Respiratory Tract Gene Transfer

TRANSPLANTATION OF GENETICALLY MODIFIED T-LYMPHOCYTES DIRECTLY TO THE RESPIRATORY EPITHELIAL SURFACE*  

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To evaluate the strategy for potentially treating respiratory disorders with genetically modified T-lymphocytes, the interleukin-2 (IL-2)-dependent murine T-cell line, CTLL2, was genetically altered with the Escherichia coli β-galactosidase (β-gal) gene (lacZ). In vitro with a retroviral vector and the modified T-cells were transplanted directly to the respiratory epithelial surface of syngeneic C57Bl/6 mice. Southern and Northern analyses confirmed that the neomycin-selected modified T-cells contained and expressed the lacZ gene. The fate of the modified T-cells (CTLL2/lacZ) was followed by flow cytometry with T-cell surface marker Thy1.2 and fluororescent β-gal analysis. One day after transplantation (7.5 × 10⁶ CTLL2/lacZ T-cells/g of body weight), 95 ± 3% of the Thy1.2+ T-cells recovered from respiratory epithelial lining fluid (ELF) were β-gal+. Importantly, the modified T-cells remained in the lung for some time; at 3 days, Thy1.2+ β-gal+ T-cells represented 63 ± 12% of ELF Thy1.2+ T-cells and 59 ± 6% of Thy1.2+ T-cells recovered from the whole lung. At 7 days, 33 ± 8% of the Thy 1.2+ cells in ELF and 75 ± 6% of the Thy 1.2+ cells in whole lung were Thy1.2+ β-gal+. In contrast, the proportion of the Thy1.2+ β-gal+ T-cells in the spleen, the major extrapulmonary lymphatic organ, never rose above 3 ± 1% of the total Thy1.2+ cells. The number of Thy1.2+ β-gal+ T-cells in the lung could be modified by the systemic administration of IL-2, with whole lung Thy1.2+ β-gal+ T-cells increasing 4.6-fold 3 days after transplantation, compared with non-IL-2-treated animals. These studies suggest that direct transplantation of genetically modified T-cells into the lung is feasible and represents a viable strategy for lung-specific gene transfer.

A variety of human disorders can potentially be treated by modifying the genetic composition of autologous T-lymphocytes in a fashion relevant to the specific disease and directing the modified T-cells to the site of disease (1–3). Although the process of inserting new genes into lymphocytes can be accomplished using vectors such as retroviruses (1–8), one limitation of this treatment strategy is the problem of targeting the modified T-cells to the site of disease. Since the major manifestations of most disorders are usually limited to specific anatomic locations, systemic administration of genetically modified T-lymphocytes requires that, unless the modified T-cells "home" to the disease site; otherwise potential therapeutic efficacy may be limited. This, together with the risk of having potentially "dangerous" genetically modified T-cells circulating throughout the body and reaching organs with no disease, argues strongly for targeting the modified T-cells directly to specific organs.

Theoretically, targeting of T-cells could be achieved by capitalizing on the inherent (or engineered) properties of T-cells to home specifically to the site of disease. This concept is the underlying premise of modifying tumor infiltrating lymphocytes and reinfecting the modified, expanded population of cells with the expectation they will localize to the neoplasm (6, 8). Alternative strategies for homing are to capitalize on specific T-cell antigen receptors or specific homing ligands (9, 10).

In the present study, we have explored a different approach to the problem of targeting, that of directly transplanting the genetically modified T-cells to the organ of interest. As a model for this strategy, we have evaluated the consequences of directly transplanting genetically modified T-cells to the epithelial surface of the lung. To do so, the genome of the murine T-cell line CTLL2 was modified using a retroviral vector carrying the Escherichia coli lacZ (β-galactosidase) gene (11–13). The modified T-cells transplanted to the respiratory epithelial surface of syngeneic C57Bl/6 mice, and the relative numbers of modified T-cells on the respiratory epithelial surface and in the lung parenchyma quantified over time.

