Two human melanoma cell lines were transduced with the human interleukin (IL)-7 and IL-2 genes using retroviral-mediated gene transfer. Stable, high-level cytokine expression was achieved. The in vitro growth of transduced tumors was unaltered. Neither of the IL-2-transduced melanoma lines grew in athymic mice, whereas one IL-7-transduced melanoma line showed retarded in vivo growth. This is consistent with animal studies suggesting a predominantly T-cell response to IL-7-transduced tumors and a more nonspecific response to IL-2-transduced tumors. Both IL-7- and IL-2-transduced melanoma cell lines could induce cytotoxic lymphocytes in mixed lymphocyte-tumor cultures. The expression of putative melanoma antigens (MAGE)-1 and MAGE-3 was unaltered by cytokine transduction. In one cell line, IL-7 transduction resulted in a marked inhibition of the immunosuppressive peptide transforming growth factor (TGF)β. The results allow a comparison of immunobiologic properties of IL-7- and IL-2-transduced human melanoma cell lines in consideration of their use in genetically engineered tumor vaccines. IL-7 transduction results in stable cytokine expression and phenotypic alterations that appear to be favorable for enhanced immunogenicity and it deserves clinical testing. © 1993 by The American Society of Hematology.

MATERIALS AND METHODS

Melanoma cell lines The melanoma cell lines M-14 and M-24 have been previously described and were originally established from surgical biopsy specimens of patients treated at the UCLA Medical Center.25

Retroviral vectors. Vectors used for transduction of the M-14 and M-24 cell lines included the L-N-based neomycin phosphotransferase gene (neoR)-containing G1Na vector (titer range, 0.5 to 3.6 x 10⁶ cfu/mL), G1Na-CVIL-2 (titer range, 1 to 5 x 10⁶ cfu/mL) vector in which the neoR selectable marker was driven by the Mloey murine leukemia virus long terminal repeat (LTR), and the human IL-2 cDNA driven by the cytomegalovirus (CMV) early enhancer/promoter (Fig 1A). These vectors were provided as frozen supernatants from Genetic Therapy, Inc (Gaithersburg, MD). The JZEN hIL-7/tk neo (titer range 1 to 10 x 10⁶ cfu/mL) vector was constructed with the neoR gene driven by the thymidine kinase promoter, and the hIL7 cDNA under the transcriptional control of
Fig 1. Linear maps of the G1Na CV IL-2 and JZEN/hIL-7/tk neo retroviral vectors.

the myeloproliferative sarcoma virus LTR. This construct was packaged in the GP + env AM 12 amphotropic cell line and was subeloned to produce high-titer stocks of 104 cfiU/ml.27 (Fig 1B).

Animals. Experiments were performed on male and female congenitally athymic (nu/nu) BALB/c mice which were maintained in a defined-flora, pathogen-free colony at UCLA. All manipulations were approved by the UCLA Chancellor's Animal Research Committee.

Transduction and selection conditions. Transduction of the melanoma cell lines was performed using retroviral supernatants at a multiplicity of infection ranging from 1:6 (IL-2) to 4:1 (G1Na, IL-7). Infections were performed on 3 successive days in the presence of protamine sulfate 10 ag/ml, with 24 hour exposure times. Cells were washed and resuspended in maintenance media consisting of RPMI 1640 (ICN, Costa Mesa, CA), glutamine, antibiotics, and 10% fetal calf serum. After 48 hour incubation, cells were selected in G418 (Geneticin; GIBCO-BRL, Grand Island, NY) at a total concentration of 0.7 mg/ml, which was previously determined to be the minimum concentration required to kill all nontransduced cells after 10 days. Bulk cell populations were maintained under constant selection, but passaged for up to 1 week before being used in any experiments.

In vitro growth rate. To compare relative in vitro growth rates, 2 x 10 cells of each cell population were placed in duplicate 16-well diameter wells containing 2 ml maintenance media. Cells were stained in a 1:1 volume ratio with trypan blue and counted in a hemacytometer at various intervals ranging from 2 to 11 days following plating. Cell counts were analyzed using a computer generated program that applied appropriate (linear, logistic, or exponential) equations to each cell group to detect variations from the predicted program that applied appropriate (linear, logistic, or exponential) equations to each cell group to detect variations from the

DNA PCR analysis. Chromosomal DNA was isolated by cell lysis in 1% sodium dodecyl sulfate (SDS), 200 pg/ml protease K, 100 mol/L NaCl, 100 mmol/L Tris-HCl, and 25 mmol/L EDTA at 55°C for 16 hours, following which DNA isolation was accomplished by phenol-chloroform-isomyl alcohol (25:24:1) extraction and ethyl alcohol precipitation.

