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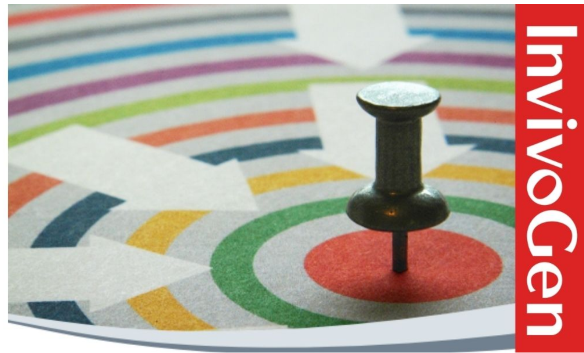

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ENHANCED PRODUCTION OF IL-6 IN TUMOR-BEARING MICE AND DETERMINATION OF CELLS RESPONSIBLE FOR ITS AUGMENTED PRODUCTION[1]

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IL-6 is a cytokine secreted in normal individuals by monocytes, fibroblasts, and endothelial cells. We have found increased levels of IL-6 in the sera from MH134 hepatoma- and CSA1M fibrosarcoma-bearing mice. Concerning the capacity of these tumor cells themselves to produce IL-6 in vitro, they exhibited the distinct contrast, i.e., the MH134 tumor cells produced high levels of IL-6 whereas the CSA1M generated a marginal level of IL-6. It was, however, demonstrated that appreciably enhanced IL-6 production was observed in spleen cell culture supernatants from both types of tumor-bearing mice when compared to those obtained from normal mice. More importantly, in contrast to the production of IL-6 by non-T cell compartment of normal spleen cells, enhanced IL-6 production of spleen cells from tumor-bearing mice was ascribed to T cell compartment. Analysis of T cell phenotype has revealed that enhanced IL-6 production was mediated predominantly by Lyt-2$^+$ but not by L3T4$^+$ T cell subset. Thus, these results indicate that increased circulating IL-6 is elicited in the tumor-bearing state and that irrespective of the potential of tumor cells themselves to produce IL-6, T cells, especially Lyt-2$^+$ T cells from tumor-bearing mice are responsible for such a high level of IL-6 production.

A number of situations such as infection and injury produces significant alterations in host metabolic and immune homeostasis. It is increasingly evident that several groups of cytokines play an integral role in the response to the homeostasis-altered situations. Of these cytokines, IFN-β_2/B cell-stimulatory factor 2 is attracting increasing attention for its potential. This cytokine with pleiotropic biologic activities has been isolated in the past few years by various investigators and the term IL-6 has been proposed (1, 2).

IL-6 is known to be produced by a variety of cells including monocytes/macrophages, fibroblasts, keratinocytes, endothelial cells, and some types of tumor cells such as myeloma cells (3–8). It has also been shown that several categories of inducing signals such as infectious agents or its products and cytokines (TNF-α/IL-1) are required for the expression of the IL-6 mRNA (3–8). Along this line of basic studies, increased levels of circulating IL-6 were found in hosts administered i.v. with endotoxin (9) or bearing autoimmune disease involving tissue injury (collagen-induced arthritis) (10).

Although biologic actions of IL-6 in vivo and its significance remain to be understood, the fact that IL-6 is produced in response to the generation of inflammatory lesions or tissue injury raised a question of whether an augmented IL-6 production is also observed in tumor-bearing hosts. Thus, the present study was performed to investigate synthesis and secretion of IL-6 in tumor-bearing hosts and to determine cell type(s) responsible for its production in the tumor-bearing state. The results demonstrate that tumor cells themselves in some, but not all cases as well as T cells (especially Lyt-2$^+$) from tumor-bearing hosts produce excessive IL-6 to exhibit its increased circulating levels.

MATERIALS AND METHODS

Mice and tumors. Female C3H/He and BALB/c mice were obtained from Charles River Laboratory, Kanagawa, Japan and Shizuoka Experimental Animal Laboratory, Hamamatsu, Japan, respectively. These mice were used at 7 to 9 wk of age. MH134 hepatoma (11) and CSA1M fibrosarcoma (12), which were induced in C3H/He and BALB/c strains with the respective carbon tetrachloride and Rous sarcoma virus, were utilized.

rIL-6 and anti-IL-6 mAb. rIL-6 was kindly provided by Dr. T. Yokota, DNAX Research Institute, Palo Alto, CA. Hybridoma producing anti-murine IL-6 mAb (6B4) (13) was a generous gift of Dr. J. Van Snick, Ludwig Institute for Cancer Research, Brussels, Belgium. 6B4 culture supernatant was used as a source of anti-murine IL-6 antibody.

Culture medium. Medium used for culturing tumor cells or spleen cells was RPMI 1640 supplemented with 5% F.C.S.

IL-6 assay. IL-6 activity was measured by IL-6-dependent cell lines, PIL-6 (10) and 7TD1 (13). PIL-6 and 7TD1 cells were cultured in 96-well flat-bottomed microtiter plates at 2×10^3 cells per well in triplicate cultures with serial twofold dilutions of samples for 5 and 3 days, respectively. Cells were pulsed with [^{3}H]thymidine (20 KBq/well) for 4 h, harvested onto a glass filter, and the incorporated radioactivity was measured with a scintillation counter. IL-6 activity in each sample was measured in parallel by using these two cell lines.

