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Genetics

Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer

(retroviral vector/gene expression/gene therapy/immunodeficiency)

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ABSTRACT **A retroviral vector called SAX, containing the cloned human cDNA for adenosine deaminase (ADA), has been constructed and used to introduce the ADA gene into cultured T- and B-lymphocyte lines derived from patients with ADA deficiency. DNA analysis showed that the SAX vector was inserted intact into the T and B cells at approximately one copy per cell. The treated cells produced the characteristic isozymes of human ADA at a level similar to normal T and B lymphocytes. It is known that ADA-deficient lymphocytes are unusually sensitive to high levels of 2′-deoxyadenosine, and this is the mechanism thought to underlie the selective lymphocytotoxicity associated with ADA deficiency *in vivo*. Expression of the introduced ADA gene was sufficient to reverse the hypersensitivity of these genetically deficient lymphocytes to 2′-deoxyadenosine toxicity. These results support the suggestion that retroviral vector gene-delivery systems show promise for application to human gene therapy.**

Retroviruses are able to transfer their genetic information at high efficiency into eukaryotic cells. These viruses can be genetically manipulated to replace their own genes with exogenous genes and thereby become vectors for gene insertion (1). Reports from several laboratories employing retroviral vectors (2–13) have demonstrated successful transfer and, in many cases, expression of exogenous genes in various hematopoietic cell types *in vitro* as well as in murine bone marrow stem cells.

Successful human gene therapy will require efficient gene transfer as well as adequate expression of the delivered gene in appropriate target cells (1). An initial candidate for gene therapy is adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) deficiency, a rare genetic disorder that underlies approximately one-quarter of all cases of severe combined immunodeficiency (SCID), a disease with profound defects of both cellular and humoral immunity (14, 15). ADA catalyzes the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively (16). The molecular basis for the deficiency of ADA in most cases is the production of a catalytically defective enzyme molecule (17). The deficiency of ADA leads to selective toxicity affecting primarily T cells and, to a lesser and variable extent, B cells, with consequent severe immunologic dysfunction. This dysfunction is most likely caused by a toxic intracellular accumulation of deoxyadenosine and its metabolites, particularly deoxyadenosine 5′-triphosphate (18, 19).

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We report here the use of a retroviral vector to study transfer and expression of the human ADA gene in ADA-deficient T- and B-lymphocyte lines.

MATERIALS AND METHODS

Generation of Viral Particle-Producing Cell Lines. The helper virus-free 3T3 packaging cell line PA-12 (5) was used to generate replication-defective viral particles containing the vector SAX. This cell line provides all the functions necessary for encapsidation of the vector RNA into a viral envelope capable of infecting a wide (i.e., amphotropic) host range without producing replication-competent virus (5). PA-12 cells were transfected with SAX plasmid DNA by the method of Wigler *et al.* (20). Stably transformed clones were isolated by their resistance to the neomycin analogue G418 (GIBCO), conferred by the function of the SAX *neo*^{R} gene. These clones were then analyzed for viral production by serially diluting the supernatant from their cultures and adding it to 3T3 cells in the presence of Polybrene (8 μg/ml; Aldrich). The medium was changed to selective medium (i.e., with G418 at 400 μg/ml) 48 hr later, and G418-resistant colonies were scored after 10–12 days. More than 50% of the clones analyzed produced viral particles containing SAX vector at titers greater than 5×10^5 colony-forming units (cfu)/ml. The clone used in these experiments had a viral titer of $2–5 \times 10^6$ cfu/ml.

