Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 473–477, January 1990
Medical Sciences

Human gene transfer: Characterization of human tumor-infiltrating lymphocytes as vehicles for retroviral-mediated gene transfer in man

(gene therapy/neomycin-resistance gene)

ATTAN KASID*, SHOSHANA MORECKI*, PAUL AEBERSOLD*, KENNETH CORNETTA†, KENNETH CULVER‡, SCOTT FREEMAN‡, ELAINE DIRECTOR*, MICHAEL T. LOTZE*, R. MICHAEL BLAESE‡, W. FRENCH ANDERSON†, AND STEVEN A. ROSENBERG*

*Surgery and ‡Metabolism Branches, National Cancer Institute, and †Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Christian B. Anfinsen, October 26, 1989

ABSTRACT **Tumor-infiltrating lymphocytes (TILs) are cells generated from tumor suspensions cultured in interleukin 2 that can mediate cancer regression when adoptively transferred into mice or humans. Since TILs proliferate rapidly *in vitro*, recirculate, and preferentially localize at the tumor site *in vivo*, they provide an attractive model for delivery of exogenous genetic material into man. To determine whether efficient gene transfer into TILs is feasible, we transduced human TILs with the bacterial gene for neomycin-resistance (*Neo*^{R}) using the retroviral vector N2. The transduced TIL populations were stable and polyclonal with respect to the intact *Neo*^{R} gene integration and expressed high levels of neomycin phosphotransferase activity. The *Neo*^{R} gene insertion did not alter the *in vitro* growth pattern and interleukin 2 dependence of the transduced TILs. Analyses of T-cell receptor gene rearrangement for β- and γ-chain genes revealed the oligoclonal nature of the TIL populations with no major change in the DNA rearrangement patterns or the levels of mRNA expression of the β and γ chains following transduction and selection of TILs in the neomycin analog G418. Human TILs expressed mRNA for tumor necrosis factors (α and β) and interleukin 2 receptor P55 but not for interleukin 1β, granulocyte/macrophage colony-stimulating factor, interleukin 6, and interferon γ when grown with high-dose interleukin 2 without subsequent activation with mitogen or specific antigen. This pattern of cytokine-mRNA expression was not significantly altered following the transduction of TILs. The *Neo*^{R} gene-transduced TILs could thus be used to follow the trafficking and survival of TILs *in vivo*, and clinical protocols using these transduced TILs in cancer patients have begun. The studies demonstrate the feasibility of TILs as suitable cellular vehicles for the introduction of therapeutic genes into patients receiving autologous TILs.**

Tumor-infiltrating lymphocytes (TILs) are lymphoid populations found associated with solid tumors and can be selectively grown in the presence of interleukin 2 (IL-2) (1, 2). Recent clinical trials have shown that the adoptive transfer of TILs in conjunction with IL-2 can result in cancer regression in some patients with advanced, otherwise refractory, cancers (1). Studies of the administration of human TILs labeled with ^{111}In have shown that TILs recirculate and preferentially localize at tumor sites by several days after infusion (3). There has been no method available, however, to study long-term distribution and survival of autologous human TILs *in vivo*. We have proposed to use an inserted neomycin-resistance (*Neo*^{R}) gene (4, 5) into human TILs as a cell label to answer these questions. It has recently been shown that the *Neo*^{R} gene can be inserted into murine lymphocytes and human TILs (K. Culver, K. Cornetta, S.M., P.A., S.F., A.K., R. Morgan, M.T.L., S.A.R., W.F.A., and R.M.B., unpublished results). This report represents results of studies to transduce and characterize the *Neo*^{R}-transduced TIL populations resulting from retroviral-mediated gene transfer.

MATERIALS AND METHODS

Transduction of Human TILs. Human TILs from six patients with melanoma were prepared as described (1, 2). Early cultures of TILs were transduced by exposing them to culture supernatant from the packaging cell line PA317 that produced N2 containing virions (5, 7, 8). The titer of the virus ranged from 2 to 10 × 10^5 colony-forming units/ml and the producer line expressed high levels of neomycin phosphotransferase (NPT). The cells were cultured with the viral supernatant in the presence of protamine sulfate (5 μg/ml) for 2 hr at 37°C. The transduced cells were selected for the *Neo*^{R} gene expression by exposure to the neomycin analog G418 (300–400 μg/ml of active drug) for 10–15 days beginning 2 days after infection. The resistant TIL populations were expanded from five patients; TIL cultures from patient number 2 could not be analyzed because of contamination problems. The supernatants from the transduced TIL lines contained no infectious virus as assessed by S$^+$L$^-$ assay.

