Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 703–707, February 1985
Biochemistry

Expression of human adenosine deaminase using a transmissable murine retrovirus vector system

(recombinant DNA/ψ2 cells/immunodeficiency)

RICHARD L. FRIEDMAN

Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

Communicated by Paul Berg, September 28, 1984

ABSTRACT **Human adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) was expressed at high levels in cultured mouse cells using a transmissable murine retrovirus vector system. A cDNA clone encoding ADA has been inserted into a plasmid vector containing retroviral transcription and packaging signals as well as a selectable gene for G418 resistance. The constructions were transfected into ψ2 cells, which package the recombinant retroviral genomes into replication-defective virus particles. Isoenzyme analysis for ADA in G418-selected ψ2 cells showed at least 20-fold more human ADA activity than endogenous mouse ADA activity. A mouse T-cell lymphoma line, BL/VL$_3$, was cocultured with transformed ψ2 cells producing human ADA, and some of the cocultured cells were selected for resistance to G418. Both G418-selected and unselected cocultured cells expressed human ADA activity at 25%–50% the level of the endogenous enzyme. Thus, efficient retroviral transduction of ADA expression was obtained.**

Adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) catalyzes the irreversible deamination of adenosine and deoxyadenosine. It is present in all human tissues, but lymphoid cells, particularly thymocytes, express the highest levels (1, 2). Deficiency of ADA activity is associated with ≈50% of autosomal recessive cases of severe combined immunodeficiency disease (SCID) (3–5), which is characterized by profound depletion of lymphoid tissue. The metabolic consequences of ADA deficiency have been investigated extensively (6, 7). Based on inhibitor studies and attempts to improve immune function by adding ADA, it appears likely that ADA deficiency causes SCID (6, 7, 8–10). Recently, several groups have isolated cDNA clones for human (11–13) and mouse (14) ADA.

It was of interest to develop a system to express ADA as a model for possible introduction of a functional ADA gene in ADA-deficient SCID. Several laboratories have now used retrovirus vector systems to introduce genes into cultured cells (15, 16–22). Retrovirus vector systems are advantageous because retroviruses infect a wide variety of cell types and transduce genes very efficiently. The SVX vector [pZIP-Neo SV(X)1] (described in ref. 23) was used for this work because it permits the simultaneous expression of a cDNA clone of interest and the Tn*5* *neo* gene, which confers resistance to the antibiotic G418 to mammalian cells (24). The SVX vector was used in conjunction with ψ2 (25), an NIH 3T3-derived cell line that contains an endogenous Moloney murine leukemia virus (MuLV) that lacks the sequence necessary for viral RNA packaging. Thus, ψ2 cells provide retroviral proteins in *trans* for packaging and reverse transcription of SVX genomes, allowing helper-free stocks of replication-defective recombinant retroviruses to be produced. A similar packaging system has now been developed for packaging recombinant SVX genomes into viruses that will infect human cells (26).

This paper describes the use of the SVX system to express human ADA in cultured mouse cells.

MATERIALS AND METHODS

Plasmid Construction. The retroviral expression vector pZIP-Neo SV(X)1 was generously provided by Richard Mulligan. *Eco*RI and *Eco*RI methylase, and T4 ligase were gifts from Paul Modrich and Stewart Scherer, respectively. All other enzymes were from Boehringer-Mannheim and New England Biolabs. *Eco*RI and *Bam*HI adapters, and *Eco*RI linkers were from Collaborative Research (Waltham, MA). All manipulations were according to established techniques (27).

Tissue Culture. The ψ2 cell line was a gift from Richard Mulligan and was maintained as described (25). Mouse 3T6 and NIH 3T3 cells were provided by Paul Berg and were grown in Dulbecco's modified Eagles medium supplemented with 5% newborn calf serum and 10% calf serum, respectively. BL/VL$_3$ cells, a radiation-induced leukemia virus (RadLV)-transformed mouse T-cell lymphoma line, were a gift from Irving Weissman and were grown as described (28). Virus titrations were performed as described (23). Cocultivation of ψ2-derived lines with BL/VL$_3$ cells was performed in RPMI-1640 medium supplemented with 10% fetal calf serum.