One microgram of DNA was amplified by polymerase chain reaction (PCR) in a total volume of 50 ml, which contained 0.2 mol/L of deoxyribonucleotide triphosphates, 0.25 mmol/L of S' and 5' oligonucleotide primers, 50 mmol/L KCl, 5 mmol/L Tris-Cl (pH 8.3), 1.5 mmol/L MgCl2, and 2.5 U AmpliTaq (Perkin Elmer Cetus, Norwalk, CT). PCR was performed in a DNA thermal cycler (Perkin Elmer). The amplification profile for neoR and IL-7 consisted of 28 cycles of denaturing for 1 minute, annealing of primers at 60°C for 2 minutes, and extension at 72°C for 2 minutes. For IL-2 amplification, the thermal cycle conditions included 40 cycles of 1 minute denaturing at 94°C, and 2 minutes of annealing/elongation at 50°C. The nucleotide sequences of the oligonucleotide primers were neoR: S'-GAT-AGA-AGG-GGA-TGC-GCT-GC-3', 3' primer S'-GAT-GGA-GAG-GCT-ATT-CG3-T-3', PCR fragment size, 790 bp; IL-7: 5' primer S'-ATG-TTC-CAT-GAT-TCT-TTT-AGG-TAT-ATC-T-3', 3' primer S'-GTT-TTC-TTT-AGT-GGT-CAT-CAA-3', PCR fragment size, 551 bp; IL-2: 5' primer S'-ACT-CAG-CAG-GTC-CAC-AT-3', 3' primer S'-AGG-TAA-TCC-ATC-TGT-CA-GA-3', PCR fragment size, 246 bp.

RNA PCR analysis. Messenger RNA was isolated using the Micro-FastTrack Isolation Kit (Invitrogen, San Diego, CA). Briefly, 4 x 10 cells were washed in phosphate buffered saline and lysed in preheated (45°C) detergent-based buffer containing DNase and proteinase for 15 minutes. The lysate was incubated for 20 minutes with oligo (dT) 20-30 cellular rinse and washed with a high salt buffer. A low salt buffer was used to remove tRNA and rRNA. The poly(A) RNA was eluted in salt-free buffer, and precipitated with glycogen, 2 mol/L NaOAc and 100% ethanol at 70°C.

Reverse transcription of the mRNA and PCR amplification of cDNA was performed using the GeneAmp Kit (Perkin Elmer). This protocol combined 0.25 pg of mRNA, 2.5 U reverse transcriptase (RT), 2.5 mmol/L oligo (dT), 1 mmol/L dNTP's, 5 mmol/L MgCl2, 50 mmol/L KCl, and 10 mmol/L Tris-HCl, and was incubated at 42°C for 30 minutes and 99°C for 5 minutes. For PCR, MgCl2 was adjusted to 2.5 mmol/L, and the suspension was mixed with AmpliTaq and appropriate oligonucleotide primers. The thermal cycler profile was as follows: 40 cycles of 1 minute denaturing at 94°C and 2 minutes of annealing/elongation at 50°C for the IL-2 primers. For IL-7 and neoR PCR, annealing was performed at 60°C for 2 minutes, and elongation was accomplished at 72°C for 2 minutes in the 40 cycle profile.

Northern analysis. Total cellular RNA was extracted from approximately 5 x 10 cells of each cell line by the guanidinium isothiocyanate method. A 20 pg aliquot of RNA was denatured for 10 minutes at 65°C in 50% (vol/vol) formamide, 2.2 mol/L formaldehyde, 20 mmol/L morpholinepropane sulfonic acid (MOPS; pH 7.0), 8 mmol/L sodium acetate, 1 mmol/L EDTA, and size fractionated by electrophoresis through a 1% agarose gel containing 2.2 mol/L formaldehyde. The 18S and 28S rRNA bands were identified on the gel by ultraviolet shadowing, and their positions recorded by tracing the bands and gel edges onto overlying cellophane. The RNA was transferred onto a nylon membrane (HYBOND-N, 0.45 pm; Amersham, Arlington Heights, IL) by blotting in 20% SSC (1 SSC is 150 mmol/L sodium chloride and 15 mmol/L sodium citrate) for 36 hours. The RNA was immobilized by ultraviolet crosslinking (Stratalinker 1800; Stratagene, La Jolla, CA).