Treatment of spleen cells with antibody plus C. mAb to Thy-1.2, L3T4 or Lyt-2 Ag were used. Anti-Thy-1.2 was purchased from Cerotec Ltd., Blackthorn, Bicester, England. The GK1.5 mAb-producing hybridoma line, specific for the L3T4 molecule (14), was a gift of Dr. F. Fitch, University of Chicago, Chicago, IL and the 3.155 mAb-producing hybridoma line, specific for the Lyt-2 molecule was obtained from the American Type Culture Collection, Rockville, MD. Spleen cells (10^8) from normal or tumor-bearing mice were incubated at room temperature for 30 min with appropriate dilutions of anti-

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Thy-1.2, anti-L3T4, or -Lyt-2 mAb. Cells were washed and incubated at 37°C for 45 min with rabbit C preabsorbed with syngeneic mouse spleen cells at a final dilution of 1:20. The efficacy of these antibody-treatments was confirmed in flow microfluorometric analysis by demonstrating that the treatment with anti-L3T4 or -Lyt-2 antibody results in almost complete elimination of the respective $Lyt-2^+$ or $L3T4^+$ T cell subset without damage onto the other alternative T cell subset, as shown in the previous studies (15).

Preparation of $Thy-1^+$ cell-enriched fraction. Spleen cells were panned onto a polystyrene dish (Corning Glass Works, Corning, NY) coated with goat anti-mouse (IgM + IgG + IgA) antibody (Cappel Laboratories, Malvern, PA) by the method of Mage et al. (16), and nonadherent cells (T cell-enriched population) were gently collected.

RESULTS

Detection of increased levels of IL-6 activity in sera from tumor-bearing mice. C3H/He and BALB/c mice were implanted with 10^6 MH134 and 2×10^5 CSA1M syngeneic tumor cells, respectively. The MH134 and CSA1M were used as models of the respective rapidly growing and slowly growing tumors. Thus, they formed an approximately 20-mm diameter of tumor mass 15 days (MH134) or 60 days (CSA1M) after tumor cell implantation. Serum samples were obtained various days after the tumor cell implantation and submitted to IL-6 assay with the use of IL-6-dependent cell line 7TD1.

The results of Figure 1 demonstrate that sera from normal mice caused only marginal (background) proliferation of 7TD1 cells. In contrast, sera from MH134 or CSA1M tumor-bearing mice exhibited potent IL-6 activity. These findings show that enhanced production of IL-6 is induced in vivo in tumor-bearing mice.

Differential potentials in tumor cells to produce IL-6 in vitro. To examine the possibility that enhanced production of IL-6 is ascribed to the capacity of tumor cells themselves to generate IL-6, both types of tumor cells were cultured without stimulation. The results of Figure 2 illustrate that culture supernatants from MH134 and CSA1M tumor cells caused the respective potent and only marginal proliferation of PIL-6 cells. The results of Figure 3 also show that the reduced capacity of CSA1M tumor cells to produce IL-6 was observed irrespective of the concentration of culture supernatant tested in the IL-6 assay (Fig. 3*A*) or the period for obtaining culture supernatant Fig. 3*B*). It was also confirmed that the addition of various concentrations of LPS or rTNF-α to the CSA1M tumor cell cultures failed to induce the production of IL-6 (data not shown). These results suggest differential capabilities of tumor cells themselves to produce IL-6.

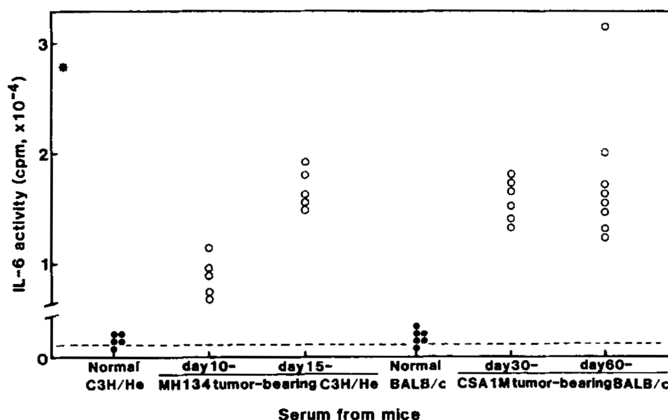

Figure 1. Increased levels of IL-6 activity in sera from tumor-bearing mice. Serum samples were obtained from normal (●) or the indicated stages of tumor-bearing mice (O). $^3H[TdR]$ uptake by 2×10^3 7TD1 cells (IL-6 activity) in the presence of these samples at the final concentration of 6% each serum was individually measured and plotted. *Dotted line* indicates background $^3H[TdR]$ uptake of 7TD1 cells. Symbol (*) indicates $^3H[TdR]$ uptake of 7TD1 cells stimulated with 3 U/well of rIL-6.

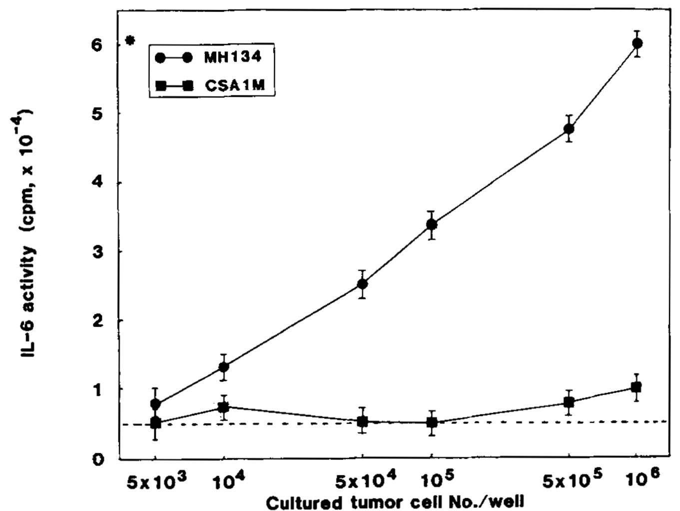

Figure 2. IL-6 activity in unstimulated culture supernatant of various tumor cells. Various numbers of MH134 or CSA1M tumor cells were cultured in 24-well culture plate for 1 day. Culture supernatant was tested for IL-6 activity at the final concentration of 25% with the use of PIL-6 cells. *Dotted line* indicates background $^3H[TdR]$ uptake of PIL-6 cells. Symbol (*) indicates $^3H[TdR]$ uptake of PIL-6 cells stimulated with 3 U/well of rIL-6.

