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INFLAMMATORY BOWEL DISEASE IN SEVERE COMBINED IMMUNE DEFICIENT (SCID) MICE: HISTO- AND IMMUNO-PATHOGENESIS

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SUMMARY

Over the recent years several murine models for the study of inflammatory bowel disease (IBD) have been developed. These models play an important role in the search for new insights in mucosal immunology and for the development of curative regimes in humans.

Transplantation of immunodeficient SCID mice with low numbers of $CD4^+$ T-cell from immunocompetent donors leads to the development of a chronic, lethal IBD. The histopathological changes of this disease resembles closely those of human IBD. The present review will focus on the histopathology of IBD in SCID recipients of $CD4^+$ T-cells, the accompanying changes in the recipients innate immune system and characterisation of the disease-inducing cell type in this murine model of IBD.

INTRODUCTION

Over the last decades the incidence and prevalence of the idiopathic human inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's disease (CD) have shown a inclining tendency (*Langholz* et al., 1991; *Munkholm* et al. 1992). This fact combined with the increased cancer risk of people suffering from IBD (*Gillen* et al., 1994; *Langholz* et al., 1992) make the inflammatory bowel diseases major hazards for the public heath in the industrialised world. Despite a massive research effort in the field of mucosal immunology during the later years, the aetiology of IBD remains obscure (*Podolsky*, 1991a,b). The currently accepted hypothesis is that IBD results from an uncontrolled or inadequately down regulated immune response in the gut towards a hitherto unknown pathogenic agent or parts hereof, probably derived from the gut flora (*Reinecker* et al., 1994; *MacDermott*, 1996). Also, genetic predisposition might play a role in the onset and development of the diseases (*Satsangi* et al., 1996; *Polito II* et al., 1996). A wider understanding of the immune modulatory and disease promoting mechanisms in the gut is obviously necessary for the development of therapeutically regimes in the treatment of IBD. Recently, a reductionistic approach to the investigation

of IBD has been made possible through the development of several animal disease models, for review see (*Elson* et al., 1995; *Bregenholt* et al., 1997a). These include mice with deletion in genes encoding certain cytokines (*Shull* et al., 1992; *Sadlack* et al., 1993; *Kühn* et al., 1993), T cell receptor chains (*Mombaerts* et al., 1993), signal transducing molecules (*Rudolph* et al., 1995), mice spontaneously developing IBD (*Sundberg* et al., 1994) and SCID mice reconstituted with T-cells from immunocompetent donors (*Powrie*, 1995; *Claesson* et al., 1996).

As the result of an autosomal recessive mutation in the gene encoding the recombinase necessary for B and T cell antigen receptor rearrangement (*Bosma* et al., 1983), SCID mice do not contain mature lymphocytes in their central and peripheral lymphoid organs, including mucosa and gut-associated lymphoid tissue (GALT).

Due to the lack of an active adoptive immune system, SCID mice are well suited as host for transplantation of various tissues, organs and cell types (*Bosma* and *Carroll*, 1991). We have used SCID mice to study the re-population of the central and peripheral lymphoid organs (*Reimann* et al., 1991) and the development of IBD following injection of T-cell subsets from allogeneic, semi-syngeneic or syngeneic immunocompetent donors. Also, this model of murine IBD has been used to characterise the disease-inducing cells phenotypically and functionally *ex vivo*. This review will describe the induction and development of IBD in SCID mice, and focus on the histo- and immunopathological features in the colon of diseased animals.

