Jpn. J. Cancer Res. 82, 950–957, August 1991

Antitumor Effect of Recombinant Human Interleukin-2 on the Growth of Murine Hemangioendothelioma D14 in Nude Mice: Occurrence of Large Granular Cells in the Tumor

Yasufumi Sakura, Takashi Houkan, Koichiro Ootsu and Akio Shino

Biology Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd., 17-85 Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532

The antitumor effect of recombinant human interleukin-2 (rIL-2) on murine hemangioendothelioma D14 (D14) in female BALB/c-nu/nu mice was examined histologically. D14 cells which had been maintained *in vitro* were transplanted subcutaneously into nude mice on day 0 (1×10^7 cells/mouse). The mice with established tumor on day 28 received rIL-2 subcutaneously at a dose of 20 μg/mouse/day for 35 days. On day 63, the mice were killed, and the tumor, spleen and bone marrow were examined histologically. In the mice that had received rIL-2, tumor growth was significantly suppressed. Histologically, there was marked infiltration of large granular cells (about 15–30 μm in diameter) in the tumors. In the adjacent areas, there was a significant increase in the number of tumor cells showing karyorrhexis. The large granular cells (LGC) contained periodic acid Schiff-positive round granules in the cytoplasm and were stained positively for Thy-1.2 surface antigen. The LGC were also positive for asialo GM1 surface antigen but not for Lyt-1, Lyt-2 or IgG surface antigens. This evidence suggests that the LGC are lymphokine-activated killer-like cells which were derived from a natural killer cell lineage. The concomitant increases in the number of LGC and the number of cells showing karyorrhexis in the tumors of the mice treated with rIL-2 suggest that LGC play an important role in the destruction of tumor cells.

Key words: Interleukin-2 — Hemangioendothelioma D14 — Antitumor effect — Nude mice — Large granular cell

Recombinant human interleukin-2 (rIL-2) has been applied in experimental therapy of patients with various malignant tumors to assess its clinical benefits for several years, and therapeutic effects have been reported against some kinds of tumors, including renal cancer and melanoma.[1] Recently, Masuzawa *et al.* demonstrated that rIL-2 has a marked therapeutic effect on human malignant hemangioendothelioma.[2] They found marked infiltration of CD8-positive cells in the regressing tumor tissue. Some of these cells were very similar to lymphokine-activated killer (LAK) cells. Therefore, it is suggested that these cells play an important role in the destruction of the tumor. In experimental studies of the antitumor effects of rIL-2 on murine hemangioendothelioma cells, Naruo *et al.* showed that LAK cells induced by rIL-2 were cytotoxic to hemangioendothelioma D14 cells *in vitro*.[3] Ootsu *et al.* showed that daily administration of rIL-2 for 30 consecutive days suppressed growth of D14 in nude mice.[4] In this study, we histologically examined the antitumor effect of rIL-2 on D14 in nude mice.

MATERIALS AND METHODS

Drug rIL-2 (TGP-3) was provided by the Applied Microbiology Laboratories of Takeda Chemical Ind. Ltd. (Osaka). The specific activity of the rIL-2 used corresponds to 1.17×10^7 JRU/mg (JRU: Japan Reference Unit, established by NIH of Japan in April 1989). The purification procedure and the biochemical and biological properties of the rIL-2 have been described in detail elsewhere.[5–13] The rIL-2 was dissolved in saline containing 5% normal mouse serum, and the concentration of the solution was adjusted as required.

Animals Female BALB/cAnNCrj-nu/nu (BALB/c-nu/nu) mice were purchased from Charles River Japan Inc. (Kanagawa) and used for the experiment at 6 weeks of age. The mice were kept in a clean rack (CLEA Japan, Inc., Osaka) under specific pathogen-free conditions, fed a chow diet (CE-2; CLEA Japan, Inc.) and given water *ad libitum*.

Tumor The murine hemangioendothelioma cell line D14 was established *in vitro* by Sato *et al.* from an angiosarcoma in the liver of a BALB/c mouse which had been given subcutaneous injections of 1,2-dimethylhydrazine dihydrochloride.[14] The cell line was kindly supplied by Prof. Kokichi Kikuchi of the Department of Pathology, Sapporo Medical College, in 1989, and has since been maintained *in vitro* in the Biotechnology Laboratories of Takeda Chemical Ind. Ltd.[3]

D14 cells were cultured in Dulbecco's minimum essential medium (Flow Labs., UK) containing 10% fetal calf

serum with 100 U/ml penicillin G, 100 μg/ml streptomycin and 3.7 mg/ml sodium hydrogencarbonate at 37°C under a humidified atmosphere of 5% CO_2 in air throughout serial passages. The growing D14 cells were harvested and washed and then re-suspended in the medium, and the concentration was adjusted to 1×10^8 cells/ml. The tumor cell suspension (0.1 ml) was inoculated into the left abdominal subcutaneous tissue of nude mice on day 0. Ten mice with established tumors were obtained, divided into 2 groups and used for the experiment on day 28. Tumor diameter was measured with calipers throughout the experiment, and the size of the tumor is expressed as the mean value of the maximal longitudinal diameter and the maximal transverse diameter.

