Efficient gene delivery to the inflamed colon by local administration of recombinant adenoviruses with normal or modified fibre structure

S Wirtz, P R Galle, M F Neurath

Abstract

***Background/Aims*—Replication deficient recombinant adenoviruses represent an efficient means of transferring genes in vivo into a wide variety of dividing and quiescent cells from many different organs. Although the gastrointestinal tract is a potentially attractive target for gene therapy approaches, only a few studies on the use of viral gene transfer vehicles in the gut have been reported. The prospects of using recombinant adenoviruses for gene delivery into epithelial and subepithelial cells of the normal and inflamed colon are here analysed.**

***Methods*—An E1/E3 deleted recombinant adenovirus (denoted AdCMVβGal) and an adenovirus with modified fibre structure (denoted AdZ.F(pk7)) both expressing the bacterial *lacZ* gene under the control of a human cytomegalovirus promoter were used for reporter gene expression in vitro and in vivo. β-Galactosidase activity was determined by specific chemiluminescent reporter gene assay.**

***Results*—Intravenous or intraperitoneal injection of AdCMVβGal into healthy Balb/c mice caused strong reporter gene expression in the liver and spleen but not in the colon. In contrast, local administration of AdCMVβGal resulted in high reporter gene expression in colonic epithelial cells and lamina propria mononuclear cells. A local route of adenovirus administration in mice with experimental colitis induced by the hapten reagent trinitrobenzenesulphonic acid was next evaluated. Interestingly, rectal administration of AdCMVβGal caused a higher β-galactosidase activity in isolated lamina propria cells from infected mice with experimental colitis than in those from controls. Furthermore, isolated lamina propria cells from mice with colitis infected in vitro showed a significant increase in reporter gene activity compared with controls. Finally, AdZ.F(pk7) adenoviruses with modified fibre structure produced 10- to 40-fold higher reporter gene activity in spleen T cells and lamina propria mononuclear cells of colitic mice compared with standard AdCMVβGal vectors.**

***Conclusions*—Local administration of recombinant adenoviruses with normal or modified fibre structure could provide a new reliable method for targeted gene expression in the inflamed colon. Such gene delivery could be used to specifically express signal transduction proteins with therapeutic potential in inflamed colonic tissue. In particular, adenoviruses with modified fibre structure may be useful in T cell directed therapies in intestinal inflammation.**

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Keywords: adenovirus; gene transfer; colitis; colon

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Most biological functions of cells and tissues of the gastrointestinal tract have been characterised by in vitro assay systems. Recently, various genetically engineered transgenic and knockout animal models of inflammatory bowel disease have been established that have provided valuable insights into the pathogenesis of chronic intestinal inflammation.[1–4] Data derived from these models clearly show that dysregulated overexpression—for example, interleukin-7[5]—or lack—for example, interleukin-2, interleukin-10[6 7]—of several key regulatory proteins causes disruption of the intestinal immune balance and severe colonic pathology in vivo. However, the use of this approach requires stable germline transmission and is not applicable to treatment of established intestinal disease. Therefore the ability to express normal and modified genes in the colon in vivo could provide a novel powerful tool for experimental studies and therapy of intestinal diseases such as inflammatory bowel disease.

As the practical use of somatic gene therapy is highly dependent on safe and efficient transfer methods, several different gene delivery systems have been recently developed.[8–10] Of the different types of viral and non-viral vector systems, recombinant human adenoviruses of serotype 5 (Ad5) have shown promising results.[8 9] Many studies have shown that the replication defective Ad5 vector has a highly efficient mode of entry into a broad spectrum of eukaryotic cells of many different species and can, unlike retroviruses, infect both dividing and non-dividing cells. Another disadvantage of retroviral vectors in the gut is the relatively low transduction efficiency of rat intestinal epithelial cells.[11] In contrast, adenoviruses are known to yield high transduction

Abbreviations used in this paper: PBS, phosphate buffered saline; pfu, plaque forming units; TNBS, trinitrobenzenesulphonic acid; LPMC, lamina propria mononuclear cell.

rates in intestinal epithelial cells.[10 12] Furthermore, they are biochemically and genetically well characterised, comparatively easy to handle, and do not readily integrate into the host genome.[9]

The benefits of recombinant adenoviruses for a wide variety of gene therapy applications in vitro and in vivo have clearly been demonstrated.[9 13] With regard to the latter approach, Ad5 vectors have been successfully used for gene delivery in animals as well as in preliminary clinical studies in humans.[14–19] When injected into the circulation, replication deficient Ad5 can efficiently transduce hepatocytes and thus has been frequently used in therapeutic studies of various animal models of liver disease.[20 21] However, the gastrointestinal tract has not been widely used for gene transfer studies in vivo. Using reporter vectors and different administration routes, we analyse in this study the potential for using recombinant adenoviruses for gene delivery into epithelial and subepithelial cells of the normal and inflamed colon. We show that local administration of adenoviruses results in efficient gene expression in the inflamed colon. These data may provide a rational basis for local adenoviral gene therapy in patients with inflammatory bowel disease.

