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# Current Status of Gene Delivery and Gene Therapy in Lacrimal Gland using Viral Vectors

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## Abstract

Gene delivery is one of the biggest challenges in the field of gene therapy. It involves the efficient transfer of transgenes into somatic cells for therapeutic purposes. A few major drawbacks in gene delivery include inefficient gene transfer and lack of sustained transgene expression. However, the classical method of using viral vectors for gene transfer has circumvented some of these issues. Several kinds of viruses, including retrovirus, adenovirus, adeno-associated virus, and herpes simplex virus, have been manipulated for use in gene transfer and gene therapy applications. The transfer of genetic material into lacrimal epithelial cells and tissues, both in vitro and in vivo, has been critical for the study of tear secretory mechanisms and autoimmunity of the lacrimal gland. These studies will help in the development of therapeutic interventions for autoimmune disorders such as Sjögren's syndrome and dry eye syndromes which are associated with lacrimal dysfunction. These studies are also critical for future endeavors which utilize the lacrimal gland as a reservoir for the production of therapeutic factors which can be released in tears, providing treatment for diseases of the cornea and posterior segment. This review will discuss the developments related to gene delivery and gene therapy in the lacrimal gland using several viral vector systems.

### Keywords

Viral vectors; Lacrimal gland; Gene therapy; Gene delivery; Sjögren's syndrome; Exocytosis

### Abbreviations

Ad,adenovirus; IL,interleukin; IFN,interferon; TNF,tumor necrosis factor; β-gal,β-galactosidase

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## 1. Introduction

Gene transfer and gene therapy in principle involve the development of efficient means for delivering gene(s) to the nuclei of somatic cells to replace a defective gene with a functionally normal one. This approach offers the hope of cures for various genetic and autoimmune diseases, including sickle cell disease [1], X-linked severe combined immunodeficiency disorder [2], Sjögren's syndrome [3], rheumatoid arthritis [4], type I diabetes [5], multiple sclerosis [6], cystic fibrosis [7], and hemophilia [8]. Additionally, it also provides hope for long-term therapeutic benefits in contrast to the transient relief provided by conventional drug therapy.

One of the basic methods of gene transfer is to modify viruses into genetic shuttles which will deliver the gene of interest into the target cells. Much progress in gene delivery and therapy has been achieved with viral vectors due to their high transduction efficiency in cells in vivo. Viral vector systems, including retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses, currently offer the best choice for efficient gene delivery [9,10]. The most commonly used DNA viral vectors are adenoviruses (Ad) and adeno-associated viruses. These vectors have been extensively used in gene therapy of the eye [11]. They have been successfully used to mediate gene transfer for ocular neovascularization [12,13], age-related macular degeneration [14], uveitis [15], diabetic retinopathy [16] corneal wound healing [17] and experimental autoimmune lacrimal gland disease [18,19]. In this review, we discuss the viral gene delivery approaches performed in the lacrimal gland to understand and modulate lacrimal gland functions for therapeutic purposes.

## 2. Gene delivery by viral vectors in primary cultures of lacrimal gland tissue

Genes can also be introduced into cells to produce beneficial substances for therapeutic purposes. The lacrimal gland is responsible for the production and regulated release of tear proteins into ocular surface fluid. These proteins include nutrient factors which nurture the cornea, as well as factors which protect the ocular surface from pathogens. Delivery of genes for necessary products the lacrimal gland would chronically secrete could be a potential therapeutic approach for patients suffering from various diseases of the eye, including, glaucoma, dry eye, keratitis, and uveitis. Currently, these diseases require long-term administration of therapeutic preparations in the form of topical eye drops and eye ointments. Although this approach has the advantage in minimizing systemic side effects, the major drawback is the short residence time of the medication on the eye and hence the need for frequent applications which in some cases, could become a functional disability that would affect the patient's quality of life. Many studies on gene therapy to the eye have been reported but the delivery and expression of foreign genes in the lacrimal gland for therapeutic purposes has not been extensively explored. As well, gene therapy to the lacrimal gland to specifically treat disorders of the lacrimal gland is of great interest, considering the large numbers of people who suffer from severe dry eye syndromes including the autoimmune disease, Sjögren's syndrome.

Recently, Banin et al. demonstrated the first feasibility study of gene transfer ex vivo in rat lacrimal gland tissue fragments using viral vectors [20] such as vaccinia, Ad, and herpes simplex. The results showed that all the vectors were capable of delivering a reporter gene (β-galactosidase or β-gal) to the lacrimal gland but with different transduction efficiencies and tropisms. After 7 days of modified lacrimal gland fragment organ culture technique [21], β-gal expression was observed in 77% of tissue fragments exposed to vaccinia vector, 41% of fragments exposed to Ad and 13% of fragments exposed to herpes vectors. Upon histologic examination, vector-specific expression patterns of reporter genes were observed. The vaccinia vector preferentially delivered the β-gal gene to the lacrimal duct cells and acini while Ad

vectors expressed β-gal mainly within the myoepithelial cells surrounding the lacrimal acini. It was also noted that β-gal expression in acinar cells transduced with Ad vectors was accompanied by degradation of these cells, possibly due to vector toxicity.