Drug injection The 10 tumor-bearing mice received daily subcutaneous doses of vehicle (5% normal mouse serum in saline, vehicle group n=5) or 20 μg of rIL-2 (rIL-2 group n=5) from day 28 to day 62. The total volume of the daily dose was adjusted to 0.1 ml/mouse/day in both groups. The daily dose was given as 2 injections of 0.05 ml in both groups: one into subcutaneous tissue very close to the tumor mass and the other into the opposite side. Our previous study had shown that this dose of rIL-2 is sufficient to suppress the growth of subcutaneously established D14 tumors in nude mice.[4]

Histological examination All mice in both groups were autopsied on day 63. The tumor, spleen and left femur were removed, and the tumors were weighed. The femurs were cut at both ends to fix the bone marrow tissue. All tissues were fixed in 10% buffered formalin (pH 7.4). The femurs were decalcified for histological examination according to the method of Plank and Rychro.[15] A tissue block was obtained from a whole-cut section through the largest longitudinal dimension of the tumor mass. A tissue block of the spleen was obtained from the largest cross section, and a tissue block of the femur was obtained from the section of longitudinal dimension. All tissue blocks were embedded in Tissue Prep (Fisher Scientific, USA). Two thin sections were made from all blocks and stained with hematoxylin and eosin (HE) or periodic acid-Schiff (PAS) and were examined under a light microscope.

For all mice the incidence of large granular cells, cells undergoing karyorrhexis and cells showing mitosis was measured at the top, center, bottom and both sides of each tumor section stained with PAS. In each of these 5 areas the measurement was done in 5 fields, and the values are given as the total number/25 fields (1.66 mm^2) in a tumor section. The correlation between incidences of large granular cells and cells showing karyorrhexis was examined.

Immunohistochemical examination After removal, the tumors from 3 mice in each group were cut into two pieces through the largest longitudinal dimension. One piece was fixed in 10% buffered formalin for the above histological examination. The other half was cut into 3 pieces. Two of the 3 pieces were used for immunohistochemical examination and the remaining piece was prepared for ultrastructural examination. For immunohistochemical examination, one piece was sliced into sections (2 mm thick), fixed in acetone at 4°C overnight, and embedded in Tissue Prep according to the AMeX method.[16] The other piece of the tumor was frozen in isopentane which had been precooled in an acetone and dry ice mixture. The frozen tumor tissue was sectioned using a cryostat and stored in a freezer at −30°C until immunohistochemical examination. The cryostat sections were air-dried and fixed in acetone at 4°C for 30 min before immunostaining. A tissue block from the spleen of these same mice was also embedded in Tissue Prep according to the AMeX method.[16]

To examine the cell surface antigens of lymphoid cells in the tumor and spleen, fluorescein isothiocyanate (FITC)-conjugated anti-Thy-1.2, anti-Lyt-1 and anti-Lyt-2 monoclonal antibodies (Becton Dickinson, USA), FITC-conjugated goat anti-mouse IgG antibody (Jackson Immuno Research Laboratories, USA), unlabeled anti-Thy-1.2 monoclonal antibody (Becton Dickinson) and rabbit anti-asialo GM1 antibody (Wako Pure Chemical Ind., Osaka) were used.

Cryostat sections of the tumor were incubated with the above FITC-conjugated anti Thy-1.2, Lyt-1, Lyt-2 and mouse IgG antibodies (1:100). Additional cryostat tumor sections which were pre-incubated with rabbit anti-asialo GM1 antibody (1:100) and unlabeled anti-Thy-1.2 monoclonal antibody (rat antibody, 1:100) were incubated with FITC-conjugated goat anti-rabbit IgG antibody (Zymed Laboratories, Inc., USA; 1:100) and FITC-conjugated goat anti-rat IgG antibody (Organon Teknika Corporation, USA; 1:100), respectively. These immunostained sections were examined under a fluorescence microscope.

The sections of tumor and spleen which were processed according to the AMeX method were deparaffinized and endogenous peroxidase was eliminated. They were then incubated with anti-Thy-1.2 monoclonal antibody (1:1000) and stained using a Vectastain ABC kit (Vector Laboratories, Inc., USA). These sections were counterstained with hematoxylin and examined under a light microscope.

Ultrastructural examination of the tumor Pieces of the tumor tissue from 3 mice which were examined immunohistochemically in each group were cut into small blocks and fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide in the same buffer, and embedded in epoxy resin (Epok 812). Ultrathin sections were made, doubly stained with uranyl acetate and lead citrate, and observed under a

JEM-1200EX electron microscope (Japan Electron Optics Lab., Tokyo).