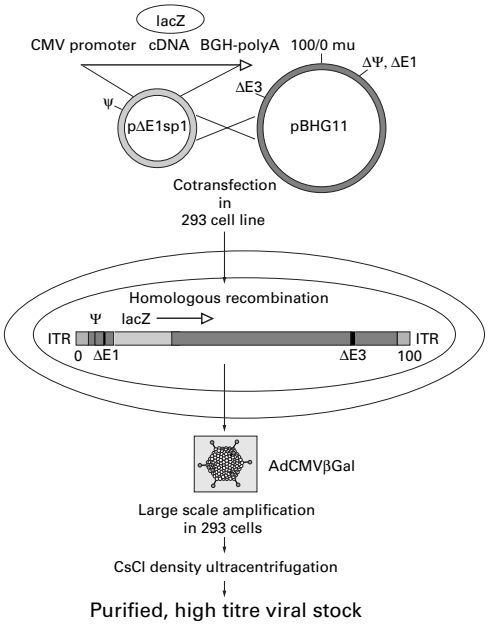

Figure 1 Strategy for generation and propagation of Ad5 vectors. This vector can be used to obtain expression vectors for cytokine signalling proteins (S Wirtz, P R Galle & M F Neurath, unpublished work) or reporter genes such as β-galactosidase. To obtain the AdCMVβGal vector, the entire β-galactosidase coding region was inserted into the Ad5 pBHG11 vector via homologous recombination using a shuttle vector (pΔE1sp1) and cotransfection strategies in 293 cells. After recombination, large scale amplification of AdCMVβGal was performed in 293 cells. Finally, high titre viral stocks were generated and used for in vitro and in vivo studies. CMV, cytomegalovirus; ITR, inverted terminal repeat.

Methods

PROPAGATION AND PURIFICATION OF RECOMBINANT ADENOVIRUSES

An E1/E3 deleted recombinant adenovirus type 5 with normal fibre structure expressing β-galactosidase was constructed as described in fig 1. The entire bacterial β-galactosidase coding region positioned downstream of a human cytomegalovirus promoter and upstream of the bovine growth hormone polyA site was subcloned into pΔE1sp1 and cotransfected together with pBHG11 into 293 cells for homologous recombination as described.[22 23] The resulting vector was denoted AdCMVβGal. In addition, an adenovirus with modified fibre structure expressing β-galactosidase, denoted AdZ.F(pk7), was kindly provided by Dr Wickham (GenVec, Rockville, Maryland, USA).

For large scale production, virus was added at a multiplicity of infection of 5–15 to confluent 293 cells growing in Dulbecco's modified Eagle's medium/F12 medium supplemented with 5% fetal calf serum and antibiotics. When cytopathic effects were completed, the cells were harvested and viral particles were released by five cycles of freezing/thawing in dry ice/ethanol. Crude viral lysates were subsequently applied twice to discontinuous caesium chloride gradients (lower layer 1.45 g/ml, upper layer 1.2 g/ml) and centrifuged overnight at 25 000 rpm at 4°C. Banded viral particles were dialysed several times against Tris/HCl (pH 8.0) and stored in aliquots after the addition of 10% glycerol.

The concentrations of plaque forming units (pfu) of individual stocks were determined by plaque assays on 293 cells essentially as described.[23 24] In brief, 293 cells growing at about 90% confluency in 60 mm plates were incubated with serial dilutions of viral stocks. After one hour, virus-containing medium was aspirated and the cells were overlaid with 10 ml prewarmed complete medium containing 1.25% seaplaque agarose (FMC; Biozym, Hessich Oldendorf, Germany). To count plaques, cells were overlaid overnight with medium containing agarose and 0.33% neutral red (Sigma, Munich, Germany).

IN VIVO GENE TRANSFER STUDIES

Six to eight week old specific pathogen-free Balb/c mice were used for the entire set of experiments. For intravenous or intraperitoneal administration, 1×10^9 pfu AdCMVβGal was injected in a total volume of 100 µl into the lateral tail vein or peritoneal cavity respectively, using a 30 gauge needle.

For rectal administration, mice were anaesthetised with avertine, and the colon was flushed several times with phosphate buffered saline (PBS) to remove faeces. A small 3.5 F catheter was carefully introduced 4 cm into the rectum and 1×10^9 pfu AdCMVβGal slowly injected in a total volume of 100 µl. To prevent rapid outflow of the viral suspension through the anus, the mice were placed vertically for 30 minutes and the rectum was inflated by a balloon connected to a 2 F catheter (Mansfield, New York, New York, USA).

In experiments with colitic mice, adenovirus administration was performed, as described above, two days after induction of experimental colitis by intrarectal administration of 0.5 mg of the hapten reagent trinitrobenzenesulphonic

acid (TNBS; obtained from Sigma) in 50% ethanol as described.[25] In some experiments mice received 10 μg/g cyclosporin A (Sigma), which was injected intraperitoneally at various time points after adenovirus administration.