Membranes were probed with radiolabeled melanoma antigen (MAGE)-1, MAGE-3, transforming growth factor (TGF)β, and β-actin cDNA's. A cDNA construct of MAGE-1 was a gift from T. Boon. MAGE-3 was cloned by PCR amplifying the reverse transcribed cDNA from M-14 mRNA. The primers were synthetic oligonucleotides homologous to the sequences CHO-8 and CHO-9. Prehybridization was performed for 4 hours at 42°C in the following solution: 50% formamide (vol/vol), 10% Denhardt's solution (1X Denhardt's solution is 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinylpyrrolidone) 1% sodium pyrophosphate, 0.05 mol/L tris (pH 7.5), 1 mol/L sodium chloride, 1% SDS, 1% dextran sulfate (wt/vol), and 0.5 mg/ml sonicated herring sperm DNA. Radiolabeled probe was added to the prehybridization buffer

From www.bloodjournal.org by guest on September 24, 2017. For personal use only.
to give a specific activity of at least $10^7$ cpm/mL. Hybridization was performed for 16 to 20 hours at 42°C. The membrane was washed twice for 15 minutes at room temperature with 2× SSC and 0.1% SDS. Autoradiographs were produced by exposing the membrane to film (Hyperfilm-MP, Amersham) overnight at 80°C using an intensifying screen. Following exposure, the membranes were stripped of probe by boiling in 0.1% SDS until radioactivity was negligible.

Densitometric scanning of Northern blots was performed using the Gelscan XL software package (Pharmacia, Uppsala, Sweden), and analyzed by an Ultrascan XL Laser Densitometer (Pharmacia). Films were scanned and absorbance readings were measured and converted into curvilinear areas. The area under the absorbance curve was integrated. TGFP, MAGE-1, and MAGE-3 readings were divided by β-actin readings to normalize band intensities.

**Cytokine enzyme-linked immunosorbent assay (ELISA).** Cytokine production was quantitated with immunoabsorbent assay kits (R & D Systems, Minneapolis, MN), for IL-7 and IL-2, which are sensitive to 15 and 31 pg/mL, respectively.

**Mixed lymphocyte-tumor reactions.** Peripheral blood lymphocytes (PBL) were obtained from heparinized volunteer blood and cultured in 24-well Costar (Cambridge, MA) plates with irradiated (10,000 cGy) M-14 or M-24 cell lines, both transduced and untransduced. In parallel cell groups, recombinant IL-2, 12 ng/mL (specific activity, 1.2 × 10^6 NU/mg; Hoffman-La Roche, Nutley, NJ), or IL-7, 16 ng/mL (specific activity, 4 × 10^6 U/mg protein, protein concentration 0.76 mg/mL; Sterling, Malvern, PA) was added. A total of 6 × 10^5 PBL and 3 × 10^6 melanoma stimulator cells were added in a volume of 3 mL RPMI 1640, 10% human AB serum, and antibiotics. Each experiment also included 6 × 10^5 PBL cultured alone with and without exogenous cytokines. In some experiments, AIM V medium (GIBCO) was used in the absence of human serum. After 7 days, cytotoxicity was measured in 4-hour chromium release assays using parental M-14 or M-24 targets.

**Growth in nude mice.** Populations of 2 × 10^6 M-14 and M-24 cells were procured from tissue culture flasks, washed, and subcutaneously injected into the flanks of congenitally athymic (nu/nu) mice. Bidirectional tumor diameter was measured using a caliper at regular intervals for up to 100 days.

**Flow cytometric analysis.** Cell populations representing all groups were analyzed by flow cytometry using a FACScan (Becton Dickenson, Mountain View, CA). Fluorescein-conjugated monoclonal antibodies were used to stain cells for the presence of class I and class II major histocompatibility antigens. Class I analysis was performed using the W6/32 hybridoma (American Type Culture Collection, Rockville, MD), which reacts with monomorphic elements on the HLA A, B, or C molecules. Class II antibody was detected using the anti-HLA-DR fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (Becton Dickenson). Cells were removed from culture flasks using 2.5 mmol/L EDTA, washed, and incubated with appropriate antibodies at recommended concentrations.

