The pathophysiology of Crohn's disease (CD) is characterised by an overriding cell mediated immune response which results in accumulation and activation of T lymphocytes and macrophages. By orchestrating the inflammatory process, proinflammatory cytokines such as interleukin (IL)-2, IL-12, and interferon-γ (IFN-γ) are thought to promote the development of the disease. IL-12, predominantly produced by antigen-presenting cells, is secreted in mucosal tissues and contributes to the predominance of the Th1 response in patients with CD. Mucosal inflammation can be abrogated by neutralising IL-12 antibodies in experimental models such as in 2,4,6-trinitrobenzene sulphonic acid (TNBS) colitis model and in lamina propria mononuclear cells (LPMNC) from patients with CD in vitro. Consequently, Th1 development is enhanced in the IL-12(p40) deficient mice compared with IL-12(p35) deficient mice, suggesting that IL-12(p40) dimers act contra-inflammatory. On the other hand, IL-12(p40) heterodimeric protein composed of the p35 and p40 subunits. The IL-12(p40) subunit specifically inhibits IL-12 secretion and lymphocyte proliferation thereby acting as an IL-12 antagonist at high concentration.

Methods: We generated a fusion protein consisting of the IL-12(p40) subunit fused to the constant region of IgG2b. IL-12(p40)-IgG2b was tested in a murine 2,4,6-trinitrobenzene sulphonic acid (TNBS) colitis model and in lamina propria mononuclear cells (LPMNC) from patients with CD in vitro. Results: Dimeric IL-12(p40)-IgG2b fusion protein bound specifically to the IL-12 receptor. In concentrations \(10^{-7} \text{M}\), it acted as an IL-12 antagonist as it inhibited interferon-γ (IFN-γ) secretion, suppressed proliferation, and increased apoptosis of LPMNC from patients with CD. However, in concentrations \(10^{-6} \text{M}\), IL-12(p40)-IgG2b increased IFN-γ secretion and lymphocyte proliferation thereby acting as an IL-12 agonist. In TNBS colitic mice, IL-12(p40)-IgG2b decreased mortality (10% vs 68%), prevented body weight loss, reduced tumour necrosis factor α, and increased IL-10 secretion.

Conclusions: The IL-12(p40)-IgG2b fusion protein has dichotomic properties as a specific IL-12 antagonist and selective repressor of mucosal inflammation at low concentration and as an IL-12 agonist at high concentration.

MATERIALS AND METHODS

Patients

Between July 1998 and July 2003, 32 patients treated at the University Hospital of the Saarland were included in our prospective study after written consent. Intestinal tissue specimens were obtained from two groups of patients: the control group included 15 specimens of macroscopically normal colonic mucosa from unaffected areas of colonic resections performed for adenocarcinoma; the second group included 17 affected colon specimens from patients with CD (see table 1).

Cells, cell lines, and antibodies

The hybridoma OKT3 produces the anti-CD3 monoclonal antibody (mAb) (American Type Culture Collection, ATCC CRL 8001; Manassas, Virginia, USA) and the hybridoma 15E8 produces the anti-CD28 mAb (kindly provided by T van Lier, NCB, Amsterdam, the Netherlands). The anti-IL-12 receptor β1 and β2 chain mAbs were kindly provided by D Presky (Hoffmann-La Roche, Nutley, New Jersey, USA). Anti-CD4 (clone MT310) and anti-CD8 (clone DK25) antibodies were purchased from Dako (Hamburg, Germany). Recombinant IL-12 was prepared in our laboratory as previously described. 5-ASA, 5-aminosalicylic acid; CD, Crohn’s disease; TNBS, 2,4,6-trinitrobenzene sulphonic acid; LPMNC, lamina propria mononuclear cells; PBMC, peripheral blood mononuclear cells; IL, interleukin; IFN-γ, interferon-γ; mAb, monoclonal antibody; PMA, phorbol myristate acetate; PBL, peripheral blood lymphocytes; TNF-α, tumour necrosis factor α.

