

Animal models of mucosal inflammation and their relation to human inflammatory bowel disease

Richard S Blumberg*, Lawrence J Saubermann† and Warren Strober‡

Animal models of inflammatory bowel disease (IBD) have been useful in the identification of those immune responses uniquely involved in IBD pathogenesis and in defining the important roles of environmental influences, such as normal luminal bacterial flora and the genetic composition of the host, in modifying IBD-associated inflammation. Recent studies have focused particular attention on CD4⁺ T cells which produce excessive quantities either of Th1 cytokines (IFN- γ and TNF) directed by IL-12 or of aTh2 cytokine (IL-4), relative to the production of suppressive cytokines such as IL-10 and transforming growth factor β . Such insights will be extremely beneficial in the development of novel approaches to the control of IBD-type inflammation, such as the use of anticytokine therapies and gene therapy, and, finally, in the identification of the genetic abnormalities and the antigens driving the inflammation that underlies the human disease.

Addresses

*† Gastroenterology Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA and the Harvard Digestive Diseases Center, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA

‡ Mucosal Immunity Section, National Institutes of Health, Building 10, 9000 Rockville Pike, Bethesda, MD 20892, USA

Correspondence: Richard S Blumberg;
e-mail: rblumberg@rics.bwh.harvard.edu

Current Opinion in Immunology 1999, 11:648–656

0952-7915/99/\$ – see front matter © 1999 Elsevier Science Ltd.
All rights reserved.

Abbreviations

APC	antigen-presenting cell
CD	Crohn's disease
CMV	cytomegalovirus
IBD	inflammatory bowel disease
IEC	intestinal epithelial cell
IFN-γ	interferon γ
LPS	lipopolysaccharide
Stat3	signal transducer and activator of transcription 3
TGF-β	transforming growth factor β
TNBS	trinitrobenzene sulphonic acid
TNF	tumor necrosis factor
TNFR1	TNF receptor 1
UC	ulcerative colitis

Introduction

Human inflammatory bowel disease (IBD) is a chronic, relapsing and remitting inflammatory condition of unknown origin that afflicts individuals of both sexes throughout life. The disease is clinically characterized by two overlapping phenotypes — ulcerative colitis (UC) and Crohn's disease (CD) — which predominantly affect the colon (UC and CD) and/or the distal small intestine (CD) in either a superficial (UC) or transmural (CD) manner. Clinical and laboratory studies in humans with IBD have long suggested that genetic and environmental factors play an inter-related role in the

pathogenesis of these disorders. These studies complement the more recent immunologic studies of the disease that have focused attention on the possibility that IBD is due to a dysregulated mucosal immune response to one or more unknown antigens present in the normal, indigenous bacterial flora. These lines of research meet in the hypothesis that IBD is due to a dysregulated response that has its origin in genetic factors that are activated by a variety of environmental factors, including those that affect the nature of the bacterial flora itself [1].

A major advance in the study of IBD and one that provides strong support for the above concept has been the discovery and subsequent analysis of a number of models of mucosal inflammation that resemble IBD [2–4]. As shown in Table 1, these models fall into four main categories and each provides unique opportunities to discover insights into the nature of the pathogenesis of IBD.

One major category consists of experimental colitides in which the mucosal inflammation develops spontaneously. These models offer the best possibility of defining genetic factors that lead to mucosal inflammation; the factors leading to inflammation can be studied in animals (e.g. mice) with relatively defined genetic backgrounds.

Another main category involves mucosal inflammation occurring in otherwise normal animals that are exposed to an exogenous agent, usually an agent that induces an immune response. This category affords the ability to relate particular kinds of immune responses to particular histopathologic reactions and to delineate the relation of immunopathogenesis to treatment.

A third category of experimental models embraces those with particular genetic disturbances produced by either gene targeting or the introduction of a transgene. These can be subgrouped into those in which a particular cytokine or cytokine receptor is involved, those in which the TCR and/or the antigen-presenting complex is involved or those in which the intestinal epithelial cell (IEC) layer is targeted in various ways. The major advantage of these models is that they allow one to identify how and why particular immunologic defects lead to mucosal inflammation and how contributions of epithelial cell function and/or barrier activity lead to bowel inflammation.

A fourth and historically important category of experimental inflammation (it was in fact the first experimental inflammation to be fully analyzed with modern immunologic tools) comprises 'transfer models' in which inflammation is induced by transferring particular cell populations into a 'neutral' host lacking lymphoid tissue, for

Table 1

Animal models of IBD.

Spontaneous	Induced		
	Administration of exogenous agents	Gene targeting: knockout or transgenic	Transfer of cells into immunodeficient animals
Cotton top Tamarin C3H-HeJBir mouse SAMP1/Yit mouse	Enema TNBS Oxazolone Acetic acid Immune-complex-formalin Oral Indomethacin Carageenan Dextran sodium sulfate Subcutaneous Cyclosporin A Intracolonic Peptidoglycan polysaccharide	Cytokine function IL-2 ^{-/-} IL-2R α ^{-/-} IL-10 ^{-/-} TGF- β ^{-/-} TNF Δ ARE IL-7-transgenic CRFB4 ^{-/-} Stat4-transgenic T cell function G α i2 ^{-/-} TCR α ^{-/-} TCR β ^{-/-} MHC class II ^{-/-} HLA-B27-transgenic rat IEC barrier function *Trefoil factor ^{-/-} N-cadherin dominant negative mdr1a ^{-/-}	CD4 ⁺ CD45RB ^{hi} into <i>scid</i> or <i>Rag</i> ^{-/-} mice Bone marrow into Tge26 mice

*Requires environmental stress such as concomitant dextran sulfate sodium. IL-2R, IL-2 receptor.

example *scid* or *Rag*^{-/-} animals. This category is in a sense similar to the one involving gene targeting in that it allows dissection of the abnormal responses leading to bowel inflammation; however, in transfer models, the focus is more on cell populations responsible rather than molecules.

