

REVIEW

Gene transfer approaches for the treatment of inflammatory bowel disease

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The pathogenesis of Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel disease, involves a complex interplay between certain genetic, environmental and immunological factors. Considerable research progress in the last decade defined key inflammatory pathways in the inflamed gut and identified new potential therapeutic targets. Since the current medical treatment with corticosteroids and anti-inflammatory drugs is often associated with undesired side effects and cannot completely cure IBD, these current

advances in our understanding of intestinal pathology may now allow the development of new biologic treatment strategies including gene therapy. In this review, we will give a brief overview of potential gene therapy target molecules related to chronic intestinal inflammation. Furthermore, we summarize the results of recent preclinical studies for intestinal gene transfer and discuss future perspectives.

Gene Therapy (2003) 10, 854–860. doi:10.1038/sj.gt.3302013

Keywords: inflammatory bowel disease; Crohn's disease; ulcerative colitis; gene transfer; animal models

Introduction

The pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of inflammatory bowel disease (IBD), involves a complex interplay between certain genetic, environmental and immunological factors. The aetiology of these incurable and relapsing diseases still remains largely unclear. Long-term medical treatment with corticosteroids and other immunosuppressive drugs is often associated with undesired side effects, and moreover, a substantial part of IBD patients becomes steroid refractory and is likely to have one or more surgical treatments during the course of the disease.^{1,2}

Gene therapy was considered for a long time appropriate only to treat monogenetic diseases by means of a therapeutic gene transfer for replacement of a defective homologous gene. However, the field of gene therapy now also includes the treatment of autoimmunity and chronic inflammatory diseases by expression of immunologically relevant proteins with the intention of downregulating pathogenic or inducing protective immune responses. The development of a potential gene therapy for chronic intestinal inflammation is still at a very early stage. In contrast to cystic fibrosis, severe combined immunodeficiency or various kinds of cancer, there are currently no clinical trials on the way. Since CD and UC are probably multifactorial diseases, gene therapy represents an enormous challenge requiring significant advances in our knowledge of the gene defects, target cells, suitable vector systems and methods for targeted delivery of therapeutic gene expression.

However, the considerable research progress in the last decade has led to an increase in our understanding of molecular mechanisms of intestinal inflammation. Particularly, the detailed analysis of genetically engineered animal models suggests that a deregulated immune response driven by the normal luminal microflora is probably of critical importance for both the onset and chronification of the pathophysiologic process.^{3,4} Molecular and cellular analysis of normal and deregulated immune responses elucidated the biological function of intestinal immune cells, and defined key inflammatory pathways and new potential therapeutic targets.⁵ In this review, we will give a brief overview of potential gene therapy target molecules related to IBD. Furthermore, we summarize the results of recent animal studies for intestinal gene transfer and discuss future perspectives.

Molecular pathogenesis and current treatment of IBD

Recent studies have provided strong evidence that CD is characterized by a T_H1 type immune response.^{6–8} It has been suggested that the abnormal reactivity to luminal or mucosal antigens may be the result of an imbalance between proinflammatory and regulatory $CD4^+$ T lymphocytes and T_H1 -associated inflammatory cytokines, most notably tumour necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), interleukin-12 (IL-12) and interleukin-18 (IL-18) and anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), respectively.^{9–11} In addition, advances of our knowledge of IBD genetics came from systematic genome searches for IBD-associated gene loci. Recently, genetic variations in the Nod2 (CARD15) gene