Abbreviations: 5-ASA, 5-aminosalicylic acid; CD, Crohn’s disease; TNBS, 2,4,6-trinitrobenzene sulphonic acid; LPMNC, lamina propria mononuclear cells; PBMC, peripheral blood mononuclear cells; IL, interleukin; IFN-γ, interferon-γ; mAb, monoclonal antibody; PMA, phorbol myristate acetate; PBL, peripheral blood lymphocytes; TNF-α, tumour necrosis factor α.
human IL-2 and IL-12 were purchased from Pharmingen (Heidelberg, Germany). Peripheral blood mononuclear cells (PBMC) and LPMNC were isolated from the large bowel, as described previously.\textsuperscript{21} Immunomagnetic beads (Dynal, Oslo, Norway) were used to isolate CD4\textsuperscript{+} or CD8\textsuperscript{+} peripheral blood lymphocytes (PBL) or LPMNC. Cell preparations with a purity of >95% were used.

**Generation of the IL-12(p40)-IgG2b fusion protein**

DNA coding for the IL-12(p40)-IgG2b fusion protein was generated by ligation of the mouse IL-12 p40 cDNA to the DNA coding for the IL-12(p40)-IgG2b fusion protein, as described previously.\textsuperscript{21} Immunomagnetic beads (Dynal, Oslo, Norway) were used to isolate CD4\textsuperscript{+} or CD8\textsuperscript{+} peripheral blood lymphocytes (PBL) or LPMNC. Cell preparations with a purity of >95% were used.

**Table 1 Patient characteristics**

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Duration (y)</th>
<th>Surgical treatment</th>
<th>Medical treatment</th>
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<tbody>
<tr>
<td>1-15</td>
<td>53-72</td>
<td>BM/6F</td>
<td>Adenocarcinoma of ascending (n = 4) or descending (n = 11) colon</td>
<td>—</td>
<td>Hemicolectomy (right or left)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>31</td>
<td>M</td>
<td>CD, stricture in sigma</td>
<td>7</td>
<td>Resection of stricture</td>
<td>None</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>F</td>
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<td>5-ASA, glucocorticoids</td>
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<tr>
<td>18</td>
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<td>M</td>
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<td>Glucocorticoids, azathioprine</td>
</tr>
<tr>
<td>19</td>
<td>35</td>
<td>M</td>
<td>CD, 2 strictures in terminal ileum</td>
<td>5</td>
<td>Resection of ileocecal part</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>20</td>
<td>21</td>
<td>F</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>7</td>
<td>Resection of ileocecal part</td>
<td>5-ASA</td>
</tr>
<tr>
<td>21</td>
<td>23</td>
<td>F</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>5</td>
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<td>None</td>
</tr>
<tr>
<td>22</td>
<td>35</td>
<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
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<td>Resection of ileocecal part</td>
<td>Glucocorticoids</td>
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<tr>
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<tr>
<td>24</td>
<td>44</td>
<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
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<td>Resection of ileocecal part</td>
<td>Glucocorticoids</td>
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<tr>
<td>25</td>
<td>43</td>
<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>7</td>
<td>Resection of ileocecal part</td>
<td>Glucocorticoids</td>
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<tr>
<td>26</td>
<td>42</td>
<td>M</td>
<td>CD, stricture in ileum</td>
<td>5</td>
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<td>5-ASA, glucocorticoids</td>
</tr>
<tr>
<td>27</td>
<td>34</td>
<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>3</td>
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<td>Glucocorticoids</td>
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<tr>
<td>28</td>
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<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>1</td>
<td>Resection of ileocecal part</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>29</td>
<td>43</td>
<td>M</td>
<td>CD, ileocecal conglomerate tumour</td>
<td>12</td>
<td>Resection of ileocecal anastomosis</td>
<td>Glucocorticoids, azathioprine</td>
</tr>
<tr>
<td>30</td>
<td>27</td>
<td>F</td>
<td>CD, stricture in ileum</td>
<td>12</td>
<td>Resection of ileocecal part</td>
<td>Glucocorticoids, azathioprine</td>
</tr>
<tr>
<td>31</td>
<td>30</td>
<td>M</td>
<td>CD, colorectal anastomosis</td>
<td>3</td>
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<tr>
<td>32</td>
<td>21</td>
<td>M</td>
<td>CD, stricture in ileum</td>
<td>4</td>
<td>Resection of ileocecal part</td>
<td>5-ASA</td>
</tr>
</tbody>
</table>

5-ASA 5-aminosalicylic acid, CD Crohn’s disease, F female, M male.