MATERIALS AND METHODS

Cells and Experimental Animals—The murine T-lymphocyte line CTLL2 (TIB214; American Type Culture Collection, Rockville, MD), a cytotoxic T-cell derived from the C57Bl/6 murine strain, was chosen for this study because they are depend on interleukin-2 (IL-2) for survival (14, 15), permitting evaluation of the concept that the modified T-cell might be expanded in vivo following the administration of IL-2. CTLL2 cells were maintained in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD), 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10% fetal calf serum (all from Bio-Fluids Inc., Rockville, MD), and 50 units/ml pooled recombinant human IL-2 (Cetus, Emeryville, CA). The syngeneic animals used as T-lymphocyte recipients were C57Bl/6 mice (18–20 g; Jackson Labs, Bar Harbor, ME).

Retroviral Vector and Modification of T-cells—The retroviral vector, pL-BgSN (kindly provided by P. Tolstoshev, Genetic Therapy Inc., Gaithersburg, MD), carrying the E. coli lacZ gene, was constructed from pLXSN (16), a retroviral vector containing 5' and 3' long terminal repeats with an ampicillin resistance gene. Five milliliters of 0.5% calcium chloride solution was mixed with 20 ml of 0.2 M sodium acetate pH 5.2, 5 ml of a stock of helper virus (Cv-II; American Tissue Culture Collection, Rockville, MD) at 100 units/ml, and 20 μg of pL-BgSN. The mixture was incubated at 37 °C for 2 hr and then 120 μl of the transfection mixture was added to 106 CTLL2 cells cultured in 24-well plates. The transfected CTLL2 cells were resuspended with RPMI 1640 and 5% fetal calf serum and incubated for 18–24 hr in vitro. The cells were then washed and placed into 10 ml of fresh RPMI 1640 at a density of 1 × 10⁶/ml. After 24 hr in vitro, the supernatant was removed and replaced with fresh RPMI 1640. The supernatant was collected at 48 hr and used to transduce NIH-3T3 cells (17). The NIH-3T3 cells were plated at 1 × 10⁵/ml in 35-mm dishes in RPMI 1640 supplemented with 10% fetal calf serum, and the supernatant was removed after 24 hr. The cells were stained with X-gal and scored for those colonies expressing β-gal.

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* The abbreviations used are: IL, interleukin; β-gal, β-galactosidase; ELF, epithelial lining fluid; kb, kilobase; PBS, phosphate-buffered saline; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; FDG, fluorescein di-β-galactopyranoside.

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long terminal repeats of the Moloney murine leukemia virus and the neomycin resistance gene driven by the SV40 early promoter. The BamHI fragment of pMC1871 (Pharmacia LKB Biotechnology Inc.) containing the lacZ gene was altered at the ends and inserted into the XhoI site of pLXSN to create pLBgSN. This final construct contains the 5' long terminal repeat driving the lacZ gene followed by the SV40 early promoter and the neomycin resistance gene (Fig. 1A).

An infectious amphotropic virus-producing packaging cell line was produced by infecting PA317 cells (17) with supernatant from a pLBgSN transfected 293 cell clone and 1.0 mg/ml G418 (Geneticin, GIBCO). pLBgSN-infected PA317 cells were maintained in growth medium containing 1.0% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1.0 mg/ml G418. One of the monoclonal antibodies to the pLBgSN-infected PA317 cells, pLBgSN17 (viral titer of 10^10 colony-forming units/ml), was used for all subsequent experiments.

To infect CTL2 cells, 10 ml of infectious retroviral supernatant of the virus-producing pLBgSN17 clone was added to a suspension of 1 \times 10^7 CTL2 cells in 5 μg/ml Polybrene (Sigma), and 2000 units/ml IL-2 in a 50-ml culture flask and cultured overnight at 37°C. Retroviral supernatant was then replaced with nonselective IL-2 (2000 units/ml) containing medium for an additional 24 h. The infected cells were selected with 0.5 mg/ml G418 and 50 units/ml IL-2, and the numbers of T-cells were expanded for 2 weeks. The selected cells (CTL2/lacZ) were maintained with 0.5 mg/ml G418 and 50 units/ml IL-2.

