Murine intracerebral interleukin-2 injection: pathological and immunological effects


Department of Neurosurgery, Medical School, and Department of Pathology, Institute of Virus Research, Kyoto University, Kyoto, Japan

The authors have investigated whether specific pathological changes and antibodies against interleukin-2 (IL-2) are induced after intracerebral administration of recombinant IL-2 (rlL-2). In addition, IL-2 receptor (IL-2R) expression was checked on the cell surface of normal brain tissues before and after the intracerebral infusion. Reconstituted rlL-2 (specific activity $1.2 \times 10^7$ U/mg protein) was injected into the right cerebral hemisphere of normal adult C57BL/6 mice in three different dose groups, each receiving single or multiple infusions of 8, 32, or 80 U. In sham control experiments, mouse albumin purified by gel filtration and ion exchange chromatography and adjusted to the same concentration of protein as rlL-2 was injected into mice at various doses. Anti-IL-2 antibodies were measured by an enzyme-linked immunosorbent assay concurrently with assessment of IL-2 activity in serum. The IL-2R expression was determined by using immunofluorescence techniques with monoclonal antibodies against mouse IL-2R. Since histological alteration after rlL-2 injection did not differ from that in the sham control preparations, it seems that there is no direct toxic action of rlL-2 on normal brain tissues. Interleukin-2 antibodies were produced at low levels only in mice injected repeatedly at the maximum dose, and levels were insignificant in other groups. Serum levels of IL-2 activity remained low. The IL-2R expression within the brain was not enhanced within 8 weeks following the intracerebral administration of rlL-2, suggesting that direct intracerebral infusion of rlL-2 may be safely used in the immunotherapy of brain tumors.

KEY WORDS • interleukin-2 • brain neoplasm • immunotherapy

HIGHLY purified interleukin-2 (IL-2) expressing the gene for IL-2 in Escherichia coli (clone) is now available through recombinant deoxyribonucleic acid (DNA) technology. Recombinant IL-2 (rlL-2) has been shown to possess a broad range of immunoregulatory activities, and the in vivo administration of rlL-2 has been reported to possess the antitumor activity of adoptive transfer of immune lymphocytes expanded in IL-2.

On the other hand, recent preliminary clinical and animal trials have shown that systemic infusion of high-dose rlL-2 may produce toxic side effects such as pulmonary and hemodynamic abnormalities and neurological dysfunction. The causative mechanisms have not been clarified, although they appear to be directly related to systemic capillary permeability. It has recently been suggested in animal models that, following systemic high-dose injection of rlL-2, brain vascular permeability is also increased, accompanied by profound morphological vasculature and parenchymal change. Furthermore, it has been reported that in two of 12 patients without central nervous system disease (with intact blood-brain barrier (BBB)), barrier disruption followed systemic administration of rlL-2, probably related to increased vascular permeability in the normal brain. It is not known, however, whether direct intracerebral injection of rlL-2 affects neuronal or glial cells of normal brain. In addition to histological changes, there remain other very important problems that should be resolved; these include the possibility of IL-2 receptor (IL-2R) being induced on the cell surface of normal brain tissue and of antibodies formed against IL-2 being produced after direct intracerebral injection of rlL-2.
Effects of intracerebral injection of IL-2 in mice

The aim of the present study was to examine whether pathological changes and anti-IL-2 antibodies are produced after single or repeated injections of rIL-2 into a normal adult mouse brain. Furthermore, the expression of IL-2R on the cell surface of brain tissue was ascertained before and after intracerebral injection of rIL-2, using immunofluorescence techniques with monoclonal antibody (MAb) against mouse IL-2R. The possibility of clinical application of rIL-2 for brain tumors is also discussed.

Materials and Methods

Preparations

Male 6- to 8-week-old C57BL/6 mice were supplied from the Animal Center of Kyoto University for all experiments. Highly purified rIL-2 (TGP-3) derived from Escherichia coli and purified to homogeneity was used.* The rIL-2 had a specific activity of $3.5 \times 10^4$ U/mg protein in Takeda units.12,23 It corresponded to 1.2 x $10^4$ U/mg protein, calculated on the basis of the Biological Response Modifiers Program reference reagent human IL-2 (Jurkat).6 After lyophilization and reconstitution with phosphate-buffered saline (PBS) (0.02 M sodium phosphate buffer, pH 7.0, containing 0.15% NaCl), the rIL-2 was given as an intracerebral or intraperitoneal infusion. The level of endotoxin in the preparation was less than 0.1 ng/mg of rIL-2 as measured in a standard Limulus amebocyte lysate assay.