T- and B-Cell Lines. An ADA-deficient T-cell line (TJF-2) and two control normal T-cell lines (K7 and HM) were established by using the procedure of Mitsuya *et al.* (21) (see *Results*). T cells were maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, gentamicin (50 μg/ml), 10% heat-inactivated fetal bovine serum (FBS), and 10% (vol/vol) interleukin 2 (Cellular Bioproducts, Buffalo, NY) at 37°C in 5% CO_2. Fluorescence-activated cell sorter (FACS) analysis of the TJF-2 cell line showed it to be uniformly positive for T3 (pan-T-cell marker), T9 (transferrin receptor), T11 (sheep erythrocyte receptor), Tac (interleukin 2 receptor), and Ia (activated-T-cell marker). Approximately half the cells were T4-positive and half were T8-positive. The cells were uniformly negative for B1 (pan-B-cell marker) and for T6 and T10 (intrathymic lymphocyte markers). GM2756 and GM4258A, well-characterized ADA-deficient human B-cell lines, were obtained from the Human Genetic Mutant Cell

Abbreviations: ADA, adenosine deaminase; HTLV-I, human T-lymphotrophic virus type I; LTR, long terminal repeat; SCID, severe combined immunodeficiency; SV40, simian virus 40; bp, base pair(s); kb, kilobase(s).

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Repository (Camden, NJ). Control ADA-positive B-cell line JM was established from cells of a normal donor by Epstein–Barr virus infection. CEM cells, also used as an ADA-positive control, are a well-characterized lymphoblastoid T-cell line.

SAX Vector Transduction of ADA-Deficient Cells. Confluent monolayers of PA-12 cells producing SAX-containing viral particles were changed from Dulbecco's modified Eagle's medium with 10% FBS to RPMI 1640 with 10% FBS 24 hr before transduction. The SAX-producing cells were irradiated (1500 rads; 1 rad = 0.01 Gy) just prior to transduction, in order to eliminate fibroblast contamination of T and B cells after cocultivation. ADA-deficient T or B cells (10^5–10^7) were cocultured with the monolayers (containing 5×10^6 SAX-producing PA-12 cells) for 24 hr in the presence of Polybrene (2 μg/ml). After transduction, the lymphocytes were removed from the monolayer, grown in culture for 24 hr to allow adherence of any residual fibroblasts, pelleted, and resuspended in fresh medium. These cells were then either grown in mass culture or, in some cases, grown in medium containing G418 (1.0 mg/ml) for 3 weeks, to enrich for SAX-containing cells, prior to analysis.

Southern Blotting. DNA was prepared from cell lines by the method of Gross-Bellard *et al.* (22). Thirty micrograms of genomic DNA was digested with *Sac* I or *Eco*RI restriction endonuclease under the conditions specified by the supplier (*Sac* I, Boehringer Mannheim; *Eco*RI, New England Biolabs) and then subjected to Southern blot analysis (23) as previously described (11). The probe was a nick-translated 1.6-kilobase (kb) *Hin*dIII–*Bam*HI fragment containing the neo^R gene isolated from pNeo (P-L Biochemicals). The copy number of approximately one was estimated based on band intensity compared with the intensity of other single-copy genes run in comparison.

Assays for ADA Activity. The [^{14}C]adenosine assay for ADA activity was performed essentially as described by Van der Weyden and Bailey (24). The results were calculated as nanomoles of inosine produced per min per 10^8 cells. The starch gel electrophoretic analysis for ADA isozymes was performed by the method of Spencer *et al.* (25).

Assay of 2′-Deoxyadenosine Resistance. As a functional assay for restoration of ADA activity, the inhibition of cell proliferation by 2′-deoxyadenosine was compared between ADA-deficient T (or B) cells before and after ADA gene transduction. Exponentially growing cells were plated in 96-well flat-bottomed microwell plates (Costar, Cambridge, MA) at 50,000 cells per well in 200 μl of RPMI 1640 with 10% heat-inactivated horse serum, 10% (vol/vol) interleukin 2, and various concentrations of 2′-deoxyadenosine (Sigma) in triplicate. Cells were then incubated for 24 hr at 37°C in 5% CO_2, after which [^{3}H]thymidine incorporation over 4 hr was measured. Results are expressed as percent inhibition of [^{3}H]thymidine incorporation by cells in the presence of deoxyadenosine compared to untreated cells. The concentration of deoxyadenosine that led to 50% inhibition (IC_{50}) was calculated from the dose–response curves.

