The Pathogenesis of Mucosal Inflammation in Murine Models of Inflammatory Bowel Disease and Crohn Disease

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In recent years, it has become apparent that overproduction of the Th1 cytokines interleukin-12 and interferon-γ is the probable driving force behind murine models of intestinal inflammation resembling Crohn disease and intestinal inflammation in humans with Crohn disease. In addition, studies of murine models strongly suggest that this overproduction is associated with inadequate secretion of the counter-regulatory and anti-inflammatory cytokine transforming growth factor-β. Thus, mucosal inflammation in models (and possibly in humans) may result from an imbalance between normally occurring positive (immunogenic or inflammatory) responses and negative (tolerogenic or anti-inflammatory) mucosal immune responses. These new findings and the hypotheses that arise from them are being used to construct new approaches to the treatment of Crohn disease that are based on the administration of anti-inflammatory cytokines and anti-cytokine antibodies.


Dr. Warren Strober (Mucosal Immunity Section, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH], Bethesda, Maryland): Recent advances in knowledge of T-cell differentiation and cytokine production are providing new insights into the nature of autoimmune diseases and other diseases involving immune dysregulation (1). These advances are often based on studies of various spontaneous or induced animal models of immune dysfunction, particularly models that resemble human disease.

We focus on Crohn disease, a major form of inflammatory bowel disease in which murine models have been used to great advantage (2-4). Studies of T-cell and macrophage function in patients with Crohn disease and parallel studies of various newly described murine models of inflammation resembling Crohn disease can be combined to provide important insights into the underlying mechanisms of disease (5-9). Furthermore, murine models can be used to devise potential cytokine- or anti-cytokine-based treatments for disease.

Crohn disease manifests primarily as a transmural inflammation involving the full thickness of the bowel wall that frequently leads to bowel obstruction, fistulas, and abscess formation. It thus differs from ulcerative colitis, which is a relatively superficial inflammation of the mucosa that leads to early ulcer formation. These two forms of inflammatory bowel disease also differ with regard to their distribution in the intestine. Crohn disease can occur anywhere in the gastrointestinal tract and has a predilection for the terminal ileum and ascending colon; it is discontinuous, with areas of inflammation alternating with normal areas. Ulcerative colitis, in contrast, is limited to the colon and usually begins in the rectal and sigmoid areas and progresses upward continuously (10).

Histologic studies of Crohn disease show a dense accumulation of activated T cells and macrophages, which in some cases are organized into typical granulomas. The earliest microscopic lesion in Crohn disease consists of a focal accumulation of lymphocytes and macrophages located next to an intestinal crypt. When this lesion is associated with epithelioid cells, it has the appearance of a protogranuloma; when it is associated with crypt epithelioid-cell destruction, it leads to the formation of an aphthous ulcer (11). Thus, it seems that interactions between lymphocytes and macrophages are at the core of the pathology of Crohn disease. In contrast, in ulcerative colitis, the cellular infiltrate is more variegated and acute inflammatory events, such as neutrophils forming crypt abscesses, are prominent. Lymphocytes and macrophages are present, but granulomas are not (12).

The pathologic findings in Crohn disease seem to result from the interplay of environmental and genetic factors. The former are evident from the observation that the incidence of Crohn disease is greatly increased in urbanized, economically advanced societies, whereas the latter are expressed by the fact that Crohn disease (and ulcerative colitis) manifests a strong familial pattern and can be linked to disease susceptibility loci on chromosomes 3, 6, 7, 12, and 16 (10, 13-16). We believe that these factors...
may work through the formation of a dysregulated mucosal immune response that leads to chronic mucosal inflammation.

The clinical course of Crohn disease is characterized by recurrent episodes of abdominal pain and other symptoms arising from bowel inflammation and the sequelae of inflammation (bowel fistulae and abscesses). In more than half of patients, surgery is required to remove diseased tissue but is rarely curative, and 60% of surgical patients have recurrence within 10 years (17, 18). Medical therapy for Crohn disease consists of anti-inflammatory agents, such as aminosalicylates or steroids, and immunosuppressive agents, such as 6-mercaptopurine. These drugs, however, suppress rather than cure the inflammatory disease. Clearly, new treatment approaches are needed for patients with Crohn disease.

