Gene Therapy for Human Severe Combined Immunodeficiencies

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In principle, gene therapy is an attractive option for inherited blood-borne diseases, as gene transfer into hematopoietic stem cells with a self-renewal capacity should lead to cure. In addition, although still poorly characterized, these cells can easily be retrieved in relatively large numbers in the bone marrow or by mobilization in blood and are thus physically accessible to gene transfer. However, several clinical trials based on gene transfer into hematopoietic cells did not lead to efficient transduction rates of these cells [1]. The clinical grade-approved vectors enabling gene integration into the genome of hematopoietic cells are all derived from murine oncoretroviruses. Such vectors were found to be effective in the transduction of murine HSC but much less so for human HSC [2]. Two factors account for this discrepancy. Human HSC express low numbers of membrane receptors for amphotropic envelopes and, above all, DNA provirus integration complexes derived from such viruses do not cross the nuclear membrane of quiescent cells, the state of most HSC. Despite these major limitations, the issue of gene therapy for inherited immunodeficiencies appears timely for two reasons: a) the advent of significant advances in the technology of gene transfer into hematopoietic precursor cells, and b) the determination in the last 10 years of the genetic basis and molecular mechanisms of many primary immunodeficiencies. Some forms of severe combined immunodeficiency appear to be the ideal model(s) to assess gene therapy feasibility. This assumption is based on the potential selective advantage provided to lymphocyte progenitor cells by transgene expression combined with the expected long life of differentiated T cells.

Technological advances of gene transfer into hematopoietic precursors

Moloney retroviruses and related oncoretroviruses are suitable for infection and integration of transgene into cycling target cells. The potential risk of recombination events generating a replication-competent virus is prevented by using "disarmed" packaging cell lines [3–5]. As stated above, however, transduction rates of human HSC by these vectors are very low. Long-term expression of transgene in myeloid cells from patients who received *ex vivo* transduced hematopoietic precursor cells in the absence of myeloablative treatment is in the order of one cell in a few thousand [6]. In addition, transgenes placed under the transcriptional control of the viral locus control region can be silenced *in vivo* [7]. Some of these limitations have now been overcome. Packaging cell lines have been designed to produce a high titer of retroviral particles, while pseudotyping of virions with envelopes from monkey or feline retroviruses substantially increases the transduction rate of CD34(+) hematopoietic precursor cells [8]. During the *ex vivo* infection phase, the use of cytokines, i.e., flt3-ligand, stem cell factor and megakaryocyte differentiating factors enables cell division without differentiation of early hematopoietic progenitor cells. Coating of culture bags by a fibronectin fragment (CH296) also increases the transduction rate by bringing together cells and viral particles [9]. These advances should lead to a much better gene transfer efficacy in the setting of clinical trials.

Gene therapy for SCID

Some forms of severe combined immunodeficiency generally represent suitable conditions for gene therapy. These are lethal disorders with a still partially unsatisfactory treatment, e.g., allogeneic stem cell transplantation [10]. They are characterized by inherited blocks in T cell development, variably associated with defects in other lymphoid lineages [Figure 1]. The genetic bases of many forms of SCID have now been identified. The normal products of these genes are involved in one of the following: a) providing survival/growth signals to lymphocyte precursors upon γc-dependent cytokine binding, i.e., γc cytokine receptor subunit, interleukin-7 receptor chain, JAK-3 kinase; b) the V(D)J recombination process, namely Rag-1, Rag-2 and Artemis; c) signal transduction, i.e., CD45, CD3 subunits or ZAP-70 kinase; and d) protection against cell apoptosis, i.e., enzymes of purine metabolism-adenosine deaminase and purine nucleoside phosphorylase [11].

In theory, expression of any of these normal genes, even in a small set of lymphocyte precursor cells, should restore the production of a large number of mature T lymphocytes that will be long-lived. This is based on the assumption that a selective