INDUCTION OF IBD IN SCID MICE

Initially, IBD in SCID mice was reported to be inducible by transfer of purified $CD45RB^{high}$ virgin type $CD4^+$ T-cells from congenic immunocompetent donor mice (*Powrie* et al., 1993; *Morrissey* et al., 1993), whereas transfer of both $CD45RB^{high}$ cells and $CD45RB^{low}$ cells would reconstitute the SCID mice but not lead to disease. Subsequently, we demonstrated that IBD can be induced in SCID mice by intraperitoneal injection of limited numbers ($<10^5$) of syngeneic highly purified $CD3^+$ $CD4^+$ spleen T-cells containing both the $CD45RB^{high}$ and $CD45RB^{low}$ subsets (*Claesson* et al., 1996). Thus, in both experimental models, non-fractionated spleen cells are unable to induce IBD (*Claesson* et al., 1996; *Powrie* et al., 1993; *Morrissey* et al., 1993). The tissue origin of the injected cells does not seem to be important as both $CD4^+$ T-cells isolated from thymus, spleen, lymph nodes and lamina propria also have disease-inducing potentials (*Reimann* et al., 1994). Non-traumatic transplantation of small pieces of gut-wall from syngeneic donors onto the back of SCID mice, likewise induces an IBD indistinguishable from the disease induced by purified $CD4^+$ T cells (*Rudolphi* et al., 1994). Furthermore, IBD can be induced in SCID mice by injection of purified $CD3^+$ $CD4^+$ T-cells expressing a transgenic T cell receptor (TCR) specific for the H-Y male epitope (*Reimann* et al., 1995), suggesting that the disease develops independently of specific antigen recognised by the disease inducing T-cells.

In agreement with this suggestion, attempts to clone the disease-inducing cell type by adoptive transfer of lamina propria T-cells from diseased mice into normal SCID hosts have proven unsuccessful, as IBD in secondary or tertiary