RESULTS

Antitumor effect The tumor sizes in mice from day 28 to day 63 are shown in Fig. 1. After day 40, tumor growth was significantly suppressed in the mice that received rIL-2. The tumor weight in the rIL-2 group was markedly suppressed on day 63 (vehicle: 6150±730 mg vs. rIL-2: 1600±85** mg mean±SE, $P<0.01$).

Histological findings D14 tumor cells were spindle-form and proliferated with mitotic figures. The cells had a tendency to form bundles, and the bundles were intertwined. The tumor cells occasionally formed capillary-like structures.

Marked infiltration of large granular cells was observed in and around the tumors in mice given rIL-2 (Fig. 2). The large granular cells (LGC) were 10–30 μm in diameter and contained many granules in the cytoplasm which were vividly stained with PAS. Cells undergoing karyorrhexis were present in the areas infiltrated by LGC. Slight infiltration of LGC was also observed in the tumors in the vehicle group.

The numbers of LGC and cells undergoing karyorrhexis markedly increased, while mitosis had a tendency to reduce in the tumors in the rIL-2 group as shown in Table I. Also, a highly significant correlation was noted between the numbers of LGC and cells undergoing karyorrhexis (Fig. 3; $r=0.87$, $P<0.01$).

Infiltration of lymphocytes, histiocytes and neutrophils was observed to the same extent in both the vehicle and rIL-2 group.

Immunohistochemical findings In the D14 tumors in the rIL-2 group, there were many Thy-1.2-positive large cells. Examination of the adjacent sections revealed that the Thy-1.2-positive large cells were LGC (Fig. 4). A nerve fiber showed positive staining for Thy-1,2, too. Asialo GM1-positive cells were also frequently observed in these tumors, and their size and number were very similar to those of the Thy-1.2-positive large cells. Sur-

Table I. Incidence of LGC, Karyorrhexis and Mitosis in D14 Tumors in Mice Treated with rIL-2

Group	n	LGC (mean±SD)	Karyorrhexis (mean±SD)	Mitosis (mean±SD)
Vehicle	5	6.6±7.5	33.2±9.5	63.4±32.7
rIL-2	5	429.8±153.5*	107.2±19.5*	42.4±16.8

Mice received vehicle or rIL-2 (20 μg/mouse/day) for 35 days. Incidences of large granular cells, cells undergoing karyorrhexis and cells showing mitosis were measured. The values are given as the total number/25 fields (1.66 mm^2) of the tumor section. * Significantly different from vehicle group ($P<0.01$, Student's t test)

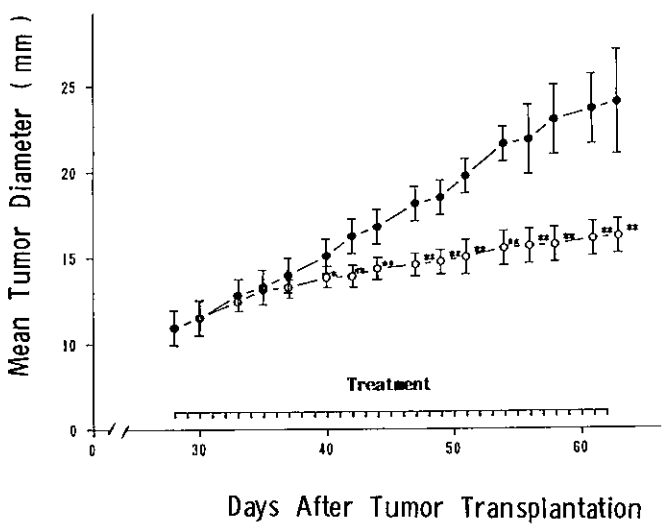


Fig. 1. Changes in mean diameter of D14 tumors during treatment with rIL-2. Mice received a daily subcutaneous dose of vehicle (5% normal mouse serum, ●) or rIL-2 (20 μg/mouse, ○) from day 28 to day 62 after the transplantation of D14 cells. Mean±SD (n=5). *, **: Significantly different from vehicle group (* $P<0.05$, ** $P<0.01$; Student's t test).

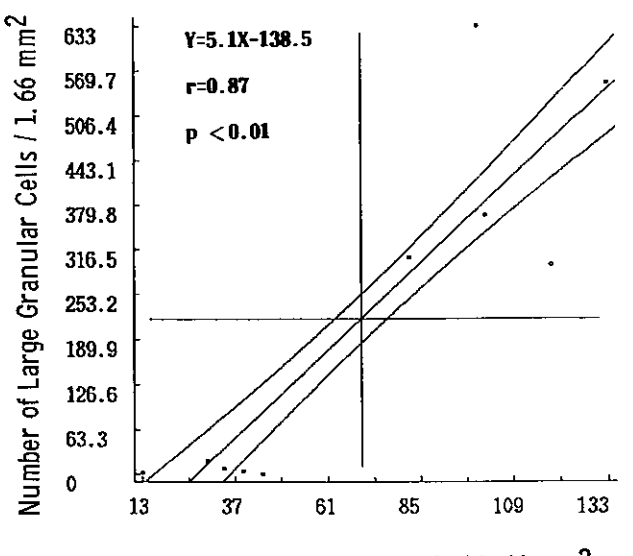


Fig. 3. Correlation between incidence of LGC and incidence of karyorrhexis in D14 tumors in mice in both groups.