Cytokine Production in Crohn Disease

Dr. Ivan J. Fuss (Mucosal Immunity Section, NIAID, NIH): Intestinal lesions in Crohn disease are replete with activated T cells. Investigators in our laboratory and others have attempted to characterize the function of these T cells with regard to their most cogent immunologic function: their capacity to produce various cytokines. We knew of recent studies showing that if naive CD4+ T cells are cultured under conditions that favor interleukin-12 production, Th1 T cells that produce IFN-γ and tumor necrosis factor are induced. In contrast, if naive CD4+ T cells are cultured under conditions that favor interleukin-4 production, Th2 T cells that produce interleukin-4, interleukin-5, interleukin-6, and interleukin-13 emerge. Furthermore, this pattern of T-cell differentiation is associated with distinct functional activities: Th1 T cells are the key players in delayed-type hypersensitivity reactions, whereas Th2 T cells are potent inducers of antibody-mediated immunologic reactions (1). We sought to determine whether cytokine secretion in Crohn disease (or ulcerative colitis) allows characterization of T cells in the lesions as Th1-type or Th2-type T cells (19).

In our studies (2), we extracted purified CD4+ T cells from lesional intestinal tissue obtained at surgery from patients with Crohn disease and ulcerative colitis or from controls who came to surgery for cancer and other noninflammatory diseases. We cultured the cells in a polyclonal T-cell stimulant, the anti-CD2-anti-CD28 antibody pair that has been shown in previous studies (20, 21) to be a particularly good stimulant of activated T-cell populations, such as those in the intestinal lamina propria. As shown in Figure 1, stimulated CD4+ T cells from patients with Crohn disease produced approximately two times more interferon-γ than did CD4+ T cells from controls or patients with ulcerative colitis. In parallel studies, we enumerated stimulated CD4+ T cells that produce interferon-γ by using an enzyme-linked immunospot assay technique (2) in which cells that produce a particular cytokine are identifi-
fied by the footprint that they leave on a surface coated with anticytokine antibody. As in the case with interferon-γ secretion, the number of cells secreting interferon-γ was increased in the inflamed lamina propria of patients with Crohn disease but not patients with ulcerative colitis. Finally, we found that mature (CD45RO+) CD4+ T cells in the peripheral circulation of patients with Crohn disease, when cultured and stimulated as described above, secreted three times more interferon-γ than did mature CD4+ T cells from controls. This circulating T-cell subpopulation is likely to contain mucosal cells that have re-entered the circulation; thus, the increased interferon-γ production seen in cultures of this subpopulation is probably a peripheral reflection of secretion by cells in the inflamed mucosa.

To obtain estimates of CD4+ T-cell production of interleukin-4 and interleukin-5 in the lamina propria, we analyzed culture supernatants (obtained as described above) for their interleukin-4 and interleukin-5 content. We found that production of interleukin-4 by cells from tissues of Crohn disease and ulcerative colitis was greatly decreased compared with production of interleukin-4 by cells from control tissues. By contrast, production of interleukin-5 by CD4+ T cells in the lamina propria of patients with Crohn disease decreased, whereas production of interleukin-5 by CD4+ T cells in the lamina propria of patients with ulcerative colitis increased substantially (Figure 1). As with the interferon-γ studies, these results were corroborated by studies using the enzyme-linked immunospot technique, which showed that CD4+ T cells obtained from the lamina propria of patients with ulcerative colitis contain an increased number of cells secreting interleukin-5.

These data, in conjunction with data from previous studies of inflammatory bowel disease, establish that the cytokine secretion profile of lesional Crohn CD4+ T cells (that is, production of large amounts of interferon-γ and small amounts of interleukin-4 and interleukin-5) is most consistent with the Th1 category of T cells (2, 3, 22, 23). As studies of several human diseases and experimental models of inflammation show (24-29), this T-cell category is associated with granulomatous inflammatory reactions. Thus, its presence in Crohn disease is consonant with and probably responsible for granulomatous inflammation. The cytokine data also show that T cells extracted from lesional colonic tissue in ulcerative colitis are qualitatively different from those in Crohn disease in that their reduced production of interferon-γ and their elevated production of interleukin-5 makes them more like Th2-type T cells. In this regard, only their unexplained lack of high interleukin-4 production prevents the definite assignment of lesional CD4+ T cells to this category.

