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Antitumor Activity of Recombinant Human Interleukin-1 against Heterotransplanted Human Non-Hodgkin Lymphomas in Nude Mice

Tadaaki Miyamoto[1] and Shu-guang Wu

Hospital, National Institute of Radiological Sciences, Anagawa 4-9-1, Chiba 260

Antitumor activity of recombinant human interleukin 1α (IL-1) against seven human non-Hodgkin lymphomas grown in athymic nude mice was studied. Growth of the lymphomas was markedly inhibited after an injection of 0.4 mg/kg IL-1. The growth inhibition of Burkitt lymphoma was found to be dose-dependent up to 0.4 mg/kg, reaching a plateau thereafter. The loss of colony-forming ability of the cells and the loss of cell viability showed the same type of dose-dependence and progressed during 24 h following an injection of IL-1. In accordance with these observations, histopathologic examination revealed progressively spreading coagulative necrosis without bleeding. Little infiltration of inflammatory cells into the tumor tissue was observed. IL-1 growth inhibition of T lymphoma in beige nude mice having low natural killer activity was similar to that in nude mice. These findings suggested that the antitumor effects might not be produced through cell-mediated antitumor actions. Immunocytological examination with anti-IL-1 antibody revealed that administered IL-1 was bound to the lymphoma cells, suggesting that IL-1 receptor is probably expressed on these cells *in vivo*. The antitumor action of IL-1 on the lymphomas may be exerted directly through the IL-1 receptor.

Key words: Interleukin-1 — Human lymphoma — Antitumor effect

Interleukin-1α (IL-1), originally defined as a monocyte-derived factor mitogenic for thymocytes, is now known to show many biological activities. It is produced by a wide variety of cell types and has a broad range of target cells.[1,2] The early studies showed that this cytokine can increase natural killer cell activity,[3] antibody-dependent cytotoxicity[4] and macrophage tumoricidal killing.[5] Recently, IL-1 has been shown to be cytotoxic and cytostatic to some tumor cell lines *in vitro* and to have antitumor activity *in vivo*. Now that recombinant IL-1 can be obtained in sufficient amounts for clinical use, the potential role of IL-1 in antitumor immunotherapy is an important issue.

There are a number of observations suggesting that IL-1α can mediate *in vitro* antitumor effects. Both human and murine tumor cell lines *in vitro* can be inhibited by IL-1.[6–10] However, studies of the *in vivo* antitumor effect of IL-1 have been limited to mouse syngeneic tumors.[11–18] Its action against human tumors *in vivo* has not been explored. The tumor growth inhibition by IL-1 in mice was reported to be rather weak in the case of less immunogenic tumors,[13,14] suggesting a host-mediated action in addition to the direct action observed *in vitro*. Braunschweiger *et al.*[12] reported the pathophysiological effects of IL-1, including acute hemorrhagic necrosis, restricted blood flow and clonogenic cell killing, in RIF-1 and panc O2 solid tumors in syngeneic mice,[12] but little information is available about the *in vivo* cell kinetics of tumors after IL-1 treatment. Detailed experiments on various types of tumors are needed to characterize the IL-1 antitumor activities.

Recently, our laboratory has succeeded in establishing six nude mouse tumor lines from patients with non-Hodgkin malignant lymphoma.[15] We used these tumor lines to test the effect of IL-1 on human non-Hodgkin lymphomas (NHL). We show herein that human recombinant IL-1 has antitumor activity against human NHL grown in nude mice and we describe the cell-kinetic behavior and morphologic changes.

MATERIALS AND METHODS

Nude mouse tumor lines Seven human non-Hodgkin's malignant lymphomas transplanted into nude mice were used throughout these experiments. Six of them are tumor lines newly established in our laboratory, including one T-lymphoma (HS-T) and five B-lymphomas (IH-B, IS-B, KM-B, KT-B and OK-B).[15] The other is a Burkitt lymphoma transplanted into nude mice,[19] which was used for both *in vivo* and *in vitro* experiments because of its high plating efficiency in agar plates.

IL-1 and treatment Recombinant human IL-1α (rhIL-1α, IL-1) was supplied by Dainippon Pharmaceutical Co., Ltd.[20] The mouse thymocyte comitogenic activity (LAF) is 2×10^7 U/mg protein. The protein concentration of this preparation was 2.11 mg/ml (determined by the micro-Kjeldahl method), and the endotoxin content was less than 0.05 ng/mg protein (*Limulus* test kit Pyrodick, Seikagaku Kogyo, Tokyo). For treatment,

[1] To whom correspondence should be addressed.

IL-1 in 0.1 ml of PBS was injected into the femoral muscle of the hind limbs.