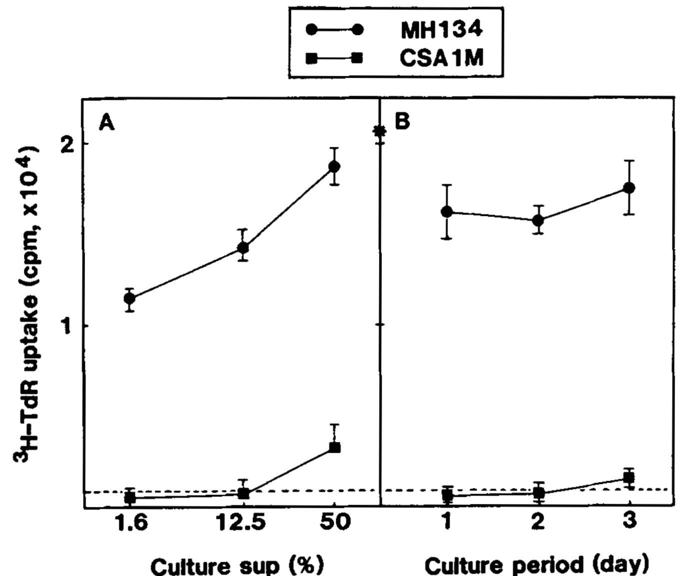

Figure 3. Differential abilities of various tumor cells to produce IL-6. (*A*): Culture supernatants used in Figure 2 (5×10^5 tumor cells/well) were tested for IL-6 activity at various concentrations (indicated) with the use of 7TD1 cells. (*B*): Culture supernatants of MH134 and CSA1M tumor cells (5×10^5/well) were obtained various days (indicated) after culturing. These supernatants were tested at the final concentration of 25% with the use of 7TD1 cells.

Enhanced production of IL-6 by spleen cells from tumor-bearing mice. The fact that increased levels of circulating IL-6 were observed in tumor-bearing mice irrespective of whether the tumor cells themselves produce high magnitude of IL-6 implied that IL-6 may also be produced by some type(s) of host's cells. To examine this, spleen cells from normal or tumor-bearing mice were cultured without any stimulation (Fig. 4). Culture supernatants from normal C3H/He and BALB/c mice caused an appreciable magnitude of PIL-6 proliferation, especially when cultures were performed at higher den-

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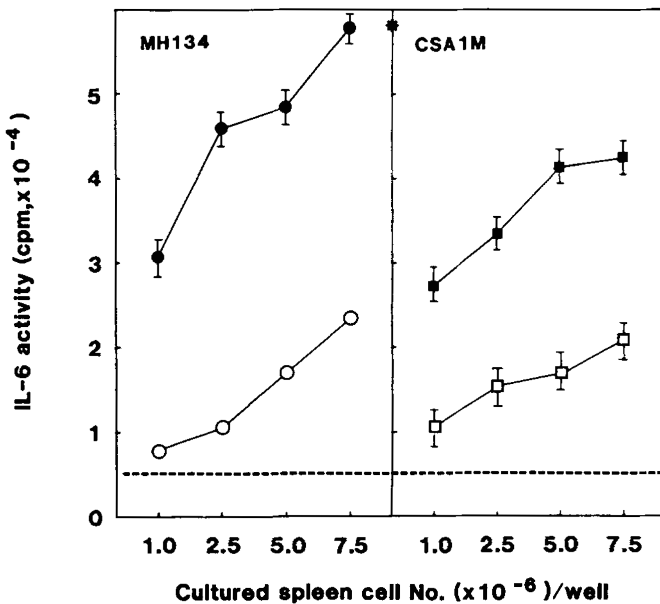

Figure 4. Enhanced production of IL-6 by spleen cells from tumor-bearing mice. Various concentrations (indicated) of spleen cells from normal (O——O) or MH134 or CSA1M tumor-bearing mice (●——●) were cultured in 24-well culture plates without any stimulation. Supernatants collected 1 day later were submitted to IL-6 assay by using PIL-6 cells.

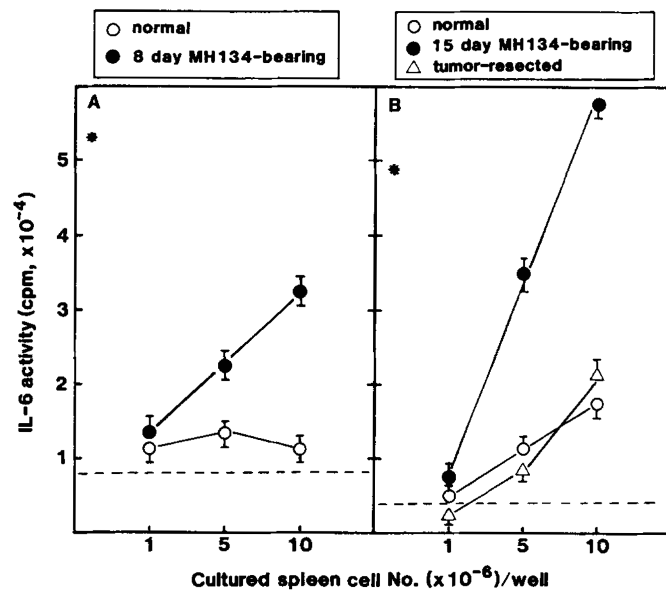

Figure 5. Enhanced IL-6 production depends on the presence of tumor (IL-6-high producer tumor model). C3H/He mice were implanted i.d. with 10^6 MH134 tumor cells, and divided into three groups. Group 1 of mice were used as a source of 8-day tumor-bearing mice (*panel A*). Group 2 of mice were used as 15-day tumor-bearing mice. Group 3 of mice received the resection of their tumor mass at 8-day tumor-bearing stage and used 7 days thereafter together with group 2 of mice (*panel B*). Spleen cells (5 × 10^6/well) from these groups of mice or normal control mice were cultured without stimulation for 1 day. Culture supernatants obtained were tested for IL-6 activity by using PIL-6 cells.

sities of spleen cells. However, strongly enhanced IL-6 production was observed in culture supernatants from tumor-bearing mice. This was the case even at lower densities of cultured spleen cells.

