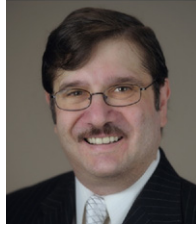


## Proinflammatory Cytokines in the Pathogenesis of Inflammatory Bowel Diseases



Warren Strober



Ivan J. Fuss

Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

The cytokine responses characterizing the inflammatory bowel diseases are the key pathophysiologic elements that govern the initiation, evolution, and, ultimately, the resolution of these forms of inflammation. Studies during the last 2 decades now provide a detailed (but not yet complete) picture of the nature of these responses. The first tier of cytokine responses are governed by the T-cell differentiation patterns dominating the disease. In Crohn's disease, the major cytokines arise from T-helper cell (Th) 1 and Th17 CD4<sup>+</sup> T-cell differentiation and consist of interferon- $\gamma$  and interleukin (IL)-17/IL-22 generated by these types of differentiation. The relative importance of these cytokines to Crohn's inflammation is still unclear, although evidence is mounting that interferon- $\gamma$  is *primus inter pare* (first among equals). In contrast, in ulcerative colitis, a Th2-like differentiation process is paramount, which results in expansion of natural killer T cells producing IL-13 (and perhaps IL-5). These disease-specific cytokine patterns give rise to a second tier of cytokines that span the Th1/Th17–Th2 divide and act as upstream facilitators and downstream mediators of inflammation. These cytokines include the well-known tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , IL-6, tripartite, as well as a more recently studied cytokine known as TL1A (tumor necrosis factor-like ligand). In this review, we will explore this cytokine landscape with the view of providing an understanding of how recent and future anticytokine therapies actually function.

**Keywords:** Crohn's Disease; Ulcerative Colitis; Cytokines; IL-12; IL-23; IFN- $\gamma$ ; IL-17; IL-22; TL1A.

In the past 2 decades, studies of the cytokines driving the inflammatory bowel diseases (IBDs) and other forms of mucosal inflammation has borne ample fruit, both in providing us with major insights into the mech-

anism of these diseases and in pointing us in the direction of new therapies. In this review, we will focus on the main cytokine responses in Crohn's disease (CD) and ulcerative colitis (UC) that initiate and sustain inflammation, leaving the task of discussing the regulatory cytokines that oppose such inflammation to other reviewers.

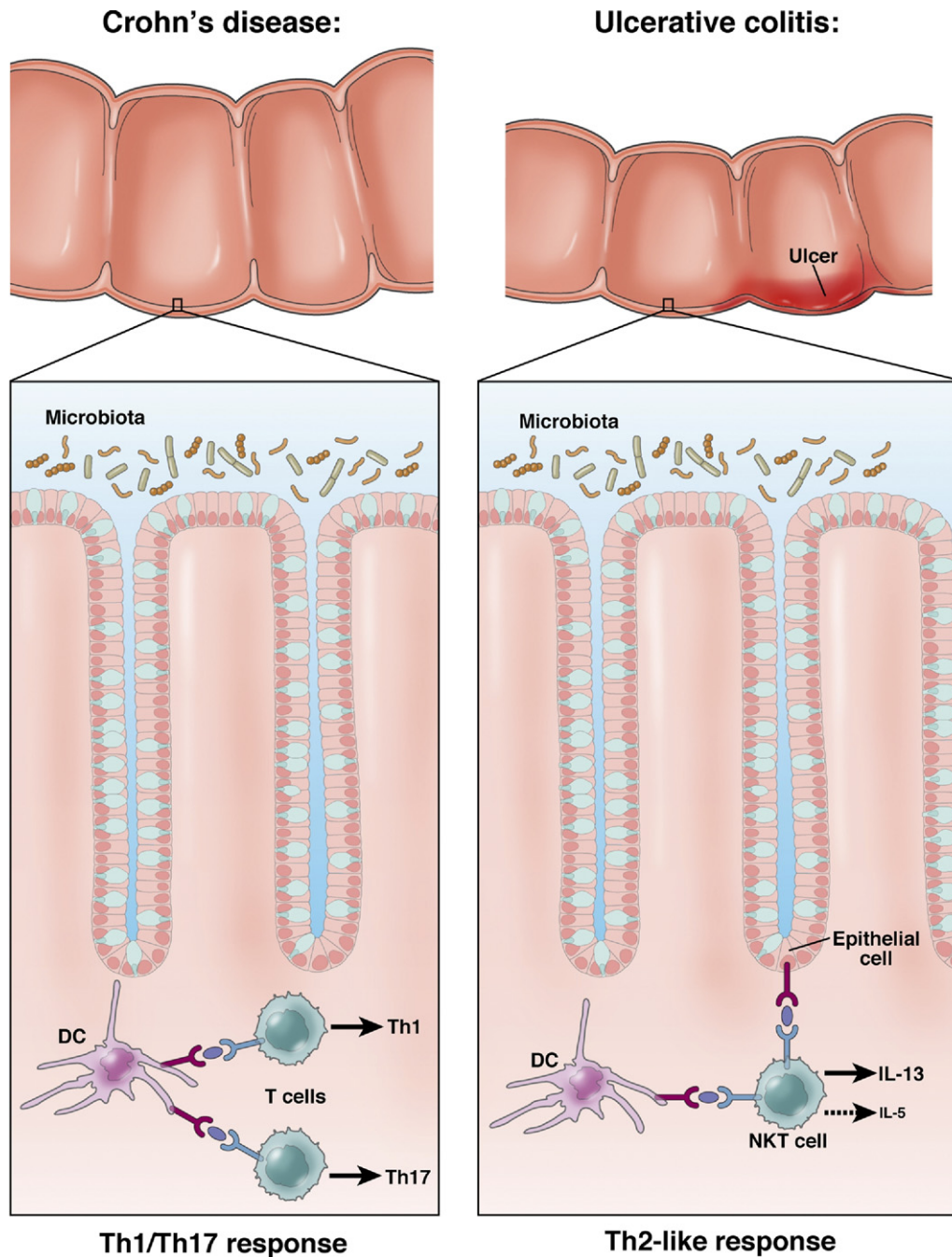
### T-Cell Differentiation Pathways and Gut Inflammation

With the discovery in the late 1980s that T helper (Th) cells differentiate into Th1 and Th2 cells,<sup>1</sup> producing different sets of cytokines, it was quickly established that CD differed from UC in that CD seemed to be a Th1 cytokine-mediated disease characterized by increased production of interferon (IFN)- $\gamma$ , whereas UC seemed to be a Th2 cytokine-mediated disease characterized by increased production of interleukin (IL)-5 production and normal IFN- $\gamma$  production.<sup>2,3</sup> One caveat, however, was that production of the signature cytokine of the Th2 response (IL-4) was not increased in UC and it was clear that the latter was a "Th2-like" disease rather than a fully Th2 disease (see Figure 1).

Support for these concepts came from studies of several murine models of IBD resembling CD, particularly trinitrobenzene sulfonic acid (TNBS)-induced colitis and cell transfer-induced colitis, which showed that inflammation was reversed by treatment with

**Abbreviations used in this paper:** CD, Crohn's disease; DSS, dextran sodium sulfate; IFN- $\beta$ , interferon- $\beta$ ; IL, interleukin; oxa-colitis, oxazolone-colitis; NKT, natural killer T cell; RAG, recombination activating gene; SCID, severe combined immune-deficient; TCR, T-cell receptor; TGF- $\beta$ , transforming growth factor- $\beta$ ; TL1A, tumor necrosis factor-like ligand; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; UC, ulcerative colitis.