Nude mice and irradiation Nude mice (BALB/c *nu/nu*) and beige nude mice (B57 B6/6, *bg/bg, nu/nu*)[21] are propagated in our institute and maintained under specific pathogen-free (SPF) conditions. Male nude mice (6–8 weeks old) were used for experiments. The mice were given 5-Gy whole-body irradiation from a ^{137}Cs source immediately before tumor transplantation and kept under SPF conditions.

Tumor take and serial transplantation When implanted tumors began growing and attained 15–20 mm in diameter, mice were killed by cervical dislocation and their tumors were cut into small pieces with scissors in a Petri dish. About 8 mm^3 pieces of tumors thus prepared were implanted into new mice subcutaneously with a trocar. For Burkitt tumors, 10^7 cells taken from a tumor were injected as a single-cell suspension.

IL-1 antitumor effect and growth delay assay The effect of IL-1 on the growth of HS-T, IH-B, IS-B, KM-B, KT-B, OK-B and Burkitt lymphoma transplanted in nude mice was assessed in terms of the tumor volume as a function of time after IL-1 treatment. The tumor volume was calculated as a hemiellipsoid form according to the formula $V = 1/3$ length $\times$ width $\times$ height and expressed as relative tumor volume at the time of the treatment. The regrowth delay was measured as the difference in time for treated and control tumors to reach 3 times the pretreatment tumor volume.

Measurement of reproductive cell death and interphase death Clonogenic cell survival in Burkitt lymphoma was assayed as described previously.[19] Briefly, Burkitt lymphoma was resected under sterile conditions, minced with scissors and pipetted to produce a single-cell suspension in F-10 medium supplemented with 10% calf serum. After staining with 0.4% erythrosine B, viable cells were

