Interleukin 4 Retards Dissemination of a Human B-Cell Lymphoma in Severe Combined Immunodeficient Mice

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Abstract

We have examined the antitumor activity of murine interleukin 4 (IL-4) on development of a human B-cell lymphoma (Daudi) in severe combined immunodeficient (SCID) mice. The progression of Daudi cells in SCID mice was followed by histological staining and by flow cytometric analysis of CD20+ cells in spleen, liver, bone marrow, and kidneys. By day 35, CD20+ Daudi cells populate the majority of space in the bone marrow and kidney in vehicle-treated mice. Mice receiving i.p. injections of IL-4, commencing 7 or 14 days after tumor inoculation, exhibit a reduction in tumor burden as well as a decrease in CD20+ cells in both compartments. The antitumor activity of IL-4 does not appear to be due to an antiproliferative effect, since the cytokine does not alter the growth of Daudi cells in vitro, nor does it correlate with any marked cellular infiltration in tumor-bearing tissues. In 51Cr-release assays, we observed that splenocytes from IL-4-treated mice were capable of lysing YAC-1 but not Daudi cell targets. Our findings demonstrate that: (a) systemic administration of IL-4 retards dissemination of a human B-cell lymphoma in SCID mice; and (b) antitumor activity elicited by IL-4 may not involve a direct effect on proliferation of Daudi cells or on the induction of cytolytic activity.

Introduction

IL-4, a pleiotropic cytokine capable of affecting diverse cell types of hematopoietic and nonhematopoietic origin (1), has been shown to elicit potent antitumor activity in vivo (2–6). Tepper et al. (2) and Golumbek et al. (3) demonstrated that IL-4 abrogated development of transplantable murine tumors when administered locally at the site of injection. In these experiments, cytokine was delivered using implants composed of myelomas (2) or renal cell carcinomas (3) transfected with the IL-4 gene. In the latter case, mice bearing IL-4-secreting tumor cells were capable of eliciting an immune response sufficient to reject challenges with the parental tumor (3). In general, the tumors sensitive to IL-4 treatment developed as solid masses, which exhibited only marginal dissemination to distant anatomical sites. However, other murine models have been described in which tumor development and progression display growth patterns that more closely resemble malignant disease in humans (7–9). In one example, Ghetie et al. (7) showed that a human B-cell lymphoma, Daudi, when injected into C.B.-17 SCID mice, could be detected sequentially in the bone marrow, lung, kidney, and spinal cord. Migration of Daudi cells to the thoracolumbar region was accompanied by paralysis of the hindlimbs. The utility of this model for assessing the efficacy of therapeutic modalities soon became apparent when it was demonstrated that administration of immunotoxin conjugates, prepared by coupling anti-CD 19 or anti-CD22 mAb to ricin A chain, delayed the onset of hindlimb paralysis.

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The abbreviations used are: IL-4, interleukin 4; SCID, severe combined immunodeficiency; NK, natural killer.

(10–12). Our goal in the present study was to use this model to evaluate the antitumor efficacy of IL-4.

Materials and Methods

Animals. Male SCID mice (C.B-17 scid/scid) were obtained from Charles River. Mice were maintained in a specific pathogen-free facility.

Cells. Human Burkitt lymphoma cell lines (Daudi) were obtained from the American Type Tissue Collection and maintained by serial passages in RPMI (JRH Biosciences) medium containing 20% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 50 μg/ml streptomycin (Grand Island Biological). Cells were grown in a humidified atmosphere of 5% CO2.

Cytokines. Murine IL-4, having a specific activity of 2–4 × 106 units/mg protein, was obtained from Schering-Plough (Union, NJ).

Induction of Daudi Tumors in SCID Mice (SCID-Daudi Mice). Daudi cells were grown to a density of 0.5–1 × 106 cells/ml. Cells were collected by centrifugation and washed with sterile PBS. SCID mice, ages 6–8 weeks, were given an i.v. inoculum of 1 × 106 Daudi cells in 0.2 ml sterile PBS. Survival was monitored daily.

Cytotoxicity Assays. Expression of cytolytic activity in splenocytes from SCID-Daudi mice was measured using a 51Cr-release assay as described previously by Mulé et al. (13).

Cell Growth Assays. Daudi cells were seeded into 24-well plates (Falcon Plastics) at a concentration of approximately 2.5 × 105 cells/ml in RPMI with 20% FCS. Medium was supplemented with 0.1, 1.0, or 5.0 μg/ml murine IL-4. After 24, 48, and 72 h incubation, viable cell counts were determined by trypan blue dye exclusion.

Therapy of SCID/Daudi Mice. Treatment with once daily i.p. injections of murine IL-4 (1 or 5 μg/day) in 1% normal mouse serum/PBS or vehicle control (total volume, 0.2 ml) were commenced 7 or 14 days after i.v. inoculation of Daudi cells.