## 3. Ad-mediated gene therapy

### 3.1 Dry eye syndrome

Dry eye has been defined as "a disorder of the tear film due to tear deficiency or excessive evaporation that causes damage to the interpalpebral ocular surface and is associated with symptoms of discomfort" [22]. Systemic autoimmune diseases like Sjögren's syndrome, rheumatoid arthritis, lupus erythematosus, and thyroiditis are considered as the major initiating factors in some kinds of dry eye disease. One of the most severe forms of dry eye is found in patients with Sjögren's syndrome, an inflammatory autoimmune disorder characterized by lymphocytic infiltration and affecting approximately 4% of the population in the United States [23]. The immune-related lacrimal insufficiency reduces the quality and quantity of tear production below the level required to maintain a healthy and comfortable ocular surface. It is believed that a combination of immunologic, genetic, hormonal and environmental factors play a crucial role in the development of autoimmunity in the lacrimal gland [24,25]. The inflammatory infiltrates produce toxic factors that act as immune mediators, resulting in reduced secretory function caused by secretory tissue atrophy and dysfunction of the surviving tissue [26,27]. There is evidence that these lymphocytic infiltrates produce proinflammatory cytokines, including interleukin (IL)-1, -6, -12, and -18; interferon (IFN)-γ; and tumor necrosis factor (TNF)-α [28–32]. However, in addition to these proinflammatory cytokines, anti-inflammatory cytokines including IL-10, transforming growth factor (TGF)-β and IL-4 have also been detected [33,34]. Numerous studies indicate that regulation of anti-inflammatory cytokines (IL-10, IL- 4) or specific inhibitors of proinflammatory cytokines (sTNFR, IL-ra, anti-TNF-α) can play an important immunoregulatory role in inhibiting a disease process [35,36]. Among these cytokines, the local up-regulation of IL-10 and/or inhibition of TNF-α binding to target cells have received increased attention as promising therapeutic potentials against an immunopathological disease [37]

### 3.2 Autoimmune dacryoadenitis in animal models

The pathogenesis of dry eye syndrome has been difficult to fully elucidate due to the limited availability of lacrimal gland tissue samples from patients with Sjögren's syndrome, thereby prompting the need for animal models of this disease. Murine models of autoimmune disease resembling secondary Sjögren's syndrome have been established [38,39]. An induced lacrimal gland disease, autoimmune dacryoadenitis, has also been induced in mice and rats which to a certain extent resembles primary Sjögren's syndrome in humans [40]. However, compared to studies of rodent models with induced lacrimal disease, fewer studies have been conducted on larger animals to effectively evaluate the efficiency of ocular therapies. Keratoconjunctivitis sicca has been studied in dogs [41] however, a spontaneous disease in dogs is challenging and they also require expensive maintenance care. Hence a rabbit model developed in this institute [42,43] was pursued for dry eye disease and experimental validation of therapies as an alternative option.

Adenoviruses are the most common cause of acute viral infections of the cornea [44]. A rabbit model was used to determine the effects of an ocular Ad virus infection on lacrimal gland histopathology. Studies revealed that an Ad infection of the cornea resulted in increased numbers of $RTLA^+$ and $CD18^+$ cells and increased expression of MHC class II molecules in the lacrimal gland. However, Ad was not detected in the lacrimal tissue explanted 21 days after post-inoculation and it was unclear whether the changes were caused by the inoculated virus or through an aberrant expression of Class II histocompatibility antigens which involved T cell

activation that led to autoimmunity in the lacrimal gland [44,45]. The possibilities suggested by these early studies for T-cell modulation of lacrimal gland function were instrumental in the establishment of a rabbit model for autoimmune dacryoadenitis which we have subsequently used for studying the mechanism of Sjögren's syndrome and for identifying and evaluating therapeutic interventions. Autoimmune dacryoadenitis in rabbits was established through autologous mixed cell reactions that involved incubation of purified acinar cells prepared from one surgically excised inferior lacrimal gland and peripheral blood lymphocytes from the same animal [44]. Disease is then induced by injecting the donor rabbit's remaining inferior lacrimal gland with the activated peripheral blood lymphocytes after 5 days of co-culture with the autologous acinar cells in the mixed cell reaction. After 2 weeks, abundant periductal foci of lymphocytes resembling the autoimmune lesions characteristic of Sjogren's syndrome are seen [43]. The induced adenitis is accompanied by lacrimal gland dysfunction characterized by reduced tear production, reduced tear stability, abnormal corneal staining and an increased presence of CD4+ cells in the gland; features that mimic the clinical manifestations of Sjögren's syndrome [46]. The severity of the induced disease shows no signs of abatement and increases with time over a period of 6 months after disease induction (unpublished data). Disease in the rabbit model has also been established through remote site injection of the activated lymphocytes (unpublished data).

