Anti-tumor Effects of Interleukin-4 and Interleukin-5 against Mouse B Cell Lymphoma and Possible Mechanisms of Their Action

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We investigated the anti-tumor effects of recombinant mouse interleukin (IL)-4 and IL-5 by using a transplantable B cell lymphoma 38C13 cell line as a model. Daily local administration of either IL-4 or IL-5 produced moderate but significant inhibition of the rate of local tumor growth and prolongation of mean survival time (MST) in syngeneic C3H/HeJ mice; these anti-tumor effects appeared to plateau at low doses. Histopathologic and immuno-histochemical examination revealed necrotic changes in the cytokine-treated tumors, associated with infiltration of inflammatory cells such as eosinophils, macrophages, and lymphocytes. The infiltrating lymphocytes were found to be Thy-1.2$^+$ T cells. To elucidate the importance of T cells, the rate of tumor growth and the MSTs were compared between athymic T cell-deficient BALB/c nude mice and immunocompetent C3H/HeJ mice. In the nude mice the transplanted tumor grew more rapidly and the MST was shorter than in the normal mice, suggesting a significant contribution of infiltrating T cells in the anti-tumor effects of the interleukins. Lastly, *in vitro*, growth inhibition of the 38C13 cells was observed in a dose-dependent manner at relatively high concentrations of either cytokine. Therefore, we conclude that both IL-4 and IL-5 have moderate anti-tumor effects against 38C13 B cell lymphoma both *in vivo* and *in vitro*, and that the observed *in vivo* anti-tumor effects are probably mediated both by tumoristatic action of infiltrating cells, such as eosinophils, macrophages and T lymphocytes, and by direct anti-proliferative action of the recombinant cytokines.

Key words: Anti-tumor effect — Interleukin-4 — Interleukin-5 — B cell lymphoma — Anti-proliferative effect

Anti-tumor immune response is mediated by various types of inflammatory cells such as T helper (Th)[5] cells, cytotoxic T lymphocytes (CTL), B cells, macrophages, and eosinophils, and by substances released from them. Interleukin-4 (IL-4) and interleukin-5 (IL-5) are multifunctional cytokines produced by Th cells. Both have pleiotopic effects on various aspects of the immune system. Some of the biological activities associated with IL-4 include: co-stimulation of B cell proliferation along with anti-immunoglobulin antibodies,[1] enhanced synthesis of IgG1 and IgE from B cells and proliferation of activated B cells,[2,3] stimulation of T cell proliferation,[4] induction of CTLs,[5] augmentation and/or suppression of lymphokine-activated killer cell activity and proliferation,[6,7] increase and/or decrease in cytokine production

and tumoricidal activity of macrophages/monocytes.[8-11] Another cytokine, IL-5 was first reported as a soluble factor derived from the supernatant of a T cell hybridoma capable of replacing some functions of the T cells,[12] such as the optimal induction of CTLs.[13] IL-5 can also potentiate the proliferation of activated B cells and induce the synthesis of IgA from some B cell populations.[14,15] Also, IL-5 is a potent eosinophil-colony-stimulating factor capable of augmenting the production of eosinophils and activating their function.[16]

Recently, mouse IL-4 was reported to possess a potent anti-tumor activity in a system using IL-4 gene-expressing tumor cell lines.[17] In the present report, we describe the *in vivo* and *in vitro* anti-tumor activities of the purified recombinant IL-4 and IL-5, adopting a well-studied mouse B cell lymphoma, 38C13, as a model, and discuss the various possible mechanisms of their action.

MATERIALS AND METHODS

Mice C3H/HeJ mice and BALB/c *nu/nu* mice were purchased from Charles River Japan (Atsugi) and from Japan CLEA Inc. (Tokyo), respectively. The mice were maintained at the Center for Bio-medical Research, Fac-

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[5] Abbreviations used in this paper: Th, helper T lymphocytes (CD4$^+$); CTL, cytotoxic T lymphocytes (CD8$^+$); IL-4, interleukin-4; IL-5, interleukin-5; PBS, phosphate-buffered saline; CM, complete medium; MTV, mean tumor volume; MST, median survival time; ABC staining, avidin-biotin-complex immuno-histochemical staining; MTT assay, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide proliferation assay.

ulty of Medicine, University of Tokyo. All the mice used were female mice at 5 weeks of age at the beginning of experiments.

Interleukin preparations Purified recombinant mouse IL-4 was kindly provided by Ono Pharmaceuticals, Inc. (Osaka). Purified recombinant mouse IL-5 was a generous gift from the Bio-Medical Institute of Suntory Inc. (Osaka). Purity of the cytokine preparations was determined by gel electrophoresis. They had specific activities of 1.65×10^6 U/ml and 3.0×10^6 U/ml, respectively, as measured by standard bioassays. The cytokine solutions were diluted in phosphate-buffered saline (PBS) and stored at 4°C, and the diluted preparations were used within 4 weeks.

Cells 38C13 is a transplantable B cell lymphoma line derived from a carcinogen-induced lymphoma which occurred in a C3H/HeJ mouse.[18] The cell line has been used in mouse tumor studies. The 38C13 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 10 μM 2-mercaptoethanol and antibiotics (CM) at 37°C in a humidified 5% CO_2 incubator.

Tumor cell transplantation and the treatment protocols C3H/HeJ mice underwent subcutaneous transplantation of 38C13 cells by injection of 1×10^5 38C13 cells in 0.2 ml of PBS in the left axillary region. Treatment with IL-4 or IL-5 was started 6 h after the subcutaneous injections of the lymphoma cells; this timing was adopted to mimic the possible local conditions used in a previous paper using IL-4 gene-transferred tumor cell lines.[17] The mice were given daily injections of IL-4 or IL-5 at doses of 2 ng, 20 ng, or 200 ng in 0.2 ml of PBS into the tumor inoculation site; control groups of mice received no treatment or were treated with PBS alone. In another study, the same number of 38C13 cells were injected subcutaneously into the left axillary region of immunocompetent C3H/HeJ and athymic BALB/c nude mice. These two groups of mice were left untreated. After the tumor inocultion, tumor masses were estimated daily by measuring the width and the length of the locally developing tumor using a caliper. The tumor volume was calculated by using the formula: $1/2 \times (\text{width})^2 \times (\text{length})$. The median survival time (MST) of the experimental animal groups was calculated as the period between tumor transplantation and death of the tumor-bearing mice.

