lack of α-TTP specifically affects the bone resorption axis of bone remodeling.

Fujita *et al.*[11] then showed that dietary α-tocopherol supplementation rescued the abnormal bone phenotype in the *Ttpa*$^{-/-}$ mice. Furthermore, osteoclast formation in bone marrow cell cultures from wild-type mice was inhibited by sera from *Ttpa*$^{-/-}$ mice. In contrast, osteoclast precursors from *Ttpa*$^{-/-}$ mice formed normal osteoclasts when cultured in wild-type sera. Addition of α-tocopherol to RANK ligand–treated bone marrow cultures increased osteoclast formation, osteoclast size and the number of nuclei per osteoclast. Notably, α-tocopherol affected only osteoclast formation during the maturation phase and did not affect the proliferative phase of osteoclast development. α-tocopherol also induced foreign-body giant cell formation by macrophages, demonstrating that it can induce macrophage fusion as well as osteoclast fusion. Interestingly, of all the vitamin E isoforms the authors tested, only α-tocopherol stimulated osteoclast precursor fusion. Other antioxidants had no effect on osteoclast fusion. These results suggest that the effects of α-tocopherol on osteoclast fusion are independent of its antioxidant effects.

The authors then analyzed osteoclast differentiation markers after α-tocopherol treatment and found that α-tocopherol increased the expression of a key molecule involved in osteoclast fusion, dendritic cell–specific transmembrane protein (DC-STAMP)[12], in osteoclast precursors[11]. In addition, DC-STAMP expression was decreased in *Ttpa*$^{-/-}$ mice. Using gain- and loss-of-function experiments for DC-STAMP, Fujita *et al.*[11] confirmed that this protein was responsible for mediating the effects of vitamin E on osteoclast precursor fusion. They also defined a pathway through which vitamin E increases DC-STAMP expression via p38 mitogen-activated protein kinase signaling to activate the transcriptional regulator microphthalmia-associated transcription factor, which binds the promoter of the gene encoding DC-STAMP. Finally, the authors found that rats or mice fed α-tocopherol for eight weeks at doses that are present in the vitamin E supplements used by many people showed a 20% decrease in bone mass and had increased bone resorption and osteoclast size[11]. Together, these results show that the major effect of vitamin E on bone remodeling is to enhance osteoclast size, which results in increased bone resorption per osteoclast (**Fig. 1**).

The results of the study by Fujita *et al.*[11] raise several important questions. Should people, especially those at risk for osteoporosis, continue to take vitamin E supplements based on these current results? Will the benefits of vitamin E on cardiovascular health outweigh the risks for bone metabolism that the authors associated with high vitamin E intake? Given the previous studies that have suggested that vitamin E can influence bone formation and bone resorption, only carefully conducted clinical trials in humans will be able to resolve these questions.

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1. Ahmadieh, H. & Arabi, A. *Nutr. Rev.* **69**, 584–598 (2011).
2. Gao, X., Wilde, P.E., Lichtenstein, A.H., Bermudez, O.I. & Tucker, K.L. *J. Nutr.* **136**, 1021–1026 (2006).
3. Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids* (National Academy Press, Washington, DC, 2000).
4. Sandhu, S.K. & Hampson, G. *J. Clin. Pathol.* **64**, 1042–1050 (2011).
5. Lee, J.H. *et al. J. Biol. Chem.* **284**, 13725–13734 (2009).
6. Soeta, S., Higuchi, M., Yoshimura, I., Itoh, R., Kimura, N. & Aamsaki, H. *J. Vet. Med. Sci.* **72**, 951–957 (2010).
7. Maniam, S., Mohamed, N., Shuid, A.N. & Soelaiman, I.N. *Clin. Pharmacol. Toxicol.* **103**, 55–60 (2008).
8. Pasco, J.A. *et al. J. Womens Health (Larchmt)* **15**, 295–300 (2006).
9. Zhang, J., Munger, R., West, N.A., Cutler, R.D. & Wengreen, J.H. *Am. J. Epidemiol.* **163**, 9–17 (2006).
10. Hamidi, M.S., Corey, P.N. & Cheung, A.M. *J. Bone Miner. Res.* published online, doi:10.1002/jbmr.1566 (2012).
11. Fujita, K. *et al. Nat. Med.* **18**, 589–594 (2012).
12. Yagi, M., Miyamoto, T., Toyama, Y. & Suda, T. *J. Bone Miner. Metab.* **24**, 355–358 (2006).