ADA Assays. Hemolysates were prepared as described (29) and extracts of tissue-culture cells were prepared as in ref. 30 except a ball-bearing homogenizer (W. E. Balch and J. E. Rothman, personal communication) was used instead of a motorized tissue grinder. Isoenzyme analysis was performed on 1% agarose gels (SeaKem Laboratories, Rockland, ME) using the buffer system and histochemical staining protocol described (29).

RESULTS

Construction of a Retrovirus Vector to Express Human ADA. Fig. 1 shows the strategy for vector construction. The ADA 211 cDNA clone described in ref. 11 has been shown by DNA sequencing (32) to contain a 1089-base-pair open reading frame starting with ATG, and a poly(A) addition site (A-A-T-A-A-A) at its extreme 3′ end. It has been shown that RNA polyadenylylation signals inserted into retrovirus vectors reduce the transduction efficiency of the recombinant genomes (15). A unique *Acc* I site in the 3′ untranslated region of ADA 211 was therefore used to delete the A-A-T-A-A-A sequence. Because ADA 211 contains a *Bam*HI site in the coding region for ADA, it was not possible to use

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency disease; MuLV, murine leukemia virus; RadLV, radiation-induced leukemia virus; LTR, long terminal repeat; kb, kilobase(s); env, envelope.