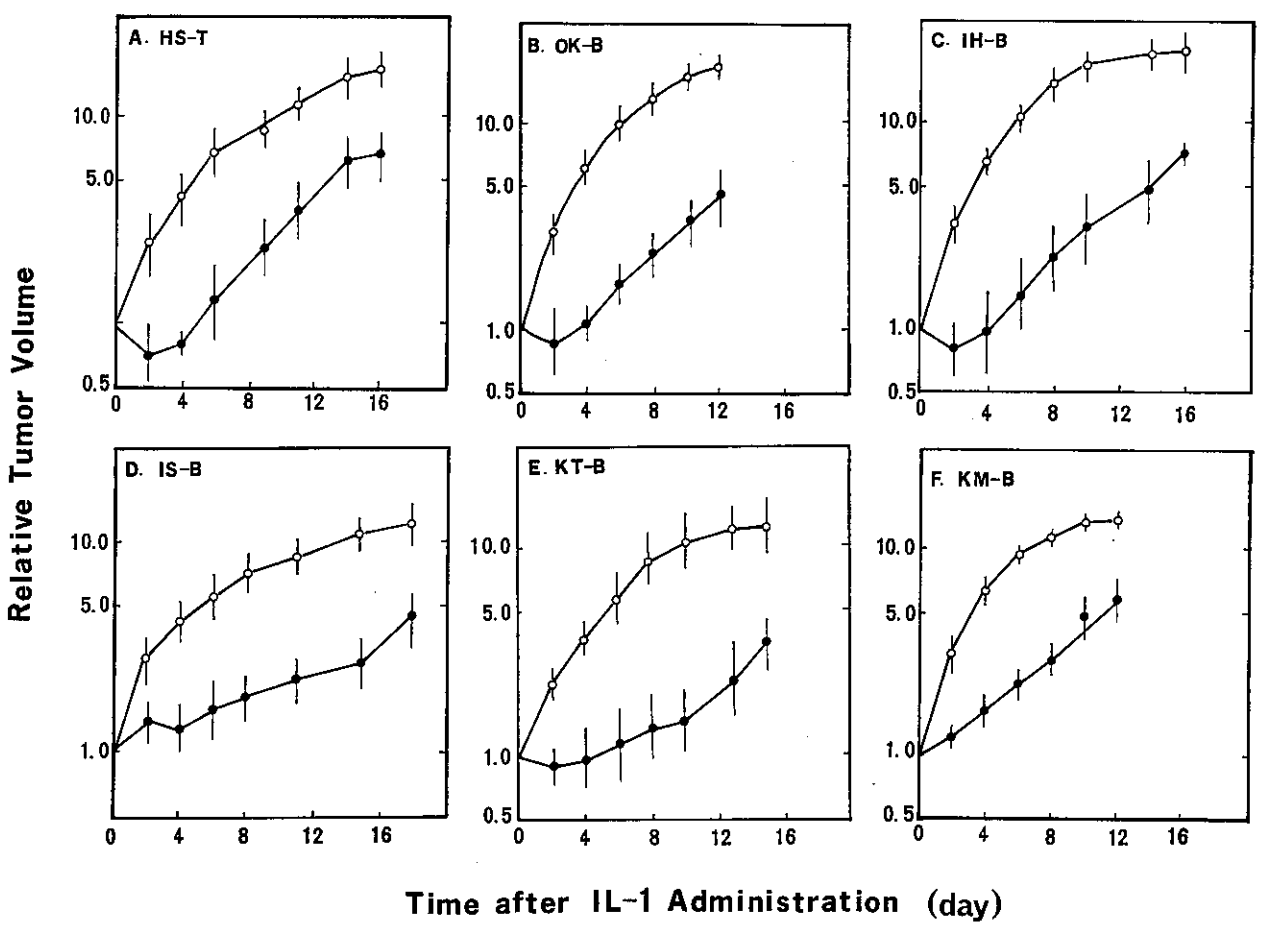

Fig. 1. The growth inhibition of six newly established human lymphomas in nude mice after injection of 0.4 mg/kg IL-1. All of the lymphomas showed a good response to the drug. Bars indicate SE of 3 to 5 tumor-bearing nude mice. ○, untreated; ●, IL-1-treated.

Table I. Growth Delay of Non-Hodgkin's Malignant Lymphoma in Nude Mice after IL-1 Treatment (0.4 mg/kg)

Cell line	HS-T	OK-B	IH-B	IS-B	KT-B	BK-B	Km-B	Mean ± SD
Growth delay (day)	8.2	7.0	8.0	13.1	11.2	6.0	6.2	8.5 ± 2.7

counted using a hemocytometer and a suitable number of viable cells was inoculated into double soft agar plates and incubated for 12 days in a 5% CO_2 incubator at 37°C. The plating efficiency was 20 to 50%. On the other hand, some of the cell suspensions were mixed with 0.4% erythrosine B in PBS (phosphate-buffered saline solution, pH 7.4) at a ratio of 1 to 0.2 for 20 s and then an aliquot of the mixture was placed on the hemocytometer with a cover glass and counted to determine the percentage of interphase death. This was calculated by means of the following formula: (Total cells − viable cells)/(total cells) × 100%.

Morphologic studies on IL-1-treated tumors HS-T lymphoma expressing T markers was chosen for morphologic examination. At various times after 0.4 mg/kg IL-1 injection, the tumor was resected, fixed with 10% formalin and paraffin-embedded. Specimens taken from the paraffin-embedded materials were stained with hematoxylin and eosin for examination of morphological changes induced by IL-1. Another specimen was reacted with anti-rhLI-1α polyclonal rabbit antiserum (Dainippon Pharmaceutical Co.), followed by application of the avidin-biotin-peroxidase complex method to determine whether or not IL-1 administered was bound to the tumor cells.

RESULTS

Growth delay of the six human non-Hodgkin lymphomas caused by rhIL-1α Nude mice bearing human non-Hodgkin malignant lymphoma were im injected with IL-1 at 0.4 mg/kg at about 2 weeks post-transplantation, when the tumors were growing exponentially and had attained a size of about 8 to 10 mm in diameter. As shown in Fig. 1, the growth of all tumor lines was significantly inhibited by IL-1; tumor growth in the IL-1-treated group slowed down or ceased for 4 days or more, then recovered. The average growth delay of the seven tumors, including BK-B (Fig. 2), caused by IL-1 at 0.4 mg/kg was about 8.5 days (Table I), and the variation in growth delay time was rather small.

Dose-response of the growth delay of Burkitt lymphoma to IL-1 To study the dose-dependence of growth delay of Burkitt lymphoma treated with IL-1, the drug was injected at doses of 0.1 to 0.8 mg/kg into tumor-bearing nude mice. As shown in Fig. 2a, the tumor growth was inhibited in a dose-dependent manner. The actual delay time was plotted against the dose of IL-1 (Fig. 2b). The