RESULTS

Construction of the SAX Vector. The ADA gene-containing vector SAX was cloned in a multistep fashion. The parental plasmid vector N2 is a Moloney murine leukemia virus-based vector with the region coding for viral structural genes deleted and the bacterial neomycin-resistance gene (neo^R) inserted as a dominant selectable marker (11). A fusion gene was created between the simian virus 40 (SV40) promoter and the ADA structural gene as described in the legend to Fig. 1. This SV40 promoter-ADA gene fusion product was inserted into the single *Xho* I site of the N2 vector with the same polarity as the parental vector, so that the initiation of transcription of the ADA gene would occur in the SV40 promoter and terminate in the 3′ long terminal repeat (LTR) of the vector (Fig. 1). The name SAX thus stands for S (SV40 promoter), A (human ADA gene), X (inserted into the *Xho* I site of N2).

FIG. 1. Map of SAX vector. SAX was made by inserting a SV40-promoted human ADA cDNA into the previously described (11) parental vector N2. A fusion gene was created between the SV40 promoter and the ADA structural gene by placing the 400-base-pair (bp) *Kpn* I–*Hin*dIII fragment containing the enhancing and promoting elements of the SV40 early promoter immediately upstream of a 1300-bp sequence containing the full-length ADA cDNA [*Eco*RI–*Acc* I fragment of clone ADA 211 (26)]. The following regions are indicated: 0–1.5 and 4.7–5.5 kb, Moloney murine leukemia virus sequences; 1.5–2.8 and 4.6–4.7 kb, neomycin-resistance gene (neo^R) from Tn*5* transposon (the hatched area is the coding sequence); 2.9–3.3 kb, *Kpn* I–*Hin*dIII fragment of the SV40 early promoter; 3.3–4.6 kb, human ADA cDNA (hADA, black box); LTR, viral long terminal repeat; ψ, viral packaging signal. Restriction sites: S, *Sac* I; P, *Pst* I; E, *Eco*RI; C, *Cla* I.

Characterization of the SAX Vector. The SAX vector was initially used to infect 3T3 cells. After infection and selection in medium containing 0.5 mg of G418 per ml, the cells were biochemically analyzed. By blot hybridization analysis of electrophoretically fractionated poly(A)$^+$ RNA, three vector-specific transcripts were shown to be present, two presumably initiating within the 5′ LTR and the other, shorter transcript presumably initiating within the SV40 promoter (data not shown).

Gene Transfer into T and B Cells. This SAX retroviral vector was used to transduce the human ADA gene into ADA-deficient T and B cells. The ADA-deficient T-cell line TJF-2 was established from a 2-year-old patient (JF) with ADA-deficient SCID by infection of peripheral blood mononuclear cells with human T-lymphotrophic virus type I (HTLV-I), using the procedure of Mitsuya *et al.* (21). Two control normal T-cell lines (K7 and HM) were similarly established by HTLV-I infection. Southern blot analysis of the transduced T- and B-cell lines showed that the integrated proviral vector DNA is intact (Fig. 2). Since *Sac* I cuts once in each LTR (Fig. 1), an intact provirus would release a 4.9-kb fragment. This fragment was present in *Sac* I-digested DNA from SAX-transduced TJF-2 (lane 1) and two SAX-transduced B-cell lines (lanes 2 and 3) but was absent in the nontransduced T-cell line (lane 5). Further analysis with the restriction endonuclease *Eco*RI, which produces a 3.3-kb internal fragment (lanes 6–8), confirmed that at this level of analysis the vector DNA present in the transduced cells is intact. Quantitative analysis of Southern blots from a population of SAX-transduced TJF-2 cells revealed that they contain, on the average, one proviral copy per cell. The overall efficiency of infection ranged between 23% and 50% in different experiments as determined by DNA dot blot analysis of individual clones of T cells derived by limiting dilution following SAX infection (data not shown).