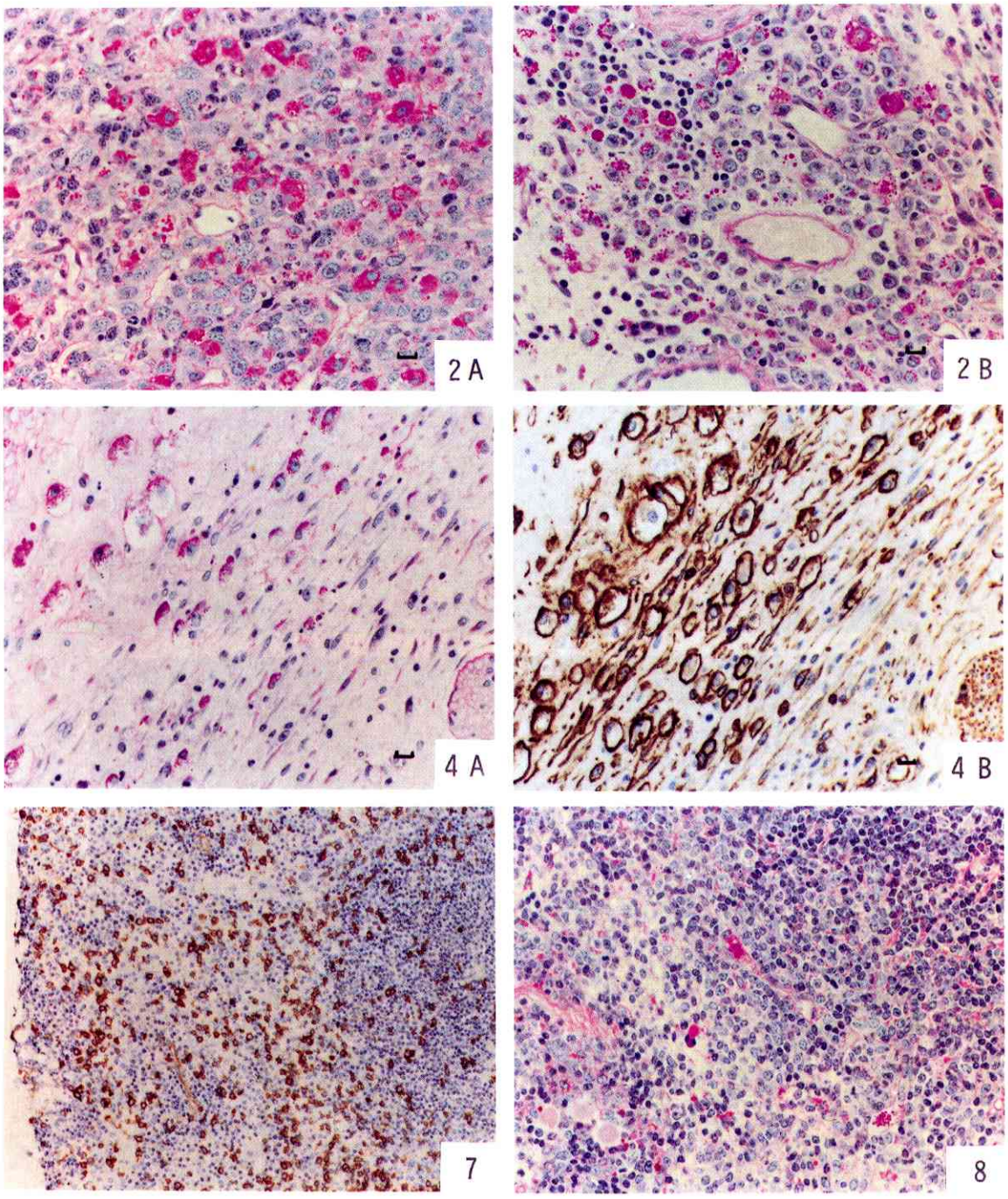
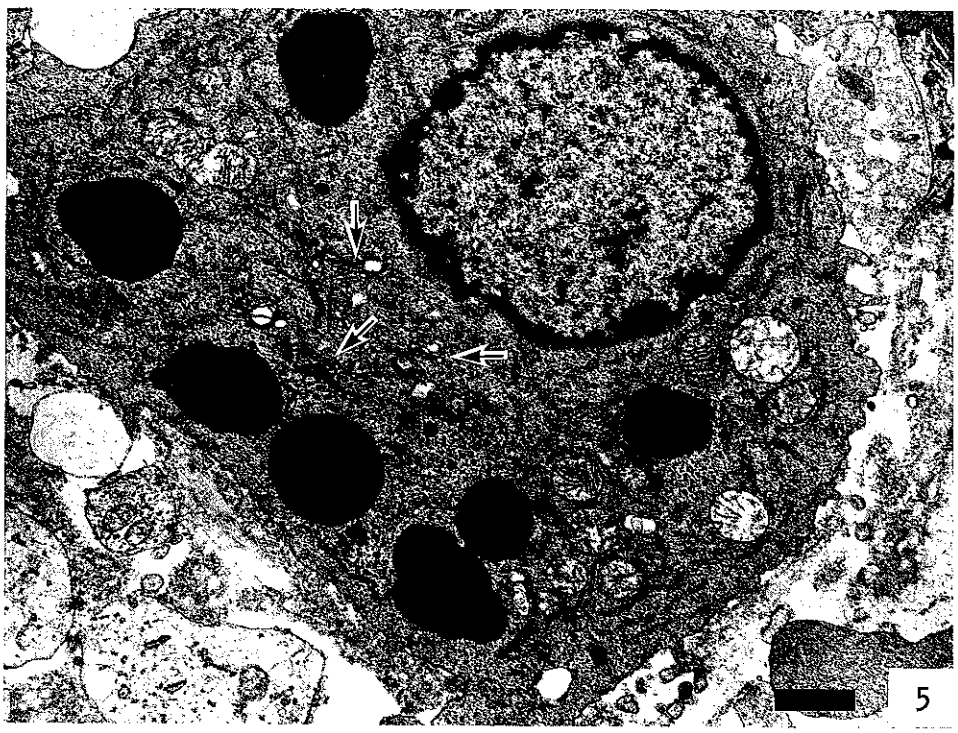


