Transfection of Interleukin 2 Gene into Human Melanoma Cells Augments Cellular Immune Response

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Abstract

A prcclinical model was used to determine if transfection of the interleukin-2 (IL-2) gene into human melanoma cells would augment the response of autologous and allogeneic peripheral blood lymphocytes (PBLs) from melanoma patients. IL-2 gene was transfected into three human melanoma cell lines; secretion of IL-2 from stable transfected cells was confirmed by enzyme-linked immunosorbent assay. The PBL response to these melanoma cells was then examined in a mixed-lymphocyte tumor reaction using PBLs from eight melanoma patients. The PBL response to autologous (P < 0.01) or human leukocyte antigen A cross-reactive (P < 0.05) transfected melanoma cells was significantly higher than it was to nontransfected melanoma cells. These data suggest that IL-2 gene transfection may be an important strategic approach to enhance specific immune responses induced by a polyvalent melanoma cell vaccine.

Introduction

Interleukin 2 plays an important role in activating antigen-specific T-cells (1, 2). Recent animal model studies show that murine tumor cells transfected with IL-2 gene can secrete IL-2 and, when challenged in animals, can induce systemic antitumor immunity and protection against tumor challenge (3–6). Cytokine gene transfection into tumor cells is applicable to the production of a cancer vaccine using whole cells. Development of a parallel clinical model for human melanoma would increase the efficiency of MCV therapy (7) and potentially eliminate the problems of toxicity associated with exogenous administration of IL-2 (8). Active specific immunotherapy using MCV has been shown to be one of the most promising approaches of inducing antitumor protective immunity (9, 10) and management of malignant melanoma patients (7, 11).

We recently demonstrated that T-cells can be stimulated in vivo and in vitro by allogeneic melanoma cells bearing shared or cross-reactive HLA-A antigens (12, 13). This finding is particularly important in the development of a polyvalent allogeneic melanoma cell vaccine for activating melanoma patient immune responses. In this study, full-length human IL-2 complementary DNA was transfected into human melanoma cell lines. We analyzed the cellular immune response to transfected and nontransfected melanoma cell lines, using HLA-typed autologous and allogeneic PBLs obtained from eight melanoma patients.

Materials and Methods

PBLs. Blood was obtained from eight melanoma patients. PBLs were separated by ficoll-hypaque gradient centrifugation, resuspended in AIM-V medium (Gibco, Grand Island, NY) containing 10% human AB (Irvine Scientific, Santa Ana, CA) heat-inactivated serum, and then used immediately in a MLTR or cryopreserved for HLA typing (14).

Tumor Cell Lines. Mcx, M10, and M101 melanoma cell lines were established and characterized in our laboratory. The HLA was typed by Dr. Paul Terasaki’s laboratory (UCLA School of Medicine, Los Angeles, CA) (14). The HLA types of these melanoma lines were: Mcx (HLA-A25; B27, 62); M10 (HLA-A24; B35, 38); and M101 (HLA-A2; B29; B44).

Transfections. The IL-2 expression vector pBcl2/RSV-IL-2 (15) contains a rat preproinsulin II gene under the control of RSV long terminal repeat sequences. The entire insulin leader region and insulin sequences encoding a translation initiation codon have been incorporated. This chimeric IL-2 mRNA produces significantly more IL-2 protein than does IL-2 mRNA containing the natural IL-2 leader and initiation codon (15).

Cultured melanoma cells were made into single-cell suspension by trypsinization, washed with phosphate-buffered saline three times, and resuspended in 0.8 ml of phosphate-buffered saline. Sixty μg of PBC12/RSV-IL-2 vector were added to each cell line suspension, and the suspension was transfected into a plastic electroporation cuvette and incubated on ice for 10 min. Electroporation was carried out at 750 V/cm and 25 μfarad using a standard electroporation protocol with a Gene-pulser electroporation unit (Bio-Rad, Richmond, CA). After pulsing, the cuvet was incubated in ice for 10 min. Cells were then transferred into flasks and cultured. Transfected lines were selected and evaluated for IL-2 secretion.