ISOLATION OF LAMINA PROPRIA MONONUCLEAR CELLS

Colonic lamina propria mononuclear cells (LPMCs) were isolated from resected large bowel specimens using a previously described technique.[26] In brief, after removal of Peyer's patches, the colon was longitudinally opened, rinsed several times in PBS to remove faeces and debris, and cut into small pieces (about 0.1 cm). Tissues were incubated at 37°C in PBS supplemented with 0.145 mg/ml dithiothreitol and 0.37 mg/ml EDTA for 15 minutes. The tissue was subsequently further digested in RPMI 1640 containing 0.15 mg/ml collagenase (Worthington, Munich, Germany) and 0.1 mg/ml DNase (Boehringer Mannheim, Mannheim, Germany) for 75–90 minutes at 37°C on a shaking platform. LPMCs were finally isolated from the interface of a discontinuous 40%/100% Percoll gradient (Biochrom, Berlin, Germany).

ISOLATION OF SPLEEN CD4 LYMPHOCYTES

For cell isolation, spleens were aseptically removed, cut into pieces and squeezed through a 40 μm nylon mesh. Red blood cells were removed by hypotonic lysis in ACK lysis buffer (4.1 g NH_4Cl, 0.5 g $KHCO_3$, 18.6 mg EDTA, 500 ml water, pH 7.2). CD4 T lymphocytes were isolated using immunomagnetic beads specific for CD4 (Dynal, Oslo, Norway) with subsequent bead detachment according to the manufacturer's instructions. The resulting cell population was more than 95% CD4 as assessed by FACS (Coulter, Krefeld, Germany) analysis.

IN VITRO INFECTION EXPERIMENTS

In vitro experimental studies were performed with freshly isolated LPMCs. Cells were stimulated with 50 ng/ml phorbol 12-myristate 13-acetate (obtained from Sigma) and 10 μg/ml phytohaemagglutinin (obtained from Pharmacia, Uppsala, Sweden) for 48 h in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum, 4 mM L-glutamine, 10 mM HEPES, and 100 U/ml each penicillin and streptomycin (Biochrom). For infection, cells were resuspended in 0.5 ml culture medium containing 10% fetal calf serum and AdCMVβGal at a multiplicity of infection of 1000, and incubated at 37°C in the presence of 5% CO_2. After 18 hours, viral particles were washed away with medium, and cells were incubated in 2 ml RPMI medium for an additional 24 hours before cell lysis.

ANALYSIS OF THE REPORTER GENE EXPRESSION

A 100 mg portion of tissue frozen in liquid nitrogen was homogenised in Reporter Lysis Buffer (Boehringer Mannheim) supplemented with 1 mM dithiothreitol, 0.5 mM phenylmethanesulphonyl fluoride, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 μg/ml aprotinin (Boehringer) as protease inhibitors. Isolated cells were lysed without homogenisation. After centrifugation to remove debris, the samples were incubated for 45 minutes at 50°C to quench endogenous β-galactosidase activity. Then 50 μl of individual cell lysates was added to chemiluminiscent reaction buffer (Clontech, Heidelberg, Germany) and incubated for one hour at room temperature. β-Galactosidase activity was determined in a tube luminometer (Berthold, Bad Wildbad, Germany).

HISTOLOGICAL ANALYSIS

Organs (spleen, liver, kidney, pancreas, lung, heart, small and large bowel) were removed at various time points after local administration of recombinant adenoviruses to mice with TNBS induced colitis. Cryosections (10 μm) were cut, dried, and stained with haematoxylin/eosin for pathological assessment.

IMUNOCYTOCHEMISTRY AND DOUBLE STAINING ANALYSIS

Immunocytochemistry was performed on isolated LPMCs from mice with TNBS induced colitis. Briefly, intestinal cells from colitic mice were fixed in 4% paraformaldehyde and washed in 0.01 M PBS. Cytospins were made and pretreated with 10% serum in PBS and incubated overnight at 4°C with the primary antibody (polyclonal rabbit anti-mouse CD4 antibody; Pharmingen, San Diego, California, USA). On the following day, sections were rinsed in PBS and incubated with a biotinylated secondary IgG antibody (1:100; obtained from Vector, Burlingame, California, USA) for one hour at room temperature followed by incubation with streptavidin conjugated Cy2 (Dianova, Hamburg, Germany) (1:500) for two hours at room temperature. Sections were rinsed with PBS and subjected to a second cycle of staining by using the chromogenic substrate for β-galactosidase, 5-bromo-4-chloro-3-indolyl-β-D-galactosidase, for 16 hours at 37°C. Slides were mounted with mounting medium and analysed with a Zeiss microscope. CD4 and β-galactosidase positive cells were counted in randomly selected high power fields (0.25 mm^2).

Results

β-GALACTOSIDASE ACTIVITIES IN MICE AFTER ADMINISTRATION OF REPORTER VECTOR BY DIFFERENT ROUTES

Recombinant adenoviruses have been used for a wide variety of gene therapy applications in vitro and in vivo. However, only limited information on targeted gene expression in intestinal cells is available. In an initial approach to this problem, we focused on gene delivery to the normal colon using adenoviral vectors with β-galactosidase reporter genes. For this purpose, an E1/E3 deleted adenoviral vector (denoted AdCMVβGal) expressing β-galactosidase under the control of a cytomegalovirus promoter was used which was generated by homologous recombination (fig 1; see Methods).