Histological examination For histological studies, the tumor-bearing mice were killed at various time points after tumor inoculation. The histological samples were fixed with 2% paraformaldehyde in PBS at 4°C for 4–12 h and embedded in paraffin. Sections (2 μm) were stained with hematoxylin-eosin.

Avidin-biotin-complex (ABC) immuno-histochemical staining for cell-surface markers For detection of surface marker antigens on lymphocytes and macrophages,

the ABC assay was performed.[19] In brief, tissue specimens were snap-frozen in liquid nitrogen and cryostat sections (4–6 μm-thick) were made from the frozen tissues. After air-drying, the sections were fixed with acetone for 10 min at 4°C and then hydrated with PBS. The sections were blocked with rat serum and treated with anti-Thy-1.2 (Becton Dickinson, USA), anti-CD4 (anti-L3T4, GK-1.5), anti-CD8 (anti-Lyt-2, Becton Dickinson), anti-Mac-1 (Boehringer, Germany), or with S5A8 monoclonal antibody, which is reactive with an idiotypic epitope on the IgM of the 38C13 cells. After 2 h, the slides were washed and the bound monoclonal antibodies were detected by using a commercially available immuno-histochemical staining kit (Vector Lab. Inc., USA), according to the instructions. The bound second antibody was detected by peroxidase reaction, using diaminobenzidine tetrahydrochloride (DAB) as a coloring agent. After immunostaining, the specimens were post-stained with 1% methyl green.

Assays for *in vitro* cell proliferation and cell cycle analysis The proliferation of 38C13 lymphoma cells was measured *in vitro* by using a colorimetric MTT method, as described by Mosmann[20] with minor modifications. The tumor cells were incubated in a 96-well microculture plate at a cell density of 10^5 cells/ml in CM containing varying concentrations of the recombinant IL-4, IL-5 or both. After 24 h, 10 μl of 5 mg/ml MTT solution (MTT; Sigma, USA) was added and the cells were incubated for a further 4 h. The insoluble purple reaction product produced by MTT reduction in live cells was then dissolved in isopropanol containing 0.04 M HCl, and the optical density was measured using an ELISA reader (Dynatech Lab., USA) with test and reference wavelengths set at 570 and 630 nm, respectively. For cell cycle analysis, the 38C13 cells were cultured *in vitro* under the same conditions as for the MTT proliferation assays. After a 24-h incubation, the cells were washed and the nuclei of the cells were stained by using a commercially available cell cycle analysis kit (Cycle TEST DNA Accessory Kit, Becton Dickinson).[21] The DNA content in individual cells was assayed by using a FACScan flow cytometer with scanning of fluorescence emission wavelengths longer than 580 nm; 2×10^4 events were routinely collected and cell cycle analysis was performed using the CELLFIT Ver 1.2 software program (Becton Dickinson). Populations in G_0/G_1, S, and G_2/M phases were also calculated.

Statistical analysis The experimental data were expressed as mean values ± standard deviation. Student's t test was employed for the statistical analysis of the mean tumor volume (MTV) and *in vitro* cellular proliferation. For statistical comparison of MST of the treated mice, the U-test was used. A P value of <0.05 was considered to be statistically significant.

RESULTS

Effects of IL-4 and IL-5 on local tumor growth and MST
In all four groups of mice transplanted with the 38C13 lymphoma, large tumors formed at the site of injection by day 10. However, a significant difference in tumor growth rate was observed in the two groups of mice treated with either IL-4 or IL-5, compared with the two control groups of mice left without treatment or treated with PBS injection alone. The MTVs of PBS-control, IL-4-treated, and IL-5-treated groups were 94.9%, 41.2%, and 47.1%, respectively, of the control untreated group at day 10 after tumor inoculation and the start of cytokine treatment, and 95.8%, 47.3%, and 53.9% of the control, respectively, at day 20. There were statistically significant differences in the MTVs in the IL-4- or IL-5-treated groups at both day 10 and day 20, compared to the untreated or PBS-treated groups ($*$ $P<0.05$, $**$ $P<0.01$) (Fig. 1), demonstrating the anti-tumor effects of both IL-4 and IL-5.

The effect of the cytokines on the MST of tumor-transplanted mice is shown in Fig. 2. The MSTs of the untreated, PBS-treated, IL-4-treated, and IL-5-treated

groups were 20.5 days, 22.0 days, 33.5 days, and 31.8 days, respectively. The MSTs of the cytokine-treated groups were significantly longer than those of the two control groups ($*$ $P<0.05$, $**$ $P<0.05$).

Next, we determined the dose-response relationship of the anti-tumor effects of recombinant cytokines. Three 10-fold-different daily doses of IL-4 or IL-5 were given locally into the 38C13 injection site. Again, treatment with either IL-4 or IL-5 showed significant inhibitory effects on MTV both at day 10 and at day 20 (Fig. 3 A and B, $*$ $P<0.05$, $**$ $P<0.01$). However, a statistically significant dose-response relationship was not observed among the groups treated with either cytokine, suggesting that the observed anti-tumor actions of these recombinant cytokines may plateau at a dosage lower (<2 ng/mouse/day) than what we employed in these studies.

Histopathology of tumor tissues treated with IL-4 or IL-5
Histopathological examinations of tumor tissues from the untreated, PBS-treated, IL-4-treated, and IL-5-treated mice were performed. In the tissues obtained from the untreated and the PBS-treated mice, there was progressive lymphomatous growth of the injected 38C13
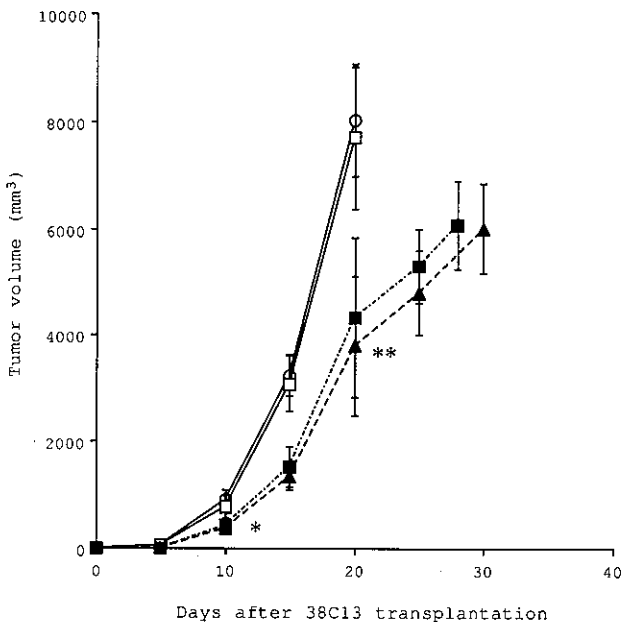