Polymerase Chain Reaction. Total cellular RNA was prepared according to the single-step method, and PCR was performed as previously described (16, 17). Briefly, cell pellets were lysed with guanidine thiocyanate, extracted with acid phenol, and precipitated with isopropanol to 2 μg of total RNA. To synthesize complementary DNA, 20 units of avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) were incubated for 20 min at 37°C with 50 pmol of 3'-IL-2 primer in a 20-μl volume of 1X Taq buffer with 800 nmol deoxyxynucleotide triphosphate. Fifty pmol of 5’-IL-2 primer and 2 units of Taq polymerase (Promega) were added in 1X Taq buffer for a total volume of 80 μl and run for 25 cycles in a thermocycler (Precision Scientific, IL). The following are the IL-2 primers used based on published sequences (18): 5' primer = 5’-GAAATGGAATATATATTTACAGATACGATCC-3' 3' primer = 5’-CTTTCTCAGATCCCTTATGGTAC-3'

In Situ Hybridization. A synthetic oligodeoxynucleotide sequence complementary to the mRNA for IL-2 was end-labeled with digoxigenin and used to detect mRNA in IL-2-transfected melanoma cells. In situ hybridization was performed as recently described (17). Briefly, the cells fixed on glass slides were prehybridized for 1 h at 42°C in a solution containing deionized formamide, 20X standard saline citrate (SSC) (1X SSC = 0.15 M NaCl, 0.015 M Na₃C₆H₅O₇). Denhardt’s solution (Sigma), heat-denatured sheared herring sperm DNA, yeast transfer RNA, and dextran sulfate. Primers (3’ end) of IL-2 mRNA synthesized for PCR were tail-labeled with digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN) using the DNA tailing kit (Boehringer Mannheim). Digoxigenin-labeled probe was placed on the cells and incubated at 42°C overnight.

Cells containing IL-2 mRNA were detected using the Genius nonradioactive nucleic acid detection kit (Boehringer Mannheim). Briefly, slides were incubated with 2% normal sheep serum and 0.3% Triton X-100 at room temper-
Table 1. Autologous lymphocyte response to Mcx-IL-2 versus Mcx melanoma cells

<table>
<thead>
<tr>
<th>MLTR</th>
<th>Exogenous IL-2 (pg/ml)</th>
<th>[3H]Thymidine incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL</td>
<td>0</td>
<td>496 ± 77</td>
</tr>
<tr>
<td>PBL + Mcx</td>
<td>0</td>
<td>793 ± 22</td>
</tr>
<tr>
<td>33</td>
<td>932 ± 73</td>
<td></td>
</tr>
<tr>
<td>330</td>
<td>7771 ± 502a</td>
<td></td>
</tr>
<tr>
<td>3300</td>
<td>19635 ± 735a</td>
<td></td>
</tr>
<tr>
<td>PBL + Mcx-IL-2</td>
<td>0</td>
<td>8077 ± 706a</td>
</tr>
</tbody>
</table>

a Proliferative response of PBL to melanoma cells was expressed as the average cpm ± SEM of quadruplicate experiments in MLTR.

b Significance of the difference was compared with the cpm of PBL plus Mcx without IL-2 (P < 0.01).

increase in response to Mcx-IL-2 versus Mcx cells (P < 0.01). For comparison purposes we examined the effect of adding exogenous IL-2 to MLTR with Mcx and compared to MLTR with Mcx-IL-2 (Table 1). When 330 pg/ml or more exogenous IL-2 was added to MLTR, there was a significant enhancement of stimulation. MLTR with Mcx-IL-2 was slightly higher than the addition of 330 pg/ml IL-2 to MLTR with Mcx. As a control, PBL stimulated with 1650 pg/ml of IL-2 alone gave a 1.9-fold increase (cpm) in proliferative response above PBL alone. This response was significantly less than PBL stimulation with Mcx-IL-2.

Six allogeneic patients' PBLs were stimulated with Mcx cells. MLTR showed that three of these patients (EW, JE, MC) had a significantly (P < 0.01) higher response to Mcx-IL-2 versus Mcx cells (Fig. 2A). The other three patients (LO, DO, VA) had no significant difference in response to Mcx-IL-2 or Mcx. Importantly, on the evaluation of the three patients PBL with a differential stimulated response to Mcx-IL-2 had HLA-A antigens that cross-reacted with HLA-A25 of Mcx; the other three patients (LO, DO, VA) did not have cross-reactive HLA-A antigens. The allogeneic PBL and Mcx cells did not share any HLA-B antigens.

