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# Part I

## Chapter 1

### Introduction Part I

Gene and cell therapy based treatment strategies for  
inflammatory bowel diseases

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## **Abstract**

Inflammatory bowel diseases (IBD) are a group of chronic inflammatory disorders most commonly affecting young adults. Currently available therapies can result in induction and maintenance of remission, but are not curative and have sometimes important side effects. Advances in basic research in IBD have provided new therapeutic opportunities to target the inflammatory process involved. Gene and cell therapy approaches are suitable to prevent inflammation in the GI tract and show therefore potential in the treatment of IBD. In this review, we are presenting the current progress in the field of both gene and cell therapy and future prospects in the context of IBD. Regarding gene therapy, we focus on viral vectors and their applications in preclinical models. The focus for cell therapy is on regulatory T lymphocytes and mesenchymal stromal cells, their potential for the treatment of IBD and the progress made in both preclinical models and clinical trials.

## Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory diseases most commonly affecting young adults [1-3]. The exact pathogenesis is unknown, but it is widely accepted that IBD result from an inappropriate response of a defective mucosal immune system to the intestinal flora and other luminal antigens [4-6].

IBD include two major disorders: ulcerative colitis (UC) and Crohn's disease (CD). These disorders have distinct and overlapping pathologic and clinical characteristics [7]. UC is a relapsing non-transmural inflammatory condition that is limited to the colon [8]. Patients characteristically present with bloody diarrhoea, passage of pus, mucus, or both, and abdominal cramping [8]. CD is a relapsing, transmural inflammatory disease of the gastrointestinal (GI) mucosa that can involve the entire GI tract from the mouth to the anus [8]. Patients characteristically present with discontinuous involvement of various portions of the GI tract and the development of complications including strictures, abscesses, or fistulas [8]. IBD are associated with a considerable reduction in quality of life of the patients [9-11] and currently no curative treatment options are available. Conventional therapeutics cannot prevent complications in IBD and although novel treatment strategies, including TNF-neutralizing antibodies, have greatly increased the therapeutic armamentarium, many patients still have to undergo surgery [12]. For this reason, the development of new treatments to prevent initiation of inflammation and more important allow for long term remission is required.

Gene and cell therapy approaches are more and more considered in relation to the prevention of inflammation in the GI tract. Gene therapy consists of the insertion or alteration of genes within an individual's cells to treat disease. Cell therapy describes the process of introducing new cells into a tissue in order to treat a disease. Both approaches have been applied successfully in a clinical setting for a broad range of diseases either separately or together, including

early stage clinical development for IBD [13-23]. Here we discuss current progress in the field and future treatment prospects in the context of IBD.

## Gene therapy as treatment for IBD

To facilitate the uptake and the expression of the transgene in the target cell, a vector is required. Vectors can be non-viral or viral. The choice of a safe and reliable vector that can mediate long term gene transfer to both dividing and non-dividing cells is of vital importance for a gene therapy approach. Although viral vectors are created from pathogenic viruses, they are modified in such a way as to minimize their pathogenicity. This usually involves the deletion of a part of the viral genome critical for viral replication. Such a virus can efficiently infect cells and has the potential for long term stable gene expression. In the gene therapy section of this review we will focus on viral vectors that have been used successfully in gene therapy applications in recent years [14, 15], are able to target the gut [24-32] and can therefore be considered for gene delivery in the GI tract, namely retroviral, lentiviral, adenoviral and adeno-associated viral vectors (for an overview see: **Table 1** or **Figure 1**).

For an overview of non-viral delivery methods to the intestine we recommend the review from O'Neill et al [33].

### *Retro- and lentiviral vectors*

Retroviral vectors were used for the first time in a clinical setting over 20 years ago [34-36] and are among the most commonly used vectors in gene therapy. Retroviral particles require disruption of the nuclear membrane to gain access and therefore need cell division for entering the cell [37]. Retroviruses have been demonstrated to be able to transduce intestinal epithelial cells [24-26], although at a low efficiency. At the contrary, intestinal epithelial cells can be transduced efficiently by lentiviruses [27] which are a sub-class of retroviruses. The lentiviruses have an advantage over retroviruses as vectors in gene therapy because of their ability to transduce non-dividing cells [38, 39]. Furthermore the lentivirus did not induce mucosal damage or distribute beyond the

distal colon [27] and appeared therefore as a potential vector for gene delivery in the treatment of IBD.