HSC = hematopoietic stem cells

SCID = severe combined immunodeficiency

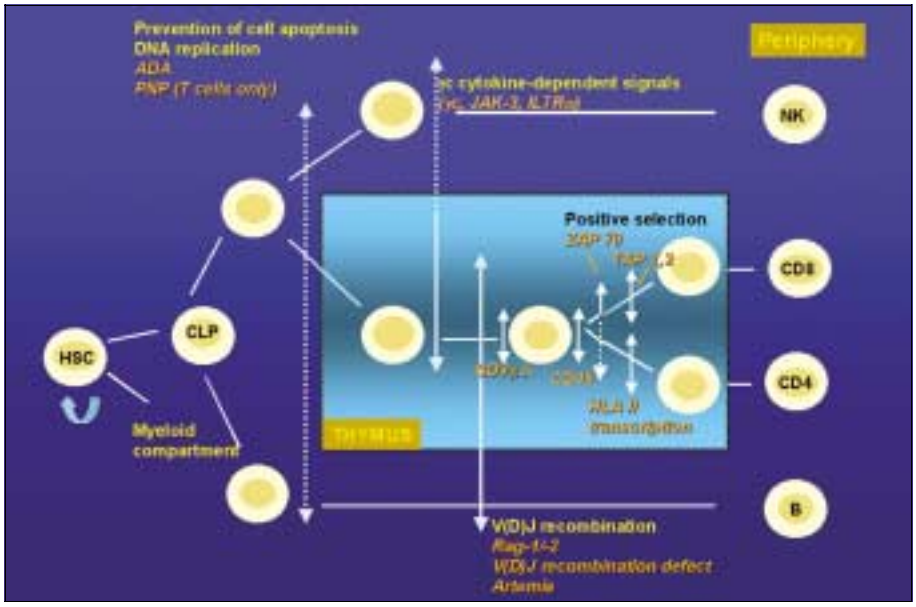

Figure 1. Scheme of human lymphocyte development pathway with indication of SCID conditions. Each arrow represents a block in lymphocyte development, causing SCID. Interrupted line indicates partial block.

growth advantage is conferred to the normal gene-transduced lymphocyte precursors. This hypothesis has received strong support from unique clinical observations and experimental data. In two patients – one with X-linked SCID (SCID-X1, γc deficiency), the second with ADA deficiency – a "natural gene therapy" resulted from a likely single spontaneous reverse mutation that occurred in a T cell precursor [12,13]. It was found in the former case that at least 1,000 T cell clones, as defined by the expression of differentially rearranged T cell receptor-β chains, were generated and persisted over 2–5 years [12]. These results indicate that multiple cell divisions of the γc(+) T cell precursor occurred prior to TCR gene rearrangement. Analysis of the immune system of this unique patient thus revealed the *in vivo* capacity of a single T cell precursor to generate a diverse T cell repertoire (about 1% of the normal one). Its relative stability over time (up to the age of 5 years) serves as strong evidence to support the rationale of gene therapy for SCID-X1. Efficient generation of normally functioning T cells with a clinical benefit has also recently been observed in a patient with Wiskott-Aldrich syndrome [14]. In parallel, Sorrentino's group [15] demonstrated that in JAK-3(-) mice, following *ex vivo* JAK-3 gene transfer into HSC, a selective advantage was provided to JAK-3(+) lymphocyte precursors, since, in the periphery, the frequency of JAK-3(+) differentiated lymphocytes was found to be much higher than the frequency of transduced myeloid cells [15]. To some extent, a similar finding was made by Halene and Kohn [3] in a clinical trial of ADA gene therapy.

ADA = adenosine deaminase
TCR = T cell receptor

Gene therapy of SCID-X1 (γc deficiency)

Based on this rationale, we selected SCID-X1 as a potentially optimal model. Indeed, besides the above mentioned observations, the fact that there is little γc gene transcription regulation, that γc expression pattern is ubiquitous and constitutive among the different hematopoietic lineages, while γc exerts no autonomous function, were all positive factors minimizing the potential advent of deleterious effects of γc gene transfer. In addition, it was found – both in *in vitro* gene transfer experiments in γc-deficient B cells and in an *in vivo* murine model – that the expression of an abnormal endogenous γc protein at cell surface did not exert a detectable dominant negative effect on the expression and function of the γc transgene product [16,17]. *In vitro* gene transfer experiments [18] and correction of γc deficiency in several γc(-) mice models [19–21] positively completed a set of preclinical experiments suggesting potential efficacy. A clinical protocol was then built based on the recent advances of the technology of *ex vivo* gene transfer into human hematopoietic progenitor cells [22]. A rate of 30–40% of CD34(+) cell transduction was readily achieved. A clinical trial was initiated in March 1999 and the preliminary results were reported a year later [22]. The first two patients have shown sustained correction of most aspects of the immunodeficiency and no adverse effects. Similar findings, with a shorter follow-up, have been observed in another two patients. The pattern of γc transgene integration and expression confirmed that γc was necessary to induce T and natural killer lymphocyte development, but much less so for B lymphocytes. The efficacy of gene transfer into hematopoietic progenitor cells was sufficient to generate a normal size T lymphocyte pool in the periphery, the characteristics of which cannot be distinguished from age-matched controls. T cell repertoire diversity, the predominant presence of naive T cells and antigen-specific T cell responses were all within normal range one year after treatment [22]. B cell immunity was also restored, at least in part; although it is yet unclear whether only transduced B cells are functional. Antigen-specific immune responses in these children have developed, as their existing infections were cleared prior to treatment, and the children have since grown normally without serious infections while free of any treatment.