### 3.3 Prophylactic effect of IL-10 in vivo

IL-10 is a pleiotropic cytokine produced by Th-2 type T cells, B cells, monocytes and macrophages and is considered an immunoregulatory cytokine because of its inhibitory effects on the expression of a large spectrum of proinflammatory cyokines (such as chemokines, MHC-II molecules, costimulatory molecules and other inflammatory mediators [47–49]. IL-10 was also found to suppress antigen– stimulated proliferation of murine Th-1 cells [50,51] suggesting that endogenous IL-10 down regulates cell-mediated immune responses in the development of autoimmune diseases. Its effector functions include induction of a shift of T-cell cytokine expression from a Th1 to a Th2 profile [51], and attenuation of the production of pro-inflammatory cytokines by macrophages [52–54] and polymorphonuclear neutrophils [55]. IL-10 has proven to be useful in several preclinical models of autoimmune diseases [56,57], but its administration is difficult as it needs multiple injections. IL-10 gene therapy using viral vectors stands out as an alternative method. Several reports have established that IL-10 gene therapy inhibits autoimmune diseases [58,59]. To determine whether the expression of the interleukin-10 gene suppressed lymphocytic proliferation in an in vitro autologous mixed cell reaction [37], lacrimal gland acinar epithelial cells were transduced with Ad vector encoding viral IL- 10 (vIL-10) (Fig.1). The transduction of lacrimal epithelial cells with IL-10 diminished lymphocytic proliferation in the mixed cell reaction. IL-10 product was transiently expressed with maximal production during the first week, after which detectable amounts declined with each successive week. Using the rabbit model of induced dacryoadenitis we reported that Ad-mediated gene transfer and expression of viral IL-10 resulted in prophylaxis, with diminution of lacrimal gland immunopathology and ocular surface disease [19]. The transduced vIL-10 encoded by the Epstein-Barr virus shares 84% sequence homology with human IL-10 and mimics several of its immunosuppressive activities. However, unlike human IL-10, it lacks the stimulatory effects on natural killer cells and cytotoxic T cells [60,61]. Ad-mediated IL-10 gene transfer to the lacrimal gland of rabbits with induced disease resulted in transient expression and secretion of vIL-10 in tears for less than 2 weeks. Short-lived Admediated vIL-10 expression has also been reported by De Kozak et al in treatment of experimental autoimmune uveoretinitis induced in mice and rats [62]. In that study, expression of the vIL-10 gene was associated with significantly decreased size and number of immune infiltrates, including $CD4^+$, $CD18^+$, $RTLA^+$ cells and MHC class II molecule expressing cells. However, a significant increase in the number of $CD8^+$ cells was observed [19]. These effects are in agreement with published data showing that vIL-10 exerts its immunosuppressive

properties by down-regulating the MHC class II molecule and proinflammatory cytokine expression without stimulating cytotoxic T cells [37,46,63]. Prophylactic treatment of rabbits with vIL-10 before injection of activated autoreactive lymphocytes protected tear production and tear stability compared to animals with induced disease that did not receive Ad encoding vIL-10 [19].

### 3.4 Prophylactic effect of TNF-α in vivo

TNF-α, an interesting targets of immunotherapy, has been reported to play an important role in the pathogenesis of several immune mediated disorders, including rheumatoid arthritis [64,65] and Sjögren's syndrome [66]. It is a pleiotropic inflammatory cytokine that promotes mononuclear cell infiltration in glands by inducing the secretion of several proinflammatory cytokines, expression of endothelial adhesion molecules, release matrix metalloproteinases from glandular epithelial cells, all of which promote the influx of mononuclear cells [67]. Also, TNF-α secretion by infiltrating T cells has been associated with apoptosis of glandular epithelial cells [68]. Since then many innovative strategies targeting TNF-α for the treatment of autoimmune diseases have been established. Inhibition of TNF-α has been used as an effective therapy in patients with rheumatoid arthritis and Crohn's disease [69–71]. Neutralization of TNF-α was initially achieved using chimeric monoclonal antibodies in patients with rheumatoid arthritis. Kolls et al. reported the construction of an Ad vector encoding a chimeric TNF inhibitor, TNFRIp55-Ig [72]. The expression of TNFRIp55-Ig inhibitor gene suppressed lymphocytic proliferation in an in vitro autologous mixed cell reaction when the lacrimal epithelial cells were transduced with an Ad vector [37]. Expression of the AdTNFRIp55-Ig gene has been reported to successfully block the effect of TNF-α in several animal models [72–74]. The transgene product, a fusion protein formed by joining the human 55-kDa TNF receptor extracellular domain to a mouse IgG heavy chain, binds TNF by engaging two of its three receptor sites. Using this Ad vector we demonstrated a gene therapy treatment of rabbit lacrimal glands with established autoimmune dacryoadenitis [18,75]. The expression of TNFRIp55-Ig resulted in improvement of clinical features, which included increased basal tear production, increased tear stability and a reduction of corneal surface defects. These results suggest that the TNF inhibition altered the spectrum of cytokines in the local infiltrates to one that did not impair tear secretion. The therapeutic effect also reduced the intensity of immune cell infiltration in the gland (Fig.2).

