

EUROPEAN CONCERTED ACTION ON HUMAN HEMATOPOIETIC STEM CELL: GENE TRANSFER INTO HEMATOPOIETIC STEM CELLS

Patrice Mannoni,* Marco Bregni,^o Antonio Tabilio

*Department of Gene Therapy, Institut J. Paoli-I, Calmettes, Marseille, France; ^oIstituto Nazionale per lo Studio e la Cura dei Tumori, Milan; #Sezione di Ematologia e Immunologia Clinica, Policlinico Monteluce, Perugia, Italy

Human gene therapy has progressed from theoretical to practical reality in a short time. Because transduction of specific target cells is the critical step in gene therapy, improvement of gene transfer methods has been the principal aim of research. Retroviral-mediated gene transfer, which was developed the early 1980's in animal models, is the procedure most used today. Vectors need to be engineered that will target specific cell types, insert their genetic information into a safe site on the genome, and be regulated by normal physiological signals. When efficient vectors of this type are produced (retroviral, viral, synthetic, or a combination of the three), gene therapy will probably have a profound impact on medical practice.

The first approved gene transfer (only a marker gene) protocol was begun in May 1989 and the first clinical gene therapy trial, which was for the correction of adenosine deaminase (ADA) deficiency, was initiated in September 1990. Many had assumed that hematopoietic stem cells would be the first target of gene therapy because they persist long term and involve numerous diseases of all lineages.

The *European Concerted Action (ECA) workshop on Gene Transfer into Hemopoietic Cells* took place in Marseille on October 17th and 18th, 1995. Eighteen European laboratories participated in this workshop: 2 from the UK, 2 from Germany, 2 from Italy, 2 from Spain, 1 from the Netherlands, 1 from Austria and 8 from France.

Five main topics were discussed:

- Vectors for transduction of hemopoietic cells.

- Functional assays defining progenitors and stem cells.
- Methods of transduction.
- Gene transfer into lymphoid and dendritic cells.
- Clinical protocols based on gene transfer in hemopoietic or lymphoid cells.

The session devoted to vectors developed for hematopoietic cell transduction showed that retroviral vectors are still the most frequently employed and appropriate tools. Only one group reported not using viral vectors but plasmids complexed to liposomes to transduce murine stem cells prior to reinfusing the cells into mice (A. Thierry, Bethesda). Most retroviral vectors used are based on Moloney murine leukemia virus backbone. Interestingly, some laboratories (W. Ostertag, Hamburg) proposed adopting other viruses such as MPSV or FFV to modify the U3 region of pre-existing virus, or designing new viral vector backbones to improve gene expression. New vectors have also been designed to interact with growth factor receptors to increase gene transduction efficiency (B. Nilson, Cambridge). This goal has been achieved with viral vectors expressing the ligand for the epidermal growth factor (EGF) receptor. Viral particles preferentially bind to EGF receptors on the target cell, and it has been shown that the addition of proteases that specifically cleave the EGFr-viral particle interaction increases transduction efficiency through the amphotropic virus receptor by up to one thousand times. This *two-step targeting* approach could open up interesting prospec-

tives for *in vivo* gene therapy. Different groups have obtained coexpression of a selective gene, such as NeoR, and a functional gene by either fusing coding sequences (C. Bagnis, Marseille) or linking the two coding sequences with an internal ribosomal entry site (IRES). For example, using the IRES approach the Perugia group (A. Tabilio, M. Di Ianni) have demonstrated the expression of both the *Herpes simplex virus-thymidine kinase* (HSV-TK) and the LacZ gene in U-937 transduced cells. New packaging cell lines, including human ones (e.g. FLY cell line) have been produced (F.L. Cosset, Lyon) and shown to yield high titres of amphotropic retrovirus. Use of the green fluorescent protein (GFP) as a new marker was reported (F. Rueda, Barcelona), but preliminary results indicate that expression of the transgene has a deleterious effect on the behavior of the producing cell line. Identification of hematopoiesis-associated inducible promoters and genes was documented by an elegant gene-trap approach combining self-inactivating retroviral vector and growth factor dependence of the TF-1 cell line (P. Dubreuil, Marseille). The reports and the discussions pointed out the need to improve vectors and design retroviral backbones able to ensure stable or specific inducible expression. Coexpression of genes still remains a challenge, even though interesting prospects can be foreseen with polycistronic IRES-vectors.

The session devoted to functional assays of stem cells focused on both the identification of hematopoietic progenitors from different sources, including mobilized blood cells, bone marrow and cord blood, and the consequences of culture conditions on the integration of retroviral vectors in these progenitors. A number of presentations (C. Bello-Fernandez, Vienna), (A. Schütz and A. Fauser, Idar-Oberstein), (D. Bossy, Marseille) drew attention to the fact that a clear definition of stem cells, as opposed to their progeny, is still needed. J. Hatzfeld (Villejuif) proposed a modified methylcellulose assay that detects HPP-GEMM (high proliferative potential – granulocytes –

erythrocytes – monocytes – megakaryocytes); this assay detects cells with both lineage multipotentiality and high proliferative capacity, two properties that characterize stem cells. Improvement in the phenotypic identification of progenitors, including a better understanding of the ectopic diversity of CD34 (D. Bossy, Marseille) and the expression pattern of cytokine receptors along the differentiation pathway (C. Bello-Fernandez), is required. Phenomena underlying both the differences in gene integration in cells from different sources and their individual variability remain poorly understood (A. Schütz, Idar-Oberstein).