Recent work has shown that Th1 T-cell differentiation occurs when T cells interact with antigen-presenting cells and the latter produce interleukin-12, the key inductive cytokine of Th1 T-cell differentiation and growth (30). Thus, if T cells in the lesional tissues of Crohn disease are in fact Th1 T cells, production of interleukin-12 in these tissues should also be increased. To explore this possibility, Neurath and colleagues performed immunohistochemical studies of frozen tissues from patients with Crohn disease and ulcerative colitis and from controls. They found that inflamed Crohn disease tissue stained positive for interleukin-12, whereas tissue with ulcerative colitis and control tissue did not (Neurath M, Fuss I, Pettersson S, Schurmann G, Herfarth C, Meyer zum Buschenfelde KH, et al. Up-regulation of the interleukin-12/stat-4 pathway distinguishes Crohn disease from ulcerative colitis. In preparation). These in situ data agree with data from studies done by Monteleone and colleagues (31), in which lipopolysaccharide-stimulated mononuclear cells extracted from the lamina propria of patients with Crohn disease had increased production of interleukin-12.

These results allow us to piece together a probable final common pathway of the immunologic process that results in Crohn disease. First, when the mucosal immune system in patients who develop Crohn disease is exposed to an initiating antigenic stimulus, it mounts a dysregulated and excessive Th1 T-cell response characterized by increased interleukin-12 production by antigen-presenting cells followed by increased interferon-γ production by CD4+ T cells. Interferon-γ then acts on macrophages to induce the production of proinflammatory cytokines, such as tumor necrosis factor-α, interleukin-6, and interleukin-1β, which are the proximal causes of inflammation in Crohn disease (32). Finally, tumor necrosis factor-α and interferon-γ produced during the Th1 T-cell response act in synergy to “back-stimulate” further production of interleukin-12 by antigen-presenting cells; this positive feedback effect amplifies and sustains the Th1 T-cell-mediated inflammatory response (33).

This proposed final common pathway of Crohn disease immunopathogenesis is an important step forward because it provides a blueprint for cytokine- or anti-cytokine-based treatment of the disease. However, by establishing the existence of this pathway, we necessarily define new and more precise questions about the origin of Crohn disease. For instance, what is the nature of the antigenic trigger that initially induces or sustains the abnormal Th1 response, and what is the nature of the underlying immunologic abnormality or abnormalities that lead to its presence? Answers to these questions are
emerging from recent studies of murine models of mucosal inflammation that resemble Crohn disease.

**Interleukin-2 Knockout Mice: A Model of Colitis Associated with Central and Peripheral Dysregulation of the Th1 T-Cell Response**

Dr. Björn R. Lúdvíksson (Mucosal Immunity Section, NIAID, NIH): The murine models of mucosal inflammation now available are, by their very diversity, providing important new clues to the various possible mechanisms of inflammatory bowel disease (5–9, 34). Because patients with Crohn disease have T cells in lesional tissue of the lamina propria that produce reduced amounts of interleukin-2 (2, 35, 36), we reasoned that the model of murine colitis in interleukin-2 knockout mice—which do not produce interleukin-2 because of a mutation in the interleukin-2 gene—may be particularly important to the understanding of Crohn disease.

Initial studies of interleukin-2 knockout mice found that these mice develop colonic inflammation when reared in a conventional environment with normal bacterial flora but remain healthy when reared in a sterile or pathogen-free environment (6). This observation suggests that exposure of these mice to one or more antigens present in the normal microflora is necessary to trigger colitis and led us to determine whether parenteral administration of various normally innocuous antigens to interleukin-2 knockout mice reared in a pathogen-free environment would induce colitis on demand. One such antigen, trinitrophenyl-keyhole-limpet hemocyanin (TNP-KLH), and other trinitrophenylated proteins (given intraperitoneally in complete Freund adjuvant) induce severe transmural colitis in pathogen-free interleukin-2 knockout mice within days of administration. By contrast, normal mice remain free of disease after similar treatment (7). The ability of trinitrophenyl-substituted protein to induce colitis is probably related to the cross-reactivity between trinitrophenyl-substituted protein and antigens in the gut microflora and therefore the ability of trinitrophenyl protein to induce an ongoing response to such microflora.