DNA and RNA Analyses. Restriction enzyme-digested DNA (15 μg per lane) was electrophoresed in 0.8% agarose, and the separated fragments were transferred to nitrocellulose membranes and hybridized overnight at 42°C with ^{32}P-labeled probes (specific activity, >10^8 dpm/μg) in 6× SSC/10% dextran sulfate/50 mM Tris·HCl, pH 7.5/1 mM EDTA containing denatured salmon sperm DNA at 100 μg/ml (1× SSC = 0.15 M NaCl/15 mM sodium citrate, pH 7). Blots were washed three times in 1× SSC for 90 min, followed by a high stringency wash in 0.1× SSC at 55°C for 30 min. Autoradiography was carried out at −70°C using Kodak XAR-5 films with intensifying screening.

For Northern blots, RNA was extracted from 10^8 cells by the guanidinium isothiocyanate/cesium chloride ultracentrifugation method (9); this was followed by electrophoresis on a 1% agarose/2.2 M formaldehyde gel and blot transfer to a nitrocellulose membrane. The quality, equivalent loading, and RNA transfer were assured by ethidium bromide staining of the gels before and after the Northern transfers and rehybridization of RNA blots with ^{32}P-labeled human γ-actin probe (10).

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: TIL, tumor-infiltrating lymphocyte; NPT, neomycin phosphotransferase; IL, interleukin; TCR, T-cell antigen receptor; C, constant; D, diversity; J, joining; V, variable.

