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Interleukin 10 gene therapy ameliorates TNBS induced colitis

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resubmitted with revisions to Gene Therapy
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Abstract

Background & Aims: The transfer of genes encoding immunomodulatory proteins to the intestinal mucosa is a promising new approach to the treatment of Crohn's disease (CD).

Methods: This study investigates the therapeutic efficacy of an adenoviral vector encoding IL-10 (AdvmuIL-10) in experimental colitis. BALB/c mice were treated with a single intravenous injection of AdvmuIL-10, empty cassette virus (Adv0) or PBS prior to the induction of trinitrobenzene sulfonic acid (TNBS) colitis.

Results: AdvmuIL-10 treatment prevented the severe loss of body weight associated with TNBS administration. In addition, AdvmuIL-10 therapy led to a significant reduction in both stool pro-inflammatory protein levels (IL-1β and TNFR-II) and acute phase response (serum amyloid protein). Finally, the histological scores of mice with TNBS colitis treated with AdvmuIL-10 were significantly lower than Adv0 or PBS treated controls. The therapeutic efficacy of AdvmuIL-10 was associated with a decrease in the IFN-γ and IL-6 levels detected in colonic homogenates from mice with TNBS colitis, whereas no effect was observed on cytokine release from stimulated systemic lymphocytes.

Conclusions: AdvmuIL-10 is an effective therapy in the TNBS model of colitis. Gene therapy strategies using adenoviral vectors encoding IL-10 may prove to be a potent therapy for chronic inflammatory conditions such as Crohn's disease.
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Introduction

Studies of IL-10 deficient (IL-10−/−) and IL-10 receptor-2 deficient mice show that these mice develop a T helper (Th)-1 cell mediated intestinal inflammation and indicate that IL-10 is an important regulatory cytokine within the mucosal immune system.1, 2 The activity of IL-10 in counter-regulating mucosal inflammation is likely to be multifactorial. IL-10 is a potent downregulator of IL-12 production and thus acts at the level of Th1 cell induction.3 In addition, IL-10 suppresses production of other pro-inflammatory cytokines and chemokines including TNF-α, IL-1β, IL-6 and IL-8.4 Finally, there is substantial evidence that IL-10 acts both to promote the differentiation and augment the activity of regulatory T cells.5,8

The observations in IL-10 mice have laid the foundation for therapeutic trials of IL-10 in several other models of colitis. These studies have shown that systemic IL-10 administration is able to prevent intestinal inflammation by downregulating the intestinal pro-inflammatory Th1 response.1, 9, 10 Based on these successful experimental findings, recombinant (r)IL-10 was administered by subcutaneous injection to patients with either mild/moderate or steroid refractory Crohn’s disease, as well as in patients undergoing ileal resection to prevent postoperative recurrence.11, 12 Although the data indicated that systemic rIL-10 therapy is safe and well tolerated, this therapy did only have a modest therapeutic effect compared with placebo. Explanations for this lack of efficacy include the short half-life of rIL-10,14 local delivery of insufficient amounts of rIL-10 to inhibit mucosal Th1 responses and the side effects associated with high dose rIL-10.15

Sustained IL-10 delivery may prove more effective than daily systemic injections. Indeed, daily intragastric administration of IL-10-secreting Lactococcus lactis caused a 50% reduction in disease severity in mice with dextran sulfate sodium induced colitis and prevented the onset of colitis in IL-10−/− mice.16 Alternatively, adenoviral vectors could be used to deliver the IL-10 gene and secure constant expression of high levels of IL-10 within the gastro-intestinal tract. Adenoviral vectors can infect intestinal epithelial cells with high efficiency17 and after intravenous administration they target the liver and the spleen, but also the colon.18, 19 Adenoviral transfer of IL-10 has been shown to be beneficial in murine experimental arthritis, another Th1 cell mediated disease.20, 22 Finally,
we have recently demonstrated the efficacy of adenovirus mediated IL-10 gene transfer in the treatment of established colitis in IL-10 mice.21

In the present study, we have investigated the therapeutic efficacy of murine (m)IL-10 adenovirus mediated (AdvmuIL-10) gene transfer in murine TNBS colitis, which has many histological and immunological similarities to Crohn's disease, and is also relatively unresponsive to daily systemic injections of rIL-10.22 Intradigital TNBS administration induces a transmural colitis, which results from the rapid induction of an IL-12 driven, Th1 cell mediated response that is not balanced by the prompt appearance of a regulatory response.23 The TNBS colitis model is associated with increased concentrations of IFN-γ and TNF-α in the intestinal mucosa.24 In this paper we demonstrate that systemic administration of adenovirus expressing mIL-10, but not the control adenovirus or PBS vehicle, reduces both the clinical and histopathological signs of TNBS colitis. These data suggest that adenovirus-mediated gene transfer may be a valuable therapeutic alternative in Crohn's disease.

