Regulation of experimental mucosal inflammation

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Studies conducted over the past 10 years have provided ample evidence that many types of inflammations arising from basic abnormalities of immune regulation are ultimately 'funneled' through a Th1 or Th2 T cell-mediated immune reaction. Thus, by understanding these types of reactions and, in particular, by identifying their natural checkpoints, one can control the inflammation regardless of its more basic causes. A case in point is the inflammatory disease of the intestine known as Crohn disease, a disease now thought to be due to one or more abnormalities leading to an excessive immune response to elements of the bacterial microflora of the gut. Both in murine models and by study of Crohn disease itself, we have shown that Crohn inflammation is due to a Th1 T-cell abnormality involving overproduction of interleukin (IL)-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α. In addition, we and others have shown that treatment of mice with anti-IL-12 or other agents that downregulate the level of IL-12 secretion can have a dramatic effect on the inflammation. This is because anti-IL-12 administration leads to apoptosis of activated Th1 T cells. A second checkpoint of Th1 T-cell-mediated inflammation involves its downregulation by the suppressor cytokine, transforming growth factor (TGF)-β. We have been delivering TGF-β to mice with experimental intestinal inflammation, using several novel approaches. In particular, we have successfully treated such mice with intranasally administered DNA encoding active TGF-β. Another approach currently under investigation is delivery of TGF-β by gene therapy. These and other developments in the understanding of inflammation paint a bright future for cytokine-based therapeutic agents. It is now apparent that these therapies are not only effective and safe but also potentially long-lasting. □ *Crohn disease; cytokines; Th cells*

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The advent of both spontaneous and induced models of mucosal inflammation in the last ten years has led to many new insights into the regulation of normal mucosal immune responses and the factors responsible for the development of inflammatory diseases of the mucosa (1). One major insight that has become apparent from the study of these models is that regardless of the immunologic basis of the experimental inflammation, the latter is dependent on the presence of a normal bacterial microflora (1–3). This is seen in the fact that in every case in which it has been studied, the inflammation does not develop under germ-free conditions. The significance of this fact is that human inflammatory bowel diseases are likely to be due to an abnormal response to a normal intestinal constituent rather than to an intestinal pathogen. Other quite independent studies showing that patients with this disease react immunologically to their own microflora support this concept (4). A second insight that derives from the models is that mucosal inflammation can arise from many different discrete immunologic defects. This implies that human inflammatory bowel diseases can be caused by several different underlying abnormalities and yet appear to be the same disease. Recent genetic studies of the human disease support this view in that they show that several different genetic loci can be identified and that disease in any given patient may be genetically distinct from disease in another patient (5). A third insight from the animal models, and one that has the most relevance to the treatment of human diseases, is that regardless of its etiology, the inflammation is due to a relatively small number of stereotypic immune responses that serve as the final common pathway of the inflammation. Thus, in the vast majority of the models the final common pathway is a Th1 T-cell-mediated immune response leading to the generation of Th1 inflammatory factors such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Similarly, in most of the remaining models the final common pathway is a Th2 T cell-mediated immune response leading to the generation of inflammatory factors induced by that response (1). The significance of this lies in the fact that one need not know the underlying genetic defect of the human inflammatory bowel to devise successful treatments for it but merely devise treatments that block one of the final common pathways. For example, the induced model of inflammation caused by the intrarectal instillation of trinitrobenzene sulfonic acid (TNBS), a pure Th1 T-cell-mediated inflammation in SJL/J mice, is promptly treated by the parenteral administration of anti-interleukin (IL)-12 and, in addition, responds to anti-TNF-α (6). The latter has already been shown to be an effective therapy of Crohn disease, and the former is currently being tested both in patients with this disease and in patients with other Th1 T-cell-mediated inflammations.