In subsequent studies, we characterized the immune response in interleukin-2 knockout mice to colitis induced by TNP-KLH (7). We found that the colonic lesional tissue was infiltrated predominantly by CD4+ T cells and that such cells were necessary for disease because in vivo depletion of CD4+ T cells by administration of anti-CD4 antibody prevented inflammation. Furthermore, we found that the CD4+ T cells produced large amounts of interferon-γ but little or no interleukin-4, whether they were evaluated by studies of cytokine mRNA production in vivo or interferon-γ protein production in vitro. Finally, we used immunohistologic staining methods to show that the inflamed tissue of interleukin-2 knockout mice contained markedly increased amounts of interleukin-12 and that treatment with anti-interleukin-12, either prospectively (at the time of TNP-KLH administration) or retrospectively (after TNP-KLH administration) prevented or cured the induced colitis (7).

These observations suggest that the likely immunopathologic scenario leading to colitis in interleukin-2 knockout mice begins with exposure to an antigen that elicits an ongoing, unregulated Th1 T-cell response. The Th1 cytokine interferon-γ is then produced and acts on macrophages to induce the production of the proinflammatory cytokines that are the immediate cause of the inflammation. Thus, the final common pathway of disease pathogens in interleukin-2 knockout mice is similar, if not identical, to that in humans with Crohn disease.

In further studies, we focused on the question of why TNP-KLH elicits a Th1 T-cell response in interleukin-2 knockout mice and not in normal mice. One possibility is that in the absence of interleukin-2, the mice do not develop a Th2 T-cell response and thus do not produce Th2 cytokines, such as interleukin-4 and interleukin-10, that are normal counter-regulators of Th1 responses. The presence of a deficient Th2 T-cell response in interleukin-2 knockout mice is made likely by the fact that interleukin-2 is necessary for T-cell growth during the early stage of antigen-driven Th2 T-cell differentiation (19, 30, 37, 38). In addition, in the studies described earlier, interleukin-2 knockout mice manifested poor interleukin-4 responses after administration of TNP-KLH (7). A second possibility is that in the absence of interleukin-2, the mice are unable to elaborate various suppressor cytokines, such as transforming growth factor-β (39), at least under certain conditions.

In our investigation of these possibilities, we sought to explain the surprising observation that intraperitoneal administration of anti-CD3, a powerful polyclonal T-cell stimulant, in interleukin-2 knockout mice did not result in colitis and that concomitant administration of anti-CD3 and TNP-KLH prevented colitis induced by the administration of TNP-KLH alone. In an initial series of studies, we found that administering anti-CD3 to interleukin-2 knockout mice elicited both T cells that produced interleukin-4 and T cells that produced transforming growth factor-β, whereas administering TNP-KLH alone did not elicit T cells that produced either of these cytokines. We then found that administration of anti-CD3 and TNP-KLH was similar to administration of anti-CD3 alone in that it also elicited T
cells that produced interleukin-4 and transforming growth factor-β. Finally, to determine which of these two cytokines might be responsible for the ability of anti-CD3 to prevent colitis, we conducted studies in which we administered either anti–transforming growth factor-β or anti-interleukin-4 to interleukin-2 knockout mice given both anti-CD3 and TNP-KLH. We found that the mice given anti–transforming growth factor-β, but not those given anti-interleukin-4, developed colitis (40). These results are compatible with the view that the development of colitis after TNP-KLH administration is caused by the inability of interleukin-2 knockout mice to mount a transforming growth factor-β response after antigen administration and not by their inability to produce interleukin-4.

The idea that T-cell dysregulation leading to the development of colitis can ultimately be traced to a deficiency in the ability to mount a counter-regulatory (suppressive) transforming growth factor-β response holds for other models of experimental colitis as well (41) and can be applied to mucosal homeostasis in normal mice because the latter develop colitis (albeit transient colitis) if they receive TNP-KLH along with anti–transforming growth factor-β (40). In addition, this concept is consonant with the fact that in normal mice, oral tolerance (the immunologic mechanism by which the mucosal immune system ordinarily establishes a state of unresponsiveness to ingested soluble protein) is also at least partly mediated by transforming growth factor-β (42–45). Thus, transforming growth factor-β emerges as a common denominator in the negative regulation of mucosal responses in both normal and pathologic states, and dysregulation of this factor may be an underlying cause of Crohn disease.

