



Perspective

Politically correct gene therapy? A “clean environment” improves gene delivery to the brain!

The Holy Grail of neurological gene therapy is achieving long term transgene expression in the brain. Many neurological disorders evolve over 10–40 years. Most current gene therapy strategies, particularly those utilising adenoviral vectors, offer only shorter-term expression, although in some cases more persistent expression has been detected. This limits gene therapy applications to those diseases where relatively restricted gene delivery could still make a major therapeutic impact. Cancer gene therapy requires only short-term gene expression to achieve tumour cell killing. Thus, in neurology, direct virus-mediated gene transfer is mostly used to treat brain tumours,^{1,2} whereas neurodegenerative disorders which require long term therapy, have currently mainly been approached by engineered cell transplantation.³

Adenoviral vectors have been used for gene transfer into the brain, achieving a generally restricted gene transfer, both anatomically and temporally, although some important exceptions have been reported.^{4,5} However, gene delivery to the brain is prolonged when compared with delivery to peripheral organs like the liver. While in the liver, transgene expression is completely eliminated within 2–3 weeks, low level expression persists in the brain for substantially longer periods of time. Nevertheless, viral genomes can still be found in the liver as late as 6 months post-virus administration, indicating that even in the liver the inflammatory and immune cells may

have limitations in physically eliminating cells containing adenoviral vector genomes.

One hypothesis to explain why transgene expression persists for longer in the brain is that inflammatory and immune cells do not clear virus genomes from the brain as efficiently as from other organs. This may be in part due to the well documented “immune-privilege” of the brain. However, it is immune presentation, rather than effector mechanisms, that are impaired during antigen administration to the brain. The absence of dendritic cells from the brain parenchyma, the lack of a well developed lymphatic drainage, and the blood brain barrier, are all thought to contribute to the impaired immune priming in the brain. Effector mechanisms, as evidenced by powerful delayed type hypersensitivity responses, are very active in the brain; a self-limited and dose-dependent inflammation is generally seen after a single administration of adenovirus to the striatum, however, peripheral restimulation results in large numbers of lymphocytes infiltrating the brain and “turning off” any remaining transgene expression.⁶

What are the factors that affect long term transgene expression in the brain following infections of recombinant adenoviruses? Different researchers have reported differing longevities of transgene expression. Reasons for such variations remain unclear. Animal strains, the transgene itself, or even surgical technique may influence the duration of transgene expression. Furthermore, Stevenson *et al.* (1997), have demonstrated that the brain ventricles and parenchyma constitute “immunologically” separate compartments: while virus injected into the ven-

Table 1

| | <i>Primed vs unprimed</i> | <i>Conventional vs SPF</i> | <i>C3H vs C57B1/6</i> |
|-----------------------------------|--|--|------------------------------------|
| T cells | Higher counts of T cells in primed mice; higher CD8 ⁺ :CD4 ⁺ cell ratio in primed mice; higher CD4 ⁺ :CD8 ⁺ ratio in unprimed mice | Not determined | Not determined |
| Antibodies | Higher antibody levels in primed mice | Higher antibody levels in conventional mice | Higher antibody levels in C3H mice |
| Amount of transgene expression | Less expression in primed mice | Less expression in conventional mice | Similar expression |
| Longevity of transgene expression | Shorter expression in primed mice | Apparently reduced expression in conventional mice | Comparable persistence |

tricles elicited an immune response, it did not do so when injected into the parenchyma.⁷ Cytokine responses to adenovirus injection also differ in each compartment.⁸

The paper by Ohmoto *et al.* (1999)⁹ has now examined in much detail the effects of priming animals with a peripheral administration of adenovirus, keeping the animals under strict restricted exposure to environmental 'pathogens', and mouse strain, on T cell responses, antibody production, levels of transgene expression, and longevity of transgene expression. Their results are summarised in Table 1 (and see refs. in Ohmoto *et al.* 1999 to previous work on adenovirus-induced inflammation in the brain by these authors).

Ohmoto *et al.* (1999) have now formally demonstrated that not only is lymphocyte infiltration higher in primed animals, but the response is qualitatively different. While in unprimed animals CD4⁺ T cells predominate, it is the CD8⁺ cells which constitute the majority of infiltrating T cells in primed animals. Thus, CD8⁺ lymphocytes are likely to be responsible for decreasing transgene expression after interacting with transduced brain cells. Whether this involves direct cell killing or indirect mechanisms remains to be determined.

Importantly, Ohmoto *et al.* (1999) also demonstrate a major difference in the response of naïve animals raised under either conventional lab conditions, or within a 'specific pathogen free' environment. Antibody levels were higher in animals raised in a conventional environment, the amount of transgene detected in their brains was lower, and its longevity of expression, appeared significantly shorter. Differences between SPF-raised mice and conventionally housed mice still exist in primed animals, indicating that a clean environment and specific priming act as two independent factors. This suggests that raising the animals in an SPF environment does more than protect them from sporadic exposure to human adenoviruses. What factors are responsible for the enhanced reactivity of 'conventionally' raised animals remain to be determined.

In conclusion, these data demonstrate that the environment in which animals are raised plays an important role in determining the outcome of gene transfer experiments in the brain. A "clean" environment clearly reduces inflammatory and immune responses to adenoviral vectors, and is also likely to facilitate gene transfer mediated by other means. Importantly, this paper addresses some of the issues gene therapists will have to confront when moving into clinical arenas. While it is possible to raise animals under 'pathogen free' conditions, it is clearly much more difficult to do so with humans. Thus, this paper succeeds in modelling one of the challenges that clinical gene therapists will have to face. It is expected that a better understanding of the factors affecting inflammatory and immune responses against adenoviruses will facilitate the design of less toxic and less immunogenic viral vectors with increased gene transfer efficiency and longevity.

Paradoxically, recent evidence suggests that in certain cases, inflammatory and immune cells may secrete neuronal growth factors, and have beneficial effects on neuronal survival. Thus, it has been recently shown that human T-cell lines specific for myelin autoantigens, and which are present in the brain of patients with inflammatory brain lesions produce biologically active BDNF;¹⁰ equally, autoimmune T cells can protect rodent retinal neurons from axotomy-induced cell death.¹¹ Thus, inflammation may, at least in some cases, promote neuronal survival. How viral vector induced inflammation relates to vector encoded transgene longevity, and whether the beneficial role of certain inflammatory cells could be harnessed to achieve long term transgene expression in the brain, remains to be explored.

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