Although the above categories of mucosal inflammation make a distinction between spontaneous and induced models, this distinction is more apparent than real. Thus, in many of the 'spontaneous' or induced models, hidden (subtle) environmental and/or genetic factors must be present for inflammation to occur. This is illustrated by the fact that in some of the induced models, one sees evidence of an underlying genetic defect. Moreover, in some of the gene-targeted models, an appropriate environmental stimulus must be present (such as a particular type of bowel flora). These facts emphasize once again the inter-relation between the immune system and the genetic and environmental influences that operate in the development of experimental mucosal inflammation and, by extension, in human IBD. In the present review, we will summarize the major insights provided by recent studies of models of mucosal inflammation.

Functionally important cell types in experimental mucosal inflammation

T cells and B cells

The CD4⁺ T cell plays a key role in the pathogenesis of experimental mucosal inflammation. Thus in many of the models described in Table 1 — including the *scid* and CD ϵ Tg26 (also known as Tge26) transfer models, several

of the knockout mice (IL-10^{-/-}, IL-2^{-/-} and TCR α ^{-/-} models) and finally the induced model of inflammation associated with TNBS (trinitrobenzene sulphonic acid) administration — infiltration of lamina propria by CD4⁺ T cells is a key feature of the immunopathology. More importantly, direct inhibition of CD4⁺ T cells by either CD4 mimetics or anti-CD4 monoclonal antibodies prevents development of disease [5]. By contrast, the CD8⁺ T cell — although present in the lamina propria infiltrate of many of the same models — is not an essential element since its deletion does not result in amelioration of inflammation [6]. If B cells have any role in experimental mucosal inflammation, it is to promote downregulation of the inflammatory response [7]. Finally, $\gamma\delta$ T may function to prevent colitis or hasten its healing by regulating the IEC barrier function through the production of factors such as keratinocyte growth factor [8].

Although (as noted above) CD4⁺ T cells are responsible for the initiation of colitis, they also play a role as counter-regulatory T cells that limit or abrogate mucosal inflammation. In the initial description of the *scid* transfer model, for instance, the autoaggressive cells were shown to be contained in the CD4⁺ CD45RB^{hi} (naïve) T cell subset whereas inhibitory or counter-regulatory cells were found to be present in the CD4⁺ CD45RB^{lo} (memory) T cell subset [2]. Thus whereas transfer of CD45RB^{hi} T cells resulted in colitis, co-transfer of CD45RB^{hi} and CD45RB^{lo} cells resulted in no colitis. More recently, this simple dichotomy has been refined in studies that show that the

CD45RB^{lo} T cell subset contains cells capable of causing progressive colitis; however, in this case the colitis exhibits delayed kinetics in comparison with that induced by the CD45RB^{hi} T cell subset, indicating that the CD45RB^{lo} T cell subset in reality contains a mixture of autoaggressive and counter-regulatory cells [9•]. In any case, the nature of the autoaggressive CD4⁺ T cell in this model and indeed in most models of mucosal inflammation is as an IL-12-driven Th1 T cell that produces TNF [10••,11,12] and IFN- γ [13•,14,15••,16]. On the other hand, the inhibitory or counter-regulatory T cell is a cell producing suppressor cytokines — either Th3 T cells producing transforming growth factor β (TGF- β) or so-called Tr1 cells producing IL-10 and TGF- β [17••,18,19••,20,21].

Finally, it should be noted that Th2 T cells can also subserve effector functions in certain models of inflammation. Thus in the TCR $\alpha^{-/-}$ mice already mentioned, as well as in an induced model of inflammation in which the contactant oxazolone is administered to SJL/J mice, one sees a relatively superficial inflammation (resembling UC rather than CD) that is mediated by IL-4-producing Th2 T cells and which is abrogated by inter-crossing with IL-4^{-/-} mice (in TCR $\alpha^{-/-}$ mice) or administration of anti-IL-4 (in oxazolone-induced colitis) [22•,23••,24•].

Macrophages

In the various experimental models of mucosal inflammation, as in human IBD, recruitment and enhanced activation of macrophages is a constant feature of the immunopathology [25]. This raises the possibility that functional disturbances of macrophages may be important in disease pathogenesis. In support of this possibility one can point to recent findings in mice with myeloid-specific deficiency of Stat3 (signal transducer and activator of transcription 3) that results from gene targeting; such mice exhibit an inability to produce several Stat3-dependent cytokines, most notably IL-10 — an important downregulating cytokine for macrophages [26••]. Macrophages from such mice exhibit the phenotype of IFN- γ -primed macrophages that cannot be inhibited by regulatory cytokines (such as IL-10). As a result, triggering of macrophages from these mice by lipopolysaccharide (LPS) *in vitro* results in excessive proinflammatory macrophage activity characterized by increased IL-12, TNF, IL-6 and IL-1 β secretion; administration of LPS to these mice results in the occurrence of inflammation. These studies thus show quite clearly that a primary defect in macrophage function can result in mucosal inflammation.

IECs

There are several features of IECs that make them relevant to the pathogenesis of chronic mucosal immune inflammation both in mice and humans [27]. One of these is that IECs form a barrier to the passage of organisms and restrict the passage of macromolecules into the lamina propria, where the macromolecules might be taken up by antigen-presenting cells (APCs) and thus stimulate lymphocytes. The relation of

this barrier function to the occurrence of mucosal inflammation is shown quite dramatically in two quite different mouse models of experimental mucosal inflammation. In mice that express a 'dominant negative' N-cadherin gene, N-cadherin function is defective and thus the integrity of tight junctions (of which they are a critical part) is compromised [28]. The result is a UC-like inflammation that closely correlates in location with epithelial cells expressing the defective gene function. Similarly, mice with disruption of the multidrug resistance gene 1a (*mdr1a*^{-/-}), as a result of gene targeting, manifest IECs and lymphocytes that cannot pump out potentially deleterious substances from within the cell. It is thought that this may lead to premature cell death [29•]. This defect also leads to a UC-like inflammation most probably due to defective IEC function (rather than defective lymphocyte function) since irradiated bone-marrow-chimeras of mutant mice that are repleted with normal lymphocytes still develop colitis whereas normal mice repleted with mutant *mdr1a*^{-/-} lymphocytes do not.

