

Life at the beginning: perturbation of the microbiota by antibiotics in early life and its role in health and disease

Sebastian Zeissig & Richard S Blumberg

This Commentary discusses how treatment with antibiotics in infancy shapes host immunity and influences susceptibility later in life to diseases mediated by the immune system.

“Sow a thought, and you reap an act;
Sow an act, and you reap a habit;
Sow a habit, and you reap a character;
Sow a character, and you reap a destiny.”
—Anonymous (quoted by Samuel Smiles
in *Life and Labor*, 1887)

The advent of commercial antibiotics therapy in the early twentieth century revolutionized the medical treatment of infectious diseases. Antibiotics, together with other measures such as vaccination and the broad introduction of hygienic practices, have since led to a steady decrease in the morbidity and mortality associated with infectious diseases and, remarkably, still reflect an exception to current pharmacological treatment in that they allow the cure of established disease¹. Such advances in the treatment of infectious diseases have fueled a pathogen-centric view of microbes and the emergence of further efforts to minimize contact with the microbial world. As a result, society has become overly obsessed with obliterating every microbe. However, this view has now been challenged by a wealth of studies documenting an unforeseen complexity of the human-associated microbiota that coexists and has coevolved in a mutualistic relationship with its human host and contrib-

utes to host biology and health through the provision of resistance to colonization, by harmful organisms, participation in metabolism and maturation of the immune system, among other pathways^{2,3}. Increasing knowledge of such symbiotic traits thereby continues to shift the view of microbes from a pathogen-dominated perspective to an integrated view that acknowledges the beneficial aspects of the microbiota.

This is not a new discussion but a continuous one that dates its origins to 150 years ago during the earliest days of the scientific investigation of immunity, as espoused by Pasteur, Koch, Mechnikov, Ehrlich and others, who actively considered both the beneficial and the detrimental aspects of microbes. In the most recent iteration of this dialectic through the lens of current observations, it has become increasingly clear that although less exposure to the microbiota as a consequence of changes in lifestyle (hygiene) and medical treatment (antibiotics) is associated with improved control of infectious diseases, it may be an important contributor to the observed rise in a variety of complex diseases mediated by the immune system (‘immune-mediated’ diseases) as well as metabolic disorders and, potentially, neoplastic diseases^{1,4–6}. This may be especially consequential during early life, when the relationship between commensalism and the host’s immune system is just being established^{7–9}. In this Commentary, we discuss how treatment with antibiotics in early life affects the balance between health and disease later in life through persistent effects on the human commensal microbiota.

Dynamics of microbial colonization

Upon delivery, neonates leave a sterile environment and are rapidly exposed to and colonized by so-called ‘pioneer’ organisms whose composition is determined by the type of delivery^{7–9}. Thus, vaginal delivery is associated with neonatal colonization by microorganisms dominant in the vaginal tract and feces of the mother, while delivery by Cesarean section leads to a different set of pioneer organisms acquired mainly from microbial communities that colonize the skin of caregivers^{7–9}. The latter circumstance is associated with a divergent microbial and, presumably, immunological starting point for the host at the earliest days of life, with potential implications for susceptibility later in life to immune-mediated diseases. Following initial colonization, the subsequent development of the host-associated microbiota is characterized by a considerable degree of dynamic variation in its composition, especially during the initial years of life^{7–10} (Fig. 1). The ensuing trajectory toward an increase in the richness and diversity of the colonizing (commensal) microbiota is strongly influenced by environmental factors and ultimately converges on a unique adult-associated microbial configuration by around 3 years of age^{7–10}. The development of microbial commensalism in each person is tightly linked to maturation of the host’s immune system. At the time of birth, the host’s immune system is immature. Colonization with nonpathogenic microorganisms serves an important role in establishing and molding immunological competence or, potentially, incompetence and dysfunction (Fig. 1)¹¹. Thus, the ‘choreography’ of the acquisition of microbes that colonize the

Sebastian Zeissig is in the Department of Internal Medicine, University Medical Center Schleswig-Holstein, Kiel, Germany. Richard S. Blumberg is in the Division of Gastroenterology, Hepatology, and Endoscopy, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA.
e-mail: rblumberg@partners.org

host, as well as their composition, no doubt has major consequences for the function of the host's immune system. This is especially true during early postnatal life, which represents a uniquely vulnerable period during which the host is susceptible to environmental influences on microbial colonization, the consequences of which may reverberate in the host's life through durable epigenetic effects¹². In line with such considerations, perturbations of the commensal microbiota by antibiotics during infancy have been linked to the development later in life of many complex immune-mediated diseases, as discussed below.

