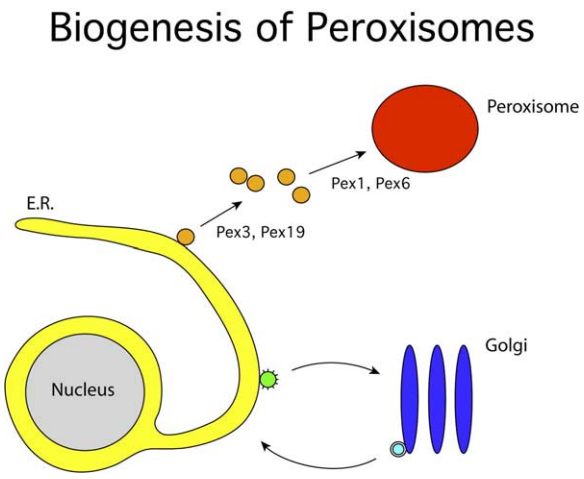

Figure 1. Where Do New Peroxisomes Come from?

Peroxisomes may form by the budding of vesicles (pale orange) from the ER in a pathway that is distinct from that producing secretory transport vesicles (green) from the ER. In the secretory pathway, vesicles carry membrane and cargo proteins to the Golgi apparatus; other vesicles retrieve some of the membrane material from the Golgi and return it to the ER. Pex3, an integral protein required for peroxisome formation, appears to originate in the ER and be packaged into vesicles (pale orange) by a budding or blebbing process that requires the Pex19 protein. Small precursor vesicles may fuse under the direction of two AAA ATPase proteins, Pex1 and Pex6, to form the functional large peroxisome (dark orange).

bly other peroxisomal proteins into a patch for budding from the ER now becomes accessible. How would such a budding mechanism sort peroxisomal from secretory cargo (membrane and luminal) proteins? An obvious experiment would be to evaluate the clustering of Pex3p tagged with yellow fluorescent protein in cells harboring mutations in coat proteins such as COPI, COPII, and clathrin. If such mutations produced an effect on peroxisome biogenesis, the role of the standard budding machinery would have to be re-evaluated. However, if no effect is observed with these mutants, one could screen the collection of yeast deletion mutants for candidate genes involved in peroxisome biogenesis. Alternatively, a purely biochemical approach based on a cell-free Pex3p budding reaction may reveal a new cellular machinery for budding of Pex3p from the ER. Clearly, many new genetic and biochemical studies will flow from the Hoepfner et al. work. Who said yeast cell biology was dead?

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Sugar-Coated Regulation of T Cells

The human gut is host to hundreds of different species of commensal bacteria that live in peaceful partnership with the host immune system. These commensal bacteria are far from neutral bystanders as they are intimately involved in the development of the immune system. Reporting in this issue of *Cell*, Kasper and colleagues (Mazmanian et al., 2005) reveal that a bacterial polysaccharide, PSA, produced by the commensal bacterium *Bacteroides fragilis* directs development of the immune system of the mouse host.

We are surrounded by billions of bacteria. Our own gut is host to hundreds of different species of bacteria, although, for the most part, we are oblivious to their presence. These gut commensal bacteria are not neutral bystanders as the proper development of both the gastrointestinal tract and the immune system of their mammalian hosts is dependent on their presence. "Germ-free" rodents raised in an environment devoid of microbes exhibit malformed intestinal epithelia, improperly developed gut-associated lymphoid tissues (GALT), in addition to general deficiencies in their immune systems (Hooper, 2004). Although it has been long recognized that introduction of commensal flora into the gut of these animals induces maturation of the immune system, the exact bacterial determinants mediating these changes have remained elusive. In this issue of *Cell*, Kasper and colleagues now reveal the identity of one of these molecular determinants (Mazmanian et al., 2005).

Kasper and colleagues previously showed that zwitterionic polysaccharides from the commensal bacterium *Bacteroides fragilis* are taken up by antigen-presenting cells, such as dendritic cells, of the host. These sugar molecules are then processed, loaded onto MHC class II molecules, and presented to CD4 T cells of the host immune system (Cobb et al., 2004). The CD4 T

cells are activated via their T cell receptors in an apparently polyclonal fashion, similar to their activation by mitogens except that the bacterial sugar molecules first need to be processed. In the new work, Kasper and colleagues reveal that the interaction of a commensal bacterial product with T cells is important for T cell regulation (Mazmanian et al., 2005). These authors link development of the immune system to a specific product of commensal bacteria, PSA.