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**Figure 1.** The basic dichotomy of cytokine function in inflammatory bowel disease (IBD). Although Crohn's disease and ulcerative colitis are both forms of IBD and exhibit overlapping genetic profiles, these disease are characterized by very different T-cell responses. Crohn's disease is driven by a T-helper cell (Th) 1/Th17 response in which IL-12 and IL-23 cytokines play key roles. Ulcerative colitis, in contrast, is driven by a Th2-like response in which NKT cells producing IL-13 (and IL-5) is the major response. In addition, in both forms of IBD, cytokines that have functions both "upstream" and "downstream" of these basic cytokines also play important inflammatory roles.

anti-IL-12p40—an antibody directed against a cytokine initially identified as IL-12, the master cytokine driving the Th1 response.<sup>4,5</sup> These findings, along with the fact that patients with CD exhibited increased lamina propria IL-12 production as compared to controls,<sup>6-8</sup> ultimately became the basis for development of a humanized anti-IL-12p40 antibody for treatment of patients with CD. When such an antibody became available, it was

shown in clinical trials that anti-IL-12p40 had a level of therapeutic efficacy similar to that of anti-tumor necrosis factor (TNF)- $\alpha$  and, moreover, could induce remission in patients with anti-TNF- $\alpha$  resistance.<sup>9,10</sup> These results not only formed the basis of a new therapy for CD, they also represented incontrovertible evidence that a cytokine containing a p40 chain played a major pathogenic role in this disease.

Parallel studies of UC, which will be discussed here at greater length, verified that UC was a Th2-like disease because it was associated with increased IL-13 production (but not IL-4 production) by natural killer T (NKT) cells, rather than by conventional T cells.<sup>11</sup> As is the case with CD, analysis of the pattern of T-cell differentiation was predictive of the basic cytokines causing UC-type inflammation.

### Th17 Response in CD Pathogenesis

The concept that CD was an IL-12-driven Th1 inflammation did not remain unchallenged for long: about the time anti-IL-12p40 was shown to be effective in the treatment of CD, a new set of cytokines, the Th17 cytokines (IL-17 and IL-23), was shown to function as effectors in various autoimmune disease models.<sup>12–15</sup> Among the latter was the cell-transfer colitis model in which it was shown that development of colonic inflammation was apparently more dependent on IL-23 than IL-12.<sup>16,17</sup> The idea that a Th17 response rather than a Th1 response was the major engine of inflammation in CD did not contradict the previously observed effect of anti-IL-12p40 in experimental and human CD because both IL-12 and IL-23 are heterodimers of which one chain is p40<sup>12</sup>; thus, anti-IL-12p40 can neutralize both IL-12 and IL-23.

To fully understand how Th17 T-cell responses are involved in experimental colitis or CD, it is important to take a moment to review certain salient features of Th17 immunobiology. First, IL-12 has a very different relation to IFN- $\gamma$  than does IL-23 to IL-17. IL-12 is the major inducer of Th1 cells producing IFN- $\gamma$  via direct interaction with IL-12 receptors on undifferentiated T cells<sup>18</sup>; in contrast, transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-6 (or IL-21) are the major inducers of Th17 cells producing IL-17 (and IL-22 as well) and the function of IL-23 is to interact with already differentiated Th17 cells (now expressing an IL-23 receptor) to cause stabilization and/or expansion of Th17 cells.<sup>19–22</sup> There is evidence that differentiation of Th17 cells in the absence of IL-23 leads to Th17 cells producing IL-10, an anti-inflammatory cytokine, which are therefore poor inducers of inflammation.<sup>23</sup> In addition, it has recently been shown that Th17 cells induced in the presence of IL-1 $\beta$  have a unique messenger RNA profile and an increased capacity to induce inflammation.<sup>24</sup> Thus, not all Th17 cells are equal.

Second, because Th17 cells usually require TGF- $\beta$  for differentiation, a cytokine also involved in regulatory T-cell differentiation, it is not surprising that Th17 cells and regulatory T cells have a “ying-yang” relationship, wherein development of one type of cell is reciprocal to development of the other type of cell. This is seen in the fact that induction of Foxp3 expression, the signature protein of regulatory T cells, inhibits ROR $\gamma$ t function, the main IL-17 transcription factor. Similarly, the induc-

tion of Th17 cells inhibits Foxp3 expression.<sup>25,26</sup> Another manifestation of the relationship between Th17 cells and regulatory T cells is that the latter cells produce large amounts of TGF- $\beta$  and can induce naïve CD4<sup>+</sup> cells to become IL-17-producing cells in an inflammatory milieu that contains IL-6, or can themselves be converted into Th17 cells under these circumstances.<sup>27–30</sup> This “plasticity” of IL-17-producing cells and regulatory T cells indicates that Th17 responses in IBD may morph into regulatory responses (and vice versa), depending on the character of the inflammation.

A third and final feature of Th17 development is that induction of IL-17 gives rise to IL-17-producing T cells, as well as cells producing both IL-17 and IFN- $\gamma$ ; in addition, there is evidence that in a milieu lacking TGF- $\beta$ , IL-12 and IL-23 tend to act on cells initially producing IL-17 to become cells producing IFN- $\gamma$ .<sup>31–33</sup> This capacity of at least some Th17 T cells to produce IFN- $\gamma$  is consonant with the heterogeneity of Th17 cells mentioned here and may facilitate their pathologic potential.

### First Wave of Th17 Studies

The initial studies assessing the importance of Th17 responses in experimental colitis and CD used the previously mentioned cell-transfer colitis model. This model consists of inflammation developing in immunodeficient mice (either recombination activating gene [RAG]-deficient or severe combined immune-deficient [SCID] mice) after adaptive transfer of naïve CD4<sup>+</sup> T cells (CD45RB<sup>high</sup> T cells) that develop into proinflammatory effector cells in the absence of a mature (CD45RB<sup>low</sup> T cells) cell population that contain regulatory T cells.<sup>34,35</sup> To put these studies into prospective, one should be aware that anti-IFN- $\gamma$  administration leads to complete amelioration of cell-transfer colitis and transfer of CD45RB<sup>high</sup> (colitis-inducing) cells unable to synthesize IFN- $\gamma$  because they lack a key factor (T-bet) necessary for IFN- $\gamma$  production, does not initiate colitis.<sup>36,37</sup> In addition, the percentage of cells in the inflamed lamina propria of this model producing IFN- $\gamma$  alone is 15–25 times higher than the percentage of cells producing IL-17 alone and 10 times higher than those producing both IL-17 and IFN- $\gamma$ .<sup>38,39</sup> These facts strongly suggest that the dominant effector cell driving cell-transfer colitis is an IFN- $\gamma$ -producing cell that is likely originating mainly from a Th1 response not a Th17 response. Nevertheless, certain influential studies have appeared presenting compelling evidence that a Th17 response is, in fact, the key effector cell response in this colitis model.

