Cytokine mRNA expression in intestinal tissue of interleukin-2 deficient mice with bowel inflammation

I B Autenrieth, N Bucheler, E Bohn, G Heinze, I Horak

Abstract

***Background*—Mice deficient in interleukin-2 (IL-2) develop inflammatory bowel disease resembling ulcerative colitis in humans. Recent studies provided evidence that αβ T cells, particularly CD4 T cells, rather than B cells, are involved in the pathogenesis of bowel inflammation of IL-2 deficient mice.**

***Aim*—To analyse the pattern of expression of cytokine mRNA in intestinal tissue of normal and IL-2 deficient mice.**

***Methods*—Expression of β-actin, IL-1α, IL-1β, IL-6, IL-10, tumour necrosis factor α (TNF-α), interferon γ (IFN-γ) and transforming growth factor β1 (TGF-β1) mRNA was analysed in colon and small intestinal tissue of both IL-2 deficient (IL-2−/−) mice and normal (wild type) litter mates (IL-2+/+) at different ages by using qualitative, as well as semiquantitative, competitive reverse transcription polymerase chain reaction (RT-PCR). Results were correlated with the phase of progression of the disease, as determined by histology.**

***Results*—IL-2−/− mice had expressed low levels of IL-1α, IL-1β, IL-6, TNF-α, and IFN-γ mRNA in the colon by 1.5 weeks of age. In advance of the development of histologically and clinically detectable bowel inflammation, expression of IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, and IL-10, but not TGF-β1, mRNA increased in the colon of IL-2 deficient mice. In contrast, IL-2+/+ mice expressed TGF-β1 mRNA in colon tissue at 13 and 23 weeks of age, but not IL-1α, IL-1β, IL-6, TNF-α, IL-10, or IFN-γ mRNA. Levels of expression of cytokine mRNA in tissue from the small intestine were comparable in IL-2−/− and IL-2+/+ mice.**

***Conclusions*—Bowel inflammation in IL-2 deficient mice is preceded by an increase in IL-1α, IL-1β, TNF-α, and IFN-γ mRNA expression in colon tissue. Low levels of TGF-β1, but high levels of IL-1α, IL-1β, IL-6, TNF-α, IFN-γ, and IL-10 mRNA expression correlate with the manifestation of severe colitis, and suggest that T cells and macrophages are involved in bowel inflammation of IL-2 deficient mice.**

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Keywords: cytokine; mRNA expression; interleukin-2 deficient mice; bowel inflammation

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Ulcerative colitis and Crohn's disease are characterised by chronic inflammation of the gastrointestinal tract.[1–3] The cause and pathogenesis of both of these processes as well as efficient therapy have not yet been elucidated.[1–5] A number of clinical and experimental studies, however, have suggested that an imbalance of the immune system may account for the manifestation of these diseases.[1] In the past decade several studies in humans considered the pathogenic events occurring during bowel inflammation, including—for example, characterisation of lymphocyte populations and cytokine expression patterns.[6–15] Based on these studies it is commonly accepted that lymphocytes and mononuclear phagocytes are probably essential components of the pathogenic events that occur during chronic bowel inflammation. Moreover, it is well established that cytokines are important mediators of inflammatory processes, and that the various cytokines acting together are crucial for lymphocyte differentiation and function in normal and pathological conditions. Furthermore, it has been postulated that the presence of a certain luminal microflora or microbial products, such as superantigens or cell wall polymers, may be important co-factors in the pathogenesis of bowel inflammation.[16–18]

Recent efforts have been made to establish animal models relevant to the study of the pathogenesis of bowel inflammation (for reviews, see[19–22]). In principle, three different types of animals models for inflammatory bowel disease are currently available: (*a*) mouse models with altered T cell populations—for example, T cell receptor α chain deficient mice,[23] SCID mice reconstituted with $CD45RB^{high}$ CD4 T cells,[24] or Tgε26 mice which are transgenic for human CD3ε reconstituted with wild type bone marrow cells[25]; (*b*) mouse models with imbalanced cytokine functions, such as interleukin (IL) 2,[26 27] IL-10[28] or transforming growth factor (TGF) β1[29 30] deficient mice; and (*c*) mouse models with altered cell signalling proteins, such as $G\alpha_i2$ deficient mice[31 32] or (*d*) mice expressing a dominant negative N-cadherin, which disrupts cadherin mediated cell–cell contact.[33] Interestingly, all these altered animals have an imbalanced immune system, and all of these mice develop bowel inflammation in addition to other pathological processes.