## 4. Ad-mediated gene transfer: In the study of lacrimal gland physiology

Since the initial reports of Ad-mediated expression of β-gal in lacrimal gland, we and others have subsequently optimized Ad-mediated gene transfer into lacrimal acini in primary culture, reaching a transduction efficiency of 80–90% in many cases with a relatively low multiplicity of infection (1–5). This achievement has been invaluable in the exploration of the function of different effectors in the lacrimal gland, suggesting that aspects of adenoviral uptake are unusually efficient in lacrimal acinar cells. Highlights from such studies are described below.

### 4.1 Androgen responses in primary cultures of lacrimal epithelial cells

Sjögren's syndrome is most prevalent in women, with a gender ratio of 9:1. This huge gender difference may be partially attributable to the role that androgen-regulated transcription plays in lacrimal gland physiology [76–78]. Furthermore, androgens have been shown to suppress the inflammation in lacrimal glands of mouse models of Sjögren's syndrome [79]. Studies also demonstrated the expression of various endogenous proteins including secretory component (SC), cystatin-related protein 1 and the C3 component of the prostatic binding protein in the primary lacrimal gland epithelial cell cultures were androgen responsive in male rats [80–82]. Vanaken et al. demonstrated androgen regulation in primary cultures of rat lacrimal gland through the use of recombinant adenoviral vectors [83]. The rat lacrimal gland primary cultures

under androgen control were used as a homologous test system for tissue-specific transcription studies. By the use of two recombinant adenoviral vectors containing genomic fragments of the SC gene, they demonstrated the functionality of the *sc* promoter as well as its androgen regulation in this culture system.

### 4.2 Role of cytoplasmic dynein in apical secretory traffic in lacrimal acini

Conventional cytoplasmic dynein is a large multisubunit complex of ~1400 kDa consisting of two heavy chains and several intermediate and light chains [84]. The dynein heavy chains contains sites for MT binding and ATP hydrolysis, and is responsible for generation of mechanochemical force. Directed transport of vesicles by dynein requires a multiprotein complex called the dynactin complex [85]. Ad vectors with dynamitin constructs (Ad-Dynt) were used to study the participation of dynein-driven vesicle transport in stimulated secretory traffic to the apical membrane in lacrimal acini [86]. It was revealed that a cholinegeric agonist, carbachol, induced microtubule-dependent recruitment of cytoplasmic dyein and the dynactin complex into the subapical region and was inhibited by Admediated overexpression of dynamitin, suggesting that dynein activity drives this recruitment. Overexpression of dynamitin depletes subapical stores of rab3D, a member of the rab family of small GTP binding proteins that participate in membrane trafficking in eukaryotic cells, in resting acini, suggesting that dynein may also maintain this secretory vesicle population at the apical membrane. These data implicate cytoplasmic dynein in stimulated traffic to the apical plasma membrane in these secretory epithelial cells [86].

### 4.3 Role of PKC-α and PKC-ε on apical exocytosis of lacrimal acini

Protein kinase C (PKC) plays a major role in cholinergic and $\alpha_1$-adrenergic-stimulated lacrimal gland protein secretion [87]. However, as PKC is a family of at least 10 different isozymes, its role is somewhat complicated. The lacrimal gland contains at least four PKC isoforms, -α, -δ, -ε, and –λ [88]. Using adenoviral-mediated expression of PKC effectors and/or modified forms of these proteins, the major roles of PKC-α and PKC-ε on the apical exocytosis of lacrimal acini have been elucidated [89,90]. Hodges et al. demonstrated that PKC-α can be overexpressed using an adenoviral vector carrying the myristoylated PKC-α α constructs (myr-PKC α) in rat lacrimal gland acini [89]. Results showed that the overexpression of a constitutively active form of PKC-α increased basal protein secretion without altering $Ca^{2+}$ handling in the lacrimal gland. The increase was dependent on the concentration of Ad used and therefore the amount of PKC-α expressed, implying that secretion can be stimulated by circumventing the release of neurotransmitters and instead through the activation of their receptors.