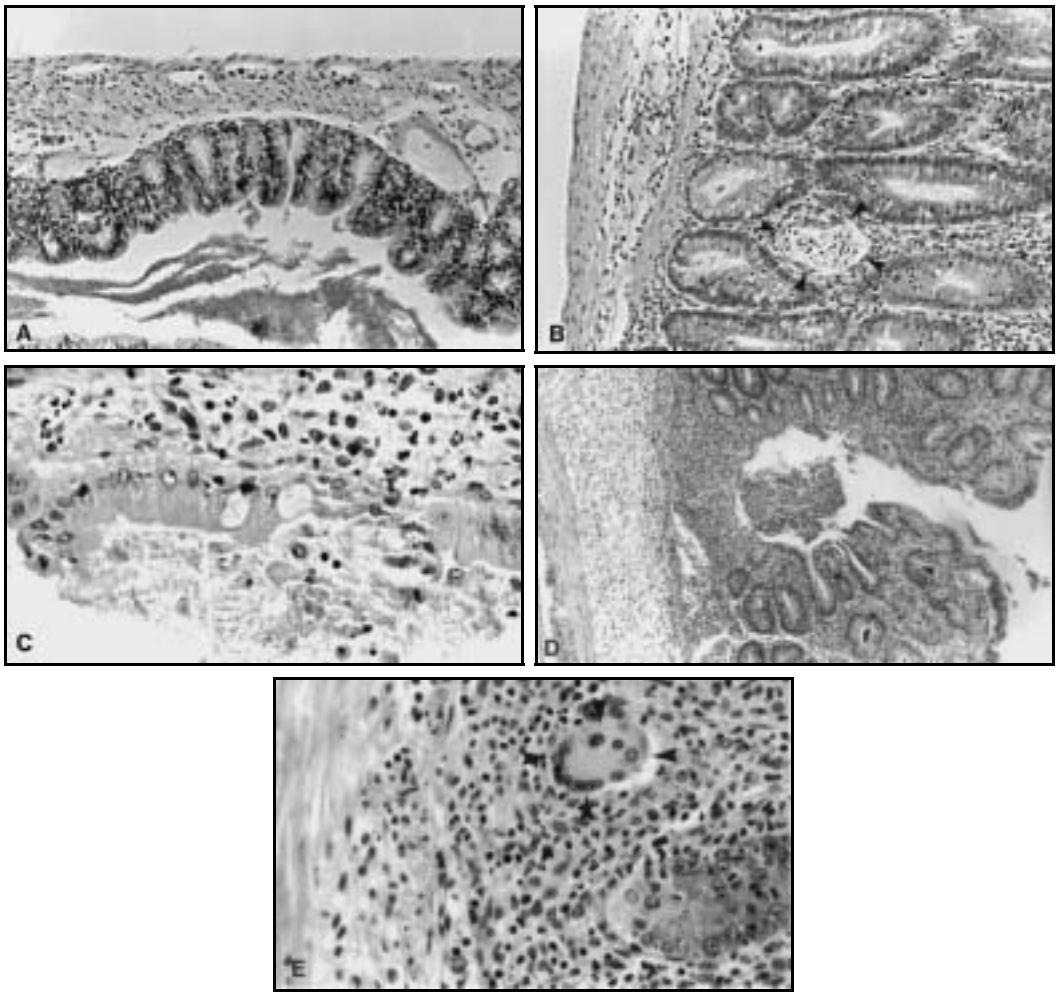

Figure 1: Histopathology of T-cell induced IBD. (A) Colon from a normal SCID mouse, magnification x100. (B) Mononuclear cell infiltration and epithelial proliferation in the colon of a SCID mouse with IBD. Arrowheads show crypt abscesses (x100). (C) Epithelial lesion in the colon of a SCID mouse with IBD, notice the many polymorphonuclears in the subepithelial lamina propria (x200). (D) Large ulcerative lesions in the colon of a SCID mouse with IBD (x50). (E) Giant cell formation (arrowheads) in the deep colonic lamina propria of a SCID mouse with IBD (x200).

transplanted SCID mice did not develop faster than in the primarily transplanted animals (*Reimann* et al., 1995).

However, the colonic microbial flora of the recipient plays a major role, because under specific pathogen free conditions, $CD4^+$ T-cell transplanted SCID mice fail to develop IBD (*Aranda* et al., 1997).

RECONSTITUTION OF LYMPHOID ORGANS

Upon transplantation, the $CD4^+$ T-cells selectively repopulate the spleen, mesenteric lymph node, peritoneal cavity, and the GALT of SCID recipients, whereas the thymus and regional peripheral lymph nodes are not repopulated (*Rudolphi* et al., 1991a,b; *Reimann* et al., 1991a, 1993a). A similar re-population pattern is seen when $CD4^+$ T-cell clones specific for the make H-Y antigen are transplanted into SCID mice (*Rudolphi* and *Reimann*, 1993).

Other organs such as such as liver, lungs, gonads, and adrenals do not get infiltrated or show signs of damage post $CD4^+$ T-cell transplantation (*Reimann* et al., 1991).

CLINICAL FEATURES OF IBD IN SCID MICE

Within three to six months following injection of purified $CD4^+$ T-cells SCID mice begin to show signs of a chronic inflammatory bowel disease, although some mice stay disease free for up to eight months. The mice exhibit a hunched back appearance, ruffled fur, and distended abdomen. The clinical disease is characterised by a prominent weight loss and softened stools. In the later stages of disease development further weight loss, diarrhoea, and in severe cases prominent rectal prolaps and bloody diarrhoea are seen. The disease is lethal within two months after onset.

HISTOPATHOLOGY OF IBD IN SCID MICE

On gross examination, the entire large intestine is enlarged and exhibits a whitely inflamed appearance. Occasionally, in severe cases the distal parts of the small intestine is macroscopically affected.