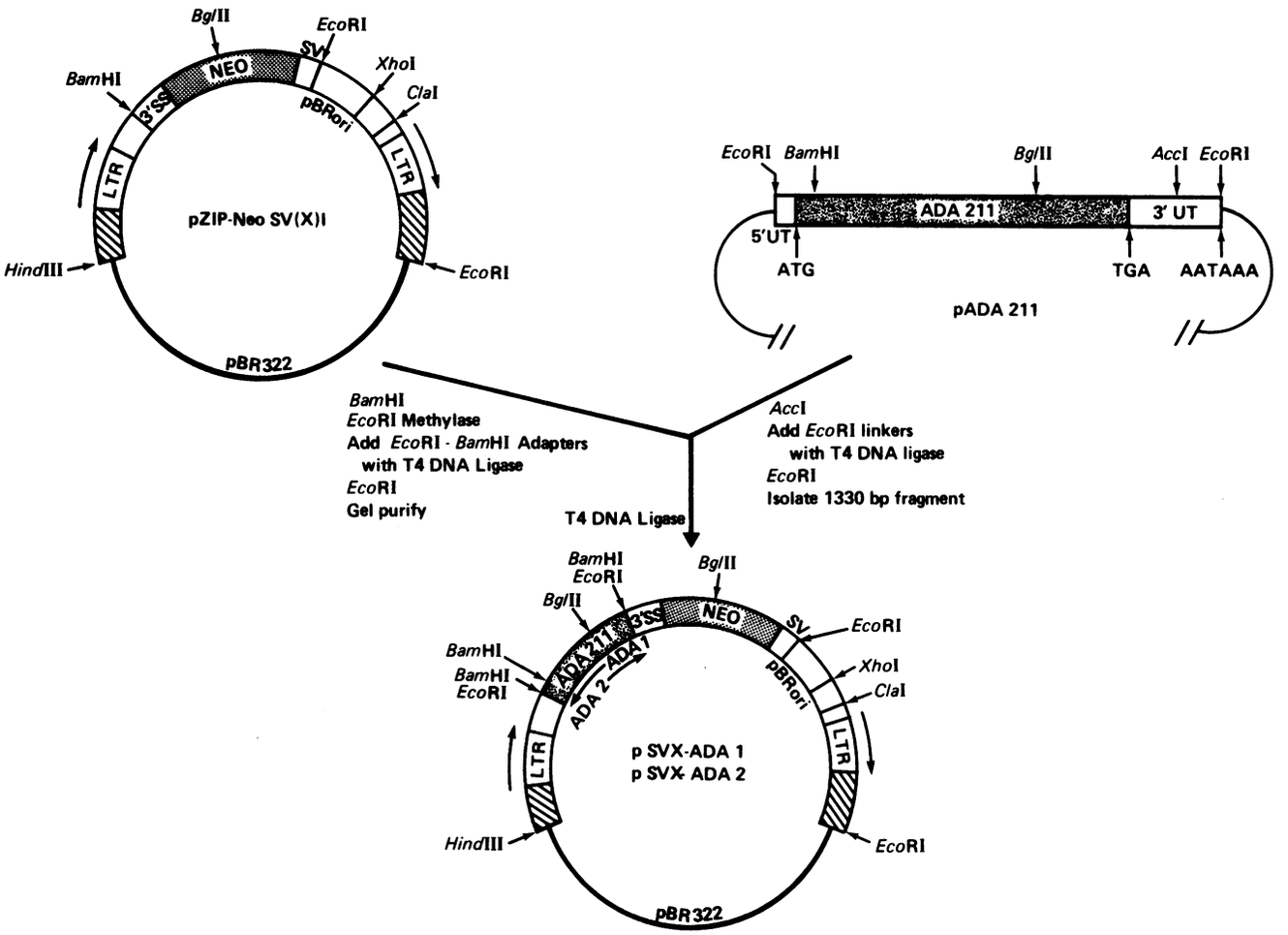

FIG. 1. Construction of retrovirus vectors containing human ADA sequences. pZIP-Neo SV(X)1 is described in ref. 23. pADA 211 (11) contains the ADA 211 cDNA subcloned in pBR325 (31). Orientations of ADA sequences in pSVX-ADA 1 and 2 were determined by digestion with *Bgl* II. NEO, Tn*5* neomycin resistance gene; 3'SS, Moloney MuLV 3' splice site; SV, simian virus 40 origin of replication; UT, untranslated region; bp, base pairs; pBRori, pBR322 origin of replication.

*Bam*HI linkers to insert the cDNA into the unique *Bam*HI site in the SVX plasmid. Instead, an *Eco*RI linker was added to the 3' end of ADA 211, and the *Bam*HI site of SVX was converted to an *Eco*RI site after *Eco*RI methylation and addition of *Eco*RI/*Bam*HI adapters. After transfection into *Escherichia coli* HB101, clones that contained the deleted ADA 211 cDNA in both the forward (ADA 2) and reverse (ADA 1) orientations with respect to the upstream long terminal repeat (LTR) of SVX were selected.

Transfected Mouse Fibroblasts Express High Levels of Human ADA. ψ2 cells were transfected (33) with pSVX-ADA 1 and ADA 2 as well as with the SVX vector. An isoenzyme analysis for human ADA was performed 48 hr post-transfection and the human protein was faintly detected only in cells that received pSVX-ADA 2. It was not possible to detect transient production of the SVX-derived RNAs (data not shown). The transfected ψ2 cells were selected with 400 μg of G418 per ml and the efficiency of transformations was $\approx$0.1% with SVX and SVX-ADA 1 and 0.01%–0.001% with SVX-ADA 2. The basis for the reduced transformation efficiency with ADA 2 constructs is not yet clear.

Production of human ADA was assayed in the G418-resistant populations to assess the integrity of the recombinant transcription units and to verify that the ADA 211 cDNA clone actually contained the entire protein-coding sequence. Each population contained $\approx$10 independent transformants. Fig. 2 shows the results of isoenzyme assays of G418-resistant cells that had been transfected with the three plasmids. No ADA activity in extracts of ψ2, ψNeo (SVX transformed), or ψADA 1 (pSVX-ADA 1 transformed) cells comigrates with the human erythrocyte ADA standard. All the transformed lines express equivalent amounts of mouse ADA. The ψADA 2A cells express $\approx$20-fold more of the human isoenzyme than the endogenous mouse isoenzyme and ψADA 2B cells express $\approx$5-fold more. Thus, ADA 211 must contain the entire coding sequence for ADA and it is highly likely that its expression is driven by the LTR promoter. Because simultaneous expression of ADA and the Tn*5* *neo* gene in SVX requires a functional LTR promoter, correct splicing, and correct termination and polyadenylylation of at least some of the RNAs, the recombinant transcription unit must also be intact.