Fig. 1. Mouse IL-4 and IL-5 mediated inhibition of local tumor growth in B-lymphoma cell line. Tumor growth rates in four different groups of C3H/HeJ mice bearing B cell lymphoma were compared after subcutaneous injection. Each point represents the MTVs of each group of 8 to 11 animals measured on the day indicated. Error bars indicate ±SEM ($*$ $P<0.05$, $**$ $P<0.01$). ○, No IL-4 and IL-5; □, PBS control; ▲, treated with IL-4; ■, treated with IL-5.
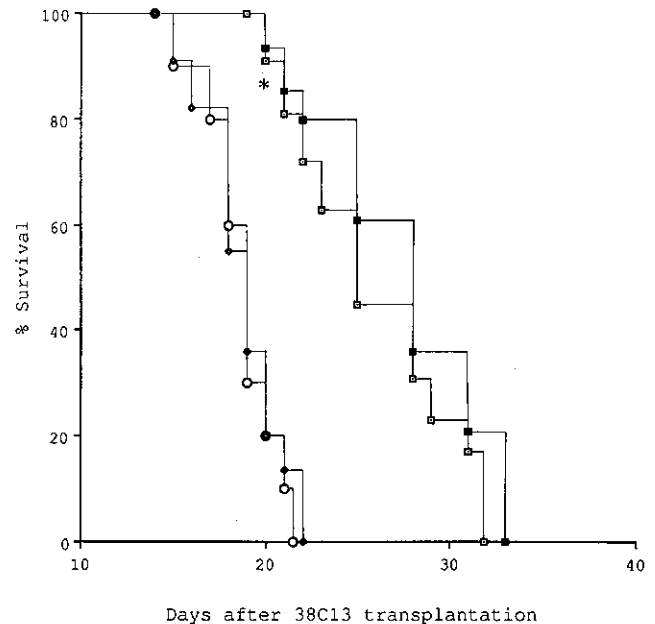

Fig. 2. MST after subcutaneous injection of 38C13 cells. MSTs of the four groups from 8 to 11 mice after inoculation of 38C13 cells. MSTs of both the IL-4 and IL-5 groups (20 ng/mouse/day) were longer than those of the untreated and PBS groups. $*$ indicates significant differences between IL-4 and IL-5 treated groups and the two control groups at the same time point ($*$ $P<0.05$). ○, No IL-4 and IL-5; ◇, PBS control; ⊞, treated with IL-4; ▣, treated with IL-5.
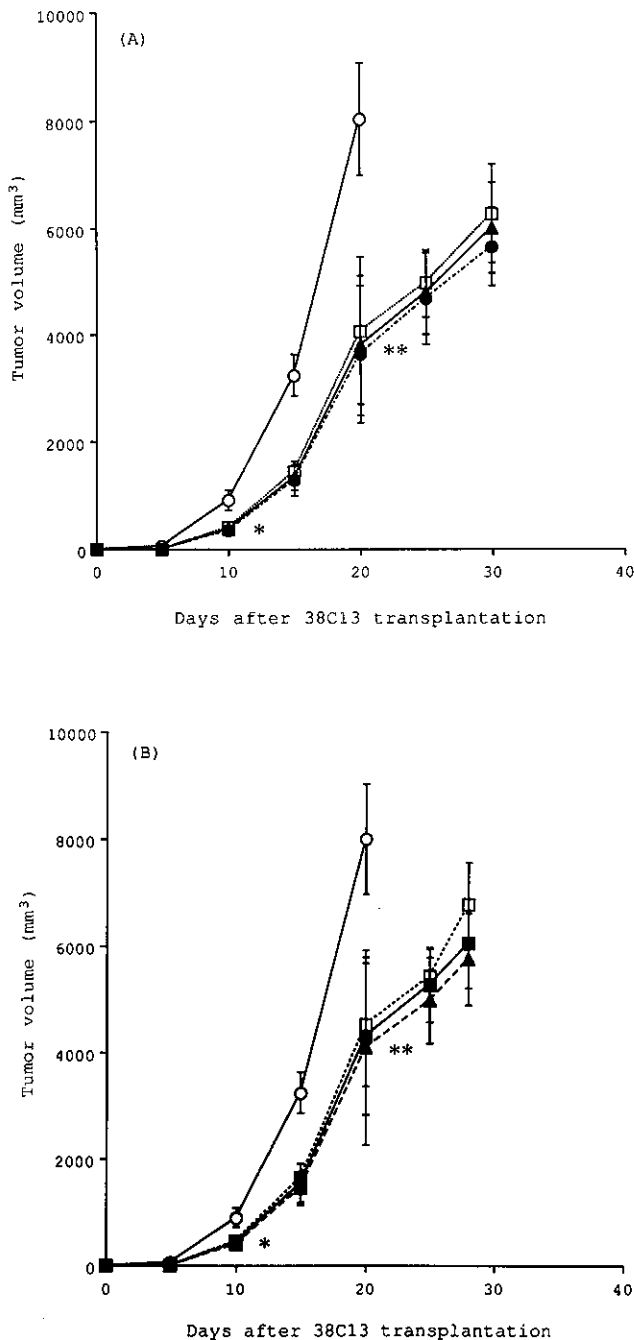
Fig. 3. IL-4 or IL-5 mediated inhibition of local tumor growth of B cell lymphoma. MTVs in the mice bearing B cell lymphoma were compared among the three groups treated intra-tumorally with different dosages of IL-4 (A) and IL-5 (B) (2 ng, 20 ng, 200 ng/mouse/day). Each point represents the MTVs of each group of 8 to 11 mice. No significant difference in tumor inhibition was identified among IL-4/IL-5 treated groups at the three dosages employed ($P > 0.05$). (A) ○, No IL-4 and IL-5; □, IL-4 2 ng; ▲, IL-4 20 ng; ●, IL-4 200 ng. (B) ○, No IL-4 and IL-5; □, IL-5 2 ng; ■, IL-5 20 ng; ▲, IL-5 200 ng.

cells at the local site with numerous mitotic cells on both day 10 and day 20, with few cellular infiltrates (Fig. 4 A). In contrast, examinations of tumor tissues from the IL-4- and IL-5-treated mice revealed the presence of tumor cell necrosis and degeneration associated with infiltrating cells; these infiltrates were composed primarily of eosinophils, macrophages, and lymphocytes as revealed by hematoxylin-eosin stain (Fig. 4 B and C). On day 10, a band of infiltrating eosinophils could be seen predominantly in the outer region of small blood vessels in the tumor tissue, and macrophages were also present. Histological sections obtained from mice treated with IL-4 or IL-5 for 20 days revealed, in comparison to day 10, more pronounced infiltration of eosinophils, macrophages, and lymphocytes along with tumor cell degeneration.

