In Vivo Effects of Locally Secreted IL-10 on the Murine Antitumor Immune Response

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Background: Interleukin-10 (IL-10) is a cytokine secreted by the TH2 class of murine lymphocytes that suppresses the secretion of interferon-γ (IFN-γ) by TH1 lymphocytes and inhibits macrophage-mediated T-cell stimulation and cytotoxicity. The observation that IL-10 is produced by human carcinomas in vitro and in vivo led to the hypothesis that this cytokine plays a role in the suppression of the human anti-tumor immune response. We tested this hypothesis in a murine model.

Methods: To evaluate the effect of IL-10 on the induction of an anti-tumor immune response, mice were immunized with tumor cells transfected with the IL-10 gene and then challenged with parental tumor. The effect of the local secretion of IL-10 on an established immune response was tested by immunizing mice with parental tumor and then challenging with IL-10-secreting tumors.

Results: IL-10-secreting tumors were as effective immunogens as control tumors. Immune mice rejected IL-10-secreting tumors as readily as control challenge tumors. In an in vitro assay, IL-10 did not inhibit CD8 lymphocyte secretion of IFN-γ in response to tumor stimulation. One IL-10-secreting tumor clone regressed when injected into naive mice and induced an antigen-specific immune response capable of protecting mice from subsequent tumor challenge.

Conclusions: The local secretion of IL-10 did not inhibit either the induction of an anti-tumor immune response or the ability of established effector cells to reject challenge tumors. In contrast to its effect on TH1 lymphocytes, IL-10 does not inhibit IFN-γ secretion by CD8 lymphocytes.

Key Words: IL-10—Immunosuppression—CD8+ lymphocyte—Tumor transfection—Vaccine.

In vitro studies have shown that interleukin-10 (IL-10) inhibits many aspects of murine cell-mediated immunity. First defined by its ability to inhibit the synthesis of cytokines [IL-2, interferon-γ (IFN-γ)] by TH1 lymphocytes in the presence of antigen-presenting cells, IL-10 also impairs macrophage costimulatory signals needed for TH1 lymphocyte proliferation (1–4). Exposure of TH1 cells to IL-10–pretreated Langerhans cells induces clonal anergy in the TH1 population (5). IL-10 also suppresses the effector function of macrophages: macrophage killing ability and IFN-γ–induced macrophage cytokine secretion are both markedly impaired (6,7).

These inhibitory properties of IL-10, coupled with evidence that a TH1-pattern cytokine response is an important mechanism of T cell–mediated tumor regression (8,9), has led to the hypothesis that the local secretion of IL-10 in tumors may suppress the host anti-tumor immune response.

IL-10 has been found to be secreted by some human melanoma, colon carcinoma, and renal carcinoma cell lines (10) and is present in human ovarian carcinoma biopsies (11). Our study examined the effect of local secretion of IL-10 on the murine anti-tumor immune response in vivo to understand better the role of IL-10 found at the site of human tumors.
MATERIALS AND METHODS

Tumor Transfection

The MCA 203 and 205 tumors are methylcholanthrene-induced tumors generated in C57BL/6 mice (12). Plasmid pCD-SRαIL-10, which contains the SV40 early promoter and the RUS segment of human T-cell leukemia virus type 1 long terminal repeat (13), along with the gene for murine IL-10, was obtained from the American Type Culture Collection (ATCC; 68027). Plasmid pCDSRαIL-10 was cotransfected with plasmid pSV2hph, containing a hygromycin-resistance gene (14) at a 40:1 ratio by using a modified calcium phosphate-transfection method (5). The cells were selected in hygromycin (100 μg/ml).

IL-10 Assays

Transfected clones were expanded and tested for mIL-10 secretion by using a mIL-10 enzyme-linked immunosorbent assay (ELISA; Endogen, Boston, MA, USA). IL-10 assays were done on supernatants from 1 million cells in 2 ml of complete medium (16) in a 24-well Costar plate at 48 h. By using this ELISA, 1 unit/ml corresponds to 262 pg/ml. The mast cell line H7 was a gift of Dr. Bruce Hess (Immunex, Seattle, WA, USA). These cells were cultured in McCoys medium supplemented with heat-inactivated 10% fetal calf serum, minimal essential medium (MEM) amino acids (0.1 mM), MEM nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), 2-ME (100 μM), penicillin 50 IU/ml, streptomycin (50 μg/ml), gentamicin (50 μg/ml), and mIL-3 (100 ng/ml; Genzyme, Cambridge, MA, USA) washed thrice, and then added to samples of tumor-cell supernatant along with 0.3 ng/ml IL-4. They were incubated for 18 h, pulsed with tritiated thymidine for 6 h, and then counted. Under these conditions, H7 mast cells proliferate in response to IL-10 (Hess, unpublished data). Murine IL-10 was kindly provided by Dr. Satish Menon (DNAX, Palo Alto, CA, USA). The specific activity of this mIL-10 was 5 × 10^6 U/mg.

CD8 Lymphocyte Cytokine Secretion

Two clones, 0.3.1 and 3F5, specifically lytic for the murine lymphomas B6 1710 and B6 1153, respectively (17), and one cell line, αEmale G2, which is specifically lytic for the murine lymphoma Emale G2, are 100% CD8+ by fluorescence-activated cell sorting (FACS; 17 and unpublished observations). CD8 lymphocytes (2.5 × 10^6) were stimulated with an equal number of tumor cells in 1 ml of media in the absence of IL-2. Supernatants were harvested at 24 h and tested for IFNγ by ELISA (Genzyme).

Animal Experiments

Female C57BL/6 mice were obtained from the Charles River Breeding Laboratories, Wilmington, MA, USA. C. parvum was the gift of Dr. James Yang (NCI, Bethesda, MD.) and was used at 1 mg/ml in 0.05 ml Hanks' balanced saline solution (HBSS) containing 20 million tumor cells per milliliter. Footpad amputations were done at day 7 by using pentothal anesthesia. Statistical calculations were done by using Fisher's exact test.

RESULTS

Transfected Tumors Stably Secrete Biologically Significant Levels of IL-10

The methylcholanthrene-induced sarcoma, MCA 203, was cloned by limiting dilution. One clone (203.5) was cotransfected with the plasmid pCD-SRαIL-10, containing the murine IL-10 gene, and the plasmid pSV2hph, which confers resistance to the antibiotic hygromycin. Selection in hygromycin yielded two clones, 203.5IL-10A and 203.5IL-10B. The initial clone 203.5 was also transfected solely with pSV2hph and selected in hygromycin to generate a control clone, 203.5HY. The in vitro growth rates of the parental clone and the transfecants were equivalent.

Tumor cell supernatants were assayed for the presence of IL-10 by ELISA. As shown in Table 1, biologically significant levels of IL-10 were produced by the transfected tumor cells, whereas no IL-10 was detected in the parental or control transfected clone supernatants. The tumor cells were stably transfected; the amount of IL-10 detected in the cell supernatants during 15 weeks of continuous culture

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Initial test</th>
<th>Time in culture (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>203.5IL-10A</td>
<td>3,686</td>
<td>4,864</td>
</tr>
<tr>
<td>203.5IL-10B</td>
<td>7,680</td>
<td>5,376</td>
</tr>
<tr>
<td>203.5HY</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>203.5HY</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

IL-10, interleukin-10.

aPicograms per milliliter IL-10 per 5 × 10^5 cells/ml for 48 h by enzyme-linked immunosorbent assay.