macrophages from FMF patients when compared to cells from healthy individuals. It remains unclear, however, what the physiological role of PYRIN is in the activation of caspase-1 and the ASC-dependent pathway that PYRIN regulates. These important questions will be the subject of future investigation.

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A Narrow Circle of Mutual Friends

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DOI 10.1016/j.immuni.2011.05.008

Commensal microbiota confers a goldilocks state of alertness to pathogens, yet restrains deleterious inflammation. In this issue of *Immunity*, Geuking et al. (2011) demonstrate that a minimal bacterial community of the Schaedler flora establishes a balance between pro- and anti-inflammatory T cells in the gut.

Coevolution of metazoans and microorganisms has been driven by multifaceted cross-kingdom symbioses affecting nutrient and energy consumption, immune defense, detoxification, tissue repair, and organ morphogenesis. Evolutionarily conserved mutualistic relationships between metazoan hosts and their symbiotic and commensal microbiota have been most intensely studied in the context of mammalian gut because of their immediate medical relevance. The gut harbors highly complex, species-specific microbial communities whose composition both influences and is influenced by the innate and adaptive immune system. "Tonic" stimulation afforded by the immune recognition of commensal microorganisms facilitates the development of the mucosal immune system and confers resistance to infection. Besides induction of secreted mediators of the immune responses (antimicrobial peptides, chemokines, and cytokines) and intracellular immune effectors and recruitment and activation of innate immune cells, the adaptive arm of the immune system is also profoundly affected by the commensal microbiota (Garrett et al., 2010). The latter notion comes from observations in germ-free mice where $CD4^+$ T cells exhibit pronounced skewing toward production of T helper 2 (Th2) cell cytokines, whereas Th1 cell cytokine production is sharply diminished. Most notable, however, is the essentially complete absence of IL-17- and IL-22-producing Th17 cells and diminished expression of IL-23. In agreement with these findings, prolonged antibiotic treatment of conventionally housed animals leads to a diminished expression of proinflammatory Th17 and Th1 cell cytokines. Consequently, decreased amounts of IL-17, IL-22, and IL-23 lead to increased susceptibility to infection with intestinal pathogens such as *Citrobacter rodentium* (Ivanov et al., 2009).

Commensal microbiota is also required for TGF-β-dependent IgA production. In addition to immune response and inflammation-promoting effect, commensal microbiota facilitates elaboration of anti-inflammatory mechanisms in the gut, including IL-10 production and increase in numbers of suppressive T cells, i.e., both Foxp3-expressing regulatory T (Treg) cells and Foxp3-negative, IL-10-producing Tr1 cells. When the immunological balance in the gut is tipped toward immune responses against commensal and opportunistic microbes, unwanted local and systemic maladies ensue, as suggested by several recent studies in experimental mouse models (Garrett et al., 2010). In particular, Th17 cell generation can facilitate diverse pathologies including colitis, inflammatory bowel disease, and arthritis (Ivanov et al., 2009; Wu et al., 2010). These unwanted consequences of overexuberant effector responses or inadequate negative regulation of the immune stimulation by gut microbiota are dependent on a genetic background of the host and thus are likely inconsequential at a species level. Thus, under physiologic conditions, the bacterial community in the gut promotes inflammatory responses capable of

keeping invading pathogens and opportunistic infections at bay and buffers these responses by inducing cells and soluble mediators, restraining their pathological consequences. In addition to a key role in the immune defense function and in limiting inflammation in the gut, signaling pathways engaged by commensal flora affect the homeostasis and repair of intestinal epithelium and its barrier function.

With the realization of the complexity of the gut microbiota came an important question of whether regulation of inflammation is dependent on specific bacterial species or their related groups, or whether there is a minimal microbial community that can provide necessary regulation that includes both tonic stimulation of the immune system and negative regulation of potentially harmful responses.