PKC-ε was first identified in association with actin as an effector of exocytosis in hippocampal neurons [91]. PKC-ε is known to influence cell adhesion and motility as it is associated with actin cytoskeleton reorganization [92–94]. However, little is known about the contribution of PKC-ε in regulation of actin filament remodeling in acinar exocytosis in epithelial cells. To investigate the involvement of apical actin remodeling in carbachol-stimulated exocytosis in reconstituted rabbit lacrimal acinar cells, Ad vectors with a dominant-negative (DN) PKC-ε constructs were used [90]. It was found that carbachol-stimulation increases PKC-ε association with apical actin filaments and actin-coated structures in lacrimal acini. To inhibit PKC-ε activity and probe its functional role in exocytosis, lacrimal acini were transduced at high efficiency with Ad vectors carrying the DN-PKC-ε constructs. Overexpression of PKC- ε resulted in profound changes in apical and basolateral actin filament organization in parallel with inhibition of the carbachol-stimulated secretion of protein and β- hexosaminidase. These data confirm the role of PKC-ε as an actin-binding protein recruited transiently to apical actin filaments and actin-coated structures, possibly, representing fusion intermediates, in carbachol-stimulated lacrimal acini. It was further established that its inhibition, through overexpression

of DN-PKC-ε, stabilized actin-coated structures and correspondingly inhibited stimulated exocytosis of secretory products at the apical plasma membrane.

### 4.4 Role of actin and non-muscle myosin II in apical exocytosis of tear proteins

Green fluorescent protein (GFP)-actin has been used to measure the dynamics of actin in live cells [95–98]. Ad vectors encoding GFP-actin have been utilized to label the actin filament of lacrimal acini to obtain qualitative and quantitative measures of its dynamics [99]. The GFP-labeled apical actin filament array in lacrimal acini showed rapid carbachol-induced remodeling of the sub-apical actin network. Also, additional functional and morphological analyses of lacrimal acini exposed to the general myosin ATPase inhibitor, 2,3-butanedione monoxime and the more selective myosin light chain kinase inhibitor, demonstrated that the filamentous actin array beneath the apical plasma membrane of stimulated lacrimal acini participates actively in exocytosis, in conjunction with nonmuscle myosin II.

## 5. Ad-mediated gene transfer: Modulation of secretory functions by Adv capsids in lacrimal epithelia

Although replication-deficient Ad vectors are leading candidates for gene therapy, there is a paucity of data on the cellular effects associated with Ad binding, internalization and trafficking to the nucleus, particularly in epithelial cells that represent normal targets for Ad infection. To study these effects, lacrimal gland acinar cells were exposed to replication-defective Ad serotype 5 (Ad5) containing a reporter gene (green fluorescent protein (GFP) or β-galactosidase (LacZ) or UV-inactivated Ad virus in vitro [100]. The organization and function of the lacrimal acinar secretory pathway in the reconstituted acinus-like structures were investigated. Exposure of lacrimal acini to replication-defective Ad constructs at high transduction efficiency (>80%) with a multiplicity of infection (MOI) of 5 for 16–18 h elicited a marked dispersal of rab3D, from its normally apical enrichment, a change independent of altered rab3D expression or membrane association. Rab3D is associated with the large pool of mature secretory vesicles beneath the apical plasma membrane in lacrimal gland as well as pancreas and parotid gland [86,101,102]. The dispersal of apical rab3D occurred independently of effects on the cytoskeleton or other membrane compartments or decreased protein synthesis. Stimulation of the Ad-transduced cells with carbachol resulted in a significant decrease in the release of protein and the secretory product, β-hexosaminidase. Furthermore, exposure of lacrimal acini to UV-inactivated Ad also depleted rab3D-enriched secretory vesicles in parallel with the inhibition of carbachol-stimulated release of protein and β-hexosaminidase, though the extent of the dispersal and inhibition, respectively, by UV-inactivated virus was slightly less than that caused by mock UV-inactivated Ad. However, the effects on acinar secretory functions were directly related to the duration of exposure to the Ad capsid, as lacrimal acini exposed to Ad constructs for a period of 4 h at MOI of 5 and ~80% transduction efficiency caused a 50% reduction in apical rab3D labeling.