Microbial perturbation by antibiotics

Antibiotics continue to be among the most frequently prescribed medications^{13,14}. Children represent the age group most commonly administered antibiotics, with an estimated 50% of prescriptions attributed to inappropriate use^{14,15}. Not surprisingly, the rate of prescriptions for antibiotics correlates with resistance to antibiotics, which reflects an increasingly relevant medical problem¹³. In addition, antibiotics are ubiquitously present in the food supply, which further intensifies these problems¹⁶.

Only recently has there been a focus on understanding the consequences of the administration of antibiotics on the structure and, implicitly, the function of the commensal microbial community. Profiling of 16S rRNA has revealed that such treatment is associated with broad and profound changes in the composition of the commensal intestinal microbiota. Such perturbations are largely transient and, for most communities, are followed by recovery to the pretreatment state^{17–20}. However, persistent alterations in microbial composition have been documented for a wide range of antibiotics, with each cycle of antibiotic treatment potentially introducing novel and stable changes in microbial composition (Fig. 1)^{17–20}. Although there has been little study of the immunological consequences of the administration of antibiotics in the general population, evidence exists that antibiotics-induced changes in microbe composition are associated with immunological alterations in the mucosa⁶. Thus, despite the well-documented resilience of the commensal microbiota, treatment with antibiotics is associated with persistent changes in microbial composition and with potential long-term consequences for host immunity (Fig. 1).

Antibiotics and the hygiene hypothesis

In 1989, Strachan reported an inverse relationship between household size and the

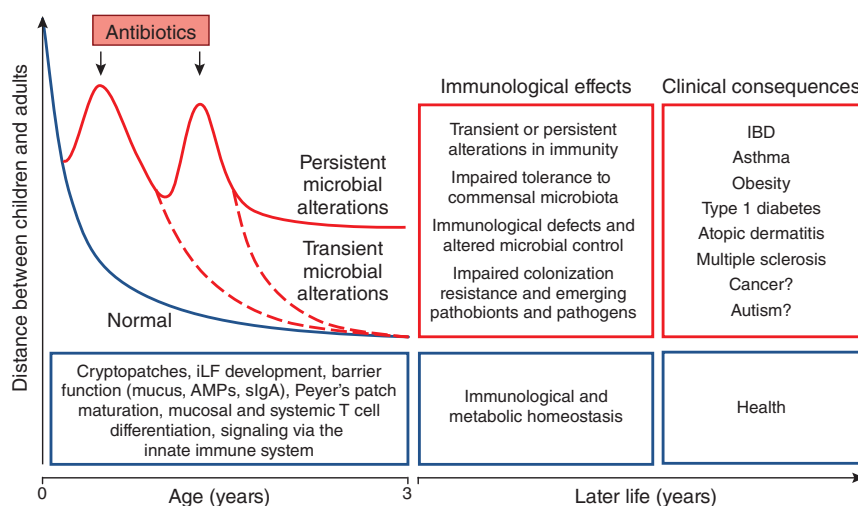


Figure 1 Microbial colonization, development of the immune system and their perturbation by treatment with antibiotics early in life. Microbial colonization during early postnatal development represents a dynamic process, which evolves toward an adult-like configuration within 3 years after birth. Colonization by commensals is associated with and required for the maturation of host immunity (blue boxes, bottom); this leads to immunological and metabolic homeostasis of the host. The influences of microbes on specific pathways of host immunity are often confined to windows of opportunity, with a particular role for specific microbes whose effects may be persistent and durable in their presence or absence during neonatal life. Treatment with antibiotics is associated with alterations in the host-associated microbiota that, even if transient, may lead to persistent alterations in immunological function if they occur during such windows of opportunity. Consequently, such events may regulate susceptibility later in life to atopic, immune-mediated, metabolic and potentially neoplastic diseases, as well as neuropsychiatric disorders. Durable epigenetic changes probably contribute to persistent effects of microbial regulation of host immunity. The proposal of strategies to reverse epigenetic changes, well illustrated by the ability to convert differentiated cells into pluripotent stem cells, thereby suggests that the therapeutic manipulation of epigenetic modifications may provide a suitable approach for restoring immunological defects acquired as a consequence of missed opportunities for host-microbe interactions. Distance of microbial communities between children and adults (vertical axis) is reported according to ref. 10. AMPs, antimicrobial peptides; iLF, isolated lymphoid follicle; slgA, secretory IgA.