Expression of the Transferred Genes in T and B Cells. Starch gel electrophoresis for ADA isozymes was performed on cell lysates to assess the expression of the introduced gene (Fig. 3). The control B- (lane 1) and T- (lane 7) cell lines show a normal pattern of three human ADA isozymes. ADA-deficient B (lane 2) and T (lane 4) cells show no ADA activity. However, after transduction with the SAX vector, the nonselected uncloned population of cells shows ADA activity

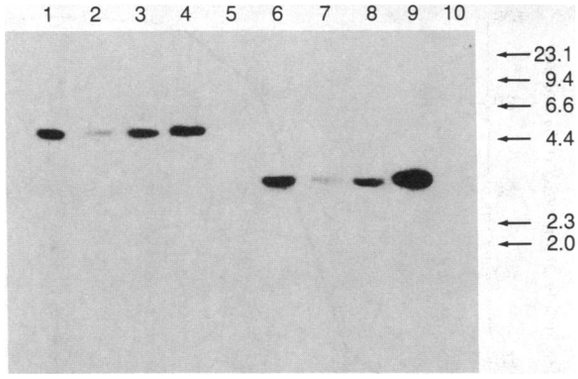

FIG. 2. Southern blot of ADA-deficient and ADA-deficient, SAX-transduced T- and B-cell lines. Thirty micrograms of genomic DNA was loaded per lane except for lanes 2 and 7, which received 10 μg. Lanes 1–3: *Sac* I-digested DNA from TJF-2 (lane 1), GM4258A (lane 2), and GM275 (lane 3) cells transduced with SAX virus and selected with G418. Lane 4: SAX plasmid DNA (30 pg) digested with *Sac* I. This amount of plasmid gives a band intensity equivalent to ≈1 gene copy per genome. Lane 5: *Sac* I-digested DNA from uninfected TJF-2 cells. Lanes 6–8: *Eco*RI-digested DNA from TJF-2 (lane 6), GM4258A (lane 7), and GM2756 (lane 8) cells transduced with SAX virus and selected with G418. Lane 9: SAX plasmid DNA (30 pg) digested with *Eco*RI. Lane 10: *Eco*RI-digested DNA from uninfected TJF-2 cells. Markers at right show migration positions and sizes (kb) of *Hin*dIII fragments of phage λ DNA.

comparable to the ADA$^+$ B- and T-cell normal controls (lanes 3 and 5). Therefore, the transduced ADA-deficient T and B cells produce apparently normal ADA enzyme. The production of enzymatically active protein is not due to the activation of the endogenous mutant ADA gene as a nonspecific consequence of retroviral infection, since cells transduced with a vector similar to SAX but not containing the ADA gene (viz., the parental N2 vector) do not produce enzymatically active ADA protein (Table 1).

ADA enzymatic activity in lysates of the T- and B-cell lines was quantitated by measuring the conversion of [^{14}C]adenosine to [^{14}C]inosine (Table 1). ADA-deficient T- and B-cell lines produced ≈1% of the adenosine-deaminating activity of normal T and B cells, in agreement with previous reports (27, 28) in which a non-ADA aminohydrolase was detected in ADA-deficient cells with ≈1% of normal activity. After introduction of a normal ADA gene by the SAX vector, these cells produced ADA activity at levels approximately half that of normal cell lines (Table 1). The expression of the introduced human ADA gene in human T and B cells is dependent upon the type of promoter used to facilitate transcription of the ADA gene. The ADA vector SAX, containing the early promoter of SV40 (a virus with tropism for primate cells), produced a considerably greater increase in ADA activity in TJF-2, GM2756, and GM4258A cells than similar vectors promoting ADA gene transcription with either the mouse metallothionein or the Moloney LTR gene promoter (data not shown).

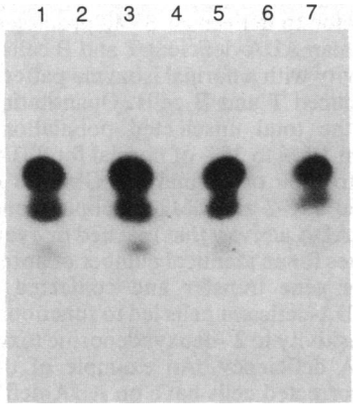

FIG. 3. Starch gel electrophoresis of ADA-deficient and ADA-deficient, SAX-transduced T- and B-cell lines. Lysate from 2 × 10^6 cells was added per lane. *In situ* chemical staining for human isozymes of ADA was done according to the method of Spencer *et al.* (25). Lane 1: ADA-positive B-cell line VSD-O. Lane 2: ADA-deficient B cells (GM2756). Lane 3: ADA-deficient B cells (GM2756) transduced with SAX virus and selected in G418. Lane 4: ADA-deficient T cells (TJF-2). Lane 5: ADA-deficient T cells (TJF-2) transduced with SAX virus and selected in G418. Lane 6: blank. Lane 7: ADA-positive T-cell line CEM.

