The phenotype of mice deficient in PTG suggests that the PTG gene is a candidate gene for type 2 diabetes and insulin resistance in humans. However, previous studies have indicated that PTG gene polymorphism does not contribute to insulin resistance or glucose intolerance (16, 17). Given that G_M gene polymorphism has been associated with insulin resistance in some human populations (18–20), it will be important to reexamine the possible relation between the PTG gene and insulin resistance in humans.

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Connecting the dots from Toll-like receptors to innate immune cells and inflammatory bowel disease

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**Conflict of interest:** The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: inflammatory bowel disease (IBD); CTL antigen-4 (CTLA-4); suppressor of cytokine signaling (SOCS); Src homology protein-1 (SHP-1); IL-10 receptor (IL-10R); Toll-like receptor (TLR); pathogen-associated molecular product (PAMP).

The etiologies of inflammatory bowel diseases (IBD) are not known but are thought to involve a genetic predisposition toward exaggerated inflammatory responses to enteric flora. Effective treatments for IBD are therefore predicated on the regulation of inflammatory responses in the intestine. Most current therapeutic agents for IBD, including 5-ASA, prednisone, and anti–TNF antibody are directed at the reduction of proinflammatory molecules. Recently, a number of negative regulatory molecules (e.g., IL-10, TGF-β, CTL antigen-4 [CTLA-4], Fas, suppressor of cytokine signaling [SOCS] proteins, A20, and Src homology protein-1 [SHP-1]), which either bind to effector immune cells and inhibit their activation (e.g., IL-10, TGF-β, and CTLA-4), induce programmed cell death (e.g., Fas), or regulate intracellular signaling pathways (e.g., SOCS proteins, SHP-1, and A20), have been identified. These negative regulatory molecules may provide novel therapeutic targets for the treatment of IBD.

IL-10, Stat3, and IBD
Among these negative regulators of inflammation, IL-10 inhibits multiple cell types, including macrophages (1, 2). The physiological importance of IL-10 is highlighted by the spontaneous development of bowel inflammation in IL-10–deficient (*IL-10*^{−/−}) mice (3, 4). The inflamed mucosa of *IL-10*^{−/−} mice contains elevated num-
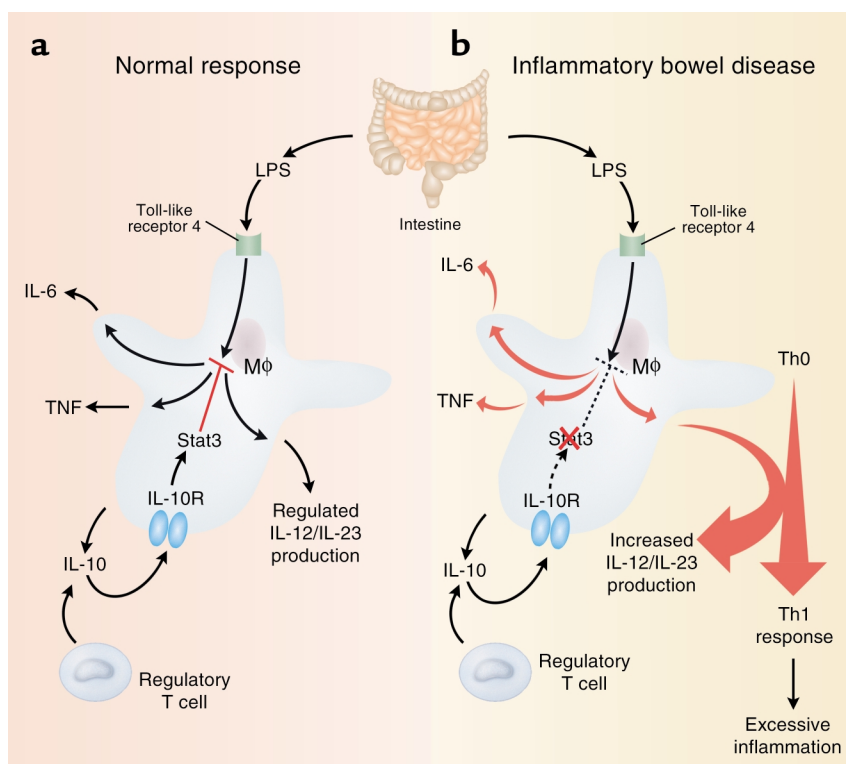
Figure 1
Model illustrating the roles of the Toll-like receptor (TLR) and Stat3 in IBD. Myeloid cells respond to LPS-mediated TLR stimulation. Normally, inflammation is controlled by IL-10, which stimulates myeloid cell Stat3 activation to suppress TLR-induced IL-12/IL-23 production (**a**). Kobayashi et al. (7) demonstrate that in *LysMCre/Stat3flox–* mice, IL-10 does not effectively suppress IL-12/IL-23 production. IL-12/IL-23 release activates lymphocytes, causing an exaggerated bias toward Th1-type inflammation (**b**).