Efficient Retroviral Transduction of Human ADA into Mouse Lymphoid Cells. The RadLV-transformed mouse T-cell lymphoma line BL/VL_3 (28) was cocultured for 4 days with ψADA 2A cells. Because ψADA 2A cells adhere to tissue culture flasks and BL/VL_3 cells do not, they are easily separated. Some of the non-adherent lymphoid cells were split into 24 wells (4×10^3 cells per well) and selected with 300 μg of G418 per ml, a concentration at which untransformed BL/VL_3 cells are killed. There was little cell death and no appreciable reduction in growth rate after 3 weeks of selection. Populations of cells were grown from 12 of the 24 wells and tested for ADA isoenzymes. All 12 produced human ADA. Fig. 3 shows the results for 5 of the 12 populations. The figure also shows an isoenzyme analysis of BL/VL_3 cells that were cocultured with ψADA 2A cells

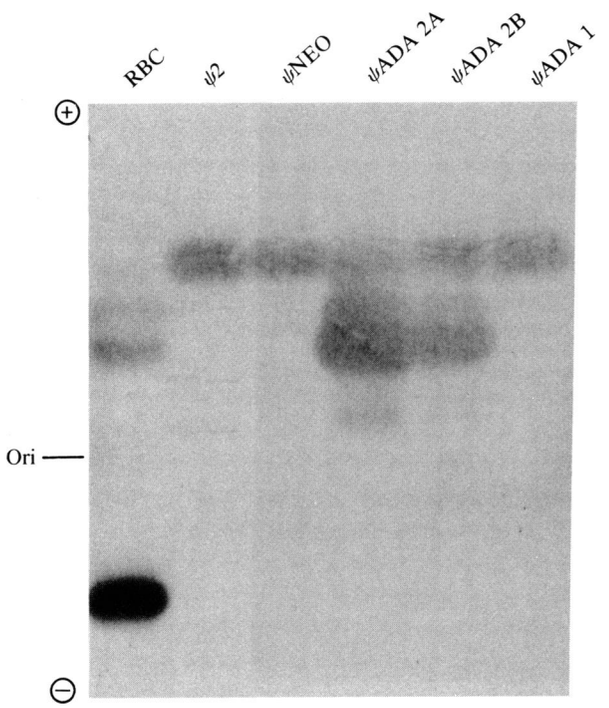

FIG. 2. ADA isoenzyme analysis of ψ2 cells transfected with SVX-derived plasmids. ψNeo, ψADA 2A and 2B, and ψADA 1 cells were transfected with pZIP-Neo SV(X)1, pSVX-ADA 2, and pSVX-ADA 1, respectively. RBC indicates human hemolysate used as a standard for migration of human ADA isoenzymes. Ori marks the origin of the gel, and the dark band in the RBC lane, near the negative electrode, is hemoglobin. Note that all of the ψ2 and ψ2-derived cell lines express similar levels of the mouse isoenzyme, which migrates more rapidly than the human isoenzyme.

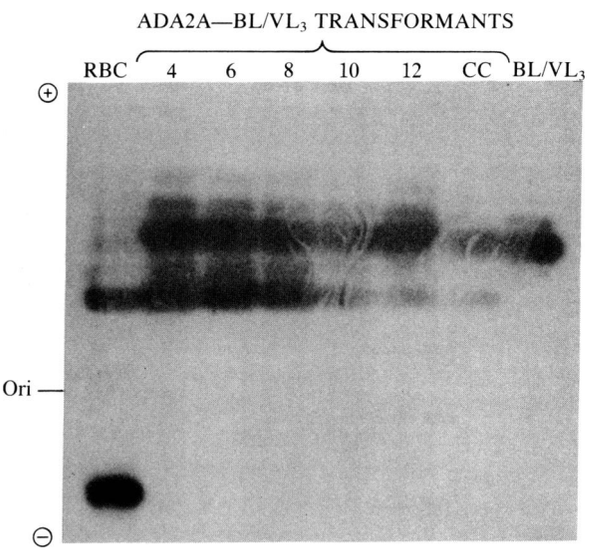

FIG. 3. ADA isoenzyme analysis of BL/VL_3 cells cocultured with ψADA 2A cells. RBC indicates human hemolysate used as a standard for human isoenzymes. The transformants labeled 4, 6, 8, 10, and 12 were assayed after selection with G418. CC indicates cells that were cocultured but not drug selected (see text).