For the lymphocyte preparation, mesenteric lymph node cells from normal adult mice were prepared by dispersing the cells with scissors. The lymphoid cells were suspended at $5 \times 10^6$ cells/ml in RPMI-1640 medium supplemented with 2% heat-inactivated fetal calf serum (FCS) and concanavalin A (5 µg/ml),† and cultured at 37°C for 3 days in an atmosphere of 95% air and 5% CO2. The mitogen-activated lymphocytes were used as a positive control for immunohistochemical staining of IL-2R.

Intracerebral Injection of IL-2

The reconstituted rIL-2 was injected into the right cerebral hemisphere of adult mice with a 25-µl Hamilton microinjector and a Yaoi needle. The intracerebral injection point was performed under semi-stereotactical guidance, 5 mm to the right of the midline and 5 mm posterior to the midorbital line. The injected mice were divided into three groups according to the dose of administered rIL-2 titer: 8, 32, or 80 U. These three groups were further divided into two subgroups each, one receiving single and the other multiple (serial 4-weekly) injections of rIL-2. The volume of the intracerebral injection was kept less than 10 µl per mouse in order to avoid the production of increased intracranial pressure.14 Mouse albumin was purified by gel filtration (Sephadex G-200) and successive ion exchange chromatography (diethylaminoethyl cellulose). The purified mouse albumin was adjusted to the same concentration of protein as rIL-2 in each group of the injected mice, when used as a sham control preparation for intracerebral administration of rIL-2. For systemic administration of rIL-2 as a control, reconstituted rIL-2 was injected intraperitoneally at the same time as the intracerebral injection.

After rIL-2 administration, IL-2 and anti-IL-2 antibody activity in the serum and histological examination including IL-2R expression were chronologically investigated in each group of treated mice. Groups of three to 10 adult mice were used for each experiment.

Antisera

In order to obtain both IL-2-specific capture antibody and indicator antibody for determination of IL-2 activity by enzyme immunoassay (EIA) using a double-antibody sandwich, rIL-2 (1 mg/ml in PBS) was emulsified in an equal volume of Freund's complete adjuvant,‡ and an aliquot containing 1.0 mg of rIL-2 was given subcutaneously to goat and rabbit. Antisera were collected after successive 3-weekly injections of rIL-2 emulsified with incomplete Freund's adjuvant. For purification of anti-IL-2 antisera, immunoglobulin was precipitated with (NH₄)₂SO₄ at 40% saturation. After dialysis, specific antibodies were isolated by affinity chromatography on rIL-2-Sepharose 4B. Affinity-purified antisera were prepared by adsorption of hyperimmunized antisera on Sepharose 4B columns with the antigen (rIL-2) covalently linked with subsequent elution. The titer of each serum was then measured by EIA. The eluate containing immunoglobulin was neutralized to pH 7.0 with NaOH and frozen until use. Goat anti-IL-2 antibody was used as IL-2-specific capture antibody. Rabbit anti-IL-2 antibody was used as indicator antibody, and was labeled directly with horseradish peroxidase.10,18 In order to obtain the standard positive control with definite IL-2 neutralizing activity, mice were immunized subcutaneously with 0.2 mg of TGP-3 emulsified with complete Freund's adjuvant. Antisera were collected after successive 3-weekly injections of TGP-3 emulsified with incomplete Freund's adjuvant. The titer of each serum was measured by enzyme-linked immunosorbent assay (ELISA) after purification by affinity chromatography on rIL-2-Sepharose 4B.

