: the continuum theory

We have proposed earlier that the definition of mutualist and pathogenic microbes, and of regulatory and pro-inflammatory immunity, reflects a dual and Manichean view of the world (Eberl, 2010). Instead, we have suggested that the pathogenicity of microbes is defined by the context in which the microbe interacts with its host: mutualists can become pathogens when penetrating the wrong niche, and notorious pathogen can become mutualists when restricted to the right niche. Facing an evasive definition of pathogenicity and a continuum of microbial qualities, the host and its immune system have developed an array, if not a continuum, of types of immune responses that allow establishing a stable coexistence with thousands of different microbes and maintain a profitable homeostasis. In that regard, pro-inflammatory immunity is only one type of immune responses that is used by the host in its interactions with microbes, yet a powerful tool to deny penetration of the host by microbes. Nevertheless, when unleashed during overwhelming injury and infection, the balance between benefits and costs may tip over to immunopathology, arguably the most visible part of the pro-inflammatory iceberg.

References

Agarwal, S., and Mayer, L. (2009) Pathogenesis and treatment of gastrointestinal disease in antibody deficiency syndromes. *J Allergy Clin Immunol* **124:** 658–664.

Aujla, S.J., Chan, Y.R., Zheng, M., Fei, M., Askew, D.J., Pociask, D.A., *et al*. (2008) IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med* **14:** 275–281.

Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. (2005) Host-bacterial mutualism in the human intestine. *Science* **307:** 1915–1920.

Bouskra, D., Brezillon, C., Berard, M., Werts, C., Varona, R., Boneca, I.G., and Eberl, G. (2008) Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456:** 507–510.

Brandl, K., Plitas, G., Mihu, C.N., Ubeda, C., Jia, T., Fleisher, M., *et al*. (2008) Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. *Nature* **455:** 804–807.

Brandl, K., Sun, L., Neppl, C., Siggs, O.M., Le Gall, S.M., Tomisato, W., *et al*. (2010) MyD88 signaling in nonhematopoietic cells protects mice against induced colitis by regulating specific EGF receptor ligands. *Proc Natl Acad Sci USA* **107:** 19967–19972.

Brandtzaeg, P., Carlsen, H.S., and Halstensen, T.S. (2006) The B-cell system in inflammatory bowel disease. *Adv Exp Med Biol* **579:** 149–167.

Buonocore, S., Ahern, P.P., Uhlig, H.H., Ivanov, I.I., Littman, D.R., Maloy, K.J., and Powrie, F. (2010) Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* **464:** 1371–1375.

Cella, M., Fuchs, A., Vermi, W., Facchetti, F., Otero, K., Lennerz, J.K., *et al*. (2009) A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* **457:** 722–725.

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., *et al*. (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421:** 744–748.

Davis, C.D., and Milner, J.A. (2009) Gastrointestinal microflora, food components and colon cancer prevention. *J Nutr Biochem* **20:** 743–752.

Dethlefsen, L., McFall-Ngai, M., and Relman, D.A. (2007) An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* **449:** 811–818.

Duerr, R.H., Taylor, K.D., Brant, S.R., Rioux, J.D., Silverberg, M.S., Daly, M.J., *et al*. (2006) A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* **314:** 1461–1463.

Eberl, G. (2010) A new vision of immunity: homeostasis of the superorganism. *Mucosal Immunol* **3:** 450–460.

Eberl, G., Marmon, S., Sunshine, M.J., Rennert, P.D., Choi, Y., and Littman, D.R. (2004) An essential function for the nuclear receptor RORγt in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol* **5:** 64–73.

Fagarasan, S., Muramatsu, M., Suzuki, K., Nagaoka, H., Hiai, H., and Honjo, T. (2002) Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* **298:** 1424–1427.

Gaboriau-Routhiau, V., Rakotobe, S., Lecuyer, E., Mulder, I., Lan, A., Bridonneau, C., *et al*. (2009) The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31:** 677–689.

Gareau, M.G., Wine, E., Rodrigues, D.M., Cho, J.H., Whary, M.T., Philpott, D.J., *et al*. (2011) Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* **60:** 307–317.