prevalence of hay fever (allergic rhinitis) and eczema⁵. He suggested that the increasing incidence of atopic diseases may result from reduced opportunities for infection⁵. This theory, later termed the 'hygiene hypothesis', gained considerable attention and has since served as a possible mechanistic explanation for the rise in the incidence of atopic disease such as allergic rhinitis, atopic dermatitis and asthma¹. In addition, more than two decades before the publication of Strachan's work, similar correlations between hygiene and disease were made for multiple sclerosis and were later extended to other so-called autoimmune or immune-mediated diseases, including type I diabetes and inflammatory bowel disease (IBD)¹. The hygiene hypothesis has since been supported by various additional lines of epidemiological evidence, including, most notably, the observation of inverse trends between the incidence of infectious and immune-mediated disorders, as well as opposite patterns in their geographical distribution¹. Intriguingly, similar concepts seem to apply to antibiotics-mediated interference with the commensal microbiota.

Thus, many studies have demonstrated that the use of antibiotics, particularly during infancy and childhood, correlates with the occurrence of asthma, atopic dermatitis, multiple sclerosis and IBD (Fig. 1)^{21–24}. This suggests that alterations in the human-associated microbiota as a consequence of changes in lifestyle and antibiotics use may influence susceptibility to immune-mediated diseases.

Antibiotics and immune-mediated diseases

The human-associated microbiota not only provides resistance to colonization with invading pathogens but also is critically involved in the postnatal maturation of mucosal and systemic immunity^{3,11}. Perturbation of the commensal microbiota due to changes in hygiene or treatment with antibiotics may affect those processes and lead to persistent alterations in host immunity and an increased occurrence of immune-mediated diseases. In keeping with such findings, mice raised in a germ-free environment exhibit hallmarks of atopy, including a T helper type 2 signature, airway eosinophilia and increased produc-

tion of immunoglobulin E (IgE) upon antigen challenge²⁵. While immunotolerance can be restored in germ-free mice upon colonization of the intestinal tract with microbes, such restoration requires exposure to microbes during the neonatal period and is considerably less effective when performed in adult mice²⁵. Furthermore, early and persistent effects of the microbiota on host immunity and susceptibility to immune-mediated diseases are not specific to atopic diseases only. Thus, exposure of neonatal female mice of the nonobese diabetic strain to the intestinal flora of male mice protects them from islet inflammation and type 1 diabetes in a testosterone-dependent manner²⁶. Moreover, microbial colonization of neonatal germ-free mice is associated with reduced recruitment of invariant natural killer T cells (iNKT cells) to the mucosa and proliferation of these cells, which protects the mice from intestinal inflammation then they reach adulthood. Such protective effects are not observed when adult germ-free mice are colonized with commensal microbes and are epigenetically determined^{12,27}.

Notably, treatment of mice with antibiotics, particularly during early postnatal development, elicits alterations to the immune system similar to those observed in germ-free mice, including increased production of interleukin 4 and IgE^{28–30}, diminished numbers of intestinal regulatory T cells (T_{reg} cells)³¹, increased infiltration of the colon with iNKT cells^{12,27} and susceptibility later in life to allergen-induced airway hyper-reactivity²⁹ and colitis¹². Such observations suggest the existence of limited windows of opportunity for microbiota-dependent regulation of host immunity and further indicate that interference with this process through treatment with antibiotics may alter susceptibility to immune-mediated diseases. Consistent with the concept of such windows of opportunity, exposure to the microbiota in adulthood may not compensate for opportunities missed early in life but may in fact promote inflammation rather than tolerance. This is reflected in the observation that while exposure to microbial elements early in life provides the host with durable protection from subsequent development of immune-mediated disorders, adult animal models of immune-mediated diseases are often dependent on the presence of the microbiota, and infections exacerbate established disease or function as risk factors for the development of disease in humans.