In vitro Evaluation of CTL2/lacZ Cells—Integration of the lacZ cDNA into the genome of CTL2/lacZ cells was evaluated by restriction enzyme analysis following PvuII digestion of 10 pg of genomic DNA. After agarose gel electrophoresis and transfer to nylon membrane (Schleicher and Schuell), the 2.5-kb PvuII fragment of lacZ was detected using a labeled lacZ probe generated by the random priming method (Promega Biotech, Madison, WI) and subsequent autoradiography (18). Expression of lacZ transcripts in the T-cells was performed by Northern analysis following formaldehyde-agarose gel electrophoresis of 10 μg of total RNA, transfer to nylon membrane, hybridization to the labeled lacZ probe, and autoradiography (19).

To determine the expression of the lacZ protein product, β-galactosidase (β-gal), colorimetric, and cytometric analyses were used. The colorimetric assay of β-gal activity in cell lysates was performed using o-nitrophenyl β-D-galactopyranoside (Boehringer Mannheim) as a substrate and detection at 420 nm (20). Cytotoxic staining of β-gal in CTL2/lacZ cells was carried out by fixing cells in 4% paraformaldehyde, washing with phosphate-buffered saline (PBS, pH 7.4; Whittaker Bioproducts, Inc.), and incubating with 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 50 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) (Boehringer Mannheim) at 37°C for 4 h (21). Cells were considered positive for β-gal if visible blue staining was observed within the cell.

To visualize the location of transplanted CTL2/lacZ cells in vivo, 1 × 10^7 lacZ-containing thymocytes were transplanted intrapleurally into 10 to 12-week-old nude mice (nu/nu) by intratracheal administration. To evaluate the effect of transplantation, control CTL2 cells and CTL2/lacZ cells were kept in culture for at least 1 week in nonselective media at a density of less than 5 \times 10^6/ml, conditions which maintain viability of the cultivated cells at ≥90% as determined by trypan blue exclusion. The cultivated cells were collected by centrifugation at 200 × g for 5 min and suspended in staining media (4% formalin, 40 μM HEFES, pH 7.0 (Biofluids, Inc.) in PBS) for analysis by flow cytometry. For convenience, the cells recovered from the respiratory epithelial fluid will be referred to as cells in ELF, and the cells extracted from the minced lung will be referred to as cells in whole lung. To evaluate CTVII2/lacZ cell trafficking to extrapulmonary lymphoid tissue, the spleen was removed in a tissue culture dish, and the cells were recovered as described for the whole lung.

To evaluate the location of transplanted CTL2/lacZ cells in the lung, staining of Thy1.2 and β-gal was performed on cryostat mouse lung sections. The lungs were perfused with PBS from the right atrium and then with 4% paraformaldehyde. Fixed lungs were excised, submerged in 4% paraformaldehyde overnight, and then frozen. Cryostat sections were stained with biotin-labeled monoclonal mouse antibody to Thy1.2 followed by peroxidase-labeled avidin-biotin complex (Vector Laboratories, Burlingame, CA) and then incubated at 37°C for 5 min with chromogen 3,3'-diaminobenzidine (Sigma), yielding a brown reaction product. Lung sections were then stained for the blue β-gal product using X-gal as described above.

Quantification of Thy1.2+ / β-Gal+ T-cells—Flow cytometry analysis was used to simultaneously quantify T-cell surface phenotype Thy1.2 and cytotoxic β-gal in CTL2/lacZ T-cells recovered in respiratory ELF, whole lung, and spleen. Following cell recovery, a minimum of 1 × 10^6 cells were pelleted and resuspended in 100 μl of staining media. The cells were next warmed (37°C, 10 min) by incubating (30 min, 4°C) with biotin-conjugated anti-Thy1.2 monoclonal antibody (Becton Dickinson, Immunocytometry Systems, Mountain View, CA), washing with staining media, and incubating with phycoerythrin-conjugated streptavidin (Becton Dickinson) for 30 min at 4°C. As a control irrelevant antibody, CTL2 cells were incubated with a monoclonal antibody to murine helper T-cell phenotype LYT2 (Becton Dickinson). For the measurement of β-gal activity, the cells were then washed (37°C, 10 min) and then exposed to 1 mM fluorescein isothiocyanate-labeled anti-β-galactosidase (FDG; Molecular Probes, Inc., Eugene, OR) for 1 min, after which the reaction was stopped by adding ice-cold staining media (13).