Recovery of Tissues/Cells. On days 28 and 35 after inoculation of Daudi cells, SCID/Daudi mice were euthanized. Spleens, liver, and kidneys were excised. To facilitate flow cytometric analyses, cell suspensions were prepared from tissue pieces that were minced immediately after excision. In the case of bone marrow cells, single-cell suspensions were prepared from aspirants of femurs as described previously by Fine et al. (14).

Immunofluorescence Analysis. The CD20 marker was used to enumerate Daudi B-cell lymphomas. Single-cell suspensions prepared as described above were stained with phycoerythrin-conjugated anti-human CD20 or isotype-matched (mouse IgG1) control antibody (Becton Dickinson Immunocytometry Systems). Cells were analyzed by FACScan using LYSIS II or CELLQUEST software (Becton Dickinson). In preliminary experiments, mouse IL-4 was shown to have no effect on CD20 expression on Daudi cells in vitro (data not shown). In addition, no detectable binding of the anti-human CD20 to mouse tissues was observed (data not shown).

Histopathology. A gross examination of various tissues was performed after laparotomy. Samples of spleen, liver, kidney, lung, and vertebral column were fixed in 10% buffered formalin and paraffin embedded. After overnight fixation, samples of vertebral column were decalcified with RDO solution (APEX Engineering Products) according to the manufacturer’s protocols. Sections (5 μm) were prepared using a Leitz 1512 microtome and stained with hematoxylin and eosin. Stained sections were examined by light microscopy.

Results and Discussion

Implantation of tumor cells engineered to secrete cytokines (e.g., IL-2, IL-4, and IL-7) has been used as a delivery system in evaluating...
antitumor activity in murine models (2–4, 15). Although certain cytokines, such as IL-4, have demonstrated antitumor activity, the tumors targeted in these studies usually developed as solid masses that did not disseminate. In this report, we have used a SCID mouse model (7) to examine the effect of IL-4 on development of a human B-cell lymphoma. SCID mice were given i.v. inoculations of 1 × 10⁶ Daudi cells, followed by once daily injections of mouse IL-4 (1 or 5 μg/mouse) starting 7 or 14 days after Daudi tumor transfer. A similar dosing regimen was adopted by Katz et al. (16) in assessing efficacy of IL-6 in a murine melanoma model. On days 28 and 35, single-cell suspensions from bone marrow aspirants were prepared and analyzed by flow cytometry for CD20⁺-staining Daudi cells. In Fig. 1, we show approximately 6% CD20⁺ cells in bone marrow aspirants recovered on day 28 from vehicle control-treated mice. Mice receiving injections of IL-4 (1 or 5 μg/day) started 7 days after inoculation with Daudi cells displayed less than 1.3%. A similar reduction in the percentages of CD20⁺ cells in the bone marrow was also seen when IL-4 administration was started 14 days after tumor inoculum.

Gross examination at autopsy of Daudi tumor-bearing SCID (SCID-Daudi) mice on days 28 and 35 after inoculation revealed discrete tumor masses on kidneys with little or no involvement of the liver, spleen, pancreas, pericardium, lungs, and mediastinum. For the most part, these observations have been corroborated by histological examination of tissue sections. In Fig. 2a, we show that SCID-Daudi mice receiving vehicle injections reveal foci of Daudi cells in the cortex of kidneys recovered between days 28 and 35 after inoculation. At the higher magnification (Fig. 2b), darkly staining Daudi cells are readily identified within the renal cortex (Fig. 2a, arrow). In vehicle-treated mice, by day 35, infiltrating Daudi cells account for ≥50% of the renal parenchyma (Fig. 2c). Ultimately, Daudi lymphomas progress to a stage of engraftment where normal kidney parenchyma is displaced by tumor cells and normal tubular architecture is obliterated. In contrast, no tumor foci are seen in kidneys recovered from an IL-4-treated mouse (Fig. 2d). Interestingly, we have been unable to identify a consistent increase in any cellular infiltrate in IL-4-treated SCID-Daudi mice. This stands in marked contrast to previous reports (2, 3), where infiltrates of eosinophils, CD8⁺ cells, and macrophages have been noted.

The most significant reductions in dissemination of Daudi lymphomas were seen at daily doses of 1 or 5 μg IL-4. Treatment with 0.1, 0.25, or 0.5 μg/day were not effective (data not shown). Interestingly, the dosages of IL-4, which were therapeutic, approximate the levels used by Hillman et al. (17) in studies where BALB/c mice given daily injections of 1–2 μg IL-4 exhibited a dramatic decrease in the formation of pulmonary metastases by murine renal carcinomas.

IL-4 completely ablated colonization of the kidney in mice given an i.v. inoculum of 1 × 10⁶ Daudi cells. In subsequent experiments, it became apparent that the potency of IL-4 antitumor activity was dependent upon the size of the initial tumor burden. Mice given inocula of 5 × 10⁶ Daudi cells exhibit tumor growth by day 35 in the kidney cortex, despite receiving daily injections of IL-4. However, the number and size of foci were markedly reduced when IL-4 treatment was initiated 7 days after tumor inoculum (data not shown).