Additional experiments were performed to determine whether enhanced IL-6 production of spleen cells depends on the presence of tumor. The results of Figure 5*A* again illustrate enhanced IL-6 production by spleen cells from 8-day MH134 tumor-bearing mice. Portions of the same batch of MH134 tumor-bearing mice received the resection of the tumor on day 8 of tumor-bearing state, and the rest of animals were left without the tumor resection. Seven days later, spleen cells from either normal mice, the above tumor-resected mice, or 15-day MH134 tumor-bearing mice were cultured for the production of IL-6. The results are shown in Figure 5*B*. Spleen cells from 15-day tumor-bearing mice produced more potent levels of IL-6 than those from 8-day tumor-bearing mice, whereas cells from tumor-resected mice failed to exhibit enhanced IL-6 production, although the magnitude of IL-6 production was almost comparable to that observed by normal spleen cells. Similar patterns of IL-6 production of tumor-bearing and tumor-resected mice were obtained in the CSA1M model (Fig. 6). Thus, these results indicate that spleen cells from tumor-bearing mice produces enhanced levels of IL-6, and that such an enhanced IL-6 production is reversible and disappears after the surgical removal of tumor.

Phenotypes of cells responsible for enhanced production of IL-6 in tumor-bearing mice. We next examined which population(s) of spleen cells is responsible for augmented IL-6 production that is associated with tumor-bearing state. First, spleen cells from normal or MH134 or CSA1M-bearing mice were treated with anti-Thy-1.2 plus C. The resulting Thy-1$^+$ cell-depleted spleen cells were tested for their ability to produce IL-6. The results of Table I demonstrated that the Thy-1$^+$ cell depletion from normal spleen cells induced slight but significant enhancement of IL-6-producing capacity, whereas the same treatment for spleen cells from tumor-bearing mice resulted in a striking decrease of IL-6 production. It should, however, be noted that the capacity of Thy-1$^+$ cell-depleted normal spleen cells to produce IL-6 is almost comparable to the IL-6 producing capacity of Thy-1$^+$ cell-depleted spleen cells from tumor-bearing mice.

We also tested the ability of Thy-1$^+$ cell-enriched spleen cell suspension from normal or tumor-bearing mice to produce IL-6. The results of Figure 7 illustrate that en-

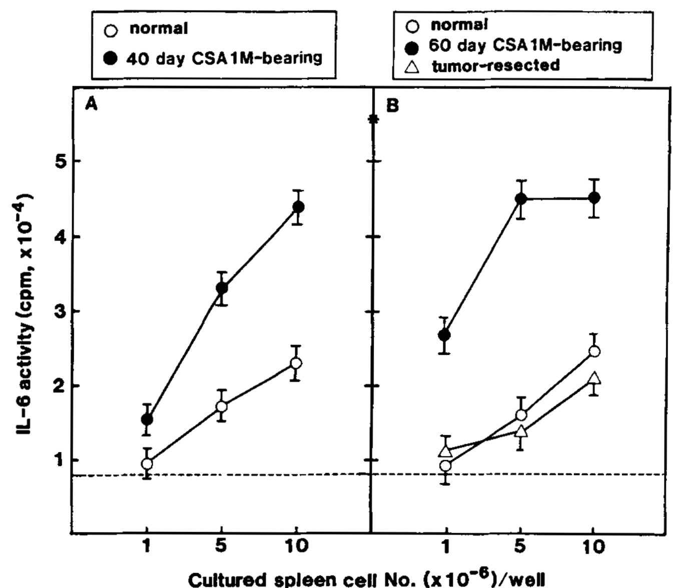

Figure 6. Enhanced IL-6 production depends on the presence of tumor (IL-6-low producer tumor model). BALB/c mice were implanted i.d. with 2 × 10^5 CSA1M tumor cells. Spleen cells from 40-day tumor-bearing mice (*panel A*). 60-day tumor-bearing mice (*panel B*) or mice 20 days after CSA1M tumor resection on day 40 (*panel B*) were cultured, and culture supernatants were tested for IL-6 activity by using PIL-6 cells.

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TABLE I

T cells from tumor-bearing mice are responsible for enhanced IL-6 production

Spleen Cells		IL-6 Activity[a] (cpm, × 10^{-3})	
From mice[b]	Treatment[c]	Expt. 1	Expt. 2
Normal	C	7.6 ± 0.1	7.2 ± 0.7
	anti-Thy-1.2 + C	14.5 ± 1.6	12.3 ± 1.3
Tumor-bearing	C	26.0 ± 1.0	29.6 ± 3.1
	anti-Thy-1.2 + C	11.8 ± 1.3	12.1 ± 1.3

[a] Spleen cells treated (7.5 × 10^6/well) were cultured for 24 h and supernatants (25%) were submitted to IL-6 assay with the use of PIL-6. Background cpm of PIL-6 cells were 5.7 × 10^3 (expt. 1) and 5.0 × 10^3 (expt. 2). ^{3}H[TdR] uptakes (cpm) of PIL-6 cells in the presence of 3 U/well of rIL-6 were 24.6 × 10^3 (expt. 1) and 28.8 × 10^3 (expt. 2).

[b] Normal or 15-day MH134 tumor-bearing C3H/He mice were used.

[c] Spleen cells from normal or tumor-bearing mice were treated with C or anti-Thy1.2 + C.