Correlation of tumor-inhibitory activity with T lymphocytes In order to determine the phenotype of the infiltrating lymphocytes, immuno-histochemical staining of frozen tumor tissues was performed, using monoclonal antibodies against T cells and macrophages. In the tumor tissue obtained on day 10 after tumor inoculation, some of the infiltrating cells were shown to be positive for the Mac-1 macrophage antigen and a few other cells were positive for the T lymphocyte markers, Thy-1.2 , CD4, or CD8. By day 20 and thereafter, a majority of infiltrating cells was also positive for the Mac-1 antigen, but a considerable number of cells which bear T cell markers were detected perivascularly in the frozen sections of the cytokine-treated groups, compared with those from untreated and PBS-treated control groups (Fig. 5). There was one noticeable difference between the IL-4-treated and IL-5-treated tumor tissues, namely that fewer CD4$^+$ T cells were detected in tissue sections from IL-5-treated mice compared to IL-4-treated mice, although there was comparable infiltration of CD8$^+$ T cells in tissue sections taken from both groups of mice. Therefore, it is likely that the anti-tumor effects of IL-4 and IL-5 are mediated by these infiltrating macrophages, T lymphocytes, as well as eosinophils.

In a previously published report,[17] the effect of IL-4 was assessed by using T cell-deficient nude mice, so the contribution of immunocompetent T cells was not studied. Therefore, we decided to investigate the importance of T cell-mediated cellular immunity in anti-tumor defense against the 38C13 tumor. The lymphoma cells were transplanted into two groups of mice, immunocompetent C3H/HeJ mice and T cell-deficient athymic BALB/c nu/nu mice. The MTV was significantly larger and the MST was significantly shorter in the immunodeficient athymic nude mice than in the immunocompetent C3H/HeJ mice (* $P < 0.05$) (Fig. 6). These results strongly imply the importance of T cell-mediated cellular immunity in host defense against the lymphoma and argue for
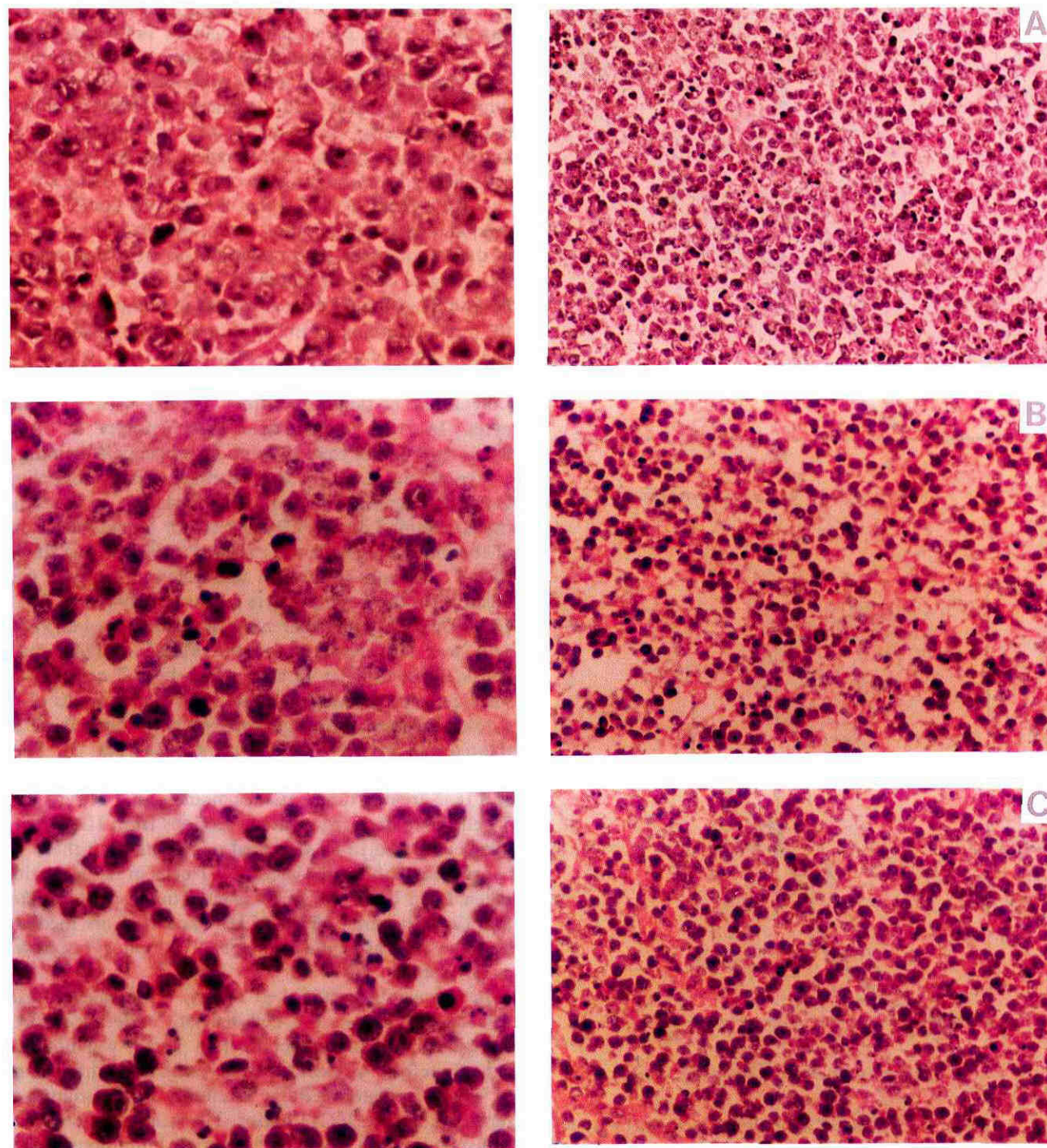
Fig. 4. Representative histopathology in the tumor-bearing mice treated with IL-4, IL-5 or control. (A) C3H/HeJ mouse on day 20 after subcutaneous injection, treated with PBS only. Note the presence of conspicuous B lymphoma cells. Mitoses can be easily seen. (B) and (C) C3H/HeJ mouse on day 20 after subcutaneous injection, treated with IL-4 or IL-5 20 ng/mouse/day intratumorally. In marked contrast to (A), eosinophils are easily seen in most fields of the sections from the interleukin injection site. Macrophages and lymphocytes are also seen. More eosinophils are seen in the IL-5 section than in the IL-4 section. In addition, note the presence of necrotic and degenerating lymphoma cells in the sections of the experimental groups (subcutaneous tissues, ×200 & ×400, H & E stain).