Cytokines and IBD in myeloid Stat3–deficient mice

In this issue of the *JCI* Kobayashi et al. (7) perform multiple genetic manipulations of the *LysMCre/Stat3flox–* mouse to elucidate the sequential innate and adaptive immune processes that lead to the development of this Th1-type IBD. First, they address the questions of whether IFN-β, TNF, or IL-12p40 contribute to the enterocolitis and Th1 profile of *LysMCre/Stat3flox–* mice by interbreeding those mice to *Stat1–/–*, *TNF–/–*, and *IL-12p40–/–* mice, respectively. These experiments show that Stat1 and TNF are dispensable, whereas IL-12p40 is required for enhanced Th1 responses and enterocolitis in *LysMCre/Stat3flox–* mice. The deletion of IL-12p40 ablates both IL-12 and IL-23, a proinflammatory cytokine that shares the IL-12p40 subunit (8, 9), so it is not clear whether the lack of IL-12, IL-23, or both results in the amelioration of enterocolitis in *IL-12p40–/– × LysMCre/Stat3flox–* mice. Kobayashi et al. also find that disease is prevented in *RAG-2–/– × LysMCre/Stat3flox–* mice, despite high levels of IL-12p40, demonstrating a requirement for adaptive lymphocytes in this model of IBD.

LPS, TLR4, and IBD in myeloid Stat3–deficient mice

The first and perhaps critical step in initiating immune responses is typically the engagement of host Toll-like receptor (TLR) molecules by conserved pathogen associated molecular products (PAMPs) (10). TLR ligation by PAMPs induces the activation of NF-κB and other transcription factors resulting in the production of multiple proinflammatory molecules. Recently, it has been appreciated that TLRs also influence the nature of the immune response, in particular T cell skewing toward a Th1 or Th2 profile. Myeloid cells, which are exquisitely sensitive to TLR ligands and produce significant IL-12p40, are therefore poised to play key roles in the initiation and possibly the Th1/Th2 skewing of inflammatory responses. The potency of myeloid cell TLR responses also warrants their effective negative regulation to prevent pathological inflammation. Kobayashi et al. (7) address the potential role of TLRs in the IL-12–driven IBD of

bers of myeloid cells, IFN-γ–producing CD4+ T cells, and high levels of proinflammatory cytokines such as IL-1, IL-12, IL-6, and TNF (4). Antibody-induced depletion of IFN-γ or IL-12 abrogates or prevents, respectively, spontaneous inflammation in *IL-10–/–* mice (4). Furthermore, *RAG-2–/–* mice reconstituted with *IL-10–/–* CD4+ T cells develop bowel inflammation while *RAG-2–/– IL-10–/–* double-mutant mice do not. Therefore, Th1 biased CD4+ T cells appear to be pathogenic in *IL-10–/–* mice (4). Finally, *IL-10–/–* mice do not develop disease when raised in gnotobiotic conditions but do develop IBD when transferred to conventional facilities, indicating a role for enteric flora in the pathology of this IBD model (4, 5). Taken together, these data suggest that CD4+ T cell production of IL-10 prevents the development of a CD4+ T cell–mediated, IL-12–driven, Th1-type inflammation in the intestine that is initiated by the presence of microbes in the gut lumen.