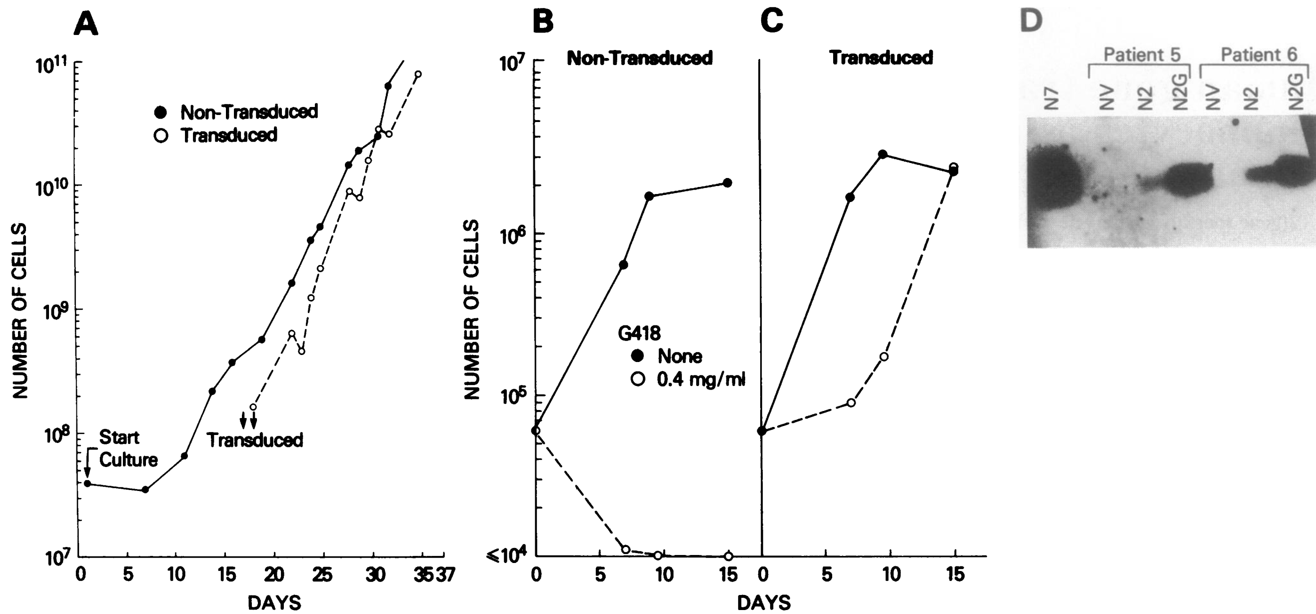

FIG. 1. Long-term growth (*A*), effect of G418 on the proliferation (*B* and *C*), and NPT activity (*D*) of transduced TILs. TIL cultures established from malignant melanomas were grown at a density of 2–5 × 10^5 cells per ml in 1000 units of recombinant IL-2 per ml and exposed to N2 supernatant (multiplicity of infection = 3) in the presence of protamine sulfate at 5 μg/ml. A portion of the infected culture was placed under selection (400 μg of G418 per ml) for 10 days, beginning 2 days after infection. For *in situ* NPT assay, cell lysates were electrophoresed on a nondenaturing SDS/polyacrylamide gel. The gel was then overlaid with agarose containing kanamycin (25 μg/ml) and 2 nM [γ-^{32}P]ATP (6000 Ci/mmol; 1 Ci = 37 GBq) followed by filter transfer of ^{32}P-labeled kanamycin to Whatman P81 paper and autoradiography. N7, an N2-transfected G418-selected PA317 producer line (2 × 10^5 cells); NV, nontransduced (5 × 10^6 cells); N2, transduced unselected (5 × 10^6 cells); N2G, transduced G418-selected (5 × 10^6 cells).

RESULTS

A characteristic example of the growth of nontransduced and transduced TILs in IL-2 is shown in Fig. 1*A*. An aliquot of TILs from this patient was transduced on days 17 and 18 of growth. The *Neo*^{R} gene-transduced TILs remained dependent on IL-2 for growth, and their proliferation was similar to that of nontransduced TILs. To confirm the *Neo*^{R} gene expression in transduced TILs, cells were analyzed for their growth in medium containing G418 (Fig. 1*B* and *C*) and also for the presence of intracellular NPT activity (Fig. 1*D*). Whereas nontransduced TILs did not survive G418 selection, transduced TILs continued to proliferate in toxic concentrations (400 μg/ml) of G418 at a rate similar to that of the nontransduced TILs. Similar results were obtained by comparing the growth of nontransduced and transduced selected cells from other patients. The transduced TILs expressed high levels of NPT activity (Fig. 1*D*), which was undetectable in lysates of nontransduced cells. The NPT activity was also detectable in transduced but unselected cells, although at a lesser level (Fig. 1*D*).

We next confirmed the presence of proviral sequences in transduced TIL populations by hybridization of *Sac* I-digested DNA with the ^{32}P-labeled *Neo*^{R} probe. Results in Fig. 2 show that a single copy of the expected 3.2-kilobase (kb) *Neo*^{R} hybridizing fragment was present without rearrangement or deletion in all five transduced TIL populations but was undetectable in nontransduced cells. The integration of the vector DNA and NPT activity of the transduced TILs was stable and invariant during extended cell cultures (tested at 8–10 weeks after transduction) even in the absence of G418 (Figs. 1*D* and 2). DNA was also digested with *Sca* I, a restriction enzyme that does not cut within the provirus; thus a unique fragment reflecting its integration in the host genome is specific for each transduced cell, because viral integration is an apparently random process (8). As expected, *Sca* I-digested DNA revealed multiple clones of transduced TILs containing proviral DNA integrated into different sites of the host chromosome (Fig. 2). Some transduced TILs grown in G418 beyond 8–10 weeks did develop prominent *Neo*-hybridizing bands on a smeared background (patients 4 and 6) that may be due to several reasons, including the selected growth advantage of some clones, toxicity of the gene expression in some clones, and preferred integration sites. The fact that vector DNA and NPT activity could be detected, though less distinctly in transduced TILs that were never selected for growth in G418 (Figs. 1*D* and 2), suggests that TIL cultures were transduced at an efficiency >10–20%.

Since the effective use of the *Neo*^{R} gene-transferred TILs in clinical studies in humans is dependent on the fact that the transduced TILs are not substantially altered in their properties, we performed studies of phenotypic and functional characterization of TILs. The antigen specificity of T lymphocytes is derived from the specific rearrangement and expression of T-cell antigen receptors (TCRs) (11–13). In addition to the α/β heterodimer that codes for the complete TCR molecule in thymocytes and peripheral blood T cells, two additional TCR genes, γ/δ, have recently been identified (14–18). To test for the presence of specific T-cell lineage and clonal rearrangements as well as the expression of TCR genes, we used human genomic and cDNA probes specific for the β and γ subunits of the TCR.