To determine the most efficient way to target recombinant gene expression in the colon using adenoviruses, we injected 1×10^9 pfu

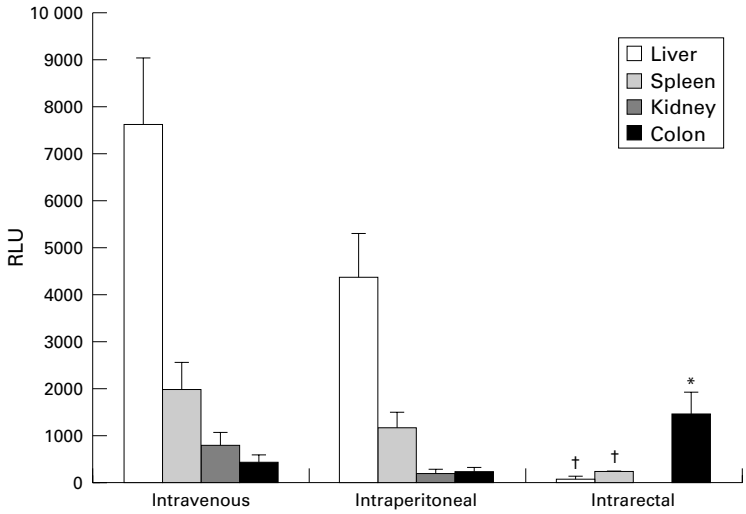

*Figure 2 β-Galactosidase activities after different routes of reporter vector administration. Six to eight week old healthy Balb/c mice were infected with 1×10^9 pfu AdCMVβGal by the indicated administration routes. After three days, organs were removed and homogenised, and luminescent β-galactosidase activity was determined. Results are expressed as mean (SD) from three independent experiments (three mice/group) and are reported as increase in enzyme activity (relative light units (RLU))/100 mg of tissue) compared with untreated control mice. * and † indicate significantly ($p<0.05$) increased or decreased respectively RLU values compared with the other two administration routes. nd, not determined.*

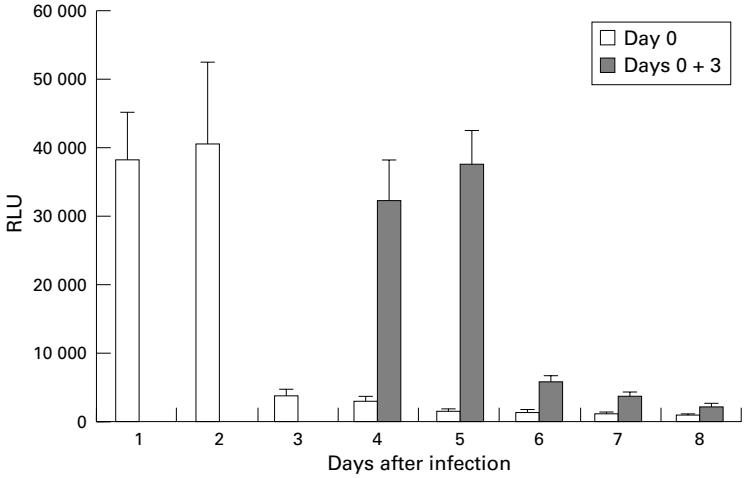

Figure 3 Time course of β-galactosidase expression in the colon of Balb/c mice. Six to eight week old Balb/c mice were infected at days 0 and 3 with 1×10^9 pfu AdCMVβGal via the rectum. The colon was removed at the indicated time points, and luminescent β-galactosidase activity was determined in colonic homogenates. Results are expressed as mean (SD) from three independent experiments (three mice/group) and represent an increase in enzyme activity (relative light units (RLU)) compared with uninfected control mice.

AdCMVβGal into six to eight week old Balb/c mice intravenously, intraperitoneally, or locally by rectal injection through a catheter (fig 2). After injection into the lateral tail vein, strong chemiluminiscent reporter gene activity was observed after three days in the spleen and particularly in the liver, whereas only low amounts of β-galactosidase were found in colonic specimens. Similar results were obtained after injection of the same amount of reporter vector into the peritoneal cavity, although in the latter case overall transduction efficiency to liver and spleen was lower. High β-galactosidase expression was also seen close to the injection site (tail after intravenous injection; peritoneal cavity and diaphragm after intraperitoneal injection) (data not shown).

We next explored whether local administration of adenoviruses would result in higher reporter gene expression in the colon. When 1×10^9 pfu AdCMVβGal was administered locally into the colonic lumen via a catheter, significantly higher β-galactosidase activity compared with the intravenous and intraperitoneal routes was observed after three days in the colon (fig 2). Interestingly, the level of transduction was low in the liver and spleen after local administration of AdCMVβGal, suggesting that this approach may result in a relatively selective and high expression of the reporter protein in the colon.