for 3 days but not selected with G418 (CC in the figure). To ensure that there were no contaminating ψADA 2A cells, the non-adherent cells were split 1:10 into a fresh flask and cultured for 2 days to allow any contaminating fibroblasts to reattach. Microscopic examination showed that the cocultured BL/VL_3 cells were not contaminated with the much larger ψADA 2A cells. The isoenzyme analysis in Fig. 3 was intentionally overdeveloped so it would reproduce clearly, and the left side of the gel developed more than the right. Shorter development showed that, depending on the isolate, 25%–50% of the total ADA activity was in the human isoenzyme. There was little difference between cells that were selected with G418 and cells that were assayed without drug selection. Thus, transduction must be extremely efficient. The fact that the human enzyme constitutes only 25%–50% of the ADA activity in BL/VL_3 cells does not mean that ADA expression is less efficient in BL/VL_3 cells than in NIH 3T3 cells. The specific activity of the endogenous ADA in thymocyte extracts is considerably higher than that in fibroblasts (1, 2). In fact, ADA is a marker for differentiation of T-cell malignancies (34, 35).

The retroviral RNAs in the BL/VL_3 transductants were analyzed to determine whether the newly integrated proviruses had a structure consistent with that predicted from the organization of pSVX-ADA 2. Fig. 4*A* shows an RNA blot of RNAs from ψ2 and ψNeo cells. The 7.9-kilobase (kb) MuLV and 2.9-kb env mRNAs in ψ2 cells serve as convenient size standards and the lower of the two additional bands in ψNeo RNA is the spliced *neo* mRNA (the upper band is unspliced). Fig. 4*B* shows an RNA blot of total RNA from ADA-transformed and untransformed BL/VL_3 cells probed with pSVX-ADA 2. Three bands are apparent in the BL/VL_3 RNA and correspond to the full-length RadLV RNA, a 5.6-kb RadLV-derived mRNA, and the RadLV envelope mRNA (40). These RNAs are seen because of homology between RadLV and Moloney MuLV LTRs (Martin Scott, personal communication). Two additional bands, as well as some additional hybridization to the band at 5.6 kb, are seen in all 12 ADA 2A-BL/VL_3 transformants. The smaller of the new bands, at about 4.3 kb, corresponds to the spliced *neo* mRNA seen in the ψNeo cells. It is not yet clear whether the band just above the *neo* mRNA in the ADA-transformed lymphocytes is an artifact due to the presence of the 28S rRNA or represents an aberrant splicing product of the recombinant SVX-ADA 2A genome. The additional hybridization at 5.6 kb represents the full length ADA-containing retroviral mRNA expected to arise from the SVX-ADA 2A provirus. When the blot shown in Fig. 4*B* was washed and reprobed with pADA 211 (Fig. 1), the 5.6-kb band remained in all of the ADA 2A-BL/VL_3 transformants but could not be seen in untransformed BL/VL_3 cells (not shown). It is clear, however, that the 5.6-kb ADA-containing mRNA is not nearly as abundant as the spliced *neo* mRNA.

While transduction of ADA into BL/VL_3 cells by cocultivation was extremely efficient, efforts to introduce human ADA into mouse fibroblasts (3T6, NIH 3T3) by transduction with the retrovirus have not been successful. The lack of infectivity appears to be specific for ADA-containing constructs. Greater than 10^4 G418-resistant 3T6 cell colony-forming units/ml are readily obtained from tissue culture supernatants from populations of ψNeo cells. The infectivity of ψADA 2 supernatants is at least 2 orders of magnitude lower. Since ψ2 cells express a substantial level of the MuLV genomic and env mRNAs, it is not likely that inadequate production of retroviral proteins by the ψ2 cells explains the poor fibroblast infectivity of ADA 2 transformants. It is also conceivable that high level expression of ADA from the LTR promoter is toxic to some mouse fibroblast cell lines.