Fig. 2. D14 tumor in a mouse given rIL-2. Many large granular cells (LGC) are observed in (A) and around (B) the tumor. The bar indicates 10 μm. PAS, $\times$350.

Fig. 4. D14 tumor in a mouse given rIL-2. (A) Many LGC are observed in the periphery of the tumor tissue. PAS, $\times$350. (B) The same area of an adjacent section. The LGC are Thy-1.2 positive. A nerve fiber also shows specific staining for Thy-1.2, because Thy-1 antigen is usually present in nerve fibers. The bar indicates 10 μm. ABC method, $\times$350.

Fig. 7. Section of the spleen from a mouse given rIL-2. Many Thy-1.2-positive cells varying in size are observed in the red and white pulp. ABC method. $\times$170.

Fig. 8. Section of the spleen from a mouse given rIL-2. The number of LGC is small. PAS. $\times$350.



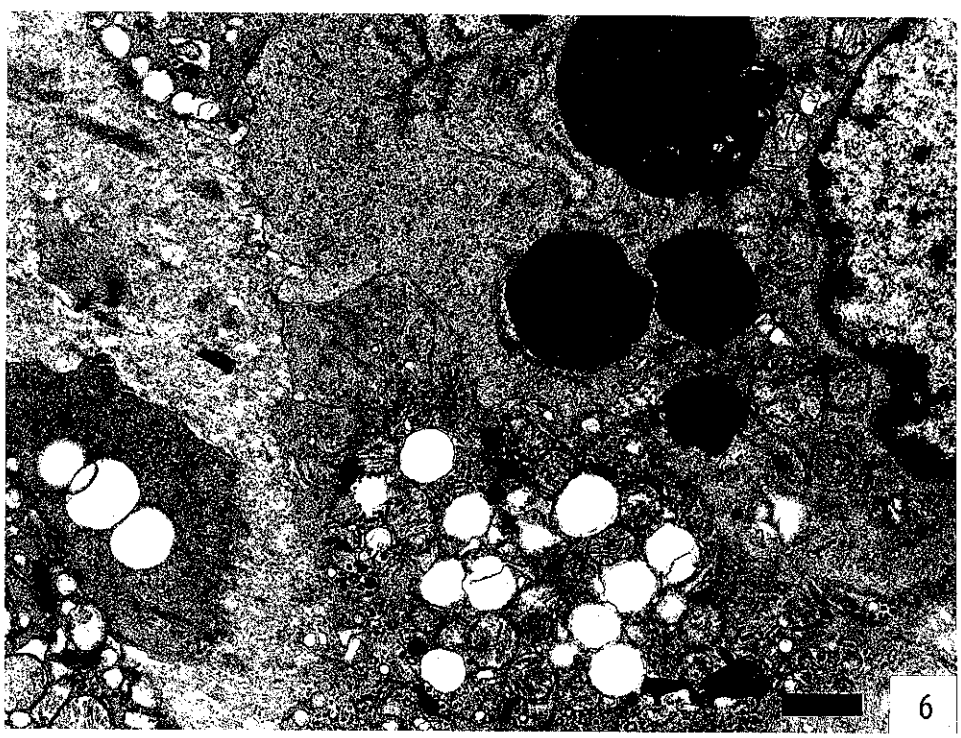


Fig. 5. Electron micrograph of an LGC in a tumor from a mouse given rIL-2. The LGC contains many electron-dense granules and well-developed Golgi complexes (arrows) in the cytoplasm. The bar indicates 1 μm. $\times$13000.