For immunohistochemical analysis of IL-2R, rat anti-mouse IL-2R MAb was used.§

Measurement of IL-2 Activity

Two assay procedures were used for IL-2 activity in serum. A conventional bioassay for IL-2 and a double

---

* Recombinant IL-2 kindly supplied by Takeda Chemical Industries, Ltd., Osaka, Japan.
† Concanavalin A supplied by Pharmacia Fine Chemicals, AB Uppsala, Sweden.
‡ Freund's complete adjuvant supplied by DIFCO Laboratories, Detroit, Michigan.
§ Monoclonal antibody was the generous gift of Dr. J. Yodoi, Institute for Immunology, Kyoto University, Faculty of Medicine, Kyoto, Japan.
antibody sandwich EIA in which a solid phase is coated with IL-2-specific antibody. For bioassay, IL-2 activity was evaluated by its ability to maintain an IL-2-dependent murine cell line, as reported previously.\(^{16,33,36}\) In the present study, however, the double antibody method was used exclusively because the latter method showed much higher sensitivity and absolute specificity for IL-2.\(^{7}\) A solid phase is coated with IL-2-specific capture antibody. This is reacted with the test sample containing IL-2, then with enzyme-labeled IL-2-specific indicator antibody constituting a different species from the capture antibody, followed by the enzyme substrate. The antigen (or IL-2) in the test sample is captured and immobilized on the sensitized solid phase where it can itself then fix the enzyme-labeled antibody. The microtiter plates were precoated with the capture antibody by incubating them overnight at room temperature with a solution of antibody in PBS (100 µl/well at 15 µg/ml). The optimum concentrations of capture antibody were determined by titration. After two washes with PBS, 100 µl of 5% serum in PBS was added to each well for 1 hour to block nonspecific binding during subsequent steps. The serum used in this step and in the diluent for subsequent steps was from the same species of animal as the indicator antibody. Two washes with PBS were followed by the addition (100 µl/well) of the serially diluted test samples, of a positive control solution (10% rIL-2 diluted in newborn calf serum) and a negative control solution (newborn calf serum alone diluted in PBS). After incubation at room temperature for 90 minutes the plates were washed five times with PBS, and then indicator antibody at a dilution of 1:1000 in PBS was added to each well (100 µl/well). A further 90 minutes of incubation followed, then the plates were washed five times with PBS. The amount of IL-2 bound was then detected by the addition of 100 µl of substrate solution containing 0.2% of O-phenylenediamine, 6 mM H$_2$O$_2$, and 0.01% thimerosal in 0.1 M phosphate-citrate buffer, pH 5.0. These wells were incubated at room temperature for 10 minutes. The reaction was stopped by the addition of 4 M H$_2$SO$_4$. Absorbance (optical density value) was measured by spectrophotometry at a wavelength of 492 nm. When the titer of anti-IL-2 antibody activity in test samples was determined, both positive (mouse antiserum with definite IL-2 neutralizing activity) and negative (serum from normal mice) controls were simultaneously tested at serial fourfold dilutions at between 1:4 and 1:4096. The results generated in this assay were evaluated by the ratio of the optical densities of the test sample to a group of known reference negative and positive control samples. The titer of anti-IL-2 antibody activity in test samples was then calculated by the reciprocal of dilution at which the test sample shows more than twofold optical densities of the reference negative samples. The protein concentrations were calculated by reference to a semilogarithmic standard curve derived from nine dilutions of mouse IgG (Fig. 1).

**Histological Study**

Parallel experiments of mice treated with rIL-2 and purified mouse albumin were performed in which mice were sacrificed by cervical spine dislocation at specific time points, and their brains were submitted for histological processing. The paraffin-embedded tissues were sectioned at 4 µm and stained with hematoxylin and eosin. The site of injection into the right cerebral hemisphere was easily confirmed at the time of sacrifice because injection was performed under semi-stereotactic guidance.

**Immunohistochemical Study**

The brains were harvested and embedded in Optimal Cooling Temperature (OCT) compound. The brain tissues were snap-frozen in liquid nitrogen, cut as frozen sections at 4 µm, and placed on plain glass slides. After brief storage at -50°C, the slides were air-dried and incubated with the primary mouse IL-2R MAb for 30 minutes at room temperature in a humidified chamber. Subsequently, the sections were washed in PBS for 10 minutes. The fluorescein isothiocyanate-labeled rabbit anti-rat IgG was applied to the sections for 30 minutes.