Finally, it is important to mention that TNP-KLH in interleukin-2 knockout mice also has central, intrathymic effects that probably play a role in the development of colitis in these mice. In particular, we have shown that interleukin-2 knockout mice given TNP-KLH rapidly develop a thymocyte profile marked by decreased numbers of CD4^+ CD8^- (double-positive) thymocytes and increased numbers of CD4^+ (or CD8^+) single-positive thymocytes (Figure 2). In further studies, we found that the single-positive cells are activated cells that manifest a Th1 T-cell cytokine ability and that adoptive transfer of the CD4^+ (single-positive) cells to a naive recipient leads to induction of colitis (46). These and previous studies of interleukin-2 knockout mice introduce the possibility that a central thymic defect contributes to and is perhaps fundamental to the dysregulated T-cell response underlying colitis in interleukin-2 knockout mice (47, 48). Therefore, central defects in T-cell differentiation may also play a role in Crohn disease.

Trinitrobenzene Sulfonic Acid Colitis: An Experimental Model of Induced Colitis Responsive to Treatment with Anti-Interleukin-12 and Oral Tolerance Induction

Dr. Warren Strober (Mucosal Immunity Section, NIAID, NIH): Studies of interleukin-2 knockout mice showed that an uncontrolled Th1 T-cell-mediated inflammation, such as that likely to be present in Crohn disease, can arise from a fundamental defect in the immune response, such as interleukin-2 deficiency. Further insight into the possible causes of Th1 T-cell-mediated inflammation came from studies of mice with intact immune systems that were subjected to an antigenic challenge that, in effect, subverted normal regulatory mechanisms (49). This is the murine model of chronic inflammation, induced in immunocompetent SJL/J or BALB/c strains of mice by intrarectal administration of trinitrobenzene sulfonic acid (TNBS), is known as TNBS colitis.

Trinitrobenzene sulfonic acid is a haptenating agent that couples trinitrophenyl groups to endogenous (self) proteins more or less indiscriminately; the altered self-proteins stimulate a local immunologic response usually called a delayed hypersensitivity reaction (5, 50, 51). In the colon, this reaction leads to an inflammation marked by a transmural cellular infiltration with T cells and macrophages in a pattern like that described in interleukin-2 knockout mice and Crohn disease (5—7, 11, 12).

An initial immunologic analysis of mice with TNBS colitis showed that the lesions are dominated by CD4^+ T cells. When cultured in vitro with anti-CD3/anti-CD28 stimulant, these cells produce greatly increased amounts of interferon-γ and de-
creased amounts of interleukin-4. Because this response profile indicates a Th1 T-cell response, we examined macrophage production of interleukin-12 in the lamina propria. Using a histochemical technique to determine interleukin-12 production in situ, we showed that TNBS colitis was also associated with increased production of interleukin-12 (5).

On the basis of these immunologic findings, we proposed the following sequence. First, colonic proteins haptenated by TNBS are taken up, processed, and presented to T cells by antigen-presenting cells throughout the colonic lamina propria (dendritic cells or macrophages). Second, this presentation, occurring in the absence of adequate counter-regulatory mechanisms, leads to excessive secretion of interleukin-12 and induction of an unbalanced Th1 T-cell response. Finally, the Th1 cytokines, particularly interferon-γ, act on macrophages to induce the production of additional proinflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β, and interleukin-6, which then serve as the immediate causes of the inflammation.

In subsequent studies, we verified key elements of this proposed sequence. First and perhaps most important, we found that neutralization of the mucosal interleukin-12 response by systemic administration of anti interleukin-12 led to complete abrogation of TNBS colitis, whether interleukin-12 was given at the time of TNBS administration as a preventive measure or several weeks later as a treatment (5). Second, we found that interferon-γ is a necessary mediator of colonic inflammation in that TNBS colitis could be downregulated by the administration of antibodies to interferon-γ. Finally, we found that tumor necrosis factor-α and other proinflammatory cytokines are critical components of the effector phase of colonic inflammation because the inhibition of the synthesis of these cytokines at the messenger RNA level also blocked the development of colitis (52). Together, these findings probably indicate that a poorly regulated Th1 T-cell response is the key mechanism underlying the pathogenesis of TNBS colitis.