Non-immune cells

In any discussion of cells potentially involved in the causation of experimental mucosal inflammation or indeed in human IBD, it is easy to overlook cell types that at first glance have little relation to mucosal immune function. However, the mucosa is a complex environment whose physiology is regulated by a variety of cell types, not just lymphoid/myeloid cells or epithelial cells, that could also be a nexus involved in inflammation. This possibility is supported by recent studies showing that transgenic mice that express herpes simplex virus thymidine kinase, under the control of a glial fibrillary acid protein function, develop colitis when ganciclovir is administered to induce DNA chain termination [30••]. Interestingly, the glial cell death occurs selectively in the jejunal nerves — not in the colonic nerves or in other nerve tissues. This leads to reduced intestinal motility of affected jejunal segments and secondary bacterial overgrowth in these segments. However this outgrowth does not appear to be the cause of the colitis, since the colitis is not ameliorated by antimicrobial agents. In fact the lesion produced resembles ischemic bowel disease, suggesting that the colitis has its origin in vascular dysfunction. Interestingly, ischemia has been suggested as a pathogenic mechanism in human CD affecting the small bowel; however, how such ischemia would lead to inflammation in humans or indeed in mice is still not understood [1].

Effector mechanisms in models of mucosal inflammation

IL-12/IFN- γ -mediated pathways

It was mentioned above that the major effector cell in experimental inflammation, the CD4⁺ T cell, causes disease through the excessive secretion of either Th1 cytokines (e.g. IFN- γ and TNF) or Th2 cytokines (e.g. IL-4, IL-5 and IL-13). It is of interest that the histopathology of the inflammation associated with these different cytokine production patterns is quite distinct. The

Th1-cell-induced mucosal inflammatory diseases are generally transmural inflammations that are more or less granulomatous in character and contain relatively few acute inflammatory cells such as neutrophils and eosinophils. In contrast, Th2-cell-induced diseases are characterized by more superficial inflammations associated with an acute inflammatory cell exudate and/or the presence of mucosal edema. This has led to the notion that experimental inflammations dominated by Th1 cytokines are models of CD whereas those dominated by Th2 cytokines are representative of UC. This concept obtains support from a recently described mixed model in which both Th1 and Th2 cytokine overproduction is seen. This is a TNBS-induced colitis model in which mice that are given TNBS per rectum are from the BALB/c strain (i.e. a Th2-oriented mouse) rather than the SJL/J mouse strain that is usually employed to obtain TNBS-induced colitis. In the case of BALB/c mice both IL-4 and IFN- γ overproduction occurs, with the former predominating over the latter and the histopathology is more like that seen in UC than in CD [24*].

The Th1 cytokine pathway is initiated by IL-12, a p40-p35 heterodimer produced by macrophages, B cells and follicular dendritic cells. IL-12 signaling occurs through a heterodimeric IL-12 receptor which, when cross-linked, leads to activation of the kinases Jak2 and Tyk2 followed by Stat3 and Stat4 activation and nuclear translocation and then induction of IFN- γ mRNA synthesis.

Given the central role of IL-12 in the Th1 cytokine pathway, it is not surprising that this cytokine has been shown to be a key factor in the pathogenesis of virtually all experimental models of mucosal inflammation that have been appropriately studied, most notably the *scid* and Tg ϵ 26 transfer models and the TNBS-induced colitis model [2]. Thus, in each of these models, either inter-crossing with IL-12^{-/-} mice or administration of anti-IL-12 has a profound ability to either prevent development of colitis or, in the case of anti-IL-12 administration, to treat already established colitis. This is in some contrast with the fact that anti-IFN- γ administration is a less effective treatment and it has been shown that IFN- γ ^{-/-} mice can be induced to develop TNBS-induced colitis. The reasons for this discrepancy may be at least two-fold. First, it is now known that anti-IL-12 administration to mice with TNBS-induced colitis leads to death of activated Th1 cells by Fas-mediated apoptosis whereas anti-IFN- γ does not have this effect [13*]. Thus, while anti-IL-12 leads to loss of Th1 cells, anti-IFN- γ merely blocks the function of IFN- γ . Second, Th1 inflammation also results in TNF production and it is likely that the latter cytokine can cause experimental mucosal inflammation even in the absence of IFN- γ . This suggestion is supported by a recently described model of inflammation in which the sole defect is TNF overproduction that is probably driven by IL-12 [10**].

Experimental mucosal inflammation due to excessive Th1 cell activity and overproduction of IL-12 can have several possible underlying causes. One is the presence of an abnormality leading to intrinsic hyperactivity of the Th1 pathway. This is exemplified by a recently described mouse model in which the mice have a Stat4 transgene under the control of a cytomegalovirus (CMV) promoter [14]. Such mice are free of inflammation unless they are administered LPS, which causes activation of the CMV promoter. Alternatively, the mice develop inflammation when they are exposed to nonindigenous bacterial microflora and are therefore exposed to bacteria to which they have not been previously tolerized; the immune response thus evoked again leads to activation of the CMV promoter. Overall, this model offers proof that intrinsic overactivity of the Th1 pathway can lead to inflammation that can be driven by bacteria in the mucosal bacterial microflora.

Another and perhaps more important cause of excessive Th1 cell activity and overproduction of IL-12 is that the latter is not appropriately counter-regulated. As discussed in detail below this can be due to inadequate suppressor cytokine responses or, more subtly, to an abnormality in the ability of Th1 cells to be regulated by normal suppressor cytokine responses. Finally, as also discussed below, Th1 overactivity can be due to intrinsic overproduction of a Th1 cytokine component such as TNF.