TIME COURSE OF β-GALACTOSIDASE EXPRESSION

Recent studies have suggested that the colonic epithelium of immunocompromised mice can be transduced for a long time after intravenous injection of recombinant adenoviruses.[27] As the intestinal epithelium has a high turnover rate, this long lasting effect may indicate persistent transduction of cryptic stem cells, although Ad5 is believed not to readily integrate into the host cell genome.[27] To study the duration of recombinant gene expression after intrarectal virus administration in mice with an intact immune system, we performed time course experiments over a period of eight days after infection. It was found that reporter gene expression in the colon decreased in a time dependent manner (fig 3). The highest levels of β-galactosidase activity were found within the first 48 h after virus administration, indicating strong expression of the reporter gene shortly after entry of the adenovirus into the cells. After three days, reporter gene expression decreased considerably day by day. On day 8, reporter gene activity was still detectable but was only just above background β-galactosidase activity.

To determine the time course after repeated administration of the adenoviral vector, we next performed a secondary challenge with another 1×10^9 pfu AdCMVβGal three days after the first administration (fig 3). This second administration resulted in similar kinetics to the first treatment, suggesting that repeated applications of adenoviral vectors are feasible but do not have synergistic effects on reporter gene expression in vivo.

ADENOVIRAL MEDIATED GENE EXPRESSION IN THE COLON OF MICE WITH EXPERIMENTAL COLITIS

Inflammatory bowel disease is associated with severe colonic injury and histopathological alterations such as epithelial cell hyperplasia, damage of the crypt architecture, massive infiltration with lymphocytes, and formation of inflammatory foci.[28] To determine whether the disruption of the mucosal barrier in the inflamed colon has any effect on adenoviral transduction efficiency, we injected adenoviruses intrarectally into healthy mice and mice with experimental colitis. In these experiments, we used the hapten reagent TNBS to induce colitis in Balb/c mice as previously described.[26] Three days after local administration of 1×10^9 pfu AdCMVβGal, β-galactosidase activity in colonic specimens from TNBS treated mice

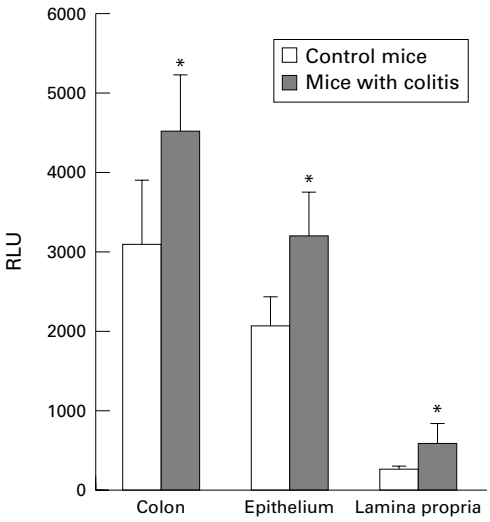

Figure 4 β-Galactosidase activities in the colon of mice with experimental colitis. Six to eight week old Balb/c mice with or without trinitrobenzenesulphonic acid (TNBS) induced colitis were infected with 1×10^9 pfu AdCMVβGal via the rectum. After three days the colon was removed and homogenised. In addition, epithelial and lamina propria cells were isolated and luminescent β-galactosidase activity was determined. Results are expressed as mean (SD) from three independent experiments (three mice/group) and represent an increase in enzyme activity (relative light units (RLU)) compared with untreated control mice. Asterisks indicate significantly ($p<0.05$) increased RLU values compared with the control group.

was significantly higher than in those from untreated control mice (fig 4). Within the colon a higher reporter gene expression was seen in epithelial cells than LPMCs, suggesting that this approach could be particularly useful for modulating gene expression in the former cell type.

In further studies, we analysed potential toxic effects of local adenoviral gene delivery to the inflamed gut. Accordingly, 1×10^9 pfu AdCMVβGal was administered intrarectally to mice with TNBS induced colitis. Organs (spleen, liver, lung, pancreas, small bowel, kidney, heart) were removed after seven days and analysed histologically. As shown in fig 5, there were no pathological findings. Furthermore, there were no apparent signs of systemic toxicity seven days after local administration of adenoviruses as determined by serum levels of creatinine (0.35 *v* 0.35 mg/dl in untreated mice), urease (19 *v* 17 mg/dl), bilirubin (0.95 *v* 1.01 mg/dl), alkaline phosphatase (88 *v* 126 U/l), and lipase (<190 *v* <190 U/l), suggesting that local administration of adenoviruses is a relatively safe method for adenoviral gene delivery in the gut.