**TGFP, bioassay.** TGFP, was measured by bioassay using the MV 1 Lu mink lung cell line (ATCC-CCL-64), as previously described. Briefly, cells were washed twice and suspended in EMEM media (GIBCO) with 2% fetal bovine serum (Hyclone Laboratories, Logan, UT). Viability was determined by Trypan Blue dye exclusion and adjusted to 4 × 10^5/mL. The assay was performed in triplicate with 100 μL of cells and 100 μL of acid-activated conditioned media in a 96-well flat-bottomed microtiter plate. Recombinant TGFP, (R & D Systems) in serial dilutions was used as the standard. IL-2, IL-7, and the combination of both cytokines were used as additional standards to control for the effect of this cytokine on the MV 1 Lu mink lung cell line. The plates were incubated at 37°C with 5% CO₂ for 24 hours and pulsed with 1 μCi of ³H-thymidine (Amersham) during the final 4 hours. Cells were harvested using a semiautomatic cell harvester, and ³H-thymidine uptake was measured in a scintillation counter as an index of cell proliferation. TGFP, content was determined based on percent decrease in ³H-thymidine incorporation compared with a rTGFP, standard. To confirm the specificity of this bioassay for TGFP, turkey antihuman TGFP, IgG (10 μg/mL; Becton Dickenson) was added to the supernatant to inhibit the activity.

**Cell irradiation.** Cells were removed from tissue culture flasks with 1× AV buffer, washed in RPMI, counted and adjusted to 10^6 cells/mL in sterile 15 mL polypropylene centrifuge tubes (Corning, Corning, NY). A total of 10,000 cGy was administered to all cell groups using a Gammacell 220 Cobalt-60 (Atomic Energy of Canada) irradiator at a dose rate of approximately 400 cGy/minute.

**RESULTS**

**Proviral DNA amplification and gene expression.** The human melanoma cell lines M-14 and M-24 were transduced with IL-7 or IL-2 using the respective amphotropic retroviral vectors shown in Fig 1 and selected in G418. As seen in Fig 2A, amplification of the respective proviral cytokine genes and neoR selectable marker was shown by PCR of genomic DNA samples. Expression of proviral mRNA was shown using RT PCR (Fig 2B). Both cell lines were also transduced with the neoR vector, G1Na, and selected in G418. G1Na (neoR)-transduced cell lines were similarly shown to have integrated and expressed neoR (not shown).

**Cytokine production.** Bulk populations of the melanoma cell lines transduced with the IL-7 vector [M-14 (IL-7) and M-24 (IL-7)] produced high levels of IL-7 protein as measured by ELISA. M-14 (IL-7) produced an average of 14,200 pg/10^6 cells/24 h, and M-24 (IL-7) produced 92,600 pg/10^6 cells/24 h. Stable, high-level expression has been noted for over 6 months. Untransduced (parental [p] cell lines), or those transduced with a neoR-containing retroviral vector (G1Na), did not produce any IL-7. IL-2-transduced M-14 and M-24 produced an average of 9,500 and 35,300 pg/10^6 cells/24 h, respectively.

**In vitro proliferation of IL-7-transduced melanoma.** Transduction and expression of IL-7 did not appear to significantly affect the in vitro growth rate of either melanoma cell line. The proliferation rates of all parental and transduced cell groups for M-14 and M-24 were found to conform best to logistic growth curves. The in vitro doubling times of these groups were as follows: M-14 (p, IL-2, and IL-7) = 35.3 hours, and M-24 (p, IL-2, and IL-7) = 43.2 hours. Morphology, as assessed by phase-contrast light microscopy, was unaltered in transduced cells.

**Cytokine production by irradiated tumor cells.** Because genetically-engineered live tumor cell vaccines may need to be irradiated before use in clinical trials, the viability and level of cytokine production of M-14 (IL-7) and M-24 (IL-7) were serially measured after 10,000 cGy irradiation. Cytokine production persisted at levels that were within 50% of levels secreted by nonirradiated cells for up to 10 days after irradiation, by which time all cells had died.