Materials and methods

Adenoviral vectors
The adenovirus encoding mIL-10 under an RSV promoter (AdvmuIL-10) and the empty cassette adenovirus (Adv0) were a gift from Professor Dallman (Imperial College, London, UK). Viruses were propagated in the 293 human embryonic kidney cell line (Quantum Biotechnology, Montreal, Canada) and purified by ultracentrifugation through two cesium chloride gradients (Boehringer Mannheim, Lewes, UK). The titre of the adenoviral vectors was determined by plaque assay on 293 cells.25 Viral stocks were aliquoted and stored in 10% glycerol at -80°C until use.

Mice and induction of colitis
BALB/c mice were purchased from Charles River (Charles River, Someren, the Netherlands) and maintained in filter-top cages under specific-pathogen free conditions at our animal care facility. The Animal Studies Ethics Committee of the
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Table I  Experimental set-up

<table>
<thead>
<tr>
<th>Group (number of mice)</th>
<th>TNBS/control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=12)</td>
<td>TNBS</td>
<td>PBS</td>
</tr>
<tr>
<td>2 (n=12)</td>
<td>TNBS</td>
<td>Ado</td>
</tr>
<tr>
<td>3 (n=12)</td>
<td>TNBS</td>
<td>AdvmulL-10</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>saline</td>
<td>PBS</td>
</tr>
<tr>
<td>5 (n=6)</td>
<td>saline</td>
<td>Ado</td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>saline</td>
<td>AdvmulL-10</td>
</tr>
</tbody>
</table>

University of Amsterdam, the Netherlands approved all experiments. The experiments were conducted in 8-week old female mice. Colitis was induced by rectal administration of 0.5 mg 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma Chemical Co, St Louis, MO, USA) dissolved in 40% ethanol (Merck, Darmstadt, Germany), using a vinyl catheter that was positioned 3 centimetres from the anus, as described previously.29 Control mice underwent identical procedures, but were instilled with similar volumes of saline (NaCl 0.9%). One day prior to the first instillation of TNBS/saline, mice received a tail vein injection of 1x10⁸ PFU of AdvmulL-10, Ado or saline vehicle (table 1).

Assessment of inflammation

Body weights were recorded and wasting disease was determined by percentage of weight loss from initial body weight. Mice were sacrificed 9 days after the first TNBS/saline instillation (i.e. 48 hours following the second TNBS challenge) by cardiac puncture. Serum was separated by centrifugation (10,000g for 10 min) from whole blood and stored at -20°C until use. The spleen, caudal lymph nodes, liver and colon were harvested through a midline incision. The colon was opened longitudinally and the stools were collected. The wet weight of the distal 6 cm of colon was measured and used as an index of disease related intestinal wall thickening. Subsequently, each colon was divided longitudinally into two parts: one for histology, the other was snap-frozen in liquid nitrogen and stored at -80°C for cytokine measurement. Liver and colon homogenates were made with a tissue homogenizer in 9 volumes of Greenberger lysis buffer (300 mM
NaCl, 15 mM Tris, 2 mM MgCl₂, 2 mM Triton (X-100), Pepstatin A, Leupeptin, Aprotinin (all 20 ng/ml), pH 7.4). Tissue was lysed for 30 minutes on ice followed by two times centrifugation (10 min 14000 g). Homogenates were stored at -20°C until further use.

**Histological analysis**

The longitudinally divided colons were rolled up and fixed in 4% formalin. Fixed tissues were embedded in paraffin, and 4-6 μm sections were stained with haematoxylin and eosin for histological evaluation. An experienced pathologist blinded to treatment allocation scored the following parameters: 1) percentage of colon involved, 2) fibrosis, 3) edema, 4) erosions and ulcerations, 5) crypt loss, 6) infiltration of mononuclear cells and 7) polymorphonuclear cells as described previously. The total score ranges from 0 (normal colon) to a maximum of 20 points (severe inflammation).