Representative Southern blot analyses of a single patient's nontransduced, transduced, and transduced selected TILs (number 6) are shown in Fig. 3 *A*, *B*, and *C*, respectively. Results from four other preparations including transduced selected cells were similar and TILs from each patient showed a novel pattern of gene rearrangement that was not altered by *Neo*^{R} gene insertion (data not shown). The germ-line fragment of 11.5 kb in *Eco*RI-digested DNA (containing the Jβ1-Cβ1 gene clusters) was replaced by bands of 8.5 and 7.5 kb representing the rearranged β-chain gene and suggest oligoclonal origins of the TIL population (Fig. 3*A*). Similarly, the 24-kb germ-line band of *Bam*HI-digested DNA is replaced by a distinct rearranged band (≈19 kb) and two other less distinct bands (≈14 and ≈12 kb), further suggesting the oligoclonality of the TIL cultures (Fig. 3*C*). The apparent loss

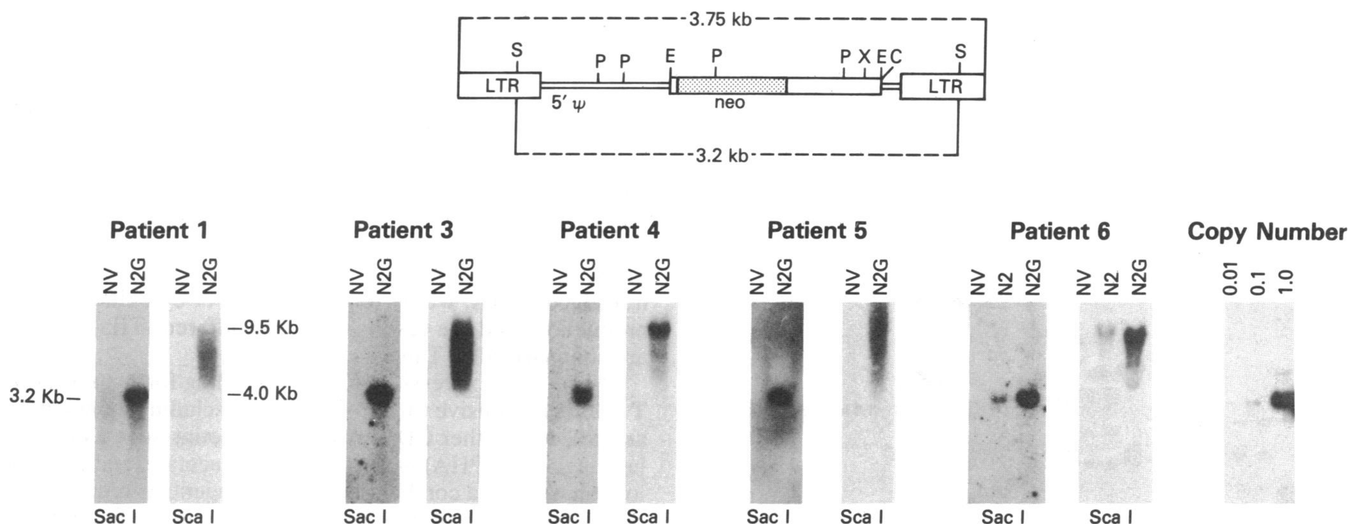

FIG. 2. Detection and integration of vector DNA (N2) in transduced human TILs. Total cellular DNA (15 μg) was isolated from TILs and was digested to completion with appropriate restriction enzymes. The DNA was then electrophoresed in 0.8% agarose gels. The fragments were transferred to nitrocellulose filters and hybridized to a ^{32}P-labeled *Neo*^{R} probe encompassing the coding region. *Sac* I cleaves once in the long terminal repeats (LTRs) flanking the inserted proviral sequence, thus excising a 3.2-kb *Neo*^{R}-containing proviral DNA fragment. *Sac* I does not cleave within the proviral sequences; thus a unique fragment reflecting its integration in the host genome is detected. The copy number was determined by mixing nontransduced DNA (15 μg) with the N2 plasmid DNA in proportions corresponding to 0.01, 0.1, and 1.0 viral genome copy per cell before digesting with *Sac* I enzyme. NV, no vector—i.e., nontransduced; N2, transduced unselected; N2G, transduced G418-selected TILs.