DISCUSSION

Human ADA has been expressed successfully in mouse fibroblasts transfected with SVX-ADA vector constructs and transduced into a mouse T-cell lymphoma, BL/VL_3. It is not yet clear why infection of the BL/VL_3 is so much more

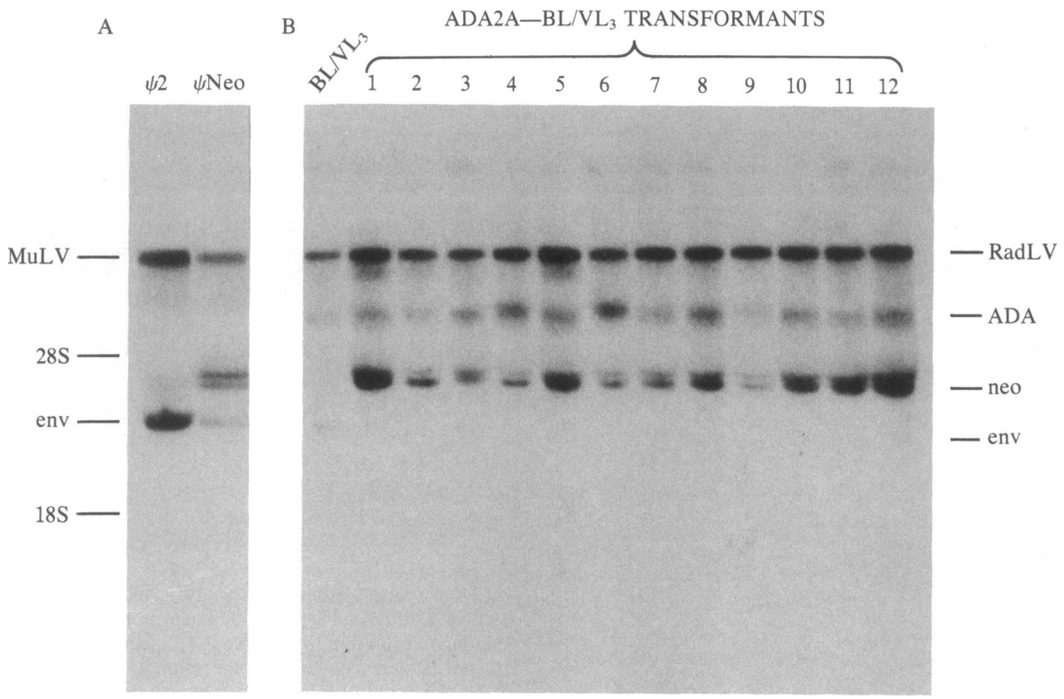

FIG. 4. RNA blot analysis of RNAs from BL/VL$_3$ cells that express human ADA. Total cellular RNA was prepared as described in ref. 36 from ψ2 cells, ψNeo cells, and BL/VL$_3$ cells that had been cocultured with ψADA 2A cells (see text). A fraction of each isolate (20 μg) was denatured, electrophoresed on a 1.2% agarose gel, and transferred to nitrocellulose as described (37). The blots were probed with nick-translated (38) pSVX-ADA 2 DNA at a specific activity of 10^8 cpm/μg and were washed as described (27). The mouse 28S and 18S rRNA size standards, at 5.0 and 1.95 kb, respectively (39), migrated identically on the two gels shown; thus, the RNA sizes in *A* and *B* may be compared directly. (*A*) RNA from ψ2 and ψNeo cells. The packaging-defective 7.9-kb Moloney MuLV genomic mRNA and the 2.9-kb spliced envelope mRNA produced in ψ2 cells are labeled MuLV and env, respectively, and are convenient size markers. The lower of the two additional bands in ψNeo cells is the 4.3-kb spliced neo mRNA and is labeled neo in *B*. (*B*) RNA from BL/VL$_3$ cells and G418-resistant BL/VL$_3$ cells after cocultivation with ψADA 2A cells. The locations of the 7.9-kb RadLV genomic mRNA, the 4.3-kb spliced neo mRNA derived from the transduced SVX-ADA 2A provirus, and the 2.9-kb RadLV envelope mRNA are labeled RadLV, neo, and env, respectively. The location of the 5.6-kb ADA mRNA derived from the transduced provirus is labeled ADA. As explained in the text, there is also a 5.6-kb RadLV-derived RNA in BL/VL$_3$ cells that shares homology with the Moloney virus LTR and can thus be seen in the control BL/VL$_3$ lane at the left of the figure.