**Cell cultures**

Cell suspensions of the spleens and caudal and mesenteric lymph nodes were prepared by filtration through a cell strainer (Becton Dickinson) and red cell lysis of splenocyte suspensions. Cells were suspended in RPMI 1640 medium (Biowhittaker, Walkersville, MD, USA), supplemented with 10% FCS and 1% penicillin-streptomycin-glutamine solution (GibcoBRL, Grand Island, NY, USA). Cells (1 x 10⁵/well) were seeded on 96-well round bottom plates (Costar) precoated with anti-(α)CD3 antibody (1:30 concentration; 145.2C11 clone) in a final volume of 200 μl in the presence of soluble αCD28 monoclonal antibodies (mAb) (1:1000 concentration, Pharmingen, San Diego, CA, USA) under standard conditions (37°C, 5% CO₂). Culture supernatants were collected from 3 wells after 48 hours (h), pooled and stored at -20°C until use.

**Stool samples**

Stool samples were collected upon sacrifice from all animals and weighed. Samples were emulsified in 500 μl per 100 μg stool weight of a solution of 1 mg/ml soy trypsin inhibitor and 1 mg/ml phenylmethylsulfonyl fluoride in PBS. Supernatants were collected after centrifugation at 10,000g for 15 minutes and stored at -20°C.
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Cytokine analysis and serum amyloid protein measurement

Cytokine concentrations (IL-10 and TNF-α (BD Pharmingen, San Diego, CA, USA)); IFN-γ (Genzyme Diagnostics, Cambridge, MA, USA); IL-1β (R&D Systems, Abingdon, Oxon, UK); and IL-6 (Sandoz, Basel, Switzerland) were measured by sandwich ELISA using paired Abs according to the manufacturers recommendations. Serum amyloid protein (SAP) levels were measured in serum samples by ELISA as described previously.23

Statistical analysis

Differences between treatment groups were analyzed by the Mann-Whitney U test. Results are expressed as mean ± SEM. A p value of less than 0.05 was considered to represent a significant difference.

Results

Biodistribution of IL-10 expression

Intravenous administration of adenoviral vectors predominantly results in transduction of hepatocytes.19 To confirm that AdvmIL-10 infection was successful, IL-10 expression was assessed in liver homogenates of BALB/c mice with TNBS colitis treated with 1x10⁷ PFU AdvmIL-10, Adv0 or PBS vehicle (n=5 tested/group, figure 1). The mean IL-10 concentration ten days after AdvmIL-10 injection was 760 ± 99 pg/mg compared with 331 ± 80 pg/ml in PBS (p=0.031) and 224 ± 32 pg/ml in Adv0 (p=0.008) treated mice. Ten days after injection, IL-10 (400 (50-1230) pg/ml) was detected in the serum of 6 out of the 15 surviving mice that had received AdvmIL-10. In all the other mice IL-10 levels in the serum were below the detection limit. There were no differences between the treatment groups in the concentration of IL-10 detected in colon homogenates (data not shown).
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**Figure 1** AdvmulL-10 administration induces hepatic IL-10 expression

Twenty-four hours prior to induction of TNBS colitis, BALB/c mice received $1 \times 10^8$ PFU AdvmulL-10, Adv0 or PBS by tail vein injection. After 10 days, mice were sacrificed and IL-10 levels were determined in liver homogenates. Results are expressed as mean ± SEM (n=5/group). *p=0.031 (AdvmulL-10 versus PBS) and p=0.008 (AdvmulL-10 versus Adv0).

**AdvmulL-10 treatment reduced body weight loss in TNBS colitis**

To determine whether AdvmulL-10 was able to prevent TNBS colitis, disease was induced in BALB/c mice 24 h after tail vein injection of $1 \times 10^8$ PFU of AdvmulL-10. Control mice with TNBS colitis received Adv0 or PBS alone. There was 25% mortality in each TNBS group. As expected, intrarectal instillation of TNBS resulted in diarrhea and wasting in mice treated with PBS and Adv0 (figure 2). However, recipients of AdvmulL-10 suffered less severe weight loss and by the end of the experiment, the body weights of AdvmulL-10 treated mice were significantly higher than PBS treated (p=0.015) or Adv0 treated mice (p=0.020). All negative control mice that received intrarectal saline 24 h after systemic injection with PBS, Adv0 or AdvmulL-10 survived and gained weight throughout the experiment (data not shown). These results indicate that administration of AdvmulL-10 significantly attenuated body weight loss associated with TNBS-induced colitis.
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Figure 2 AdvmuIL-10 treatment prevents body weight loss