Measurement of Anti-IL-2 Antibody Activity

The anti-IL-2 antibody activity of test samples was determined at serial dilutions run in duplicate by an ELISA. The microtiter plates were coated by incubation for 2 days at 4°C with a solution of TGP-3 in 0.01 M Na$_2$HPO$_4$ and 0.01 M NaCl, pH 8.0 (100 µl/well at 10 µg/ml). After two washes with PBS, 100 µl of 2% bovine serum albumin in PBS was added to each well. The wells were incubated overnight at 4°C to block nonspecific binding during subsequent steps. A 250-µl aliquot of the serially diluted test sample in RPMI-1640 medium containing 10% heat-inactivated FCS was added to the precoated microtiter plates with TGP-3 and incubated overnight at room temperature. After the solution was decanted, the wells were rinsed five times with RPMI-1640 medium containing 10% FCS. A 100-µl aliquot of 5000-fold diluted horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Ig)M and IgG (Fab fragment) was added to each well and incubated at room temperature for 3 hours. After this solution was decanted, the wells were rinsed six times with PBS. The amount of anti-IL-2 antibody bound was then detected by the addition of 100 µl of substrate solution containing 0.2% of O-phenylenediamine, 6 mM H$_2$O$_2$, and 0.01% thimerosal in 0.1 M phosphate-citrate buffer, pH 5.0. These wells were incubated at room temperature for 10 minutes. The reaction was stopped by the addition of 4 M H$_2$SO$_4$. Absorbance (optical density value) was measured by spectrophotometry at a wavelength of 492 nm.

In the present study, histology and immunohistochemistry were used to confirm that the injection was performed under stereotactic guidance. The results generated in this assay were evaluated by the ratio of the optical densities of test sample to a group of known reference negative and positive control samples. The protein concentrations were calculated by reference to a semilogarithmic standard curve derived from nine dilutions of mouse IgG (Fig. 1).

Histological Study

Parallel experiments of mice treated with rIL-2 and purified mouse albumin were performed in which mice were sacrificed by cervical spine dislocation at specific time points, and their brains were submitted for histological processing. The paraffin-embedded tissues were sectioned at 4 µm and stained with hematoxylin and eosin. The site of injection into the right cerebral hemisphere was easily confirmed at the time of sacrifice because injection was performed under semi-stereotactic guidance.

Immunohistochemical Study

The brains were harvested and embedded in Optimal Cooling Temperature (OCT) compound. The brain tissues were snap-frozen in liquid nitrogen, cut as frozen sections at 4 µm, and placed on plain glass slides. After brief storage at -50°C, the slides were air-dried and incubated with the primary mouse IL-2R MAb for 30 minutes at room temperature in a humidified chamber. Subsequently, the sections were washed in PBS for 10 minutes. The fluorescein isothiocyanate-labeled rabbit anti-rat IgG was applied to the sections for 30 minutes.

Measurement of Anti-IL-2 Antibody Activity

The anti-IL-2 antibody activity of test samples was determined at serial dilutions run in duplicate by an ELISA. The microtiter plates were coated by incubation for 2 days at 4°C with a solution of TGP-3 in 0.01 M Na$_2$HPO$_4$ and 0.01 M NaCl, pH 8.0 (100 µl/well at 10 µg/ml). After two washes with PBS, 100 µl of 2% bovine serum albumin in PBS was added to each well. The wells were incubated overnight at 4°C to block nonspecific binding during subsequent steps. A 250-µl aliquot of the serially diluted test sample in RPMI-1640 medium containing 10% heat-inactivated FCS was added to the precoated microtiter plates with TGP-3 and incubated overnight at room temperature. After the solution was decanted, the wells were rinsed five times with RPMI-1640 medium containing 10% FCS. A 100-µl aliquot of 5000-fold diluted horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Ig)M and IgG (Fab fragment) was added to each well and incubated at room temperature for 3 hours. After this solution was decanted, the wells were rinsed six times with PBS. The amount of anti-IL-2 antibody bound was then detected by the addition of 100 µl of substrate solution containing 0.2% of O-phenylenediamine, 6 mM H$_2$O$_2$, and 0.01% thimerosal in 0.1 M phosphate-citrate buffer, pH 5.0. These wells were incubated at room temperature for 10 minutes. The reaction was stopped by the addition of 4 M H$_2$SO$_4$. Absorbance (optical density value) was measured by spectrophotometry at a wavelength of 492 nm.