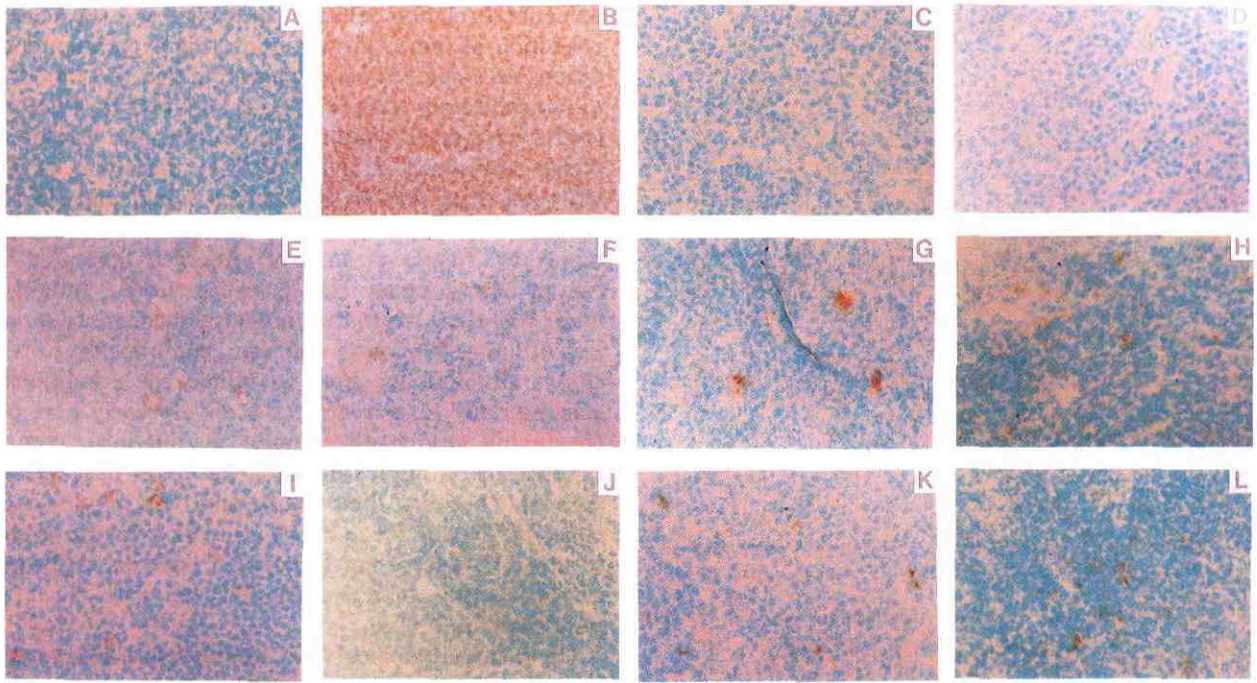
Fig. 5. Immuno-histochemical (ABC) staining using monoclonal antibodies against Thy-1.2, CD4, CD8, Mac-1, and S5A8 of frozen tumor sections from injection sites in the four groups. (A) B-lymphoma cells of untreated group stained with PBS alone (×200). (B) Prominent membrane staining of 38C13 cells from untreated group by S5A8 (×200). (C) Thy-1.2 staining of frozen tumor sections of untreated group showing no reactivity (×200). (D) Thy-1.2 staining of frozen tumor sections of PBS group showing no reactivity (×200). (E) Thy-1.2 staining of frozen tumor sections of the IL-4 group showing special reactivity for T cells (×200). (F) CD4 staining of Th cells on frozen tumor sections of the IL-4 group (×200). (G) CD8 staining of CTLs in frozen tumor sections of the IL-4 group (×200). (H) Mac-1 staining of frozen tumor sections of the IL-4 group, also showing special reactivity for macrophages (×200). (I) Thy-1.2 staining of frozen tumor sections of the IL-5 group (×200). (J) No staining in frozen tumor sections of the IL-5 group with CD4 antibody (×200). (K) CD8 staining of CTLs on frozen tumor sections of the IL-5 group (×200). (L) Mac-1 staining on frozen tumor sections of the IL-5 group (×200).

the possible significance of the role played by tumor-infiltrating T cells in the anti-tumor response of IL-4-treated and IL-5-treated normal, immunocompetent C3H/HeJ mice.

Effects of IL-4 and/or IL-5 on *in vitro* proliferation of 38C13 cells From the foregoing observations, infiltrating host inflammatory cells are likely to play a predominant role in the favorable anti-tumor response in IL-4- and IL-5-treated mice. However, both IL-4 and IL-5 have been shown to possess potent activities towards B cells, and the 38C13 lymphoma cell line is also of B cell origin. Therefore, the direct effects of the recombinant cytokines on 38C13 cell proliferation were investigated *in vitro* using a colorimetric proliferation assay (MTT assay) and by cell cycle analysis. After a 24-h exposure to either IL-4 or IL-5, moderate dose-dependent inhibition of 38C13 proliferation was evident at high concentrations of the cytokines (Fig. 7). This anti-proliferative activity of the interleukins was not synergistic, however, when the two

interleukins were tested in combination. Cell cycle analysis of the 38C13 cells exposed to the cytokines *in vitro* for 24 h, showed a dose-dependent reduction in the percentages of cells in the S and $G_2 + M$ phases of the cell cycle (Fig. 8), suggesting that the observed *in vitro* anti-proliferative effects are due to a block (or delay) in cell cycle progression of 38C13 cells.