As an extension of the hygiene hypothesis, concepts such as 'old friends' and the 'disappearing microbiota' have been suggested to support an alternative mechanism: the 'microbe' hypothesis. They propose that not increased hygiene itself but instead reduced

exposure to particular microorganisms that have coevolved with humans, but are gradually disappearing due to eradication by antibiotics (or potentially vaccines), would be responsible for the increased incidence of immune-mediated diseases^{4,32}. In line with this concept, the occurrence of immune-mediated diseases is indirectly related to exposure to particular bacteria (for example, *Helicobacter pylori*), bacterial constituents (for example, lipopolysaccharide), viruses (for example, hepatitis A virus), fungi (for example, eurotium taxon) and parasites (for example, *Toxoplasma gondii*)^{4,33–35}. While such correlations do not prove causality, published evidence suggests that some such bacteria and viruses do regulate susceptibility to immune-mediated diseases. Thus, exposure of mice to *H. pylori* or to influenza A virus, particularly during the neonatal period, protects them from airway hyper-reactivity through population expansion of T_{reg} cells and a subset of iNKT cells^{36,37}. In intestinal immunity, colonization of neonatal but not adult mice with *Bacteroides fragilis*, an abundant commensal in the intestinal ecosystem, protects them from intestinal inflammation in adult life²⁷. Intriguingly, not only particular bacterial species but also single molecular constituents derived from those bacteria are sufficient to 'program' host immunity and regulate susceptibility to immune-mediated diseases. The administration of individual polysaccharides (such as polysaccharide A, PSA) or sphingolipids (such as Bf717) derived from *B. fragilis* is associated with persistent effects on host immunity and protection in mouse models of IBD and multiple sclerosis^{27,38,39}. Similarly, protection from intestinal inflammation and predisposition to atopic disorders observed after the administration of *Clostridium* strains to neonatal mice is mediated by short-chain fatty acids, which are metabolic products of those bacterial strains and act through peripheral induction of T_{reg} cells^{31,40,41}. Thus, persistent loss of dominant microbial regulators of host immunity after treatment with antibiotics may provide an explanation for the occurrence of atopic and immune-mediated diseases.

Alternative explanations to the hygiene hypothesis have been proposed. It is thus conceivable that the presence of particular microorganisms during infection, rather than treatment with antibiotics itself, would be the basis for a correlation between antibiotic use and immune-mediated diseases. This would be in line with the association of recurrent infections with asthma or IBD and with the observation of primary immunodeficiency in a subset of patients with immune-mediated diseases, which may result in predisposition to recurrent infections. Along similar lines, knockout mice

with deletion of the genes encoding Toll-like receptor 5, the transcription factor T-bet or the pattern-recognition receptor NLRP6 exhibit alterations in the intestinal microbiota that are transmissible to wild-type mice and that increase susceptibility to immune-mediated diseases³. This suggests that genetic alterations in the host, and particularly those associated with immunodeficiency and susceptibility to environmental incursions, can regulate predisposition to immune-mediated diseases in a manner dependent on alterations in the commensal microbiota. Chronic inflammatory diseases such as IBD may thus result from aberrant immunological responses to an altered commensal microbiota induced by both host genetic factors and environmental influences (including infections with enteropathogens). In addition, the inflammation associated with IBD may induce further alterations in the composition of the microbiota, including the enhanced presence of bacteria such as Proteobacteria that are normally a minor population in the intestines and may be proinflammatory. Under these conditions, pathogenic microbes may gain a foothold in disease through the induction of inflammation or modulation of the secretion of antimicrobial peptides, which creates niches for those pathogens and other phyla that function as 'inflammatory allies', such as Proteobacteria⁶. Treatment with antibiotics may further support the generation of such niches, either as a direct consequence of impaired resistance to colonization, as in the antibiotics-associated diarrhea linked to infection with *Clostridium difficile*, or indirectly, through effects on the secretion of antimicrobial peptides, as noted for vancomycin-resistant *Enterococcus*⁴². Thus, pathogens, antibiotics and inflammation itself all contribute to dysbiosis and the pathogenesis of chronic, immune-mediated diseases.

Metabolic diseases and the commensal microbiota

The metabolic pathways of the human host and its microbiota are tightly linked, which has probably provided strong selective forces for their coevolution^{2,3}. Intestinal microorganisms utilize ingested or host-derived carbohydrates as their principal energy source and thereby contribute to the extraction of energy from food². Such fermentation processes generate metabolic products with beneficial roles in host biology, such as short-chain fatty acids². The central role of the intestinal microbiota in host metabolism is reflected in the observation that germ-free rodents exhibit increased excretion of caloric material in the feces, a compensatory increase in food intake and resistance to diet-induced obesity². Furthermore, it is likely that many of the metabolic effects on germ-free animals are