Germ-free mice are characterized by a decreased number of CD4 T cells in the spleen. This number increases to normal after colonization of the mouse gut with wild-type *B. fragilis*. However, this return to normal numbers of CD4 T cells does not occur if the intestines of these mice are colonized by a mutant form of *B. fragilis* that cannot express PSA. The spleens of germ-free mice colonized by the wild-type form but not the PSA-deficient form of *B. fragilis* regain a normal architecture as determined by an increase in follicle size and organization. Interestingly, not only is administration of purified PSA sufficient to restore normal numbers of CD4 T cells in germ-free mice, but it also increases the population of CD4 T cells in the spleens of "conventional" animals (that is, those with normal gut flora). This finding may reflect the mitogenic effect of CD4 T cell expansion previously observed in vitro (Brubaker et al., 1999; Tzianabos et al., 1999).

What is the mechanism of this CD4 T cell expansion? It remains to be determined if the increased number of T cells is due to expansion of a specific subset of CD4 cells, such as that observed upon exposure to bacterial superantigens, or whether it results from an increase in all CD4 cells, such as that induced by the potent mitogen concanavalin A. Examining changes in the activation status and T cell receptor repertoire of CD4 T cells after colonization by *B. fragilis* will aid in distinguishing between these two possibilities. Furthermore, biochemical studies to examine how the zwitterionic polysaccharide antigen interacts with the T cell receptor and the signaling pathways that are activated by this interaction should shed light on the mechanisms responsible for T cell expansion.

Another important matter to resolve is how PSA presentation, which appears to be limited to the mesenteric lymph nodes, affects the CD4 T cell population as a whole. The subset of T cells that resides in the mesenteric lymph nodes of the gut expresses $\alpha 4/\beta 7$ integrin. In contrast, most T cells express an alternate molecule, L-selectin, which enables them to enter other peripheral lymph nodes (Wagner et al., 1998). Do activated CD4 T cells move out of the mesenteric lymph nodes to other peripheral organs or does the PSA-mediated activation of these cells induce them to produce cytokines and other effector molecules that alter T cell homeostasis in the host animal? Commensal bacteria are known to be one of the driving forces of the homeostatic proliferation of T cells, and PSA may be an important contributor to this phenomenon (Mazmanian et al., 2005).

CD4 T cells differentiate into effector cells that secrete cytokines. These effector cells have been classified into at least two types: T helper 1 (Th1) cells that secrete interferon-γ (IFNγ) and T helper 2 (Th2) cells that secrete interleukin-4 (IL-4). CD4 T cells from germ-free mice are skewed toward a Th2 phenotype and produce more IL-4 and less IFNγ than CD4 T cells from wild-type mice. Colonization of the mouse gut with *B. fragilis*, but not *B. fragilis* deficient in PSA, results in a stronger Th1 response with T cells secreting more IFNγ than IL-4. PSA mediates this effect by inducing antigen-presenting cells, such as dendritic cells, to produce the Th1-polarizing cytokine IL-12 (Mazmanian et al., 2005).

PSA is a unique MHC class II antigen because it is a sugar and also because it seems to activate antigen-presenting cells. In addition to IL-12 secretion, dendritic cells cultured with PSA express increased amounts of MHC class II molecules and of the co-stimulatory molecules CD80 and CD86. It remains to be determined whether dendritic cell activation is dependent on Toll-like receptors (TLRs) and, if so, which TLRs are responsible for recognition of the sugar. Recent work by Medzhitov's group has shown that recognition of commensal bacteria by TLRs is essential for maintaining intestinal homeostasis (Rakoff-Nahoum et al., 2004). PSA or other zwitterionic sugars derived from commensal bacteria may be important molecules in this interaction.

It is becoming clear that bacterial products not only induce inflammatory responses in the host during infection but also provide benefits to the host. The Kasper et al. study is the first to show that a specific bacterial product is crucial for the development of the mammalian immune system. A parallel can be drawn between this discovery and the recent finding that two bacterial products (fragments of peptidoglycan and lipopolysaccharide) of a luminescent bacterium work together to induce maturation of a light organ in the squid where the bacteria reside (Koropatnick et al., 2004). These bacterial products are able to alter the epithelial morphology of the squid organ, reminiscent of the changes wrought in the gastrointestinal epithelium of mammals colonized by commensal bacteria. Bacterial products may be responsible for proper development of not only the gastrointestinal tract and immune system but also the respiratory tract and other host tissues as well.

The Kasper et al. study prompts us to ask a whole new series of questions regarding how different subsets of T cells are formed and the influence of gut-associated bacteria in this process. Further examination of the role of PSA and other commensal bacterial products on development of the host immune response should reveal even greater complexity among the interactions of the microbial world with their unsuspecting hosts.

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