MEDICINE

Gene Therapy—New Challenges Ahead

David A. Williams and Christopher Baum

Therapeutic gene transfer for treating certain human diseases, so-called somatic gene therapy, has had some limited successes (*1*, *2*). Chief among these is the treatment of patients with X-linked severe combined immunodeficiency (SCID-X1). In a clinical trial, 10 SCID-X1 patients were transfused with a population of their own bone marrow–derived progenitor and stem cells transduced ex vivo with a retroviral vector carrying a transgene. The transgene encoded the common γ chain of the interleukin-2 receptor (γc), the protein that is defective in these patients, leading to a compromised immune system. Of the 10 patients treated, nine showed clinically significant, long-term improvements in functional immunity for a disease that would otherwise be fatal (*1*). Limited alternative therapies, such as unrelated or haploidentical hematopoietic stem cell transplantation, offer lower correction rates with higher morbidity and mortality. However, as with all new therapies, side effects should be expected. On page 415 of this issue, Hacein-Bey-Abina *et al.* (*3*) report that the two youngest SCID-X1 patients receiving gene therapy have developed T cell leukemia due to insertion of the retroviral vector near the promoter of the proto-oncogene *LMO2*.

Previous studies in animals predicted that retrovirus-mediated gene transfer poses a potential, but remote, risk of insertional oncogenesis (*4*, *5*). The surprising finding reported by Hacein-Bey-Abina *et al.* is the repeated occurrence of this side effect (2 out of 10 patients) and the independent insertion of the recombinant retrovirus at the same *LMO2* gene locus (which encodes a transcription factor required for hematopoiesis). Until this report, retroviral insertion in the context of gene therapy has been considered an untargeted and largely random event.

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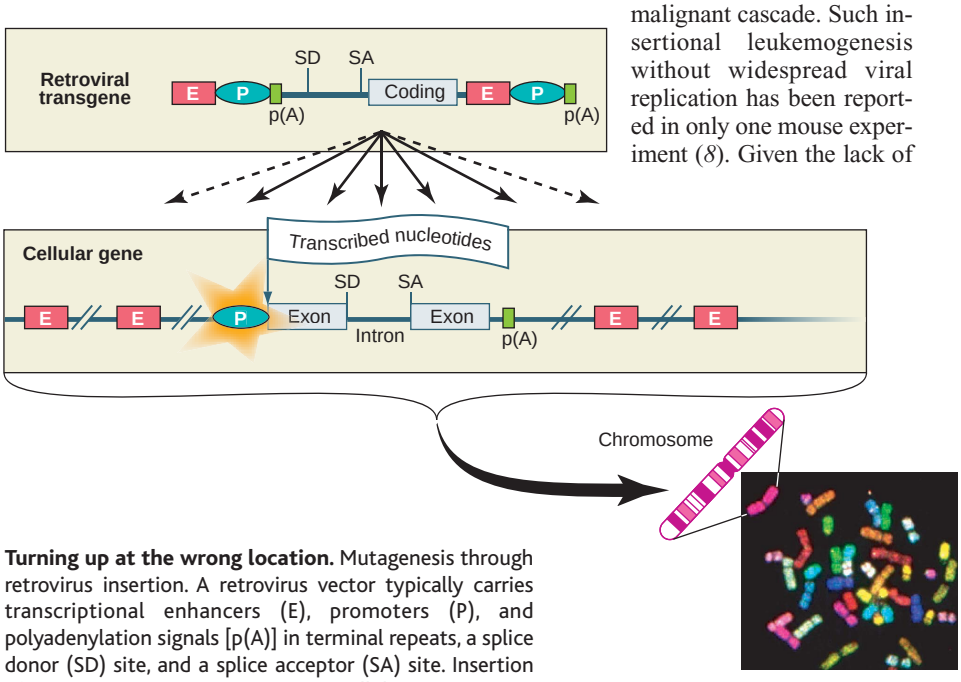
Indeed, disease- and protocol-specific issues may have played an important part in the evolution of the T cell leukemias. The two young patients, aged 1 and 3 months at the time of gene therapy, received large numbers of genetically modified, undifferentiated hematopoietic cells, with each cell carrying a different vector insertion site. The γc transgene carried by the vector encoded a potent antiapoptotic product that was expressed in an unregulated fashion. The young age of the patients, a theoretical expanded pool of target precursor T cells, and the initial presence of immune deficiency leading to selection and forced expansion of transduced cells may all be relevant to the higher than expected frequency of the insertional mutagenesis event and subsequent clonal dysregulation.