have been associated with susceptibility to CD.^{12,13} Nod2 is involved into responsiveness towards bacterial products and activates nuclear factor kappa B (NF- κ B), a key transcription factor within the framework of innate immunity.^{14,15} On the basis of these new mechanistical insights, many (immuno-) biological therapies are currently being evaluated.⁵ Increasing evidence that the proinflammatory cytokine (TNF- α) plays a key role in the immunopathogenesis of CD and several other immune-mediated diseases has led to the development of several therapeutic anti-TNF- α strategies.^{16,17} TNF- α blocking biologicals proven to be clinically effective in CD are humanized (Infliximab) and engineered human (CDP571) monoclonal antibodies to TNF- α .¹⁷ Infliximab, which binds to both soluble and transmembrane TNF- α and inhibits binding to TNF receptors, is currently used to treat moderate to severe or fistulizing CD. In contrast, a TNF- α receptor/IgG1 fusion protein (Etanercept), a decoy receptor for TNF- α , which is similarly to Infliximab beneficial in rheumatoid arthritis, did not positively affect the clinical response in colitis. One possible explanation for this different degree of clinical response is the observation that Infliximab binds to transmembrane TNF- α (mTNF) on monocytes and activated T cells, (whereas Etanercept preferentially blocks soluble TNF) and thus inhibits mTNF/TNF receptor 2 interaction.^{18–20} In addition, Etanercept binds, unlike Infliximab, to lymphotoxin- α , a cytokine capable to bind to TNF- α receptors and involved in the development of Peyer's patches and the development of experimental colitis in mice.²¹ However, the clinical studies so far suggest that this cytokine does not play a role in the pathogenesis of CD. Other immunobiological substances, which are effective in animal models and currently tested in clinic trials, include monoclonal antibodies to leucocyte adhesion molecules (α 4 integrin, α 4 β 7)²² and cytokines/cytokine receptors (IL-12, interleukin-2 (IL-2) receptor),^{5,23} recombinant cytokines (interferon- α 2A, interferon- β 1 α)^{24,25} and antisense oligonucleotides to the p65 subunit of the transcription factor NF- κ B.²⁶ Clinical trials for some other biological compounds showed limited or no efficacy (recombinant IL-10 and antisense oligonucleotides to the adhesion molecule ICAM-1).^{27–29} The success of anti-TNF- α strategies for the therapy of refractory CD demonstrated clearly the prospects of immunobiological therapy for the future treatment of IBD. However, disadvantages of such therapies are sometimes undesired side effects such as development of infections or immune reactions against non-human parts of antibodies as well as higher production costs in comparison with small molecular compounds.^{30,31} To look ahead, these new therapeutic approaches are likely to be used in combination with standard therapies, but will not be, at least in the near future, 'the cure' for IBD.

Targeting gene transfer to the alimentary tract

As a result of the presence of a large number of stem cells in intestinal crypts and the prima facie ease of access from the luminal site, the gut is suggested to be an interesting target for therapeutic gene transfer. Unfortunately, gene transfer into the bowel wall is rather demanding because protective extracellular barriers such as tight junctions, glycocalyx and mucus are potent

safeguards against the entry of extrinsic genetic information.^{32,33} For the purpose of a gene therapy for inflammatory bowel disease, targeting immune cells of the gut associated lymphoid tissue (GALT) is potentially desirable. Since the epithelial barrier prevents efficient transduction of subepithelial areas after vector administration into the gut lumen,³⁴ other administration routes or specialized vector systems are required to target enhanced numbers of mononuclear cells in the lamina propria, mesenteric lymph nodes and Peyer's patches of the ileum.

As a result of the rapid clearance of viral particles by the liver, a systemic injection of recombinant viral vectors into the circulation of immune-competent mice results mainly in the transduction of hepatocytes and spleen cells and does not transduce the intestinal mucosa efficiently.³⁴ With the aim to overcome this problem, recently Ye *et al* injected recombinant adenoviral particles into the circulation of mice with a temporary liver bypass. The outcome of this method regarding intestinal gene therapy was an increased reporter gene expression in cells of vessels and capillaries of the intestinal wall, which persisted for several weeks.³⁵

In vitro and *in vivo* studies with human and rodent cell lines and animal models using reporter genes demonstrated that transduction of the intestinal mucosa by local administration of liposomal,^{36–38} retroviral,^{39,40} lentiviral,⁴¹ adeno-associated (AAV) viral^{42,43} and adenoviral^{34,44–47} vector systems is feasible and could be significantly enhanced by the use of mucolytic substances (dithiothreitol, N-acetylcysteine, Nacystelyn^{32,48}). Taken together, the apparently most promising system for efficient gene transfer to the gastrointestinal tract are recombinant helper-dependent AAV vectors lacking all viral reading frames.⁴⁹ During *et al*⁴² demonstrated a long-lasting recombinant gene expression in the stomach, duodenum and small bowel after a single AAV delivery in fasting rats with an oral feeding tube.⁴² Reporter gene expression was detected from 6 h to 6 months after administration in both the epithelial layer and lamina propria cells. This group used this method successfully in a vaccination strategy leading to auto-antibodies targeting specific brain proteins.⁴³ In this study, transduction of colon cells after oral vector delivery was not observed, but could presumably be achieved by rectal application.