**Growth of IL-7-transduced melanoma lines in nude mice.** IL-7–transduced melanoma cell lines were inoculated into nude mice and their growth compared with un-
transduced cells and with cells transduced with G1Na and IL-2. The following pattern emerged from three separate experiments for each tumor cell line: (1) neoR-transduced melanoma lines had comparable in vivo growth rates to parental tumors, although one population of M-24 (neoR) cells that died in culture over a period of 3 months had correspondingly retarded in vivo growth; (2) neither M-14 (IL-2) nor M-24 (IL-2) grew in nude mice in any experiment for up to a 100-day observation period; (3) M-24 (IL-7) had a growth rate comparable to M-24 (p); and (4) M-14 (IL-7) had a more retarded growth rate in nude mice, and certain animals in this group failed to develop tumors. A representative experiment is presented in Fig 3. IL-7–transduced melanoma cells retrieved from these immunodeficient mice continued to produce cytokine when placed into short-term in vitro culture.

**In vitro mixed lymphocyte-tumor reaction (MLTR).** To assess in vitro interactions of PBL with transduced tumor targets, PBL were isolated from allogeneic donors and cocultured for 7 days alone or with irradiated parental IL-2, and IL-7–transduced M-14 and M-24 cell lines. PBL were assayed for cytotoxicity against the stimulator parental melanoma cell line using 4-hour chromium release assays. These results are presented in Fig 4 for M-24; those for M-14 were identical (data not shown). The findings may be summarized as follows: (1) coculture of PBL with untransduced M-14 or M-24 did not generate any cytotoxicity against either target; (2) coculture with IL-7 or IL-2–transduced M-14 or M-24 cells generated enhanced lymphocyte cytotoxicity against stimulator tumor targets compared with coculture with parental tumors; and (3) the addition of exogenous IL-7 or IL-2 (at concentrations similar to those produced by transduced tumors) to MLTR using untransduced tumor stimulators failed to induce lymphocytic cytotoxic function. The biologic potency of exogenously added IL-2 and IL-7 was confirmed by the finding that PBL cultured in IL-2 or IL-7 alone without tumor cells generated mean cytotoxicities of 14-fold and fourfold, respectively, compared with PBL cultured in media alone. This finding was confirmed in all assays performed.

**Expression of major histocompatibility complex (MHC)**
antigens. Flow cytometry was used to determine whether IL-7 or IL-2 transduction affected MHC Class I or II antigen expression. In three separate experiments, no differences were noted except in the case of M-14-(IL-7)-transduced cells, which showed decreased class II expression relative to the parental and IL-2-transduced populations. Both the percentage of cells staining positively with the HLA-DR antibody (p, 21.0 ± 3; IL-2, 14.0 ± 4; IL-7, 4.0 ± 1.5) and the mean fluorescence units (p, 7.0 ± 1; IL-2, 6.9 ± 0.7; IL-7, 1.0 ± 0.1) of IL-7-transduced M-14 cells were decreased significantly compared with parental and transduced cells.

Expression of MAGE. MAGE represent a family of genes whose protein products are variably expressed on the cell surface of approximately 40% of melanoma cell lines and several other malignancies. To determine if retroviral cytokine gene transfer altered MAGE expression, Northern analysis of parental and transduced M-14 and M-24 cell groups was performed. The blot was probed in succession with radiolabeled MAGE-1, MAGE-3, and β-actin cDNA. Band intensities were normalized to β-actin and showed no significant difference in steady-state MAGE mRNA expression by any of the cell groups tested. The results are presented for MAGE-1 expression in M-14 (Fig 5) and are similar for MAGE-3 and M-14 (not shown).

Regulation of TGFβ1 expression in IL-7-transduced melanoma cells. Expression of the immunosuppressive factor TGFβ1 may be downregulated by IL-7 in some types. Hock et al found similar results in IL-7–transduced murine plasmacytoma and mammary adenocarcinoma cell lines. Assessing the involvement of T-cell subsets by monoclonal antibody blockade, this group noted that CD4+ cells

DISCUSSION

Our group has previously shown that a mouse fibrosarcoma cell line transduced with IL-7 cDNA (FSA-IL-7) had decreased in vivo tumorigenicity that was mediated through a T-cell response. Histologic analyses of IL-7–producing FSA tumors showed CD4+ and especially CD8+ lymphocytes dominating the cellular infiltrates. These tumors stimulated the production of tumor-infiltrating lymphocytes, which were highly cytotoxic for parental FSA in vitro. FSA-IL-7 grew progressively in T-cell–depleted mice, underscoring the importance of these lymphocyte subsets. These observations corroborated previous reports showing that IL-7 is effective in stimulating specifically cytotoxic T cells.