associated with the immunological abnormalities of such states, including the consequences of impaired production of short-chain fatty acids for T_{reg} cells and of reduced production of ATP for the T_H17 subset of helper T cells^{31,40,41,43}. In addition, in humans and mice, not only is obesity itself associated with alterations in the microbiota, but also the obesity-associated microbiota actively contributes to host metabolic dysfunction, dysregulation of the immune system and inflammation^{44,45}. Such observations suggest that perturbations of the intestinal microbiota after treatment with antibiotics may affect host and microbial metabolism and, through those effects, may affect susceptibility to immune-mediated diseases. Consistent with that, treatment of mice with antibiotics is associated with changes in the composition of the intestinal microbiota, altered hepatic lipid metabolism and adiposity, which is most pronounced when such treatment is started early in life¹⁶. Moreover, while published epidemiological studies have shown that exposure of infants to antibiotics is associated with obesity later in life^{46,47}. Such results demonstrate that treatment with antibiotics affects host metabolism through modulation of the intestinal microbiota and may contribute to the pathogenesis of prevalent diseases at the interface between metabolism and immunity, including obesity, fatty liver disease, type 2 diabetes and coronary artery disease.

Conclusions and perspectives

Antibiotics have revolutionized the treatment of infectious diseases. However, both their appropriate and their inappropriate use are associated with potentially deleterious consequences. The use of antibiotics has thus contributed not only to the rise in resistance to antibiotics and the emergence of vancomycin-resistant *Enterococcus* and new biotypes of *C. difficile* but also to the increasing incidence of atopic and chronic inflammatory disorders. Many of these unintended consequences derive in particular from the problematic use of antibiotics in early life, when the host's newly acquired microbiota and immune system are just learning to live with one another. In addition to greater deliberation about the use of antibiotics in early life, future research is needed at many levels. This includes gaining better understanding of microbial community development at all mucosal surfaces in early life and how that is associated with normal devel-

opment of the immune system and 'legacy' effects on the function of the immune system later in life. Similarly, more attention needs to be paid to monitoring the structure and function of the microbial community before and after the administration of antibiotics and the correlation of that with the function of the immune system and metabolic function, as well as clinical outcomes. While rodent models have provided critical insight into the mechanisms of host-microbe interactions, additional research is needed to confirm and extend those observations to humans as a first step toward the development of clinical concepts that apply such knowledge to strategies to maintain or restore homeostasis of the immune system. In this sense, 'biobanking' of fecal materials before the administration of antibiotics may be considered as a strategy for allowing the retrieval of beneficial microbial species for later autotransplantation. In addition, therapeutic interference with specific molecular pathways that provide the basis for persistent effects of the microbiota on host immunity, such as epigenetic modifications, may allow the reversal of immunological defects in adolescents and adults that are acquired as a consequence of altered host-microbe interactions during infancy. Finally, the finding of a critical role for the commensal microbiota in malignant diseases raises the question of whether changes in the microbiota, possibly as a consequence of treatment with antibiotics in infancy, may set the stage for oncological disorders that occur many decades after those initial events. Better understanding of the mechanisms of host-microbe mutualism and strategies to prevent its perturbation may thus provide pathways to longevity and healthy aging.

ACKNOWLEDGMENTS

Supported by the US National Institutes of Health (DK044319, DK051362, DK053056 and DK088199 to R.S.B.), the Harvard Digestive Diseases Center (DK0034854 to R.S.B.), the European Research Council (Starting Grant Agreement 336528 to S.Z.), Deutsche Forschungsgemeinschaft (ZE814/4-1, ZE814/5-1 and ZE814/6-1, and the Excellence Cluster 'Inflammation at Interfaces', to S.Z.) and the European Commission (Marie Curie International Reintegration Grant 256363 to S.Z.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Bach, J.F. *N. Engl. J. Med.* **347**, 911–920 (2002).
2. Tremaroli, V. & Backhed, F. *Nature* **489**, 242–249 (2012).