IN VITRO ADENOVIRUS TRANSDUCTION OF LPMCs FROM UNINFLAMED AND INFLAMED COLON

As the above experiments also suggested an uptake of adenoviruses by LPMCs, we next focused on the capacity of adenoviruses to mediate gene expression in these cells in vitro. LPMCs were thought to be only poorly receptive to adenoviruses, because they express only low levels of receptors for virus attachment through the fibre capsid protein and have only small amounts of αvβ3/5 integrins for interaction with penton base proteins.[29 30] We therefore analysed the transduction potential of unstimulated or stimulated LPMCs in an in vitro assay system using reporter gene vectors (fig 6). In these studies, LPMCs from healthy and colitic mice were isolated and infected with AdCMVβGal. β-Galactosidase activity in freshly isolated LPMCs from TNBS treated mice infected with AdCMVβGal was significantly higher than in control LPMCs from healthy mice. Stimulation of LPMCs with phorbol ester plus phytohaemagglutinin before infection led to further increased transduction rates in LPMCs from both normal and colitic mice, suggesting that activated LPMCs from the inflamed colon are a potentially attractive target for adenoviral vectors.

EFFECT OF TREATMENT OF MICE WITH CYCLOSPORIN A ON EXPRESSION OF THE *lacZ* REPORTER GENE IN VIVO

The consecutive loss of reporter gene expression over time after in vivo injection of AdCMVβGal as described above could be the result of a high epithelial turnover rate and the replacement of transduced cells by proliferation of stem cells in the crypts.[27] On the other hand, many studies report limited transgene expression by E1 deleted Ad5 vectors because of a strong CTL mediated cellular immune response against target cells presenting adenovirus derived peptides on MHC class I.[31 32] We therefore wanted to analyse whether the decline in reporter gene expression described above was mainly or in part a result of destruction of Ad5 infected cells by the immune system. Accordingly, we performed time course experiments as above with TNBS treated mice given daily injections of the immunosuppressive drug cyclosporin A (fig 7). Interestingly, there were no major differences in colonic β-galactosidase activity between immunocompromised mice and controls, suggesting that a high epithelial turnover rather than CTL mediated immune responses could be mainly responsible for the reduction of reporter gene expression.

ADENOVIRUSES WITH MODIFIED FIBRE STRUCTURE ALLOW HIGH REPORTER GENE EXPRESSION IN LPMCs FROM MICE WITH EXPERIMENTAL COLITIS

The above data suggested a limited efficacy of local AdCMVβGal delivery for targeted gene expression in LPMCs and T lymphocytes probably because of their low numbers of receptors for virus attachment and their low expression of αvβ3/5 integrins. We therefore determined in a final series of studies the capacity of recently developed adenoviruses with modified fibre structure (denoted AdZ.F(pk7)) for β-galactosidase gene delivery to splenic T cells and LPMCs. Accordingly, we isolated spleen CD4 T cells from normal mice and compared the capacity of the AdCMVβGal and the AdZ.F(pk7) vectors to induce β-galactosidase activity in these cells. We observed that the latter adenoviral vector induced 10–40-fold higher expression of the reporter gene than the former vector (fig 8). Furthermore, in LPMCs from colitic mice the AdZ.F(pk7) vector induced a more than