efficient than infection of either 3T6 or NIH 3T3 cells. One possible explanation is that the BL/VL$_3$ cells, which harbor RadLV, were actually infecting ψADA 2A cells with RadLV, generating a pseudotyped virus or providing helper function. Two problems with this explanation are that RadLV infects fibroblasts poorly (28, 40) and BL/VL$_3$ cells would be expected to be resistant to superinfection with a RadLV-pseudotyped SVX virus. It is also possible that the cell-to-cell contact during cocultivation results in far more efficient infection than is possible with virus stocks alone. The low abundance of the full-length ADA-containing mRNA in transformed BL/VL$_3$ cells seems to favor the latter explanation. Poor production of the genomic retroviral mRNA would result in low virus titers. If the band seen to migrate just above the *neo* mRNA in ADA-transformed BL/VL$_3$ cells (Fig. 4*B*) represents an aberrantly spliced mRNA arising from the recombinant retroviral genome, it may explain the poor production of the full length mRNA.

We are currently trying to isolate cell lines that package ADA-transducing virus more efficiently. We are also attempting to use the SVX system to introduce ADA into mouse hematopoietic stem cells as described in ref. 21. These experiments lay the technical groundwork for the introduction of a functional ADA gene into human cells that lack or that are defective for that function. If ADA is expressed in high levels in erythrocytes in reconstituted mice, it will be possible to determine whether a mild inherited hemolytic anemia (41) can be caused by overproduction of ADA in erythrocytes.

Many questions must be answered before attempts to introduce the ADA gene into ADA-deficient SCID can be considered. It is not yet clear that ADA-deficient patients actually possess stem cells capable of differentiating into mature T and B cells. There is, however, evidence that ADA-deficient patients do have some T-cell precursors that can differentiate into mature T cells when cultured in the presence of normal thymic epithelial cells (9, 10). Even if ADA-deficient SCID patients have normal lymphoid stem cells, it is not clear what therapeutic effect the introduction of the ADA gene might have. One virtue of this approach in ADA-deficient SCID is that removal of the postulated block to stem-cell differentiation would allow lymphoid cells to proliferate; that is, those cells that receive the gene would have a selective advantage. On the other hand, because SCID patients are unable to reject bone marrow grafts, they are ideal candidates for marrow transplantation. Excellent results have been obtained using T cell-depleted marrow from HLA-haploidentical parental donors (42, 43). The striking success of bone marrow transplantation in SCID requires a careful assessment of risks and benefits of alternative therapies before they are attempted.

I thank Warren Liao for many helpful discussions and constructive suggestions, Irving Weissman for his helpful suggestions, and I gratefully acknowledge Richard Mulligan for providing the SVX vector and communicating results prior to publication. I am also grateful to Paul Berg for his insightful comments on this manuscript. In particular, I would like to thank George Stark for his encouragement and support, without which this work would not have been possible. This investigation was supported by Public Health Service Grant CA30124 to Dr. Stark from the National Cancer Institute.

R.L.F. is a trainee of the Medical Scientist Training Program of the National Institutes of Health.