Fig. 6. Electron micrograph of an LGC in a tumor from a mouse given rIL-2. The cytoplasmic granules are surrounded by fine, electron-dense membranous vesicles at the periphery. The adjacent tumor cell contains many cytoplasmic vacuoles suggesting degeneration. The bar indicates 1 μm. $\times$13000.

face IgG-positive cells were smaller both in number and in size than the Thy-1.2-positive large cells, and Lyt-1 and Lyt-2-positive cells were not observed. In the tumors in the vehicle group, a small number of Thy-1.2-positive cells, asialo GM1-positive cells and IgG-positive cells were observed.

Ultrastructure of large granular cells in the tumor LGC contained large cytoplasmic granules about 1 to 2 μm in diameter and well-developed Golgi complexes (Fig. 5). The cytoplasmic granules showed high electron density and surrounded by fine membranous vesicles. D14 tumor cells adjacent to LGC were sometimes vacuolated (Fig. 6).

Histological findings in the spleen and bone marrow In the spleen of the mice treated with rIL-2, a large number of Thy-1.2-positive lymphoid cells of various sizes were observed (Fig. 7). A small number of LGC were also observed (Fig. 8). In the spleen of the mice treated with the vehicle, a small number of Thy-1.2-positive cells were observed, but LGC were not observed.

In the bone marrow of the rIL-2 group, a small number of LGC and a slight increase in the number of eosinophils were observed.

DISCUSSION

Recombinant human interleukin-2 (rIL-2) has obvious antitumor[17-21] and antimetastatic[10, 17] activities in mice. rIL-2 has no direct cytotoxic effect on most target cells *in vitro*, but it has various host-mediated immunomodulatory effects such as induction of lymphokine-activated killer (LAK) cell activity[22, 23] and the augmentation of natural killer (NK) cell activity[22, 24] and cytotoxic T lymphocyte (CTL)[22] activity in mice. rIL-2 also induces LAK cell activity in spleen cells of nude mice *in vitro*[25, 26] and *in vivo*.[3] These killer cells appear to play an important role in the antitumor and antimetastatic effects of rIL-2 in mice.

The present study showed that the number of LGC and cells undergoing karyorrhexis increased significantly in D14 tumors in nude mice treated with rIL-2. The evident suppression of the growth of the D14 tumors is well explained by the significant increase in karyorrhexis and the slight decrease in mitotic figures in the tumor tissue. These results together with the evidence of the degeneration of tumor cells adjacent to the LGC suggest that a great amount of karyorrhexis occurred in D14 tumor cells. Therefore, the concomitant increase in LGC and karyorrhexis with a high correlation at the same site of the tumor suggests that LGC injured the neighboring tumor cells in the same manner as the killing of target cells by LAK cells *in vitro*.

The LGC were very similar to the large granular cells which were observed in colon 26 tumors in BALB/c mice receiving rIL-2 and/or recombinant interferon-α A/D[11] with respect to cell size, morphological features, and cell surface phenotype: Thy-1.2$^+$, Lyt-1$^-$, Lyt-2$^-$ and IgG$^-$. These phenotypical features resembled those of NK-LAK cells *in vitro*.[27, 28] It can also be postulated that the IGC were not derived from mature T cells, as the present study was carried out in nude mice which are devoid of mature T cells. Daily subcutaneous administration of 10 μg of the rIL-2 for 10 consecutive days induced LAK activity in the spleen of nude mice.[3] Thy-1.2$^+$ cells in the spleen in the rIL-2 group in this experiment appeared to have LAK activity, because murine LAK effector cells are known to express Thy-1 antigen. It has been reported that LAK cells *in vitro* are heterogeneous with respect to cell surface phenotype,[27, 28] cell size,[29] ability to adhere to the culture dish[30] and the amount of cytoplasmic granules.[31] Agah *et al.* sorted large and granule-rich cells from LAK cells *in vitro* using a cell sorter, and showed that the large cells were more cytotoxic than the unsorted LAK cells.[31] The large granule-rich LAK cells resembled LGC in their morphological features.

The kinetics and origin of the LGC in this experiment remain obscure. LGC occurred more frequently in the tumor than in the spleen or the bone marrow in the mice treated with rIL-2, while these LGC were observed rarely and only in the tumors in the mice in the vehicle group. These findings suggest that LGC and their precursor cells were mobilized from the host organs such as the spleen, bone marrow or peripheral blood into the D14 tumor to attack the tumor cells, thus indicating that the host reaction against the tumor was enhanced markedly by the treatment with rIL-2. In addition, it is possible that some sorts of lymphocytes which had infiltrated into the tumor may be transformed into LGC by the treatment with rIL-2.