When the titer of anti-IL-2 antibody activity in test samples was determined, both positive (mouse antiserum with definite IL-2 neutralizing activity) and negative (serum from normal mice) controls were simultaneously tested at serial fourfold dilutions at between 1:4 and 1:4096. The results generated in this assay were evaluated by the ratio of the optical densities of the test sample to a group of known reference negative and positive control samples. The titer of anti-IL-2 antibody activity in test samples was then calculated by the reciprocal of dilution at which the test sample shows more than twofold optical densities of the reference negative samples. The protein concentrations were calculated by reference to a semilogarithmic standard curve derived from nine dilutions of mouse IgG (Fig. 1).
Effects of intracerebral injection of IL-2 in mice

After the slides were rinsed twice in PBS, coverslips were applied with the use of 90% glycerol in PBS. All sections were then evaluated for the presence or absence of fluorescence cells in a blinded study by using a fluorescent microscope with epi-illumination optics.

Each antibody was used at optimal concentration as determined by previous titration experiments. As a positive control for IL-2R staining, activated lymphocytes with concanavalin A were prepared in a similar fashion. Negative controls included the use of complete medium containing 10% FCS in place of the primary antibody for brain tissues or the omission of the primary antibody for activated lymphocytes. Negligible background staining was observed.

Results

Kinetics of Anti-IL-2 Antibody and IL-2 Activity

Prior to the determination of anti-IL-2 antibody activity in test samples, the concentration of enzyme-labeled antibodies used in our assay system was optimized. At the 100-fold dilution of the labeled antibodies, the average absorbance for two dilutions was obtained with the linear range of the reference standard curve \( r = 0.998 \) (Fig. 1); the relationship of optical density to the logarithm of the added protein concentration of mouse IgG was almost linear for dilutions of mouse IgG from 80 ng/ml to zero. Therefore, the 100-fold diluted antibodies were used in the following experiments. From this reference curve, it was first confirmed that our assay can function sufficiently to detect the anti-IL-2 antibody activity, and thus the protein concentration of mouse serum was gained in each group of mice after intracerebral or intraperitoneal administration of rIL-2 (Fig. 2). In our IL-2 antibody detecting system, the titer of antisera from mice treated with adjuvant agents was much higher than that in mice receiving repeated intracerebral or intraperitoneal infusions to a maximum dose of rIL-2 (80 U/injection/mouse). It was noted that, while the antibodies were produced in mice treated with multiple intracerebral or intraperitoneal injections of rIL-2 (80 U/injection/mouse), the titer of anti-IL-2 antibody was insignificant in other groups with the single administration of rIL-2.

Fig. 1. Standard curves for detecting anti-interleukin-2 (IL-2) antibody activity following intracerebral administration of recombinant IL-2. Values of absorbance at a wavelength of 492 nm represent a linear relationship to protein concentrations of mouse immunoglobulin (IgG) from 100 ng/ml to zero, especially at the 100-fold dilution (open circles) of enzyme-labeled antibodies \( r = 0.998 \). Dilutions of mouse IgG are as follows: 30-fold (closed circles), \( 3 \times 10^2 \)-fold (closed triangles), \( 10^3 \)-fold (open triangles), \( 3 \times 10^4 \)-fold (closed squares), and \( 10^5 \)-fold (open squares).

Fig. 2. Measurement of anti-interleukin-2 (IL-2) antibody activity following single or multiple injections of recombinant IL-2 (rIL-2, 80 U/injection/mouse). Sera were harvested 10 days after intracerebral (closed circles) or intraperitoneal (closed triangles) administration. The fourfold diluted antisera were then evaluated for their titer of anti-IL-2 antibody by enzyme-linked immunosorbent assay, using the standard curve as described in Fig. 1. In sham control experiments, purified mouse albumin was used for intracerebral (open circles) or intraperitoneal (open triangles) infusion. Sera from untreated mice (open square) and immunized mice (closed square) by subcutaneous injection of 0.2 mg of rIL-2 emulsified with Freund's adjuvant were used, respectively. Values are expressed as the means ± standard deviations for three to six mice.
Furthermore, it was observed that the amount of anti-IL-2 antibodies in mice with multiple infusions gradually decreased and reached a plateau in untreated mice 1 month after the last intracerebral injection of rIL-2 (Fig. 3). In the case of mice treated with a single 80-U infusion of rIL-2, no significant level of anti-IL-2 antibody activity was seen during the 6 weeks after administration (Table 1). It was verified by double-antibody sandwich EIA that IL-2 activity in the sera was not augmented significantly within 8 weeks following the direct intracerebral administration of rIL-2 (Fig. 4). In contrast, it was found that the IL-2 activity in serum definitely increased at 3 and 15 minutes after the intravenous infusion of rIL-2, although no significant IL-2 activity was seen at 30 minutes or later.