Classification of models of mucosal inflammation

One way of classifying the various models of mucosal inflammation is to group them into two broad categories on the basis of whether the inflammation is due to the overproduction of a key Th1 (or Th2) effector cytokine or to the underproduction of a regulatory cytokine. This classification is based on the fact that normal mucosal responses usually represent a balance between an effector response necessary for host defense and a tolerogenic response necessary to control potentially damaging reactions that could result in mucosal inflammation (7). In recent years it has been shown that the latter or tolerogenic response is composed of at least two elements, one of which is the induction of anergic T cells or the frank deletion of the latter, and the other the induction of regulatory T cells that produce suppressor cytokines such as transforming growth factor (TGF)-β or IL-10.

Several examples of models due to overproduction of an effector cytokine can be mentioned. The first is the TNBS model already alluded to (6) above. Recently, we have found that the strain susceptible to the Th1 T-cell-mediated form of this inflammation, the SJL/J strain, differs from the resistant C57BL/6 strain by virtue of the fact that the former shows a high IL-12 response after lipopolysaccharide (LPS) administration, whereas the latter does not. Since the induction of TNBS colitis requires instillation of TNBS dissolved in ethanol, it is likely that this method of induction is accompanied by disruption of the mucosal barrier, followed by exposure of the mice to a mucosal microflora rich in LPS. Thus, in effect, the mice are induced to produce IL-12, which then supports the Th1 T-cell response necessary for the inflammation. We can therefore say that TNBS colitis depends on an overexuberant IL-12 response. The second example of overproduction is the model of colitis described by Wirtz et al. (8), occurring in mice bearing a STAT4 transgene under a cytomegalovirus (CMV) promotor. STAT4 is the major intracellular messenger induced by IL-12 signaling, so that its overexpression would be expected to result in excessive Th1 T-cell responses. In fact, LPS administration to these mice results in an abnormally high IL-12 response and colitis. In addition, chronic colitis in STAT-4 transgenic mice but not control mice could be adoptively transferred to SCID mice by colonic and splenic CD4+ T cells that were activated with antigens present in autologous bacterial flora. This again shows that an exuberant Th1 response leads to cells that mediate colitis; in addition, it shows that, as indicated above, abnormal responses to its microflora is a cause of the Th1 response in a mouse with an appropriate abnormality.

Examples of models of mucosal inflammation due to inadequate suppressor cell response can also be cited. Two rather obvious models in this category are mice with IL-10 deficiency and recently reported mice bearing a transgene encoding a dominant negative TGF-β receptor, which thus do not respond to TGF-β (9, 10). In both cases these mice lack intact suppressor systems and manifest mucosal inflammation. Perhaps the best-known model in this category, however, is the SCID transfer model, which is produced by the transfer of naïve ($CD45Rb^{hi}$) T cells to SCID (or RAG-2-deficient) mice (11). Apparently, such colitis is due to the absence of a regulatory T cell because the co-transfer of mature ($CD45Rb^{lo}$) T cells along with the naïve T cells prevents the colitis. That the mature cells are in fact the source of a regulatory cell is indicated by the fact that if such cells are obtained from IL-10-deficient mice or are administered along with anti-TGF-β, they cease to provide protection against the development of colitis. In addition, regulatory T cells producing IL-10, known as Tr1 T cells, can substitute for the mature cells.

It should be noted at this point that these two models of possible causes of mucosal inflammation may mirror the mechanisms underlying inflammatory bowel disease. Indeed, the fact that the models can be classified in this manner suggests by implication that the human disease also broadly can be due to either excessive effector cell responses or inadequate regulatory responses. Ultimately, this may have considerable clinical relevance since it may be possible to tailor treatment of patients with inflammatory bowel disease on the basis of the broad category of defect present. Patients with an excessive effector cell response may best be treated by curbing the excessive response, whereas patients with an inadequate regulatory response may best be treated by providing an exogenous source of regulation.