3. Hooper, L.V., Littman, D.R. & Macpherson, A.J. *Science* **336**, 1268–1273 (2012).
4. Blaser, M.J. & Falkow, S. *Nat. Rev. Microbiol.* **7**, 887–894 (2009).
5. Strachan, D.P. *Br. Med. J.* **299**, 1259–1260 (1989).
6. Willing, B.P., Russell, S.L. & Finlay, B.B. *Nat. Rev. Microbiol.* **9**, 233–243 (2011).
7. Dominguez-Bello, M.G. *et al. Proc. Natl. Acad. Sci. USA* **107**, 11971–11975 (2010).
8. Koenig, J.E. *et al. Proc. Natl. Acad. Sci. USA* **108** Suppl 1, 4578–4585 (2011).
9. Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A. & Brown, P.O. *PLoS Biol.* **5**, e177 (2007).
10. Yatsunenkov, T. *et al. Nature* **486**, 222–227 (2012).
11. Renz, H., Brandtzaeg, P. & Hornef, M. *Nat. Rev. Immunol.* **12**, 9–23 (2012).
12. Olszak, T. *et al. Science* **336**, 489–493 (2012).
13. Goossens, H., Ferech, M., Vander Stichele, R., Elseviers, M. & Group, E.P. *Lancet* **365**, 579–587 (2005).
14. Hicks, L.A., Taylor, T.H. Jr. & Hunkler, R.J. *U.S. N. Engl. J. Med.* **368**, 1461–1462 (2013).
15. McCaig, L.F., Besser, R.E. & Hughes, J.M. *J. Am. Med. Assoc.* **287**, 3096–3102 (2002).
16. Cho, I. *et al. Nature* **488**, 621–626 (2012).
17. Dethlefsen, L., Huse, S., Sogin, M.L. & Relman, D.A. *PLoS Biol.* **6**, e280 (2008).
18. Jakobsson, H.E. *et al. PLoS ONE* **5**, e9836 (2010).
19. Dethlefsen, L. & Relman, D.A. *Proc. Natl. Acad. Sci. USA* **108** Suppl 1, 4554–4561 (2011).
20. Antonopoulos, D.A. *et al. Infect. Immun.* **77**, 2367–2375 (2009).
21. Ng, S.C. *et al. Gut* **62**, 630–649 (2013).
22. Murk, W., Risnes, K.R. & Bracken, M.B. *Pediatrics* **127**, 1125–1138 (2011).
23. Nørgaard, M. *et al. Am. J. Epidemiol.* **174**, 945–948 (2011).
24. Flohr, C., Pascoe, D. & Williams, H.C. *Br. J. Dermatol.* **152**, 202–216 (2005).
25. Sudo, N. *et al. J. Immunol.* **159**, 1739–1745 (1997).
26. Markle, J.G. *et al. Science* **339**, 1084–1088 (2013).
27. An, D. *et al. Cell* **156**, 123–133 (2014).
28. Oyama, N., Sudo, N., Sogawa, H. & Kubo, C. *J. Allergy Clin. Immunol.* **107**, 153–159 (2001).
29. Russell, S.L. *et al. EMBO Rep.* **13**, 440–447 (2012).
30. Noverr, M.C., Falkowski, N.R., McDonald, R.A., McKenzie, A.N. & Huffnagle, G.B. *Infect. Immun.* **73**, 30–38 (2005).
31. Atarashi, K. *et al. Science* **331**, 337–341 (2011).
32. Rook, G.A. *Proc. Natl. Acad. Sci. USA* **110**, 18360–18367 (2013).
33. Braun-Fahrlander, C. *et al. N. Engl. J. Med.* **347**, 869–877 (2002).
34. Ege, M.J. *et al. N. Engl. J. Med.* **364**, 701–709 (2011).
35. Matricardi, P.M. *et al. Br. Med. J.* **320**, 412–417 (2000).
36. Arnold, I.C. *et al. J. Clin. Invest.* **121**, 3088–3093 (2011).
37. Chang, Y.J. *et al. J. Clin. Invest.* **121**, 57–69 (2011).
38. Ochoa-Repáraz, J. *et al. Mucosal Immunol.* **3**, 487–495 (2010).
39. Mazmanian, S.K., Round, J.L. & Kasper, D.L. *Nature* **453**, 620–625 (2008).
40. Furusawa, Y. *et al. Nature* **504**, 446–450 (2013).
41. Arpaia, N. *et al. Nature* **504**, 451–455 (2013).
42. Brandl, K. *et al. Nature* **455**, 804–807 (2008).
43. Atarashi, K. *et al. Nature* **455**, 808–812 (2008).
44. Turnbaugh, P.J. *et al. Nature* **444**, 1027–1031 (2006).
45. Devkota, S. *et al. Nature* **487**, 104–108 (2012).
46. Ajslev, T.A., Andersen, C.S., Gamborg, M., Sorensen, T.I. & Jess, T. *Int. J. Obes.* **35**, 522–529 (2011).
47. Trasande, L. *et al. Int. J. Obes.* **37**, 16–23 (2013).