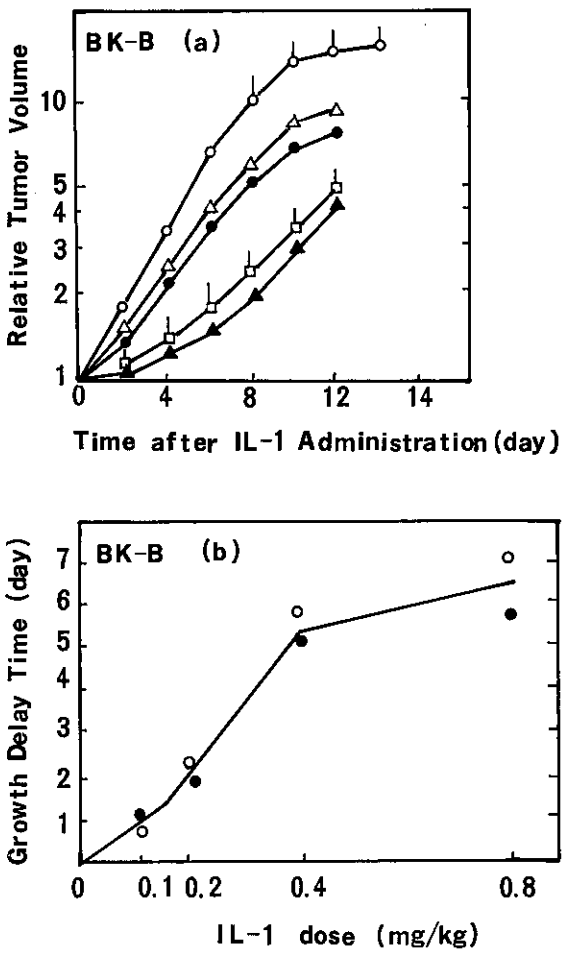

Fig. 2. The growth inhibition of Burkitt lymphoma in nude mice after injection of IL-1 at doses from 0.1 to 0.8 mg/kg. The growth was inhibited in a dose-dependent manner (a). The degree of inhibition is plotted against dose (b). The inhibition is expressed in terms of the growth delay time (additional time required for the treated tumor to reach 3 times its initial volume as compared to the time in the case of the untreated control). (a) IL-1 dose (mg/kg): ○, control; △, 0.1; ●, 0.2; □, 0.4; ▲, 0.8. Each point (○, ●) in (b) indicates the result of a separate experiment.

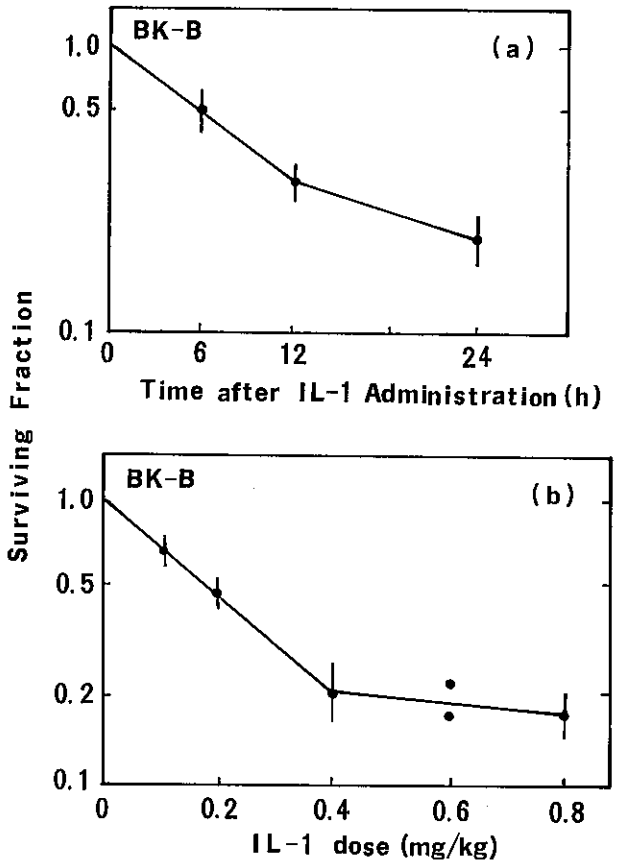

Fig. 3. The survival of clonogenic cells taken from Burkitt lymphoma grown in nude mice as a function of time after a fixed dose (0.4 mg/kg) of IL-1 (a), and at 24 h after various doses of IL-1 (b). Each point indicates the result of a separate experiment. Bars indicates SE of the 3 to 4 experimental results.