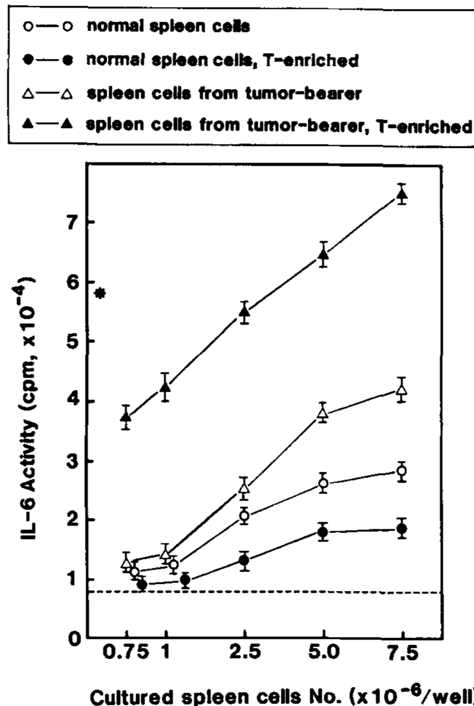

Figure 7. Enhanced IL-6 production by Thy-1$^+$ cell-enriched fraction from tumor-bearing mice. Thy-1$^+$ cell-enriched fraction was obtained by panning spleen cells from normal or tumor-bearing mice on a polystyrene dish coated by goat anti-mouse Ig antibody. The purity of Thy-1$^+$ cells was more than 90% when examined on flow microfluorometric analysis using anti-Thy-1.2 antibody. Culture supernatants obtained from these fractionated or whole spleen cells were tested for IL-6 activity by using PIL-6 cells.

richment of Thy-1$^+$ cells from normal spleen cells reduces the IL-6-producing capacity. In contrast, Thy-1$^+$ cell-enriched splenic population prepared from tumor-bearing mice exhibited appreciably enhanced IL-6 production. Thus, taken collectively with the results of Table I and Figure 7, it is likely that non-T cell populations (presumably splenic macrophages) from both normal and tumor-bearing mice can produce similar levels of IL-6 that are slight but significantly detectable. In contrast, enhanced IL-6 production by spleen cells from tumor-bearing mice is responsible for T cell compartment.

Which subset of T cells is responsible for enhanced IL-6 production in tumor-bearing mice was finally determined. The results of Table II demonstrate that the major capacity to produce IL-6 is ascribed to Lyt-2$^+$ T cell subset in both MH134 and CSA1M tumor systems. Thus, irrespective of whether tumor cells themselves have the potential to produce IL-6, the Lyt-2$^+$ T cell subset from tumor-bearing mice, in general, exhibits enhanced capacity for the production of IL-6.

Inhibition of IL-6 activity by anti-IL-6 mAb. IL-6 activity in sera from tumor-bearing mice, culture supernatants of tumor cells, or culture supernatants from spleen cells of tumor-bearing mice have been assessed with the use of two IL-6-dependent cell lines that have been independently established. To test the specificity of these IL-6-dependent cell lines, we examined whether this activity is blocked by anti-IL-6 mAb. The results of Table III show that the IL-6 activity contained in various samples is reduced to almost background levels in the presence of anti-IL-6 mAb. These results confirm that the activities that have been detected by using IL-6-dependent cell lines are ascribed to IL-6 itself.

DISCUSSION

Although IL-6 (IFN-β_2/B cell-stimulatory factor 2) was identified more than eight years ago, the multiplicity of its biological activities has only recently begun to be recognized (17, 18). New biologic activities obtained for this intriguing cytokine have, therefore, raised the more general question of how IL-6 with its multitude of biologic activities fits into the network of interactive cytokines involved in homeostasis-maintaining mechanisms, including immune systems.

The present study demonstrates that augmented production of IL-6 with pleiotropic biologic functions is induced in tumor-bearing hosts and that its enhanced production is attributable to T cell compartment (predominantly Lyt-2$^+$ T cell subset) in tumor-bearing hosts as well as some but not all types of tumor cells themselves. Thus, taken together with the observations obtained in human rheumatoid arthritis (19) as well as experimentally induced murine arthritis (10), the present results establish the existence of various conditions that are associated with augmented IL-6 production. In addition, our results also add to a growing list of studies that investigate cell types possessing the potential to produce IL-6.

It has been documented that in addition to untransformed normal lymphoid and nonlymphoid cells (4–7, 10, 20–22), a variety of tumor cells possesses the potential to produce IL-6. These include various carcinoma (23–25) and sarcoma cells (25) and lymphoid tumor cells (26). However, it appears that IL-6 is not produced by all types of tumor, but that some of these may fail to produce IL-6 spontaneously, although a possibility of the production in vivo by these tumor cells is not discounted. In contrast, it is noteworthy that T cell compartment is responsible for augmented IL-6 production in hosts bearing tumors with both high and low IL-6-producing phenotypes.

Among lymphoid cells, macrophages/monocytes and T cells are known to produce IL-6 (4, 5, 10, 20). However, macrophages appear to be a major source of IL-6 producer in normal state, as has also been suggested by the results of Table I. In contrast, T cells were responsible for enhanced IL-6 production in tumor-bearing state and this resembles the situations in which T cells produced augmented IL-6 in collagen-induced arthritic mice (10).