Table 1. ADA activity and susceptibility to inhibition by 2′-deoxyadenosine of normal ADA-positive, ADA-deficient, and SAX-transduced T and B cells

Cell type	ADA activity*	IC_{50},† μM
T-cell lines		
HM (normal ADA$^+$)	413 ± 55	2940
TJF-2 (ADA$^-$)		
Not transduced	4.7 ± 1.5	100
SAX-transduced	147 ± 46	280
SAX-transduced, selected‡	1020 ± 136	2600
SAX-transduced, selected;‡ + 10 μM 2′-deoxycoformycin	<1.0	<100
N2-transduced	4.5	110
B-cell lines		
JM (normal ADA$^+$)	454 ± 119	8100
GM2756 (ADA$^-$)		
Not transduced	2.7 ± 0.4	210
SAX-transduced	314 ± 99	1100
SAX-transduced, selected‡	1250 ± 134	7400

*The [^{14}C]adenosine assay for ADA activity was performed essentially as described by Van der Weyden and Bailey (24). Results are expressed as nmol of inosine produced per min per 10^8 cells (mean ± SEM for 5 determinations, except N2-transduced TJF-2, for which the value is the result of a single determination).
†Concentration of 2′-deoxyadenosine that inhibits incorporation of [^{3}H]thymidine to 50% of control value after 24 hr of culture.
‡Grown for 3 weeks in medium containing G418 at 1 mg/ml.

The T and B cells transduced with the SAX vector also express a second enzyme, neomycin phosphotransferase (the product of the *neo*^{R} gene), at levels adequate for selection in medium containing the neomycin analogue G418. Growth of cells in the presence of G418 allows the evaluation of ADA expression in an enriched population of transduced cells because cells do not survive in the presence of G418 unless they express the *neo*^{R} gene. Selection of the SAX-transduced cells with G418 (1 mg/ml) resulted in cell populations with levels of ADA activity in the normal range (Table 1).

Protection of SAX-Transduced TJF-2 Cells from 2′-Deoxyadenosine Toxicity. Proliferation of ADA-deficient lymphocytes is inhibited by much lower concentrations of 2′-deoxyadenosine than is the proliferation of normal lymphocytes (29). Populations of ADA-deficient T and B cells that were transduced with the SAX vector were tested for growth inhibition in the presence of 2′-deoxyadenosine. Both T and B cells treated with this vector showed a substantially increased resistance to 2′-deoxyadenosine (Fig. 4 and Table 1). SAX-transduced cells isolated after selection with G418 (1 mg/ml) were fully restored to normal levels of resistance to 2′-deoxyadenosine toxicity. Addition of 2′-deoxycoformycin, a specific inhibitor of ADA activity (30), rendered these transduced cells sensitive to 2′-deoxyadenosine, demonstrating that the resistance to 2′-deoxyadenosine of the SAX-transduced cells is, in fact, due to the production of functional ADA enzyme activity (Table 1).