IL-2−/− mice were generated by targeted disruption of the IL-2 gene.[26] IL-2, which is

one of the key regulatory molecules of the immune system, exerts pleiotropic effects on various cells of the immune system, including T cells, natural killer (NK) cells, B cells, and macrophages.[34] Interestingly, in addition to altered T cell functions observed in vitro, IL-2 deficient mice develop generalised autoimmune diseases, including anaemia and an unlimited inflammatory bowel disease that is similar to ulcerative colitis in humans.[26 27 35 36] Subsequent studies provided evidence that T cells, rather than B cells, are apparently required for colitis in IL-2 deficient mice.[37] More recently published work showed that CD4 T cells, in particular, have an important role during this process.[38] Hence, both intraepithelial and lamina propria αβ T cell receptor positive lymphocytes of IL-2−/− mice exhibit increased cytotoxicity. Furthermore, these cells appeared in intestinal tissue prior to manifestation of the disease.

The aim of this study was to determine whether the production of a particular cytokine pattern reflects the pathogenic events occurring in intestinal tissue during bowel inflammation of IL-2−/− mice. In this study, we show the pattern of cytokine mRNA expression which both precedes and parallels bowel inflammation in IL-2-/mice and suggests a role for both T cells and macrophages in the pathogenesis of colitis in these animals.

Methods

ANIMALS

Heterozygous IL-2+/- mice[26] from a mixed C57BL/6 H129/Ola background were crossed to obtain IL-2−/− and IL-2+/+ mice. Mice were bred under specific pathogen free conditions in a barrier sustained facility at the Institute of Virology and Immunobiology, University of Wurzburg, Germany. Offspring were screened for IL-2 mutation by using PCR.[27] In the experiments described in this study, we used litter mate controls to avoid differences between lines with a different assortment of genes from C57BL/6 and 129/Sv mice.

TABLE 1 *Sequences of primers used for PCR*

Cytokine	*Sequence (5'–3')*	*Product size (bp)*
β-actin		
Sense	TGGAATCCTGTGGCATCCATGAAAC	348
Antisense	TAAAACGCAGCTCAG TAACAGTCCG	
IL-1α		
Sense	CTCTAGAGCACCATGCTACAGAC	308
Antisense	TGGAATCCAGGGGAAACACTG	
IL-1β		
Sense	TCATGGGATGATGATAACCTGCT	502
Antisense	CCCATACTTTAGGAAGACACGGATT	
IL-4		
Sense	ATGGGTCTCAACCCCCAGCTAGT	399
Antisense	GCTCTTTAGGCTTTCCAGGAAGTC	
IL-6		
Sense	CTGGTGACAACCACGGCCTCCCCT	600
Antisense	ATGCTTAGGCATAACGCACTAGGT	
TNF-α		
Sense	GGCAGGTCTACTTTGGAGTCATTGC	307
Antisense	ACATTCGAGGCTCCAGTGAATTCGG	
IFN-γ		
Sense	TGAACGCTACACACTGCATCTTGG	460
Antisense	TGACTCCTTTTCCGCTTCCTGAG	
IL-10		
Sense	ACCTGGTAGAAGTGATGCCCCAGGCA	237
Antisense	CTATGCAGTTGATGAAGATGTCAAA	
TGF-β1		
Sense	CCTCCCCCATGCCGCCCTCG	545
Antisense	CCAGGAATTGTTGCTATATTTCTG	

MEASUREMENT OF CYTOKINE mRNA EXPRESSION IN INTESTINAL TISSUE

Mice at 1.5, 7, 13, and 23 weeks of age were killed, and the intestines removed. Each small intestine and the colon was extensively washed with phosphate buffered saline (PBS; pH 7.4). A sample of the distal part of the colon (about 20% of the total colonic tissue) was used for histological sections (see later). The remaining colon (about 80%) or small intestine was homoginised in 3 ml buffer containing 4 M guanidine isothiocyanate (Sigma Chemical Co, Deisenhofen, Germany), 25 mM sodium citrate (Serve, Heidelberg, Germany), 0.5% N-lauroylsarcosine (Sigma), and 100 mM 2-mercaptoethanol (Fluke, Buchs, Switzerland). The homogenates were stored at −70°C until further processing. RNA was purified by phenol-chloroform extraction, precipitated by isopropanol, and resuspended in diethylpyrocarbonate treated water as described previously.[39 40]

Reverse transcription (RT) was performed by mixing 20 μg RNA solution and 2 μg oligo(dT) (United States Biochemical, Cleveland, OH, USA), and incubating the solution (10 μl) for 10 minutes at 65°C. Then, 10 μl of a solution containing 2× reverse transcriptase buffer (100 mM Tris-HCI (pH 8.3), 150 mM KCI, 6 mM $MgCI_2$ (Gibco BRL, Life Technologies, Berlin, Germany), 40 U RNasin (Promega Biotec, Madison, Wl, USA), 20 mM dithiothreitol (Gibco), 200 U Superscript RNase H Reverse Transcriptase (Gibco), and 2 mM deoxynucleoside triphosphate (dNTP)) was added, and the tubes were incubated for 60 minutes at 37°C. Finally, the tubes were heated to 90°C for five minutes, and 180 μl water was added to the reaction mixture. Samples were stored at −20°C until further use. RNA extraction and reverse transcription were performed simultaneously for colon and small intestine isolated from 1.5, 7, 13 and 23 week old IL2+/+ and IL-2−/− mice.