Hock et al found similar results in IL-7–transduced murine plasmacytoma and mammary adenocarcinoma cell lines. Assessing the involvement of T-cell subsets by monoclonal antibody blockade, this group noted that CD4+ cells
Several groups have reported decreased tumorigenicity and rejection of murine malignancies engineered to produce IL-2. This antineoplastic response was shown to be predominantly mediated by CD8+ T cells. IL-7 and IL-2 have a number of similar and overlapping biologic properties. Both cytokines stimulate growth and differentiation of B-cell and T-cell subsets at various levels of maturity. IL-7 has been identified as a growth factor for B-cell progenitor cells, as well as proliferative cytokine for adult thymocytes. It also enhances the proliferation of CD4+ and CD8+ T cells in culture, and induces CTL, LAK and natural killer (NK) activity in a variety of cell systems and species.

In direct comparisons with IL-2, IL-7 has been shown to induce slightly lower LAK activity, and to be a slightly less potent stimulator of renal cell carcinoma TIL. However, in an evaluation of the ability of certain cytokines to generate antitumor CTL in a murine model, IL-7 was found to have greater activity than IL-2. When used in combination, IL-2 and IL-7 appear to stimulate the selective proliferation of CD4+ lymphocytes in culture. Because of these similarities, we were interested in comparing the effects of these cytokines in a human gene therapy tumor model. Specifically, we sought to assess the in vivo and in vitro immunobiologic alterations in human melanoma cell lines transduced with IL-2 and IL-7.

Transduction of two melanoma cell lines with IL-7 and IL-2 retroviral vectors resulted in stable, high-level expression of cytokine without the need for cell cloning. IL-7 and IL-2 production was maintained at consistently high levels by both M-14 and M-24 cell lines for at least 6 months in culture. Each vector used different promoter/enhancer elements to drive the cytokine gene, but no obvious difference in the levels of expression were noted between the CMV and LTR promoters. Moreover, no evidence of "transcriptional silencing" for IL-7-transduced cells was noted in vivo. Significant cytokine production was also found to persist for up to 10 days following irradiation, as noted by others. In accord with previous reports, neither cell morphology nor in vitro growth was altered by the introduction of these cytokine retroviral vectors.

In analyzing the in vivo behavior of these transduced tumor cells, the growth of IL-2-producing M-14 and M-24 tumors was completely inhibited in nude mice. This finding is in agreement with the work of Gansbacher et al. Although others have suggested the involvement of NK cells in the nude mouse response to IL-2-producing malignancies, it is also possible that cells of the monocyte/macrophage lineage were involved in tumor killing. But this mechanism was not addressed in the present study. In two of three experiments, the M-14 (IL-7) line showed slower growth than parental or neoR-transduced cells. The M-24 (IL-7) cells did not show different growth compared with parental cells. IL-7 transduction of murine tumors in immunologically-intact syngeneic mice is highly effective in reducing tumorigenicity and generating systemic immunity. A fivefold greater number of CD4+ and CD8+ T lymphocytes were recruited to IL-7-secreting tumor sites, both populations being required for in vivo rejection. This T-cell-dependent mechanism probably explains the incomplete effect of human IL-7 transduction in these T-cell-deficient mice.

In addition to the evidence that IL-2 and IL-7 transduction decreased in vivo tumorigenicity to varying degrees, enhanced in vitro cytotoxicity was also observed. PBL stimulated by both IL-7- and IL-2-transduced tumors acquired increased killing activity against melanoma targets com-
pared with coculture with parental tumors. Interestingly, the addition of recombinant IL-7 or IL-2 at levels comparable to those produced by engineered cells did not effectively reproduce the effect of continuous cytokine production of engineered cells. It was surprising that this admittedly artificial in vitro system identified a superior method of lymphocyte cytotoxicity, and is similar to work performed by other groups.37,42 It is noteworthy that parental M-14 and M-24 tumors did not induce in vitro lymphocyte cytotoxicity. These facts indicate that parental M-14 and M-24 may be immunosuppressive, and that cytokine gene transfer with IL-2 and IL-7 altered this property. Further, the combination of IL-2- and IL-7-transduced cells also resulted in increased cytotoxicity in the majority of experiments performed. Cooperative immune activation by IL-2 and IL-7 has been previously documented, and this combination may be worthy of study in the development of genetically engineered tumor vaccines.10,13,18