However, a safety issue to be considered with both retro- and lentiviral vectors is their potential to integrate at many sites in the human genome [40, 41]. Those genomic integrations can result in insertional mutagenesis causing cancer development as has been observed in clinical trials [19, 42-44]. Even though significant improvements in lentiviral vector safety have been achieved in recent years [45], the concern for random integration remains and needs to be addressed [46, 47] before these vectors can be considered as safe tools for gene therapy applications in IBD.

#### *Adenoviral vectors*

Despite the fact that adenoviruses are pathogenic viruses and can cause morbidity, especially in immune-compromised patients [48], adenoviral vectors have been frequently used in gene therapy due to their broad tissue tropism and lack of integration into the host genome [49]. Gene therapy using adenoviral vectors has shown potential in the treatment of colitis in preclinical models [28-30]. For example a single systemic injection of an adenoviral vector carrying the Interleukin-10 (IL-10) transgene was sufficient not only to prevent the onset of colitis but also to induce clinical and histological remission in mice with established disease [29]. Additionally Schmiiedlin-Ren and colleagues demonstrated that intestinal epithelial cells of IBD patients can be efficiently transduced *ex vivo* by adenoviral vectors [50]. All together, these results suggest that targeting of the inflamed intestine through the luminal route can be possible using adenoviral vectors [50].

However, hematologic and hepatic toxicities were observed in animal studies after injection with high vector doses [51-53], which imply that further development in generating a new type of adenoviral vector is necessary before considering clinical applications. Recently a gutted adenovirus, devoid of all viral coding sequences, was shown to induce less toxicity [54] after delivery. However, this finding, if promising for future therapeutic applications, needs further exploration.