In the first of 3 such studies, it was shown that RAG-deficient mice also deficient in IL-23p19 (and thus deficient in IL-23) did not develop colitis upon transfer of naïve T cells, whereas the same mice also deficient in IL-12p35 (and thus deficient in IL-12) did develop colitis upon transfer.<sup>16,40</sup> Correspondingly, proinflammatory cy-

tokine production (TNF- $\alpha$  and IFN- $\gamma$ ) was greatly reduced in the IL-23p19-deficient mice, whereas it was only moderately reduced in the IL-12p35-deficient mice. Interestingly, while the IL-12p35-deficient mice exhibited greatly increased levels of IL-17, they also exhibited increased levels of IFN- $\gamma$ . This suggests that the inflammation in IL-12p35-deficient mice may have been due, as least in part, to the previously mentioned IFN- $\gamma$  component of the Th17 response, which in the absence of the p35 chain comprising part of the inhibitory cytokine, IL-35, is exaggerated.<sup>41</sup> In a recent update of these findings, it was shown that transfer of naïve T cells lacking the IL-23 receptor also failed to generate colitis.<sup>42</sup> In addition, signaling through this receptor enhanced intestinal T-cell proliferation and accumulation of IL-17-producing cells, especially those producing both IL-17 and IFN- $\gamma$ , which again could be a particularly proinflammatory Th17-cell subpopulation. Finally, the lack of T-cell expansion in mice reconstituted by IL-23R-deficient T cells could be overcome by cotransfer of T cells bearing IL-23R, indicating that a factor produced by cells bearing the receptor can drive cell proliferation of cells lacking the receptor. Overall then, cell-transfer colitis was shown in studies of both IL-23-deficient mice and IL-23R-deficient mice to exhibit greatly diminished colitis.

In the second study, SCID mice were initially transferred antigen-specific (ie, flagellin-specific) T cells, ie, memory T cells rather than naïve T cells, as in the usual cell-transfer colitis study. Interestingly, in this case, the lamina propria of the inflamed colon contained a 5:1 preponderance of IL-17-producing cells vs IFN- $\gamma$ -producing cells, quite the opposite of what is seen with the transfer of naïve cells.<sup>43</sup> This could conceivably be explained by the fact that memory cells are more responsive to IL-23 than to IL-12.<sup>12</sup> Next, SCID mice were transferred previously polarized Th1 or Th17 flagellin-specific T cells producing mainly IFN- $\gamma$  and IL-17, respectively. It was found that the mice transferred Th17 cells developed severe colitis, whereas the mice transferred Th1 cells developed little if any colitis. In addition, treatment of the Th17-reconstituted mice with anti-IL-23p19 either prevented colitis or reduced already established colitis. These studies suggest that Th17 cells, but not Th1 cells, mediate inflammation in a modified cell-transfer colitis model. Two points about these results require further consideration. First, the inflamed colons of mice transferred Th17 cells and exhibiting colitis contained cells producing large amounts of IFN- $\gamma$ , even though at the time of cell administration they were producing mainly IL-17. Thus, the mice reconstituted with Th17 cells could conceivably have had inflammation driven by both IL-17 and IFN- $\gamma$ . Second, the lack of inflammation in the mice transferred IFN- $\gamma$  cells is discordant with earlier cell-transfer colitis studies already discussed in which colitis was shown to be reversed by treatment with

anti-IFN- $\gamma$ <sup>36</sup>; this could conceivably be explained by the fact that a memory rather than a naïve population was transferred to the SCID mice and that this memory cell population was subject to inhibition by regulatory T cells.

In the third study of the role of IL-17 in transfer colitis, it was shown that although transfer of T cells lacking the capacity to produce any individual Th17 cytokine (ie, IL-17A, IL-17F or IL-22) to RAG1-deficient mice led to full-blown colitis, transfer of T cells from an ROR $\gamma$ t-deficient mice, that is, mice lacking a transcription factor necessary for production of all of these Th17 cytokines fail to develop colitis. Thus, although IL-17A and IL-17F are redundant, at least one member of the IL-17 cytokine family was required for the occurrence of colitis.<sup>44</sup> Further confirmatory studies showed that RAG1-deficient mice that fail to develop colitis upon transfer of ROR $\gamma$ t-deficient cells, do develop colitis if administered exogenous IL-17A.<sup>44</sup> One possible discrepancy in these data was that transfer of ROR $\gamma$ t-deficient T cells also led to a great reduction in the production of IFN- $\gamma$ , suggesting that it was really the IFN- $\gamma$  produced during a Th17 response that was mediating the inflammation. However, this possibility seemed to be at least partly negated by the fact that RAG1-deficient mice transferred IL-17F-deficient T cells, thus manifesting colitis due to IL-17A alone, exhibit decreased colitis when treated with anti-IL-17A.<sup>44</sup>

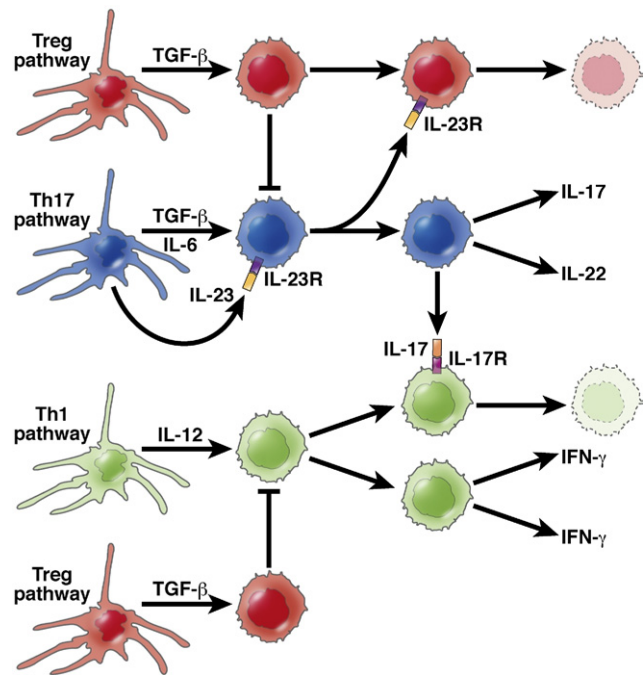
### Th17 Responses Are Essential, But Not Necessarily as Effector Cell Responses

The various studies discussed here appear to offer definitive evidence that at least one major type of experimental colitis, cell-transfer colitis, requires a Th17 response to support development of colonic inflammation. However, before we accept this conclusion we need to consider studies probing the impact of Th17 responses on regulatory T-cell responses. In an initial study of this question, it was shown that the transfer of naïve T cells to immune-deficient (RAG1-deficient) mice also lacking IL-23p19 (ie, mice able to mount a Th1 but not a Th17 response) only develop colitis if they have a concomitant IL-10 or TGF- $\beta$  deficiency and thus cannot mount a regulatory T-cell response.<sup>45</sup> In addition, IL-23p19-deficient mice exhibit increased numbers of regulatory T cells (Foxp3<sup>+</sup> T cells) in the colon and transfer of naïve T cells into RAG1-deficient/IL-23p19-deficient mice that also lack Foxp3<sup>+</sup> T cells develop colitis despite the absence of IL-23p19 (and the ability to mount a Th17 response). Similar findings were found in a second study, in which it was shown that RAG1-deficient mice reconstituted with IL-23R-deficient T cells also exhibit increased numbers of Foxp3<sup>+</sup> T cells in the colon, which was then shown to be a result of the lack of a negative effect of IL-23 on these cells.<sup>42</sup> These findings strongly suggest that IL-23 suppresses regulatory T-cell

development and thus introduce the possibility that mice that lack IL-23 fail to develop colitis, not because they cannot produce a key effector cytokine (IL-17), but rather because they have a dominant regulatory T-cell response. In the same vein, the inability of transferred polarized Th1 cells to cause colitis could be due to the fact that, in the absence of Th17 cells, Th1 cells are subject to suppression by regulatory T cells.