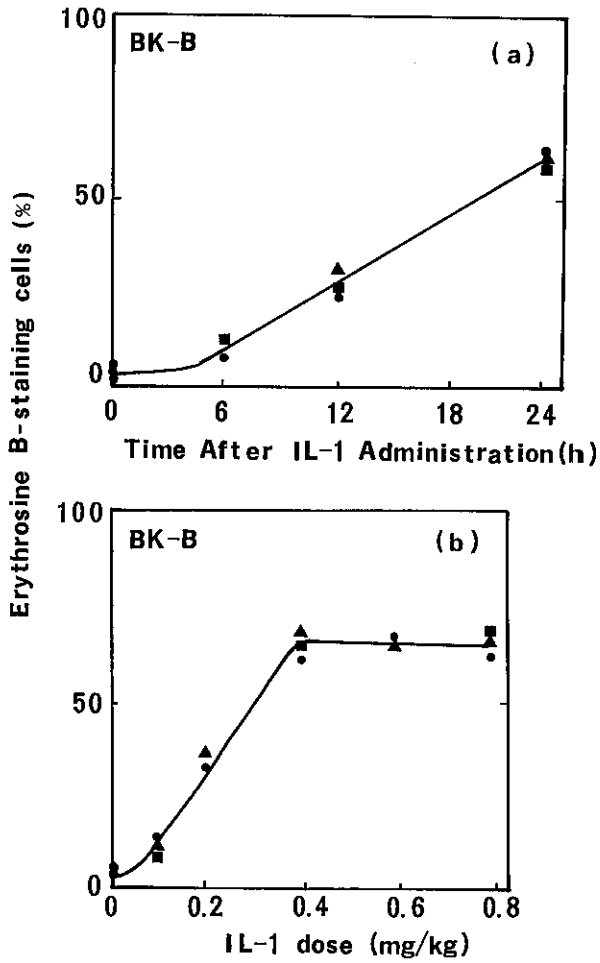

Fig. 4. The percent increase of erythrosine B-staining cells taken from Burkitt lymphoma as a function of time after a fixed dose (0.4 mg/kg) of IL-1 (a), and at 24 h after various doses of IL-1 (b). Each point ●, ▲, indicates the result of a separate experiment.

growth inhibition increased up to 0.4 mg/kg of IL-1 and then plateaued, resulting in a biphasic response pattern.

Time- and dose-response of clonogenic Burkitt lymphoma cells after IL-1 treatment To determine the kinetics of antitumor action by IL-1, a single dose of IL-1 (0.4 mg/kg) was injected into nude mice and the tumor was removed to examine the colony-forming ability of the viable cells. As shown in Fig. 3a, the cell killing by IL-1 increased with time after IL-1 treatment. The time at which IL-1 exerted maximum tumor inhibition was about 24 h after IL-1 injection, when the surviving fraction was about 0.2. Subsequently, there was a rapid repopulation of the clonogenic cells. On the other hand, when various doses of IL-1 were injected into tumor-bearing nude mice, the surviving fraction decreased with increasing dose up to 0.4 mg/kg and then plateaued (Fig. 3b).

Time course of cell death of Burkitt lymphoma after IL-1 treatment A single dose of IL-1 (0.4 mg/kg) was injected im into nude mice and the fraction of dead cells was determined at various times after IL-1 treatment. Fig. 4a shows that the fraction of dead cells as identified by the erythrosine B (EB)-staining method increased with increasing time after IL-1 injection. At 6, 12 and 24 h after IL-1 treatment, the fraction of dead cells reached about 8, 22 and 65%, but there was no further increase thereafter. On the other hand, a single IL-1 dose of 0.1 to 0.8 mg/kg was injected into nude mice to determine the dose-response of dead cell fraction at 24 h later. Fig. 4b shows that at the doses of 0.1, 0.2, 0.4 mg/kg, the

fraction of dead cells is about 10, 30 and 65%, respectively, but there was no further increase in the fraction at higher doses.

Antitumor effect of IL-1 on HST lymphoma grown in beige nude mice Beige nude mice having low NK activity were used to examine whether or not the activation of NK cells by IL-1 might be involved in the inhibition of the tumor growth. As shown in Fig. 5, growth delay was observed in HST lymphoma in beige nude mice as well as in BALB/c nude mice, to almost the same degree.

Morphological changes of HST lymphoma after IL-1 treatment Fig. 6a, b, c, d shows the histopathological changes of HST lymphoma at 0 (control), 6, 12, and 24 h after IL-1 injection, respectively. At 6 h (Fig. 6b), focal necrotic areas of the tumor appeared, in which the tumor cells no longer appeared as closely apposed sheets of pleomorphic cells but had become rounded with pyknotic nuclei and shrunken cytoplasma. At 12 h (Fig. 6c), the necrosis was progressively spreading, while individual cells became more damaged and some of them seemed to have disappeared, leaving traces of cell debris. It is noteworthy that within these necrotic areas there were many dilated capillaries engorged with red blood cells but no intra-luminal thrombi with bleeding. At 24 h (Fig. 6d), most of the tumor was devastated; necrotic cells were sparsely scattered and the dilated vessels contained degenerated red blood cells. However, detailed observation at high magnification (Fig. 6e, f) revealed that the stromal framework of tumor tissue remained, despite such severe damage to the tumor cells.

Throughout the histopathological changes following IL-1 administration, there was little indication of inflammatory cells infiltrating into the affected tumor. There were no specific findings in the normal tissues and organs of the autopsied mice.

Detection of IL-1 in HST tumor after IL-1 administration Fig. 7a shows the IL-1-untreated tumor and Fig. 7b, the IL-1-treated tumor. Both specimens were reacted in the same way with antihuman IL-1 rabbit antibody to determine whether the injected IL-1 was bound to the tumor cells or not. The IL-1-treated tumor cells were peroxidase-stained, indicating the binding of the human IL-1 to the tumor cells.