Table 1 shows the primer pairs used for PCR.[39–42]

cDNA was mixed with a solution of 1 U Taq DNA polymerase (Perkin Elmer, Branchburg, NJ, USA), 200 mM dNTP, 200–500 nM 5' and 3' primers, and Taq DNA polymerase buffer (50 mM KCI, 10 mM Tris-HCI (pH 8.3), and 1.5 mM $MgCI_2$) (Perkin Elmer). This mixture was overlaid with mineral oil, and 15–35 cycles of PCR were performed in a thermal cycler, using the following conditions: denaturation, 45 seconds at 94°C; annealing, 60 seconds at 60°C; and extension, 90 seconds at 72°C. PCR products were visualised by agarose gel electrophoresis. The data shown are representative of at least five animals per group and time point.

For semiquantitive assessment of β-actin,TNF-α and IFN-γ mRNA expression, semiquantitative competitive PCR was carried out as described previously.[39 40 42] For this purpose, PCR was carried out in parallel for the samples to be compared, using a constant

amount of target cDNA in the presence of serially diluted control competitor DNA with 5' and 3' primer sequences in a tandem array. The plasmids pmCQ (for β-actin and TNF-α PCR) and pG2PCR106γ4 (for IFN-γ PCR) were kindly provided by T Blankenstein, Freie Universitat Berlin, and I Berberich, Universitat Wurzburg. Furthermore, we ensured that equal amounts of cDNA were obtained from the samples to be compared. Thus, only samples which revealed comparable results in competitive RT-PCR for β-actin were used for the determination of IFN-γ and TNF-α mRNA expression levels.

HISTOLOGICAL EXAMINATION

Colon and small intestinal tissue was investigated both macroscopically and microscopically for signs of inflammation as described previously.[27] For histological investigations a part (see earlier) of the small intestine and colon were fixed in buffered paraformaldebyde (Merck), infiltrated and then embedded in Technovit 7100 medium (Heraeus Kulzer,