Additional questions about the role of Th17 responses in colitis models are raised by more recent studies of cell-transfer colitis, as well as studies of TNBS-colitis and dextran sodium sulfate (DSS)-colitis. In these cell-transfer colitis studies, it was found that transfer of T-cell populations from IL-17-deficient mice to immunodeficient mice led to earlier onset of colitis and higher levels of IFN- $\gamma$  production than transfer of cell populations from wild-type mice.<sup>46</sup> These findings could be explained by the fact that Th1 cells bear IL-17 receptors and IL-17 signaling via these receptors inhibits Th1 differentiation by suppressing expression of T-bet, a factor necessary for Th1 T-cell development.<sup>46</sup> Similarly, in the studies of TNBS-colitis and DSS-colitis, it was shown that although IL-17 responses accompany IFN- $\gamma$  responses in both models, induction of colitis in IL-23p19-deficient mice led to more severe inflammation than in wild-type mice.<sup>47,48</sup> Thus, both in the cell-transfer model and in the TNBS/DSS-colitis models, lack of cells producing IL-17 led to more not less disease and the latter seemed to be playing a regulatory role rather than an effector role in the inflammation.

In summary, it now seems clear that although the Th17 response has the potential to be a proinflammatory response when present in sufficient numbers (as shown in the studies of the ROR $\gamma$ t-deficient/RAG1-deficient mice discussed here), it is more likely to be functioning mainly as a regulatory response in experimental colitis models, where the number of Th17 cells may be limited (see Figure 2). Support for this thesis is not only vested in the studies discussed here, but also in the fact that IL-22, a Th17 cytokine generated under the same conditions that led to production of IL-17, has been shown to have anti-inflammatory effects in colitis models; IL-22 deficiency is associated with more severe colitis<sup>49,50</sup> and has been shown to induce signal transducer and activation of transcription (STAT) 3 in epithelial cells and to thus promote epithelial integrity in the face of inflammation.<sup>51</sup> Finally, it is important to point out that development of a relatively small Th17 response in the absence of a Th1 response does not necessarily lead to a CD-like inflammation. This is seen in recent studies of pig-tailed macaque monkeys who manifest decreased epithelial cell barrier function, entry of bacteria components into the lamina propria, and increased numbers of lamina propria IL-17<sup>+</sup> T cells in the absence of the transmural inflammation and granuloma formation characteristic of CD.<sup>52</sup>



**Figure 2.** Regulatory mechanism initiated by the T-helper cell (Th) 17 response. The role of the Th17 response in CD is under intense investigation. It is gradually emerging that in this disease this response may be more important in the regulation of the inflammation than in its induction. In this diagram, the Th17 response (depicted by the cells shown in blue) generate cells producing IL-17 and IL-22, the latter a cytokine clearly known to ameliorate gut inflammation. This response depends on the production of IL-23, which also inhibits regulatory T cell (Treg) generation (depicted by cells shown in red) and counteracts the inhibitory effect of Tregs on both Th1 (interferon- $\gamma$  [IFN- $\gamma$ ]) and Th17 (IL-17) proinflammatory responses. In contrast, IL-17 interaction with IFN- $\gamma$ -producing cells (depicted by cells shown in green) via an IL-17 receptor inhibits generation of IFN- $\gamma$ -producing cells. Thus, the Th17 response is both permissive and inhibitory of the Th1 response, probably at different phases of the inflammatory cycle.

### Concept of Inflammatory Microdomains

So far in our discussion of experimental colitis, we have focused mainly on data from the cell-transfer model of colitis. However, it is not prudent to rely too heavily on this model as a mirror of CD pathogenesis, given the fact that colitis in this model develops under the very special conditions that obtain in an initially lymphopenic host. In addition, genetic studies of CD strongly suggest that unlike the situation in cell-transfer colitis, the T-cell response directly or indirectly reflects a genetically determined tendency to hyper-respond to one or several microbial components in the commensal microflora.<sup>53</sup> For these reasons, an experimental colitis induced by tri-nitro phenyl (TNP)-modified antigens in the gut lumen, TNBS-colitis, may in some ways better approximate CD because in this model the mucosal immune response to a strong antigenic stimulus (TNP-modified protein) has been shown to be accentuated by a genetically determined hyper-responsiveness to lipopolysaccharide (LPS)

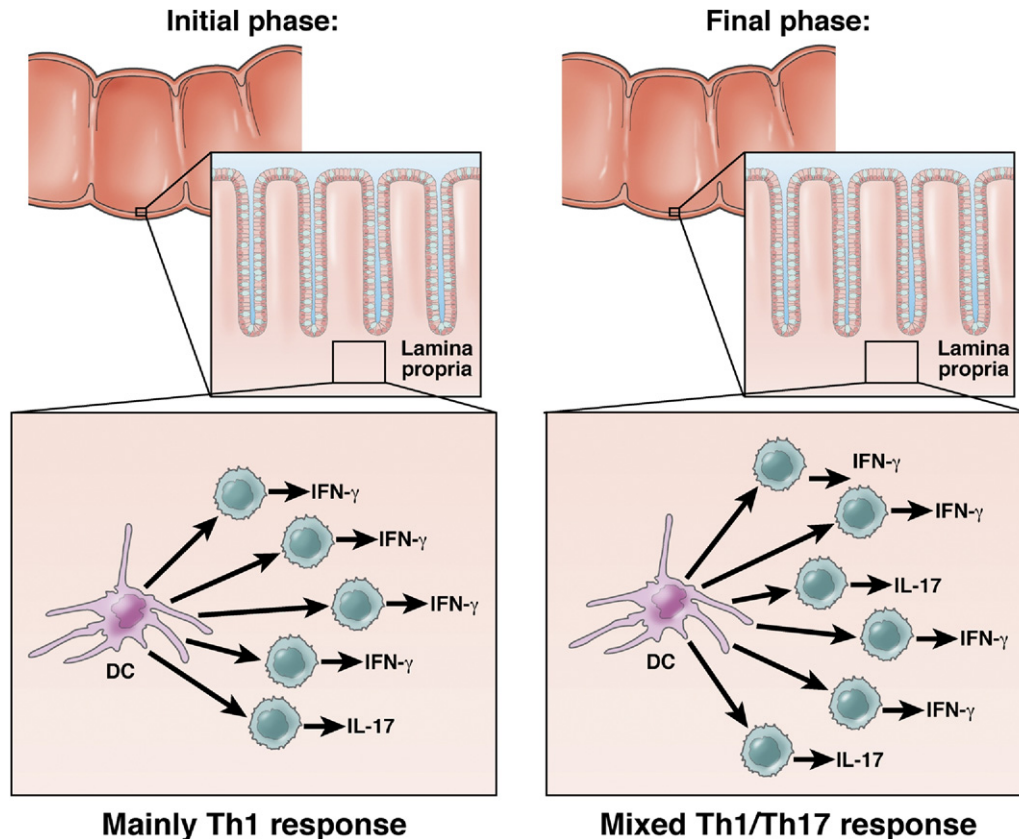
and perhaps other TLR ligands.<sup>54</sup> Thus, this model simulates the above-mentioned hyper-responsiveness.