An interesting point that has become apparent in recent studies is that while Th1-induced IL-12-mediated inflammation is largely channeled through the Stat4-IFN- γ pathway, neither of these components is entirely critical to such inflammation. For instance in the case of Stat4 (an intracellular signaling protein) its absence neither prevents IFN- γ production nor prevents inflammation; this is shown by the fact that transfer of CD45RB^{hi} T cells from Stat4^{-/-} mice to Rag^{-/-} recipients still results in induction of colitis, albeit that the colitis is diminished compared with that obtained upon transfer of normal CD45RB^{hi} cells [15**]. The latter observation accords with the fact that Stat4^{-/-} mice do produce IFN- γ when stimulated with anti-CD3 in the presence of IL-2. In the case of IFN- γ , it has already been noted that TNBS-induced colitis can be induced in IFN- γ ^{-/-} mice; in addition, it has been shown that whereas transfer of bone marrow from IL-12^{-/-} mice prevents development of colitis in the Tg ϵ 26-transfer model, such transfer from IFN- γ ^{-/-} mice does not prevent development of colitis [15**]. Thus, the data strongly support the view that IL-12 functions in both an IFN- γ -dependent and -independent manner to produce inflammation. Finally, it should be noted that in humans IFN- γ can be induced by IFN- γ itself, raising the question as to whether an IL-12-independent Th1 inflammation can exist in the species. This question may be resolved when patients with Th1-induced colitis such as those with CD are subjected to anti-IL-12 therapy.

Th1/TNF-mediated pathways of inflammation

IL-12 acts on T cells to induce the production of TNF as well as IFN- γ ; both of the latter cytokines induce positive feedback for further IL-12 production as well as 'downstream' production of a series of proinflammatory macrophage-derived cytokines that include IL-1 β , IL-6 and TNF itself. Recently, an experimental model of mucosal inflammation has been produced by creating mice that overexpress TNF (TNF Δ ARE mice) [10**]. This was accomplished by gene-targeting AU-rich elements of the TNF gene so as to produce a mutant gene that gave rise to a more stable TNF mRNA. The disease in such TNF-overproducing mice is unique in that it involves primarily the small intestine rather than the large intestine and has a markedly granulomatous histology that is remarkably similar to that in CD. The intestinal pathology in this model results from TNF signaling through the either TNF receptor I (TNFRI; also known as p55) or TNFRII (p75), suggesting that specific effector cells and/or downstream effector molecules (related to TNFRI and TNFRII) are ultimately responsible for the immunopathology of this inflammation. In recent studies it has been shown that mice resulting from crosses between IL-12 $^{-/-}$ mice and mice with the above-described altered TNF gene do not manifest disease. Thus, while TNF emerges as a key effector cytokine in inflammation of the small bowel, IL-12 remains as the 'master' cytokine necessary for initiation of such inflammation.

The TNFR is part of the TNFR family of molecules, many of which have important effects on immune functions. Thus, it is hardly surprising that other members of the family can also affect the development of experimental mucosal inflammation. Perhaps the most important of these family members is CD40, the receptor present at the surface of APCs (and B cells) that interacts with the counter-receptor (CD40 ligand) which is present on activated T cells. Such interactions lead to initiation of the Th1 differentiation pathway via backstimulation of APCs and IL-12 production. The importance of this to models of mucosal inflammation is dramatically shown by the fact that administration of anti-CD40-ligand antibody completely blocks induction of TNBS-induced colitis, presumably by preventing the signaling of APCs necessary for IL-12 production [31]. Interestingly, however, such antibody treatment is not effective in the treatment of established TNBS-induced colitis — presumably because after inflammation is initiated there is disruption of mucosal integrity and APCs produce IL-12 in the absence of CD40 ligand via stimulation by bacterial products.

Another member of the TNFR family is OX40, a receptor present on the surface of T cells that interacts with OX40 ligand (a counter-receptor on B cells and APCs). OX40 also plays a role in experimental mucosal inflammation via its signaling function. Thus administration of OX40-Fc fusion protein, a molecule that blocks T cell signaling via OX40, also ameliorates TNBS-induced colitis [11]. In a

like fashion, blockage of another TNFR family member, the lymphotoxin β receptor (LT β R), by administration of LT β R-Fc fusion protein inhibits mucosal inflammation in both the *scid* and Tge26 transfer models [12]. The mechanism here again is presumed to be the inhibition of cell-cell interactions necessary for the establishment of a Th1 response.

Anti-inflammatory cytokine pathways in experimental mucosal inflammation

As noted above, it is possible that over-activity of the Th1 pathway is caused by defective counter-regulation, that is the failure to generate suppressive T cells and their suppressor cytokines. It is in this context that experimental mucosal inflammation can be viewed as a failure of normal mechanisms of oral tolerance, particularly by the production of suppressor cytokines that mediate such tolerance; examples of such cytokines are TGF- β and IL-10 [20,32,33].

Additional evidence that TGF- β plays a key counter-regulatory role in experimental mucosal inflammation comes from the study of a Th2-cell-mediated colitis, namely oxazolone-mediated colitis [24*]. In this case, instillation of oxazolone per rectum induces not only an IL-4 response which causes the inflammation but also a TGF- β response which ultimately limits the inflammation. This is in contradistinction to TNBS-induced colitis, which is not characterized by TGF- β production unless the latter is administered orally [20]. Of interest, oxazolone-induced colitis is limited to the distal half of the colon. That this may be due to TGF- β secretion patterns is suggested by the fact that TGF- β secretion is higher in the proximal half of the colon and that anti-TGF- β administration results in pan-colitis. The level of TGF- β secretion in oxazolone-induced colitis is far higher than even the curative TGF- β levels noted in TNBS-induced colitis upon feeding trinitrophenol (TNP)-substituted protein [20,23**]. This raises the question of why the TGF- β response in oxazolone-induced colitis doesn't prevent the colitis. The answer probably lies in the greater resistance of Th2 T cells (compared with Th1 cells) to TGF- β suppression. Finally, it should be noted that oxazolone-induced colitis is a short-lived inflammation and rapidly resolves once the treated mice pass through an acute period. It is likely that this rapid resolution is due to the TGF- β response, which appears to catch up with and quell the Th2-cell-mediated inflammation in a fairly short period of time.