The majority of the *in vivo* studies have been performed with replication-defective Ad5 vectors, which have a highly efficient mode of entry into a broad spectrum of eukaryotic cells and can, unlike retroviruses, infect both dividing and nondividing cells.⁵⁰ Administration of E1/E3-deleted recombinant adenoviruses with a duodenal feeding tube was reported to result in strong concentration-dependent transduction of cells of the duodenum, jejunum and ileum,⁴⁴ whereas adenoviral particles applied by rectal enema efficiently transduced the colon mucosa.³⁴ However, the vast majority of cells transduced after intraluminal vector instillation were resorptive enterocytes. Owing to the high turnover rate of the gut epithelium, the adenovirus-mediated gene expression was, therefore, only transient and declined sharply after 2–3 days.³⁴ For perpetuation of gene expression, a repeated administration without systemic antibody responses or other enhanced immune reactions was possible.⁴⁴

Chen *et al.*⁵¹ reported successful gene transfer into the Peyer's patches by direct injection of recombinant Ad5. Within the Peyer's patches, macrophages and epithelial cells were transgene positive, whereas T- and B lymphocytes were not transduced.⁵¹ This is consistent with the observation that lymphocytes are only poorly receptive to adenovirus infection, because they express only low levels of coxsackie and adenovirus receptor (CAR) for virus attachment through the fibre capsid protein and display only small amounts of α v-integrins required for interaction with penton-base proteins and virus internalization.^{52,53} Interestingly, studies in mice with experimental colitis as a model for human IBD showed that disruption of intestinal epithelial integrity and other histopathological alterations during mucosal inflammation increased transduction of lamina propria mononuclear cells (LPMC) by adenoviral vectors.³⁴ It was shown that the capacity of recombinant Ad5 for targeting non- or semipermissive cells can be enhanced by developing adenoviral vectors with genetically altered tropism.⁵⁴ When evaluated for intestinal gene transfer, recombinant Ad5 with modified fibre structure binding to ubiquitously expressed cellular heparansulfate receptors increased transduction of LPMC *in vitro* more than 10-fold.³⁴

Intestinal stem cells

Most of the cell types of the intestinal epithelium are constantly shed into the faecal stream and must be replaced by a steady supply of cells generated by rapidly dividing multipotent stem cells. Stem cells are a prime target for gene therapeutical approaches. The successful genetic modification of intestinal stem cells has therefore outstanding clinical potential for many gastrointestinal diseases including IBD. Unfortunately, no definitive cellular markers for intestinal stem cells have been identified so far and they thus cannot be simply distinguished from other epithelial cells. Therefore, a direct proof for successful gene transfer to these cells is currently not feasible. However, strong recombinant gene expression in the gut epithelium for a prolonged time after vector administration is likely to require stable transduction of intestinal stem cells. Interestingly, oral administration of AAV vectors yielded stable transduction of the gut epithelium more than 6 months postinfection.⁴² Since AAV are able to provide long-term recombinant gene expression because of stable integration into the cellular genome or concatemerization of vector molecules,⁵⁵ and the gut is a natural host for efficient infection,⁵⁶ AAV vectors could potentially be important for transduction of intestinal stem cells. Intravenous injection of recombinant adenoviruses into mice was also associated with stable transduction of crypt cells in the colon epithelium, whereas other parts of the GI-tract were reporter gene negative.⁵⁷ Since an adaptive immune response against adenoviral components would eliminate transduced cells, this observation is only evident in immune-defective mice. The outcome of these experiments is nevertheless surprising, because the colon is not efficiently transduced after systemic adenovirus administration and moreover, Ad5 does not readily integrate into the host genome. Recent research progress in the field of intestinal stem cell biology,

particularly the development of *in vitro* cultivation methods for adult primary intestinal epithelium, may now allow detailed characterization and experimental manipulation of stem cells.^{58–60} In the future, transplantation of *in vitro* transfected intestinal stem cells could thus be an important method of delivering therapeutic genes to the gut.