Viral capsid proteins, exterior proteins associated with the protein-rich coat around the viral core, have been utilized for second- and third- generation gene delivery systems [103–106]. These systems have eliminated the presence of a complete viral vector particle but depend on the use of viral capsid proteins to interact with the host factors to facilitate entry of associated DNA. To determine the cellular effects of capsid proteins on lacrimal acinar cells, isolated Ad penton protein and knob protein, the region of the fiber protein known to interact with the cellular receptor, CAR, in the absence of the rest of the virus were investigated [107]. These proteins which have been used in non-viral gene transfer technique to enhance gene transfer to HeLa and 293 cells reflect the established infection pathway of Ad vectors [105]. It was found that treatment of lacrimal acini with recombinant Ad penton protein resulted in an almost complete loss of rab3D-enriched secretory vesicles [107]. The process occurs in parallel with

an uncoupling of the stimulated secretory response, resulting in significantly increased basal protein release and significantly decreased carbachol-stimulated protein release. Knob protein treatment did not elicit significant change in the basal and carbachol-stimulated release of bulk protein. Additionally, penton protein caused additional cytoskeletal changes over and above the effects elicited by Ad alone including loss of the abundant apical actin network and bundling/disorganization of microtubules. However, knob protein was not found to elicit detectable microtubule organization. These results suggest that the penton protein, and not knob/fiber, is responsible for the deleterious changes in lacrimal gland function associated with chronic Ad exposure and that knob/fiber are better choices potentially for non-viral gene delivery into lacrimal acini.

## 6. Retroviral-mediated gene transfer: Immortalization of rabbit lacrimal gland epithelial cells

Immortalized cell lines have been extensively used in basic research as they offer the possibility of an inexhaustible supply of cells. Immortalized cell lines possessing morphological characteristics and physiological functions similar to that of primary cells can serve as models of animal and human tissues. Many different types of immortalized cell lines have been established over the past few years through various methods and some of them have even been successfully substituted for primary cells in many bioartificial organ systems [108–111]. An immortalized lacrimal epithelial cell line will be of great value to study the intracellular signaling pathways and lacrimal gland-associated gene expression studies. Also, a successful immortalized lacrimal epithelial cell line could serve as a cellular component for the proposed bioartificial lacrimal gland device which we are attempting to create in our laboratory [112].

Earlier attempts to culture and propagate primary lacrimal gland cells for extended periods of time have met with limited success [113–115]. Nguyen et al. were the first to establish an immortalized lacrimal epithelial cell line in a rabbit model using an immortalizing amphotropic retroviral vector containing the E6 and E7 genes of the human papillomavirus by injecting the retroviral vector into the orbital lacrimal glands of normal New Zealand White rabbits [116]. Two days after injection, cells were isolated from the lacrimal glands and were plated onto Matrigel®-coated culture plates. The cultured cells flattened out and grew in a monolayer typical of epithelial cells with a cobblestone appearance and this morphology was retained even at higher passages (p36-58). Ultrastructurally, the immortalized cells showed numerous interdigitating villi in the intercellular spaces and components of the cytoplasm such as intermediate filaments were observed. They retained many characteristics of primary lacrimal gland epithelial cells, including production and secretory granules and pharmacological responses to stimulation with carbachol. The cells were also characterized by immunoreactivity and positive staining was attained for the transferrin receptor and transferrin. Another immortalized rabbit lacrimal epithelial cell line using the simian virus 40 T antigen (SV40) was also established. However, the morphological and physiological characteristics of this cell line have not been reported directly [117].

Studies have shown that generation of cell lines immortalized by the introduction of viral oncoproteins alone tend to lose the normal phenotype of primary cells over extended periods of time. Hence, the reliability of cell lines generated through ‘viral transformation’ is questionable. However, different types of human cells have been efficiently transduced, expanded and characterized through the expression of human telomerase reverse transcriptase (hTERT) [118–122]. Cells immortalized through this technique, maintain a stable genotype and retain critical phenotypic markers. Although, the expression of TERT might be species specific, Thomas et al. reported the first use of hTERT expression in experimental xenotransplantation using bovine adrenal cells immortalized by transducing plasmids encoded for hTERT and SV40 T antigen [123]. The immortalized cells transplanted into severe

combined immunodeficient (SCID) mice also formed functional tissue when replaced for the animals' own adrenal glands. However, no immortalized lacrimal epithelial cell line using hTERT has been reported so far.

## 7. Conclusions and future directions

Recombinant adenoviral vectors have been extensively evaluated in the lacrimal gland and are found to be the most efficient gene transfer technique available to transduce lacrimal epithelial cells [20,46,83,86]. However, one major drawback in using adenoviral vectors is that they provide an unstable transgene expression because they rarely integrate into the transduced cell's genome while vectors such as AAV provide prolonged transgene expression [124]. However, the limited capacity for gene delivery with respect to insert size severely limits the utility of AAV vectors. Also of concern is the demonstration of altered secretory functions associated with chronic exposure of the Ad penton protein. Retroviral vectors have been extensively used for their high efficacy; however, they do not have the ability to transduce non-dividing cells, a major limitation that has restricted their clinical use to gene therapy involving haematopoietic cells, rapidly dividing tumor cells, or ex vivo gene therapy of cells that can be propagated in cell culture [125]. Lentiviral vectors possess the ability to transduce non-dividing cells; however, safety concerns and the non-specific integration in the host cell's chromosome mitigate the usefulness of these vectors for gene delivery.