Breathe easy: microbes protect from allergies

Arya Khosravi & Sarkis K Mazmanian

Changes in gut microbial composition have been linked to inflammatory bowel disease, obesity and allergies in humans. A new study shows that pattern recognition of commensal bacteria by B cells reduces allergic inflammation in mice, adding to the mounting evidence for the 'hygiene hypothesis' (pages 538–546).

Twenty-three years ago, a study consisting of a single figure and insightful speculation redefined our relationship with microbial organisms[1]. Over a century earlier, Koch and Pasteur validated the germ theory of disease, effectively branding microbes as agents of infection. This realization promoted a campaign committed to eradicating infectious diseases by targeting their root cause. Vaccine development, antibiotic treatments, improved sanitation practices and the adoption of a hygienic lifestyle were part of the armory in the war against microorganisms. However, these very advances may be a Pyrrhic victory over infectious disease as, in turn, reduced exposure to microbes seems to contribute to the development of atopic and inflammatory disorders. In this issue of *Nature Medicine*, Hill *et al.*[2] provide experimental support for the notion that microbes may protect from allergic disease.

In 1989, David Strachan evaluated the associated risk between allergic rhinitis and sixteen perinatal, social and environmental factors[1]. He found that family size had a significant inverse correlation with the development of allergy. Extrapolating that smaller family size may reduce individual exposure to respiratory infections; Strachan suggested that such infections might be protective against the development of atopic disease. From this proposal arose the hygiene hypothesis, which redefined our relationship with the microbial world, prompting us to better understand how microorganisms benefit host health and development. In the decades that followed, multiple studies have supported Strachan's suggestion that microbial exposure may benefit host immune function. Notably, these studies have identified the commensal microbiota (consisting of over a 100 trillion microbes that colonize all environmentally exposed surfaces of a mammalian host) as an important modulator of host immune responses. In particular, specific microbes in the gut have been shown to influence intestinal immune development and protection from disease[3,4].

These effects extend beyond the gut, as microbes and their products have been shown

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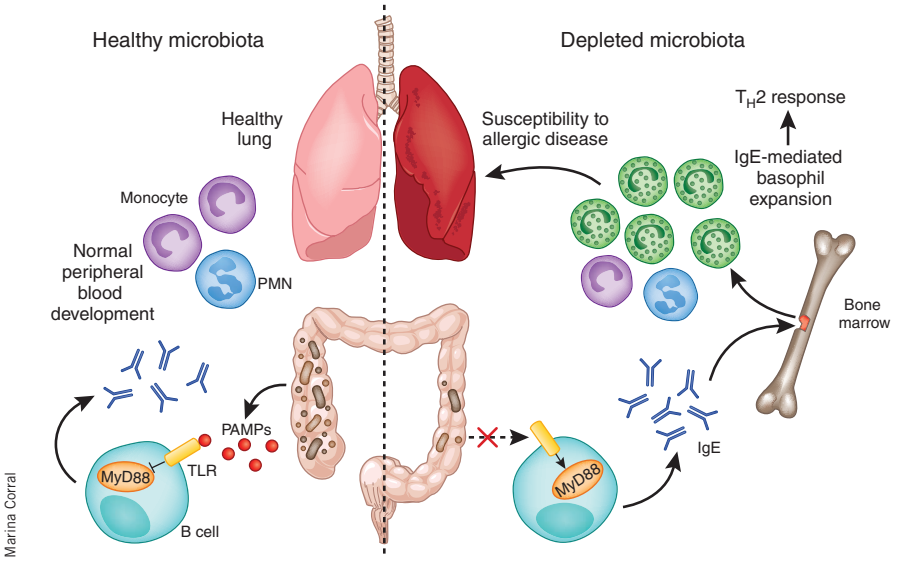

