Cancer Immunol Immunother (1993) 36: 101 – 107

Cancer Immunology Immunotherapy

Antitumor effects of recombinant interleukin-6 expressed in eukaryotic cells

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Received 15 May 1992/Accepted 19 August 1992

Abstract. In the present study we evaluate the antitumor efficacy of a glycosylated molecule of interleukin-6 (IL-6), which was cloned and expressed in Chinese hamster ovary cells. When tested with two syngeneic murine tumors, the MC38 adenocarcinoma and the MCA106 fibrosarcoma, recombinant IL-6 (rIL-6) significantly reduced the number of day-3 established MC38 lung metastases, but had no effect on MCA106 lung metastases. A similar effect of rIL-6 was seen on day-3 MC38 liver metastases. The antitumor activity mediated by rIL-6 was achieved at doses of the cytokine ranging from 6 μg to 150 μg/day. There was no correlation between the responsiveness to rIL-6 of these two tumors and their susceptibility, in vitro, to a direct cytostatic effect of the cytokine or the increase in the expression of major histocompatibility complex (MHC) antigens after exposure to rIL-6. However, a correlation was seen between the antitumor response to rIL-6 and the initial number of tumor cells expressing MHC antigens. The possible role of MHC antigens expressed on tumor cells, the generation of MHC-restricted cytotoxic cells and the responsiveness to IL-6 are discussed.

Key words: Immunotherapy – Cytokines – ADCC – Recombinant interleukin-6 – Eukaryotic cells

Introduction

It has been previously demonstrated that certain cytokines, including interleukin-2 (IL-2), interferon (IFN) α and γ, tumor necrosis factor (TNF) and interleukin-1 (IL-1) affect tumor growth by mechanisms such as direct cytostatic effects on tumor cells, expansion and activation of major-histocompatibility-complex(MHC)-restricted and non-restricted cytotoxic cells, increasing the expression of tumor-associated and MHC antigens on tumor cells, and increasing the expression of Fc receptors on cells mediating antibody-dependent cellular cytotoxicity (ADCC) [3–7, 27, 31, 32, 40].

IL-6 consists of secreted phosphoglycoproteins, ranging in size from 20 kDa to 30 kDa and coded on a single gene on chromosome 7 p21 [34, 40], elicits a broad range of immune and acute-phase responses [9, 34] and causes the differentiation of $CD3^+$ cells [11, 14, 17, 25, 37, 40]. Recombinant IL-6, cloned and expressed in *Escherichia coli* [13], mediates antitumor responses in mice with established tumors [14, 20, 21, 23] and the antitumor effects are mediated by $Lyt2^+$, $L3T4^+$ cells [14]. The antitumor effects or rIL-6 could, at least in several tumors, be attributed to its inhibitory effect on tumor growth in vitro and to the ability to cause tumor cell differentiation, either alone or together with other cytokines [1, 2, 18, 26, 38].

In an attempt to produce a molecule that is identical to the natural glycosylated form, the IL-6 gene was cloned and expressed by recombinant DNA techniques in eukaryotic Chinese hamster ovary (CHO) cells [24, 40]. To evaluate the antitumor effects of this molecule we studied its activity both in vitro and in mice bearing established liver and lung metastases.

Materials and methods

Mice. C57BL/6 ($H2^b$), Balb/c ($H2^d$) and C3H/Hen ($H2^k$) mice, 10–16 weeks old, were obtained from the Tel-Aviv University animal facility.

Antibodies. (B10.A × A/J)F1 anti-B10 (anti-$H2^b$) alloserum [33] was kindly supplied by Dr. David Sachs (Transplantation Biology Research Center, Massachusetts General Hospital, Mass.).

Recombinant cytokines. Human rIL-6 (Interpharm Laboratories Ltd. Ness-Ziona, Israel), cloned and expressed by the simian virus 40 vector in cells, was purified to homogeneity from ovary cell supernatant and had an approximate activity of 16×10^6 IU/mg, as determined by a plasmacy-

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toma growth assay. The protein concentration of the stock material was 400 μg/ml buffer (50 mM citric acid, pH 2.5) with an endotoxin level of below 0.1 ng/mg protein (by the Limulus assay test). Human rIL-2 (kindly supplied by Dr. Rosenberg, Surgery Branch, NCI) had a specific activity of 3×10^6 units/mg. The biological and biochemical activities or rIL-2 have been described elsewhere [29].

Tumors. The syngeneic sarcoma MCA106 was induced by 0.1 ml 1% 3-methylcholanthrene in sesame oil injected intramuscularly into C57BL/6 mice [28]. The MC38 colon adenocarcinoma, syngeneic to C57Bl/6 mice, was induced by dimethylhydrazine. Single-cell suspensions were prepared for injection by excising tumors growing subcutaneously in syngeneic mice. For in vitro analysis tumor cells growing in culture were employed.