In summary, the present study revealed that daily local injections of rIL-2 for 35 consecutive days induced marked infiltration of LGC in transplanted hemangioendothelioma D14 in nude mice. The LGC have characteristics similar to those of NK-LAK cells *in vitro* with respect to morphological and immunohistochemical features, and may be mobilized into the tumors to attack the tumor cells.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. Akira Imada of the Biology Research Laboratories for his encouragement and continued interest in this work. We thank Dr. Kyozo Tsukamoto and Dr. Ken-ichi Naruo of the Biotechnology Laboratories for their gift of the D14 cells maintained *in vitro*, and Mrs. Emiko Oda for her skillful technical assistance.

(Received February 12, 1991/Accepted May 25, 1991)

REFERENCES

1) Rosenberg, S. A., Lotze, M. T., Muul, L. M., Chang, A. E., Avis, F. P., Leitman, S., Linehan, W. M., Robertson, C. N., Lee, R. E., Rubin, J. T., Seipp, C. A., Simpson, C. G. and White, D. E. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N. Engl. J. Med.*, **316**, 889–897 (1987).
2) Masuzawa, M., Higashi, K., Nishioka, K. and Nishiyama, S. Successful immunotherapy for malignant hemangioendothelioma using recombinant interleukin-2. *Jpn. J. Dermatol.*, **98**, 367–369 (1988) (in Japanese).
3) Naruo, K., Shiho, O., Tsukamoto, K. and Miyachi, Y. Effect of recombinant human interleukin-2(rIL-2) on the growth of murine endothelial cell lines, and cytotoxic activity of nonspecific killer cells induced by rIL-2 against the endothelial cell lines. *Jpn. Pharmacol. Ther.*, **17**, 3781–3786 (1989) (in Japanese).
4) Ootsu, K., Houkan, T., Naruo, K., Tsukamoto, K., Maruguchi, Y., Toda, K. and Miyachi, Y. Effect of recombinant human interleukin-2(rIL-2) on the growth of murine endothelial cell lines *in vivo*. *Jpn. J. Pharmacol. Ther.*, **17**, 4259–4266 (1989) (in Japanese).
5) Hinuma, S., Onda, H., Naruo, K., Ichimori, Y., Koyama, M. and Tsukamoto, K. Translation of interleukin-2 mRNA from human peripheral blood leukocytes in *Xenopus* oocytes. *Biochem. Biophys. Res. Commun.*, **109**, 363–369 (1982).
6) Kato, K., Yamada, T., Kawahara, K., Onda, H., Asano, H., Sugino, H. and Kakinuma, A. Purification and characterization of recombinant human interleukin-2 produced in *Escherichia coli*. *Biochem. Biophys. Res. Commun.*, **130**, 692–699 (1985).
7) Naruo, K., Hinuma, S., Kato, K., Koyama, M., Tada, H., Shiho, O. and Tsukamoto, K. Comparison of the biological properties of purified natural and recombinant human interleukin-2. *Biochem. Biophys. Res. Commun.*, **128**, 257–264 (1985).
8) Yamada, T., Kato, K., Kawahara, K. and Nishimura, O. Separation of recombinant human interleukin-2 and methionyl interleukin-2 produced in *Escherichia coli*. *Biochem. Biophys. Res. Commun.*, **135**, 837–843 (1986).
9) Hinuma, S., Naruo, K., Shiho, O. and Tsukamoto, K. Characteristics of murine non-specific killer cells induced *in vivo* by recombinant human interleukin-2. *Immunology*, **59**, 251–259 (1986).
10) Hinuma, S., Naruo, K., Ootsu, K., Houkan, T., Shiho, O. and Tsukamoto, K. Suppression of pulmonary tumour metastasis in mice by recombinant human interleukin-2: role of asialo GM1-positive cells. *Immunology*, **60**, 173–179 (1987).
11) Sakura, Y., Ootsu, K. and Shino, A. Combination therapy of colon carcinoma 26 in mice with recombinant human interleukin-2 and interferon-α A/D: occurrence of large granular cells in the tumor. *Jpn. J. Cancer Res.*, **80**, 895–903 (1989).
12) Ootsu, K., Gotoh, K. and Houkan, T. Therapeutic efficacy of human recombinant interleukin-2 (TGP-3) alone or in combination with cyclophosphamide and immunocompetent cells in allogeneic, semi-syngeneic, and syngeneic murine tumors. *Cancer Immunol. Immunother.*, **30**, 71–80 (1989).
13) Sakura, Y., Houkan, T. and Shino, A. Effects of recombinant human interleukin-2 (TGP-3) on normal mice — histomorphological examinations. *Jpn. Pharmacol. Ther.*, **17**, 3025–3040 (1989).
14) Sato, N., Sato, T., Takahashi, S. and Kikuchi, K. Establishment of murine endothelial cell lines that develop angiosarcomas *in vivo*: brief demonstration of a proposed animal model for Kaposi's sarcoma. *Cancer Res.*, **46**, 362–366 (1986).
15) Plank, J. and Rychro, A. Eine Schnellentkalkungsmethode. *Zentralbl. Allg. Pathol. Pathol. Anat.*, **89**, 252–254 (1952).
16) Sato, Y., Mukai, K., Watanabe, S., Goto, M. and Shimosato, Y. The AMeX method. A simplified technique of tissue processing and paraffin embedding with improved preservation of antigens for immunostaining. *Am. J. Pathol.*, **125**, 431–435 (1986).
17) Rosenberg, S. A., Mule, J. J., Spiess, P. J., Reichert, C. M. and Schwarz, S. L. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. *J. Exp. Med.*, **161**, 1169–1188 (1985).
18) Maekawa, R., Matsumoto, M., Kitagawa, T., Harada, M. and Sato, K. Effect of recombinant interleukin 2(R-IL 2) on *in vivo* growth of murine myeloma X5563. *Cancer Immunol. Immunother.*, **23**, 25–30 (1986).
19) Bubenic, J. and Indrova, M. The anti-tumor efficacy of human recombinant interleukin-2. Correlation between sensitivity of tumours to the cytolytic effect of LAK cells *in vitro* and their susceptibility to interleukin 2 immunotherapy *in vivo*. *Cancer Immunol. Immunother.*, **24**, 269–271 (1987).
20) Vaage, J. Local interleukin 2 therapy of mouse mammary tumors of various immunogenicities. *Cancer Res.*, **48**, 2193–2197 (1988).
21) Anderson, T. D., Hayes, T. J., Gately, M. K., Bontempo, J. M., Stern, L. L. and Truitt, G. A. Toxicity of human recombinant interleukin-2 in the mouse is mediated by interleukin-activated lymphocytes. Separation of efficacy and toxicity selective lymphocyte subset depletion. *Lab. Invest.*, **59**, 598–612 (1988).
22) Chang, A. E., Hyatt, C. L. and Rosenberg, S. A. Systemic administration of recombinant human interleukin-2 in mice. *J. Biol. Response Modif.*, **3**, 561–572 (1984).
23) Ettinghausen, S. E., Lipford, E. H., III, Mule, J. J. and Rosenberg, S. A. Systemic administration of recombinant interleukin 2 stimulates *in vivo* lymphoid cell proliferation