Figure 1 The intestinal microbiota protects against allergic inflammation. Hill *et al.*[2] show that the absence of MyD88-dependent microbial stimulation promotes IgE production by B cells, resulting in basophil expansion in the bone marrow and subsequent susceptibility to allergic disease. In this model, intestinal bacteria are depicted as the source of microbial products that induce antiallergic immune responses; however, microbes at other anatomical locations may affect T helper type 2 (T_H2) immunity. PAMP, pathogen-associated molecular pattern; PMN, polymorphonuclear cell.

to affect systemic infectious and inflammatory diseases[5–7]. The microbiota has a crucial role even outside the immune compartment, modulating weight gain, various endocrine disorders and nociceptive recognition[8–10]. However, with the exception of epidemiological studies that further support Strachan's initial observations, very little convincing evidence has emerged that determines whether and how the microbiota contributes to the development of allergic disease. Hill *et al.*[2] now provide a major leap forward by characterizing a mechanism by which commensal bacteria may be preventing the development of atopic disease.

Hill *et al.*[2] show that antibiotic-mediated depletion of the microbiota is sufficient to predispose mice to allergic disease (**Fig. 1**). Mice treated with wide-spectrum antibiotics had an altered immune profile, with increases in serum concentrations of IgE and circulating basophils, two important mediators in atopic disease. Furthermore, after respiratory exposure to house dust mite allergen, antibiotic-treated mice showed a heightened allergic response characterized by increased alveolar inflammation and exaggerated immune responses consistent with allergic reaction. The phenotype in antibiotic-treated mice was similar to that in germ-free mice, suggesting the microbiota's influence on host immune function is plastic since it is reversed after pharmacologic disruption of host-microbial symbiosis.

In an elegant set of experiments, the authors decipher aspects of the molecular interplay between specific microbial-host factors that promote proper immune development, preventing allergic disease[2]. Increases in serum IgE in mice after antibiotic treatment promoted basophil expansion and subsequent susceptibility to allergic disease. Compared to conventionally raised mice—with an intact microbiota—both antibiotic-treated and germ-free mice showed a higher proportion of bone marrow basophilic precursor cells and higher expression of their proexpansion receptor CD123, as well as increased expansion of these cells after stimulation *ex vivo*. The authors then sought to determine whether there was a mechanistic link between the increased serum IgE concentration and the basophil expansion. No increases in circulating basophils were observed after antibiotic treatment of Rag1-knockout mice, which lack B cells, or in mice in which IgE was neutralized, suggesting that communication between the microbiota and B cells is crucial in preventing proallergy immune development. Intriguingly, humans with high serum IgE concentrations also have increased basophil proportions, suggesting that IgE may regulate basophil-mediated allergic disease.

Speculating that direct microbial sensing by immune cells affects allergy, the authors showed that the commensal microbiota modulates B cell production of IgE antibody in a myeloid differentiation factor 88 (MyD88)-dependent manner[2]. MyD88-deficient mice with an intact microbiota showed high circulating levels of basophils and high serum IgE concentration, similar to antibiotic-treated and germ-free mice. This phenotype was reproduced in mice in which MyD88 expression was selectively deficient in B cells. Furthermore, exposure of antibiotic-treated mice with the microbial molecule CpG, a Toll-like receptor (TLR)-dependent microbial ligand, was sufficient to reduce serum IgE as well as the frequency and total number of circulating basophils. What remains to be determined is whether these B cells reside in systemic compartments or are located in intimate association with the microbiota at mucosal surfaces. In other words, where (and how) do microbial products contact B cells?