Garrett, W.S., Lord, G.M., Punit, S., Lugo-Villarino, G., Mazmanian, S.K., Ito, S., *et al*. (2007) Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* **131:** 33–45.

Goodman, A.L., McNulty, N.P., Zhao, Y., Leip, D., Mitra, R.D., Lozupone, C.A., *et al*. (2009) Identifying genetic determinants needed to establish a human gut symbiont in its habitat. *Cell Host Microbe* **6:** 279–289.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* **6:** 1123–1132.

Hongoh, Y. (2010) Diversity and genomes of uncultured microbial symbionts in the termite gut. *Biosci Biotechnol Biochem* **74:** 1145–1151.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., *et al*. (2006) The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17^{+} T helper cells. *Cell* **126:** 1121–1133.

Ivanov, I.I., Frutos Rde, L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., *et al*. (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* **4:** 337–349.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., *et al*. (2009) Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* **139:** 485–498.

Kao, J.Y., Zhang, M., Miller, M.J., Mills, J.C., Wang, B., Liu, M., *et al*. (2010) *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. *Gastroenterology* **138:** 1046–1054.

Kastelein, R.A., Hunter, C.A., and Cua, D.J. (2007) Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* **25:** 221–242.

Khader, S.A., Gaffen, S.L., and Kolls, J.K. (2009) Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol* **2:** 403–411.

Koropatnick, T.A., Engle, J.T., Apicella, M.A., Stabb, E.V., Goldman, W.E., and McFall-Ngai, M.J. (2004) Microbial factor-mediated development in a host-bacterial mutualism. *Science* **306:** 1186–1188.

Lai, Y., Di Nardo, A., Nakatsuji, T., Leichtle, A., Yang, Y., Cogen, A.L., *et al*. (2009) Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med* **15:** 1377–1382.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., *et al*. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* **201:** 233–240.

Lee, Y.K., Menezes, J.S., Umesaki, Y., and Mazmanian, S.K. (2010) Microbes and Health Sackler Colloquium: proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* doi 10.1073/pnas.1000082107

Leppkes, M., Becker, C., Ivanov, I.I., Hirth, S., Wirtz, S., Neufert, C., *et al*. (2009) RORgamma-expressing Th17 cells induce murine chronic intestinal inflammation via redundant effects of IL-17A and IL-17F. *Gastroenterology* **136:** 257–267.

Liang, S.C., Tan, X.Y., Luxenberg, D.P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., and Fouser, L.A. (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* **203:** 2271–2279.

Lochner, M., Peduto, L., Cherrier, M., Sawa, S., Langa, F., Varona, R., *et al*. (2008) In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORγt+ T cells. *J Exp Med* **205:** 1381–1393.

Lochner, M., Berard, M., Sawa, S., Hauer, S., Gaboriau-Routhiau, V., Fernandez, T.D., *et al*. (2011a) Restricted microbiota and absence of cognate TCR antigen leads to an unbalanced generation of Th17 cells. *J Immunol* **186:** 1531–1537.

Lochner, M., Ohnmacht, C., Presley, L., Bruhns, P., Si-Tahar, M., Sawa, S., and Eberl, G. (2011b) Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORγt and LTi cells. *J Exp Med* **208:** 125–134.

Luci, C., Reynders, A., Ivanov, I.I., Cognet, C., Chiche, L., Chasson, L., *et al*. (2009) Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. *Nat Immunol* **10:** 75–82.

McFall-Ngai, M. (2007) Adaptive immunity: care for the community. *Nature* **445:** 153.

McFall-Ngai, M., Nyholm, S.V., and Castillo, M.G. (2009) The role of the immune system in the initiation and persistence of the *Euprymna scolopes – vibrio fischeri* symbiosis. *Semin Immunol* **22:** 48–53.

Macpherson, A.J., and Uhr, T. (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* **303:** 1662–1665.

Mai, V., Ukhanova, M., Visone, L., Abuladze, T., and Sulakvelidze, A. (2010) Bacteriophage administration reduces the concentration of listeria monocytogenes in the gastrointestinal tract and its translocation to spleen and liver in experimentally infected mice. *Int J Microbiol* doi 10.1155/2010/624234

Mazmanian, S.K., Round, J.L., and Kasper, D.L. (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* **453:** 620–625.