Propidium iodide was added to stain dead cells for exclusion by flow cytometry (24). The cells were kept at 4°C until flow cytometry was performed.

Flow cytometry analysis was carried out using FACScan (Becton Dickinson). The settings were kept constant for all samples and at least 10^5 events were counted. CTL2 and CTL2/lacZ cells stained with Thy1.2-phycocerythrin and FDG were used to set statistical quadrants so that >95% of the Thy1.2+ / β-gal+ cells were in the upper left quadrant and >95% of the Thy1.2+ β-gal− cells were in the upper right quadrant. Spleen cells from normal mice were used to adjust the forward scatter lower threshold so that all Thy1.2+ cells were analyzed. Background nonspecific FDG staining in the samples from ELF or whole lung was determined in parallel experiments in which noninfected CTL2 cells (Thy1.2+/ β-gal−) were transplanted via the trachea and recovered from ELF and whole lung at 1 and 7 days. The relative number of cells falling into the two statistical regions was then compared: Thy1.2+ / β-gal− (left upper quadrant) and Thy1.2+ β-gal+ (right upper quadrant). The linear relationship (r = 0.98) found between these two quadrants was used to subtract the random FDG background from samples of CTL2/lacZ cells recovered in vivo. The number of Thy1.2+ / β-gal+ T-lymphocytes was determined by multiplying the number of total cells recovered by the percentage of Thy1.2+ β-gal+ cells obtained.

In Vivo Expansion of the Number of CTL2/lacZ Cells—To assess whether systemic administration of IL-2 modified the in vivo capacity of Thy1.2+ / β-gal+ T-cells in the lungs following intrapulmonary transplantation of the CTL2/lacZ T-cells, 10,000 units of human recombinant IL-2 was administered via the peritoneum to mice 3 times daily (25) after intrapulmonary transplantation of 7.5 × 10^5 CTL2/lacZ T-cells/kg (of body weight). On day 3, cells from ELF, whole lung, and spleen of IL-2-treated and untreated mice were assessed as described above. To confirm that the IL-2 induced the numbers of transplanted CTL2/lacZ cells in the lung (and not simply the ability to recover the cells), total RNA was extracted from homogenized whole lung 3 days post-transplantation. The extracted RNA (20 μg for each animal) was evaluated by slot blot analysis, hybridization with a labeled lacZ probe and autoradiography (19).

In Vivo Evaluation—All data are expressed as mean ± standard error of the mean. All statistical analyses were carried out using the two-tailed Student's t test.
RESULTS

In Vitro Analysis of T-cells Genetically Modified with lacZ—Southern, Northern, and protein analyses confirmed that the CTLL2/lacZ T-cells contained an integrated, functional lacZ gene (Fig. 1). In this regard, genomic DNA of CTLL2 cells evaluated by Southern analysis with a 32P-labeled lacZ probe demonstrated no lacZ sequence, whereas a 2.5-kb PvuII lacZ fragment was clearly evident in the genome of CTLL2/lacZ T-cells (Panel B). Northern analysis using a 32P-labeled lacZ probe demonstrated no lacZ mRNA transcripts in CTLL2 cells but that CTLL2/lacZ cells contained the expected 6.5-kb lacZ transcripts (Panel C). Evaluation of the lysates of CTLL2 cells using a β-gal colorimetric assay was negative, but β-gal activity was clearly present in CTLL2/lacZ cells; β-gal activity in CTLL2/lacZ was 14.3 × 10^3 units/10^6 cells, corresponding to 2.4 × 10^6 molecules of β-gal/cell (20). Finally, the lacZ protein product, β-gal, was not detected by cytochemical staining with X-gal in the CTLL2 T-cells, but was readily detected in the CTLL2/lacZ T-cells (Panels D and E). Flow cytometric analysis comparison of CTLL2 and CTLL2/lacZ cells demonstrated that almost all of the CTLL2/lacZ cells were T-cells containing cytosolic β-gal activity; whereas >96% of the CTLL2 cells were Thy1.2+ β-gal− (Fig. 2A), >96% of the CTLL2/lacZ cells were Thy1.2+ β-gal+. To exclude a possibility that retroviral integration of the foreign gene (lacZ) might change the growth characteristics of the CTLL2/lacZ cells, the proliferative response of CTLL2 and CTLL2/lacZ T-cells to IL-2 were compared. Importantly, the CTLL2/lacZ showed the same in vitro growth response to IL-2 as the unmodified CTLL2 T-cells (half-maximal [3H]thymidine incorporation for CTLL2 was 0.62 ± 0.07 units/ml