After defining the major immunologic features of TNBS colitis, we sought to explain why TNBS (and, by extension, trinitrophenyl-haptenated colonic proteins) elicit a severe colonic inflammation but more common mucosal antigens (such as those associated with the intestinal microbiota or food substances) do not. The induction of a mucosal response by intrarectal administration of TNBS may be a form of mucosal immunization that bypasses the normal oral tolerance mechanisms by which the mucosal immune system limits responses to common mucosal constituents. It is important to understand that two such mechanisms of oral tolerance have so far been defined. In one mechanism, which dominates when the dose of ingested antigen is high, antigen entering the mucosal immune system via mucosal follicles induces T-cell clonal deletion or clonal anergy so that tolerance occurs because of loss of responsive T cells. In the other mechanism, which dominates when the dose of ingested antigen is low, antigen entering the mucosal immune system via mucosal follicles induces T cells to produce a suppressor cytokine, such as transforming growth factor-β, so that tolerance occurs because positive responses are suppressed (53). On the basis of these mechanisms of oral tolerance, it can be postulated that intrarectal administration of TNBS leads to an unbalanced response because this type of administration is associated with failure of deletion or anergy induction or failure of suppressor-cell induction.

To investigate this possibility, we sought to modify TNBS colitis by administering TNBS orally (that is, by a route of antigen administration that ordinarily favors oral tolerance induction). Mice were given TNBS intrarectally, alone or in combination with an oral feeding of a crude mixture of colonic proteins (including proteins derived from colonic microflora) that had been prehaptenated with TNBS in vitro. Mice that were fed TNBS-haptenated colonic proteins and were simultaneously given TNBS intrarectally did not develop colitis and did not manifest an enhanced Th1 T-cell response in the colon. In contrast, mice given TNBS only intrarectally developed Th1 T-cell-mediated colitis. We also found that when stimulated, the T cells in the lamina propria of mice that were fed TNBS-haptenated colonic proteins produced large amounts of transforming growth factor-β, which may act as sup-

Figure 3. Mechanism of colitis induction and tolerance in trinitrobenzene sulfonic acid (TNBS) colitis. Intrarectal administration of TNBS and formation of trinitrophenyl-haptenated colonic protein lead to induction of a polarized Th1 T-cell response in the lamina propria that is not counter-regulated by transforming growth factor-β (TGF-β)–producing T cells; the resultant unfettered interferon-γ production leads to activation of macrophages, production of inflammatory cytokines, and development of colitis. Oral administration of trinitrophenyl-haptenated colonic protein leads to induction of T cells producing TGF-β, which shuts down the excessive Th1 T-cell response of TNBS colitis. APC = antigen-presenting cell; TGF-α = tumor necrosis factor-α; PP = Peyer patch; T = T cell.
pressor T cells to suppress the inflammation. To examine this possibility, we administered anti-transforming growth factor-β to mice that were receiving TNBS intrarectally and TNBS-haptenated colonic protein orally; this treatment resulted in colitis (41). These results support the contention that intrarectal administration of TNBS leads to a dysregulated immune response that bypasses normal mucosal homeostatic mechanisms, such as those involved in the induction of oral tolerance (41, 54, 55) (Figure 3).

Another important conclusion from our study of TNBS colitis is that TNBS colitis is initiated by the administration of TNBS and thus by the formation of haptenated colonic protein antigens but is probably sustained by intestinal microfloral antigens that stimulate TNBS-specific T cells in a cross-reactive manner. This idea is strongly supported by the fact that inflammation is limited to the colon and that T cells extracted from inflamed tissue and transferred to naive recipients induce colitis in the absence of exposure to TNBS (41). This conclusion and earlier ones made from results in interleukin-2 knockout mice imply that pathologic inflammation in Crohn disease can be induced initially by an exogenous agent that is the equivalent of TNBS in TNBS colitis and can then be continued in the absence of the exogenous agent by continued stimulation of the T cells by cross-reactive antigens in the normal mucosal environment. It can be postulated that in Crohn disease, cross-reactive antigens are found in both the large and small intestine, providing a possible explanation for the fact that inflammation in human Crohn disease is not limited to the colon as it is in murine models.

Taken together, results of studies of mice with TNBS colitis provide further insight into the immunopathogenesis of a mucosal inflammation resembling Crohn disease by showing that such inflammation arises not only from an underlying immune defect but also from a response that bypasses normal counter-regulatory mechanisms. The TNBS colitis model and the interleukin-2 knockout model establish that an ongoing Th1 T-cell-mediated inflammation maintained by continuous stimulation by antigens in the intestinal microflora may occur in several different ways. It is therefore possible that Crohn disease has several possible origins, all of which lead to a similar final pathway.