Treatment of TNBS colitis with a DNA plasmid encoding TGF-β

As already alluded to above, efforts to treat mucosal inflammation have centered on use of anti-cytokine antibodies. These can theoretically be used in any type of inflammation, regardless of whether the underlying factor is an effector cell or a regulatory cell abnormality, since in either case the end result is an unchecked Th1 (or Th2) T cell response. The use of anti-IL-12 in this context is of particular note, since it has been shown that anti-IL-12 administration is followed by apoptosis of Th1 T cells, presumably because IL-12 acts as a growth factor for these cells (12). Evidence that anti-TNF-α has the same effect is inherent in the fact that such therapy in patients with Crohn disease leads to long-lasting remissions. However, a convincing demonstration that anti-TNF-α has this effect has yet to appear. It should be noted that anti-cytokines are by no means the only way to interrupt a Th1 T-cell response. One alternative that was recently been reported is the administration of the B subunit of cholera toxin, a substance that does not have the toxicity of the cholera holotoxin (13). Oral administration of the B subunit was shown either to prevent or to treat TNBS colitis by exerting a negative effect on IL-12 synthesis. This

treatment also results in T-cell apoptosis, as one might expect if the cells are being deprived of IL-12.

The approach of treating a model of colitis with a suppressor cytokine has also been recently reported and has been met with considerable success. This approach was first suggested by studies in which it was shown that mice that are pretreated with orally administered trinitrophenol (TNP)-haptenated colonic proteins subsequently develop TGF-β-producing cells in their lamina propria, which protects them from the development of TNBS colitis (induced by giving TNBS intrarectally (14). Thus the principle was established that the presence of increased numbers of T cells in the intestinal lamina propria capable of producing TGF-β can prevent Th1 T-cell-mediated colitis. In initial studies to exploit this finding, it was shown that TGF-β-producing cells could be created by the intranasal administration of a plasmid (naked DNA) encoding active TGF-β under a CMV promotor (15). Such administration, it could be shown, leads to the appearance of both macrophages and T cells (in both the spleen and the intestine) producing substantial quantities of TGF-β. In subsequent studies it was shown that administration of the TGF-β plasmid could both prevent the induction of colitis and, more importantly, treat colitis after it is already established.

The mechanism by which the plasmid prevents/treats a Th1 T-cell-mediated inflammation was examined by measuring cytokine production by T cells and antigen-presenting cells (APCs) after plasmid administration. It was found that, as expected, production of TGF-β by lamina propria T cells was greatly increased after plasmid administration, whereas both IL-12 and IFN-γ secretion was greatly decreased; thus, as in other situations, there was a reciprocality between TGF-β production and Th1 cytokine production. An unexpected finding was that IL-10 secretion was also increased by plasmid administration, and thus one mechanism of the TGF-β effect is the induction of a cytokine (IL-10) that has a known down-regulatory effect on IL-12 secretion. Interestingly, this was not the only mechanism, since administration of the plasmid along with a sufficient amount of anti-IL-10 monoclonal antibody to block IL-10 secretion was still accompanied by blockade of TNBS colitis induction. In this case, whereas IL-12 production was not affected by plasmid administration, IFN-γ production was greatly decreased, suggesting that IL-12 signaling was being blocked by the presence of TGF-β. Evidence that this was in fact the case came from studies in which the expression of the beta-2 chain of the IL-12 receptor was measured by flow cytometry in mice under various conditions. These studies showed that mice subjected to TNBS colitis induction who were treated with intranasal TGF-β-encoding plasmid manifested decreased expression of the beta-2 chain as compared with mice subjected to such induction and not so treated. Thus, it was likely that TGF-β-producing cells were inhibiting the Th1 response by two mechanisms: induction of IL-10 and inhibition of IL-12 signaling.