One obvious approach is to greatly reduce the number of variables in this highly complex ecological system, i.e., to monocolonize germ-free mice with a particular commensal microorganism or a microbial community comprised of few members. Recent studies implicated several specific components of gut flora in induction of pro- or anti-inflammatory T cells and production of IL-10 upon colonization of a germ-free host. In particular, segmented filamentous bacteria (SFB) were sufficient to promote potent Th17 cell differentiation whereas Clostridia belonging to clusters IV and XIVa increased numbers of colonic Treg cells (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009; Atarashi et al., 2011). Furthermore, *Bacteroides fragilis* expressing polysaccharide A (PSA) was able to promote IL-10 production and Treg cell generation, whereas *B. fragilis* lacking PSA induced Th17 cell differentiation (Mazmanian et al., 2008; Round et al., 2011).

The study in this issue of *Immunity* by Geuking et al. (2011) examines effects of introduction of a small consortium of microbes known as altered Schaedler flora (ASF) to the guts of germ-free animals by asking whether inflammatory and anti-inflammatory responses are induced in a balanced or unbalanced way. The authors find that ASF both potentiates production of inflammatory cytokines and boosts Treg cell subset. Specifically, ASF was capable of increasing the numbers of Treg cells in colonic lamina propria. Although most colonic Treg cells in colonized mice were lacking in Helios expression, a feature indicative of their peripheral origin, adoptive transfer studies showed that Treg cell population present prior to bacterial introduction expands and accumulates in the gut of colonized mice. Thus, ASF colonization can promote de novo differentiation of Treg cells in the gut and associated lymphoid tissues as well as accumulation of thymically generated Treg cells in the gut. This increase in Treg cells was dependent upon T cell receptor (TCR) specificity, because ASF colonization of mice expressing transgenic TCR specific for LCMV epitope with irrelevant specificity failed to cause an increase in $Foxp3^+$ Treg cells. In contrast, inflammatory T cells were clearly inducible by ASF in both SMARTA and B6 mice (but not other strains of gnotobiotic mice), although ASF was markedly less potent in doing so compared to SFB. At face value, this observation suggests that in contrast to TCR-dependent induction of Treg cells, Th17 cell differentiation was induced by ASF in a TCR-independent manner. Alternatively, it is possible that cross-reactivity of SMARTA TCR with an endogenous ligand(s) or limited diversity of TCR generated due to endogenous TCR gene rearrangement allows for differentiation of effector T cells, but not Treg cells, in these mice. The anti-inflammatory function of this microbial consortium was mediated by a key cytokine: IL-10. The blockade of IL-10R signaling led to a dramatic increase in the generation of inflammatory T cells in ASF-colonized mice. Although $CD4^+$ T cells were identified as a source of IL-10, it is likely that other cell types secrete IL-10 in response to microbiota. The induction of Treg cells by ASF was partially dependent on TLR signaling, although fewer Treg cells induced in MyD88-TRIF double-deficient gnotobiotic animals were fully capable of blocking the induction of inflammatory T cells. The authors also argue that Treg cell induction in ASF-colonized B6 mice could be dependent on epithelial damage and increased translocation of otherwise "benign" ASF bacteria. Indeed, more Treg cells were found in the colonic lamina propria upon epithelial damage induced by the chemical DSS.

In these experiments, ASF is viewed as a unit, a single entity with at least two functions: immune activation and immune suppression affecting adaptive immunity. At the same time, ASF is also likely to regulate innate functions such as tissue repair. Indeed, ASF-colonized mice were obviously resistant to DSS, whereas germ-free mice are highly sensitive to DSS. It is also important to note that, most likely, ASF does not induce a complete range of anti-inflammatory mechanisms. For example, in a genetic mouse model of autoimmune type 1 diabetes, germ-free MyD88-negative NOD mice colonization with ASF led to partial inhibition of disease development, whereas conventional MyD88-negative mice were fully protected (Wen et al., 2008).

One other important notion based on the Geuking et al. results is that the genetics of the host is an important component in the described regulatory mechanisms. Whereas mice of B6 origin were producing Th17 cells in response to ASF, other inbred and outbred strains tested did not. The result is consistent with the previously described failure of ASF to induce this type of response in SWR gnotobiotic mice (Ivanov et al., 2009). Interestingly, SFB is a potent inducer of Th17 cells in both strains of mice. Thus, genetics of the host can dictate sensitivity to inflammatory signals provided by different members of microbial communities.