**Detection of the IL-12(p40)-IgG2b fusion protein**

The IL-12(p40)-IgG2b protein was detected by ELISA. Briefly, 96 well microtitre plates were coated with rat antimouse Fc antiserum (5 μg/ml phosphate buffered saline (PBS); Dako, Hamburg, Germany), blocked with 3% (w/v) bovine serum albumin in PBS, and incubated with serial dilutions of cell supernatant containing fusion protein or human IgG (0.5 μg/ml) as a control. Plates were incubated with a rat antihuman Fc polyclonal antibody conjugated with alkaline phosphatase (1 μg/ml PBS; Dako). ELISA was developed by addition of the alkaline phosphatase substrate disodium p-nitrophenyl phosphate (1 mg/ml PBS; Dako). For Western blot analysis, 5 μg of purified fusion protein was electrophoresed through a 10% sodium dodecyl sulphate-polyacrylamide gel and electrophoretically transferred onto nitrocellulose membranes. Membranes were blocked with Tris buffered saline, 5% (w/v) bovine serum albumin, incubated with an anti-IL-12p40/p70 antibody (clone C8.6; 1:250) (Pharmingen), and subsequently with a goat anti-mouse Ig antibody conjugated to horseradish peroxidase (1:3000) (Dianova, Hamburg, Germany).

**Binding of the IL-12(p40)-IgG2b fusion protein to IL-12 receptor positive cells**

PBMC were stimulated for 72 hours with human IL-2 (400 U/ml) and phorbol myristate acetate (PMA) (2 ng/ml). Cells were washed, incubated with fetal calf serum (10% v/v) for 15 minutes at 4°C to block non-specific staining, and incubated with fusion protein in increasing concentrations. Bound fusion protein was monitored by an FITC conjugated

**Table 2 Primer oligonucleotides**

<table>
<thead>
<tr>
<th>Primer oligonucleotides</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12(p40)-IgG</td>
<td>gac ctc cag acg cgg tgg gag cgg gag aca cag gca</td>
</tr>
<tr>
<td>Downstream primer</td>
<td>gac ctc cgg gag att gca gac</td>
</tr>
<tr>
<td>Truncated IL-12(p40)-IgG</td>
<td>gac ctc cag acg cgg tgg gag cgg gag aca cag gca</td>
</tr>
<tr>
<td>Downstream primer</td>
<td>gac ctc cgg gag att gca gac</td>
</tr>
</tbody>
</table>
anti-IgG antibody (Dako). Specific binding of the fusion protein was confirmed by competitive inhibition with recombinant IL-12 (Pharmingen). Cells were stained for CD4, CD8, and IL-12 receptor β1 and β2 chains, respectively, fixed for 30 minutes with 1% (v/v) paraformaldehyde, and analysed by three colour flow cytometry (FACS-Vantage; Becton Dickinson, Heidelberg, Germany) using CellQuest software.

**Proliferation assays**

Cells (1×10⁶) in 0.2 ml RPMI 1640 medium and 10% fetal calf serum were incubated with 0.5 µCi [³H]thymidine (specific activity 3.64 GBq/mg) for 16 hours at 37°C. Cells were harvested on a cell harvester and [³H]thymidine incorporation was determined by liquid scintillation spectrometry. All assays were performed in triplicate with an inter-test variance of less than 15%.

**Determination of IFN-γ secretion and staining of intracellular cytokines**

IFN-γ in culture supernatant was determined by ELISA according to the manufacturer’s instructions (BD Pharmingen). Measurement of intracellular cytokines was performed as described previously.24

**Detection of caspase-3 positive cells and apoptosis**

Apoptotic cells were detected by flow cytometry as described previously.24 Briefly, cells were washed twice in PBS, incubated in 0.1% (w/v) sodium citrate, 0.1% (v/v) Triton X-100, and propidium iodide (50 µg/ml) for 4–8 hours at 4°C, and analysed by flow cytometry. Apoptotic cells were determined by monitoring cells with hypodiploid nuclei. Active caspase-3 was assayed by FACS analysis. Briefly, cells were fixed, permeabilised, and incubated with a rabbit antibody against amino acids 163–175 of murine caspase-3 (Biocarta Europe, Hamburg, Germany) and subsequently a FITC labelled goat antirabbit antibody.

