Immune Reactions in Skin and Hair Follicle Gene Therapy

Robert M. Hoffman

AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111. E-mail: all@anticancer.com

An immunological response to non-native proteins is a problem that must be overcome for successful gene therapy. A study by Ghazizadeh *et al.* in this issue of *Molecular Therapy* describes the immune response to retrovirus-mediated *lacZ* gene transfer to the skin [1]. The study shows that both CD4 and CD8 immune responses need to be eliminated to permit persistent expression of this transgene. The investigators use of transgenic mice lacking both CD4 and CD8 function was critical for this study.

Following retroviral delivery of *lacZ* to the dorsal skin of immunocompetent B6 mice and mice immunodeficient for the immunoglobulin heavy chain (Igh$^{-/-}$), frozen tissues sections were prepared and analyzed by X-gal staining to detect transduced cells. Two weeks after transduction with the virus, no X-gal positive cells were observed in tissue sections obtained from the transduced skin of B6 or Igh$^{-/-}$ mice, indicating the loss of β-gal expression. In B6 mice, the investigators showed that both CD4$^+$ and CD8$^+$ cells had infiltrated the dermal and epidermal compartments of the transduced skin—suggesting the involvement of these cells in the rejection of the transgene product. While anti-β-gal antibodies were present in B6 mice, they were absent in Igh$^{-/-}$ mice, implicating the cellular arm of the immune system in the clearance of transduced skin cells.

In order to demonstrate directly a role of CD4$^+$ and CD8$^+$ cells in the immune response to retroviral gene therapy, the authors next transduced the dorsal skin of MHC$^{-/-}$ mice, which are deficient in both class I and class II MHC molecules and are thus depleted of both CD4 and CD8 T cells [2]. Tissue sections obtained from MHC$^{-/-}$ mice had a significant number of X-gal positive keratinocytes and fibroblasts. The findings suggested that inhibition of both CD4 and CD8 T cell-mediated responses may be necessary for long-term transgene expression in the skin.

In fact, persistent transgene expression was only seen in mice lacking expression of both class I and class II MHC molecules. The presence of either CD4 or CD8 T cells could eliminate transduced cells. Therefore, long-term cutaneous gene therapy may require development of strategies to interfere with activation or function of both components of the cellular immune response. Chronic pharmacological immunosuppression is one approach, but such a strategy could have undesirable side effects. Therefore, alternative approaches to skin gene therapy should be considered.

One solution can be found in the hair follicle. The hair follicle is a highly complex skin appendage consisting of six concentric cylinders formed by distinctive cell types producing highly specialized proteins [3]. The hair follicle continuously cycles through three major stages termed *anagen,* which is the hair-growth phase, an involution phase termed *catogen,* and a resting phase called *telogen* [3]. In 1995, we demonstrated that a liposome-entrapped *lacZ* reporter gene could be selectively targeted to hair matrix cells after topical application in mice [4]. Recently, this result was confirmed in both mouse and human skin grown on immunodeficient mice, with the additional observation that the early anagen follicle is the main target for topically-administered liposome-entrapped *lacZ* [5].

Low numbers of immune cells are found in the proximal hair follicle epithelium, and very few macrophages and Langerhans cells are seen in the adjacent dermal papilla [6]. The reduced numbers of T cells and Langerhans cells, and the virtual absence of MHC class I expression, all suggest that the anagen proximal hair follicle constitutes an area of immune privilege within the hair follicle immune system [6].

Sato *et al.* [7] showed that the sonic hedgehog (*shh*) gene, delivered with an adenovirus vector, stimulated anagen development and hair shaft production in the C57BL/6 mouse. Delivery of the *shh* gene apparently did not involve acute immune responses, as seen in retrovirus-mediated *lacZ* gene transfer observed by Ghazizadeh *et al.* [1]. *shh* mRNA expression and hair cycle enhancement could be seen for at least seven days. However, there may have been a delayed immune reaction, because the effects of *shh* were diminished by day 14.

Ex vivo approaches can also be applied to the hair follicle. Mouse anagen skin fragments can be grown in histoculture and genetically modified at high efficiency [8] with adenovirus-expressed green fluorescent protein (GFP). In the latter study, transduced skin fragments were subsequently grafted onto nude mice, and GFP was readily visualized in as many as 75% of the transplanted hair follicles. Hair follicle dermal sheath cells taken from the scalp of an adult human male could form new dermal papilli and hair follicles, and produced hair shafts when transplanted onto the skin of an unrelated female [9].

In vivo and *ex vivo* gene therapy of the hair follicle can also take advantage of the high manufacturing capacity of the hair follicle as a bioreactor to produce molecules ectopically, other than those involved directly in hair shaft production and pigmentation. In a recent study, it was

shown that the topical application of both naked and liposome-entrapped plasmid vectors expressing the hepatitis surface antigen (HbsAg), resulted in antigen-specific immune responses [10]. The effectiveness of this topical vaccine depended on the presence of normal hair follicles indicating targeting and manufacture of the gene product in the follicles. This result indicates that immune reactions may occur via hair follicle gene delivery. Such reactions may vary with the nature of the product of the transgene and whether the product is secreted systemically.

Many applications for both skin and hair follicle gene therapy can be imagined. Further study of the immune reaction to individual transgene products delivered in different vector contexts to the skin and hair follicle are needed in order to optimize both strategies of gene therapy.

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