We examined the response of a melanoma patient's (SM) (HLA-A3, 23; B7, 44) PBL to two allologenic IL-2-transfected and nontransfected melanoma cell lines. The allogeneic melanoma cell lines are currently used in our MCV therapy. Both IL-2-transfected lines significantly (P < 0.05) augmented MLTR response compared to respective nontransfected lines (Fig. 2B). There was a differential difference in the MLTR response between the two transfected lines. This may be explained by the HLA-A antigen cross-reactivity as recently described (12, 13). The HLA-A23 antigen (SM PBL) strongly cross-reacts with HLA-A24 antigen (M10) and to a lesser degree with HLA-A2 antigen (M101) (12, 13). MLTR showed this patient's response to be significantly higher for transfected versus nontransfected cells of both M10 (P < 0.05) and M101 (P < 0.05) lines (Fig. 2B).

Flow cytometry analysis showed that IL-2 gene transfection did not change the Mcx expression of HLA antigens and ICAM-1. ICAM-1 and HLA class I but not HLA-DR were expressed on the surface of Mcx cells (Fig. 3).

Discussion

The MLTR with autologous PBLs revealed a markedly increased response to Mcx when these melanoma cells were transfected with the IL-2 gene. This response indicated that the small amount of IL-2 secreted from the transfected cells can augment a specific PBL response. MLTR using allogeneic PBLs from six patients showed a significant response to Mcx-IL-2 melanoma cells only when the patient had cross-reactive HLA-A. This finding supports specific stimulation by IL-2-transfected melanoma cells and confirms previous observations that human T-cells recognize allogeneic melanoma cells with shared or cross-reactive HLA-A (12, 13, 19, 20). MLTR using IL-2-transfected M10 and M101 provided further evidence for a specific T-cell response: allogeneic PBLs responded more strongly to the melanoma cell line bearing a more cross-reactive HLA-A antigen. The
IMMUNE RESPONSE TO IL-2-TRANSFECTED HUMAN MELANOMA

Patients

Cellular and humoral responses against melanoma in vivo (8–11) and can significantly improve the long-term survival of patients with metastatic melanoma (7, 11). The MCV was selected on the basis of expression of polyvalent tumor-associated antigens that are immuno-genic in humans (7) and HLA-A antigens. Selective HLA-A matched allogeneic melanoma cells can be more effectively utilized in MCV protocols compared to autologous melanoma, particularly in regard to logistic administration to patients. Transfecting MCV cell lines with the IL-2 gene should improve MCV therapy efficacy and eliminate problems associated with systemic administration of IL-2 with MCV. Previously, we have demonstrated that systemic IL-2 administered with MCV was toxic (8). Low-dose IL-2 in the microenvironment of the tumor cells used as immunogens in the vaccine may be sufficient to provide the activating signal to further augment specific T-cell responses. Previously we have demonstrated that MCV therapy can specifically augment MLTR and cytotoxic T-cell responses to autol-ogous melanoma (7). Before cytokine gene transfection into whole tumor cell lines can be used for cancer vaccines, the selection of the tumor lines and therapy with the nontransfected tumor cells must be

recognition by PBLs of allogeneic melanoma with shared or cross-reactive HLA-A antigens has previously been demonstrated by antibody and cold-target inhibition assays (12, 13).

Flow cytometry showed that IL-2 gene transfection did not change Mcx cell surface expression of HLA class I, HLA-DR, or ICAM-1. These molecules play important roles in T-cell recognition of tumor cells; however, the marked increase in PBL response was not due to modulation of these cell surface antigens.

The M10 and M101 melanoma cell lines are two of the three lines of the MCV used for active specific immunotherapy (7). This MCV is a promising form of immunotherapy that enhances tumor-specific

Fluorescence intensity

Fig. 3. Expression of HLA class I (A), HLA-DR (B), and ICAM-1 (C) on nontransfected Mcx cells (open areas) and IL-2-transfected Mcx cells (closed areas). Mcx cells were stained with anti-HLA class I, anti-HLA-DR, and anti-ICAM-1 antibodies; washed; and then stained with fluorescein isothiocyanate-conjugated goat anti-mouse IgG. The control histograms represent staining by fluorescein isothiocyanate-conjugated goat anti-mouse IgG without exposure to primary antibody. Flow cytometry analysis was performed with the FACSort flow cytometer.

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well established. From our previous studies with the established MCV, we have an excellent in vivo system to rigorously evaluate the efficacy of IL-2 transfection and confer the in vitro results. This model system will also allow us to assess other cytokine gene transfections into MCV lines to prescreen in vitro their potential effect before in vivo application.

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References

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