**Histological Examination of Brain Tissues**

As compared with sham control experiments using purified mouse albumin injections, the mice treated with rIL-2 in all intracerebral infusion groups did not show any specific in vivo abnormality during the 8 weeks after single or multiple injections. Histological study mainly focused on examining specific changes involving neuronal degeneration or necrosis, stromal necrotizing angiitis and tissue edema, the appearance of abnormal cells with neovascularization, leptomeningeal or choroid plexus inflammatory reactions, and glial alterations. Although hemorrhages due to direct infusions, thickening of arachnoid membranes, reactive gliosis, or infiltration of mononuclear lymphoid cells were occasionally seen at the injected sites or subependymal layers (Fig. 5), neuronal and vascular pathological changes were not induced following intracranial rIL-2 administration. In addition, ventricular dilatation was observed in only a few cases, probably due to intraventricular hemorrhages after the injections. It seems likely, however, that these findings are nonspecific responses because of a similar observation in the sham control experiments. The nonspecific reactive changes mostly disappeared within 2 months after intracerebral infusion. Thus, it was suggested that neither specific nor

<table>
<thead>
<tr>
<th>Time Post Infusion</th>
<th>Experiment</th>
<th>Optical Density Values (492 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td>i.c. rIL-2</td>
<td>0.718 0.387 0.188 0.119 0.088 0.081</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.817 0.446 0.221 0.123 0.095 0.071</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.615 0.455 0.262 0.134 0.109 0.088</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.612 0.484 0.292 0.132 0.168 0.084</td>
</tr>
<tr>
<td>2 wks</td>
<td>i.c. rIL-2</td>
<td>0.610 0.421 0.201 0.134 0.087 0.068</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.627 0.356 0.163 0.104 0.082 0.076</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.608 0.461 0.236 0.129 0.098 0.088</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.499 0.334 0.185 0.108 0.088 0.079</td>
</tr>
<tr>
<td>3 wks</td>
<td>i.c. rIL-2</td>
<td>0.600 0.419 0.201 0.125 0.082 0.075</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.467 0.336 0.162 0.098 0.079 0.076</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.488 0.336 0.204 0.105 0.083 0.068</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.397 0.296 0.195 0.110 0.090 0.069</td>
</tr>
<tr>
<td>4 wks</td>
<td>i.c. rIL-2</td>
<td>0.638 0.291 0.169 0.103 0.083 0.079</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.549 0.287 0.161 0.099 0.073 0.074</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.638 0.350 0.164 0.114 0.194 0.090</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.549 0.545 0.188 0.121 0.087 0.081</td>
</tr>
<tr>
<td>5 wks</td>
<td>i.c. rIL-2</td>
<td>0.468 0.324 0.191 0.091 0.068 0.119</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.449 0.353 0.188 0.095 0.075 0.162</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.610 0.377 0.198 0.127 0.088 0.083</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.474 0.323 0.190 0.115 0.089 0.083</td>
</tr>
<tr>
<td>6 wks</td>
<td>i.c. rIL-2</td>
<td>0.653 0.421 0.195 0.093 0.140 0.075</td>
</tr>
<tr>
<td></td>
<td>i.c. sham</td>
<td>0.540 0.293 0.143 0.082 0.059 0.071</td>
</tr>
<tr>
<td></td>
<td>i.p. rIL-2</td>
<td>0.509 0.310 0.184 0.121 0.088 0.080</td>
</tr>
<tr>
<td></td>
<td>i.p. sham</td>
<td>0.458 0.271 0.164 0.105 0.077 0.079</td>
</tr>
<tr>
<td>control (positive)</td>
<td></td>
<td>1.416 1.543 1.807 1.629 1.048 0.552</td>
</tr>
<tr>
<td>untreated mice</td>
<td></td>
<td>0.476 0.300 0.153 0.076 0.054 0.053</td>
</tr>
</tbody>
</table>