Culture conditions can affect the ability of target progenitor cells to integrate retroviral vectors. M. Bregni (Milan) showed that efficient transduction into mobilized blood cells may rely on elevated expression of receptors for amphotropic retroviruses, rather than on a high percentage of actively cycling cells. Increased susceptibility to retroviral-mediated gene transfer can be obtained by ex-vivo exposure to cytokines (M. Bregni, Milan; F. Lemoine, Paris), which augments both the expression of receptors for amphotropic retroviruses and the percentage of actively dividing cells (a recent publication in *Blood* demonstrates that only a small percentage of mobilized blood cells is cycling). Strategies aimed at interfering with cytokines involved in the control of the quiescent state of early progenitors were discussed. Although the use of antisense oligonucleotides or antibodies directed against transforming growth factor- β (TGF- β) (J. Hatzfeld, Villejuif), genetically modified stromal cells (P. Charbord, Besançon; W. Ostertag, Hamburg), and genetically modified packaging cell lines (C. Casimir, Cambridge) were all proposed as techniques for increasing gene transfer, they require further evaluation. There was great interest in such marker genes as nlsLacZ (J. Hatzfeld, F. Louache, Villejuif) or glycophorin-B (A. Dubart, Villejuif).

The third session focused on protocols devoted to transduction into CD34⁺ cells and, in

some instances, more primitive progenitor populations, such as CD34⁺ cells expressing the Thy-1 surface antigen or CD34⁺ CD38⁻ cells. Cytokine-mediated cell cycling activation still seems to be essential for obtaining retroviral integration. Which cytokine to use and the association with putative inhibitors of TGF- β were discussed at length. There was partial consensus on the necessity of using stem cell factor (C-Kit ligand) associated with IL3 and IL6 during transduction. The Marseille group also reported a degree of synergic activity with FLT-3 ligand. However, it is still not clear whether the addition of these cytokines can modify both the number of primitive stem cells and retroviral transduction. The apparent contradiction between cell activation and induction of cell differentiation was the subject of much debate.

P. Chabord (Besançon) presented interesting data on supporting long-term hemopoiesis by taking advantage of human (CRC) or murine (MS5) stromal cells. The same approach had been employed by I. Dube (Toronto), who reported that dog stem cells cultivated on an autologous stromal layer could be transduced.

The importance of the interaction between target cells and producers was emphasized by the Marseille group, who demonstrated a lack of correlation between gene transfer efficiency and viral titre in transduction experiments that used a cocultivation protocol. This approach was challenged by other groups (F. Louache, Villejuif) whose transduction protocol employs supernatants from human packaging cell lines that exhibit high viral titres, such as the TASAF cell line (F.L. Cosset, Lyon).

—

The fourth session debated ongoing research and prospects for gene transduction into more differentiated hematopoietic cells. C. Caux (Lyon) clearly demonstrated that dendritic cells can be generated from bone marrow or cord blood CD34⁺ cells in the presence of GM-CSF, TNF α and CD40 ligand. Supporting evidence came from Mannoni's team (Marseille), who are investigating whether CD34⁺ cells isolated from peripheral blood can be transduced with a

MFG vector that expresses the nls-LacZ gene (C. Bagnis, Marseille).

It was agreed that problems are encountered in transducing peripheral blood lymphoid cells regardless of the stimulation pathway (IL2 and CD3, IL2 and CD2/CD28, or IL2 and PHA). The best results (up to 80% of X-GAL⁺ cells after transduction with a LacZ containing vector) were obtained by cocultivation with a producing cell line screened for its ability to transduce human hematopoietic cells (A.M. Imbert, Marseille), but efficiency ranged from 5 to 40% with average cell lines. Controversy arose over whether CD4⁺ lymphoid cells from an HIV⁺ subject could be employed as target for transgene expression of factors that induce resistance to HIV, such as interferons (E. Lauret, Orsay).

—

The last session dealt with clinical trials. Projects monitoring graft versus host disease with lymphoid cells transduced with the HSV-TK gene in the allogeneic bone marrow transplantation setting were presented by P. Tiberghien (Besançon). A study which will use the nls-LacZ gene as its marker is being set up in Marseille to follow hematopoietic reconstitution after autologous stem cell transplantation in cancer patients receiving high-dose chemotherapy (P. Mannoni, Marseille). W. Ostertag (Hamburg) pointed out that CD34⁺ cells could be selected *in vivo* with Taxol derivatives and other chemotherapeutic agents with MDR genes. A clinical AIDS gene therapy protocol, which is scheduled to start next year in collaboration with the Besançon and Marseille groups, and which will use TAT inducible interferon β retroviral constructs, was introduced by M. Methali (Strasbourg). The approach is in line with the interesting data and perspectives that E. Lauret (Orsay) presented on gene transduction of a non-inducible low-producing INFB β retroviral vector into CD4⁺ cells in order to protect them from HIV infection (E. Lauret, Paris). The treatment of chronic granulomatous diseases by expressing the Phox protein (M. Grez, Frankfurt) and the treatment of chronic myeloid leukaemia by expressing interferon- α (De

Verneuil, Bordeaux) were also considered.

Most of the teams who participated in the workshop are still trying to define the best protocols for transducing progenitor cells, including stem cells and differentiated cells like lymphocytes. The meeting should result in the

exchange of materials (packaging cell lines - vectors) and the organization of short-term visits between laboratories.

A. Tabilio and *M. Bregni* will organize the next meeting in Italy and *W. Ostertag* proposed that the one after that take place in Germany.