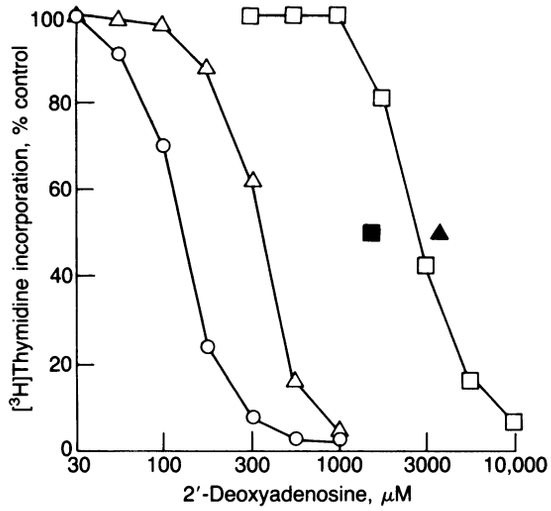

FIG. 4. Inhibition of proliferation of TJF-2 cells by 2'-deoxyadenosine. ○, TJF-2; △, SAX-transduced TJF-2; □, SAX-transduced, G418-selected TJF-2. Points ■ and ▲ represent 50%-inhibitory concentrations for K7 and HM, two nonleukemic ADA-positive T-cell lines derived by transformation with HTLV-I during one typical experiment, presented for reference. All points represent the geometric means of 3–5 separate experiments, each with triplicates.

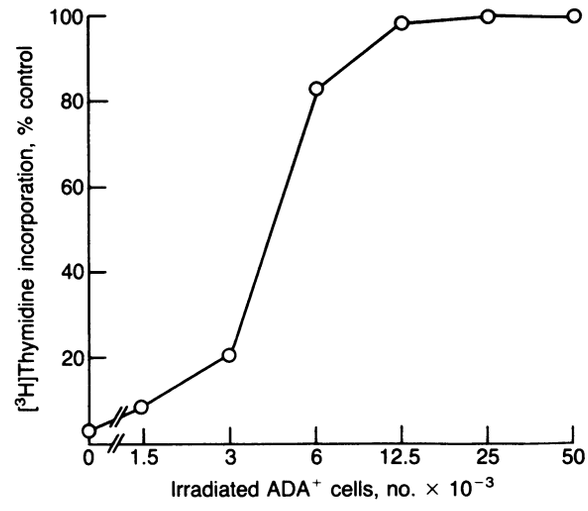

FIG. 5. Reversal of 2'-deoxyadenosine inhibition of proliferation of TJF-2 cells by addition of ADA-positive cells. The ADA-positive cells were TJF-2 transduced by SAX and selected in G418. Prior to addition of these cells to cultures of 50×10^3 ADA-deficient TJF-2 cells, they were lethally irradiated (10,000 rads). 2'-Deoxyadenosine was present at 300 μM. One hundred percent thymidine incorporation was 35,000 cpm. Fifty thousand irradiated SAX-transduced TJF-2 cells incorporated 316 cpm.

Individuals heterozygous for ADA deficiency, as well as rare individuals with partially defective ADA protein, may be immunologically normal and yet have only a fraction (5–50%) of the normal concentration of ADA enzyme activity (14, 17). Furthermore, transfusions with ADA-positive erythrocytes can occasionally improve the immune function of ADA-deficient SCID patients (31–33). It appears, therefore, that a small percentage of ADA-positive cells can partially correct the metabolic disturbances in ADA deficiency and restore immune function in some patients. To test whether a similar effect could be observed *in vitro*, we added increasing proportions of irradiated (10,000 rads; 1 rad = 0.01 Gy), SAX-transduced, G418-selected TJF-2 cells, as a nonproliferating source of detoxifying ADA activity, to ADA-deficient TJF-2 cells cultured in inhibiting concentrations of 2'-deoxyadenosine. Fig. 5 shows that in the presence of 300 μM 2'-deoxyadenosine, proliferation of the ADA-deficient T cells was restored to normal levels when 10–20% of the cells in the cultures were ADA-positive. This experiment not only demonstrates that functional ADA was obtained by gene transfer with SAX but also suggests that beneficial *in vivo* effects may be obtained when only a minority of the patient's lymphoid cells are transduced with the ADA gene.