*Anti-interleukin-2 (IL-2) antibody activity was determined by optical density values. i.c. = intracerebral; rIL-2 = recombinant IL-2; i.p. = intraperitoneal. See Materials and Methods for protocol.
Effects of intracerebral injection of IL-2 in mice

toxic pathological alteration in the normal brain tissues was produced during the 2 months following direct intracerebral administration of rIL-2. The pathological results are summarized in Table 2.

**Immunohistochemical Analysis of Cell-Surface IL-2 Receptor Expression**

The expression of IL-2R on the cell surface of brain tissues in mice was compared before and at 1, 2, and 3 weeks after single or multiple intracerebral infusions of the maximum dose of rIL-2 (80 U/injection/mouse), by using immunofluorescence techniques with anti-mouse IL-2R MAb. No significant expression of IL-2R was induced in either the rIL-2-injected groups or the sham control groups, whereas mitogen-stimulated lymphocytes were clearly stained by the MAb against mouse IL-2R (Fig. 6).

**Discussion**

The authors have previously investigated the therapeutic possibility of specific adoptive immunotherapy with immune cytotoxic T lymphocytes (CTL's) expanded in IL-2 for malignant gliomas.35-37 It was suggested that the simultaneous administration of IL-2 during adoptive immunotherapy would be more desir-
T. Yamasaki, et al.

Fl. 6. Immunohistochemical examination of interleukin-2 (IL-2) receptor in brain tissue after intracerebral administration of recombinant IL-2 (rIL-2, 80 U/injection/mouse), using rat anti-mouse IL-2 receptor monoclonal antibody (see Materials and Methods). x 100. Left: Brain injected with rIL-2. Center: Concanavalin A-activated lymphoblasts (positive control study). Right: Purified mouse albumin-injected brain (sham-treated control study).

able, because the proliferation of immune CTL's is dependent upon IL-2, and IL-2 itself plays a pivotal role in CTL generation and activation on the basis of IL-2 responsiveness.23,32,36,37 The efficacy of local injection of CTL plus rIL-2 into the tumor cavity or cerebrospinal fluid space has been reported in human patients with brain tumors.11,13 On the other hand, it has been demonstrated that systemic administration of high-dose rIL-2 can cause disruption of the BBB as well as of the cerebrovascular morphological integrity,6,30 although the causative mechanisms underlying these alterations are unclear. It has been postulated that IL-2 may act on the cerebral vasculature and, in turn, may elicit pathological changes within the brain parenchyma.

Prior to clinical trials of therapy with combined tumor-specific CTL's and rIL-2, the present study was conducted to further analyze the production of anti-IL-2 antibodies in serum, histological changes, and cell-surface expression of IL-2R within the injected mouse brain tissues during the 8 weeks after direct intracerebral administration of rIL-2. The levels of anti-IL-2 antibody activity were found to be lower in mice treated with multiple intracerebral injections of the maximum rIL-2 dose (80 U/injection/mouse) than in those receiving intraperitoneal infusions; however, the levels were insignificant in mice with single intracerebral or intraperitoneal rIL-2 infusions. The explanation for the incomplete production of antibodies in mice receiving repeated intracerebral injections is probably that the immunological reactions cannot be fully generated in the brain, because the brain is considered a partially immunologically privileged environment in which normal regulatory restraints are lacking.3,17,34 Another explanation is that the BBB in some areas of the injected brain tissues may be artificially destroyed by the direct intracerebral infusion of rIL-2 per se, and therefore systemic immunological responses may be partially induced to produce the antibodies.