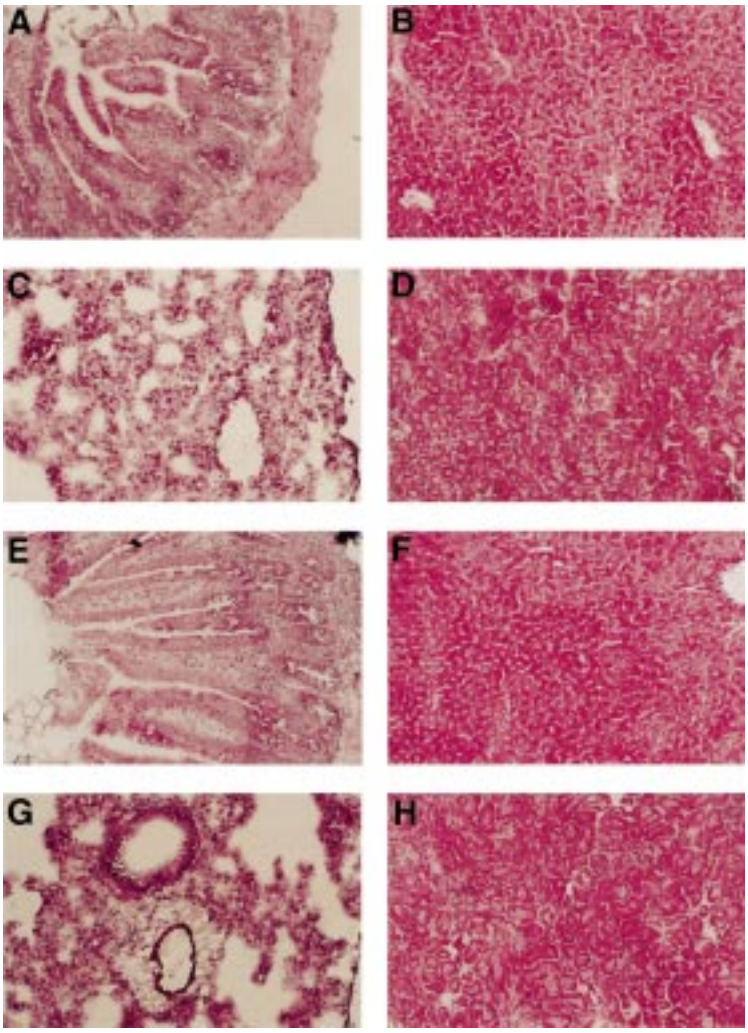

Figure 5 Histological analysis of the small bowel (A, E), liver (B, F), lung (C, G), and kidney (D, H) of mice with experimental colitis given AdCMVβGal or phosphate buffered saline (PBS). Eight week old Balb/c mice with trinitrobenzenesulphonic acid (TNBS) induced colitis were infected with 1×10^9 pfu AdCMVβGal via the rectum. After seven days, organs from AdCMVβGal treated (lower panel) and PBS treated control (upper panel) mice were removed. Cryosections were made and stained with haematoxylin/eosin. No inflammatory reaction was noted upon adenoviral gene delivery. Additional experiments after a second administration of AdCMVβGal after 14 days showed similar results (not shown). Magnification × 200.

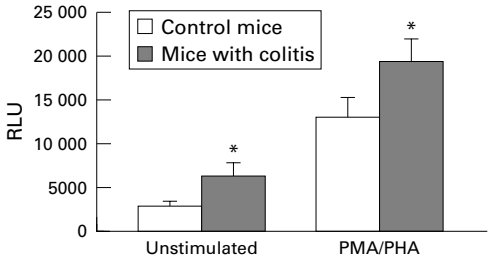

Figure 6 β-Galactosidase activities in infected lamina propria cells (LPMCs). LPMCs from healthy Balb/c mice or mice with trinitrobenzenesulphonic acid (TNBS) induced colitis were isolated. Then 1×10^6 unstimulated or phorbol ester/phytohaemagglutinin (PMA/PHA) stimulated cells were infected with AdCMVβGal at a multiplicity of infection of 1000. After three days the cells were lysed and luminescent β-galactosidase activity was determined. Results are expressed as mean (SD) from three independent experiments (three mice/group) and represent an increase in enzyme activity (relative light units (RLU)) compared with untreated control mice. Asterisks indicate significantly ($p<0.05$) increased RLU values compared with the control group.

10-fold higher expression of β-galactosidase activity than identical amounts of the standard AdCMVβGal virus (fig 8). Finally, we found in double staining studies on cytospins from LPMCs from colitic mice that more than 3% of the lamina propria CD4 T cells express β-galactosidase after administration of AdZ.F(pk7), suggesting a high transduction efficiency. Taken together, these data suggest that adenoviruses with modified fibre structure may be appropriate for the design of T cell- and LPMC-directed gene therapies in intestinal inflammation.

Discussion

Previous studies have shown that administration of recombinant cytokines, monoclonal antibodies, or antisense phosphorothioate oligonucleotides may be considered as potentially novel approaches for the treatment of inflammatory bowel disease.[33–36] Although the colon is an attractive target for somatic gene therapy approaches, adenoviral gene therapy has only recently been considered for treatment of intestinal inflammation.[37–39] In this study, we have evaluated the prospects of this new therapeutic approach using recombinant replication deficient adenoviruses as transfer vehicles for gastrointestinal gene therapy and experimental studies in vivo. We show that a single rectal administration of recombinant adenoviruses results in high target gene expression in the inflamed gut. These data may provide a rational basis for local adenoviral gene therapy in patients with inflammatory bowel disease.

Several studies have shown that other gene delivery methods such as liposome mediated gene transfer can be used to express genes in the gastrointestinal tract including the colon.[40–46] In the colon, the limitations of this technique were based on the transient expression of target genes (1–4 days) and the low transfection efficacy of the eukaryotic expression vectors in epithelial (5–10%) and subepithelial (<5%) cells.[45] Based on these observations, alternative strategies including the use of adenoviruses appeared to be desirable for intestinal gene therapy. However, recent studies have shown that the route of administration has a major influence on the transduction efficiency of adenoviruses in various tissues in rats.[47] For instance, intravenous application of adenoviruses results in strong transduction in the liver, whereas intestinal tube feeding of adenoviral vectors results in high transduction efficiency in the duodenum, jejunum, and ileum but not the colon.[10 40] The colon, however, has not been widely used for gene transfer studies in vivo. Previous studies by Jobin and coworkers[37] showed a high transduction capacity of intestinal epithelial cells in vitro, and recent data suggest that intraperitoneal administration of an interleukin 4-producing adenoviral vector can be used, in spite of a low transduction rate, to reduce intestinal pathology in rats with acute experimental colitis.[38] However, this is the first study to show high gene expression in the murine colon in vivo after local adenovirus administration.