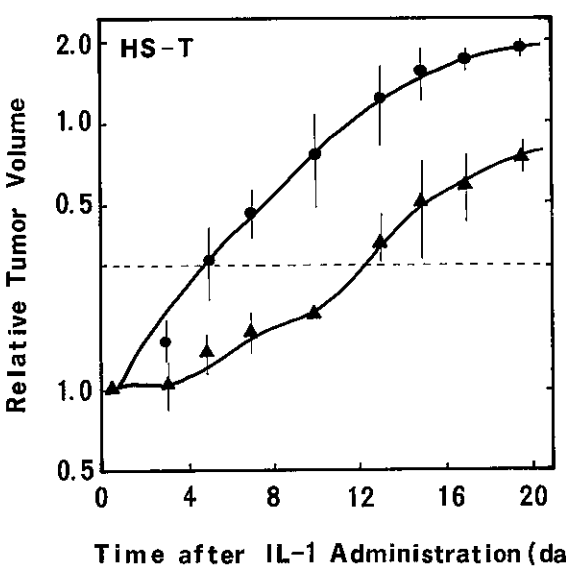

Fig. 5. The growth inhibition of HST lymphoma grown in beige nude mice after injection of 0.4 mg/kg IL-1. The growth delay time of the IL-1-treated group was about 8 days. ●, untreated; ▲, treated.

DISCUSSION

In the present paper, the antitumor action of rhIL-1α on human malignant lymphomas grown in nude mice was investigated in terms of tumor growth delay, kinetics of cell death including interphase death and reproductive death, and morphological alterations.

As shown in Fig. 1 and Table I, all of the lymphomas responded to IL-1 at the dose of 0.4 mg/ml with an average growth delay time of 8.5 ± 2.7 days. We also found that two human lung tumors grown in nude mice and syngeneic SCC VII tumor in C3H mice[22] were responsive. As far as we are aware, all experimental tumors so far examined responded to IL-1.[11-14, 16-18] The kinetics of cell survival following IL-1 administration were examined in detail using Burkitt lymphoma cells because of the high plating efficiency in soft agar plates. With increasing dose of IL-1 from 0.1 to 0.8 mg/kg, the growth delay of tumors was biphasic, increasing dose-dependently up to 0.4 mg/kg then remaining at a plateau level (Fig. 2). This is consistent with a dose-dependent increase of direct cell death (Fig. 3b) and loss of cell proliferative integrity (Fig. 4b) up to 0.4 mg/kg. The similar dose-dependencies indicated that these effects may occur through a common pathway. The plateau in tumor response is also consistent with the presence of some fraction of tumor cells insensitive to IL-1. Nakamura *et al.*[11] reported that four experimental tumors showed a dose-dependent response to IL-1 but it is not clear from their data whether or not this dose-dependency reached a plateau level. On the other hand, the time-course experiments (Figs. 3a, 4a) demonstrated that both types of cell death were increasing linearly until 24 h after IL-1 administration. Thereafter, the interphase death plateaued but the reproductive death stopped and the remaining viable cells recovered their proliferative integrity within another 24 h (data not shown). Such