Preparation of tumor cells. Single-cell suspensions were prepared for injection by excising growing, fresh tumor transplanted subcutaneously into syngeneic mice, mincing the tissue in Hanks balanced salt solution (HBSS), and stirring it in a triple-enzyme mixture (deoxyribonuclease 0.01 mg/ml, collagenase 1 mg/ml and hyaluronidase 0.1 mg/ml) [4]. The dispersed tumor cells were collected, passed through a 100-gauge nylon mesh, and washed three times in HBSS. The cells were then adjusted to the appropriate number for injection into mice.

Isolation of mononuclear cells (MNC) from various organs [5]. Spleens were excised, crushed with the hub of a syringe, passed through a 100-gauge nylon mesh and washed three times after lysis of the erythrocytes by resuspension of the cell pellet in 10% buffered ammonium chloride solution. Livers were excised, minced into 1- to 3-mm fragments and stirred in the triple-enzyme solution as mentioned above. The cells were then transferred to a Lympho-paque gradient (Nyegaard, Norway), centrifuged at room temperature for 20 min and the interphase was collected. The erythrocytes were lysed by resuspending the cell pellet in red blood cell lysing buffer (Sigma). The cells were then washed three times in HBSS and resuspended in complete medium. Peritoneal exudate cells (PEC) were collected by washing the peritoneum cavity with 10–15 ml RPMI, containing 10% fetal calf serum.

Generation of lymphokine-activated killer (LAK) cells. LAK cells were prepared as previously described [5]. Samples containing 2×10^6 splenocytes/ml were placed in a 24-well tissue-culture plate (Corning, N. Y.; 2 ml/well) in complete medium in the presence of various concentrations of cytokines. The plates were incubated for 4–6 days at 37° C in a humidified incubator containing 5% CO_2. The cells were then harvested, washed in complete medium and resuspended to be used as effector cells in the cytotoxicity assay.

^{51}Cr-release assay [5]. B16 melanoma cells, growing in culture were trypsinized, washed and labeled with 100 μCi ^{51}Cr for 1 h. The cells were then washed three times with complete medium before dilution and incubation for 4 h or 18 h with effector cells, at various ratios, in 96-well round-bottom plates. To test antibody-dependent cellular cytotoxicity (ADCC), 10 μl/well anti-H2^b allosera at 1:50 dilution was incubated with the target cells for 20–30 min at 37° C before the addition of effector cells. Spontaneous release of ^{51}Cr was measured after incubation of cells with complete medium only and total release was measured after incubation of cells in 0.1 M HCl.

Spontaneous release (cpm) was less than 20% of maximal release (cpm) for all data presented. The percentage cytotoxicity was determined as follows:

$$\text{lysis (\%)} = \frac{\text{observed release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

Cytotoxicity was expressed in lytic units (LU)/10^7 effector cells. LU_{30} (1LU) was defined as the number of effector cells that caused 30% lysis of 10^4 ^{51}Cr-labeled target cells.

Tumor therapy models [4, 6]. C57BL/6 mice were injected intravenously (to generate lung metastases) or intrasplenically (to generate liver metastases) with tumor cells suspended in HBSS (5×10^5 cells/ml). Three days after tumor injections mice received rIL-6 i. p. for 5 consecutive days and on day 14 mice were sacrificed, their liver and lungs were removed and the metastases were enumerated.

Table 1. Effect of recombinant interleukin-6 (rIL-6) on the growth in culture of several tumor cell lines[a]

Tumor		Total viable cells[b] ($\times 10^{-5}$)		
		Day 0	Day 3	Day 6
MC38	Medium	1	6	10
	rIL-6	1	11	13
MCA106	Medium	1	ND[c]	7
	rIL-6	1	ND	5

[a] Tumor cells were seeded in 24 wells and cultured in complete medium in the presence or absence of 50 000 units/ml rIL-6. After 3 and 6 days in culture, cells were harvested by trypsinization, washed in complete medium and the number of viable cells was determined after staining in 0.2% Trypan blue

[b] Mean of three wells

[c] ND, not determined

Enumeration of metastases. Enumeration of metastases was performed by intratracheal (for lung metastases) or i. v. (for liver metastases) injection of 15% India Ink followed by bleaching of the lungs or livers with Fekette's solution. By employing this procedure, distinct white tumor nodules formed on the blackened surface of the organs could be easily identified [16, 22].

Statistical analysis. Analysis was performed by the Wilcoxon rank sum test [10]. Two-sided P values are presented in all experiments.

Results

Effects of rIL-6 on growth of two murine tumors in culture

Cytokines such as IL-2, TNF and IFNγ can mediate antitumor responses by either exhibiting direct antitumor activity or generating cells with cytotoxic and ADCC activities [7, 12, 15, 27, 31, 32, 39]. To reveal the potential of rIL-6 in mediating these activities, we first analyzed the effect of relatively high concentrations of rIL-6 on tumor cell growth in culture. For this purpose we incubated two antigenically unrelated tumor cells, MC38 and MCA106, with and without 50 000 units/ml rIL-6. After 3 and 6 days in culture the number of viable cells was determined.

As shown in Table 1, rIL-6 caused a reduction (28%) in the number of MCA106 tumor cells after 6 days in culture, when compared to cells incubated in medium alone. In MC38 tumor cells, however, rIL-6 increased the yield of cells after 3 days (83%) and 6 days (30%).