These preliminary results demonstrate the validity of the concept that gene therapy based on a selective advantage of normal gene-transduced cells can work. It is also possible that

the selection process contributes to the maintenance of γc expression, thus avoiding transgene silencing, as has often been observed following gene transfer with vectors in which transgene expression is controlled by the viral LTR [7]. However, to understand the full significance of these results, a number of questions need to be answered. First of all, why were previous gene therapy trials for ADA deficiency unsuccessful, while a survival selective advantage should have been provided by ADA transgene expression into lymphocyte precursors? That all ADA-deficient patients received enzyme replacement therapy by weekly muscular injections of bovine ADA coupled with polyethylene (PEG-ADA) could have largely reduced or even abrogated the survival advantage of transduced over non-transduced cells. In addition, the clinical trials were performed in the early 1990s. As discussed above, many advances in gene transfer technology have since occurred, leading at least to higher gene transfer efficiency.

The sustained detection of thymopoiesis one year after treatment, as well as the low percentage of transduced myeloid cells (in the order of 1/1,000) together with the presence of some transduced CD34(+) cells [22], suggest that immature pluripotent progenitor cells have been transduced. These findings, together with the absence so far of any indication of γc transgene silencing, raise hope that a functional T cell pool could persist in these patients for many years. Careful long-term follow-up of the size of the T cell pool, its repertoire and function is therefore warranted.

Gene therapy for other immunodeficiencies

What about extending the treatment to other immunodeficiencies? If one assumes that the most suitable disease was chosen, then treatment of other conditions raises additional difficulties [1,18]. This means that further advances in gene transfer technology in hematopoietic precursor cells will be necessary in this setting. Nevertheless, SCID conditions with an identical or closely related disease mechanism to SCID-X1, i.e., JAK-3 and IL-7R, deficiency are the most obvious next candidate diseases for gene therapy. Experimental gene therapy of JAK-3(-) mice has provided convincing results, including proven efficacy in non-conditioned mice [15]. SCID conditions caused by Rag-1, Rag-2 and Artemis gene mutations are also worth considering, since allogeneic stem cell transplantation provides poorer results than it does for other SCID conditions. Genetic deficiencies in the immune system that impair further downstream T cell development will be more difficult to tackle for two reasons: a) a less potent selective advantage is to be expected, and b) considerations of transgene expression regulation (both spatial and temporal) will have to be addressed. In this respect however, the spectacular achievement of tissue-specific β-globin expression in erythroid cells of β-thalassemic mice represents a significant advance. This result is based on the insertion in the lentiviral vector of a minimal version of the locus control region of the β-globin gene [23].

One can conclude from the above that proof of principle has been achieved in the genetic treatment of inherited disorders of the immune system. Much remains to be done by combining an in-depth appraisal of every single disease mechanism (in other words, understanding the function of the corresponding gene product) with the development of new aspects in gene transfer technologies. It is obvious that the potentially safe use of lentiviral vectors able to efficiently target HCS would provide a major advance to the field [4].

LTR = long terminal repeat

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Humor is emotional chaos remembered in tranquility.

James Thurber (1894–1961), American humorist

*Quality is like justice: it must not only be done, it must be **seen** to be done.*

Anonymous

Capsule

A closer look at DNA replication

Replication of genomic DNA, a highly regulated process that occurs just before a cell divides, is initiated from DNA sequences called replication origins. Identifying these origins through their sequence characteristics alone has posed a challenge because not all of the matching sequences function as origins. Thus, to map all of the origins in the yeast genome, Wyrick et al. have identified every location bound by proteins of the origin replication complex (ORC), as well as by so-called minichromosome maintenance proteins that are also required for replication. Origins are found away from transcribed regions and cluster at repetitive sequences at telomeres, where they may also be involved with setting up chromatin domains, as well as at transposable elements, which pepper the genome. The authors found evidence that the processes of replication and transcription interfere with one another. Although much can be learned from such studies of populations of DNA molecules in cells, the details of how replication occurs at specific locations on individual DNA strands is also critical. Norio and Schildkraut (p. 2361) have now developed a methodology to follow the replication of single DNA molecules from the Epstein-Barr virus. Replication is initiated in specific zones rather than at predictable individual sites, and pausing plays a significant role in the pattern of duplication. Termination of replication, on the other hand, can occur anywhere.

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