DISCUSSION

In the work presented here, we evaluated the anti-tumor effects of recombinant mouse IL-4 and IL-5 in a well-studied transplantable B cell lymphoma model. In *in vivo* studies, we observed a statistically significant decrease in the rate of local tumor growth in tumor-transplanted mice, with concomitant prolongation of the MST in mice treated daily with a direct local injection of either of these two recombinant cytokines, in comparison to those of control mice. However, there was no sig-
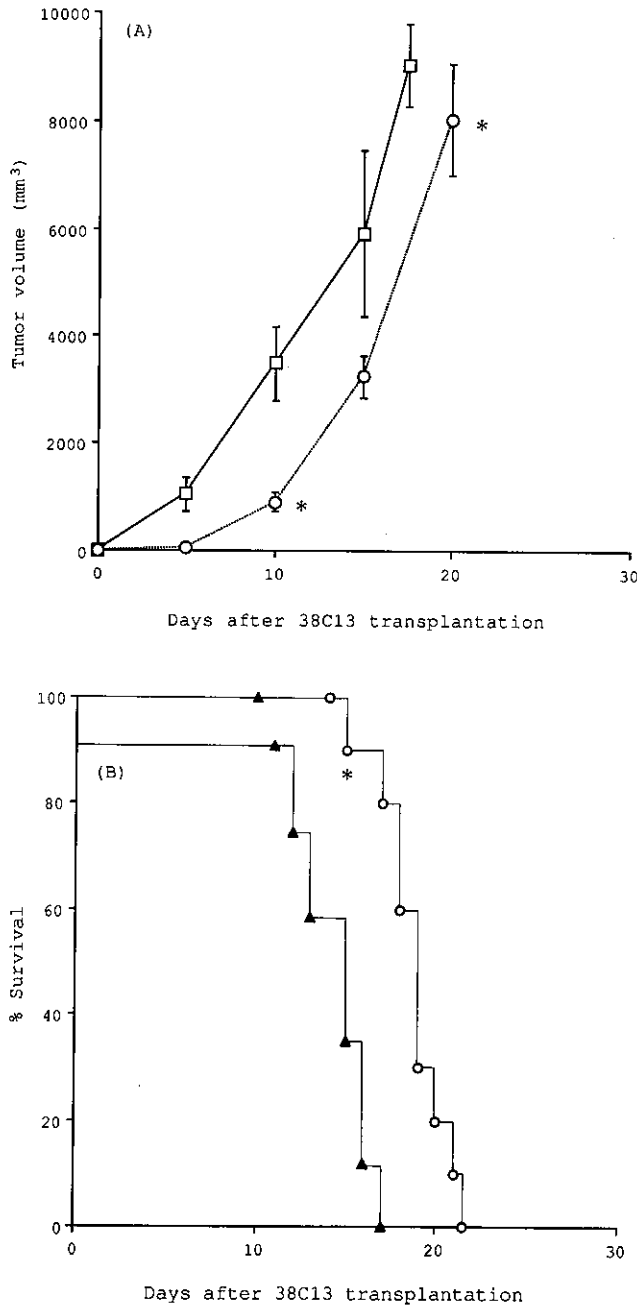
Fig. 6. Comparison of tumoricidal activity in thymic (C3H/HeJ) and athymic mice (BALB/c *nu/nu*) transplanted with 1×10^5 38C13 cells *in vivo*. Each data point corresponds to the day indicated. (A) The results of each group from 8 to 11 mice are indicated as the MTV±SEM. The MTV was significantly larger in the BALB/c *nu/nu* compared to the C3H/HeJ mice (* *P*<0.05). □, BALB/c *nu/nu* mice; ○, C3H/HeJ mice. (B) MST of the two groups after inoculation of 1×10^5 38C13 cells. Each point represents the MST of each group. The difference between athymic and thymic mice groups at the same time point was significant (* *P*<0.05). ▲, BALB/c *nu/nu* mice; ○, C3H/HeJ mice.

nificant correlation between the dosage and the degree of inhibition of tumor growth under the experimental conditions we employed here, suggesting that these moderate tumor-inhibitory actions of both IL-4 and IL-5 are maximal at very small daily doses. We observed a somewhat higher anti-tumor activity of IL-4 than that of IL-5, although there was no statistically significant difference between the two. The results are in accordance with a report by Tepper *et al.*, who observed favorable anti-tumor effects using IL-4-gene-transfected tumor cell lines.[17] In their work, they observed a complete suppression of tumor formation with some tumors; in the present study, although we tried to mimic the conditions by starting daily IL-4 treatment at a very early time point (beginning at 6 h after tumor inoculation), complete suppression of tumor formation was never observed. The difference between their results and ours may be caused either by the difference in the route of cytokine administration (IL-4-secreting tumor cell lines vs. daily intratumor injection of recombinant IL-4) or in the type of tumor cell lines used.