**Induction of TNBS colitis**

B6C3 mice (female, six weeks old) were obtained from Charles River (Sulzdorf, Germany). TNBS colitis was induced as described previously.13 25 26 Briefly, TNBS (2.0 mg) (Sigma) in 50% ethanol was slowly administered into the lumen of the colon via a 3.5 F catheter. Mice were treated by daily intraperitoneal injections of IL-12(p40)-IgG2b (20 µg/200 µl PBS and 2 µg/200 µl PBS, respectively). Treatment was started two hours after application of TNBS and continued until day 6. Mice in the control groups were injected with truncated IL-12(p40)*-IgG2b (20 µg/200 µl PBS) fusion protein.

**Grading of histological changes**

Cryostat sections (4 µm) of tissues were mounted on APS treated glass slides, air dried, fixed in 4% (wt/vol) paraformaldehyde/PBS (pH 7.4), and stained with haematoxylin- eosin. The degree of inflammation of colon specimens was graded semiquantitatively from 0 to 4, as described by Neurath and colleagues.14 Grading was performed in a blinded fashion by AS.

**Statistical analysis**

Statistical analyses were performed using the Wilcoxon rank test or χ² test. Data were considered to be significant at the p<0.05 level.

**RESULTS**

**IL-12(p40)-IgG2b fusion protein is a dimeric protein and binds specifically to IL-12 receptor positive cells**

We generated the IL-12(p40)-IgG2b fusion protein by joining the p40 domain to the Fc domain of IgG2b, as described in the materials and methods section. For control purposes, we deleted the receptor binding site of IL-12(p40) and, by fusion to Fc IgG2b, we generated the truncated fusion protein IL-12(p40)*-IgG2b. Western blot analysis revealed that the IL-12(p40)-IgG2b fusion protein had an apparent molecular weight of 140 kDa under non-reducing conditions and of 70 kDa under reducing conditions (fig 1), indicating that the IL-12(p40)-IgG2b fusion protein forms homodimers, probably via disulphide bonds between the Fc domains.
of activated LPMNC in vitro. Freshly isolated LPMNC cells were stimulated with immobilised anti-CD3 and anti-CD28 mAb plus increasing concentrations of IL-12 (20–2000 pg/ml) to secrete IFN-γ (fig 3). Addition of increasing amounts of IL-12(p40)-IgG2b fusion protein (2 pg/ml–200 ng/ml) resulted in a concentration dependent inhibition of IFN-γ secretion by activated LPMNC. In concentrations >10–8 M, however, IL-12(p40)-IgG2b increased IFN-γ secretion in LPMNC, indicating an agonistic effect of the fusion protein. The effect was mediated via the IL-12 receptor because addition of the truncated IL-12(p40*)-IgG2b fusion protein that does not bind to the IL-12 receptor did not modulate IFN-γ secretion of cells (fig 3).

To characterise the agonistic effect, LPMNC were separated in CD4+ and CD8+ T cell populations by negative purifications procedures and incubated with the IL-12(p40)-IgG2b fusion protein. IFN-γ was induced on incubation of only CD8+ T cells and not CD4+ T cells. Even high concentrations of IL-12(p40)-IgG2b did not induce IFN-γ secretion in CD4+ LPMNC. As a control, these cells secreted significant amounts of IFN-γ after stimulation with PMA plus ionomycin (data not shown).

**IL-12(p40)-IgG2b decreases proliferation and decreases apoptosis of LPMNC of patients with CD**

In the next set of experiments we studied whether the fusion protein modulates proliferation of activated LPMNC in vitro. LPMNC from patients with CD were stimulated with immobilised anti-CD3 mAb plus anti-CD28 mAb. Addition of the IL-12(p40)-IgG2b protein (0.02 pg/ml–2 ng/ml) inhibited cell proliferation in a concentration dependent manner (fig 4). However, higher concentrations of the fusion protein increased proliferation of LPMNC. Addition of recombinant IL-12 (2 ng/ml) completely abolished the IL-12(p40)-IgG2b induced growth inhibition, indicating that the effect was specifically mediated via the IL-12 receptor.