The finding of decreased HLA-DR expression in M-14 (IL-7) cells was not anticipated on the basis of previous work with this cytokine. Although cytokines such as interferon-γ upregulate MHC antigen expression on tumor cells, the effects of IL-7 on MHC expression have not been widely studied.37,43,44 Some investigators have noted IL-7-induced upregulation of the intercellular adhesion molecule-1 (ICAM-1), in melanoma and T cells, and have associated this finding with enhanced antigen presenting capabilities.46,47 There is evidence suggesting that decreased MHC class II expression in melanomas may be associated with the level of tumor invasion and reduced metastatic potential, but these correlations have yet to be clearly delineated.48 These preclinical experiments were performed to determine if the MHC antigens required for tumor antigen presentation (class I) were altered by IL-7 transduction. No difference in MHC class I expression was noted in either cell line.

A number of putative tumor-specific antigens have been described for human melanomas.48 MAGE antigens are a family of genes whose protein products are expressed on the cell surface of many malignant melanomas and other tumor types in association with HLA-A1 antigens.92,34 These proteins are capable of eliciting a cytotoxic T-lymphocyte reaction when cultured with autologous lymphocytes.34 The results of this study show that expression of these proteins, which may be important in tumor immunity, is not altered by retroviral gene transduction with either IL-2 or IL-7.

The immunosuppressive factor TGFβ1 is produced by several tumor types including melanomas; it is a potent inhibitor of antitumor responses.47 Tumors producing TGFβ1 may have a more malignant phenotype.49 IL-7 has recently been shown to downregulate TGFβ1 production in murine fibrosarcomas as well as in murine macrophages and, therefore, may play an important role in the immunomodulation of TGFβ1-producing tumors (Dubinett, submitted). The downregulation of tumor TGFβ1 production may have several beneficial effects including the reversal of immunosuppression of cytotoxic lymphocytes associated with malignancies.50 Because TGFβ1 decreases lymphocyte endothelial binding, diminished production of this cytokine may also contribute to the access of lymphocytes to the tumor site, as well as their potential for antigen recognition.51 M-24 (IL-7) cells produced less than one-third the TGFβ1 compared with neoR-transduced cells, and with parental cells cultured in media alone and in the presence of exogenous recombinant IL-7. This finding was reflected at the mRNA level as well, but was not observed for M-14. The absence of TGFβ1 regulation may be attributable to the lower level of IL-7 produced by the M-14 cell line, or to inherent differences between these tumors. Our studies with murine fibrosarcomas indicate that the degree of IL-7-mediated TGFβ1 downregulation varies with individual cell lines (Dubinett, submitted).

A primary goal of gene therapy for cancer is the immunomodulation of malignancies by the constitutive production of substances capable of inducing tumor rejection by the host. IL-2 and IL-7 have been shown to be powerful mediators of several effector arms of the cellular and humoral immune responses. Their immunobiological alterations of two well-characterized melanoma cell lines have implications for clinical vaccine gene therapy efforts.

ACKNOWLEDGMENT

The authors thank June Baumer for assistance with analyzing in vitro growth data. M-14 and M-24 cell lines were provided by Romaine E. Saxton, PhD. The technical assistance of Rosemary Martinez is greatly appreciated. The ImmuneX Corporation generously provided IL-7 cDNA.

REFERENCES

9. Welch PA, Namen AE, Goodwin RG, Armitage R, Cooper
42. Uchiyama H, Darrow TL, Seigler HF, deKernion JB: Human renal carcinoma line transfected with interleukin-2 or interferon a gene(s): Impl...


Transduction of human melanoma cell lines with the human interleukin-7 gene using retroviral-mediated gene transfer: comparison of immunologic properties with interleukin-2

AR Miller, WH McBride, SM Dubinett, GJ Dougherty, JD Thacker, H Shau, DB Kohn, RC Moen, MJ Walker and R Chiu