Fig. 1. Recombinant retroviral construct pLBgSN containing the E. coli β-galactosidase gene (lacZ) and in vitro analysis of the integration and expression of lacZ in the genetically modified T-lymphocytes (CTLL2/lacZ). A, structure of the retroviral vector, pLBgSN. Derived from the Moloney murine leukemic virus, the vector consists of 5’ and 3’ long terminal repeats (LTR), the Ψ encapsidation signal, the 5’ LTR-driven lacZ gene, and the neomycin resistance gene (NeoR) directly driven by the SV40 early promoter (SV40). The restriction enzyme sites for PvuII (P) are indicated. B, autoradiograms of Southern analysis of a PvuII digest of 10 μg of genomic DNA hybridized with a 32P-labeled lacZ probe. Lane 1, uninfected CTLL2 cells do not have lacZ gene-containing fragments. Lane 2, CTLL2/lacZ cells demonstrate the expected 2.5-kb lacZ fragment. C, autoradiograms of Northern analysis of 10 μg of total RNA hybridized with the 32P-labeled lacZ probe. Lane 3, uninfected CTLL2 cells have no detectable lacZ transcripts. Lane 4, CTLL2/lacZ cells demonstrate the 6.5-kb lacZ transcripts. Panels D and E, cytochemical evaluation of β-galactosidase (β-gal) activity in vitro. D, uninfected CTLL2 T-cells as a negative control. E, CTLL2/lacZ T-cells demonstrate the β-gal+ blue color product. Panels D and E are both inverted phase contrast microscopy (40X).

Fig. 2. Flow cytometry evaluation of Thy1.2+ and β-gal+ T-cells. Ordinate, phycoerythrin (PE)-labeled Thy1.2+ cells expressed as log fluorescence intensity. Abscissa, FDG-stained β-gal-containing cells, detected as fluorescein isothiocyanate (FITC) expressed as log fluorescence intensity. A, uninfected CTLL2 cells evaluated in vitro. The vast majority of cells are positive for Thy1.2 surface antigen and negative for β-gal. B, CTLL2 cells modified with the pLBgSN retrovirus containing lacZ (CTLL2/lacZ). Almost all cells are simultaneously positive for Thy1.2 and β-gal activity. Panels C-F, in vivo cell samples recovered from epithelial lining fluid or whole lung as indicated. C, cells recovered from ELF of an untransplanted mouse, demonstrating the presence of few Thy1.2+ cells and the absence of all Thy1.2+ β-gal+ cells. D, cells recovered from respiratory epithelial lining fluid 1 day after intratracheal transplantation of CTLL2/lacZ T-cells, demonstrating the presence of a large number of Thy1.2+ β-gal+ cells. E, cells recovered from minced lung of an untransplanted mouse, demonstrating the presence of some Thy1.2+ cells and the absence of Thy1.2+ β-gal+ cells. F, cells recovered from minced lung 1 day after intratracheal transplantation of CTLL2/lacZ T-cells, demonstrating the presence of a large number of Thy1.2+ β-gal+ cells. In panels C-F, >95% of the Thy1.2+ cells are alveolar macrophages; a very small proportion of these cells are β-gal+.
IL-2, compared with 0.84 ± 0.37 for CTLL2/lacZ; p > 0.5), i.e. the modified cells maintained the responsiveness to IL-2 demonstrated by the parent cell line, offering the possibility that numbers of CTLL2/lacZ cells might be modulated in vivo with exogenously administered IL-2.