The mechanism(s) by which IL-10 signals inhibit immune cell activation is poorly understood. IL-10 binds to a recently described IL-10 receptor (IL-10R) complex that is composed of at least two subunits, IL-10Rα and IL-10Rβ — also known as CRFB4 or CRF2-4 (2). IL-10R signaling in macrophages requires the kinase Jak-1 and the transcription factor Stat3 (2). The essential role of Stat3 in mediating IL-10 signals in myeloid cells was demonstrated by the targeted deletion of Stat3 in myeloid cells (*LysMCre/Stat3flox–* mice) that rendered neutrophils and macrophages unresponsive to IL-10 (6). This myeloid unresponsiveness to IL-10 resulted in the development of a polarized Th1-type immune response and chronic enterocolitis (6). Thus, the specific inability of myeloid cells to respond to IL-10 through Stat3 signals appears to recapitulate spontaneous inflammation seen in *IL-10–/–* mice.

LysMCre/Stat3^flox– mice by interbreeding with *TLR4^-/-* mice. They show that *TLR4^-/- × LysMCre/Stat3^flox–* mice display dramatically reduced intestinal inflammation compared to *LysMCre/Stat3^flox–* mice. This disease amelioration is consistent with the requirement for microbe or microbial products in the pathology of IBD in *IL-10^-/-* mice (4, 5).

A model for the role of IL-10, TLR4, and Stat3 in IBD

This work suggests a potential model (Figure 1) for the sequential activation and negative regulation of innate and adaptive immune cells during intestinal inflammation: LPS, perhaps from the intestinal lumen, induces the production of IL-12/IL-23 by myeloid cells, which drives a Th1-type inflammatory process in the lymphocyte population. The model suggests that inflammation would normally be controlled by myeloid or lymphocyte-derived IL-10 acting through Stat3 in myeloid cells to block further production of IL-12/IL-23. This suppressive IL-10 signal may be particularly important in the gut, where immune cells are continuously in close contact with LPS and other microbial PAMPs. In *LysMCre/Stat3^flox–* mice, IL-10 does not effectively suppress IL-12/IL-23 production by myeloid cells, resulting in the expansion and Th1 bias of CD4+ T cells. This study by Kobayashi et al. (7) clearly places TLR-induced myeloid IL-12/IL-23 production as an important target of IL-10-induced, Stat3-mediated anti-inflammatory effects. Additionally, this study points to important unanswered questions regarding the anti-inflammatory effects of IL-10 including: How is IL-10 receptor expression regulated in myeloid cells? How does IL-10 activation of Stat3 regulate TLR-induced expression of IL-12p40?

The experimental evidence above and other data suggest that IL-10 might be a promising target for alleviating inflammation in human patients with IBD. Specifically, the evidence for the potential therapeutic use of IL-10 includes the fact that IL-10 suppresses the production of inflammatory cytokines by multiple immune cell types; continuous IL-10 treatment can prevent or ameliorate disease in several mouse models of IBD; *IL-10^-/-* mice develop IBD; and small trials of treatment with IL-10 in patients with ulcerative colitis showed reduced cytokine levels and improved histological scores (4, 11). It is therefore vexing that recent clinical trials found no beneficial effect of IL-10 in the treatment of Crohn disease (12, 13). It may be that the nature of the IBD being treated, the timing of IL-10 administration, the route of drug delivery, or the specific cell types being targeted by IL-10 will affect the outcome of the therapy (14). Additionally, IL-10 may be more effective when used in consort with other novel or established treatments for IBD. It is clear that further experimental dissection, like that performed by Kobayashi et al. (7), of the cell types and/or factors that mediate the anti-inflammatory effects of IL-10 in the intestinal mucosa are warranted.

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