On histopathological and immuno-histochemical examinations, we noted considerable infiltration of tumor tissue by macrophages, eosinophils, and both CD4$^+$ and CD8$^+$ T lymphocytes, associated with tumor cell necrosis and degeneration, in IL-4-treated tumors. The results are consistent with the IL-4-gene therapy report,[17] which described infiltration of tumor tissues by eosinophils and macrophages. But, in their experiments, immunodeficient athymic nude mice were used as tumor hosts, so they did not observe T lymphocyte infiltration. Our present study was conducted using immunocompetent C3H/HeJ host mice and showed infiltration by both the Th cells (CD4$^+$) and the CTLs (CD8$^+$) into the tumor tissues after treatment with intratumor injection of recombinant IL-4. The eosinophilic infiltration of tumor tissues in the IL-4 treatment group was consistent with other reports.[17, 22] Infiltration of tumors by macrophages is also consistent with other papers[17] and the increased tumoricidal activity of IL-4 on macrophages.[9] In IL-5-treated tumors, we also noted infiltration by eosinophils, macrophages, and CTLs. IL-5 is a major eosinophil colony-stimulating factor,[16, 23] and IL-4 is also reported to have direct growth-promoting activity in these cells.[24] Therefore, it is likely that the tumor-infiltrating eosinophils are activated by IL-5 and IL-4. The major difference between IL-4-treated and IL-5-treated tissues was the presence or the absence of infiltration by CD4$^+$ T cells. IL-4 has been demonstrated to have stimulatory activities in both the Th cells and the CTLs,[5, 25] whereas IL-5 has been reported to have a CTL-inducting activity in immature thymocytes only.[13, 26] This may be the explanation for the observed difference between the two cytokine-treated tissues. The significance of this finding is
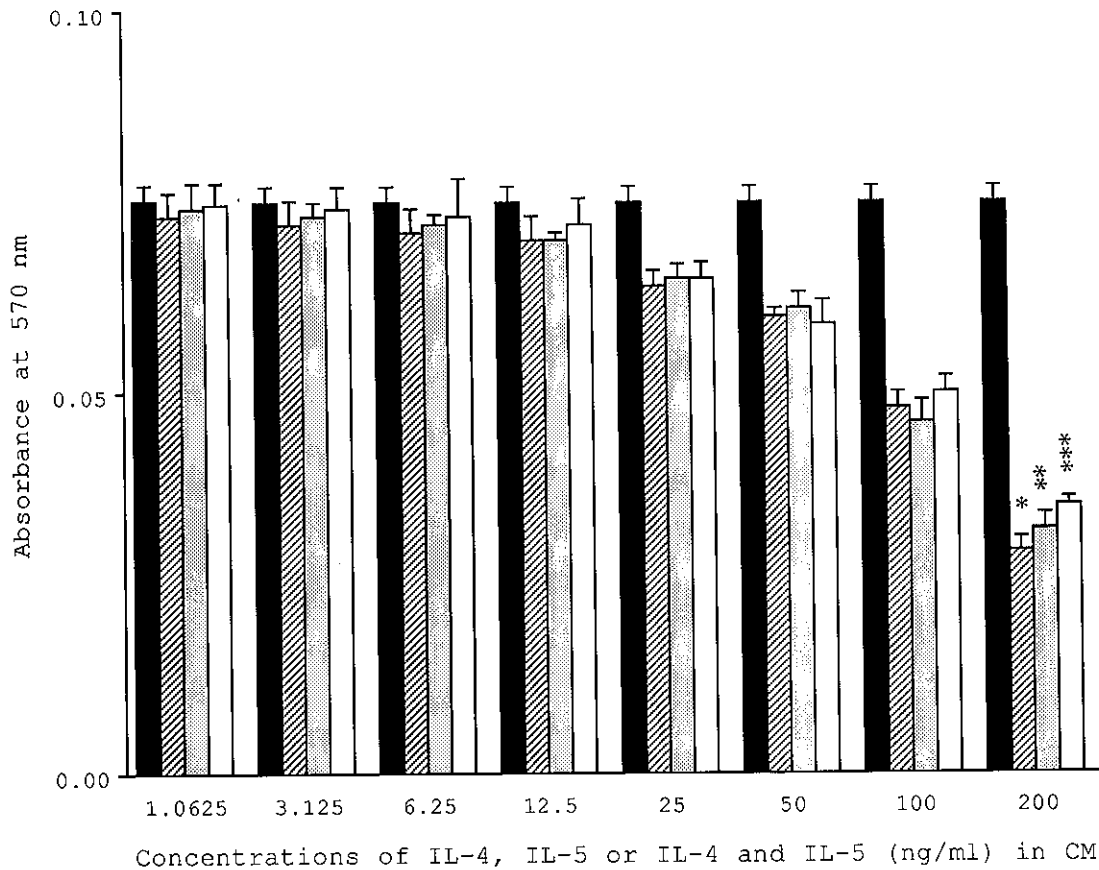
Fig. 7. Effects of IL-4 and /or IL-5 on the proliferation of 38C13 cell line *in vitro*. 38C13 cells (1×10^5/ml) were cultured for 24 h with IL-4 or IL-5 alone, or in combination. The cell proliferation was determined by a standard colorimetric assay. Each optical density is shown the mean absorbance±SD. Absorbance values of 38C13 cells treated with IL-4 and/or IL-5 were significantly lower than that of the control at 200 ng/ml (*, **, *** $P<0.05$). ■, Negative control; ▨, IL-4; ▩, IL-5; □, IL-4+IL-5.

not known. The cytokines that directly stimulate these cellular infiltrations are currently not known; it is possible that a local injection of IL-4 or IL-5 may initiate a complex set of interactions between cells and cytokines produced from them, resulting in migration of T lymphocytes, macrophages and eosinophils into the tumors.

There are still many unanswered questions with regard to the possible effector mechanisms exhibited by these infiltrating eosinophils, macrophages, Th cells and CTLs. Each of these possible effector cells displays various anti-tumor activities, by direct cell killing, by antibody-mediated cell killing, or by secreting cytotoxic cytokines, such as IL-1, TNF-α, and others.[8, 9] But we presently do not know which of these infiltrating cell types is the major determinant of IL-4 or IL-5 action. First, eosinophils are reported to exhibit potent anti-tumor action,[22, 27] so it is likely that the locally infiltrating eosinophils play some part in the anti-tumor effects, including micro-