Recently, it has been shown that a chronic form of TNBS-colitis can be induced in BALB/c mice by administration of weekly intrarectal instillation of relatively low doses of TNBS.<sup>55</sup> This form of TNBS-colitis is characterized by inflammation that is at once less intense and more prolonged than that in the acute model and thus allows detailed assessment of the evolution of the response. Of particular interest to the present discussion, the cytokine profile of mice with chronic TNBS-colitis proved to be unexpectedly dynamic: in the first few weeks of the colitis one sees a “pure” Th1 response characterized by high levels of both IL-12p40 and IFN- $\gamma$ , but no increase in the level of IL-17; by 3 weeks this response subsides, only to be replaced by the gradual increase in IL-23p19 production accompanied soon afterward by an increase in TGF- $\beta$  and IL-17 production; this Th17 response reaches a sustained plateau by 40–45 days and then subsides after 70 days. Several other cytokines making a delayed appearance, in this case shortly after the onset of the IL-23p19 response, include IL-25 and IL-13; these cytokines (along with TGF- $\beta$ ) are critical to devel-

opment of fibrosis in this model, as well as its ultimate resolution, despite continued TNBS administration. The remarkable aspect about the progression in cytokine responses in chronic TNBS-colitis is that the Th1 and Th17 responses are separate in time, suggesting that intestinal inflammation in humans may ordinarily be characterized by sequential responses that change the mix of Th1 and Th17 cells as the lesions mature. This concept is in keeping with the counter-regulatory character of Th1 and Th17 differentiation illustrated by the tendency of IL-17 to inhibit IFN- $\gamma$ -producing T cells and vice versa.<sup>31,33</sup> In addition, it is consonant with recent parabiosis and cotransfer studies showing that Th1 and Th17 are in competition and down-regulate one another.<sup>39</sup> On these bases, it is attractive to speculate that CD inflammation consists of multiple microenvironments, each reflecting different stages of a cytokine response cycle (see Figure 3).

### Th1 and Th17 Cytokine Responses in CD

Our evolving knowledge of cytokine production in CD is not unlike that in experimental models of



**Figure 3.** Inflammatory microenvironments characterizing CD. There is considerable evidence that T-helper cell (Th) 1 and Th17 responses are in an uneasy state of coexistence (see Figure 2). In this Figure, the concept is put forward that such coexistence is minimized by the fact that Crohn’s inflammation consists of innumerable microenvironments, each exhibiting a progression of inflammatory patterns. In the initial and most intense phase of the inflammation Th1 responses predominate; at this point, production of IL-23 in a nascent Th17 response inhibits regulatory T-cell (Treg) generation and feeds the inflammation. In a later phase, a mixed T-cell response prevails in which the Th1 response is still predominant but is now moderated by a Th17 response producing both interleukin (IL)-17 (which inhibits IFN- $\gamma$  T cells) and IL-22.

colonic inflammation. Thus, as in the latter case, early studies pointed to the presence of an underlying Th1 response characterized by lamina propria T cells that produced increased amounts of IFN- $\gamma$  and lamina propria antigen-presenting cells that produced increased amounts of IL-12p70.<sup>6</sup> These early findings were corroborated by later studies showing that lamina propria T cells expressed increased amounts of IL-12/IFN- $\gamma$ -induced factors, such as signal transducer and activation of transcription (STAT) 4, T-bet, and IL-12R $\beta$ 2.<sup>37,56</sup> Later, with the discovery that Th17 responses might drive experimental colitis, IL-17-producing cells were sought in the lamina propria of IBD patients. In one study, elevations were found in both CD and UC (but to higher extent in the former than in the latter) and in another study, IL-17R messenger RNA was actually higher in UC than in CD, although in the latter disease the level correlated with areas of disease activity.<sup>57-59</sup> More importantly, however, it was the discovery in 2006 that single nucleotide polymorphism in the IL-23R gene was associated with decreased susceptibility to both forms of IBD that really concentrated attention on the potential role of Th17 responses in these diseases.<sup>60</sup>

Two studies have recently appeared that address the importance of the Th17 response in CD vis-à-vis, the Th1 response. In the first, attention was focused on the function of patient mesenteric lymph node T cells and dendritic cells, that is, cell populations considered to reflect the responses occurring in the lamina propria. In initial studies, it was shown the CD4<sup>+</sup> T cells from CD mesenteric lymph node produced increased amounts of both IFN- $\gamma$  and IL-17 compared to UC mesenteric lymph node and, in this case, the IL-17 levels in UC were barely elevated. Importantly, T-cell IFN- $\gamma$  production level was some 40-fold greater than the IL-17 production level.<sup>61</sup> Thus, as in the case of cell-transfer colitis, the IFN- $\gamma$  response is far greater than the IL-17 response and one cannot assume the former was originating from Th17 cells producing both IL-17 and IFN- $\gamma$ . In further studies, the types of dendritic cells were analyzed and only minor differences were found among patients and controls; however, functional studies of one of the DC types (mature DCs) assessing their ability to induce cytokine in allogeneic T cells showed that the induction of cells producing IFN- $\gamma$  was vastly greater than those producing IL-17.<sup>61</sup> Taken together, these studies strongly suggest that, from a quantitative point of view, the Th1 response in CD is far greater than the Th17 response and thus that the Th17 response is not likely to be the major source of effector cytokines.

In a second study, in which a somewhat different picture emerged, evidence was first presented showing that the surface marker CD161 identified T cells in the peripheral blood that in the resting state produced IL-17 and IL-22, but relatively little IFN- $\gamma$ . In addition, these cells expressed the Th17-associated receptor, IL-23R.<sup>62</sup>

The authors concluded that CD161 is a surrogate marker for Th17 cells. In additional studies in which cells from CD and control peripheral circulation and lamina propria were analyzed, it was shown that CD peripheral cells contained a higher percentage of IL-23R<sup>+</sup> cells and exhibited increased IL-23-induced IL-17 and IFN- $\gamma$  production compared to control cells. In addition, while CD lamina propria harbored increased numbers of CD161<sup>+</sup> cells, the frequency of CD161 expression among CD4<sup>+</sup> cells was high in both patient and control tissue (on the order of 80%). This may be due to the fact that CD161 appears on activated cells and in the activating milieu of the lamina propria, CD161 may be associated with both Th1 and Th17 cells. Although these studies suggest that Th17 cells are, in fact, increased in the circulation and lamina propria of CD as compared to controls, they do not provide clear-cut information about the frequency of these cells vs the frequency of Th1 cells in patient intestinal tissues.