or reduction of 14- and 12-kb bands of N2G TILs may be attributed to outgrowth of a clonal population with limited rearrangement. The other TIL samples contained only the 4-kb *Eco*RI fragment; this suggests that both alleles of Cβ1 have been deleted and rearrangement into the Cβ2 region has occurred (data not shown). Similarly, a *Hin*dIII-digested germ-line fragment of 7.5 kb is replaced by several bands representing V-D-Jβ2 rearrangement in TIL cultures (Fig. 3*B*). Importantly, there was no significant difference in the patterns of β-chain gene rearrangements between the nontransduced, transduced unselected, and transduced G418-selected TIL populations. We also performed Northern analysis of RNA using the Cβ probe (Fig. 3 *D* and *E*). In each case, a 1.3-kb form of full transcript containing V-D-J-C sequences was detected. Additionally, in some TIL cultures we have also detected the 1.0-kb truncated form of the β transcript (containing D-J-C or J-C sequences), consistent with the observation of others in murine T cells (Fig. 3*E*) (19). In all the cases, there were no qualitative or quantitative changes in the expression of the more prevalent 1.3-kb as well as the truncated 1.0-kb transcript following transduction with the N2 gene (Fig. 3 *D* and *E*).

The human TCR-γ gene is composed of at least two C-region genes as well as nine V-region genes that apparently can rearrange independently with each of the Cγ genes (14, 18). Southern blots (Fig. 4) showed specific rearrangements of γ genes that were not significantly different in the transduced and selected TIL populations from all five patients tested. The nongerm-line hybridizing fragments in these TIL cultures represent rearrangements of a limited number of Vγ

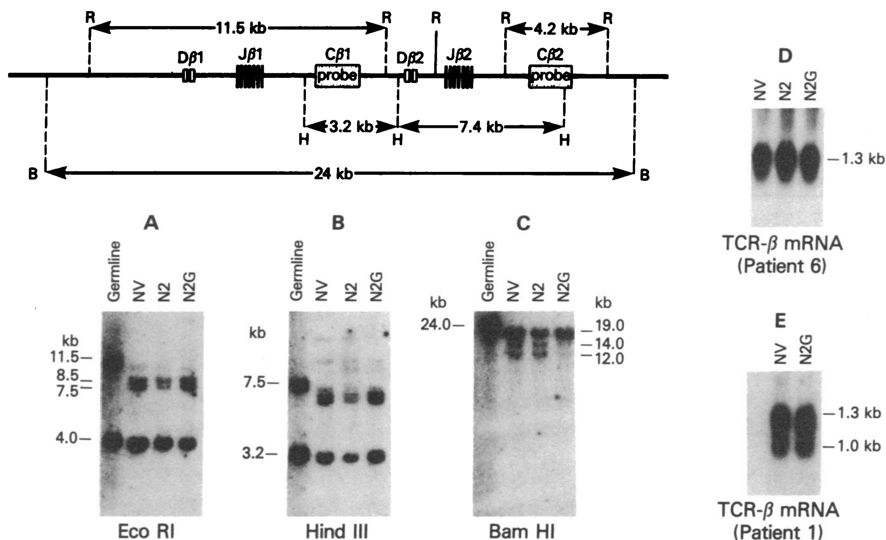

FIG. 3. Mapping and expression of TCR-β gene rearrangements in N2-transduced TILs. High molecular weight DNA was isolated, restriction enzyme digested, and hybridized with a ^{32}P-labeled human Cβ probe. Results shown here are for TILs from patient 6. Cβ probe detects rearrangements involving Cβ1 and Cβ2 loci (19). For mRNA expression (*D* and *E*) Northern blot analyses of the TCR-β gene using the Cβ probe was carried out as described in the legend to Table 1. NV, nontransduced; N2, transduced unselected; N2G, transduced G418-selected TILs. C, D, and J indicate constant, diversity, and joining, respectively.