vascular injury, tumor infarction, and fibroblastic reactions. Secondly, the role of infiltrating T lymphocyte is not clear, but both IL-4 and IL-5 have been reported to possess potent actions on T lymphocytes.[5, 6, 13, 26] We therefore propose that these T cells play some important part in the overall anti-tumor responses mediated by both IL-4 and IL-5 in our experiments using the immunocompetent host. This interpretation is supported by a comparison of the rapidity of 38C13 tumor formation in T cell-competent normal C3H/HeJ mice versus T cell-deficient BALB/c nude mice. Thirdly, with respect to macrophages, many reports on the effects of IL-4 on macrophages have been published showing either stimulatory or inhibitory effects of this potent cytokine on the secretion of TNF-α and IL-1 and on the tumoricidal activity of these cells.[8–11, 28, 29] Until now the action of IL-4 on human and murine monocytes/macrophages has generally been considered to be stimulatory,[8, 9, 28]
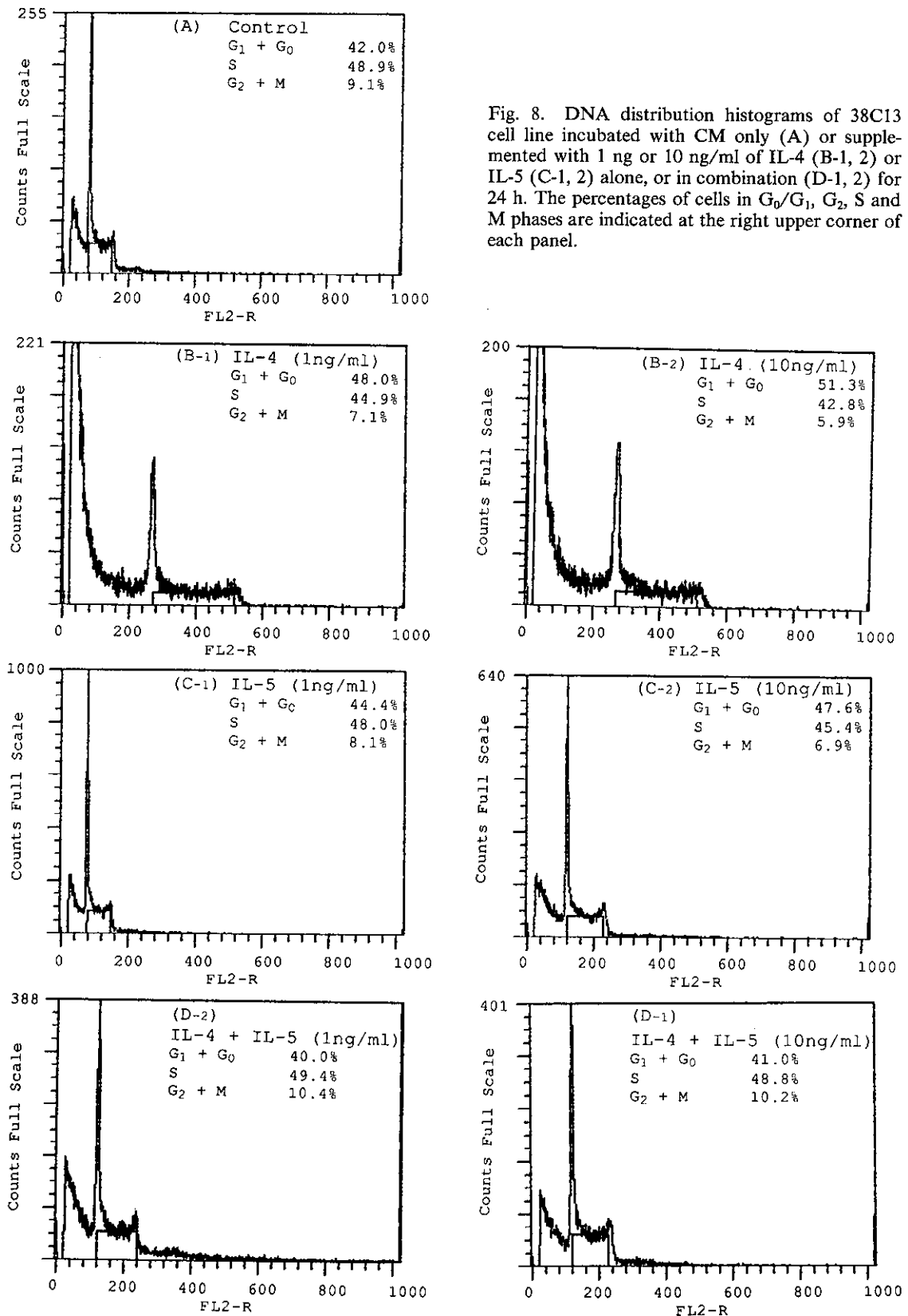
Fig. 8. DNA distribution histograms of 38C13 cell line incubated with CM only (A) or supplemented with 1 ng or 10 ng/ml of IL-4 (B-1, 2) or IL-5 (C-1, 2) alone, or in combination (D-1, 2) for 24 h. The percentages of cells in G_0/G_1, G_2, S and M phases are indicated at the right upper corner of each panel.

although the present data show that IL-4 can also inhibit some parameters of their activation.[10, 11, 29] At present, we do not understand the relative importance of these differing activities in our *in vivo* treatment system. We hope that the relative importance of these various activities reported in the literature will be clarified in the future.

Lastly, in our model tumor system, where 38C13 B cell lymphoma was chosen, direct anti-proliferative action of both IL-4 and IL-5 was documented. Other workers have reported that human IL-4 inhibits the growth of fresh multiple myeloma and lymphoma cells.[30] Our results are consistent with their observations and extend their findings to a mouse B cell lymphoma model. Cell cycle analysis demonstrated that IL-4 and/or IL-5 appeared to act by blocking or delaying cell-cycle progression of 38C13 B lymphoma cells. Therefore, it is likely that in the *in vivo* treatment experiments, which were conducted by direct local administration of recombinant cytokines, both IL-4 and IL-5 could act by decreasing the proliferation of the transplanted tumors, especially during the time period shortly after tumor inoculation. But it is unlikely that this effect has major significance at the later stages of treatment when the transplanted tumors have progressed to a macroscopic size, because this anti-proliferative effect is only statistically prominent at relatively high concentrations of the cytokines. At later stages of tumor progression, we consider it more likely that the host anti-tumor response mediated by infiltrating inflammatory cells plays a more dominant immunomodulating role. Furthermore, susceptibility of 38C13 cells

to tumoricidal activity mediated by eosinophils, macrophages and/or CTLs may differ *in vivo* versus *in vitro*.

In conclusion, in a mouse B lymphoma model system, we demonstrated that both IL-4 and IL-5 were moderately effective in suppressing the rate of tumor growth and prolonging the MST of tumor-transplanted mice when administered daily at the site of tumor inoculation in small doses. The treated tumors exhibited cell necrosis and degeneration, along with infiltration by inflammatory cells composed of eosinophils, macrophages, and T lymphocytes. Also, direct anti-proliferative actions of IL-4 and IL-5 on the lymphoma cells were observed. Therefore, the possible mechanisms of action include: direct anti-proliferative effects on the B lymphoma cells at a early time point in the treatment and cytotoxic effects mediated by inflammatory cells (eosinophils, macrophages, and T lymphocytes) attracted to the tumor tissues by the direct or indirect effects of the injected interleukins. This observation provides valuable information for the potential future clinical application of these recombinant cytokines.

ACKNOWLEDGMENTS

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