Overall, studies of CD relating to the question of the relative importance of the Th1 and Th17 response to inflammation are in agreement with the findings in murine models in that they suggest that although Th17 responses occur in human disease and potentially play some role in the inflammatory process, the Th1 response is quantitatively greater and thus more likely to be the driving force of the inflammation. This conclusion is consonant with the known pathologic effects of IFN- $\gamma$  in the intestine, the relation of Th1 responses to granulomatous disease, and the Th1 provenance of extraintestinal lesions.<sup>40,63</sup> In addition, it is consonant with results of a recent blinded clinical trial of anti-IL-17 in patients with CD, which showed that anti-IL-17 had no therapeutic effect (personal communication with W. Hueber, "Inhibition of IL-17A by Secukinumab Is Ineffective for Crohn's Disease," to be presented European Crohn's and Colitis Organisation, 2011). Finally, with respect to the protective role of the IL-23R polymorphism, it should be kept in mind that this polymorphism does not necessarily relate to the magnitude of the Th17 response because we have seen that it also can control the regulatory function of Foxp3 T cells<sup>45</sup>; it might lead to less disease because it is associated with more negative regulation than with less proinflammatory Th17 activity.

### Cytokine Responses in UC

As mentioned at the outset of this review, the cytokines driving UC were identified as having Th2-like characteristics in the initial studies attempting to place it within the Th1/Th2 spectrum. These consisted of lamina propria cells producing increased amounts of IL-5 in the conspicuous absence of increased amounts of IL-4, the more defining Th2 cytokine; hence the descriptive phrase, Th2-like.<sup>3</sup> Also absent was any hint of an increased IFN- $\gamma$  response, ruling out the presence of a Th1-driven inflammation. More recently, several studies have appeared

showing that IL-17 levels were increased in UC compared to controls, but in the most reliable studies in which protein rather than messenger RNA was measured this increase was found to be far less than that found in CD.<sup>61</sup>

In an attempt to gain insight into the origin of this kind of inflammation, investigators studied several colitis models driven by a Th2 response, such as T-cell receptor (TCR)  $\alpha$ -chain deficiency and late-phase IL-10 deficiency.<sup>64</sup> However, these models differed from UC because the dominant cytokine abnormality was an elevated IL-4 response, which is not observed in UC. A breakthrough came when it was discovered that intrarectal administration of oxazolone, an agent that, like TNBS, binds to self-proteins and renders them immunogenic, causes an intense but short-lived colitis that exhibits characteristics of UC.<sup>65</sup> The latter consisted of a superficial inflammation associated with microulcerations of the epithelium, edema of the bowel wall, and an inflammatory infiltrate containing granulocytes. In addition, the inflammation was most intense in the distal half of the colon, as frequently found in UC.

Initial cytokine analysis of oxazolone-colitis (oxa-colitis) revealed the presence of cells producing greatly increased amounts of IL-4 but not IFN- $\gamma$  and increased amounts of TGF- $\beta$ , a cytokine whose production is favored by Th2 conditions.

Furthermore, treatment of oxa-colitis with anti-IL-4 prevented disease, whereas as treatment with anti-IL-12p40 exacerbated disease. Thus, there could be no question that oxa-colitis, in contrast to TNBS-colitis, fit into the Th2 T-cell spectrum. It should be noted that these studies of oxa-colitis were conducted with SJL/J mice, a strain that proved to be more susceptible than C57BL/6 to oxa-colitis, as was the case for TNBS-colitis.

In a second study of oxa-colitis, mice were presensitized to oxazolone by skin painting and could thus be induced with a lower intrarectal dose of oxazolone, which caused a less intense and more persistent inflammation.<sup>66</sup> This modified oxa-colitis model revealed that the cytokine response, given more time, evolved from an initial IL-4 response lasting only several days into a more prolonged IL-13 response. In addition, the latter was shown to emanate from CD4<sup>+</sup> NKT cells and not from conventional CD4<sup>+</sup> T cells. Indeed, these studies proved that IL-13 and NKT cells are the driving force of oxa-colitis in that treatment of the latter with an IL-13 blocker, such as IL-13R $\alpha$ 2-Fc or with antibodies that delete NKT cells, leads to amelioration of the inflammation. In addition, mice with CD1 deficiency that lack the ability to present antigens to NKT cells as well as mice with J $\alpha$ 281-deficiency that lack the invariant TCRs that NKT cells usually use to recognize antigen were also unable to support oxa-colitis. Finally, stimulation of lamina propria T cells with CD1-expressing antigen-presenting cells loaded with  $\alpha$ -galactosyl-ceramide, the canonical antigen that has been shown to stimulate NKT cells expressing the

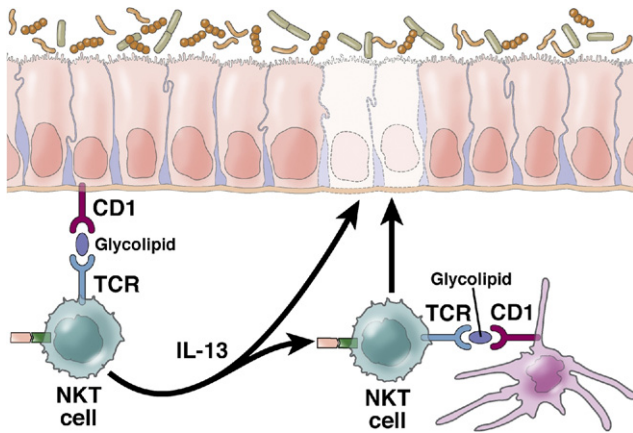
“invariant” TCR associated with NKT cells, led to high level production of Th2 cytokines, including IL-13. Taken together, these data offered definitive proof that oxa-colitis was due to NKT cells producing IL-13.

These studies of oxa-colitis set the stage for studies of UC, which would determine if an NKT cell/IL-13 abnormality was also present in this disease. Initial studies showed that stimulated lamina propria T cells from UC patients did indeed produce high levels of IL-13 but not IL-4 (or IFN- $\gamma$ ), whereas cells from CD patients produced only slightly increased amounts of IL-13 (compared to controls).<sup>11</sup> Similarly, flow cytometric studies revealed that UC patients had high levels of lamina propria CD4<sup>+</sup> T cells producing IL-13, whereas CD patients had only minor increases in such cells. In further studies, lamina propria T cells from UC patients were shown to produce IL-13 when stimulated with B cells stably transfected with CD1d, that is, cells that preferentially activate NKT cells. It was noted, however, that stimulation of UC lamina propria cells with  $\alpha$ -galactosyl-ceramide did not lead to activation of NKT cells as determined by up-regulation of V $\alpha$  and V $\beta$  chain expression. It was evident that UC lamina propria harbored increased numbers of IL-13-producing NKT cells, but that these cells were not typical NKT cells bearing invariant T-cell receptors (capable of responding to  $\alpha$ -galactosyl-ceramide).

In a final series of studies, the potential of either NKT cells and/or IL-13 to mediate tissue damage was evaluated. It was found that purified lamina propria CD4<sup>+</sup> T cells were cytotoxic to HT-29 epithelial cells prestimulated with lipopoly saccharide (LPS) to up-regulate CD1d and that such cytotoxicity was augmented by IL-13. Furthermore, it was observed that IL-13 (but not IL-4) lowers the electrical resistance of HT-29 epithelial cell monolayers by inducing epithelial cell apoptosis and increasing expression of pore-forming tight-junction protein claudin-2.<sup>67</sup> Interestingly, the latter was also observed in patients with UC. These studies suggest that the presence of NKT cells in the lamina propria of UC can lead to ulceration and inflammation by NKT-cell-mediated cytotoxicity; in addition, such tissue injury is abetted by IL-13, which augments NKT cell cytotoxicity and has effects on epithelial cell integrity (see Figure 4).