A question is raised of how T cells are stimulated for augmented IL-6 production. Several possibilities are feasible. First, it is possible that IL-6 is produced in the course of immune responses to tumor Ag or collagen by

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TABLE II

Phenotype of T cells responsible for enhanced IL-6 production

Splenic T Cells		IL-6 activity[a] (cpm, $\times 10^{-3}$)			
		Expt. 1		Expt. 2	
From Mice[b]	Subset[c]	12.5%	6.3%	12.5%	6.3%
Normal	whole T	5.8 ± 0.2	2.7 ± 0.1	3.1 ± 0.6	2.0 ± 0.2
Tumor-bearing	whole T	25.8 ± 0.3	18.7 ± 1.5	16.9 ± 1.4	14.5 ± 0.9
	L3T4$^+$ T	12.8 ± 0.4	7.3 ± 0.2	6.3 ± 1.0	4.4 ± 0.0
	Lyt-2$^+$ T	22.2 ± 4.7	20.5 ± 3.0	13.6 ± 1.7	10.5 ± 0.5

[a] Culture supernatants were collected 24 h later and indicated concentrations of supernatants were tested for IL-6 activity with the use of 7TD1 cells. Background cpm of 7TD1 were 0.9×10^3 (expt. 1) and 1.1×10^3 (expt. 2). ^{3}H[TdR] uptakes (cpm) of 7TD1 cells in the presence of 3 U/well of rIL-6 were 20.6×10^3 (expt. 1) and 17.6×10^3 (expt. 2).

[b] Fifteen-day MH134 tumor-bearing (expt. 1) or 60-day CSA1M tumor-bearing mice (expt. 2) were used as donors for splenic T cells.

[c] T cells were panned as described and T-enriched fraction was treated with either anti-Lyt-2 or -L3T4 antibody plus C to give the respective L3T4$^+$ or Lyt-2$^+$ T cells subset.

TABLE III

Inhibition of proliferation of PIL-6 cells by anti-murine IL-6 antibody

Samples for IL-6 Assay	^{3}H[TdR]uptake (cpm, $\times 10^{-3}$)[a] in the presence of		
	none	anti-IL-6	anti-IL-4
A. Sera from tumor-bearers[b]	7.8 ± 0.4	0.5 ± 0.0	7.3 ± 0.7
B. MH134 culture supernatant[c]	13.7 ± 1.8	2.8 ± 0.8	12.2 ± 0.4
C. Culture supernatant of spleen cells from tumor-bearers[d]	10.6 ± 2.5	1.2 ± 0.1	11.2 ± 1.4

[a] IL-6 activity was assessed by using 7TD1 cells. Background cpm of 7TD1 cells was 0.9×10^3. Culture supernatant of anti-IL-6 (6B4) or anti-IL-4 (11B11; ref. 40) (as control) mAb-producing hybridoma was added at the final dilution of 25%.

[b] Sera from 15-day MH134 tumor-bearing mice (five mice) were pooled.

[c] Supernatant was obtained from 24-h culture of 5×10^5/well MH134 tumor cells.

[d] Supernatant was obtained from 24-h culture of 5×10^6/well spleen cells from MH134 tumor-bearing mice.

the respective Ag-specific T cells. However, our previous study demonstrated that increased circulating IL-6 was induced by immunization with bovine type II collagen capable of eliciting tissue injury, but not with other immunogenic control protein that generates only ordinary immune responses (10). Moreover, we have found that immunization of C3H/He mice with X-irradiated (attenuated) MH134 tumor cells (10^7/mouse) or with untreated allogeneic (BALB/c) spleen cells (3×10^7/mouse) fail to induce increased levels of IL-6 production in serum (our unpublished observations). These observations may suggest that T cells specific for the corresponding Ag could have the capacity to produce IL-6 but their capacity would be limited compared to enhanced IL-6 producing ability of T cells from tumor-bearing mice.

Alternative mechanisms might be visualized as operating for increased levels of IL-6 synthesis in tumor-bearing mice. In general, it has been established that the induction of IL-6 is regulated by other inflammatory cytokines such as IL-1 or TNF-α (3, 4) as well as bacterial product (LPS) (5, 6). Inasmuch as such inflammatory cytokines are considered to be produced along with significant alterations in host's homeostasis such as infection and tissue injury (27), it is highly possible that tumor-bearing state also induces increased levels of these cytokines. Thus, one or combinations of these may be responsible for producing IL-6 constitutively and in a T cell-clonotype nonspecific way.

It is also of value to consider the phenotype of T cells responsible for augmented IL-6 synthesis. As mentioned above, two different conditions were defined for the detection of enhanced circulating IL-6 levels; i.e., collagen-induced arthritis (10) and tumor-bearing state (this study). It should be noted that there is the fundamental difference in the T cell phenotype producing IL-6 between these two situations. In contrast to the IL-6-producing capacity selective to the L3T4$^+$ T cell subset in arthritic mice, the Lyt-2$^+$ T cell subset was revealed to be predominantly responsible for IL-6 synthesis in the tumor-bearing state. It is unknown why enhanced IL-6 production is attributable to different T cell subsets at distinct situations. However, it appears that both subsets of T cells have essentially the potential for augmented IL-6 production. An earlier study from our laboratory has demonstrated that tumor-bearing state induces L3T4$^+$ T cell-selective immunosuppression (28). Based on this observation, it is tempting to speculate that the potential of L3T4$^+$ T cell subset that is otherwise capable of producing IL-6 is suppressed in the tumor-bearing state.

Thus, it is likely that various types of cells are producing IL-6 to increase its circulating levels in tumor-bearing hosts. These include i) tumor cells (except for IL-6 low or nonproducers), ii) non-T cells (probably macrophages) capable of producing IL-6 to comparable extents to those produced by non-T cells from normal hosts, and iii) T cells especially Lyt-2$^+$ T cells. It has recently been reported that T cells produce leukemia-inhibitory factor that shares some of biologic activities with IL-6 (29). Although anti-IL-6 antibody blocked IL-6 activities contained in tumor as well as spleen cell culture supernatants, more accurate analyses will be required to determine whether the IL-6 activity produced by the above three types of cells is ascribed to a biochemically identical molecule.