Transplantation of Genetically Modified T-cells to the Respiratory Epithelial Surface—Following the intratracheal transplant of 7.5 × 10⁷/g of body weight CTLL2/lacZ cells to the respiratory epithelial surface of the lung, no adverse effects attributable to the transplantation of the T-cells were noted. Flow cytometry evaluation of cells recovered from the respiratory ELF demonstrated that prior to transplantation, no Thy1.2+ β-gal+ T-cells were present (Fig. 2C). In contrast, 1 day following transplantation, large numbers of Thy1.2+ β-gal+ T-cells were evident (Fig. 2D). In the cells extracted from whole lung (which includes cells from the ELF and interstitium), pretransplantation showed primarily large, Thy1.2− cells (alveolar macrophages), with few Thy1.2+ β-gal− T-cells and no β-gal+ T-cells (Fig. 2E). Similar to evaluation of the cells from the ELF, 1 day following transplantation, large numbers of Thy1.2+ β-gal+ cells were present (Fig. 2F). Histologic evaluation of lung sections from animals 1 day after transplantation with CTLL2/lacZ cells was consistent with findings from analysis of cells recovered from ELF and whole lung. Frozen lung sections stained with peroxidase-labeled avidin-biotin complex directed to the monoclonal mouse antibody Thy1.2 and stained with X-gal for the β-gal product revealed clumps of modified cells within the alveoli (i.e. on the respiratory epithelial surface) and within the alveolar interstitium (not shown).

Quantitative assessment of the Thy1.2+ T-cells present in the lung and spleen demonstrated that transplantation of CTLL2/lacZ cells to the respiratory epithelial surface resulted in the presence of large proportions of Thy1.2+ cells that were β-gal+ in respiratory ELF and in whole lung, but not in spleen (Fig. 3). As expected, the first day (n = 4) after transplantation, most (95 ± 3%) Thy1.2+ T-cells in respiratory ELF were β-gal+. A significant number of modified T-cells persisted in the lung for at least 1 week, as evidenced by the fact that at 7 days (n = 7), 33 ± 8% of the Thy1.2+ cells in ELF were β-gal+. The same observation was made when the lung was minced and the T-cells from all compartments (ELF and the interstitial spaces) were evaluated. Interestingly, at 1 week, the proportions of Thy1.2+ cells that were β-gal+ were higher in whole lung than in ELF (p < 0.01; n = 7 for ELF; n = 5 for whole lung) suggesting progressive movement of at least some of the modified T-cells from the epithelial surface into the parenchyma. Importantly, very small proportions of Thy1.2+ β-gal+ T-cells reached the systemic circulation as evidenced by the fact that there were never greater than 3 ± 1% of Thy1.2+ cells in the spleen that were β-gal+ and, at 1 week (n = 12), no Thy1.2+ β-gal+ cells could be detected in the spleen.

Evaluation of cells from ELF at 2 weeks (n = 2) demonstrated that approximately 30% of Thy1.2+ T-cells were β-gal+, suggesting that a reasonable number remained on the epithelial surface at this time. However, the lung tissue at this time was likely repopulated by Thy1.2+ β-gal− cells, since Thy1.2+ β-gal+ cells were undetectable in the whole lung mince by 2 weeks (n = 3) after transplantation.