Prospects for Cytokine Therapy in Crohn Disease

The data presented here on the immunologic findings in patients with Crohn disease and in murine models of colitis resembling Crohn disease provide a basis for various cytokine-based treatments of Crohn disease. In particular, if Crohn disease is ultimately due to an excessive Th1 T-cell response in the intestinal mucosa, it should be possible to treat patients in one of several ways that have been shown to counteract such a response. It is important to note that treatment would be applicable to patients regardless of the immunologic abnormality leading to the possible Th1 T-cell dysregulation and could therefore be effective even if Crohn disease arose from several different defects in the regulation of T-cell differentiation into Th1 T cells.

One possible means of abrogating the Th1 T-cell response in Crohn disease is the blockade of the effects of the multifaceted cytokine tumor necrosis factor-α. Tumor necrosis factor-α is involved in the inductive phase of the Th1 T-cell response by means of its ability to synergize with interferon-γ in the feedback enhancement of interleukin-12 production (33). In addition, tumor necrosis factor-α is involved in the effector phase of the Th1 T-cell response by means of its capacity to act as a pro-inflammatory cytokine that directly mediates mucosal inflammation. In the context of Crohn disease, one important component of tumor necrosis factor-α-mediated inflammatory activity is the activation of enzymes in the lamina propria, such as collagenase, and the release of substances that increase intestinal permeability (32, 56) and thus increase the exposure of T cells in the lamina propria to mucosal antigens that induce further adverse immune responses. In keeping with these functions of tumor necrosis factor-α, we have shown that established murine TNBS colitis can be treated by the systemic administration of anti-tumor necrosis factor-α (52). In addition, we have shown that TNBS colitis can be treated with intrarectal administration of an antisense oligonucleotide that gains access to intracellular sites and blocks the synthesis of a transcription factor (NF-κB) necessary for tumor necrosis factor-α gene transcription. Of note, this antisense oligonucleotide is more effective than corticosteroids for the treatment of TNBS colitis (57) and may prove to be the treatment of choice for local, accessible forms of Crohn disease.

The above studies of the treatment of experimental murine colitis with tumor necrosis factor-α antagonists were accompanied by clinical trials of the use of anti-tumor necrosis factor-α in humans with Crohn disease. These trials have clearly shown that anti-cytokine-based therapy is well tolerated and produces excellent clinical responses in some patients (58–60). Although these findings are promising, administration of anti-tumor necrosis factor-α addresses the effector phase of the Th1 T-cell response more than it does the inductive phase. Thus, the inflammatory process of Crohn disease may still eventually shift to other effector cytokines and be-
come resistant to tumor necrosis factor-α therapy, as has been the case in the treatment of some patients with rheumatoid arthritis (61).

Another method for treating Crohn disease by means of modification of the Th1 T-cell response is the use of cytokines, such as interleukin-10, and anti-cytokines, such as anti-interleukin-12. These agents act by inhibiting interleukin-12 synthesis (interleukin-10) or function (anti-interleukin-12) and, therefore, by inhibiting Th1-mediated inflammation (30, 37). On the basis of these considerations, trials using interleukin-10 to treat patients with Crohn disease are already under way (62), and we are currently developing a human anti-interleukin-12 antibody for initial evaluation in patients with severe Crohn disease. The use of anti-interleukin-12 is of particular interest, both theoretically because of its ability to affect Th1 responses during the inductive phase and practically because of its ability to effectively treat murine models of colitis.

In conclusion, it is apparent that the above findings from clinical and basic immunology research have increased understanding of the immunologic events leading to Crohn disease. Crohn disease can now, with some confidence, be classified as part of a larger group of autoimmune diseases or diseases of immune dysregulation characterized by an excessive Th1 T-cell response. Our studies begin to locate the cause of this dysregulation and establish that its effects are localized to the gastrointestinal tract, probably because of a particular propensity (genetically determined) to react with antigens in the intestinal microflora. The big news, however, is that regardless of the underlying factors that contribute to the Th1 dysregulation, therapies to control this condition are being developed. The coming years should witness considerable advances in the control of Crohn disease.

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