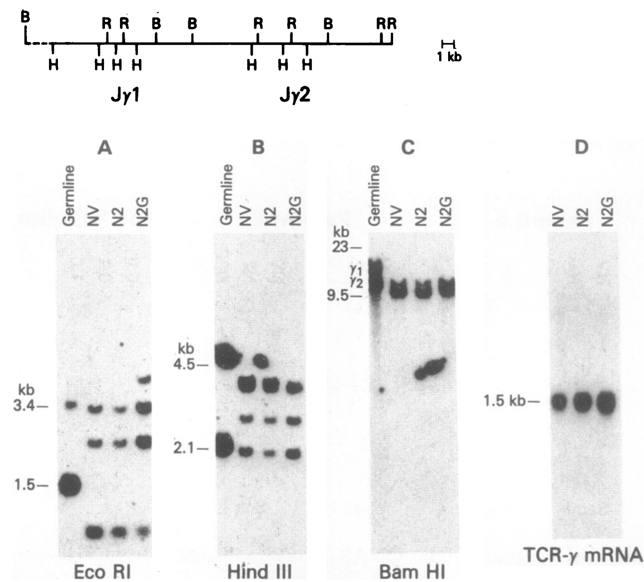

FIG. 4. Mapping of TCR-γ gene rearrangements in N2-transduced TILs. Restriction enzyme-digested DNA blots were hybridized with a 710-base-pair (bp) *Hin*dIII–*Eco*RI human Jγ probe (17). Because the two known human Jγ regions are highly homologous, the probe detects Jγ1.3 and Jγ2 loci. Northern blot analyses of the TCR mRNA (*D*) were carried out using a human γ-cDNA probe (19). NV, nontransduced; N2, transduced unselected; N2G, transduced G418-selected TILs.

genes, further supporting the oligoclonal nature of the TIL populations at the time of investigation. The higher molecular weight band seen in N2G (*Eco*RI digest) was not reproducible and may be an artifact. Although the γ gene transcript (Fig. 4*D*) was slightly smaller than the 1.7-kb γ message reported in other systems (14–18), its expression was unchanged by transduction and selection of cells in G418. The role of the γ gene is unclear because the majority of these messages in human and murine T-cell systems seem to be nonfunctional. However, human TILs express the TCR-β polypeptide on their cell surface (data not shown). Taken together these studies suggest that retroviral gene insertion was random and that the transduced populations were representative of the total TIL populations. Associated studies (Culver *et al.*, unpublished results) have shown that the phenotype and cytotoxicity profile of gene-transduced TILs was similar to those of native TILs.

The regulated and coordinated expression of the genes coding for cytokines plays an essential role in the control of the growth, differentiation, and function of immune cells. Cytokine production may also provide a useful marker for functionally characterizing T-cell subpopulations and may play an important role in mediating the antitumor effects seen *in vivo* when the cells are adoptively transferred (20–23). Thus, in evaluating the therapeutic potential of N2-transduced TILs, we performed studies of cytokine-mRNA production, and the results with five different TIL cultures are summarized in Table 1.

The *in vitro* expression of mRNA coding for cytokines in T cells is responsive to various agents, including monoclonal antibodies to either CD3 or the TCR, lectins such as phytohemagglutinin (PHA) or Con A, phorbol acetate, and calcium ionophore (20); a combination of those agents is required for optimal induction of the cytokine genes in normal peripheral blood lymphocytes and cultured T-cell lines. Here we show that high-dose IL-2 alone (1000 units/ml) was sufficient to induce mRNA for tumor necrosis factors α and β (TNF-α and TNF-β, lymphotoxin) and the IL-2 receptor, Tac (Table 1). However, under similar conditions, mRNA expression for IL-1β, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-6, and interferon γ (γ-IFN) was not detected in these TILs; the transcripts were, however, detectable in TILs cultured with phorbol myristate acetate and PHA (data not shown). This result is of interest because these TILs differ from classical cytotoxic T-lymphocyte clones, which do not release TNF-α or TNF-β upon addition of IL-2 but require specific antigen or anti-TCR antibodies (20). The pattern of cytokine-mRNA expression was not detectably altered in the transduced TILs (Table 1) following their transduction compared to nonselected cells (five patients) and cells selected in G418 (two patients). This is particularly important since insertional activation of cellular genes by retroviruses can affect normal gene expression.