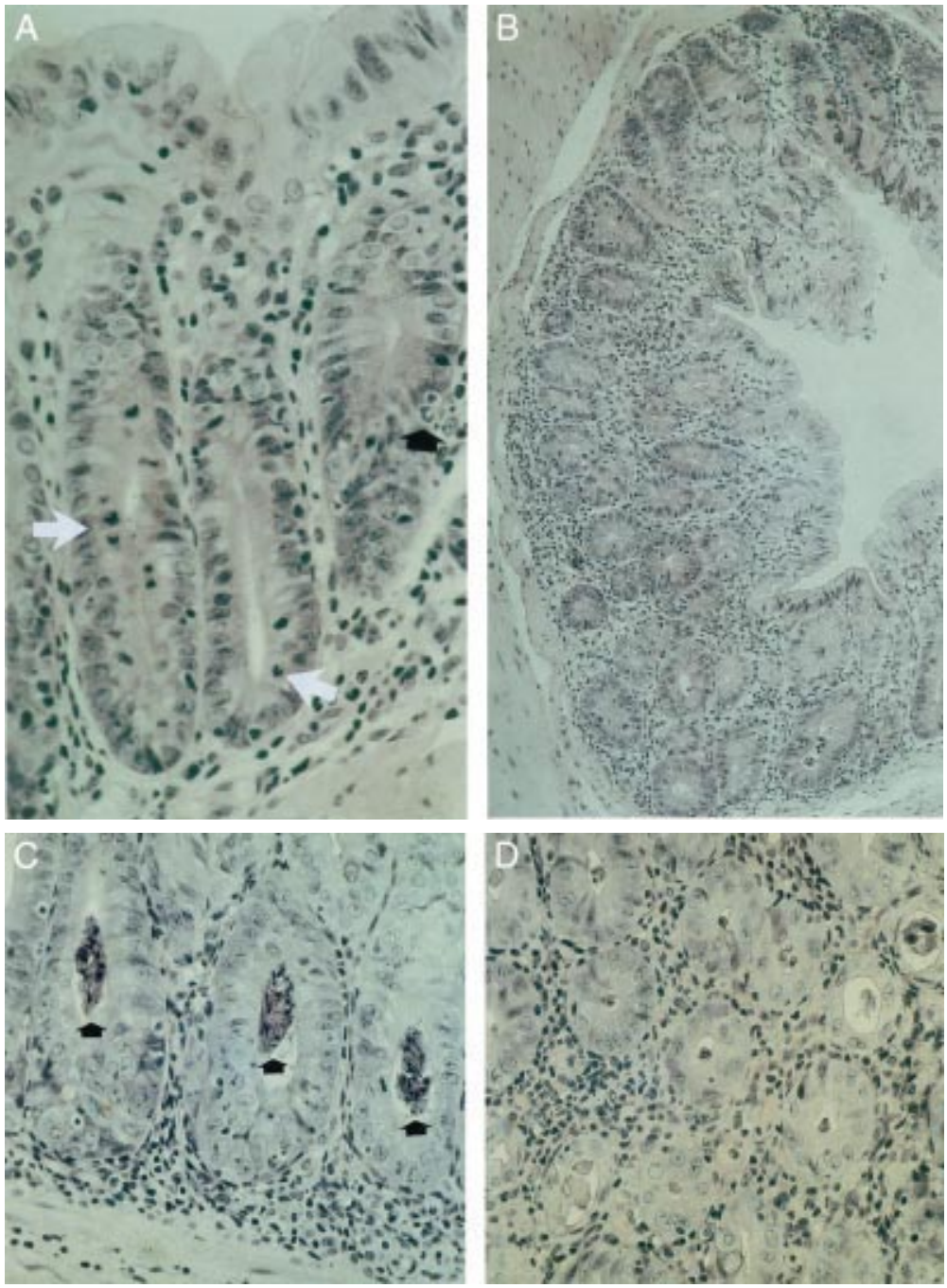

Figure 1: Colon of IL-2–/– mice at (A) 8 weeks (early phase with few alterations, mild colitis), (B–D) 13 weeks of age (later phase with severe colitis). (A) Infiltration of the lamina propria with lymphocytes, plasma cells and granulocytes. A few transmigrating polymorphonuclear leucocytes are present (black arrow). The number of mitotic epithelial cells is increased (white arrows). (B) Severe inflammation of the colon (overview). (C) Crypt abscesses and an increased number of mitotic epithelial cells (arrows). (D) Transverse section, infiltration of the lamina propria, crypt abscesses (haematoxylin and eosin).

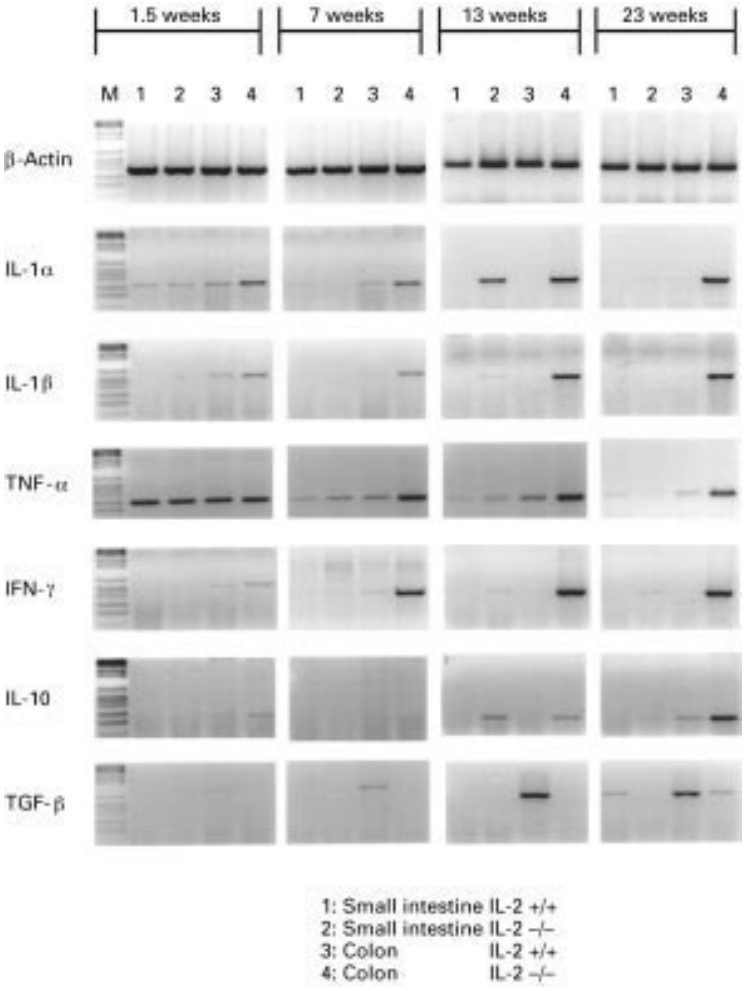

Figure 2: PCR assisted amplification of β-actin, IL-1α, IL-1β, TNF-α, IFN-γ, IL-10, and TGF-β mRNA extracted from intestinal and colonic tissue of IL-2+/'+ and IL-2−/− mice at 1.5, 7, 13, and 23 weeks of age. Amplification cycles: β-actin, 21 cycles; IL-1α, IL-1β, TNF-α, and IFN-γ, 30 cycles; IL-10 and TGF-β1, 35 cycles. M, molecular weight markers. The data shown are the results from one knockout and from one wild type mouse and are representative of five mice from each group.