In terms of gene delivery to immune cells in lamina propria and Peyer's patches, the biology of M cells is of particular interest. Intestinal M cells are distinctive epithelial cells of the follicle-associated epithelium overlying gut lymphoid tissue, which have the ability for efficient uptake of a wide range of microorganisms and macromolecules. The physiological role of M cells is apparently the rapid presentation of antigens and microorganisms to the submucosal immune cells.^{61–63} M cells are not just a 'weak point' of the mucosal barrier, but rather are specialized in transcytosis processes. Furthermore, M cells are an entry point for several pathogens into the bowel wall. Hence, the biological function of M cells could be exploited for transduction of immune cells of the GALT for immunomodulatory therapies. In this regard, transduction of M cells by AAV vectors delivered recombinant gene expression rapidly to immune cells of the lamina propria.⁴²

Viral vectors altering the levels of immunoregulatory cytokines to treat inflammation in animal models for human IBD

In the healthy gut, the mucosal immune system is characterized by tightly regulated dynamic interactions between immune- and nonimmune cells to guarantee the maintenance of an appropriate immunological balance between the host and the symbiotic luminal microflora. These interactions rely eminently upon communication between immune cells themselves and other cells involved in mucosal integrity, for example, epithelial cells. Communication of these cells can occur through direct cell surface receptor interaction or via release of soluble mediators such as cytokines. It is now well established that in the inflamed gut of patients with CD and UC, the balance of proinflammatory and regulatory cytokines is abrogated. In CD, several proinflammatory cytokines appear to play a key role for the tissue damage that accompanies IBD.⁶⁴ In contrast, many concurrent animal studies provided clear evidence that the presence of regulatory cytokines inhibiting antigen presentation and proinflammatory cytokine release or stimulating apoptosis of activated immune cells is essential for mucosal balance.^{3,65} Owing to their prominent role in the normal and inflamed gut, the development of novel therapeutic strategies focused primarily on altering the biological functions of T-cell-related cytokines. Here, we briefly summarize the results of gene therapy-based cytokine strategies in animal models for human IBD (Figure 1).

Targeting proinflammatory cytokines

Gene therapy could be an important method for the delivery of inflammatory cytokine inhibitors in IBD. It was shown recently that bioactive IL-18, a pleiotropic cytokine with structural similarities to the IL-1 cytokine

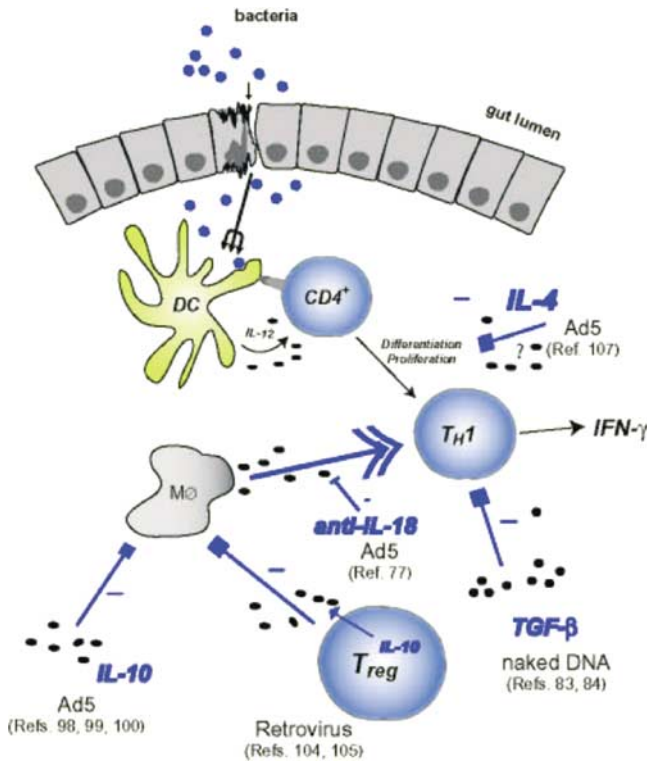


Figure 1 Schematic summary of animal gene transfer studies modulating the levels of pro- and anti-inflammatory cytokines in the inflamed gut.