in tissues. *J. Immunol.*, **135,** 1488–1497 (1985).
24) Talmadge, J. E., Herberman, R. B., Chirigos, M. A., Maluish, A. E., Schneider, M. A., Adam, J. S., Philips, H., Thurman, G. B., Varesio, L., Long, C., Oldham, R. K. and Wiltrout, R. H. Hyporesponsiveness to augmentation of murine natural killer cell activity in different anatomical compartments by multiple injections of various immunomodulators including recombinant interferon and interleukin 2. *J. Immunol.*, **135,** 2483–2491 (1985).
25) Nishimura, T., Yagi, H., Uchiyama, Y. and Hashimoto, Y. Recombinant interleukin 2 allows the differentiation of Thy 1.2^+ LAK cells from nude mouse spleen cells. *Immunol. Lett.*, **12,** 77–82 (1986).
26) Nishimura, T., Yagi, H., Uchiyama, Y. and Hashimoto, Y. The precursor cells of mouse lymphokine-activated killer (LAK) cells. *Thymus,* **9,** 131–139 (1987).
27) Ballas, Z. K., Rasmussen, W. and Van Otegham, J. K. Lymphokine-activated killer (LAK) cells II. Delineation of distinct murine LAK-precursor subpopulations. *J. Immunol.*, **138,** 1647–1652 (1987).
28) Kalland, T., Belfrage, H., Bhiladvala, P. and Hedlund, G. Analysis of the murine lymphokine-activated killer (LAK) cell phenomenon: dissection of effectors and progenitors into NK- and T-like cells. *J. Immunol.*, **138,** 3640–3645 (1987).
29) Owen-Schaub, L. B., Abraham, S. R. and Hemstreet, G. P., III. Phenotypic characterization of murine lymphokine-activated killer cells. *Cell. Immunol.*, **103,** 272–286 (1986).
30) Gunji, Y., Vujanovic, N. L., Hieserodt, J. C., Herberman, R. B. and Gorelik, E. Generation and characterization of purified adherent lymphokine-activated killer cells in mice. *J. Immunol.*, **142,** 1748–1754 (1989).
31) Agah, R., Shau, H. and Mazumder, A. Lysosome rich cells contain the lytic activity of lymphokine-activated killer cell populations. *Cancer Immunol. Immunother.*, **24,** 247–252 (1987).