

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

## One Commensal Bacterial Molecule — All We Need for Health?

Claudio Fiocchi, M.D.

Humans are not inherently endowed with a healthy immune system. The fate of the immune system depends on its interaction with a large variety of commensal microorganisms, most of which live in the lower gastrointestinal tract.<sup>1</sup> How the body maintains homeostasis with an incredibly complex enteric microflora is beginning to be discerned. For example, it was recently shown that the recognition of commensal bacteria by epithelial cells protects against intestinal injury.<sup>2</sup> Appropriate immune recognition of enteric bacteria is also essential to host-bacteria symbiosis, and a recent report by Mazmanian and colleagues implicates a single bacterial molecule as critical to this process.<sup>3</sup>

Mazmanian et al. introduced *Bacteroides fragilis*, a ubiquitous gram-negative anaerobe abundant in the mammalian gastrointestinal tract, into germ-free mice to study how the immune system responds to an immunodominant bacterial polysaccharide (abbreviated here as PSac rather than PSA to avoid confusion with prostate-specific antigen) during colonization. The spleens of animals colonized with *B. fragilis* with wild-type PSac had a normal number of CD4+ T cells and normal lymphoid architecture, whereas the spleens of animals colonized with a PSac deletion mutant ( $\Delta$ PSac) had low CD4+ T-cell counts and gross anatomical depletion, implying a role of PSac in lymphoid organogenesis (Fig. 1). Because these results suggest that PSac is necessary to stimulate the proliferation of T cells, the authors fed conventional mice purified PSac and found that it increased the numbers of splenic CD4+ T cells, demonstrating that recognition of PSac by the intestinal immune system is sufficient to induce systemic lymphocyte expansion.

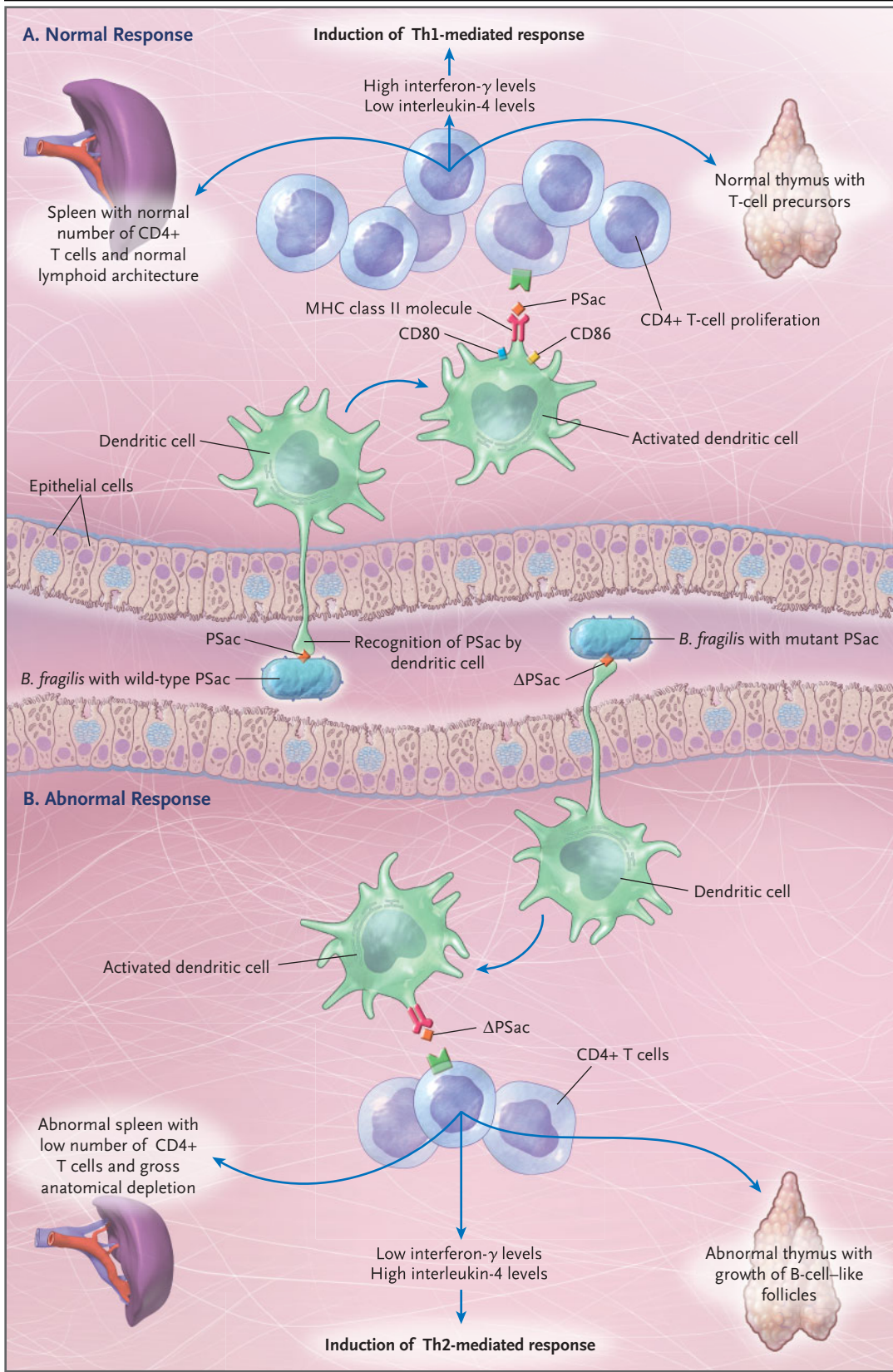
The immune response invoked by PSac involved recognition by and activation of antigen-presenting dendritic cells. This recognition also led to a dose-dependent increase in T-cell proliferation, an event that probably mimics what happens in vivo after colonization with *B. fragilis*. Antigen-specific proliferation is necessary but not sufficient for ef-

fective immunity; production of an appropriate cytokine repertoire is mandatory.