Histologically, a massive infiltration of the colonic lamina propria with mononuclear cells as well crypt elongation and crypt abscesses are observed (Figure 1B). The predominant mononuclear cell type in this infiltrate is donor derived $CD4^+$ T-cells, but increased numbers of host derived macrophages and dendritic cells are also present. At later stages in the disease development, all layers of the colon display pronounced hypertrophy and infiltration. Polymorphonuclears may dominate the lamina propria and the epithelia show spot wise signs of destruction (Figure 1C) (*Claesson* et al., 1996). The proliferative nature of the infiltrating cells is made evident by a large fraction of cells staining positive for proliferating cell nuclear antigen (PCNA) (*Claesson* et al., 1996). The massive proliferation of the colonic epithelia parallels a reduction in the number of goblet cells (see below). At the latest stages of the disease, and large transmural ulcerations are encountered (Figure 1D) accompanied by mononuclear and polymorphonuclear cell infiltration and Mac-3 positive giant cell formation (Figure 1E) (*Reimann* et al., 1995; *Rudolphi* et al., 1994; *Claesson* et al., 1996). Although lamina propria of the small intestine is densely infiltrated with donor derived $CD4^+$ T-cells, ulcerations are never found. In general, the disease is segmental, as the most severely affected animals display areas of the colon only being slightly affected by infiltration and mild hyperplasia (*Claesson* et al., 1996). The histopathological charac-

teristics described here are similar to those observed in SCID mice transplanted with CD45RB^{high} virgin T-cells (*Leach* et al., 1996). Based on the histopathology, this murine model of IBD resembles in some of the lesions human UC, in other lesions some features of CD such as crypt abscesses and transmural inflammation.

The reduction in numbers of goblet cells described above, is followed by a change in the nature of the secreted mucins. Thus, sulphomucins containing goblet cells dominate in the colon of non-transplanted SCID mice. This pattern changes towards expression of neutral mucins and subsequently to a decline in mucin containing goblet cells in severely diseased SCID mice (*Delbro*, personal communication). This observation is in agreement with a recent rapport showing, that patients suffering from UC express an altered pattern of mucin throughout the entire colon (*Smithson* et al., 1997). The cause of the change in the mucin pattern in the course of IBD in CD4$^+$ T-cell transplanted SCID mice is currently unknown, but it might reflect changes in microbial enzymatic activity such as increased sialyase activity of the luminal bacteria flora associated with disease development.

MACROPHAGES AND ANTIGEN PRESENTATION IN THE GUT OF DISEASED MICE

In human IBD a massive macrophage infiltration of the lamina propria is observed (*Rugtveit* et al., 1994). The macrophages are thought to play a central role in initiating and maintaining the inflammatory process (*Mahida* and *Jewell*, 1990).

As mentioned above, one of the first histopathological changes observed in CD4$^+$ T-cell transplanted SCID mice, is a massive mononuclear cell infiltration of the lamina propria. Immunohistochemical characterisation of this infiltrate has shown that initially, Mac-1$^+$ polymorphonuclear cell precursors, dendritic cells and activated, MHC class-II expressing, Mac-2$^+$ macrophages are infiltrating the submucosa and the basal lamina propria. In more severely affected mice, infiltration of Mac-1$^+$ polymorphonuclears is scattered through all layers of the mucosa, and clusters of activated macrophages and dendritic cells are found in the lamina propria (*Bland* et al., 1997).

In the normal gut mucosa, MHC class-II antigen-presenting molecules are expressed mainly on lamina propria macrophages and dendritic cells. During the development of IBD in SCID mice, MHC class-II expression is induced on epithelial cells whereas the MHC class-II expression on lamina propria macrophages is down regulated (*Bland* et al., 1997). The shift in antigen presentation away from the lamina propria towards the epithelium, might suggest a shift in the antigen handling of the diseased gut. This could be a determining factor in the maintenance of the intestinal inflammation as it might allow a direct presentation of luminal antigens to lamina propria CD4$^+$ T-cells.

PHENOTYPE OF DISEASE INDUCING T CELLS

IBD in SCID mice can be induced only by purified CD4$^+$ T-cells. CD8$^+$ T-cells are neither able to reconstitute the lymphoid organs of the mice nor to in-

Table 1: Characteristic of IBD-inducing T-cells in SCID mice

Surface marker / function	Suggests
TCR$\alpha\beta$ (diverse vβ repertoire), $CD4^+$, $CD8^-$	Polyclonal T-helper cells
$CD25^+$, $CD44^+$, $CD69^+$	Activated cells
$CD3^+$, $CD45RB^{low}$, L-$selectin^{low}$	Memory cells
$\alpha 4\beta 7$-$integrin^+$	Mucosa seeking
$CD2^+$, $CD28^+$	Receptive to co-stimulation
$CD95^+$ (Fas)	AICD-sensitive
Fas-L^+	AICD inducing (potentially suicidal)
IFN-γ^+, TNF-α^+, IL-2^+, IL-10^-	Th1 cells

duce IBD in SCID mice (*Rudolphi* et al., 1991b).

