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EDITORIAL

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Gene therapy: from retrovirus to triplex DNA repair

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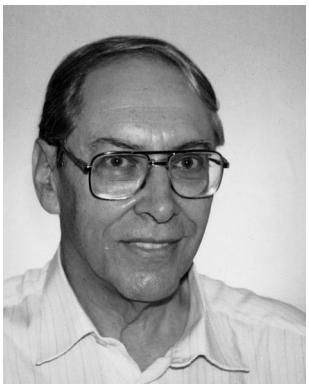
The goals of gene therapy may be summarized as the following:
– Correction of mutated genes by introduction of normal genes into the cells
– Introduction of new functional synthetic chimeric (engineered) genes into cells to endow them with new properties and functions
– Improvement of the vectoring system to target the gene-carrying vector to specific human tissue or organ
– Development of methods to introduce genes effectively into cells in vitro without viral vectors
– Direct mutagenesis of a defective gene to cause repair of the gene.

These subjects are treated in three review papers of this issue which are part of the series of papers "Gene transfer by viral vectors for gene therapy," [1] and which join previous papers published in the *Journal of Molecular Medicine* [2].

Hodgson et al. [3] deal in their review article with the need to improve the retrovirus vectors used as carriers of human genes for gene therapy. These authors observe that "the greatest shortcoming is the propensity of retroviral transducing vectors to recombine with helper virus genes within the vector producing cells that make virus particles.... Although no humans have yet been affected, testing itself is costly ($100|000/batch) and several primates infected with replication competent retrovirus died from associated neoplasms" [4]. To prevent the genetic recombination between the transducing retrovector and the helper retroviruses or the endogenous retroviral DNA in the cellular DNA genome of the virus producing cells, Hodgson et al. [3] chose retrotransposon VL30 as a vector for gene therapy: mouse VL30 is a 5-kbp LTR retrotransposon found at a level of 100–200 copies in the genomes of most mouse species and lacks viral genes but contains all the *cis*-acting signals for retroviral packaging, replication, and gene expression. Mouse VL30 retrotransposons have tissue- specific LTR transcriptional units which are necessary for transcriptional targeting. To facilitate the use of tissue specific vectors Hodgson et al. [3] are developing single LTR vectors for transferring tissue-specific transcriptional controls into single LTR vectors. The availability of new retroviruslike vectors for gene therapy will make possible the insertion and expression of genes into the genomic DNA of specified tissue cells, which may lead gene therapy into successful frontiers.

A retroviral construct was used by Altenschmidt et al. [5] to develop a new approach for gene transfer to endow naive T lymphocytes of mice or rats with synthetic chi-



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meric T cell receptors. The authors designed a strategy to "prompt T cells to lyse tumor cells which only marginally express MHC molecules and which do not express neoantigens." The absence or low level of MHC class I molecules on the cell membrane of cancer cells prevent their recognition by $CD8^+$ cytotoxic T cells (CTLs), and therefore the tumor cells escape detection by CTLs. Altenschmidt et al. [5] genetically manipulated CTLs from the donor mouse to recognize the cancer cells in a MHC-independent fashion. The specificity is determined by the new chimeric gene which in the T lymphocytes will express a T cell receptor (TCR) protein with affinity to a tumor cell protein present on the outer membrane. However, the authors note that "the safest and most efficient transduction technology for primary human T cells has not yet been established and additional parameters of CTLs responses to different tumors should be evaluated with respect to their systemic toxicity."

The use of viral vectors to transfect cells with DNA molecules carrying genes into various cells led to the search for methods of DNA transfection that would eliminate the need for viral nucleic acids. Hodgson et al. [3] review the use of liposomes for "introducing DNA, RNA, proteins, peptides, antibodies, lipids, carbohydrates, and drugs of various kinds." Cationic liposome preparations are commercially available for delivery of DNA or RNA to cells for transient expression, and the authors provide a protocol for enhancing retroviral transduction with lipofectamine [3].

While the two above reviews deal with gene transfer to cells, that by Chan and Glazer [6] focuses on potential applications of triplex DNA (targeting of specific nucleotide sequences to DNA through oligonucleotide-based triple helix formation). Their goal is to develop methodologies to cure genetic diseases by triplex-mediated mutagenesis, recombination, and transcriptional inhibition.

It may be concluded that the above techniques for gene therapy are providing new approaches for delivering or corrrecting genes in the cellular DNA by replacing retroviruses with retrolike virus elements by DNA lipofection and by direct chemical mutagenesis targeted to repair a defective genes.

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