Expansion of Thy1.2+ β-gal+ T-cells by Systemic Administration of IL-2—The decrease in the number of CTLL2/lacZ cells in the lung by 3 days post-transplantation, but with few modified T-cells in extrapulmonary lymph tissue, is consistent with the fact that CTLL2 T-cells require an exogenous source of IL-2 to remain viable. Accordant with the experience of other investigations (14, 15, 26), in vitro evaluation of CTLL2 cells cultured without IL-2 demonstrated progressive loss of the cells, such that, by 36 h, only 60% were viable and, by 48 h, only 31% were viable (as assessed by propidium iodide exclusion). Interestingly, administration of IL-2 to animals following intratracheal transplantation of CTLL2/ lacZ T-cells demonstrated an increase in the numbers of Thy1.2+ β-gal+ cells in the lung (Fig. 4). Animals treated with intraperitoneal human recombinant IL-2 post-transplantation showed no ill effects but had a higher number of CTLL2/lacZ cells in the lung. This was true when evaluated at the mRNA transcript level or by flow cytometry. Quantification of lacZ mRNA transcript levels in the lung demonstrated none were detectable pretransplantation, but abundant transcripts were detected 1 day after intratracheal transplantation of CTLL2/lacZ cells (Panel A). In contrast, under the conditions used, no lacZ transcripts were detectable by 3 days in animals receiving no IL-2. However, under the same conditions, in those animals receiving IL-2, lacZ transcripts were easily detectable at the same point in time following transplantation (compare lane 4 with lane 3). The same pattern of results were observed using flow cytometry (Panel B). Three days after transplantation, the number of Thy1.2+ β-gal+ T-cells recovered from ELF was higher in animals treated with IL-2 (n = 6) compared with that in untreated animals (n = 5), but the difference was not statistically significant. However, there was a significant increase (4.6-fold) of Thy1.2+ β-gal+ T-cells in whole lung of the IL-2-treated animals (p = 0.02; n = 8 with no IL-2; n = 8 with IL-2 treatment).

**DISCUSSION**

Genetically modified lymphocytes have the potential for treatment of a variety of human disorders, either because the modified lymphocytes have acquired an inherent lymphocyte feature that has relevance to the disease or by using the lymphocytes as a cell vehicle to synthesize and deliver a protein of therapeutic value (1–8). A major hurdle for using modified lymphocyte gene therapy is how to achieve organ specificity in order to avoid an “unnecessary” burden of...
Transplantation of Modified T-lymphocytes

The T-lymphocyte is a convenient cell to use as a cell vehicle for gene therapy. In this regard, T-cells from animals and humans have been modified by gene transfer in vitro with a variety of human, animal, and bacterial genes including: α1-antitrypsin, leukocyte adhesion glycoprotein LFA-1 (CD-18), IL-2, interleukin-4, interleukin-9, interferon-γ, interferon-α, growth hormone, adenosine deaminase, neomycin resistance, and lacZ (3, 6–8, 13, 26–36). Further, the modified cells can be expanded in large scale with the use of IL-2, and the T-cell populations can be evaluated prior to reinfusion for the presence and function of the new gene (3, 6, 7, 30, 32, 36). This approach has been used in in vivo experimental animal studies to evaluate the contribution of IL-2, interleukin 4, and interleukin 9 to T-cell growth in vivo (26, 34, 35), to track T-cells (3, 6, 30), and to evaluate the effect of lymphocytes modified with the adenosine deaminase gene (3, 30). Exogenous gene transfer of human lymphocytes has been used in humans in vivo to track tumor-infiltrating lymphocytes in individuals with melanoma and to treat adenosine deaminase deficiency (6, 36).

Despite the accessibility and ease of growing T-cells in vitro, using T-cells as a target for gene transfer in humans has limited applicability unless the problem of targeting can be solved. First, because most diseases are localized, systemic administration of the modified T-cells without targeting has the problem of limiting therapeutic efficacy, as only a fraction of the modified T-cells will randomly reach the intended target. Although this might be solved by making each modified T-cell more “potent” (e.g., with more powerful promoters driving a gene for a therapeutic protein), there is the risk that the gene product will cause systemic toxicity. Second, although T-cells have receptors that are relevant to disease specificity (e.g., antigen receptors, homing receptors), to date, there is no clear evidence that systemic administration of a genetically modified subset of lymphocytes will “home” to a specific site.

The approach used in the present study may be one strategy to circumvent this targeting problem. It is particularly applicable to the lung, which is easily accessed via the respiratory tract. However, a similar approach might be used in other closed anatomic compartments, such as the central nervous system (via the cerebrospinal fluid), the bladder (retrograde via the urethra), and the gastrointestinal tract. Further, using selective catheterization of localized arterial beds, modified T-cells could be transplanted to most organs.

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Transplantation of Modified T-lymphocytes

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