Studies implicating IL-10 as a downregulatory cytokine in experimental mucosal inflammation are also compelling. First and foremost are the studies of IL-10 $^{-/-}$ mice; these studies show that in the absence of IL-10, mice develop a Th1-mediated inflammation of the mucosal surfaces that is most severe in the colon but also involves the small intestine [2]. Additionally, disruption of *CRFB4* — the gene that encodes CRF2-4, a component of the IL-10 receptor — also leads to colitis [19**]. Thus, abnormalities of IL-10 itself and/or IL-10-mediated signaling lead to

mucosal inflammation. Further evidence of the role of IL-10 in the counter-regulation of mucosal inflammation comes from the *scid* transfer model. In this case anti-IL-10 was co-administered along with CD45RB^{lo} cells but did not abrogate the protective effect of the latter cells (unlike anti-TGF- β). In later studies it has been shown that administration of an anti-IL-10-receptor antibody does have a protective effect; thus, the previously negative result obtained with anti-IL-10 may have been due to inadequate shutoff of IL-10 function by anti-IL-10 [17**].

Just how the suppressor cytokines function in experimental mucosal inflammation is still a matter of intense investigation. As we have seen, TGF- β directly inhibits Th1 responses and — at a higher concentration — Th2 responses as well [20,23**]. The primary effect may be via its ability to inhibit IL-12 β 2 chain expression since, in the presence of TGF- β , established Th1 T cells fail to express activated Stat4 in a normal fashion. It should also be noted that there is some evidence that TGF- β is necessary for the differentiation of cells that produce TGF- β . Thus, TGF- β seems to be necessary to sustain a suppressor effect as well as to serve as an effector molecule in suppression. The activity of IL-10 in counter-regulating experimental mucosal inflammation is also likely to be multifactorial. IL-10 is a potent downregulator of IL-12 production so that it acts partly at the level of Th1 T cell induction. In addition, IL-10 suppresses production of inflammatory cytokines such as TNF at the effector stage of the inflammatory response. Finally there is now evidence that IL-10 acts as a growth factor for Tr1 T cell development and thus, as in the case of TGF- β , may be necessary for the development of suppressor T cells [21].

Cytotoxic mechanisms

Recently, cytotoxic T cell activity has been observed in a number of models of experimental mucosal inflammation; these models include the IL-2^{-/-} mouse and the colitis in the *scid* and the Tg26 transfer models. Indeed, in the latter case transfer of cells from perforin^{-/-} mice show that cytotoxic T cells do contribute to immunopathology [34]. Nevertheless, it should be recalled from the discussion above that studies with β 2-microglobulin^{-/-} mice that CD8⁺ T cells are not critical to the development of experimental mucosal inflammation [6]. Thus, although cytotoxic T cells may contribute to mucosal inflammation, the latter can occur in their absence. These findings are relative to human UC, where apoptotic intestinal epithelial cells can be found and where death of epithelial cells mediated by antibody-dependent cell-mediated cytotoxicity has long been considered an important disease effector mechanism [35]. From the animal data obtained so far, however, we must conclude that such mechanisms are contributory but nonessential factors in IBD pathogenesis.

Role of cellular traffic

CD4⁺ T cells that mediate inflammation in a number of the experimental mucosal inflammations have to migrate

from sites of sensitization to sites of effector function (in the lamina propria) to initiate and/or perpetuate the inflammatory response. Such migration is directed and depends on interaction between tissue-specific integrins and addressins, which in the case of traffic to mucosal tissues involves interactions between circulating cells bearing the α 4 β 7 integrin and the MadCAM-1 integrin on surface endothelial cells. On this basis anti- α 4 β 7 antibodies have been administered to the cotton-top tamarin model of colitis, to *scid* mice during transfer of CD45RB^{hi} T cells and to IL-2^{-/-} mice undergoing induction of colitis with TNP-KLH (i.e. trinitrophenol with keyhole limpet hemocyanin); in these cases the antibodies were shown to prevent the development of colitis [36]. Other molecules that may be relevant to recruitment and/or retention of cells within mucosal tissues include adhesion molecules such as α E β 7, P-selectin, VCAM-1 and ICAM-1 [37–40]. Thus, antibodies that retard migration to the mucosal areas can prevent mucosal inflammation.

The role of luminal bacteria in experimental mucosal inflammation

Although the various experimental models of the mucosal inflammation are quite different from one another, they have in common a remarkable dependence on the presence of normal nonpathogenic bacterial flora. This is shown by the fact that none of the mice with defects associated with mucosal inflammation develops such inflammation under germ-free conditions (this includes IL-10^{-/-} mice, TCR α ^{-/-} mice, IL-2^{-/-} mice, HLA-B7-transgenic rats and the SAMP1/Yit mice) [41,42]. In addition, animals treated with antibiotics to reduce bacterial colonization (*mdr1a*^{-/-} mice, *scid* transfer mice and the indomethacin-induced colitis in Lewis rats) manifest reduced colitis following treatment [2]. The mechanism by which the normal flora participates in experimental colitis is only now becoming clear. Mice (and humans) are normally nonresponsive to proteins in their autologous microflora but respond to the microflora of other individuals even if the latter are of the same strain, presumably as a result of oral tolerance mechanisms [43]. This, plus the fact that a germ-free environment abolishes colitis in the various models, suggests that mucosal inflammation can be due to a loss of counter-regulation of responses to autologous flora.

Although the bacterial microflora may be important in disease pathogenesis, not all bacteria have the same capacity to induce inflammation. Some bacteria that are considered to be probiotic, such as the various *Lactobacillus* species, prevent colitis from occurring in IL-10-deficient animals living under specific-pathogen-free conditions [44]. Other bacteria such as *Bacteroides vulgatus* will cause significant colitis when monoassociated in mice but this depends on the strain of mouse and type of immunodeficiency [45]. Similarly, *Helicobacter hepaticus* will cause colitis when introduced into pathogen-free *scid* and IL-10^{-/-} mice and not in controls [46,47]. In one study of Rag^{-/-} mice, colitis development

associated with *H. hepaticus* required the presence of IL-7 and was downregulated by IL-10 [48*] — suggesting that, in the appropriate context, *H. hepaticus* may be a direct stimulus of IL-7 production by IECs; these cells are known to be a key source of IL-7, which is a known cause of colitis when overexpressed in epithelial cells as a transgene [49].