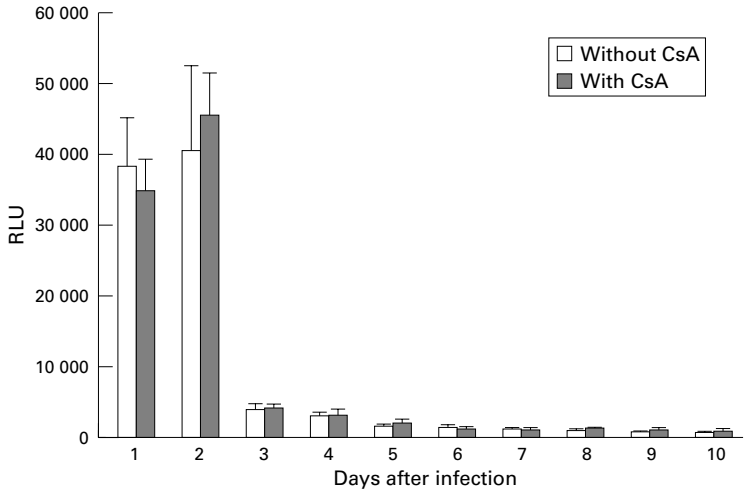

Figure 7 Time course of reporter gene expression in colitic mice with or without daily treatment with cyclosporin A (CsA). Mice with trinitrobenzenesulphonic acid (TNBS) induced colitis were infected with 1×10^9 pfu AdCMVβGal and treated with CsA as indicated. Mice were killed at the indicated time points, and luminescent β-galactosidase activity in homogenised colonic specimens was determined as specified in Methods. Results represent mean (SD) from three independent experiments (three mice/group) and represent an increase in enzyme activity (relative light units (RLU))/100 mg tissue compared with untreated control mice.

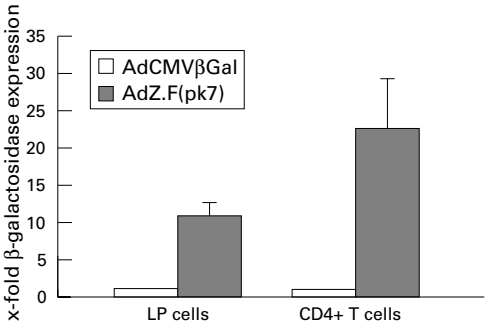

Figure 8 Adenoviral gene delivery to colonic lamina propria mononuclear cells (LPMCs) and spleen CD4 T cells using viruses with normal or modified fibre structure. β-Galactosidase activities in AdCMVβGal or AdZ.F(pk7) infected spleen CD4 T cells from healthy control mice and LPMCs from mice with trinitrobenzenesulphonic acid (TNBS) induced colitis were measured. A total of 1×10^6 cells were infected with AdCMVβGal at a multiplicity of infection of 1000. After three days the cells were lysed, and luminescent β-galactosidase activity was determined. Results represent mean (SD) from three independent experiments.

Our local adenovirus delivery system via the rectum seems to be much more effective for colonic transduction than adenovirus delivery with an oral duodenum tube, which leads to only low levels of colonic transduction in rats.[10 47] Interestingly, we could not detect high adenoviral transduction of the liver and spleen after local adenoviral delivery via the rectum. This finding suggests that several potential side effects of systemic approaches could be avoided by using local gene delivery systems. The safety of local adenoviral gene therapy in the colon was further underlined by the finding that no apparent signs of toxicity were observed in this study, as assessed by histological examination of the liver, kidney, lung, and pancreas and analysis of various blood variables. These data suggest that the inflammatory reactions in various organs that have been observed after administration of adenoviruses in mice and humans may be prevented by local administration of adenoviruses to the colon. However, our data do not exclude the possibility that repeated local administration of adenoviruses over several months may induce an immune response to viral proteins that may compound the inflammation. In this regard, it would be interesting to determine whether modified adenoviral vectors would prevent this immunological reaction. One such example is so called "gutless" vectors in which recombinant proteins were introduced by Cre-lox recombination for all of the viral genes except for those required for replication and packaging.[44] Further studies are required to determine whether these vectors could be useful for immunotherapy of intestinal inflammation.

Lymphocytes and macrophages and their secreted growth factors and cytokines have been suggested to play a central role in the pathogenesis of inflammatory bowel disease,[28] and are therefore interesting targets for gene therapeutic strategies in humans. We observed detectable levels of target gene expression in epithelial cells and LPMCs of colitic mice infected in vitro or in vivo. In particular, there were relatively high transduction rates in the colon of mice with experimental colitis. However, there was a higher transduction rate of colonic epithelial cells than LPMCs. More efficient transduction rates for LPMCs may be achieved by development of adenoviruses with a modified fibre structure or specific tagging of adenoviruses to a surface molecule on the target cells—for example, CD3.[48] Such strategies may be useful for specifically targeting recombinant gene expression to inflamed tissue sites and for the treatment of inflammatory bowel disease. The potential benefit of modified adenoviral vectors has been demonstrated in this study by the finding that adenoviruses with modified fibre structure produced much higher target gene expression in LPMCs and splenic T lymphocytes than viruses with normal fibre structure. Thus adenoviruses with modified fibre structure may be useful for T cell directed therapies in intestinal inflammation.

In summary, local administration of recombinant adenoviruses could provide a new reliable method for targeted gene expression in the inflamed colon. Such gene delivery may be used to specifically express signal transduction proteins with therapeutic potential in inflamed colonic tissue.

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