Although high transduction efficiency and a sustained transgene expression present clear advantages of a viral gene delivery system, the strong immune response that these vectors elicit may pose a safety threat to the patient and to the immediate surrounding environment [126, 127]. As immunogenicity arises from the viral capsid proteins that mediate gene delivery, engineering viral capsid proteins with altered ability to evoke immunity but with the same ability to manipulate cellular uptake would be necessary to overcome these issues. Also, the biological processes that underlie the cellular uptake and intracellular processing mechanisms need to be better understood in the lacrimal gland in particular to promote target-cell specificity of these vectors. Recent advances in improved vector design and vector purification have resulted in newer generations of viral vectors [128–130]. However, an ideal viral vector for tissue-specific transduction with regulated gene expression still remains elusive. Future progresses in vector engineering will create the means for effective gene delivery and will overcome the impediments to successful gene therapy.

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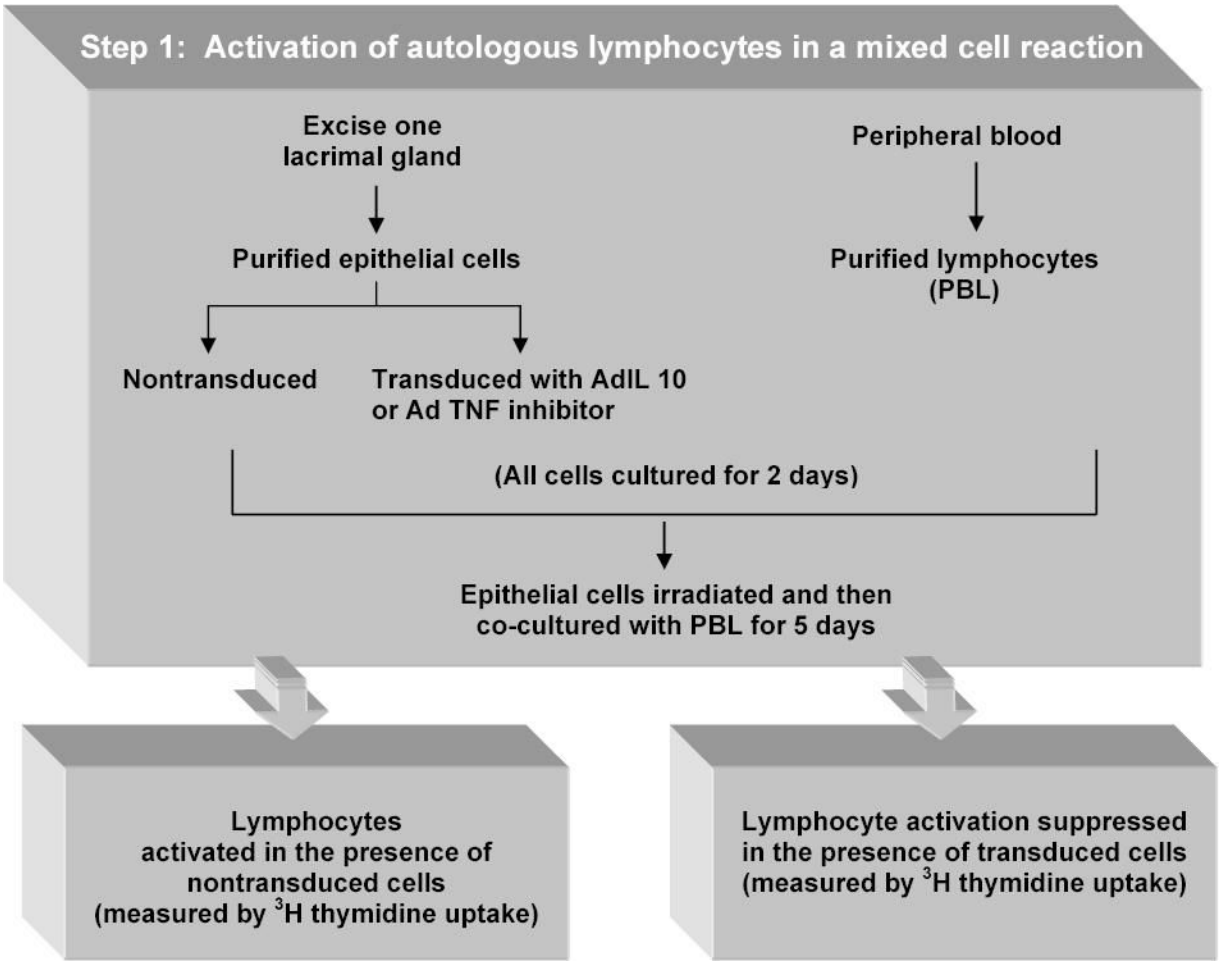


**Figure 1.**
In vitro evaluation of anti-inflammatory gene therapy. This schematic describes an in vitro method for activating autologous lymphocytes in a mixed cell reaction and suppression of the lymphocyte activation by gene transfer. [Ref: 37].