Wehrheim, Germany). Finally, six to 10 sections of small intestine and colon of each mouse were prepared using 2065 Supercut (Reichert Jung, Nuliloch, Austria), and stained with haematoxylin and eosin as described elsewhere.[43 44]

STATISTICS

Differences between mean values were analysed by using the Student's *t* test; p values <0.05 were considered significant.

Results

PROGRESSION OF BOWEL INFLAMMATION IN IL-2 DEFICIENT MICE

As described in previously published work,[27] about 50% of the IL-2−/− mice died from severe anaemia during their first 9 weeks. The cause is believed to be a B cell mediated pathomechanism.[27 37] The remaining IL-2−/− mice developed bowel inflammation that mainly affected the colon. At 7 weeks of age the first clinical and histological signs of colitis were observed in IL-2−/− mice, although there were no significant histological alterations to the tissue in younger animals. Hallmarks of colitis in older animals were the infiltration of the superficial mucosa by lymphocytes, plasma cells and granulocytes, and an increased number of mitotic epithelial cells, as well as ulcerations and crypt abscesses (fig 1). However, such pronounced tissue alterations were observed only in mice older than 13 weeks. In parallel with the development of bowel inflammation mice became severely compromised and if not killed, died from fulminant colitis between weeks 15 and 30.

CYTOKINE mRNA EXPRESSION IN SMALL INTESTINE AND COLON OF IL-2+/+ AND IL-2−/− MICE

Analysis of mRNA expression of the pro-inflammatory cytokines revealed that by 1.5 weeks of age, there was an increase in IL-1α, IL-1β, IFN-γ, and IL-10 mRNA expression in colon of IL-2−/− mice when compared with IL-2+/+ mice (fig 2). At the same time, expression of cytokine mRNA was investigated in the small intestine of each mouse as an intra-individual control. In contrast to the observation in colon tissue, there was no increase in cytokine mRNA expression in the small intestine of IL-2−/− mice.

At 7 weeks of age, expression of IL-1α, IL-1β, IL-6 (not shown), TNF-α, IFN-γ, and IL-10 in the colon of IL-2−/− mice increased further (fig 2). Although mRNA of all these cytokines was not significantly expressed in IL-2+/+ litters, a significant increase in TGF-β1 mRNA expression in colon tissue could be observed in these animals, whereas notably increased expression of TGF-β1 mRNA expression was not found in the colon of IL-2−/− mice (fig 2).

Comparable, but even more pronounced cytokine mRNA expression patterns were observed in mice at 13 and 23 weeks of age, respectively. In addition, weak but significant TGF-β1 mRNA expression was observed in the small intestine of IL-2+/+ mice, whereas there was no significant TGF-β1 mRNA expression in the intestine of IL2−/− mice. TNF-α mRNA levels decreased in an age dependent manner in the small intestine of both IL-2–/– and IL-2+/+ mice and in the colon of the latter but were increased in the colon of the former. Finally, IL-4 mRNA was not significantly expressed in intestinal tissues of either IL-2−/− or IL-2+/+ mice during the observation period (data not shown).

SEMIQUANTITATIVE ASSESSMENT OF CYTOKINE mRNA EXPRESSION

These results suggest that bowel inflammation in IL-2−/− mice is both preceded and paralleled by an increase in mRNA expression of various pro-inflammatory cytokines in colon tissue. Although we controlled the input cDNA, RT-PCR is a qualitative method in which it is difficult to compare the quantities of cytokine mRNA present in tissues. In order to compare mRNA expression of TNF-α and IFN-γ between IL-2−/− and IL-2+/+ mice we used a semiquantitative competitive RT-PCR technique.[39 40 42] Figure 3 shows the results of a representative experiment for the determina-