1. Van der Weyden, M. & Kelley, W. N. (1976) *J. Biol. Chem.* **251,** 5448–5456.
2. Adams, A. & Harkness, R. A. (1976) *Clin. Exp. Immunol.* **26,** 647–649.
3. Giblett, E. R., Anderson, J. E., Cohen, F., Pllara, B. & Meuwrissen, H. J. (1972) *Lancet* **ii,** 1067–1069.
4. Ackeret, L., Plüss, H. J. & Hitzig, W. H. (1976) *Pediatr. Res.* **10,** 67–70.
5. Hirschhorn, R., Vawter, G. F., Kirkpatrick, J. A. & Rosen, F. S. (1979) *Clin. Immunol. Immunopathol.* **14,** 107–120.
6. Martin, D. W. & Gelfand, E. W. (1981) *Annu. Rev. Biochem.* **50,** 845–877.
7. Thompson, L. F. & Seegmiller, J. E. (1980) *Adv. Enzymol.* **51,** 167–210.
8. Polmar, S. H., Wetzler, E. M., Stern, R. C. & Herschhorn, R. (1975) *Lancet* **ii,** 743–746.
9. Shore, A., Dosch, H.-M. & Gelfand, E. M. (1981) *Clin. Exp. Immunol.* **44,** 152–155.
10. Gelfand, E. M. & Dosch, H.-M. (1982) *Clin. Immunol. Immunopathol.* **25,** 303–315.
11. Wiginton, D. A., Adrian, G. S., Friedman, R. L., Suttle, D. P. & Hutton, J. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7481–7485.
12. Orkin, S. H., Daddona, P. E., Schewach, D. S., Markham, A. F., Burns, G. A., Goff, S. C. & Kelley, W. N. (1983) *J. Biol. Chem.* **258,** 12753–12756.
13. Valerio, D., Duyvesteyn, M. G. C., Meera Khan, P., Geurts van Kessel, A., de Waard, A. & van der Eb, A. J. (1983) *Gene* **25,** 231–240.
14. Yeung, C.-Y., Frayne, E. G., Muayyad, R. A.-V., Hook, A. G., Ingolia, D. E., Wright, D. A. & Kellems, R. E. (1983) *J. Biol. Chem.* **258,** 15179–15185.
15. Shimitohno, K. & Temin, H. W. (1981) *Cell* **26,** 67–77.
16. Wei, C.-M., Gibson, M., Spear, P. G. & Scolnick, E. M. (1981) *J. Virol.* **39,** 935–944.
17. Tabin, C. J., Hoffman, J. W., Goff, S. P. & Weinberg, R. A. (1982) *Mol. Cell. Biol.* **2,** 426–436.
18. Miller, A. D., Jolly, D. J., Friedmann, T. & Verma, I. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4709–4713.
19. Joyner, A., Keller, G., Phillips, R. A. & Bernstein, A. (1983) *Nature (London)* **305,** 556–558.
20. Joyner, A. L. & Bernstein, A. (1983) *Mol. Cell. Biol.* **3,** 2180–2190.
21. Williams, D. A., Lemischka, I. R., Nathan, D. G. & Mulligan, R. C. (1984) *Nature (London)* **310,** 476–480.
22. Hellerman, J. G., Cone, R. C., Potts, J. T., Rich, A., Mulligan, R. C. & Kronenberg, H. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5340–5344.
23. Cepko, C. L., Roberts, B. E. & Mulligan, R. C. (1984) *Cell* **37,** 1053–1062.
24. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1,** 327–341.
25. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33,** 153–159.
26. Cone, R. D. & Mulligan, R. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6349–6353.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
28. Lieberman, M., Decleve, A., Ricciardi-Castagnoli, P., Boniver, J., Finn, O. J. & Kaplan, H. S. (1979) *Int. J. Cancer* **24,** 168–177.
29. Harris, H. & Hopkinson, D. A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics* (Elsevier, New York).
30. Yeung, C.-Y., Riser, M. E., Kellems, R. E. & Siciliano, M. J. (1982) *J. Biol. Chem.* **258,** 8330–8337.
31. Bolivar, F. (1978) *Gene* **4,** 121–136.
32. Adrian, G. S., Wiginton, D. A. & Hutton, J. J. (1984) *Mol. Cell. Biol.* **4,** 1712–1717.
33. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31,** 360–369.
34. Coleman, M. S., Greenwood, M. F., Hutton, J. J., Lampkin, B., Krill, C. & Kastelic, J. E. (1978) *Blood* **52,** 1125–1136.
35. Chechik, B. E., Jason, J., Shore, A., Baker, M., Dosch, H.-M. & Gelfand, E. W. (1979) *Blood* **54,** 1400–1406.
36. Chirgwin, J. M., Przbyla, A. E., MacDonald, R. S. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
37. Thomas, P. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
38. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
39. Lewin, B. (1980) in *Gene Expression* (Wiley, New York), 2nd Ed., pp. 866–873.
40. Manteuil-Brutlag, S., Liu, S. & Kaplan, H. S. (1980) *Cell* **19,** 643–652.
41. Paglia, D. E. & Valentine, W. N. (1981) *Clin. Haematol.* **10,** 81–98.
42. Reisner, Y., Kapoor, N., Kirkpatrick, D., Pllack, M., Cunningham-Rundles, S., Dupont, B., Hodes, M. Z., Good, R. A. & O'Reilly, R. J. (1983) *Blood* **61,** 341–347.
43. Friedrich, W., Vetter, U., Heymer, B., Reisner, Y., Goldmann, S. F., Fliedner, T. M., Peter, H. H. & Kleihauer, E. (1984) *Lancet* **i,** 761–764.