Mazmanian et al. therefore cultured dendritic cells with CD4+ T cells in the presence of wild-type or chemically modified (Nac) PSac. They observed a dose-dependent up-regulation of interferon- $\gamma$ , a type 1 helper T cell (Th1) cytokine, but not of interleukin-4, a type 2 helper T cell (Th2) cytokine, in response to wild-type PSac, but not Nac PSac. Because the production of Th2 cytokines is often considered a default pathway associated with pathologic responses, the authors interpreted the PSac-induced up-regulation of interferon- $\gamma$  as correct and necessary to establish the proper balance of Th1 and Th2 cytokines. To support this conclusion, they conducted in vivo experiments showing

**Figure 1 (facing page). The Multiple Immunomodulatory Activities of the *Bacteroides fragilis* Polysaccharide (PSac) Molecule.**

Dendritic cells present specific bacterial antigens to immune cells in the mucosa. A recent study by Mazmanian et al.<sup>3</sup> provides important insights into the molecular events that follow the recognition by dendritic cells of wild-type PSac, an immunodominant molecule expressed by the commensal bacterium *B. fragilis*, and of a mutant PSac ( $\Delta$ PSac) molecule. In Panel A, the spleen of a mouse colonized with *B. fragilis* expressing wild-type PSac has a normal number of CD4+ T cells and normal lymphoid architecture, and the thymus is replete with normal T-cell precursors. PSac induces the activation of dendritic cells and stimulates CD4+ T cells to produce Th1 cytokines, reflected by high levels of interferon- $\gamma$  and low levels of interleukin-4. In contrast, in Panel B, the spleen of a mouse colonized with *B. fragilis* with  $\Delta$ PSac has a low number of CD4+ T cells and an abnormal thymus owing to the excessive growth of B-cell-like follicles. Instead of producing Th1 cytokines, CD4+ T cells stimulated with  $\Delta$ PSac produce little interferon- $\gamma$  and abundant amounts of interleukin-4, a typical Th2 cytokine pattern. The authors concluded that a single molecule of a common commensal bacterium can mediate multiple immunomodulatory activities essential to the development of a normal immune system. MHC denotes major histocompatibility complex.



that splenocytes from mice colonized with *B. fragilis* expressing wild-type PSac produced large amounts of interferon- $\gamma$  and little interleukin-4. The opposite was true in mice colonized with *B. fragilis* that did not express wild-type PSac: their splenocytes produced little interferon- $\gamma$  but abundant amounts of interleukin-4, a Th2 pattern also found in bacteria-free animals and precolonized human neonates. Taken together, the outcome of these in vitro and in vivo experiments indicates that a single bacterial molecule elicits an amazing variety of responses that are needed to establish and maintain immune homeostasis.

Finally, the authors observed a rare abnormality in mice colonized with *B. fragilis* that did not express wild-type PSac. The majority of these mice had excessive growth of B-cell-like follicles in the thymic medulla, whereas the thymus in mice harboring wild-type *B. fragilis* was normal (Fig. 1). Given the central role of the thymus in the development of normal cell-mediated immunity, this observation further underscores the key role of PSac in the establishment of robust systemic immunity.

What are the clinical implications of these experiments? The answer seems simple: we need only a few commensal bugs in the gut to be immunologically fit. If this is true, why do we carry billions of microbial species in our intestines? The reason may also be simple: in addition to balancing

our immunologic act, bacteria perform countless other physiologic tasks. The corollary to both conclusions is obvious: we must keep enteric bacteria happy. At the moment it appears that we are not doing a very good job, given that allergic and autoimmune diseases, including those that affect the gastrointestinal tract, are on the rise.<sup>4</sup> Using the principles of the “hygiene hypothesis,” we can try to manipulate flora with antibiotics, probiotics, and prebiotics<sup>5</sup> while awaiting the time when commensal bacterial molecules with diverse immunomodulatory activities, such as PSac, become available for therapeutic use.

Dr. Fiocchi reports having received consulting fees from VSL Pharmaceuticals.

From the Department of Pathobiology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland.

1. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005;307:1915-20.
2. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Madzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004;118:229-41.
3. Mazmanian SK, Liu C-H, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005;122:107-18.
4. MacDonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. *Science* 2005;307:1920-5.
5. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004;126:1620-33.

Copyright © 2005 Massachusetts Medical Society.

**APPLY FOR JOBS ELECTRONICALLY AT THE NEW NEJM CAREERCENTER**

Physicians registered at the new NEJM CareerCenter can now apply for jobs electronically using their own cover letters and CVs. You can now keep track of your job-application history with a personal account that is created when you register with the CareerCenter and apply for jobs seen online at our Web site. Visit [www.nejmjobs.org](http://www.nejmjobs.org) for more information.