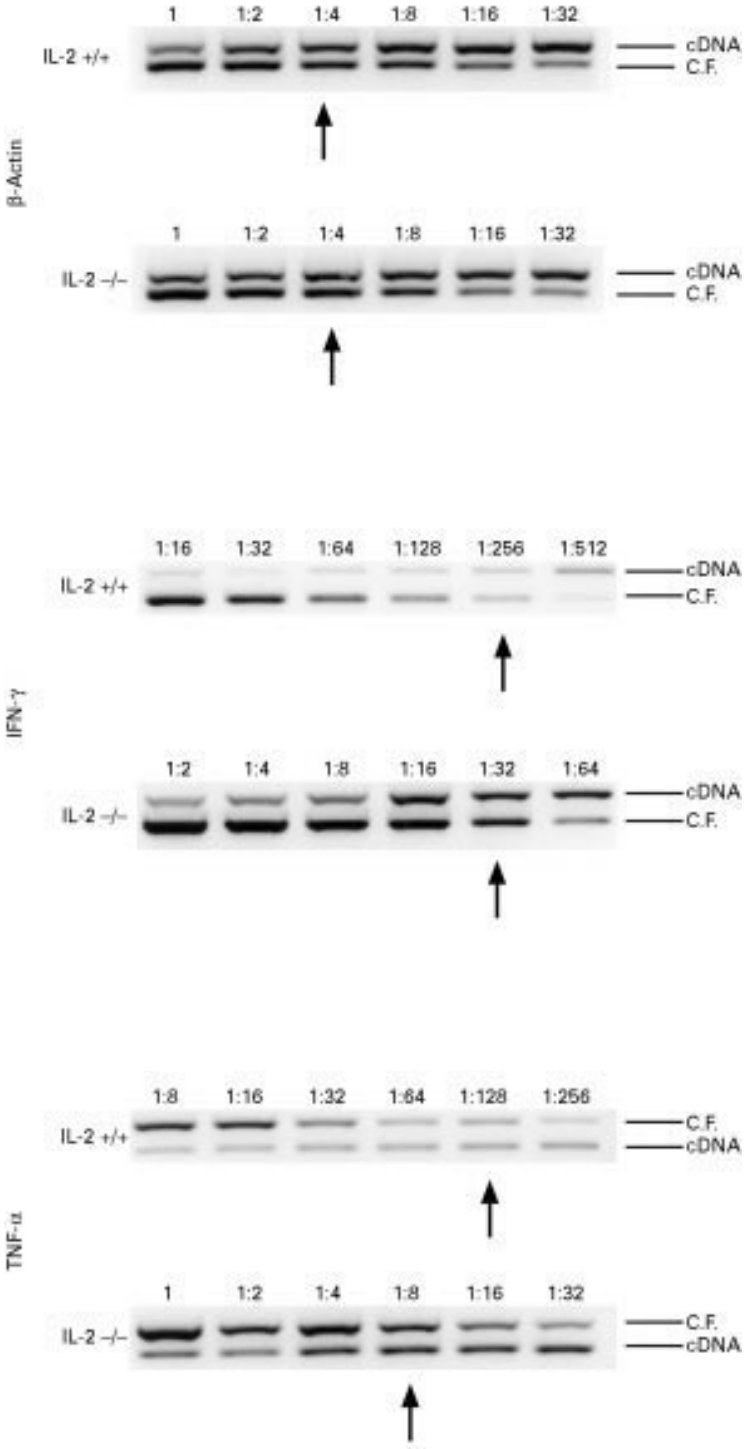

Figure 3: Semiquantitative competitive PCR assisted amplification of β-actin, IFN-γ and TNF-α mRNA extracted from colonic tissue of IL-2−/− and IL-2+/+ mice at 13 weeks of age. Constant amounts of target cDNA were amplified in the presence of serially diluted competitor control DNA (1, undiluted cDNA: β-actin, pMCQ 3 ng, 28 cycles; TNF-α, pMCQ 3 pg 35 cycles; IFN-γ, pG2PCR106g4 0.125 pg 35 cycles). The dilution at which equally dense bands for control and target DNA were obtained was used for determination of cytokine mRNA expression levels. C.F. = control fragment (competitor control DNA). Arrows indicate the dilution step at which equally dense bands were obtained. The data shown are the results from one knockout and from one wild type mouse and are representative of five mice from each group.

tion of TNF-α and IFN-γ mRNA expression in IL-2−/− and IL-2+/+ mice. The data summarised in figure 4 indicate only a slight (1.5 to 2-fold) increase in the expression of IFN-γ and TNF-α mRNA in the colon of IL-2−/− mice at

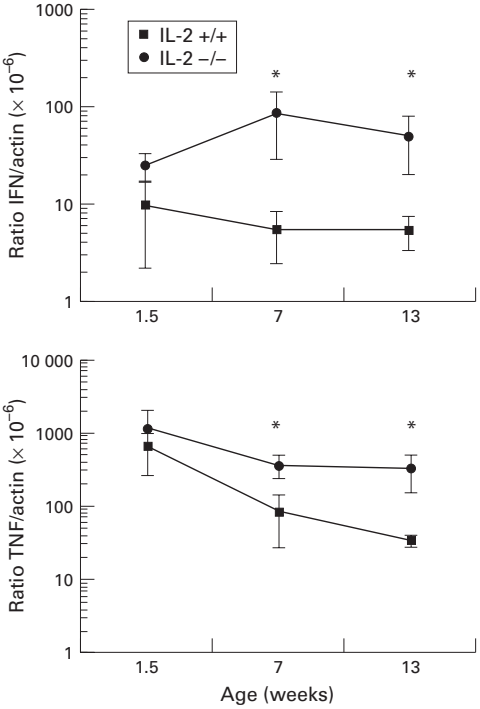

*Figure 4: Semiquantitative determination of IFN-γ and TNF-α mRNA expression in the colon of IL-2−/− and IL-2+/+ mice at 1.5, 7 and 13 weeks of age was performed as described in the legend to fig 3. The values represent the means of five mice per time point for each group. *p<0.05 between IL-2 −/− and IL-2+/+ mice. The ratio of IFN-γ or TNF-α to β-actin was calculated as follows: [concentration of IFN-γ control fragment×dilution factor of target DNA (IFN-γ or TNF-α)]÷[concentration of β-actin control fragment×dilution factor of target DNA β-actin]*

1.5 weeks of age. However, at 7 weeks of age the levels of IFN-γ mRNA and TNF-α mRNA in the colon of IL-2−/− mice were 10- and fivefold, respectively ($p<0.05$), higher than those in IL-2+/+ mice. By 13 weeks of age, INF-γ mRNA and TNF-α mRNA levels were both 10-fold higher ($p<0.05$) in IL-2−/− mice than in IL-2+/+ mice.