All gene-transfer methods that lead to integration of DNA into the chromosome carry a risk of mutagenesis. The integration of retroviruses, including lentiviruses, could lead to inactivation of tumor suppressor genes or activation of a proto-oncogene (see the figure). The former is likely to be of less clinical relevance because loss of heterozygosity (inactivation of both alleles of the tumor suppressor gene) is required for tumorigenesis to proceed. Activation of a proto-oncogene is potentially a greater risk because only one allele needs to be activated for tumor formation to be initiated. The physical and biological properties of the target DNA and the retrovirus life cycle appear to generate a preference for insertion in or near active genes (*6*, *7*). Retroviruses can up-regulate cellular genes over large distances (more than 10 kb). Considering the presence of more than 100 proto-oncogenes in the human genome, oncogene dysregulation may occur in about 0.1 to 1% of all retroviral gene-transfer events (*4*). Thus, additional patients in this or other clinical trials may have been treated with cells carrying retrovirus-vector insertions near oncogenes even though the patients themselves have remained healthy over prolonged periods of observation (*5*).

The *LMO2* gene encodes a transcription factor that is required for normal hematopoiesis. Aberrant expression of this factor has been implicated in de novo childhood T cell acute lymphoblastic leukemia. The insertional activation of this locus in two independent leukemias suggests an essential event that is required for the initiation of a malignant cascade. Such insertional leukemogenesis without widespread viral replication has been reported in only one mouse experiment (*8*). Given the lack of



Turning up at the wrong location. Mutagenesis through retrovirus insertion. A retrovirus vector typically carries transcriptional enhancers (E), promoters (P), and polyadenylation signals [p(A)] in terminal repeats, a splice donor (SD) site, and a splice acceptor (SA) site. Insertion of a retrovirus vector into or near a cellular gene appears to take place preferentially in an accessible region close to the gene promoter (star) or in regions of transcribed nucleotides (wavy box). These types of insertions have the potential to alter expression of the cellular gene. [Spectral karyogram courtesy of B. Schlegelberger and C. Rudolph]

similar side effects in previous human trials and animal studies, a combinatorial process seems to be the likely culprit. Indeed, one cannot exclude the possibility that the potent antiapoptotic effects of the γc transgene, leading to enhanced cell survival, may have conferred an abnormal proliferative advantage on some mature T cells. This possibility needs to be addressed in animal studies, particularly as introducing a selective advantage is key to the potential success of many gene therapy strategies.

In addition to the identification of disease-specific risk factors, there are three ways to limit the possible deleterious side effects of genetic interventions. The first is to develop vectors with improved safety profiles, including a reduced propensity for insertional "genotoxicity." The second is to define "safe integration sites" in the genome and to design integration vectors that are targeted to these sites. The third is to reduce the number of vector-exposed cells (and thus vector integrations) that are infused into the patient, for example, by correcting a very small number of stem cells ex vivo and genetically characterizing them before they are infused back into the patient. Molecular insertion site analysis, which uses the transgene as a tag to identify neighboring cellular sequences, is a powerful tool that should help with these three strategies (*9*). This technology is also crucial for determining the extent to which different types of integrating vectors are differentially attracted to particular areas of the genome (*6*, *7*). Combined with functional studies, such investigations will provide an important basis for future development of the gene therapy field.