The general characteristics of the IBD-inducing T-cells is shown in Table 1. The lamina propria infiltrating $CD4^+$ T-cells express L-PAM-1 and L-PAM-2 (*Reimann* and *Rudolphi*, 1995; *Rudolphi* et al., 1994), as a reflection of a mucosa seeking cell type. Obviously, this is an important feature of disease inducing T-cells, since blockade of mucosal homing can reduce intestinal inflammation in $CD45RB^{high}$ $CD4^+$ T-cell reconstituted SCID mice (*Picarella* et al., 1997). The T-cells also display high levels of CD3 and low levels of CD45RB and L-selectin, a phenotype typical for memory peripheral lymphocytes (*Reimann* and *Rudolphi*, 1995; *Reimann* et al., 1993; *Rudolphi* et al., 1994, 1996). Moreover, these cells are activated as they express interleukin (IL)-2-receptor -chain (CD25) and high levels of the activation markers CD44 and CD69 (*Rudolphi* et al., 1994). Also the cells express CD2 and CD28 which increase their responsiveness to co-stimulatory signals (*Rudolphi* et al., 1996; *Reimann* and *Rudolphi*, 1995), and CD95 and CD95-ligand making them prone to activation-induced cell death (AICD, see below) (*Bonhagen* et al., 1996).

FUNCTIONAL ANALYSIS OF DISEASE-INDUCING $CD4^+$ T CELLS

The mononuclear cells infiltrating the lamina propria of $CD4^+$ T-cell transplanted mice are highly proliferative, as shown by immunohistochemical staining for PCNA (*Claesson* et al., 1996). Likewise, a high proportion of purified lamina propria $CD4^+$ T-cells have a DNA content of >2n, indicative of mitotic activity (*Rudolphi* et al., 1996). Analysis of the T-cell receptor repertoire, shows that this in vivo proliferation leads to a polyclonal expansion of $CD4^+$ T-cells (*Rudolphi* et al., 1996), arguing against one or a few single pathogenic T-cell epitopes as the driving force in this murine model of IBD. This is in concordance with human IBD where the $CD4^+$ T-cell pool appears to be selectively but, polyclonally expanding (*Probert* et al., 1996; *Gulwani-Akolkar* et al., 1995, 1996).

When isolated, lamina propria infiltrating $CD4^+$ T-cells proliferate spontaneously *in vitro* (*Bonhagen* et al., 1996; *Rudolphi* et al., 1991). This proliferative response could be co-stimulated by exogenous IL-2 and IL-7 in combination (*Bonhagen* et al., 1996). The fact that

isolated lamina propria T-cells are responsive to IL-7 *in vitro* is interesting, since epithelial cell-derived IL-7 is though to be a central regulator of mucosal T-cells (*Watanabe* et al., 1996).

The surface molecule Fas (CD95) and its counter-receptor Fas-ligand are thought to be involved in the maintenance of the homeostasis in the immune system via the induction of AICD (*Lynch* et al., 1997). As mentioned above, our previous studies suggest that freshly isolated lamina propria infiltrating $CD4^+$ T-cells express both the Fas and Fas-ligand (FasL) molecules thus, making them sensitive to AICD. In fact, AICD can be provoked *in vitro* by ligation of the CD3 molecule on $CD4^+$ T-cells in the presence of exogenous IL-2 and Il-7, a phenomenon which might explain the large number of activated but apototic mononuclear cells seen in the lamina propria of diseased mice (*Bonhagen* et al., 1996). Since colonic epithelial cells express Fas (*Moller* et al., 1994), it could be speculated that infiltrating $FasL^+$ $CD4^+$ T-cells play a central role in the destruction of epithelial cells, by induction of Fas-mediated apoptosis, resulting in the epithelial lesions (see Figure 1C). In addition, cytotoxic reactivity of lamina propria $CD4^+$ T-cells have also been reported in IL-2 knock out mice suffering from IBD (*Simpson* et al., 1995).