TNF-α mRNA levels in the colon of 1.5 week old IL-2+/+ mice were 20-fold higher than those in the 13 week old mice. By contrast, TNF-α mRNA levels in IL-2−/− mice did not decrease significantly on aging. These findings indicate that the higher TNF-α mRNA levels in IL-2−/− mice result from an age dependent decrease in TNF-α mRNA levels in IL-2+/+ mice.

Discussion

Recently, mice with targeted disruption of cytokine genes such as IL-2, TGF-β1 and IL-10 have been bred.[26 28 29] These cytokine deficient mice show altered immune responses as a result of an imbalanced immune system, and develop bowel inflammation resembling chronic inflammatory bowel disease in humans.[26–29]

In this study we have used IL-2−/− mice in an attempt to investigate and characterise the type of cytokine expression pattern in inflamed intestinal tissue. The most salient findings of our study are as follows. Firstly, increased expression of IL-1α, IL-1β, IL-6, IL-10,

TNF-α, and IFN-γ mRNA, but decreased expression of TGF-β1 mRNA, is associated with bowel inflammation in IL-2−/− mice. Secondly, by 10 days of age mice already exhibit a shift towards the cytokine mRNA expression pattern indicated above, although bowel inflammation starts at 6 to 8 weeks of age at the earliest. This indicates that the pathomechanism that accounts for bowel inflammation in IL-2−/− mice is apparently initiated during the first few days of life. This observation is consistent with the finding that treatment of IL-2−/− mice with recombinant IL-2 prevents disorders of the immune system only when started during the first days of life (manuscript in preparation). Furthermore, 10 day old, but not 6 day old, IL-2−/− mice exhibit increased proliferation and polyclonal activation of lymphocytes,[36] suggesting that IL-2 is required for differentiation or maturation of regulatory cells.

Recent work indicated that T cells, but not B cells, are required for bowel inflammation in IL-2−/− mice.[37] More strikingly, it was shown that CD4, but not CD8, T cells infiltrating the lamina propria are involved in bowel inflammation of IL-2−/− mice.[38] That these cells are activated and exhibit increased killing activity is concluded from the expression of $CD45RB^{lo}$, L-$selectin^{lo}$ and CD69.[38] Moreover, colonic αβTCR+ CTL showed increased cytotoxic activity in IL-2−/− mice and, interestingly, appeared earlier than did manifestations of colitis.[38] However, cytokine production by these T cells was not investigated. Nevertheless, these observations, together with the data presented in this study, suggest that bowel inflammation in IL-2−/− mice is mediated by a TH1 response involving T cells and macrophages.

In patients with inflammatory bowel disease an altered TH1/TH2 cytokine profile was found in intestinal mucosal tissue.[45] For instance, IL-2, IFN-γ and IL-10 mRNA expression was increased whereas IL-4 mRNA expression was decreased. Similarly, other workers have observed increased IL-1 and IL-8, and slight increases in IL-2 and IL-6 mRNA expression even in non-diseased intestinal segments from patients with IBD.[46] Likewise, we found altered cytokine expression in 10 day old mice in which bowel inflammation developed 6 to 8 weeks later. Increased IL-10 mRNA expression, but decreased IL-2 expression, has also been detected in patients with IBD, although the latter result was not consistent for various tissue samples.[47] Earlier studies showed increased IL-2 mRNA expression in intestinal mucosal lesions of patients with Crohn's disease[12] and loss of IL-2 producing CD4 T cells in intestinal tissues of patients with IBD has also been observed.[8] Thus, the role of IL-2 in IBD in humans remains to be clarified.

In the murine model in which colitis is induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS), IL-12 was shown to be a crucial mediator of bowel inflammation.[48] Hence, administration of anti-IL-12 antibodies abrogated experimental colitis, and reduced IFN-γ production by intestinal CD4 T cells, suggesting that a TH1-like response mediates colitis.[48] Bowel disease in $CD45RB^{high}$ CD4 T cell reconstituted SCID mice, which is associated with an ex vivo TH1 response, can be inhibited by administration of anti-lFN-γ antibodies, and anti-TNF-α antibodies reduced the incidence of the disease.[24] Increased amounts of TNF-α producing cells were also found in inflamed intestine of patients with IBD, suggesting that macrophages have an important role in the disease.[6] Indeed, colitis induced by dextran sulphate sodium can also be induced in SCID mice, suggesting that T or B cells do not have a superior role in this particular disease model.[49] Interestingly, in this study, TNF-α mRNA expression in the small and large intestine of 10 day old IL-2+/+ and IL-2−/− mice was 20-fold stronger than that seen in the 7 or 13 week old mice. Although TNF-α mRNA is highly expressed in normal tissue such as spleen, lung and small intestine, this is the first study to show age dependent expression of TNF-α mRNA in intestinal tissue.[50 51] The reason for the decrease in TNF-α mRNA expression in elderly mice is not yet clear, however.