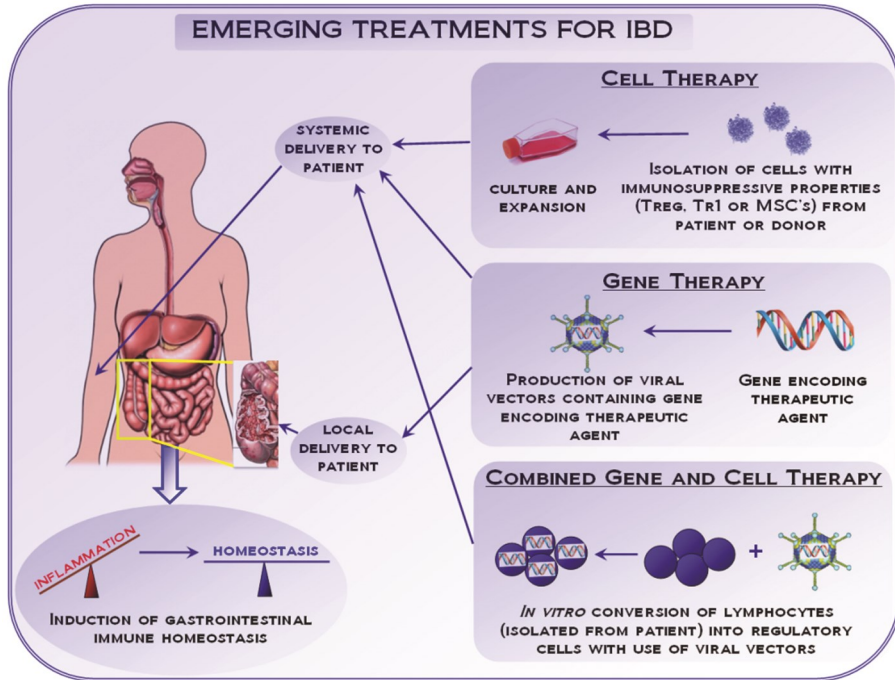
### *AAV vectors*

The non-pathogenic, replication-deficient adeno-associated virus (AAV) holds promise for gene therapy. The AAV vector has a good safety profile as it remains predominantly episomal [55]. In general, 99% of recombinant AAV are maintained as episomal copies [56], indicating a very low risk of insertional mutagenesis compared with retroviral vectors. Furthermore, AAV vectors are able to transduce both dividing and quiescent cells [57, 58] and were demonstrated to be effective as gene therapy vectors in several promising preclinical models for autoimmune and inflammatory disorders [59-66]. The therapeutic potential of the AAV as a vector in gene therapy has also been demonstrated in a clinical setting in recent studies [67-77].

AAV vectors were shown to be able to target the GI tract [31, 32] and long term transgene expression post AAV treatment was reported which in relation with the high turn-over of intestinal cells, suggests that transduction of the slow-dividing intestinal stem cells was achieved [31, 32]. However, no data are presently available about the treatment of experimental colitis with AAV vectors.

## **Cell therapy as treatment for IBD**

Cell-based therapies aim to introduce new cells into a tissue in order to treat a disease and can permit the replacement of function [78], or restore the homeostasis of the immune system [79]. In the last 50 years hematopoietic stem cell transplantation has been developed as a curative option for inherited disorders and hematologic or lymphoid cancers [13, 80], leading the way toward innovative therapies for other illnesses. Recent results obtained from animal models and early human clinical trials in graft versus host disease but also CD showed that either regulatory T lymphocytes or mesenchymal stromal cells may be of clinical relevance for the treatment of IBD (for an overview see: **Table 2** or **Figure 1**).



**Figure 1. Emerging treatments for IBD. Overview of the gene and cell therapy based treatment strategies for IBD as discussed in this chapter.** Treg: CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> regulatory T cell; Tr1: Type 1 regulatory T cell; MSC: Mesenchymal stromal cells

**Table 1 Overview of the gene therapy based treatment strategies for IBD as discussed in this chapter**

Viral vector	Gut targeting	Reference to gut targeting	Status of development	Reference for status of development
Retro- and Lentiviral	Yes	[24-27]	Not performed	N/A
Adenoviral	Yes	[28-30]	Preclinical	[28-30]
AAV	Yes	[31, 32]	Not performed	N/A

The status of development refers to research which has already been performed. AAV: Adeno-associated virus; N/A: Not Applicable



**Table 2 Overview of the cell therapy based treatment strategies for IBD as discussed in this chapter**

Cell type	Generated from	Reference for generating cell type	Status of development	Reference for status of development
Treg	Peripheral blood	[21, 101, 132-134]	Preclinical	[93, 96]
Tr1	Peripheral blood	[93, 135]	Phase I Clinical Trial	(Unpublished data, UEGW 2010-ABS-577)
MSCs	Adipose tissue/ bone marrow	[17, 22, 23]	Phase I Clinical Trial	[17, 22, 23]

The status of development refers to research which has already been performed. MSCs: Mesenchymal stromal cells; Treg: CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> regulatory T cell; Tr1: Type 1 regulatory T cell; UEGW: United Gastroenterology Week.

**Table 3 Overview of the combined gene and cell therapy based treatment strategies for IBD as discussed in this chapter**

Applied Strategy	Viral vector used	Reference for viral vector used	Status of development	Reference for status of development
<i>Ex vivo</i> generated Treg/Tr1	Retrovirus	[113, 115]	Preclinical	[113, 115]
T cell receptor transgenic Treg	Retrovirus	[116]	Preclinical	[116]

The status of development refers to research which has already been performed. Treg: CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> regulatory T cell; Tr1: Type 1 regulatory T cell.