$CD4^+$ T-cells can be divided into two functional subsets based on their cytokine production. The pro-inflammatory Th1 subset produces interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and interleukin (IL)-2, IL-12, and IL-17 whereas the anti-inflammatory subset produces IL-4, IL-5, IL-10, and IL-13 (*Mosmann* and *Sad*, 1996). Under normal circumstances this balance is strictly regulated, however, a distorted Th1/Th2 balance is observed in many human auto-inflammatory diseases (*Romagnani*, 1996; *De Carli* et al., 1994). In the normal colonic mucosa, Th2 $CD4^+$ T-cells are the dominating cell type (*Kiyono* and *McGhee*, 1994), facilitating a B-cell-mediated, rather than a T-cell-mediated immune response. Especially, IFN-γ is speculated to be a key mediator of mucosal inflammatory reactions (*Strober* et al., 1997).

By staining for intracellular cytokines in $CD4^+$ T-cells we have shown that the Th1/Th2 balance found in healthy control animals is distorted in transplanted SCID mice (*Bregenholt* and *Claesson*, 1998a,b). The levels of all the inflammatory cytokines tested (IFN-γ, TNF-α and IL-2) are increased in diseased mice. The fraction of $CD4^+$ T-cells producing IFN-γ is increased by a factor of five to six in moderately and severely diseased mice compared to healthy controls. The fractions of TNF-α and IL-2 producing $CD4^+$ T-cells are increased by a factor of two to three in moderately and severely affected mice. The production of IFN-γ and IL-2 in colonic tissue of transplanted SCID mice has also been demonstrated by PCR technique (*Rudolphi* et al., 1993). In addition to an increase in the fraction of Th1-type cells, a decrease in Th2 cell-derived cytokines is also observed in diseased mice. IL-10 producing $CD4^+$ T-cells are almost absent from moderately and severely diseased mice and the fraction of IL-4 producing $CD4^+$ T-cells is generally decreased in diseased mice as compared to healthy controls. Taken together, this means that the Th1/Th2 ratio in diseased mice is increased by up to 20 fold compared to healthy controls.

Skewing of the cytokine pattern towards a Th1-like phenotype is a common pattern of murine models of IBD (*Hörnquist* et al., 1997; *Simpson* et al., 1997; *Davidson* et al., 1996; *Mizoguchi* et al., 1996). In the human forms of IBD, CD is generally thought to be a Th1-like disease, whereas UC is a Th2-like disease (*Fuss* et al., 1996; *Breese* et al., 1993; *Niessner* and *Volk*, 1995). Thus, although the histopathology of the

SCID IBD model resembles human both UC and CD, the cytokine pattern strictly resembles that of human CD.

MUCOSAL PLASMA CELLS IN SCID MICE WITH IBD

Involvement of auto-antibodies in the immunopathogenesis of IBD has been suggested by the finding of antibodies reacting towards components of the colonic epithelium in human UC patients (*Halstensen* et al., 1990, 1993; *Biancone* et al., 1995).

Immunohistochemical staining has shown that the development of IBD is followed by infiltration of immunoglobulin containing cells in the colonic lamina propria which are totally absent in normal SCID mice (*Claesson* et al., 1996). We have attempted to correlated the local level of plasma cell infiltration with the local histopathology in individual segments of the colon: the levels of IgM, IgA, and the anti-inflammatory isotypes IgG1 and IgG2b are significantly increased in areas showing severe histopathology as compared to areas showing no, mild, or moderate histopathology (*Bregenholt* et al., 1997b, 1998). It is noteworthy that the number IgG1 and IgG2b containing cells are higher than the numbers found in normal syngeneic C.B.-17 mice (*Claesson* et al., 1996; *Bregenholt* et al., 1997b, 1998). The specificity of the produced antibodies is currently under investigation.