Another important aspect is concerned with the effects of increased levels of IL-6 on host's immune reactivity. It is generally accepted that moderate or severe immunosuppression is induced in tumor-bearing state (28, 30–34). It remains unclear how enhanced IL-6 production is related to the immune reactivity in tumor-bearing state. IL-6 produced in tumor-bearing state may function to correct the suppressive immune reactivity through its T and B cell differentiation/proliferation activities (8, 35, 36). Alternatively, this pleiotropic cytokine may be in-

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volved in the generation of immunosuppression through its capacity to stimulate the production in the liver of acute phase proteins (37), some of which are reported to exert an immunosuppressive effect (38). It is also possible that IL-6 further regulates other cytokines that have the potential to affect host's immune responses.

Our results illustrate increased IL-6 production in tumor-bearing mice and demonstrate that T cell compartment, especially Lyt-2^+ T cell subset, is responsible for this enhanced IL-6 synthesis, together with the production of IL-6 by tumor cells themselves in some cases. Our recent study has revealed that increased levels of circulating IL-6 were also found in patients bearing various types of tumor such as hepatoma and osteosarcoma (K. Utsumi, H. Fujiwara, and T. Hamaoka, manuscript in preparation). Therefore, enhanced production of IL-6 in tumor-bearing hosts is not limited to animal tumor models as described here and by others (39), but has clinical relevance. A better understanding of how a large amount of IL-6 produced in tumor-bearing hosts affects host's immune state could contribute to establishing the biologic significance of enhanced IL-6 production as well as to constructing an approach to correct immunosuppression and/or to augment tumor-specific immune responses.