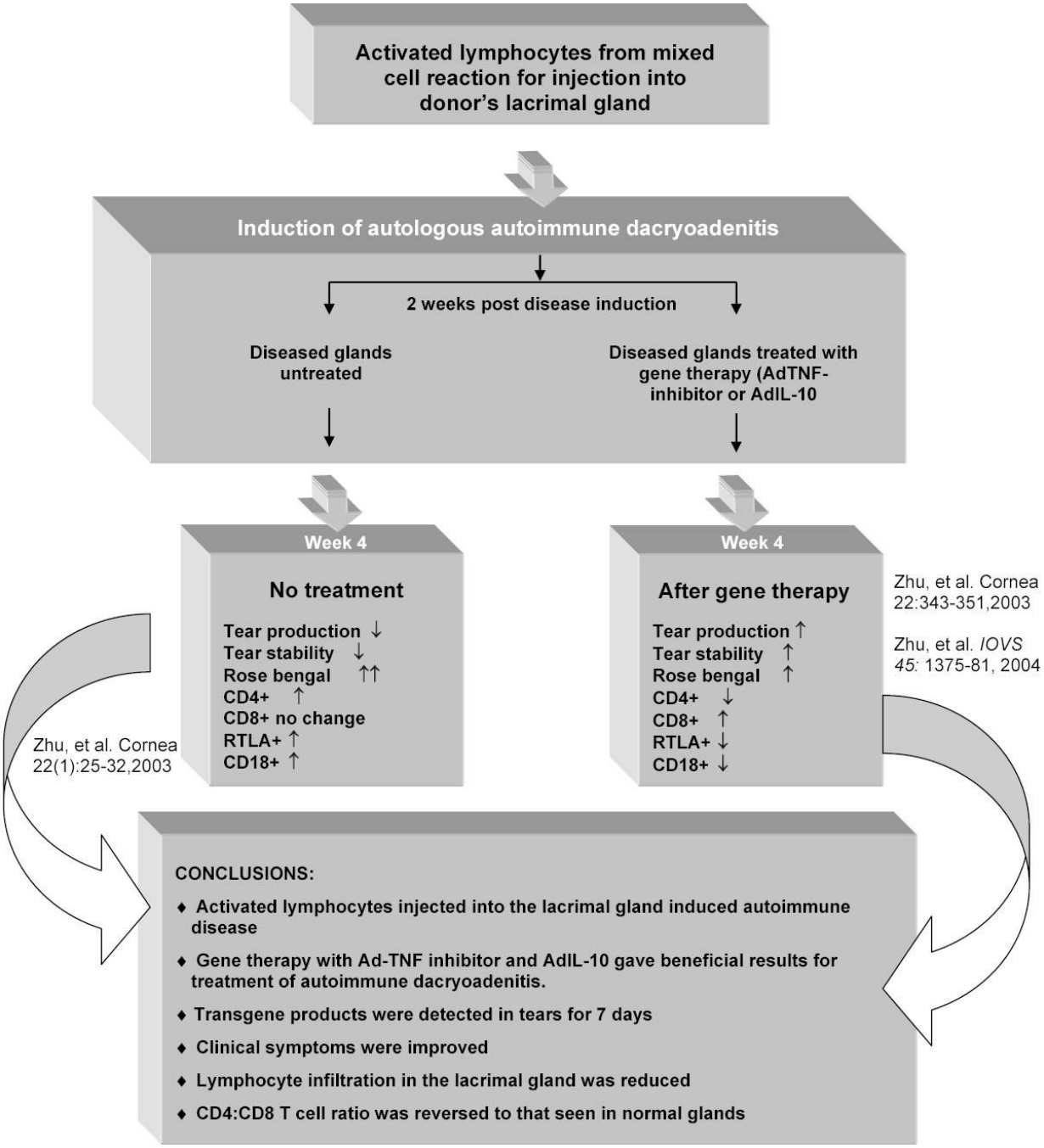


**Figure 2.**
This schematic describes the method used to induce autoimmune dacryoadenitis and the effect of gene therapy on the clinical symptoms and gland histopathology [Ref: 46,75,19].