BALB/c mice received 1x10⁸ PFU AdvmuIL-10, Adv0 or PBS by tail vein injection 24 h prior to a rectal instillation of 0.5 mg TNBS in 40% ethanol (n=12/group). All mice received a second dose 7 days later, and were sacrificed after a further 48 h. Body weights of surviving mice, measured at the end of the experiments, are expressed as a percentage of initial body weight. Results are expressed as mean ± SEM (n=9/group). Data are pooled from two separate experiments. *p=0.015 (AdvmuIL-10 versus PBS) and p=0.020 (AdvmuIL-10 versus Adv0).

AdvmuIL-10 diminished both stool and serum markers of inflammation in TNBS colitis

Both Crohn's disease and experimental colitis are associated with a profound increase in faecal levels of pro-inflammatory proteins. Therefore, stools collected upon sacrifice were assayed for IL-1β and sTNFR by ELISA (figure 3). As expected, IL-1β and TNFR-II levels were high in TNBS mice treated with PBS (731 ± 348 pg/ml and 441 ± 90 pg/ml respectively) and undetectable in all saline control mice. In stools of AdvmuIL-10 treated TNBS mice, a reduction in IL-1β level (16.9 ± 3 pg/ml) was observed compared with both PBS (see above, p=0.046) and Adv0 treated TNBS mice (135 ± 58 pg/ml, p=0.073). In addition, AdvmuIL-10 treatment significantly decreased TNFR-II levels (160 ± 29 pg/ml) compared with both PBS (see above, p<0.001) and Adv0 treatment (279 ± 14 pg/ml, p<0.001).
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Figure 3 Advmull-10 diminishes the stool cytokine levels in TNBS colitis

Stools were harvested 48 h after the second dose of TNBS from BALB/c mice treated with 1x10⁸ PFU Advmull-10, Adv0 or PBS. Levels of IL-1β (A) and TNFR-II (B) were assayed by ELISA in duplicate. Each symbol represents an individual mouse. Bars indicate mean levels for each group. Data are pooled from two separate experiments. *p=0.046 (Advmull-10 versus PBS) and **p<0.001 (Advmull-10 and versus Adv0).

Systemic IL-6 levels and SAP were determined as markers of disease activity in serum samples. Advmull-10 treatment resulted in reduced serum IL-6 levels (14.4 ± 1.4 pg/ml) compared with PBS (48 ± 24 pg/ml, p=0.040) and Adv0 treatment (47 ± 25 pg/ml, not significant) in mice with TNBS colitis. As shown in figure 4, SAP levels were significantly lower in Advmull-10 treated mice (244 ± 23 μg/ml) than in PBS (1162 ± 413 μg/ml, p<0.001) and Adv0 treated colitic mice (587 ± 141 μg/ml, p=0.004). Advmull-10 did not alter systemic IL-6 or SAP levels in the saline control mice (data not shown). Together, these findings indicate that Advmull-10 therapy diminishes both the local consequences and systemic manifestations of TNBS colitis.
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Figure 4 AdvmulL-10 therapy reduces serum amyloid protein (SAP) levels
Serum samples were collected 48 h after the second dose of TNBS from BALB/c mice treated with $1 \times 10^8$ PFU AdvmulL-10, Adv0 or PBS. The SAP concentration was assayed by ELISA in duplicate. Each symbol represents an individual mouse. Bars indicate mean levels for each group. Data are pooled from two separate experiments. **p<0.0001 (AdvmulL-10 versus PBS) or p=0.004 (AdvmulL-10 versus Adv0).

AdvmulL-10 treatment decreased intestinal inflammation in BALB/c mice with TNBS colitis
Macroscopic signs of inflammation observed in TNBS-induced colitis are marked edema and mucosal thickening, which are reflected by increased colon weight. Mice were sacrificed on day 10, and the weight of the last 6 cm of the colon was determined. There was a significant increase in the mean colon weight in all groups that received TNBS compared with the corresponding saline control groups irrespective of whether the mice had been treated with PBS (p=0.001), Adv0 (p<0.001), or AdvmulL-10 (p<0.001). However, colons from mice with TNBS colitis that had received AdvmulL-10 weighed less ($208 \pm 7$ mg) than colons from colitic mice that had received either Adv0 ($237 \pm 13$ mg, p=0.038) or PBS vehicle ($244 \pm 22$ mg, p=0.080), although this did not reach statistical significance for the latter.
TNBS mice were treated with $1 \times 10^8$ PFU AdvmullL-10, Adv0 or PBS by tail vein injection on day 0. Upon sacrifice at day 10, the extent of mucosal inflammation was examined and graded (see materials and methods). Each symbol represents an individual mouse. Bars indicate mean colitis score for each group. *p=0.038 (AdvmulL-10 versus PBS) or $p=0.012$ (AdvmulL-10 versus Adv0).