To obtain further insight into the regulation of fusion protein mediated growth arrest, we monitored the number of apoptotic LPMNC from patients with CD and from healthy controls. In controls (n = 5), activation of LPMNC with the anti-CD3 plus anti-CD28 antibody increased the number of capase-3 positive cells (+22% (median); range 5–48%), as monitored by FACS analysis. Addition of the fusion protein together with αCD3 plus αCD28 stimulation did not increase this rate significantly (median +4%; range 6–53%). In patients with CD (n = 5), activation of LPMNC by stimulation...
IL-12(p40)-IgG2b fusion protein abrogates TNBS induced colitis

In a series of in vivo studies we addressed the question of whether blocking IL-12 receptor by administration of IL-12(p40)-IgG2b fusion protein affects the clinical course of TNBS induced colitis. In a treatment trial, the IL-12(p40)-IgG2b fusion protein (20 μg and 2 μg, respectively, per mouse per day) was intraperitoneally injected into Balb/c mice starting two hours after induction of TNBS colitis. As summarised in fig 6, 68% (20.0 μg) and 63% (2.0 μg), respectively, of mice with TNBS colitis and administration of IL-12(p40)-IgG2b fusion protein survived to day 6 compared with 10% of TNBS colitis mice treated with the truncated IL-12(p40*)-IgG2b fusion protein (p < 0.01). Moreover, TNBS colitis mice that received IL-12(p40)-IgG2b fusion protein regained their initial body weight at day 6 whereas TNBS colitis mice treated with the truncated fusion protein continuously lost their body weight (data not shown). In addition, IL-12(p40)-IgG2b treated mice were more active and lost their ruffled coat appearance associated with TNBS colitis compared with untreated mice and mice inoculated with the truncated fusion protein.

The pathological changes in mice after induction of TNBS colitis have been described in detail previously. In these animals, moderate colitis is characterised by an inflammatory infiltrate in the colonic lamina propria and increased mitotic activity in the elongated epithelial crypts. In severe disease, the cellular infiltrate is composed of both mono and polymorphonuclear cells and is present in all layers of the colonic gut wall. Crypt elongations and destructions are also seen (fig 7A). After treatment with IL-12(p40)-IgG2b fusion protein (20 μg/d), TNBS induced colonic inflammation is abrogated and a nearly normal histological appearance of the colon is restored (fig 7B). This observation was confirmed by histological grading of colon sections: data from three independent experiments showed significant reduction in inflammatory activity after treatment with the IL-12(p40)-IgG2b fusion protein—that is, the score of inflammation was 3.0 (1.0–4.0) in the TNBS group compared with 1.5 (0–3.0) in IL-12(p40)-IgG2b treated mice (p < 0.05).

Administration of IL-12(p40)-IgG2b fusion protein decreases TNF-α and increases IL-10 secretion in mice with TNBS colitis

We investigated whether IL-12(p40)-IgG2b fusion protein modulates cytokine secretion of spleen T cells in mice with TNBS colitis. As summarised in fig 8, the number of tumour necrosis factor α (TNF-α) positive CD4+ T cells was decreased

Figure 6 IL-12(p40)-IgG2b fusion protein increases survival of Balb/c mice after induction of 2,4,6-trinitrobenzene sulphonic acid (TNBS) colitis. TNBS colitis was induced in Balb/c mice as described above and IL-12(p40)-IgG2b fusion protein (20 μg and 2 μg, respectively, per mouse per day) was intraperitoneally injected everyday starting two hours after induction of TNBS colitis. As controls, the truncated IL-12(p40*)-IgG2b fusion protein was administered. Survival rate is shown in a Kaplan-Meier plot. The graph represents data from four independent experiments with at least six mice in each group. Application of IL-12(p40)-IgG2b fusion protein at both concentrations decreased the mortality rate of TNBS colitis mice significantly (p < 0.01; χ² test).

Figure 7 Histological analysis of colonic specimens at day 7 after induction of 2,4,6-trinitrobenzene sulphonic acid (TNBS) colitis. Histopathology of colon specimens of mice with TNBS induced colitis (A) and TNBS plus IL-12(p40)-IgG2b treated mice (B). A pronounced inflammatory infiltrate with elongation and destruction of crypts is obvious in mice after treatment with truncated fusion protein. Treatment with IL-12(p40)-IgG2b abrogated TNBS induced inflammation and restored a normal histological appearance of the colon (microscope magnification: B, 140x; A, 200x).
in colitis mice treated with IL-12(p40)-IgG2b fusion protein (20.0 μg per mouse per day) compared with mice after treatment with the truncated IL-12(p40*)-IgG2b fusion protein (p < 0.05). In addition, a much larger fraction of CD4+ T cells from TNBS colitis mice treated with IL-12(p40*)-IgG2b fusion protein stained positive for IL-10 compared with colitis mice without treatment with fusion protein (p < 0.05).