In this study, ASF is described as a "benign" and therefore mutualistic microbiota. However, such logic must be used with caution, as host-commensal interactions can resemble host-pathogen interactions. A robust example of such relationships is found in the light-emitting organ of the Hawaiian bobtail squid *E. scolopes.* The organ is induced and colonized by luminescent Gram-negative bacterium *Vibrio fischeri* (Nyholm and McFall-Ngai, 2004). Notably, this clearly mutualistic relationship can be hardly characterized as "benign," as it exhibits many features of host-pathogen interactions including bacterial invasion and expansion within the host, lipopolysaccharide and peptidoglycan recognition by the host, and control of bacterial growth by the host. The ASF is obviously *beneficial* for the host, but being beneficial does not necessarily mean *benign.* After all, SFB that invades the epithelium (not "benign") is beneficial for the host infected by pathogens that require IL-17 for clearance. It seems important to

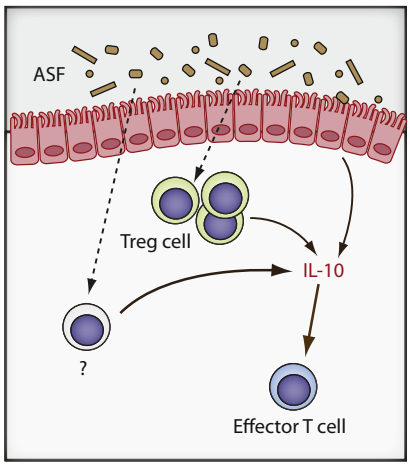

Figure 1. A Minimal Bacterial Community that Establishes a Balance between Pro- and Anti-inflammatory T Cells in the Gut
Colonization of germ-free mice with ASF promotes proinflammatory Th17 cell responses, capable of keeping invading pathogens and opportunistic infections at bay, and anti-inflammatory Treg cells. IL-10 produced by diverse cell types, including Foxp3$^+$ Treg and Foxp3$^-$ Tr1 cells, plays an important role in limiting Th17 cell responses.

determine whether Th17 cell-inducing ASF component(s) also provide some challenge to the epithelium.

The work of Geuking et al. makes an important point and elicits many more questions. The key finding is that complex host-microbe interactions leading to establishment of basic regulatory loops can be reproduced upon colonization with a small variety of microbes (Figure 1). Moreover, ASF can induce a Th17 cell response, albeit a weaker response in comparison to that elicited by a "certified" inducer of Th17 cells, the SFB. However, it is still not clear what signals can be induced by individual members of ASF, because the monocolonization experiments were unsuccessful. Although not all members were tested, a single Clostridium species tested notably failed to associate with the host. It is possible that it requires other components of ASF to either provide necessary nutrition or assist with engraftment by modifying the host tissue. Further experiments are needed to determine whether specific arms of the regulatory and effector mechanisms can be separately regulated by individual ASF members or whether the consortium as a whole is required.

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Chronic Infections Capture Little Attention of the Masses

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DOI 10.1016/j.immuni.2011.05.005

In this issue of *Immunity*, Egen et al. (2011) provide compelling evidence that only a minute fraction of mycobacteria-specific T cells present in a granuloma are actively fulfilling effector functions, an observation that may in fact be a general feature of chronic infections.

To receive activation signals and exert their effector functions, antigen-specific T cells establish interactions with antigen-presenting cells (APCs). In secondary lymphoid organs, naive T cells form contacts with antigen-bearing dendritic cells (DCs), and these interactions have been extensively studied with the help of two-photon imaging (Cahalan and Parker, 2008). Long-lived T cell:DC interactions lasting several hours are often seen during this phase, in particular in conditions of robust and efficient T cell activation (Bousso, 2008). In contrast, the way that effector T cells alter their behavior upon recognition of target cells at the site of infection has only recently been under scrutiny. In a beautifully performed study, Egen