Finally, evidence that autologous bacterial antigens are responsible for development of colitis comes from studies of the T cell response itself. Thus TCR $\alpha^{-/-}$ mice that express CD4⁺ TCR $\beta\beta^{+}$ T cells in the periphery and develop a colitis that is dependent on the presence of a normal gut flora exhibit a restricted T cell clonality and a public motif within the third complementarity-determining region (CDR3) portion of the TCR, consistent with the response to a common bacterial antigen [50*]. Interestingly, public patterns of CDR3 region usage have been observed before among CD4⁺ T cells in human CD [51]. In addition, splenic CD4⁺ T cells from the C3H-HeJBir strain of mice exhibit a skewed TCR repertoire and can transfer colitis when stimulated by protein antigens of cecal bacteria [52**]. This is consistent with previous studies describing selective antibody responses to luminal bacteria in both the TCR $\alpha^{-/-}$ and C3H-HeJBir models of experimental mucosal inflammation [53,54]. Identification of these antigens, which are likely to be central to the pathogenesis of such inflammation and which may be associated with cross-reactive mucosal antigens, will be an important goal of future studies.

The genetics of experimental mucosal inflammation

While the existence of underlying genetic factors in experimental mucosal inflammation in mice with gene deletions and transgenes is obvious, genetic factors also underlie the induction of colitis in mice presumed to be normal [2]. For example, in the dextran sulfate sodium (DSS)-induced colitis model, a hierarchy of sensitivity to colitis has been observed: C3H-HeJBir mice show greatest sensitivity — greater than NOD/LeJ, which in turn have greater sensitivity than C57BL/6J mice [55]. Although the specific genetic loci are unknown, there is reason to assume from the above discussion that the genetic makeup of a mouse is likely to influence factors affecting the occurrence of colitis via immunoregulation and/or mucosal barrier function.

Conclusions

The various animal models discussed above provide at least three important insights into the nature of human IBD. First they indicate that, just as in experimental mucosal inflammation — which can arise in the various models as a result of any number of distinct defects — a variety of defects can be the cause of IBD in humans. Second, immunologic defects leading to colitis in the animal models are defects of dysregulation and are not due to frank immunodeficiency; significantly, *scid* or Rag $^{-/-}$ mice do not normally develop mucosal inflammation. Third,

there are correlations between the final common pathways that are common to mucosal inflammation and the nature of the induced disease. The pathway in most experimental models is characterized by an excessive Th1 cell response; this type of response appears to be a good mimic of CD. The other common pathway in experimental models is overactivity of Th2 cells; this tends to produce the histologic picture of UC but there is only (at best) equivocal evidence that this disease is indeed due to a Th2-cell-mediated pathway.

What is the future of research involving animal models of mucosal inflammation? Although much has been learned, much has yet to be discovered. The models will be useful in identification of immune responses uniquely involved in IBD pathogenesis, in the development of novel approaches to control IBD-type inflammation (such as the use of novel types of anticytokine therapy or gene therapy) and, finally, in the identification of the genetic abnormalities that underlie the human disease.

Acknowledgements

RSB is funded by grants DK44319, DK51362 and DK53056 from the national National Institutes of Health (NIH). LJS is funded by grant DK02532 from the NIH.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Fiocchi C: **Inflammatory bowel disease: etiology and pathogenesis.** *Gastroenterology* 1998, **115B**:182-205.
 2. Elson CO: **Experimental models of intestinal inflammation: new insights into mechanisms of mucosal homeostasis.** In *Mucosal Immunology*. Edited by Ogro PL, Lamm ME, Bienenstock J, Mestecky J, Strober W, McGhee JR. San Diego: Academic Press; 1999:1007-1024.
 3. Snapper SB, Rosen FS, Mizoguchi E, Cohen P, Khan W, Liu C-H, Hagemann TL, Kwan S-P, Ferrini R, Davidson L *et al.*: **Wiskott-Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation.** *Immunity* 1998, **9**:81-91.
 4. Matsumoto S, Okabe Y, Setoyama H, Takayama K, Ohtsuka J, Funahashi H, Imaoka A, Okada Y, Umesaki Y: **Inflammatory bowel disease-like enteritis caecitis in a senescence accelerated mouse P1/Yit strain.** *Gut* 1998, **43**:71-78.
 5. Okamoto S, Watanabe M, Yamazaki M, Yajima T, Hayashi T, Ishii H, Mukai M, Yamada T, Noriaki W, Jameson BA, Hibi T: **A synthetic mimetic of CD4 is able to suppress disease in a rodent model of immune cells.** *Eur J Immunol* 1999, **29**:355-366.
 6. Simpson SJ, Mizoguchi E, Allen D, Bhan AK, Terhorst C: **Evidence that CD4⁺, but not CD8⁺ T cells are responsible for murine interleukin-2-deficient colitis.** *Eur J Immunol* 1995, **25**:2618-2625.
 7. Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK: **Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice.** *J Exp Med* 1997, **186**:1749-1756.
 8. Boismenu R, Havren WL: **Modulation of epithelial cell growth by intraepithelial $\gamma\delta$ T cells.** *Science* 1994, **266**:1253-1255.
 9. Claesson MH, Bregenholt S, Bonhagen K, Thoma S, Möller P, Grusby MJ, Leithäuser F, Nissen MH, Reimann J: **Colitis-inducing potency of CD4⁺ T cells in immunodeficient adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype.** *J Immunol* 1999, **162**:3702-3710.
- This paper provides evidence in support of the notion that CD4⁺ T cells in the CD45RB^{lo} subset have colitis-inducing potential.

10. Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G:
 •• **Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies.** *Immunity* 1999, **10**:387-398.
 A fascinating paper, which shows that TNF is a major factor in the development of CD-like pathology that is consistent with clinical observations in humans.
11. Higgins LM, McDonald SA, Whittle N, Crockett N, Shields JG, MacDonald TT: **Regulation of T cell activation in vitro and in vivo by targeting the OX40-OX40 ligand interaction: amelioration of ongoing inflammatory bowel disease with an OX40-IgG fusion protein, but not with an OX40 ligand-IgG fusion protein.** *J Immunol* 1999, **162**:486-493.
12. Mackay F, Browning J, Lawton P, Shah SA, Comiskey M, Bhan AK, Mizoguchi E, Terhorst C, Simpson SJ: **Both the lymphotoxin and tumor necrosis factor pathways are involved in experimental murine models of colitis.** *Gastroenterology* 1998, **115**:1464-1475.
13. Fuss IJ, Marth T, Neurath MF, Pearlstein GR, Jain A, Strober W: **Anti interleukin-12 treatment regulates apoptosis of T helper 1 T cells in experimental colitis.** *Gastroenterology* 1999, **117**:1078-1088.
 This paper provides a potential mechanism for the beneficial role of blocking IL-12 in colitis models.
14. Wirtz S, Finotto S, Kanzler S, Lohse AW, Blessing M, Lehr HA, Galle PR, Neurath MF: **Chronic intestinal inflammation in STAT-4 transgenic mice: characterization of disease and adoptive transfer by TNF- α plus IFN- γ -producing CD4⁺ T cells that respond to bacterial antigens.** *J Immunol* 1999, **162**:1884-1888.
15. Simpson SJ, Shah S, Comiskey M, de Jong YP, Wang B, Mizoguchi E, Bhan AK, Terhorst C: **T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon γ expression by T cells.** *J Exp Med* 1998, **187**:1225-1234.
 This paper examines IL-12-mediated pathways of colitis induction and shows that IFN- γ -dependent and -independent mechanisms probably exist.
16. Davidson NJ, Hudak SA, Lesley RE, Menon S, Leach MW, Rennick DM: **IL-12, but not IFN- γ , plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice.** *J Immunol* 1998, **161**:3143-3149.
17. Asseman C, Smita M, Leach MW, Coffman RL, Powrie F: **An essential role for interleukin-10 in the function of regulatory T cells which inhibit intestinal inflammation.** *J Exp Med* 1999, **190**:995-1003.
 IL-10 is an important immunoregulatory cytokine whose absence leads to colitis. This paper shows that a major source of IL-10 in protecting from colitis may be within the CD45RB^{lo} subset of T cells.
18. Stallmach A, Wittig B, Giese T, Pfister K, Hoffman JC, Bulfone-Paus S, Kundendorf U, Meuer SC, Zeitz M: **Protection of experimentally induced colitis by an interleukin-2-IgG2b fusion protein in mice.** *Gastroenterology* 1999, **117**:866-876.
19. Spencer SD, DiMarco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, Aguet M: **The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor.** *J Exp Med* 1998, **187**:571-578.
 This is a new model of colitis that is the result of knocking out a newly targeted component of the IL-10 receptor.
20. Neurath MF, Fuss I, Kelsall BL, Presky DH, Waegell W, Strober W: **Experimental granulomatous colitis in mice is abrogated by induction of TGF- γ -mediated oral tolerance.** *J Exp Med* 1996, **183**:2605-2616.
21. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo M-G: **A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevent colitis.** *Nature* 1997, **389**:737-742.
22. Mizoguchi A, Mizoguchi E, Bhan AK: **The critical role of interleukin 4 but not interferon gamma in the pathogenesis of colitis in T-cell receptor α mutant mice.** *Gastroenterology* 1999, **116**:320-326.
 Although most models of colitis are dominated by excess type 1 cytokines (e.g. IFN- γ), type 2 cytokines such as IL-4 appear to be important in the TCR α ^{-/-} model.
23. Boirivant M, Fuss IJ, Chu A, Strober W: **Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4.** *J Exp Med* 1998, **188**:1929-1939.
 This is another new model of colitis that appears to be due to excess Th2 cytokines such as IL-4.
24. Dohi T, Fujihashi K, Rennert PD, Iwatani K, Kiyono H, McGhee JR:
 • **Hapten-induced colitis is associated with colonic patch hypertrophy and T helper cell 2-type responses.** *J Exp Med* 1999, **189**:1169-1179.
 This paper investigates the role of Th1 and Th2 cytokines in TNBS-induced colitis and provides evidence in support of a pathogenic role for both. Furthermore, this study suggests that polarized cytokine responses promote different types of pathology with varying kinetics.
25. Brandtzaeg P, Haraldsen G, Rugtveit J: **Immunopathology of human inflammatory bowel disease.** *Springer Semin Immunopathol* 1997, **18**:555-589.
26. Takeda K, Kaisho T, Terada N, Akira S: **Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils.** *Immunity* 1999, **10**:34-49.
 In this model, the loss of Stat3 in myeloid cells leads to an activated phenotype of macrophages and consequently colitis, raising the possibility that primary defects in macrophages and other myeloid cells may be sufficient to initiate colitis.
27. Christ A, Blumberg RS: **The intestinal epithelial cell: immunological aspects.** *Springer Semin Immunopathol* 1997, **18**:449-461.
28. Hermiston ML, Gordon JI: **Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin.** *Science* 1995, **270**:1203-1207.
29. Panwala CM, Jones JC, Viney JL: **A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis.** *J Immunol* 1998, **161**:5733-5744.
 This new model of colitis supports other studies indicating a potential central role of intestinal epithelial cell dysfunction in the pathogenesis of colitis.
30. Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, Johnson MH, Sofroniew MV: **Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice.** *Cell* 1998, **93**:189-201.
 This is an extremely interesting study showing that interactions between nerve cells, immune cells and the vasculature may be important to mucosal homeostasis and the generation of colitis.
31. Stuber E, Strober W, Neurath M: **Blocking the CD40L-CD40 interaction *in vivo* specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion.** *J Exp Med* 1996, **183**:693-698.
32. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL: **A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4⁺ cells.** *J Exp Med* 1996, **183**:2669-2674.
33. Ludviksson BR, Ehrhardt RO, Strober W: **TGF- β production regulates the development of the 2,4,6-trinitrophenol-conjugated keyhole limpet hemocyanin-induced colonic inflammation in IL-2-deficient mice.** *J Immunol* 1997, **159**:3622-3628.
34. Simpson SJ, de Jon YP, Shah SA, Comiskey M, Wang B, Spielman JA, Podack ER, Mizoguchi E, Bhan AK, Terhorst C: **Consequences of Fas-ligand and perforin expression by colon T cells in a mouse model of inflammatory bowel disease.** *Gastroenterology* 1998, **115**:849-855.
35. Strater J, Wellisch I, Riedl S, Walczak H, Koretz K, Tandara A, Krammer PH, Moller P: **CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: a possible role in ulcerative colitis.** *Gastroenterology* 1997, **113**:160-167.
36. Hesterberg PE, Winsor-Hines D, Briskin MJ, Soler-Ferran D, Merrill C, Mackay CR, Newman W, Ringler DJ: **Rapid resolution of chronic colitis in the cotton-top tamarin with an antibody to a gut-homing integrin $\alpha 4/\beta 7$.** *Gastroenterology* 1996, **111**:1373-1380.
37. Ludviksson BR, Strober W, Nishikomori R, Hasan SK, Ehrhardt RO: **Administration of mAb against $\alpha 4\beta 7$ prevents and ameliorates immunization-induced colitis in IL-1^{-/-} mice.** *J Immunol* 1999, **162**:4975-4982.
38. Wittig B, Schwärzler C, Föhr N, Güntherth U, Zöller M: **Curative treatment of an experimentally induced colitis by a CD44 variant V7-specific antibody.** *J Immunol* 1998, **161**:1069-1073.
39. Thoma S, Bonhagen K, Vestweber D, Hamann A, Reimann J: **Expression of selectin-binding epitopes and cytokines by CD4⁺ T cells repopulating scid mice with colitis.** *Eur J Immunol* 1998, **28**:1785-1797.
40. Sams M, Panés J, Ardite E, Elizalde JI, Arce Y, Elena M, Palacín A, Fernández-Checa JC, Andeerson DC, Lobb R, Piqué JM: **VCAM-1 and ICAM-1 mediate leukocyte-endothelial cell adhesion in rat experimental colitis.** *Gastroenterology* 1999, **116**:874-883.