Michel, M.L., Mendes-da-Cruz, D., Keller, A.C., Lochner, M., Schneider, E., Dy, M., *et al*. (2008) Critical role of RORgammat in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation. *Proc Natl Acad Sci USA* **105:** 19845–19850.

Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. (2000) Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102:** 553–563.

Neufeld, K.M., Kang, N., Bienenstock, J., and Foster, J.A. (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* **23:** 255–e119.

Nimmerjahn, F., and Ravetch, J.V. (2008) Anti-inflammatory actions of intravenous immunoglobulin. *Annu Rev Immunol* **26:** 513–533.

Pan, H., Hong, F., Radaeva, S., and Gao, B. (2004) Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. *Cell Mol Immunol* **1:** 43–49.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., *et al*. (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* **6:** 1133–1141.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., *et al*. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464:** 59–65.

Rennert, P.D., James, D., Mackay, F., Browning, J.L., and Hochman, P.S. (1998) Lymph node genesis is induced by signaling through the lymphotoxin β receptor. *Immunity* **9:** 71–79.

Round, J.L., and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* **9:** 313–323.

Rouvier, E., Luciani, M.F., Mattei, M.G., Denizot, F., and Golstein, P. (1993) CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. *J Immunol* **150:** 5445–5456.

Ryu, J.H., Kim, S.H., Lee, H.Y., Bai, J.Y., Nam, Y.D., Bae, J.W., *et al*. (2008) Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. *Science* **319:** 777–782.

Sanos, S.L., Bui, V.L., Mortha, A., Oberle, K., Heners, C., Johner, C., and Diefenbach, A. (2009) RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat Immunol* **10:** 83–91.

Satoh-Takayama, N., Vosshenrich, C.A., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., *et al*. (2008) Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* **29:** 958–970.

Sawa, S., Cherrier, M., Lochner, M., Satoh-Takayama, N., Fehling, H.J., Langa, F., *et al*. (2010) Lineage relationship analysis of RORγt+ innate lymphoid cells. *Science* **330:** 665–669.

Slack, E., Hapfelmeier, S., Stecher, B., Velykoredko, Y., Stoel, M., Lawson, M.A., *et al*. (2009) Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325:** 617–620.

Talham, G.L., Jiang, H.Q., Bos, N.A., and Cebra, J.J. (1999) Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* **67:** 1992–2000.

Toledo-Arana, A., Dussurget, O., Nikitas, G., Sesto, N., Guet-Revillet, H., Balestrino, D., *et al*. (2009) The Listeria transcriptional landscape from saprophytism to virulence. *Nature* **459:** 950–956.

Turnbaugh, P.J., and Gordon, J.I. (2008) An invitation to the marriage of metagenomics and metabolomics. *Cell* **134:** 708–713.

Turnbaugh, P.J., and Gordon, J.I. (2009) The core gut microbiome, energy balance, and obesity. *J Physiol* **587:** 4153–4158.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444:** 1027–1031.

Vijay-Kumar, M., Aitken, J.D., Carvalho, F.A., Cullender, T.C., Mwangi, S., Srinivasan, S., *et al*. (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* **328:** 228–231.

Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., *et al*. (2008) Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* **455:** 1109–1113.

Wu, H.J., Ivanov, I.I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., *et al*. (2010) Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* **32:** 815–827.

Yao, Z., Painter, S.L., Fanslow, W.C., Ulrich, D., Macduff, B.M., Spriggs, M.K., and Armitage, R.J. (1995) Human IL-17: a novel cytokine derived from T cells. *J Immunol* **155:** 5483–5486.

Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Karow, M., and Flavell, R.A. (2007) Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* **27:** 647–659.

Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Stevens, S., and Flavell, R.A. (2008) Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* **29:** 947–957.

Zhang, Z., Zheng, M., Bindas, J., Schwarzenberger, P., and Kolls, J.K. (2006) Critical role of IL-17 receptor signaling in acute TNBS-induced colitis. *Inflamm Bowel Dis* **12:** 382–388.

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., *et al*. (2008) Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* **14:** 282–289.