family originally identified as (IFN- γ) inducing factor (IGIF),⁶⁶ is upregulated in the inflamed intestinal mucosa of patients suffering from CD.^{67–70} IL-18 has a variety of important immunomodulatory effects on many different cells of the immune system and is coinducer of IL-12-driven T_H1 immune responses.^{71–73} IL-18 activates in T lymphocytes the transcription factors NF- κ B and AP-1 that synergize with the IL-12-inducible transcription factor STAT-4 in activating the IFN- γ promoter in T cells.^{74,75} In addition, IL-18 augments inflammatory immune responses by upregulating the production of the proinflammatory TNF- α , interleukin-1 (IL-1) and interleukin-6 (IL-6) cytokines.⁷⁶ It is therefore believed that IL-18 expression could be a major contributing factor for pathophysiologic T_H1-related inflammatory diseases such as CD. Owing to the fact that enhanced gut IL-18 levels in myeloid and epithelial cells were present in several mouse models for human IBD,^{77–80} we investigated in our group the feasibility of an IL-18-based gene therapy approach as a potential new treatment of IBD.⁷⁷

E1/E3-deleted recombinant Ad5 vectors were constructed expressing IL-18 antisense RNA under a cytomegalovirus promoter. In order to suppress intestinal IL-18 expression, at intervals of 2 days three repeated doses of adenoviral particles were injected into C.B-17 SCID mice with chronic T-cell-mediated colitis⁸¹ by enema into the lumen of the colon. At day 6 after initial vector administration, mice were killed and subsequently analysed. As a result, the levels of endogenous IL-18 protein were significantly reduced after treatment with the IL-18 antisense construct compared with mice treated with a control virus. Using both quantitative histological assessment as well as

endoscopic scoring of colitis activity, suppression of IL-18 expression led to significant improvement of established colitis in treated SCID mice. In addition, a downregulation of mucosal IFN- γ production by LPMC was observed, whereas IFN- γ production by spleen cells was not affected indicating that local IL-18 antisense vector administration had low impact on systemic immune responses. The data from the present study show that IL-18 is important for the effector phase of chronic colitis and identified gene therapy strategies to suppress the production and/or biological function of IL-18 as a rationale for treatment of CD. These findings are further supported by recent studies demonstrating a pivotal role of IL-18 for onset of acute colitis.⁷⁹

Overexpression of regulatory cytokines

Apart from targeting of proinflammatory cytokines such as IL-12 or IL-18, overexpression of regulatory cytokines may have therapeutic relevance. Several gene therapy vectors have been successfully used to deliver the regulatory/anti-inflammatory cytokines IL-10 and TGF- β into animal models for human inflammatory or autoimmune disease.⁸²

Lately, Kitani *et al*⁸³ observed expression of recombinant TGF- β 1 mRNA in the gut after a single intranasal administration of naked plasmid DNA encoding an expression cassette for the precursor form of TGF- β 1. This TGF- β gene transfer could prevent formation of murine experimental colitis and moreover, was effective in ameliorating established disease.⁸³ Intramuscular administration of TGF- β expression plasmids has also shown therapeutic efficiency in treating colitis in rats.⁸⁴ However, the therapeutic potential of a gene transfer of TGF- β 1 or other family members is yet unclear as TGF has been implicated in numerous pathologic conditions including lung fibrosis,⁸⁵ scleroderma⁸⁶ and various infectious diseases.⁸⁷ Accordingly, intratracheal administration of recombinant Ad5 encoding the mature bioactive, but not the latent precursor form of TGF- β 1, induced severe pulmonary fibrosis in rats.⁸⁸