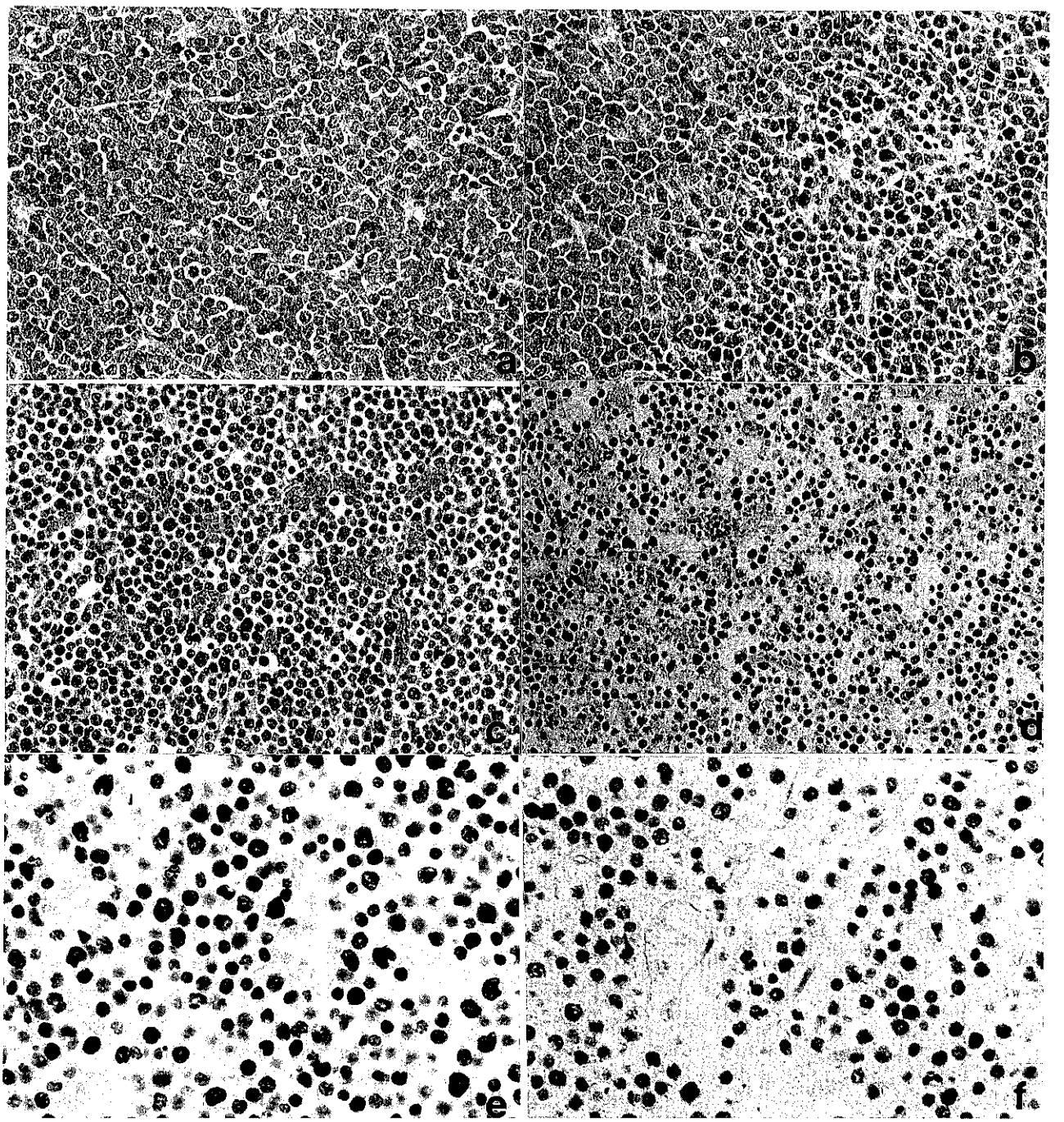

Fig. 6. Histopathological changes of HST lymphomas as a function of time after the treatment with 0.4 mg/kg IL-1. (a) sham-treated tumor, (b) tumor at 6 h after the treatment: small necrotic regions are apparent, (c) at 12 h: spreading necrosis with dilated small vessels including red cells, (d) at 24 h: extensive necrosis with sparsely scattered cells and congested capillaries. No thrombi, no extravasation of red blood cells (HE ×200). (e) at 24 h: higher magnification, and (f) 48 h: coagulative necrosis, but with little damage to the stroma (HE ×400).