Interrelation of IL-10 and TGF-β in the regulation of mucosal inflammation

Previous studies have established that both IL-10 and TGF-β are important suppressor cytokines in the regulation of mucosal inflammation (14–17). This is shown quite definitively by the fact that in the SCID-transfer model of colitis described above the protective effect of the co-administration of mature T cells is prevented when the recipient mice are given either anti-TGF-β or anti-IL-10R (17, 18). These studies, however, do not provide data on whether the IL-10 effect and the TGF-β regulatory effects were dependent on one another or independent of one another. To answer this question, we took advantage of the fact already mentioned that feeding TNP-haptenated colonic proteins prevents the induction of TNBS colitis by intrarectal TNBS administration. In particular, we reasoned that if the effect of feeding is to induce regulatory cells, the administration of anti-IL-10 or anti-TGF-β to a mouse during feeding could tell us whether the regulatory effect is mediated by IL-10, TGF-β, or both of these cytokines. We found that, in fact, the administration of either anti-IL-10 or anti-TGF-β abrogated the protective effect of feeding, suggesting that both cytokines were necessary for the regulation. However, analysis of cytokine production in this study showed that, whereas anti-IL-10 administration led to greatly decreased TGF-β secretion, anti-TGF-β administration did not affect IL-10 secretion. Thus, TGF-β could regulate inflammation in the absence of IL-10, but not vice versa.

Further studies to more clearly elucidate the interrelation of IL-10 and TGF-β regulation of mucosal inflammation consisted of adaptive transfer studies in which cells from TNP-haptenated protein-fed mice were transferred to recipient mice who were being given intrarectal TNBS to induce TNBS colitis. Here we found that anti-IL-10, administered to mice while fed TNP-haptenated colonic protein, did not prevent the emergence of cells capable of producing TGF-β (and mediating regulation of inflammation); thus, IL-10 was not necessary during the induction phase of TGF-β-producing regulatory cells. In contrast, anti-IL-10 administered to recipients of cells from fed mice did prevent the emergence of cells capable of producing TGF-β in such mice. These studies, taken together, suggest that TGF-β-producing cells are the primary suppressor cells but that IL-10 is necessary for these cells to expand in the face of a developing Th1 response that would otherwise inhibit the expansion of these cells.

Summary

The results of the studies described above have important implications for the regulation of Th1 T-cell-mediated mucosal inflammations (and, indeed, for other types of Th1 T-cell-mediated inflammations as well). For one thing, they suggest that, whereas the induction and expansion of regulatory cells (that is, cells producing

TGF-β) can prevent development of inflammation, such regulatory cells, in that they require the presence of a sufficient amount of IL-10 to reign in the Th1 T cell response during the period the TGF-β T-cell expansion, cannot easily overcome established inflammation. Stated differently, the induction of active oral tolerance via the feeding of antigens may in fact be a poor strategy for overcoming established inflammation, since active regulatory cells cannot expand in a Th1 milieu. Importantly, this limitation on regulatory cells does not apply to 'engineered' regulatory cells such as those arising from the administration of a TGF-β-producing plasmid. This follows from the fact that in the latter instance one is in effect bypassing the expansion phase of the regulatory cell, which in the natural state is dependent on a relatively low concentration of Th1 cytokine. Support for this view comes from the fact that, whereas feeding TNP-haptenated colonic protein to mice is ineffective in ameliorating established TNBS colitis, induction of TGF-β-producing cells by intranasal plasmid does have this effect.

Finally, the studies discussed here should not be construed to mean that cells producing IL-10 alone (in the absence of TGF-β) such as the recently described Tr-1 regulatory T cell, cannot act as competent regulatory cells on their own, at least under some circumstances (17). On the contrary, there is now incontrovertible evidence that IL-10 alone can and does suppress Th1 responses when present in a high enough concentration (19). This being said, it appears that if the number of Tr1 cells sufficient to affect regulation had been generated by oral antigen (TNP-haptenated colonic proteins), we would have been able to downregulate colitis in mice given anti-TGF-β (that retained an intact IL-10 response). Thus, these studies indicate that at least in a system in which regulatory cells are generated via feeding of antigen, IL-10-producing cells play a supportive rather than a primary role.

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