Severe chronic colitis develops spontaneously in gene-targeted IL-10-deficient mice⁸⁹ and IL-10 promotes the formation of antigen-specific regulatory T cells.^{90,91} Since administration of recombinant IL-10 proved therapeutic efficacy in several animal models of colitis^{92–94} and adenoviral IL-10 transfer was successful in murine models of rheumatoid arthritis, a disease with various immunological analogies to CD,^{95–97} researchers evaluated gene transfer approaches to increase IL-10 levels in the inflamed gut. Three studies demonstrated prevention of colon inflammation in experimental TNBS colitis in rats, mice^{98,99} or IL-10-deficient mice¹⁰⁰ after systemic administration of recombinant Ad5 encoding IL-10. Treatment of established disease was only possible in the IL-10^{-/-} mouse. Interestingly, *Lactococcus lactis* bacteria genetically modified to secrete murine IL-10 were used to deliver IL-10 to the intestinal mucosa. Daily administration via a feeding tube resulted in significant clinical improvement of colitis severity in the IL-10^{-/-} and the dextran sodium sulphate colitis models.¹⁰¹ Unfortunately, systemic injection of recombinant IL-10 showed in clinical trials only a modest potency in patients with active CD and surprisingly at high doses, IL-10 even induced the production of the proinflammatory cytokine IFN- γ .^{102,103} Recently, van Montfrans *et al*¹⁰⁴

transduced CD4⁺ T lymphocytes *ex vivo* with a retroviral IL-10 expression construct. These cells produced active IL-10 for a prolonged time (>4 month), expressed gut-homing integrin $\alpha 4\beta 7$ and had a regulatory/immunomodulatory phenotype. Adoptive transfer of IL-10-transduced T cells was successfully used to treat colitis in the murine CD4⁺CD45RB^{High} SCID transfer model.¹⁰⁵ It is interesting to speculate whether the local delivery of immuno-regulatory T cells stably producing IL-10 would result in more satisfactory clinical benefits than direct injection of IL-10. To conclude, despite the therapeutic experience of IL-10 in animal models of IBD is very encouraging, it remains to be seen if IL-10 gene therapy is suitable to treat human CD.

The local cytokine milieu that naive CD4⁺ T-lymphocytes cells encounter directs their differentiation into one of several functional subsets. Distinct patterns of T-cell cytokine production distinguish the two main subsets of CD4⁺ T cells. T_H1 cells predominantly produce large amounts of IL-2 and IFN- γ , whereas T_H2 cells produce IL-4, IL-5, IL-10 and IL-13.^{9,106} Since T_H1 cells are strikingly involved in the pathogenesis of CD, Hoga-boam *et al*¹⁰⁷ determined the effects of recombinant IL-4, which is a well-known differentiation factor for T_H2 cells, introduced by a recombinant Ad5 on experimental colitis in rats. In this study, it was shown that increased systemic and colonic IL-4 levels after intraperitoneal IL-4 transfer reduced acute colonic inflammation. In contrast, exogenous IL-4 was shown to exacerbate disease in a mouse model of chronic intestinal inflammation,¹⁰⁷ suggesting that IL-4 does not simply have a general anti-inflammatory role in the gut immune system. In addition, although several animal models for human IBD show clear polarization towards T_H1 or T_H2 responses, the situation in UC in humans is not fully understood.

Conclusion

As described above, several promising preclinical studies described effective therapeutic gene delivery to the inflamed animal gut. However, clinical trials with patients suffering from several 'monogenic' diseases such as cystic fibrosis demonstrated clearly that successful gene transfer to affected organs is more challenging than originally thought. Given that monogenic diseases are rather less complex diseases than IBD, it is still a long way towards a potential gene therapy for chronic intestinal inflammation. Innate and acquired immune defense mechanisms against currently used gene delivery vehicles can prevent successful long-term transgene expression and can cause, in high doses, systemic side reactions. Gutless vectors, devoid of all viral genes, allowed long-term vector persistence in hepatocytes and muscle cells and may also be useful for gene transfer to the gut.¹⁰⁸ Efficient and specific targeting of intestinal cells by means of vectors with altered tropism or by the use of intestinal cell-specific promoters may be suitable to introduce gene transfer as a new treatment option for IBD. Our final conclusions are:

(1) Currently, there are no published gene therapeutic trials in IBD patients, but many interesting potential target molecules have been identified.

- (2) Preclinical studies demonstrate that targeting recombinant gene expression to the gut with different viral and nonviral vector systems is feasible.
- (3) Overexpression of regulatory cytokines (IL-10, TGF- β) or inhibition of proinflammatory cytokines (IL-18) was beneficial in animal studies.

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