41. Song F, Ito K, Denning TL, Kuninger D, Papaconstantinou J, Gourley W, Klimpel G, Balish E, Hokanson J, Ernst PB: **Expression of the neutrophil chemokine KC in the colon of mice with enterocolitis and by intestinal epithelial cell lines: effects of flora and proinflammatory cytokines.** *J Immunol* 1999, **162**:2275-2280.
42. Contractor NV, Bassiri H, Reya T, Park AY, Baumgart DC, Wasik MA, Emerson SG, Carding SR: **Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice.** *J Immunol* 1998, **160**:385-394.
43. Duchman R, May E, Heike M, Knolle P, Neurath M, Meyer zum Buschenfelde KH: **T cell specificity and cross reactivity toward enterobacteria, bacteriodes, bifidobacterium, and antigens from resident intestinal flora in humans.** *Gut* 1999, **44**:812-818.
44. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN: **Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice.** *Gastroenterology* 1999, **116**:1107-1114.
45. Sellon RK, Tonkonogy S, Schultz M, Duieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB: **Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice.** *Infect Immun* 1998, **66**:5244-5231.
46. Li X, Fox JG, Whary MT, Yan L, Shames B, Zhao Z: **SCID/Ncr mice naturally infected with Helicobacter hepaticus develop progressive hepatitis, proliferative typhlitis, and colitis.** *Infect Immun* 1998, **66**:5477-5484.
47. Kullberg MC, Ward MJ, Gorelick PL, Caspar P, Hiery S, Cheever A, Jankovic D, Sher A: **Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma-interferon-dependent mechanism.** *Infect Immun* 1998, **66**:5157-5166.
48. Von Freeden-Jeffrey U, Davidson N, Wiler R, Fort M, Burdach S, Murray R: **IL-7 deficiency prevents development of a non-T cell non-B cell-mediated colitis.** *J Immunol* 1998, **161**:5673-5680.
- This paper provides a novel insight into interactions between microbes and immune cells and suggests that certain microbes may cause chronic colitis by inducing an immunoregulatory imbalance through effects on production of cytokines such as IL-7.
49. Watanabe M, Ueno Y, Yajima T, Okamoto S, Hayashi T, Yamazaki M, Iwao Y, Ishii H, Habu S, Uehira M *et al.*: **Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa.** *J Exp Med* 1998, **187**:389-402.
50. Takahashi I, Iijima H, Katashima R, Itakura M, Kiyono H: **Clonal expansion of CD4⁺ TCR $\beta\beta$ ⁺ T cells in TCR α -chain-chain-deficient mice by gut-derived antigens.** *J Immunol* 1999, **162**:1843-1850.
- This paper suggests that in colitis, specific antigens may be particularly relevant to disease pathogenesis.
51. Probert CS, Chott A, Turner JR, Saubermann LJ, Stevens AC, Bodinaku K, Elson CO, Balk SP, Blumberg RS: **Persistent clonal expansions of peripheral blood CD4⁺ lymphocytes in chronic inflammatory bowel disease.** *J Immunol* 1996, **157**:3183-3191.
52. Cong Y, Brandwein SL, McCabe RP, Lazenby A, Birkenmeier EH, Sundberg JP, Elson CO: **CD4⁺ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease.** *J Exp Med* 1998, **187**:855-864.
- This paper provides direct proof that dysregulated immune responses to bacterial antigens which lead to excess Th1 cytokine production may play a major role in the generation of colitis.
53. Mizoguchi A, Mizoguchi E, Tonegawa S, Bhan AK: **Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacterial antigens in TCR α mutant mice with inflammatory bowel disease.** *Int Immunol* 1996, **8**:1387-1394.
54. Brandwein SL, McCabe RP, Cong Y, Waites KB, Ridwan BU, Dean PA, Ohkusa T, Birkenmeier EH, Sundberg JP, Elson CO: **Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora.** *J Immunol* 1997, **159**:44-52.
55. Mähler M, Bristol IJ, Leiter EH, Workman AE, Birkenmeier EH, Elson CO, Sundberg JP: **Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis.** *Am J Physiol* 1998, **274**:G544-G551.