### *Regulatory T lymphocytes*

The immune system contains a population of T cells, called regulatory T lymphocytes that are specialized in immune suppression [81, 82]. Low level autoimmunity may occur in the intestine as a result of the presence of the microbial flora or auto-reactive T cells. Regulatory T lymphocytes are generated in the mesenteric lymph nodes and subsequently migrate and expand in the gut [83], thereby preventing progress to chronic autoimmune disease [84, 85]. These cells are able to suppress an immune response both by cell contact (*e.g.* killing or functional modulation of antigen presenting cells or effector T cells) and soluble factor dependent mechanisms (*e.g.* secretion of immunosuppressive cytokines or deprivation of cytokines necessary for the expansion/survival of responder T cells) [86, 87]. Antigen specific regulatory T lymphocytes have been described as having more therapeutic efficacy than polyclonal regulatory T cells [88-90]. In IBD the antigenic targets are not totally defined [6] and cell therapy would have to be restricted to polyclonal cells. However, regulatory T lymphocytes do not need to be antigen specific in order to suppress immune responses as a result of *bystander suppression* and *infectious tolerance* [91, 92]. These are general mechanism through which regulatory T lymphocytes are able to create a regulatory milieu *in vivo* [91, 92] and could introduce tolerance in IBD.

Regulatory T lymphocytes were shown to be effective in both the cure and the prevention of experimental colitis in multiple animal models [93-96]. It was shown for example that transfer of regulatory T lymphocytes into mice with colitis led to resolution of the lamina propria infiltrate in the intestine and re-appearance of normal intestinal architecture [96]. Therefore regulatory T lymphocytes could be used as a therapeutic tool in IBD where their homeostasis is disturbed [97, 98].

Among the different T cells with suppressive activity the CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> regulatory T cell (Treg) [82] and the type 1 regulatory T cell (Tr1) [81] subsets are the most well-defined so far. The Tr1 is typically characterized based on the cytokine production profile (IL-10<sup>high</sup>) [81] and Treg by the expression of the transcription factor Forkhead box p3 (FOXP3

in humans/Foxp3 in mice), which appears to function as the master regulator in their development and function [99, 100].

Treg and Tr1 have the potential to prevent or cure colitis [93-96] and a favourable safety profile in phase I clinical trials was demonstrated [21, 101]. Tr1 were shown to have a preliminary efficacy signal in patients in a phase I clinical trial for refractory CD (*unpublished data*, UEGW 2010-ABS-577). Currently the efficacy of Treg and Tr1 based cell therapy awaits further confirmation from phase II/III clinical trials but overall these results emphasize that both Treg and Tr1 are promising tools for therapeutic applications in IBD.

### *Mesenchymal stromal cells*

Mesenchymal stromal cells (MSCs) are non-haematopoietic stromal cells exhibiting multi-lineage differentiation capacity and the ability to mediate immunosuppressive and anti-inflammatory effects [102-105]. The exact mechanism by which MSCs suppress the immune system is not fully understood. It is known however that MSCs have immunosuppressive features in common with regulatory T lymphocytes, as for example preventing the maturation of antigen presenting cells (APCs) [102] or physically hinder T cells from contacting APCs [103]. Additionally it was shown that FOXP3 expression confers a greater immunosuppressive potential to MSCs [106].

MSCs can be isolated from various tissues [107-109] and were shown to ameliorate experimental colitis [110, 111]. In humans, MSCs obtained from adipose tissue induced healing in perianal fistulas in patients with CD [17]. Furthermore, in a phase I clinical trial, administration of autologous bone marrow-derived MSCs was shown to be safe and feasible in the treatment of refractory CD [22]. Additionally it was demonstrated that *ex vivo* expanded autologous bone marrow-derived MSCs are a safe and feasible approach for intrafistular injections in patients with CD [23]. These results [17, 22, 23] show potential and await further verification in phase II/III clinical trials which are currently being conducted.

## Can gene and cell therapy overlap in the treatment of IBD?

The phenotype and function of lymphocytes can be modified using viral vectors, to create tools for a cell therapy approach in the treatment of autoimmune, and inflammatory disorders [112] and by consequent IBD [113-116]. It was shown that the *ex vivo* targeting of spleen derived CD4<sup>+</sup> T cells by a retroviral vector expressing IL-10 was able to generate Tr1 that prevented colitis in an experimental model of IBD [113].