These studies of UC provide a basic framework with which to understand the immunopathogenesis of UC; nevertheless, they leave certain basic questions unanswered, including the identification of the glycolipid antigen or antigens that stimulate UC NKT cells and the mechanism by which IL-13 stimulates NKT-cell cytotoxic activity. In addition, because they focus on IL-13 as a major factor in UC, they fail to explain why a polymorphism of the gene encoding the IL-23 receptor would result in protection from development of UC (as well as CD), although one possibility here is that because IL-23R is found on NKT cells, the polymorphism may be affecting NKT-cell activity.<sup>68</sup> Finally, it is important to men-





**Figure 4.** The pathogenesis of ulcerative colitis. Inflammation in ulcerative colitis is initiated by glycolipid antigen(s) rising from either the microbiota or epithelial cells acted upon by microbiota. These antigens are presented to NKT cells in the context of CD1 on the surface of epithelial cells or dendritic cells and the NKT cells so stimulated act as effector cells mediating disease. Resultant epithelial cell damage and ulcer formation is caused by the cytotoxic activity of the NKT cells, which recognize epithelial cells bearing antigen-loaded CD1 as targets or by IL-13, which has been shown to cause epithelial cell apoptosis and loss of epithelial barrier function; in addition, IL-13 enhances NKT cell cytotoxicity.

tion that these studies offer no formal proof that either IL-13 or NKT cells (or both) are pathologic factors in UC, as they manifestly are in oxa-colitis. Nothing short of clinical studies in which the efficacy of IL-13 or NKT cell inhibitors is tested under blinded conditions will suffice to establish this possibility. That said, one recently published clinical study does offer promising correlative evidence that IL-13 is a pathologic factor in UC. This consisted of a study of a group of patients with UC given IFN- $\beta$  therapy in which it was found that significant response to this therapy was clearly correlated with decreased IL-13 production by lamina propria T cells.<sup>69</sup>

### Effector Cytokines “Bridging” the Th1/Th17/Th2 Spectrum—Tumor Necrosis Factor–Like Ligand

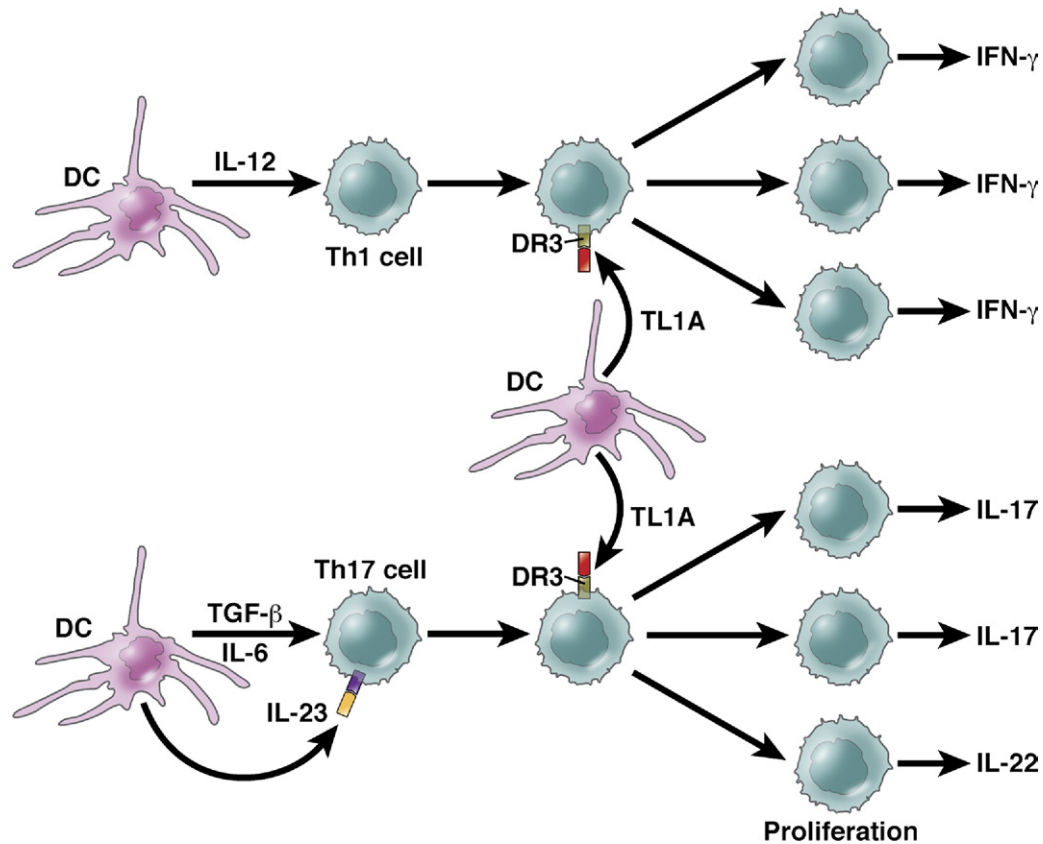
The cytokines discussed so far are those that are clearly located somewhere on the Th1/Th17/Th2 spectrum and that are mainly responsible for the distinctive type of inflammation characterizing the form of IBD with which they are associated. There are, however, a well-known group of additional cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, that are more promiscuous in their function because they are associated with both forms of IBD to a lesser or greater degree.<sup>53,64</sup> These cytokines generally arise secondary to the primary Th1/Th17 or Th2-like response as a result of stimulation of innate immune cells (macrophages, epithelial cells, mast cells, etc), which are drawn into the inflammatory milieu. In addition, each of these cytokines activate NF- $\kappa$ B and the mitogen-activated protein kinases, and thereby in-

duce various “downstream” proinflammatory effects that are the immediate precursors of tissue and organ pathology in IBD. It is important to bear in mind, however, that these cytokines are also involved in important “upstream” roles. IL-6 and possibly IL-1 $\beta$ , for instance, are essential for initial induction of Th17 responses.<sup>19,70</sup> In addition, TNF- $\alpha$  enhances production of IL-12, a function that might account for the particular (but not exclusive) association of this cytokine with Th1 responses.

