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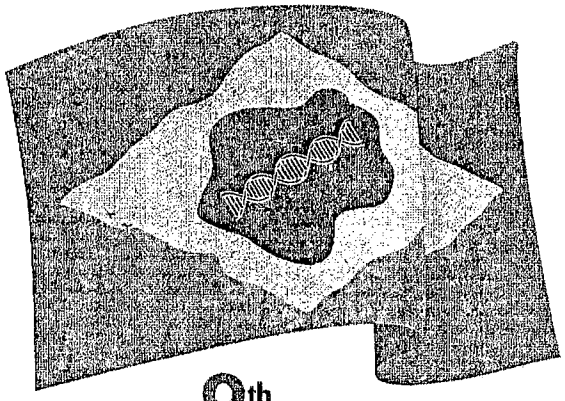

8th PEACe Conference
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P2 GENE DELIVERY

P2.1 Evidence for in vivo persistence of mGH-secreting human keratinocytes by immunohistochemical staining of grafted organotypic cultures

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The epidermis is a very attractive target for therapeutic gene delivery because it is accessible and capable of delivering the gene product to the systemic circulation. We set up a cutaneous gene therapy model in which primary human keratinocytes were transduced with the mouse growth hormone (mGH) gene under control of the retroviral LTR promoter. These genetically modified cells presented a high and stable in vitro secretion level of the order of 10 μg mGH/106cells/day, which is higher than that previously obtained in our laboratory for human growth hormone.

A number of difficulties, however, have emerged in attempting to establish GH gene therapy as a routine option in the clinic and one of the major obstacles is the inability to sustain in vivo therapeutic protein delivery. In this study organotypic raft cultures were prepared with the mGH-secreting keratinocytes and were grafted onto immunodeficient dwarf mice (lit/scid). Circulating mGH levels revealed a peak of 5-20 ng/ml in the first 5 hours after grafting, but unfortunately these levels rapidly fell to baseline values. The presence of human keratinocytes up to at least the seventh day after grafting was revealed by immunohistochemical techniques based on anti-human involucrin and anti-human high molecular weight cytokeratin (34βE12) antibody. This confirmed previous data showing that, one week after grafting, excised implants recovered in culture a great fraction of their original in vitro mGH secretion efficiency.

Taken together, these results led us to analyse different factors possibly involved in the maintenance of the transgenic protein in vivo, such as the persistence of the human cells in grafted organotypic cultures. They also open the way to study alternative techniques (different vectors, grafting procedures and implantation sites, naked DNA administration, etc.) in view of the clinical utilization of this model of cutaneous gene therapy.