Induction of immunoglobulin leakiness in T-cell transplanted SCID mice and the subsequent occurrence of serum IgM has been observed previously (*Riggs* et al., 1991, 1992; *Rudolphi* et al., 1992). Transfer of $CD4^+$ T-cells from congenic dm2 mice (IgM-allotype) into SCID mice (IgM-allotype) have shown that the resulting plasma cells are of the host IgM-allotype (*Rudolphi* et al., 1992). Thus, the plasma cells in the mucosa of $CD4^+$ T-cell transplanted SCID mice, most probably originate from leakiness in the SCID-mutation and not from donor B-cells contaminating the inoculated $CD4^+$ T-cells.

Although a possible role for B-cells in other animal models of IBD has been severely questioned (*Ma* et al., 1995; *Davidson* et al., 1996), these results in combination with several other reports (*Hörnquist* et al., 1997; *Mizoguchi* et al., 1996a,b) suggest a role for B-cells in the immunopathology of IBD.

THERAPEUTIC CONSIDERATIONS

In this and several other animal models of IBD, $CD4^+$ T-cells are shown to be the disease-inducing cells type (*Powrie* et al., 1993; *Morrissey* et al., 1993; *Simpson* et al., 1995; *Davidson* et al., 1996; *Bregenholt* et al., 1997a). $CD4^+$ T-cells also play a key role in human IBD (*Probert* et al., 1996; *Gulwani-Akolkar* et al., 1995, 1996), making them or their products obvious targets for immune therapies.

Immune therapy with infusion of monoclonal antibodies against the CD4 molecule, have shown varying results (*Emmrich* et al., 1991; *Canva-Delcambre* et al., 1996; *Stronkhorst* et al., 1997) and might today be considered as a questionable approach (*Nielsen* et al., 1997). Instead, the neutralisation of inflammatory mediators and the restoration of the Th1/Th2 balance should be brought into focus. Neutralisation of pro-inflammatory mediators such as IL-1, IL-8 and TNF-α

have shown promising results in animal models of intestinal inflammation (*Powrie* et al., 1994; *Casini-Raggi* et al., 1995). In human IBD, the blockade of TNF-α have recently shown good clinical results (*van Dullemen* et al., 1995). Treatment of IBD by reestablishment of the Th1/Th2 balance has proven useful in a number of animal models. This has been accomplished either by neutralisation of the pro-inflammatory cytokine mediators or by reconstitution of the anti-inflammatory cytokine pool (*Ehrhardt* et al., 1997; *Halstensen* et al., 1990; *Neurath* et al., 1995; *Powrie* et al., 1994). This approach has not yet been tried in human IBD, but might prove useful especially in CD, which is thought to be a Th1-mediated disease (*Romagnani*, 1996). Until the potential disease-inducing pathogen, agent or antigen have been isolated and the involvement of genetic factors have been established, cytokine directed therapy might be one of the only alternative candidates to conventional drug therapy (*Murch* and *Walker-Smith*, 1994).

CONCLUDING REMARKS

The development of several new animal models has made a reductionistic approach towards studies of the disease-inducing and -maintaining mechanisms of IBD possible. This might be valuable in defining cell types, cytokines and pathogens involved in human CD and UC.

In the present murine model of IBD, activated, mucosa seeking, memory $CD4^+$ T-cells of the Th1 type have been defined as the disease-inducing cell type. In this model the development of therapeutic regimes to restore the immunological balance of the gut mucosa should be possible. Germ-free techniques combined with the adoptive transfer of $CD4^+$ T-cells might prove very useful in the characterisation of exogenous agents important for the development of IBD.

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