DISCUSSION

Genetic diseases in which the primary pathology is localized to the lymphohematopoietic system are likely to be early candidates for gene therapy, since the tissue can be readily explanted, manipulated *ex vivo*, and reimplanted. Retroviruses provide an efficient means by which this type of genetic manipulation can occur. Part of the retroviral life cycle involves the infection of susceptible cells, followed by the reverse transcription of its RNA into double-stranded circular DNA and then the integration of this DNA into the host cell's genome. This system has been manipulated to facilitate the transduction of exogenous genes into hematopoietic cells several orders of magnitude more efficiently than other gene-transfer systems such as, for example, calcium phosphate precipitation (1). Retroviral vectors have been utilized to successfully introduce the bacterial neo^R (2, 4), human hypoxanthine phosphoribosyltransferase (3, 5, 7), human ADA (6), murine granulocyte/macrophage colony-stimulating factor (8), and murine dihydrofolate reductase (13) genes into murine (2, 6, 8) or human (3, 7) hematopoietic cells *in vitro* or into mouse bone marrow *in vivo* (4, 5, 13). Retroviral vectors have also been used to introduce the neo^R gene at high efficiency into long-lived stem cells of murine bone marrow *in vivo* (9–11). Recently, we have obtained preliminary data demonstrating that the SAX vector can successfully transfer the ADA gene into primates by means of an autologous bone marrow transplantation protocol (12).

Two prior reports (6, 34) have demonstrated construction of retroviral vectors containing the human ADA gene. Both of the vectors produced ADA transcripts using the Moloney LTR promoter. Introduction of these vectors into murine lymphoid (6) or 3T3 cells (34) resulted in levels of human ADA activity equal to or greater than the level of murine enzyme activity. In this report, we have constructed and used the SAX vector to deliver an SV40-promoted human ADA gene into human ADA-deficient T and B cells. Human ADA enzyme activity with a normal isozyme pattern was detected in the transduced T and B cells. Quantitation of the ADA activity in the total unselected population indicated an increase from 1.1% to 35% of normal for TJF-2 (T cells) and from 0.6% to 69% of normal for GM2756 (B cells). The G418-selected TJF-2 and GM2756 populations both demonstrated total ADA activity that reached or even exceeded the range of values for an identical number of normal human T or B cells. The gene transfer and conferred ADA enzyme activity in ADA-deficient cells led to functional correction of the hypersensitivity to 2'-deoxyadenosine toxicity characteristic of ADA deficiency. An example of the effects that genetically corrected cells have on ADA-deficient cells was shown when irradiated, SAX-transduced cells were used as a source of enzyme to detoxify 2'-deoxyadenosine in the medium of ADA-deficient cells.

For gene therapy to be clinically useful, the transferred gene must be capable of expressing enzymatically active protein in physiologic quantities in the proper target cells. T cells or their precursors are the cells that are most severely affected in ADA deficiency. Peripheral lymphoid cells, especially T lymphocytes, are not readily obtainable from patients with ADA deficiency due to the severe lymphopenia

that is a characteristic finding in SCID. To our knowledge, the TJF-2 cell line represents the first ADA-deficient T-cell line available to assess ADA functional activity. It is not clear, however, what relation HTLV-I-immortalized T cells have to normal T cells, much less to the pluripotent stem cell (or the lymphoid-lineage stem cell) that would be the cell that needs to be corrected *in vivo* in ADA deficiency. Nonetheless, the experiments described here, together with the studies previously published (2–13), do provide encouragement that retroviral-based vectors may provide the clinical means for correcting ADA deficiency and other genetic diseases.