Moreover, monocyte chemoattractant protein 1 is constitutively expressed in normal intestinal surface epithelium, but increased expression was observed in intestinal tissue of patients with IBD.[13] Mononuclear phagocytes isolated from lamina propria of patients with IBD produce increased amounts of IL-1β and TNF-α with a decreased IL-1 receptor antagonist to IL-1 ratio.[15] Furthermore, IL-4, an anti-inflammatory cytokine has a diminished inhibitory activity on these cells in patients with IBD, suggesting disturbed IL-4 mediated regulation of mononuclear phagocyte effector functions in IBD.[14] Interestingly, equal IL-10 concentrations can be found in both normal and IBD intestinal tissue although administration of exogenous IL-10 downregulates IL-1β and TNF-α production.[14] In keeping with these results, a beneficial effect of anti-TNF-α antibodies was also observed in IL-2−/− mice with colitis, suggesting that macrophages are involved in bowel inflammation in these mice (unpublished observations). In IL-2−/− mice we observed a broad spectrum of increased intestinal cytokine mRNA expression, whereas in IL-2+/+ mice only significant expression of TGF-β1 mRNA levels was observed, with no expression of pro-inflammatory cytokines. TGF-β1 is a ubiquitous cytokine that elicits diverse effects depending on cell type, state of differentiation and culture conditions.[30] TGF-β1 can induce both inhibitory and stimulatory effects, including collagen synthesis or degradation, monocyte activation, chemotaxis and induction of IgA production, as well as inhibition of lymphocyte proliferation.[30] Our observation suggests that a certain level of TGF-β1 production is associated with the absence of inflammation in the colon. This finding is in keeping with other models of IBD showing that treatment with anti-TGF-β antibody induces colitis.[52 53] In vitro studies on epithelial cell lines showed that

IL-2 can induce expression of TGF-β.[54 55] Therefore, we postulate that IL-2 may upregulate expression of TGF-β in IL-2+/+ mice, which obviously cannot occur in IL-2−/− mice. Downregulation of TGF-β expression in IL-2−/− mice may facilitate IL-2 induced production of IFN-γ. Likewise, colitis in TNP-KLH immunised IL-2−/− mice is associated with overexpression of IL-12, which in turn induces high levels of IFN-γ expression.[56] IL-10 mRNA expression was higher in IL-2−/− mice with end stage disease than in IL-2+/+ mice, possibly because of a negative regulatory feedback mechanism controlling and maintaining steady state expression of IFN-γ. Alternatively, the lack of increased IFN-γ mRNA expression at the later stages of colitis could result from a relative decrease in T cell numbers in end stage colitis. This observation is in keeping with the finding that TGF-β seems to be expressed constitutively, although at a low level, by intestinal epithelial cells. Furthermore, it must be stressed that future studies need to demonstrate whether the results shown in this work correlate with the functional cytokine protein concentration. Whether TGF-β1 can be used as an immunomodulatory drug for the treatment of IBD remains to be established. Interestingly, a previous report provided evidence for increased TGF-β1 (and IL-1β) mRNA expression in intestinal tissue from patients with IBD, which may argue against TGF-β1 as a target for therapeutic intervention.[10]

In addition to abnormal regulation of the immune system, exogenous factors such as micro-organisms and their products are obviously required for bowel inflammation.[16 27] IL-2−/− mice bred in a germ free environment do not develop bowel inflammation.[27] Likewise, the germ free state of HLA-B27 transgenic rats prevents the development of bowel and joint inflammation.[57] Preliminary data from our laboratory suggest that colonisation of IL-2−/− with a single bacterial species is sufficient stimulus to induce the disease (unpublished observations). At present, however, neither the bacterial component, nor the primary target cell that is stimulated and the subsequent pathomechanism induced are known.

Taken together, we found that pro-inflammatory cytokines such as IL-1 and TNF-α, as well as TH1-like cytokines such as IFN-γ are increased in intestinal tissue of IL2−/− mice suggesting that both T cells as well as macrophages are involved in bowel inflammation. Although a role of the normal gut flora in triggering bowel inflammation has been established, the mechanisms by which the flora "turns on" the pathomechanism remain to be elucidated. Whether an abnormal T cell response to microbial antigens, or microbial stimulation of innate components of the immune system initiates this process is currently being investigated in our laboratory.

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