In addition to a lower incidence of kidney colonization by Daudi lymphomas, IL-4-treated mice exhibited a significant increase in survival as well as a delay in the onset of hindlimb paralysis. In one representative experiment, 8 of 10 mice receiving vehicle injections display hindlimb paralysis by day 35. In contrast, paralysis was demonstrated in only 2 of 11 mice treated with 5 μg IL-4 (data not shown).

No visible toxicities were observed in IL-4-treated mice, even at the highest dose of IL-4 used (5 μg/day). This dosage corresponds to approximately 250 μg/kg/day. At necropsy, IL-4-treated SCID-Daudi mice revealed a significant degree of splenomegaly with an accompanying increase in splenic cellularity (data not shown). The absence of toxicities in mice is in sharp contrast to those seen in humans, where dose-limiting toxicities of IL-4 have been reported in recent Phase I clinical trials (17).

Although abrogation of dissemination of Daudi tumors in SCID mice by IL-4 has been reproducible over seven separate experiments, a definitive mechanism for these effects remains to be determined. The expression of high affinity receptors for human IL-4 on human renal cell (18) and colorectal (19) carcinoma cell lines appears to correlate with inhibition of tumor cell growth by human IL-4 observed in vitro. The fact that murine IL-4 does not bind with high affinity to human cells (1) should not exclude the possibility that low affinity ligand receptor interactions could affect the growth of Daudi cells in vitro. To assess direct antiproliferative effects of IL-4, we exposed Daudi cells to mouse IL-4 at concentrations of 0.1, 1, or 5 μg/ml. After 24, 48, and 72 h of incubation, viable cell counts were determined by trypan blue dye exclusion. As shown in Fig. 3, untreated Daudi cells exhibit a cell doubling time of between 24–36 h. Over the course of this experiment, IL-4 did not affect the growth rate. Thus, a definitive mechanism for these effects remains to be determined.
Fig. 2. Effect of IL-4 treatment on colonization of kidneys in SCID-Daudi mice. Sections of kidneys from vehicle-treated (A-C) and IL-4-treated (D) SCID-Daudi mice. A. Daudi cells (arrow) can be observed in the peripheral kidney cortex enveloping glomeruli and renal tubules on day 28 after tumor inoculation. B. Higher magnification of A. Darkly staining Daudi cells are easily distinguished from kidney parenchyma. C. On day 35, Daudi cell infiltrates account for ≥50% of renal tissue. D. no Daudi foci are detected in the kidney of a SCID-Daudi mouse treated with 5 μg IL-4 starting 1 week after tumor implantation. A, C, and D, × 40; B, × 400.
observed in splenocytes from control mice against Daudi cell targets. These levels were slightly elevated (5–6% specific lysis) in IL-4-treated mice. To rule out the possibility that the C.B.17 strain used for this study was defective in expression of cytolytic activity, splenocytes were also tested against NK-sensitive YAC-1 cells. In this case, greater than 10% specific lysis was observed in control-treated mice. In comparison, splenocytes from mice treated with IL-4 displayed almost 2-fold greater activity (25–30%; Fig. 4b). These results are reminiscent of those reported by Zhai et al. (21), where treatment with recombinant IL-2 suppressed growth of MCF-7 human breast carcinoma in SCID mice (18) by a mechanism unrelated to an enhancement of lymphokine-activated killer activity against MCF-7 cells. These investigators suggested that suppression of lymphokine-activated killer activity was mediated by activated macrophages. However, confirmation of this hypothesis awaits further investigation.

Tumor cells extravasate via the binding of cell surface molecules (e.g., β-integrins) to counter ligands located on vascular endothelial cells (e.g., VCAM-1) or to components of the extracellular matrix (e.g., fibronectin or laminin; Refs. 22 and 23). Incubation of human umbilical vein endothelial cells with IL-4 decreases the expression of the vascular adhesion molecule, VCAM-1 (1). One might speculate that IL-4 could affect the transendothelial migration and tissue dissemination of Daudi cells in SCID mice by altering adhesive interactions in vivo. Blase et al. (24) have presented evidence recently correlating the functional expression of the fibronectin receptor α5β1 (CD49e/CD29) with dissemination of malignant human B lymphocytes in SCID mice. Another putative mechanism enabling tumor cells to traverse tissue barriers in vivo involves the secretion of matrix metalloproteinases that can degrade basement membranes, facilitating transit through the subendothelium (25). It would be of interest to examine whether the expression of α5β1 or the activity of specific metalloproteinases in Daudi or other malignant human cells can be regulated by IL-4.

It has become apparent that the use of SCID mouse models provides a unique opportunity for characterizing the development of human neoplasms in vivo. Furthermore, the possibility of partially reconstituting SCID mice with human lymphocytes permits one to assess the biological activity of immunoregulatory agents, such as IL-4, on cell subsets that may mediate host response to tumors (17). The experiments described here may provide a first step toward such studies.

**References**

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