Previous work has shown that germ-free mice show T helper type 2 skewing, an immune profile consistent with increased allergic reactions[11]. Hill *et al.*[2] uncover a link between the microbiota and B cells, mediated through MyD88-dependent ligand reception that promotes appropriate host immune development. The indication that MyD88-deficient mice seem phenotypically similar to germ-free and antibiotic-treated mice suggests two things. First, if the microbiota promotes systemic immune development and function through MyD88 signaling, some of the immunological defects reported by studies in MyD88-knockout mice may reflect the absence of this beneficial influence. Second, certain TLRs might have, in part, evolved to aid in communication with the commensal microbiota rather than to recognize and respond to infectious agents[12].

Hill *et al.*[2] provide compelling evidence that perturbations to the microbiota are sufficient to promote the development of allergic disease. However, future studies should address whether the susceptibility to allergic disease persists after cessation of antibiotic treatment. Are there lasting effects to host immune function after disruption of the microbiota? If not, it would suggest multiple factors are at play in the development of hygiene-meditated atopic disease, as it does not seem likely that allergies simply arise after an unfortunate coincidence of allergen exposure during antibiotic treatment. Rather, allergy susceptibility may reflect a combination of multiple environmental 'hits' to the microbiota, including antibiotics, infection and diet changes, paired with possible genetic defects in the host that prevent appropriate recovery of the microbiota after disruption. This study, supported by dozens of epidemiological reports, strongly urges the consideration of host genetic factors associated with allergic disease in the context of their possible role in mediating host-microbe symbiosis[13]. On the basis of these emerging findings, consideration should be given to the microbiota hypothesis as a basis for observations linking microbes to

immune-mediated diseases rather than a view of infectious agents affecting allergy.

Although infectious diseases remain a substantial threat to human health, especially in this age of antibiotic-resistant 'superbugs', we are becoming ever more aware of the dramatic contribution of microbes to host physiology. Appropriately, we are now starting to realize a role for various immune factors, such as TLRs, previously considered only in the context of pathogen eradication, in maintaining and promoting host-microbe symbiosis. With these new insights compelling us to consider the microbiota as part of the organismal unit, we have the potential of redefining and discovering many aspects of our biology.

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1. Strachan, D.P. *Br. Med. J.* **299**, 1259–1260 (1989).
2. Hill, D.A. *et al. Nat. Med.* **18**, 538–546 (2012).
3. Ivanov, I.I. *et al. Cell* **139**, 485–498 (2009).
4. Mazmanian, S.K., Round, J.L. & Kasper, D.L. *Nature* **453**, 620–625 (2008).
5. Clarke, T.B. *et al. Nat. Med.* **16**, 228–231 (2010).
6. Lee, Y.K., Menezes, J.S., Umesaki, Y. & Mazmanian, S.K. *Proc. Natl. Acad. Sci. USA* **108** (suppl. 1), 4615–4622 (2011).
7. Ichinohe, T. *et al. Proc. Natl. Acad. Sci. USA* **108**, 5354–5359 (2011).
8. Bäckhed, F., Manchester, J.K., Semenkovich, C.F. & Gordon, J.I. *Proc. Natl. Acad. Sci. USA* **104**, 979–984 (2007).
9. Petruzzelli, M. & Moschetta, A. *Cell Metab.* **11**, 345–346 (2010).
10. Amaral, F.A. *et al. Proc. Natl. Acad. Sci. USA* **105**, 2193–2197 (2008).
11. Mazmanian, S.K., Liu, C.H., Tzianabos, A.O. & Kasper, D.L. *Cell* **122**, 107–118 (2005).
12. Round, J.L. *et al. Science* **332**, 974–977 (2011).
13. Garn, H. & Renz, H. *Immunobiology* **212**, 441–452 (2007).

In cancer drug resistance, germline matters too

Emily H Cheng & Charles L Sawyers

Cancer genome sequencing projects focus exclusively on the discovery of somatic changes. A new study shows that germline alterations in the proapoptotic protein BIM can have a crucial role in how a tumor responds to treatment (pages 521–528).