DISCUSSION

We generated the fusion protein IL-12(p40)-IgG2b that consists of the IL-12(p40) subunit fused to the IgG Fc domain which specifically binds to the IL-12 receptor and represses IFN-γ secretion and cell proliferation of prestimulated LPMNC in vitro. The IL-12 antagonistic activity of IL-12(p40)-IgG2b is dose dependent in the range 2 pg/ml–0.2 μg/ml (that is, 10−4–10−6 M). In concentrations >10−6 M however the IL-12(p40)-IgG2b fusion protein exhibits IL-12 agonistic activities, indicated by induction of IFN-γ secretion. This is at least partially based on induction of STAT-4 phosphorylation (data not shown) resulting in transactivation of the IFN-γ promoter. The concentration dependent dichotomic properties of the fusion protein are specific because the IL-12(p40*)-IgG2b protein without the IL-12 receptor binding site does not exhibit these activities.

One of the most striking observations is that TNBS induced colitis in mice can be successfully prevented by the IL-12(p40)-IgG2b fusion protein. The survival rate at day 6 increased from 10% without treatment to more than 60% with IL-12(p40)-IgG2b treatment. This was accompanied by significant alterations in the number of cytokine secreting spleen cells that however may not be representative of LPMNC. In TNBS colitis mice treated with the IL-12(p40*)-IgG2b fusion protein however the number of TNF-α secreting CD4+ T cells was similar compared with that in non-colitis mice—that is, 30% of CD4+ T cells compared with 50% of CD4+ T cells in colitis mice treated with the truncated IL-12(p40*)-IgG2b protein produced TNF-α. The decrease in TNFα positive cells was paralleled by a substantial increase in the number of IL-10 secreting cells. A number of experimental models indicate that IL-10 is involved in the prevention of T cell mediated inflammation in the gut. However, recent studies question the role of IL-10 as an anti-inflammatory therapeutic agent in human inflammatory bowel disease. Interestingly, IL-12(p40)-IgG2b treatment resulted in an increase in IFN-γ positive cells (see fig 8). Similar effects were observed in TNBS colitis after protective treatment with anti-CD44V7 antibodies. The IL-12(p40)-IgG2b fusion protein moreover increased substantially the number of apoptotic lamina propria cells from patients with CD, thereby limiting the inflammatory immune response. Although several studies indicate that lamina propria T cells are resistant to apoptosis, our results clearly demonstrated induction of caspase-3 positive cells and apoptosis by the IL-12(p40)-IgG2b fusion protein.

Alternative strategies for the treatment of CD include TNF-α neutralising antibodies. Clinical trials to date have been disappointing as only a minority of patients have undergone stable clinical remission and the rate of complications, such as lethal tuberculosis, increased. It is tempting to speculate that these complications may not be as frequent after treatment with IL-12(p40)-IgG2b fusion protein. Moreover, IL-12(p40) is protective particularly in mycobacterial infections. IL-12(p40) deficient mice are more susceptible to infection with Listeria monocytogenes than p35 deficient mice. Clearance of an intracellular bacterium, Francisella tularensis, is dependent on the IL-12 p35-p40 heterodimer and the p40 dimer, the latter activate macrophages, but not CD4+ or CD8+ T cells or natural killer cells. This indicates that IL-12(p40) does not inhibit reactions of innate immunity. Taken together, administration of IL-12(p40)-IgG2b fusion protein is not expected to be associated with eruption of lethal bacterial infections.

Although IL-12 neutralisation by antibodies is highly effective in experimental models of Th1 dominated inflammation, blocking the IL-12 receptor with IL-12(p40)-IgG2b would offer several advantages: (i) syngeneic domains of the fusion protein will avoid immune reactions against the protein itself, and (ii) due to the 140 kDa dimer, an expected long serum half life time compared with certain cytokine fusion proteins.

In summary, we have demonstrated that the engineered fusion protein IL-12(p40)-IgG2b profoundly alters the immune response in a murine Th1 model of intestinal inflammation and inhibits proinflammatory reactions of human lamina propria lymphocytes of patients with CD. As a cautionary note however high concentrations of the IL-12(p40)-IgG2b fusion protein act agonistic to IL-12 as they promote IFN-γ secretion and increase T cell proliferation. Further investigations on the molecular action of the IL-12(p40)-IgG2b fusion protein may provide us with a more robust agent to modulate chronic inflammatory bowel diseases with tolerable side effects.

ACKNOWLEDGEMENTS

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REFERENCES