TNBS administration results in an acute colitis that is characterized by mucosal ulceration and an influx of inflammatory cells. A pathologist blinded to treatment allocation performed histological analysis of colons harvested from mice in the above experiments (figure 5). The total histological score was decreased in AdvmulL-10 treated TNBS mice ($7.1 \pm 0.9$) compared with PBS ($10.8 \pm 1.3$, $p=0.038$) and Adv0 ($10.4 \pm 1.0$, $p=0.012$) treated TNBS mice. As illustrated by the pictures in figure 6, mice with TNBS colitis that had been treated with AdvmulL-10 displayed less mucosal ulceration, crypt loss and fibrosis, as well as a diminished influx of polymorphonuclear cells. The colons harvested from saline instilled mice showed only mild edema. There were no significant differences in histological scores between PBS, Adv0 and AdvmulL-10 treated mice (mean scores $2.0 \pm 0.7$, $0.8 \pm 0.5$ and $1.7 \pm 1.0$ respectively). Thus, the AdvmulL-10 treatment prevented development of severe TNBS colitis.
Figure 6  AdvmuIL-10 treatment attenuated colonic inflammation
H&E staining of the colons of mice with TNBS induced colitis. A) Severe colitis in mouse treated with PBS, characterized by an extensive inflammatory cell infiltrate, ulcerations and crypt loss. B) Severe colitis in mouse treated with Adv0, with histological features similar to photo A. C) Colon of a mouse treated with AdvmuIL-10. Inflammation was present but significantly less severe, with no ulcerations, less crypt loss, fibrosis and polymorphonuclear cell influx. Original magnifications: 33 x.

AdvmuIL-10 treatment decreases local cytokine production
To investigate whether the therapeutic efficacy of AdvmuIL-10 treatment was related to a generalized reduction of T cell responsiveness, cells from caudal lymph nodes and spleens were isolated and stimulated in vitro for 48 h with αCD3/CD28 Abs. IFN-γ and TNF-α levels were quantified by ELISA. No significant differences were found in IFN-γ and TNF-α concentrations in supernatants of stimulated caudal lymph node and spleen cell cultures harvested from both TNBS and saline control mice treated with PBS, Adv0 or AdvmuIL-10 (data not shown). By contrast, AdvmuIL-10 therapy did affect local pro-inflammatory cytokine release within the colon. IFN-γ and IL-6 concentrations in colonic homogenates from AdvmuIL-10 treated TNBS mice (262 ± 41 pg/ml and 172 ± 18 pg/ml) were lower than those from TNBS mice treated with PBS (429 ± 79 pg/ml, p=0.04 and 1000 ± 600 pg/ml, p=0.038) and Adv0 (430 ± 118 and 428 ± 201 pg/ml, both not significant). TNF-α concentrations were similar in the AdvmuIL-10 and Adv0 treated mice (66 ± 9 pg/ml and 72 ± 10 pg/ml), although both were lower than in the PBS treated mice (160 ± 75 pg/ml). These results suggest that AdvmuIL-10 reduced
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colon inflammation by modulating local production of pro-inflammatory cytokines such as IFN-γ and IL-6.

Discussion

The results presented here show that a single systemic dose of AdvmuIL-10 attenuated TNBS colitis. This effect was related specifically to IL-10 transgene expression, since both the empty cassette vector and PBS vehicle were unable to modify the course of disease. Furthermore, AdvmuIL-10 suppressed the pro-inflammatory cytokine levels in stool samples and colonic homogenates as well as inhibiting the acute-phase response and systemic IL-6 release.