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1. Anderson, W. F. (1984) *Science* **226,** 401–409.
2. Joyner, A., Keller, G., Phillips, R. A. & Bernstein, A. (1983) *Nature (London)* **305,** 556–558.
3. Miller, A. D., Jolly, D. J., Friedmann, T. & Verma, I. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4709–4713.
4. Williams, D. A., Lemischka, I. R., Nathan, D. G. & Mulligan, R. C. (1984) *Nature (London)* **310,** 476–480.
5. Miller, A. D., Eckner, R. J., Jolly, D. J., Friedmann, T. & Verma, I. M. (1984) *Science* **225,** 630–632.
6. Friedman, R. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 703–707.
7. Gruber, H. E., Finley, K. D., Hershberg, R. M., Katzman, S. S., Laikind, P. K., Seegmiller, J. E., Friedmann, T., Yee, J.-K. & Jolly, D. J. (1985) *Science* **230,** 1057–1061.
8. Lang, R. A., Metcalf, D., Gough, N. M., Dunn, A. R. & Gonda, T. J. (1985) *Cell* **43,** 531–542.
9. Dick, J. E., Magli, M. C., Huszar, D., Phillips, R. A. & Bernstein, A. (1985) *Cell* **42,** 71–79.
10. Keller, G., Paige, C., Gilboa, E. & Wagner, E. F. (1985) *Nature (London)* **318,** 149–154.
11. Eglitis, M. E., Kantoff, P. W., Gilboa, E. & Anderson, W. F. (1985) *Science* **230,** 1395–1398.
12. Kantoff, P., Eglitis, M., Kohn, D., McLachlin, J., Zwiebel, J., Gillio, A., Hutton, J. J., O'Reilly, R., Blaese, M., Gilboa, E. & Anderson, W. F. (1985) *Blood* **66,** 268a (abstr.).
13. Williams, D. A., Hsieh, K. & Mulligan, R. C. (1985) *Blood* **66,** 138a (abstr.).
14. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii,** 1067–1075.
15. Hirschhorn, R., Vawter, G. F., Kirkpatrick, J. A., Jr., & Rosen, F. S. (1979) *Clin. Immunol. Immunopathol.* **14,** 107–120.
16. Fox, I. H. & Kelley, W. N. (1978) *Annu. Rev. Biochem.* **47,** 655–686.
17. Adrian, G. S., Wiginton, D. A. & Hutton, J. J. (1984) *Hum. Genet.* **68,** 169–172.
18. Cohen, A., Hirschhorn, R., Horowitz, S. D., Rubinstein, A., Polmar, S. J., Hong, R. & Martin, D. W., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 472–476.
19. Coleman, M. S., Donofrio, J., Hutton, J. J., Hahn, L., Daoud, A., Lampkin, B. & Dyminski, J. (1978) *J. Biol. Chem.* **253,** 1619–1626.
20. Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C. & Axel, R. (1977) *Cell* **11,** 223–232.
21. Mitsuya, H., Guo, H., Megson, M., Trainor, C., Reitz, M. S., Jr., & Broder, S. (1984) *Science* **223,** 1293–1296.
22. Gross-Bellard, M., Oudet, P. & Chambon, P. (1973) *Eur. J. Biochem.* **36,** 32–38.
23. Southern, E. (1975) *J. Mol. Biol.* **98,** 503–517.
24. Van der Weyden, M. B. & Bailey, E. (1978) *Clin. Chim. Acta* **82,** 179–184.
25. Spencer, N., Hopkinson, D. A. & Harris, H. (1968) *Ann. Hum. Genet.* **32,** 9–14.
26. Adrian, G. S., Wiginton, D. A. & Hutton, J. J. (1984) *Mol. Cell. Biol.* **4,** 1712–1717.
27. Schrader, W. P., Pollara, B. & Meuwissen, H. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 446–450.
28. Daddona, P. E. & Kelley, W. N. (1981) *Biochim. Biophy. Acta* **658,** 280–290.
29. Hirschhorn, R., Bajal, S., Borkowsky, W., Kowalski, A., Hong, R., Rubinstein, A. & Papageorgiou, P. (1979) *Cell. Immunol.* **42,** 418–423.
30. Agarwal, R. P., Spector, T. & Parks, R. E., Jr. (1977) *Biochem. Pharmacol.* **26,** 359–367.
31. Polmar, S. H., Stern, R. C., Schwartz, A. L., Wetzler, E. M., Chase, P. A. & Hirschhorn, R. (1976) *N. Engl. J. Med.* **295,** 1337–1343.
32. Hutton, J. J., Wiginton, D. A., Coleman, M. S., Fuller, S. A., Limouze, S. & Lampkin, B. C. (1981) *J. Clin. Invest.* **68,** 413–421.
33. Dyminski, J. W., Daoud, A., Lampkin, B. C., Limouze, S., Donofrio, J., Coleman, M. S. & Hutton, J. J. (1979) *Clin. Immunol. Immunopathol.* **14,** 307–326.
34. Valerio, D., Duyvesteyn, M. G. C. & van der Eb, A. J. (1985) *Gene* **34,** 89–94.