Amidst the euphoria over targeted cancer therapies, there is increasing recognition that tumor regressions, although substantial, are rarely complete. Drug-sensitive tumors are temporarily stunned by most targeted therapies but generally resume growth within 6–12 months. Intense efforts are now focused on understanding mechanisms of resistance, as this will guide the development of next-generation compounds and appropriate combination therapies. Nearly all of the focus to date has been on tumor cells, and with good reason—tumors are genetically unstable and an ideal breeding ground for drug-resistant subclones. Examples of the success of this approach include the discovery of second-site mutations in the drug target (for example, BCR-ABL kinase domain mutations in imatinib-resistant chronic myeloid leukemia), amplification of genes encoding proteins that bypass the blocked target (for example, androgen receptor amplification in castration-resistant prostate cancer) and activation of signaling pathways through relief of negative feedback (for example, AKT kinase activation in response to mammalian target of rapamycin inhibition)[1].

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In the current issue of *Nature Medicine*, Ng *et al.*[2] report another mechanism where the culprit is not the tumor but the host. The key finding is that many individuals with chronic myeloid leukemia (CML) or epidermal growth factor (EGFR)-mutant lung cancer with suboptimal clinical response to tyrosine kinase inhibitors have a germline polymorphism or deletion in the *BCL2L11* (hereafter referred to as *BIM*) gene, which encodes a key proapoptotic member of the B cell lymphoma 2 (BCL-2) protein family. The polymorphism deletes a sequence in intron 2, altering the RNA splicing in a manner that impairs generation of the potent, death-inducing isoform of BIM. Consequently, the tumor cells in these individuals are not 'poised to die' when confronted by the relevant tyrosine kinase inhibitor.

The BCL-2 family proteins control crucial checkpoints of mitochondrion-initiated apoptosis[3]. Multiple apoptotic signals, including most conventional chemotherapy or targeted anticancer agents, culminate in permeabilizing the mitochondrial outer membrane, resulting in the release into the cytoplasm of apoptogenic factors such as cytochrome *c* and SMAC to activate caspases. BAX and BAK are essential effectors responsible for the permeabilization of the mitochondrial outer membrane, whereas antiapoptotic BCL-2, BCL-X_L and myeloid leukemia cell differentiation protein-1 (MCL-1) preserve mitochondrial integrity. The BCL2-homology domain 3-only molecules (BH3s) interconnect with the upstream apoptotic signals to promote apoptosis—some BH3s, including BIM, BID and PUMA, directly activate BAX and BAK, and others, such as BAD and NOXA, inactivate BCL-2, BCL-X_L and MCL-1 (refs. 4–6) (**Fig. 1**). Understandably, the pro-death activity of BID, BIM and PUMA is tightly controlled at the transcriptional and/or post-translational levels, with activation in response to various apoptotic stimuli[3].

Ng *et al.*[2] used paired-end DNA sequencing to identify structural variations in samples from individuals with CML. Among the many variants found in this survey, a 2,903-bp germline deletion in intron 2 of *BIM* stood out because it was only found in individuals resistant to ABL kinase inhibitors such as imatinib. Among BCL-2 family proteins, BIM has a central role in imatinib-induced apoptosis in CML and gefitinib- or erlotinib-induced apoptosis in non–small-cell lung cancer (NSCLC) harboring activating mutations of EGFR[7–10]. Tyrosine-kinase inhibitors (TKIs) induce transcriptional upregulation of *BIM* mRNA as well as stabilize BIM protein through inhibition of the MEK-ERK kinase pathway, which phosphorylates BIM, targeting it for β-transducin repeat–containing protein (TCRP)–mediated ubiquitination and degradation.

Normally, the most abundant isoform is BIM_{EL}, which contains the BH3 domain as well as the phosphorylation sites that regulate BIM protein stability. The deletion found by Ng *et al.*[2] results in preferential splicing of exon 3 over exon 4, generating the BIM-γ isoform that lacks the BH3 domain. Because the BH3 domain is essential for the proapoptotic activity of BIM, BIM-γ was predicted to be ineffective in promoting cell death. Indeed, human