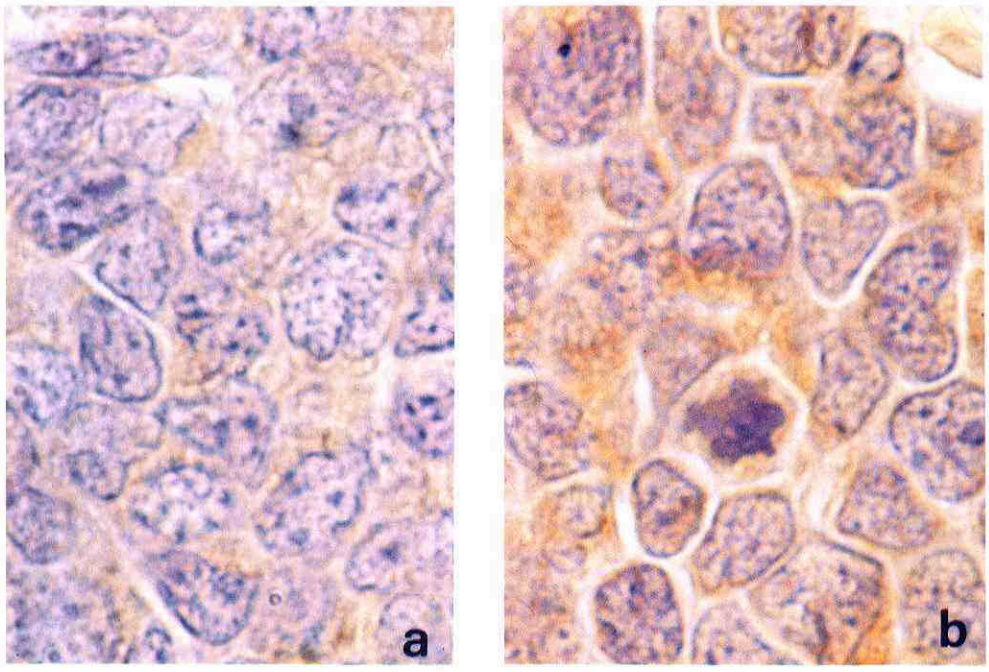

Fig. 7. Histopathology of HST lymphoma at 12 h after an injection of 0.4 mg/kg IL-1 (ABC staining, ×1000). (a) untreated tumor, (b) IL-1-treated tumor. Peroxidase-positive spots or areas were clearly observed in the IL-1-treated tumor, demonstrating that antihuman IL-1α polyclonal antibody was bound to the tumor cells, and in turn indicating the expression of IL-1 receptor.

rapid recovery of clonogenic cells was also observed in RIF-1 tumor.[12]

Time-course morphological studies were carried out on specimens taken from the HST tumors. In the 3-h specimen (data not shown), small degenerative and necrotic changes were firstly recognized, and subsequently spread, leading to the formation of large necrotic areas with congestion as a function of time after IL-1 treatment (Fig. 6b, c, d). At 24 and 48 h, most of the tumor was devastated, exhibiting necrosis and congestion. These morphological changes, reflecting a process of coagulative necrosis, are consistent with the kinetic data shown in Figs. 2 and 3.

The above findings seem to be different from those observed in TNF-induced tumor necrosis. Based on the pathophysiological findings, Belardelli *et al.*[17] and Braunschweiger *et al.*[12] suggested that the primary target of IL-1 is endothelial cells of vessels. In the case of HST tumors, however, the vascular congestion was detected after the specific cell damage. In addition, we did not observe thrombi in vessels or extravasation of blood cells (actual bleeding) throughout the observation period. The same finding was also observed in Meth A-induced fibrosarcoma in BALB/c mice,[16] which did not show thrombosis with cell necrosis after treatment with IL-1α but did show thrombosis after treatment with tumor necrosis factor (TNFα). These differences in the morphological effects of the two cytokines suggest that IL-1-induced tumor effects may not necessarily be mediated by TNF.

Le and Vilček[23] have obtained evidence suggesting that incubation of monocytes with IL-1 can result in the induction of TNF synthesis, and this mechanism may be responsible for the enhanced cytotoxicity of monocytes incubated in the presence of IL-1. Actually, many macrophages were found to be accumulated around RIF tumor tissues in C3H mice after IL-1 treatment.[12] Thus, it is generally considered that macrophages are the most promising candidates for effector cells against tumors after IL-1 treatment. In contrast, as shown in Fig. 6, few macrophages or monocytes were found around the damaged HST tumor cells after treatment with IL-1. In our experiment, macrophages are not thought to play an important role.

The cell-mediated cytotoxic action of IL-1 was also clearly seen in plasmacytoma X 5563-bearing C3H mice[14] and Meth A-induced fibrosarcoma grown in BALB/c mice.[18] In nude mice deficient in T cell functions, the IL-1-induced cell death could not involve T cell-mediated cytotoxic actions. However, IL-1 may en-

hance natural killer (NK) activity against tumors in nude mice.[3, 14] Beige nude mice having low activity of NK and T cells were used to examine this possibility. As shown in Fig. 5, the growth of HST lymphoma in beige nude mice was also inhibited by IL-1, indicating that NK activity was little implicated in the response of HST tumor to IL-1. Overall, the results strongly suggest that IL-1 manifests its antitumor activity through several pathways besides cell-mediated cytotoxic actions.

In our experiments, IL-1 was found to bind to the HST tumor until at least 12 h after IL-1 administration (Fig. 7) suggesting that the IL-1 receptor is probably expressed on the tumor cells, though the question remains whether the cells could intrinsically express the receptor *in vivo* or whether the receptor could be induced by IL-1 injection. Onozaki *et al.*[8] first demonstrated the cytostatic and cytocidal effects of IL-1 on human melanoma cell line A 375 in culture, and showed that these actions were not mediated by PG. Endo *et al.*[9] proved that the cells expressed IL-1 receptor, through which these direct tumor cell effects must be exerted. In addition, Lachman *et al.*[6] showed that the cytocidal effect of IL-1 on A 375 cells was not mediated by TNF or by PG. These *in vitro* experimental results indicate that the IL-1 binding to HST cells *in vivo* may exert antitumor activity as well.

Since IL-1 appears to have multiple mechanisms of antitumor action, it may prove to be efficacious against cancers incurable by conventional methods.

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