TL1A (tumor necrosis factor–like ligand), a cytokine more recently shown to contribute to intestinal inflammation, also belongs to the category of cytokines that bridge the T-cell differentiation spectrum. This cytokine is secreted by both antigen-presenting cells and T cells (as well as by endothelial cells) and signals through DR3, a TNF-family receptor that is found primarily on T cells; in addition, it is induced in antigen-presenting cells by toll-like receptor (TLR) ligands and FcR cross-linking and in T cells by TCR stimulation.<sup>71</sup> The relevance to TL1A to colitis is shown by the fact that exogenous administration of TL1A to mice with DSS-colitis up-regulated both Th1 and Th17 responses of T cells extracted from the inflamed colonic tissue.<sup>72</sup> Furthermore, administration of anti-TL1A at least partially ameliorated DSS-colitis and, in a subsequent study, this antibody (or a DR3 blocker [DR3-Fc]) completely prevented development of TNBS-colitis.<sup>73,74</sup>

In recent studies of DR3-deficient mice, it was found TL1A-DR3 interactions costimulate cells subject to conventional TCR stimulation, but is not required for T-cell differentiation or for host-defense against toxoplasma infection.<sup>75</sup> However, it is required for the full expression of inflammation in experimental allergic encephalopathy and experimental asthma. The increased role of DR3 signaling in these latter situations is facilitated by increased TL1A expression induced by innate factors such as toll-like receptor (TLR) stimulation and Fc stimulation.<sup>71,75</sup> Recent studies of the function of TL1A in mice bearing a TL1A transgene expressed in T cells indicate that TL1A enhances baseline T-cell and B-cell activation by TCR stimulation and can induce spontaneous inflammation of the small bowel, most evident in the terminal ileum. Surprisingly, this inflammation was accompanied by greatly increased IL-13 synthesis, which proved to be driving the inflammation because the latter was prevented by anti-IL-13 administration.<sup>74</sup> Yet another new set of findings concerning TL1A is that while TL1A inhibits the induction of new Foxp3<sup>+</sup> regulatory T cells, it might induce expansion of existing Foxp3<sup>+</sup> regulatory T cells.<sup>76</sup> Overall, these studies in mice suggest that TL1A is a co-stimulatory cytokine that optimizes both Th1 and Th17 responses characterizing murine models of inflammation and, in addition, can induce its own unique form of inflammation (see Figure 5).

Studies of TL1A in IBD are consistent with the mouse studies discussed here. First, elevated TL1A levels have



**Figure 5.** TL1A function. TL1A is a tumor necrosis factor family member that acts exclusively via DR3. Its proinflammatory function bridges the T-helper cell (Th) 1/Th17–Th2 dichotomy in that it enhances both types of T-cell responses. As shown in this Figure, Th1 or Th17 differentiation results in T cells that express DR3 (the TL1A receptor) and are then subject to TL1A costimulation, the latter necessary for optimal proliferative and cytokine responses. Th2 cells are costimulated in a similar manner (not shown). TL1A has other effects, such as those of regulatory T cells (Tregs), which are not depicted here, that may also contribute to inflammation.

been noted in both CD and UC, indicating that TL1A is not associated with a particular form of T-cell differentiation.<sup>71,72,77,78</sup> Second, lamina propria CD14<sup>+</sup> macrophages in CD produce increased amounts of TL1A and the latter enhance alloantigen-induced T-cell production of both IFN- $\gamma$  and IL-17, but had little effect on its own<sup>72</sup>; thus, as in the mouse studies, TL1A appears to have an enhancing effect rather than a primary effect on inflammatory responses. Finally, it has been shown that polymorphisms in the TL1A gene are associated with increased risk for CD, indicating that the enhancing effects are not trivial and may in fact be necessary for disease expression in some patients.<sup>79</sup>

### Anticytokine Agents Likely to Be Useful in the Treatment of IBD

The review of the cytokine responses causing experimental and human intestinal inflammation presented here offers some guideposts relating to the type of new anticytokine therapy likely to be of use in the future treatment of CD and UC. Anti-TNF- $\alpha$  therapy has been and is likely to continue to be a major form of therapy for IBD. However, anti-TNF- $\alpha$  therapy is ineffective in

about 50% of initially treated CD patients and becomes ineffective in another 50% of patients over time; thus, the need for additional anticytokine therapies is clear.<sup>80</sup> The reason for such treatment failure is presently unknown, despite considerable study, but most likely relates to the fact that anti-TNF- $\alpha$  is either primarily or secondarily unable to cause apoptosis of T cells in many patients.<sup>81,82</sup>

Anti-IL-12p40 is a major new line of therapy that was proven to have therapeutic efficacy in initial phase 1 and 2 clinical trials.<sup>9,10</sup> However, in the most recent trial involving the largest group of patients, it had significant but rather unspectacular effects, at least during the induction phase, and only marginal effects during the maintenance phase.<sup>83</sup> It should be noted, however, that in this latter study, doses of antibody administered during induction were considerably separated in time and it may be necessary to provide a more concentrated dosing schedule to see clear-cut therapeutic effects. Although anti-IL-12p40 has the potential to neutralize both Th1 and Th17 responses, its ability to neutralize the former may be its most important function, assuming that, as discussed here, the main function of the Th17 response is to restrain the regulatory T cells that would otherwise

inhibit Th1 response. For this reason, anti-IL-23p19 is, theoretically, likely to be a less effective anti-inflammatory agent as anti-IL12p40, particular if it inhibits the anti-inflammatory effects of IL-22 and the down-regulatory effects of IL-17 on Th1 response.<sup>49,50</sup> Another anti-Th1 agent, anti-IFN- $\gamma$ , has already been subjected to trial and has been found to have little effectiveness, as has anti-IL-17.<sup>84</sup> This may be because only anticytokine agents that cause apoptosis of effector elements, such as anti-IL-12p40 and anti-TNF- $\alpha$ , are likely to have major therapeutic benefit. In the absence of this quality, a therapeutic anti-cytokine (such as anti-IFN- $\gamma$ ) must accomplish the daunting task of constantly neutralizing newly produced inflammatory cytokines.<sup>85</sup>

Several years ago, anti-IL-6 was proposed as a useful anti-inflammatory agent in IBD and other autoimmune diseases and some success in the treatment of rheumatoid arthritis with this agent has been reported.<sup>86,87</sup> This form of anticytokine therapy may owe its effectiveness to the fact that antibody binding to IL-6 interferes with the ability of this cytokine to bind to soluble IL-6 and to thus engage in “trans-signaling” via insertion of the IL-6/IL-6 receptor into the cell, which ordinarily delivers an anti-apoptotic signal to the cell. Thus, anti-IL-6 prevents apoptosis of effector cells and prolongs inflammation.<sup>88</sup>

Finally, anti-TL1A has been proposed as a useful anticytokine therapy, owing to the ability of this anticytokine to potentially block the ability of TL1A to enhance proliferation of both Th1 and Th2 effector cells.<sup>72-77</sup> However, because TL1A has no effect on T cells in the absence of a primary stimulus, anti-TL1A is most likely to work in tandem with other forms of anticytokine therapy.

## Conclusions

This analysis of the cytokine responses mediating intestinal inflammation in IBD calls attention to the multilayered and complex nature of these responses, which point with ever more clarity to a rational anticytokine therapy of IBD that holds great promise for providing an effective approach to long-term control of IBD inflammation.

## Supplementary Material

Note: The first 50 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of the article. Visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org), and at [doi:10.1053/j.gastro.2011.02.016](https://doi.org/10.1053/j.gastro.2011.02.016).

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#### Reprint requests

Address requests for reprints to: Warren Strober, MD, Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Dr. CRC Bldg., 5 West Labs Rm 3940, Bethesda, Maryland 20892. e-mail: [wstrober@niaid.nih.gov](mailto:wstrober@niaid.nih.gov); fax: 301-402-2240.

#### Conflicts of interest

The authors disclose no conflicts.

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