It would be unrealistic not to expect genetic therapies to produce side effects. Gene therapy continues to require informed use in controlled clinical studies with a clear consideration of the risks and potential benefits. Current vector systems may need to be modified, and additional efforts are required to better understand the biology of the diseases that are candidates for therapeutic genetic intervention. Together this information will enable risk classifications for specific vectors and transgenes, as well as assessment of the risk factors that are unique to each clinical trial. With this approach, the therapeutic potential of somatic gene transfer may be realized through the application of appropriate prevention strategies.

References and Notes

1. S. Hacein-Bey-Abina *et al.*, *N. Engl. J. Med.* **346**, 1185 (2002).
2. A. Aiuti *et al.*, *Science* **296**, 2410 (2002).
3. S. Hacein-Bey-Abina *et al.*, *Science* **302**, 415 (2003).
4. C. Baum *et al.*, *Blood* **101**, 2099 (2003).
5. D. Kohn *et al.*, *Mol. Ther.* **8**, 180 (2003).
6. A. R. Schroder *et al.*, *Cell* **110**, 521 (2002).
7. X. Wu, Y. Li, B. Crise, S. M. Burgess, *Science* **300**, 1749 (2003).
8. Z. Li *et al.*, *Science* **296**, 497 (2002).
9. M. Schmidt *et al.*, *Blood* **100**, 2737 (2002).
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PLANT SCIENCES

Will GM Rapeseed Cut the Mustard?

John Heritage

A nationwide assessment in the U.K. of the likelihood that an important crop, oilseed rape (*Brassica napus*), forms hybrids with its wild relative, *Brassica rapa*, is presented by Wilkinson *et al*. (*1*) on page 457 of this issue. The authors draw on a combination of sources including population surveys, satellite imaging, pollen dispersal profiles, herbarium data, local floras, and other databases. Their study is a first step toward a more quantitative assessment of the risk of gene flow from transgenic plants into natural plant populations at the national level. Why is this important?

Oilseed rape (canola) is a crop used for the production of vegetable oil and diesel fuel. The pressed seed is also an important component of animal feed. Oilseed rape belongs to the Cruciferae, a family that includes cabbage, cauliflower, brussel sprouts, turnip, swede, radish, and mustard plants. Competition from weeds, especially grasses (see the figure), necessitates the use of herbicides when growing oilseed rape crops.

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The need for herbicides has made oilseed rape a prime candidate for genetic modification. The introduction of genes that confer herbicide tolerance makes it easier to manage this crop. Oilseed rape plant lines have been engineered to include the *pat* and *bar* genes encoding resistance to phosphinothricin (glufosinate ammonium); the *epsps* gene encoding resistance to glyphosate; and the *oxy* gene encoding tolerance to the oxynil family of herbicides. Other genetic modifications include introduction of a transesterase gene to alter the fatty acid content of the oil, and of a phytase gene to reduce the level of antinutrients in material destined to become animal feed. (Antinutrients reduce the nutritional value of the feed but are nontoxic.)

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Outcompeting the neighbors. A conventional oilseed rape (*B. napus*) crop growing in a field in Sussex in the southeast of England. Situated next to the crop field is a field of grass. Grasses readily outcompete oilseed rape, prompting genetic modification of this crop to render it resistant to herbicides and to provide it with a growth advantage.

The Organisation for Economic Cooperation and Development (*2*) lists 17 transgenic lines of oilseed rape for which approval for growing has been granted worldwide in the period 1995–1999. Of these, only three are not modified to express herbicide tolerance. Of the remaining 14 lines, four carry genes that modify fertility as well as those that confer herbicide tolerance. Thus, 10 lines of transgenic oilseed rape have the ability to pass on the herbicide tolerance trait to plants with which they breed. This is particularly problematic because oilseed rape forms hybrids with some of its wild relatives. Were such hybrids to mate with genetically modified (GM) varieties of *B. napus* engineered to tolerate herbicides, their offspring would express the tolerance phenotype, making agricultural management of the hybrids more difficult.

Because varieties of oilseed rape have been engineered to tolerate different herbicides, it is now considered inevitable that, where these varieties are grown on a commercial scale, gene stacking will occur, resulting in plants that exhibit toler-