As expected, the systemic administration of adenoviral vectors targeted hepatocytes, since high levels of IL-10 were detected in liver homogenates of AdvmuIL-10 treated mice 10 days after administration. In addition, IL-10 was detected at high levels in the serum of several mice that had received AdvmuIL-10. This agrees with our previous observation that serum mIL-10 levels are raised for approximately one week after AdvmuIL-10 administration in IL-10^+^ mice. In contrast, there was no difference in the levels of IL-10 detected in colon homogenates from AdvmuIL-10 or control treated mice with TNBS colitis. Finally, in this study we did not assess the host anti-adenovirus response, which may shorten the duration of transgenic IL-10 expression. However, in other animal models, we and others^2^ have shown that the delivery of immunoregulatory genes is able to suppress the development of anti-adenoviral antibodies resulting in extended transgene expression.

Previous studies of IL-10 therapy in TNBS colitis have yielded mixed results. Pre-treatment of BALB/c mice with high dose intra-peritoneal mIL-10, restored the tolerance of lamina propria mononuclear cells to autologous colonic bacterial sonicates, whilst not affecting the proliferative response to heterologous bacterial sonicates. However, the effect of IL-10 on the histological severity of colitis was not reported. A separate study that assessed the effect of daily IL-10 injections on rats with TNBS colitis demonstrated no effect on the histological disease severity even if the IL-10 injections
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were commenced prior to colitis induction. Finally, Barbara et al examined the therapeutic efficacy of an adenoviral vector encoding human IL-10 administered by intraperitoneal injection to rats with TNBS colitis. In the latter study only 12 pg/ml IL-10 was detected 24 h after injection in the serum, and no changes were demonstrated in histological scores when the virus was administered one hour after colitis induction. However, they did demonstrate a small but significant improvement in colitis if the adenoviral vector was injected 24 h prior to TNBS administration.

The experiments reported here demonstrate that a single systemic dose of AdvmuIL-10 prior to TNBS administration results in significantly less weight loss and lower histological scores than control virus. We extended the findings of Barbara et al by delineating the effect of IL-10 gene therapy on the cytokine network within the inflamed mucosa. The mechanisms by which IL-10, induced by AdvmuIL-10 therapy, could have diminished disease severity in the intestine include downregulation of antigen presenting cell activity, inhibition of pro-inflammatory cytokine production and activation of regulatory T cells. Our experiments did not address these mechanisms directly. However, AdvmuIL-10 therapy was found to decrease the levels of Th-1 cytokines detected in the colonic mucosa, which suggests an effect on T cell differentiation and cytokine release. This effect was localized to the mucosa, as AdvmuIL-10 had no effect on the ability of systemic lymphocytes to respond to T cell receptor stimulation.

The 34% drop in histological score seen with AdvmuIL-10 therapy in TNBS colitis is not as impressive as the drop seen in IL-10 mice. However, this is not surprising given the breadth of the inflammatory cascade that is activated by TNBS administration, compared with the isolated defect in IL-10 mice. In fact, the results compare favourably with other therapeutic strategies reported in mice with TNBS colitis; for example, IL-18 binding protein (42% fall in histological score) and the chemokine receptor antagonist Met-RANTES (57% fall in histological score).

We recently showed that systemic administration of high dose rIL-10 may have pro-inflammatory effects via induction of IFN-γ. In this study we found no indications for pro-inflammatory effects of IL-10, since spleen cell production of IFN-γ and TNF-α was not altered after AdvmuIL-10 treatment and serum levels of IL-6 were decreased. However, systemic administration of AdvmuIL-10 leading to high serum levels of IL-10
may cause systemic side effects. To minimize exposure of non-target organs to both the transgene and the adenoviral vector, local gene therapy is the next step in the development of targeted biological therapy. In fact, a recent study has demonstrated that intra-rectal administration of an E1 deleted adenoviral vector expressing IL-18 antisense mRNA inhibits mucosal IFN-γ release and suppresses inflammation in the transfer model of colitis. Alternative strategies to reduce the systemic side effects of immunoregulatory gene therapy include the use of less common adenovirus serotypes, or modifications that alter the tropism of the virus. Local administration of adenoviruses with normal fiber structure to the inflamed mucosa mainly targeted epithelial cells, whereas adenoviruses with a modified fibre structure infected lamina propria T cells and mononuclear cells. Further studies are required to determine the most appropriate vector for immunotherapy of intestinal inflammation.

Immunoregulatory gene delivery offers the prospect of local downregulation of the immune response in Crohn's disease. The results of the present study, showing that transient overexpression of IL-10 by prior administration of the adenoviral vector attenuates the signs of TNBS colitis, confirm the therapeutic potential of IL-10 gene therapy in intestinal inflammation.

Acknowledgements

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