Using the same approach, fully functional Treg were generated by transduction of T cells with a Foxp3 transgene. These cells were able to suppress autoimmunity and graft rejection *in vivo* [89, 115, 117, 118]. Furthermore, Hori and colleagues showed that the *in vitro* generated Treg prevented colitis in a mice model of IBD [115].

Additionally it was demonstrated that Treg can be efficiently transduced to express functional antigen-specific receptors [116]. Adoptive transfer of small numbers of these transduced Treg was associated with antigen-specific, dose-dependent amelioration of experimental colitis in mice (**Table 3**).

### General considerations relevant for IBD

The route of therapeutic delivery is important when considering gene or cell therapy in relation with IBD. The mucus lining in the intestine is a barrier for gene transfer via the luminal route [119] and the clearance of viral particles by the liver represents a problem for the systemic delivery [120]. Nonetheless, as described above it has been shown that transduction via these routes is possible and that long term transgene expression can be achieved. Possible viral vectors, as for example the AAV based viral vectors seem to have the potential to transduce the GI tract, but the optimization of gene targeting to the gut needs to be further explored. This could be achieved by testing different AAV serotypes [121] or modifying the AAV capsid [122]. A promising method is the so called *DNA shuffling* method. DNA shuffling is a method whereby genes are rearranged to form hybrid genes with new properties [123]. This can

be done using polymerase chain reactions, as described by J. Cohen [123]. If this approach is used for genes encoding AAV capsid proteins it can allow for the development of cell type specific vectors [124] and thereby shows promise for creating a gut targeting AAV. Furthermore chemical redirection of the AAV capsid shows potential in engineering vectors with novel tissue tropisms [125]. Chemical engineering refers to a process whereby the amino acids on the surface of the AAV capsid are changed [125]. This method has proven to be successful in redirecting the AAV from liver to skeletal and cardiac muscle following systemic administration in mice [125] and could therefore have potential in directing the AAV to the GI tract.

Due to the presence of stem cells in the intestinal crypts [126] the gut is suggested to be an interesting target for therapeutic gene transfer. Every crypt in the intestine contains four to six independent stem cells [126]. Stem cells are believed to divide very rarely [126]. Therefore, these cells could have the potential to permit long term, stable transgene expression after transduction. It has been shown that intestinal stem cells can be transduced *in vitro* using a retroviral vector [127]. Long term transgene expression observed in the gut after AAV vector delivery in mice suggests that transduction of intestinal stem cells is possible *in vivo* [31, 32].

## Future prospects

The knowledge about the pathophysiology of IBD is growing and it has become clear that significant genetic as well as phenotypic heterogeneity exists within both CD and UC [128]. These findings offer opportunities for more specifically targeted interventions. Gene or cell therapy based treatment strategies can be adapted and exclusively targeted at certain subgroups within the IBD patient population with characterized genetic defects linked to the impairment of their gut physiology.

Strategies to optimize gene therapy approaches include the use of a tissue specific promoter enabling site specific expression of a transgene. Recently, gut specific promoters have been described [129-131]. The A33-antigen promoter

for example strictly depends on the presence of the intestine-specific transcription factor Cdx1 which is essential for the unique intestinal expression pattern of the A33-antigen gene [129, 131]. Therefore this promoter is a promising candidate to induce intestine specific expression of a transgene [131].

## **Concluding remarks**

IBD are a group of chronic inflammatory disorders most commonly affecting young adults and currently there is no curative treatment available. A gene therapy approach for the local expression of therapeutic agents in the gut or a cell therapy approach using regulatory T cells or mesenchymal stromal cells may offer an alternative treatment for gastrointestinal inflammation. Both gene and cell therapy approaches have shown promising results in preclinical models of IBD. Cell therapy approaches have been translated to a clinic setting and currently phase II/III clinical trials for the treatment of refractory CD are in progress. Concerning gene therapy, further development of viral vectors delivery to the gut as well as long term efficacy are still needed, but pre-clinical data are promising.

Overall, both gene and cell therapy have the potential to become important players in the next generation of therapeutic agents that will be aimed at unmet medical needs as those that exist in IBD.

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