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REFERENCES

1. **Poupart, P., P. Vandenabeele, S. Cayphas, J. V. Snick, G. Haegeman, V. Kruys, W. Fiers, and J. Content.** 1987. B cell growth modulating and differentiating activity of recombinant human 26-kd protein (BSF-2, IFN-β_2, HPGF). *EMBO J. 6:1219.*
2. **Yasukawa, K., T. Hirano, Y. Watanabe, K. Muratani, T. Matsuda, S. Nakai, and T. Kishimoto.** 1987. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. *EMBO J. 6:2939.*
3. **Tatter, S. B., U. Santhanam, A. Ray, D. C. Helfgott, J. Ghrayeb, L. T. May, and P. B. Sehgal.** 1988. Interferon-β_2/B-cell differentiation factor BSF-2/Hepatocyte stimulating factor. *UCLA Symposium on Growth Inhibitory and Cytotoxic Polypeptides.* H. L. Mozes, P. Lengyel, and C. D. Stiles, eds. Alan R. Liss Inc., New York, p. 189.
4. **May, L. T., J. Ghrayeb, U. Santhanam, S. B. Tatter, Z. Sthoeger, D. C. Helfgott, N. Shiorazzi, G. Grieninger, and P. B. Sehgal.** 1988. Synthesis and serection of multiple forms of "β_2-interferon/B-cell differentiation factor BSF-2/hepatocyte stimulating factor" by human fibroblasts and monocytes. *J. Biol. Chem. 263:7760.*
5. **Tosato, G., K. B. Seamon, N. D. Goldman, P. B. Sehgal, L. T. May, G. C. Washington, K. D. Jones, and S. E. Pike.** 1988. Monocyte-derived human B-cell growth factor identified as interferon-β_2 (BSF-2, IL-6). *Science 239:502.*
6. **Helfgott, D. C., L. T. May, Z. Sthoeger, I. Tamm, and P. B. Sehgal.** 1987. Bacterial lipopolysaccharide (endotoxin) enhances expression and secretion of β_2 interferon by human fibroblasts. *J. Exp. Med. 166:1300.*
7. **Sehgal, P. B., D. C. Helfgott, U. Santhanam, S. B. Tatter, R. H. Clarick, J. Ghrayeb, and L. T. May.** 1988. Regulation of the acute phase and immune responses in viral disease: enhanced expression of the "β_2-interferon/hepatocyte-stimulating factor/interleukin-6" gene in virus-infected human fibroblasts. *J. Exp. Med. 167:1951.*
8. **Kishimoto, T., T. Taga, K. Yasukawa, Y. Watanabe, T. Matsuda, K. Nakajima, and T. Hirano.** 1987. Molecular structure and immunological function of human B cell differentiation factor (BSF 2). In *Molecular Basis of Lymphokine Action.* D. R. Webb, C. W. Pierce, and S. Cohen, eds. Humana Press, Clifton, New Jersey, p. 123.
9. **Fong, Y., L. L. Moldawer, M. Marano, H. Wei, S. B. Tatter, R. H. Clarick, U. Santhanam, B. Sherris, L. T. May, P. B. Sehgal, and S. F. Lowry.** 1989. Endotoxemia elicits increased circulating β_2 IFN/IL-6 in man. *J. Immunol. 142:2321.*
10. **Takai, Y., N. Seki, H. Senoh, T. Yokota, F. Lee, T. Hamaoka, and H. Fujiwara.** 1989. Enhanced production of interleukin 6 in mice with type II collagen-induced arthritis. *Arthritis Rheuma. 32:594.*
11. **Sato, H., M. Belkin, and E. Essner.** 1956. Experiments on an ascites hepatomas. III. The conversion of mouse hepatomas into the ascites form. *JNCI 17:1.*
12. **Yoshida, T. O., S. Haraguchi, H. Miyamoto, and T. Matsuo.** 1979. Recognition of RSV-induced tumor cells in syngeneic mice and semi-syngenic reciprocal hybrid mice. *Jpn. J. Cancer Res (Gann) 23:201.*
13. **Vink, A., P. G. Coulie, P. Wauters, R. P. Nordan, and J. Van Snick.** 1988. B cell growth and differentiation activity of interleukin-HP1 and related murine plasmacytoma growth factor: synergy with interleukin 1. *Eur. J. Immunol. 18:607.*
14. **Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev. 74:29.*
15. **Yoshioka, T., S. Sato, M. Ogata, K. Sakamoto, H. Sano, J. Shima, H. Yamamoto, H. Fujiwara, and T. Hamaoka.** 1988. Role of tumor-specific Lyt-2^+ T cell in tumor growth inhibition in vivo. I. Mediation of in vivo tumor-neutralizing activity by Lyt-2^+ as well as L3T4^+ T cell subsets. *Jpn. J. Cancer Res. (Gann) 79:91.*
16. **Mage, M. G., L. L. McHugh, and T. L. Rothstein.** 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods 15:47.*
17. **O'Garra, A., S. Umland, T. De France, and J. Christiansen.** 1988. "B-cell factors" are pleiotropic. *Immunol. Today 9:45.*
18. **Wong, G. G., and S. C. Clark.** 1988. Multiple actions of interleukin 6 within a cytokine network. *Immunol. Today 9:137.*
19. **Hirano, T., T. Matsuda, M. Turner, N. Miyasaka, G. Buchan, B. Tang, K. Sata, M. Shimizu, R. Maini, M. Feldmann, and T. Kishimoto.** 1988. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur. J. Immunol. 18:1797.*
20. **Van Snick, A. Vink, S. Calphas, and C. Uyttenhove.** 1987. Interleukin-HP1, a T cell-derived hybridoma growth factor that supports the in vitro growth of mature plasmacytomas. *J. Exp. Med. 165:641.*
21. **Bazin, R., and R. Lemieux.** 1987. Role of the macrophage-derived hybridoma growth factor in the in vitro and in vivo proliferation of newly formed B cell hydridomas. *J. Immunol. 139:780.*
22. **Rawle, F. C., J. Shields, S. H. Smith, V. Iliescu, M. Merkenschlager, P. C. L. Beverley, and R. E. Callard.** 1986. B cell growth and differentiation induced by supernatants of transformed epithelial cell lines. *Eur. J. Immunol. 16:1017.*
23. **Hirano, T., T. Taga, K. Yasukawa, K. Nakajima, N. Nakano, F. Takatsuki, M. Shimizu, A. Murashima, S. Tsunasawa, F. Sakiyama, and T. Kishimoto.** 1987. Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. *Proc. Natl. Acad. Sci. USA 84:228.*
24. **Kirnbauer, R., A. Köck, T. Schwarz, A. Urbanski, J. Krutmann, W. Borth, D. Damm, G. Shipley, J. C. Ansel, and T. A. Luger.** 1989. IFN-β_2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. *J. Immunol. 142:1922.*
25. **Van Damme, J., G. Opdenakker, R. J. Simpson, M. R. Rubira, S. Cayphas, A. Vink, A. Billian, and J. Van Snick.** 1987. Identification of the human 26-kD protein, interferon β_2 (IFN-β_2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J. Exp. Med. 165:914.*
26. **Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S-I. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, K. Koyama, A. Iwamatsu, T. Taniguchi, and T. Kishimoto.** 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature 324:73.*
27. **Moldamer, L. L., S. F. Lowry, and A. Cerami.** 1988. Cachectin: its impact on metabolism and nutritional status. *Annu. Rev. Nutr. 8:585.*
28. **Tada, T., H. Sano, S. Sato, J. Shima, H. Fujiwara, and T. Hamaoka.** 1990. Immune dysfunction expressed selectively on L3T4^+ T cells in the tumor-bearing state. *J. Leukocyte Biol. 47:149.*
29. **Baumann, H. and G. G. Wong.** 1989. Hepatocyte-stimulating factor III shares structural and functional identity with leukemia-inhibitory factor. *J. Immunol. 143:1163.*
30. **Treves, A. J., C. Carnaud, N. Trainin, M. Feldman, and I. R. Cohen.** 1974. Enhancing T lymphocytes from tumor-bearing mice suppress host resistance to a syngeneic tumor. *Eur. J. Immunol. 4:722.*
31. **Naor, D.** 1979. Suppressor cells: permitters and promoters of malignancy? *Adv. Cancer Res. 29:45.*
32. **Kirchner, H., T. M. Chused, R. B. Herberman, H. T. Holden, and D. H. Lavrin.** 1974. Evidence of suppressor cell activity in spleens of mice bearing primary tumors induced by Moloney sarcoma virus. *J. Exp. Med. 139:1473.*
33. **Kamo, I., and H. Friedman.** 1977. Immunosuppression and the role of suppressive factors in cancer. *Adv. Cancer Res. 25:271.*
34. **Tamerius, J., J. Nepom, I. Hellstrom, and K. E. Hellstrom.** 1976. Tumor-associated blocking factors: isolation from sera of tumor-bearing mice. *J. Immunol. 116:724.*
35. **Garman, R. D., K. A. Jacobs, S. C. Clark, and D. H. Raulet.** 1987. B-cell-stimulatory factor 2 (β_2 interferon) functions as a second signal

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for interleukin 2 production by mature murine T cells. *Proc. Natl. Acad. Sci. USA 84:7629.*
36. **Takai, Y., G. G. Wong, S. C. Clark, S. J. Burakoff, and S. H. Herrmann.** 1988. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. *J. Immunol. 140:508.*
37. **Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Barmann.** 1987. Interferon β_2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl. Acad. Sci. USA 84:7251.*
38. **Petersen, C. M., E. Ejlersen, S. K. Moestrup, P. H. Jensen, O. Sand, and L. Sottrup-Jensen.** 1989. Immunosuppressive properties of electrophoretically "slow" and "fast" form-α_2-macroglobulin: effects on cell-mediated cytotoxicity and (allo-) antigen-induced T cell proliferation. *J. Immunol. 142:629.*
39. **McIntosh, J. K., D. M. Jablons, J. J. Mule, R. P. Nordan, S. Rudikoff, M. T. Lotze, and S. A. Rosenberg.** 1989. In vivo induction of IL-6 by administration of exogenous cytokines and detection of de novo serum levels of IL-6 in tumor-bearing mice. *J. Immunol. 143:162.*
40. **Ohara, J., and W. E. Paul.** 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor 1. *Nature 315:333.*

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