In addition to the pharmacokinetics of anti-IL-2

<table>
<thead>
<tr>
<th>Histological Findings</th>
<th>rIL-2 single</th>
<th>rIL-2 multiple (80 units)</th>
<th>sham control single</th>
<th>sham control multiple</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of samples studied</td>
<td>56</td>
<td>36</td>
<td>80 units</td>
<td>16</td>
</tr>
<tr>
<td>hemorrhages (intracerebral or intraventricular)</td>
<td>14 (25.0%)</td>
<td>10 (27.8%)</td>
<td>9 (25.7%)</td>
<td>5 (31.3%)</td>
</tr>
<tr>
<td>thickening of arachnoid membrane</td>
<td>10 (17.9%)</td>
<td>6 (16.7%)</td>
<td>6 (17.1%)</td>
<td>5 (31.3%)</td>
</tr>
<tr>
<td>reactive gliosis</td>
<td>9 (16.1%)</td>
<td>10 (27.8%)</td>
<td>8 (22.9%)</td>
<td>6 (37.5%)</td>
</tr>
<tr>
<td>infiltration of mononuclear lymphoid cells</td>
<td>8 (14.3%)</td>
<td>9 (25.0%)</td>
<td>7 (20.0%)</td>
<td>4 (25.0%)</td>
</tr>
</tbody>
</table>

* rIL-2 = recombinant interleukin-2. The activity of IL-2 was nonspecific. See Materials and Methods for protocol.

738 J. Neurosurg. / Volume 71 / November, 1989
Effects of intracerebral injection of IL-2 in mice

antibody production, we were also interested in the chronological change in IL-2 activity itself following intracerebral injection of rIL-2. Recently, EIA based on the ELISA or radioimmunoassay technique has been developed for quantification of IL-2 using highly purified monoclonal IL-2 antibodies or IL-2. In lower concentrations, IL-2 has been detected by means of a linear dose-response relationship. In this study, both highly purified capture and indicator IL-2 antibodies were employed for the purpose of detecting IL-2 in the test samples. The double-antibody sandwich EIA, which showed as much as 100 times higher sensitivity than a conventional assay, could detect IL-2 activity in concentrations as low as 30 pg/ml with good correlation (r = 0.998) to a bioassay. The assay also detected native IL-2 in serum. With the use of these assay techniques, it was found that the IL-2 activity in mouse serum did not rise significantly after intracerebral injections of rIL-2. In contrast, a high level of IL-2 activity was seen in mice treated with intravenous infusion of rIL-2. Concerning the fate of endogenous IL-2 or of IL-2 injected intravenously, it has been determined that the serum half-life of IL-2 is approximately 3 and 6 minutes in mice and humans, respectively, and that IL-2 is primarily cleared from the circulation by the kidney. 2,5,14,19

Pathological examination showed no direct toxic action of rIL-2 on normal brain tissues, although a temporary, nonspecific infiltration of mononuclear lymphoid cells or a reactive gliosis was occasionally observed at the injected site. These nonspecific reactions, however, mostly disappeared within 2 months after intracerebral rIL-2 infusion. In view of the importance of rIL-2 in therapeutic trials as well as in the pathological situation, it was also important to ascertain the presence or absence of IL-2R on the cell surface of normal brain tissues, and to investigate the possibility of IL-2R induction after the injection of rIL-2. The current immunohistochemical analysis suggested that the expression of IL-2R was not enhanced on the cell surface of normal parenchymal brain tissues after intracerebral infusion of rIL-2.

In association with lymphokines, especially interleukin-1 (IL-1) and IL-2 in the brain, it has been indicated that IL-1 is a potent factor for astrogial proliferation but has no effect on oligodendrogial proliferation and maturation that IL-2 does not alter the growth of either type of glial cells. Benveniste, et al., reported that rIL-2 can influence the growth of oligodendrocytes, specifically their proliferation and maturation, but does not affect astrocytes. Furthermore, they have reported that oligodendrocytes can functionally absorb IL-2, although it is unknown whether IL-2 acts on oligodendrocytes via the same type of cell-surface receptor as that required to activate T cells and B cells. Conversely, Saneto, et al., have demonstrated that when IL-1 was added together with IL-2, IL-2 inhibited the proliferation of neonatal rat oligodendrocyte progenitor cells. The action of IL-2 was specifically mediated by the IL-2R. These discrepancies may be a result of different culture conditions, isolation procedures, and the age of the cells tested.

Acknowledgments

The authors express appreciation to Prof. Dr. George Klein and Dr. Klas Kärre, Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden, for their helpful comments and valuable discussions.

References