DISCUSSION

Retroviral-mediated gene transfer has provided an efficient means for transferring genes with stable integration and high expression in several cell types, including bone marrow cells (6, 8, 30). We describe here the successful use of a retroviral

Table 1. Expression of cytokine-mRNA in transduced TILs

Patient	Vector	TNF-α (1.6 kb)	LT (1.3 kb)	Tac (1.5 kb and 3.5 kb)	IL-6 (1.3 kb)	GM-CSF (1.0 kb)	IL-1β (1.8 kb)	γ-IFN (1.2 kb)	γ-Actin (2.0 kb)
1	NV	+	+	+	–	–	–	–	+
	N2	+	+	+	–	–	–	–	+
3	NV	+	+	+	–	–	–	–	ND
	N2	+	+	+	–	–	–	–	ND
4	NV	+	+	1.5 kb only	–	–	–	–	ND
	N2	+	+	1.5 kb only	–	–	–	–	ND
5	NV	+	+	+	–	–	–	–	ND
	N2	+	+	+	–	–	–	–	ND
	N2G	+	+	+	–	–	–	–	ND
6	NV	+	+	+	–	–	–	–	+
	N2	+	+	+	–	–	–	–	+
	N2G	+	+	+	–	–	–	–	+

NV, nontransduced; N2, transduced unselected; N2G, transduced G418-selected. Human probes used were as follows: tumor necrosis factor α (TNF-α) (24), the 800-bp *Eco*RI cDNA fragment; TNF-β, lymphotoxin (LT) (25), the 950-bp *Eco*RI cDNA fragment; granulocyte/macrophage colony-stimulating factor (GM-CSF) (26), the 800-bp *Xho* I cDNA fragment; IL-6 (G. C. Wong and S. C. Clark, Genetics Institute), the 1.2-kb *Xho* I cDNA fragment; Tac (27), the 937-bp *Eco*RI cDNA fragment; IL-1β (D. Carter, Upjohn Company), the 810-bp *Pst* I cDNA fragment; interferon γ (γ-IFN) (28), the 960-bp *Hin*dIII/*Bst*EII cDNA fragment; γ-actin cDNA, pHFγA-1 (10). The fragments were excised, purified, and ^{32}P-labeled by a random priming method (29). The size(s) of the transcripts detected is given in parentheses beneath each probe. +, Detected; –, not detected; ND, not determined.

vector to transfer the *Neo*^{R} gene efficiently into human TILs and provide a biological system whereby the feasibility of human gene transfer can be tested in man. The data presented here have a number of important implications. Since we have been able to stably infect all of the five TIL cultures with high efficiency and the *Neo*^{R} gene is efficiently expressed in transduced TILs, it appears that TILs from most individuals will be susceptible to gene transfer by retroviral vectors. The procedure of gene transduction has not significantly altered the growth rate, IL-2 dependence, phenotype, and normal cytokine gene expression of TIL populations. In other studies we show that the lytic potential and the expression of cell surface markers are not significantly altered following transduction and selection of TILs in G418 (Culver *et al.*, unpublished results). Moreover, the transduced TILs do not appear to represent a restricted population originating from a few clones of transduced cells but appear generally to be representative of the total TIL population and should therefore be useful for *in vivo* studies of the survival and distribution of TILs.

At present there are no reliable methods available for long-term marking of TILs in the body. Following their infusion, the *Neo*^{R} gene-transduced TILs may be readily detectable by the polymerase chain reaction technique in blood or the tumor biopsy samples, even if the transduced TILs are present as a fraction of the total cells. The information obtained from this technique may be important to optimize TIL immunotherapy. Finally, efficient expression of the *Neo*^{R} gene in TILs and our previous demonstration that TILs selectively traffic to tumor sites in the few days after their transfer (3) suggest that TILs may be an ideal delivery system for the introduction and tumor targeting of other genes capable of augmenting TIL immunotherapy, perhaps, for example, by enhanced local production of selected cytokines.

We thank the scientists and firms cited for their probes and reagents.

1. Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Topalian, S. L., Toy, S. T., Simon, P., Lotze, M. T., Yang, J. C., Seipp, C. A., Simpson, C., Carter, C., Bock, S., Schwartzentruber, D., Wei, J. P. & White, D. E. (1988) *N. Engl. J. Med.* **319,** 1676–1680.
2. Topalian, S. L., Solomon, D. & Rosenberg, S. A. (1989) *J. Immunol.* **142,** 3714–3725.
3. Fisher, B., Packward, B. S., Read, E. J., Carrasquillo, J. A., Carter, C. S., Topalian, S. L., Yang, J. C., Yolles, P., Larson, S. M. & Rosenberg, S. A. (1989) *J. Clin. Oncol.* **7,** 250–261.
4. Eglitis, M. A., Kantoff, P., Gilboa, E. & Anderson, W. F. (1985) *Science* **230,** 1395–1398.
5. Miller, D. A. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6,** 2895–2890.
6. Dzierzak, E. A., Pabayannopoulou, T. & Mulligan, R. C. (1988) *Nature (London)* **331,** 35–38.
7. Cornetta, K. & Anderson, W. F. (1989) *J. Virol. Methods* **23,** 187–194.
8. Anderson, W. F. (1984) *Science* **226,** 401–409.
9. Chirgwin, J. M., Przybyla, R. Y., MacDonald, R. Y. & Rutter, W. J. (1977) *Biochemistry* **18,** 5284–5299.
10. Gunning, P., Ponte, P., Okayama, H., Engel, J. & Blau, H. (1983) *Mol. Cell. Biol.* **3,** 787–795.
11. Kronenberg, M., Siu, G., Hood, L. E. & Shastri, N. (1986) *Annu. Rev. Immunol.* **4,** 529–591.
12. Caccia, N., Toyonaga, B., Kimura, N. & Mak, T. K. (1988) in *The T-Cell Receptors*, ed. Mak, T. W. (Plenum, New York), pp. 9–45.
13. Marrack, P. & Kappler, J. (1987) *Science* **238,** 1073–1079.
14. Strominger, J. L. (1989) *Cell* **57,** 895–898.
15. Brenner, M. B., McLean, J., Dialynas, D. P., Strominger, J. C., Smith, J. A., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. (1986) *Nature (London)* **322,** 145–149.
16. Chien, Y., Iwashima, M., Wettstein, D. A., Kaplan, K. B., Elliott, J. F., Born, W. & Davis, M. M. (1987) *Nature (London)* **330,** 722–727.
17. LeFranc, M. P., Forster, A., Baer, R., Stinson, M. A. & Rabbitts, T. H. (1986) *Cell* **45,** 237–246.
18. Quertermous, T., Murre, C., Dialynas, D. P., Duby, A. D., Strominger, J. L., Waldmann, T. A. & Seidman, J. G. (1986) *Science* **231,** 252–255.
19. Sangster, R. N., Minowada, J., Suciu-Foca, N., Minden, M. & Mak, T. W. (1986) *J. Exp. Med.* **163,** 1491–1508.
20. Taniguchi, T. (1988) *Annu. Rev. Immunol.* **6,** 439–451.
21. Kasid, A., Director, E. P. & Rosenberg, S. A. (1989) *J. Immunol.* **143,** 736–739.
22. Kasid, A., Bell, G. I. & Director, E. P. (1988) *J. Immunol.* **141,** 690–698.
23. Belldegrun, A., Kasid, A., Uppenkamp, M., Topalian, S. L. & Rosenberg, S. A. (1989) *J. Immunol.* **142,** 4520–4526.
24. Pennica, D., Nedwin, G. E., Haytlick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. V. (1984) *Nature (London)* **312,** 724–729.
25. Gray, P. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarrett, J. A., Leung, D. W., Moffat, B., Ng, P., Svedersky, L. P., Palladino, M. A. & Nedwin, G. E. (1984) *Nature (London)* **312,** 721–725.
26. Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A. & Clark, S. C. (1985) *Science* **228,** 810–815.
27. Leonard, W. J., Depper, J. M., Grabtree, G. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Kronke, M., Svetlik, P. B., Peffer, N. J., Waldman, T. A. & Greene, W. C. (1984) *Nature (London)* **311,** 626–631.
28. Hauber, J., Perkins, A., Heimer, E. P. & Cullen, R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6364–6368.
29. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
30. Hock, R. A. & Miller, A. D. (1986) *Nature (London)* **320,** 275–277.