Effects of rIL-6 on the generation of cells mediating ADCC in vitro

It has been previously shown that IL-2 induced, both in vitro and in vivo, cells that mediate ADCC [3–6]. To study the effect of rIL-6 to induce in vitro cells with ADCC activity, splenocytes from Balb/c mice were incubated at

Table 2. Effect of rIL-6 on the generation of antibody-dependent cellular cytotoxicity (ADCC) in vitro[a]

Cytokine	Effector: target ratio	Lysis, SEM (%)	
		No Ab	+Ab (ADCC)
rIL-6	100	2.5	6.2
	20	2.5	4.4
rIL-2	100	37.8	50.6
	20	10.0	19.3
rIL-6+rIL-2	100	23.6	21.0
	20	12.3	7.8

[a] Mononuclear cells, prepared from spleens of BALB/c mice, were cultured at 2×10^6 cells/ml with either 5000 units/ml rIL-6, 1000 units/ml rIL-2 or their combination. After 5 days in culture, cells were collected, washed and served as effector cells in a 4-h ^{51}Cr-release assay against ^{51}Cr-labeled B16 melanoma targets, in the presence or absence of anti-H2^b allosera (10 µl/well). Cell yield after 5 days in culture: rIL-6, 11%; rIL-2, 46%; rIL-6+rIL-2, 27%

2×10^6 cells/ml with 5000 units/ml rIL-6. Cultures of splenocytes incubated in 1000 units/ml IL-2, which produced cells mediating ADCC [5], served as positive controls. After 5–6 days, cells were tested against B16 melanoma tumor targets (H2^b), coated with anti-H2^b serum, which mediate ADCC [3, 5]. As shown in Table 2 rIL-2, as previously shown, induced both LAK activity (direct lysis) and ADCC in the presence of antibodies. rIL-6, on the other hand, did not generate either LAK or ADCC activity and when combined with IL-2, reduced both LAK and ADCC activities and the number of viable cells recovered at the end of the culture.

Effects of rIL-6 on the generation of cells mediating ADCC in vivo

It has been shown that, in vivo, IL-2 generates cells that mediate ADCC [7, 8]. These cells were induced in the injection site, namely in the peritoneal cavity, and systemically in various organs including the liver, spleen and lungs. To test the ability of rIL-6 to generate cells mediating ADCC when injected alone or together with IL-2, mice were injected i.p. three times daily for 3 consecutive days with 50 µg rIL-6, 100000 units IL-2, or their combination. On day 4, mice were sacrificed and the mononuclear cells, from both the peritoneal cavity and liver, were harvested as described in Materials and methods. As shown in Table 3, rIL-6, similar to the effect in vitro, did not generate LAK and ADCC activity either in the peritoneal exudate or liver MNC. IL-2, as previously shown, generated these activities and the addition of rIL-6 to IL-2 did not increase the generation of LAK and ADCC activities when compared to IL-2 alone, but increased the number of MNC in the liver.

rIL-6 reduces the number of established MC38 lung metastases

rIL-6 has been shown to act as a differentiation factor in MHC-restricted CD3$^+$ cells [14, 25]. Since CD3$^+$, specific cytotoxic cells (TIL) were isolated from MC38 tumors [31, 36], we studied the effect of rIL-6 on established lung metastases of this tumor. For this purpose we injected C57BL/6 mice i.v. with 3×10^5 cells followed 3 days later by rIL-6 at various doses, given three times daily for 5 consecutive days. Mice in the control group received HBSS according to the same time schedule as the rIL-6-injected mice. On day 14 mice were sacrificed, the lungs removed and the metastases enumerated. As illustrated in Table 4, rIL-6 at 2–50 µg significantly reduced the number of MC38 lung metastases, whereas rIL-6 at 1 µg/injection had no statistically significant effect.

Table 3. Effect of rIL-6 on the generation of ADCC in vivo[a]

Treatment	Lysis (%)			Cytotoxicity (LU/10^7 effectors)	
	E:T	No Ab	+Ab	No Ab	+Ab
Expt. 1					
rIL-6	20	0.0	–1.9	<1.0	7.1
rIL-2	20	31.8	55.9	54.0	192.3
rIL-6+IL-2	20	29.8	53.8	50.0	190.0
Expt. 2					
HBSS	20	12.8	22.4	11.9	20.0
rIL-6	20	2.8	16.6	2.0	25.0
rIL-2	20	16.8	46.7	26.3	100.0
rIL-6+IL-2	20	17.7	32.3	18.5	55.5

[a] C3H mice (three or four in each group) were injected i.p. three times daily for 4 consecutive days with either 50 µg rIL-6 (700000 units), 100000 units rIL-2 or their combination. Peritoneal exudate cells (PEC; Expt. 1) were collected from the peritoneal cavity in RPMI medium containing 10% fetal calf serum, whereas liver mononuclear cells (MNC; Expt. 2) were prepared by enzymatic digestion as described in Materials and methods. The cells were then washed and tested against ^{51}Cr-labeled B16 melanoma targets, in an 18-h cytotoxicity assay. PEC yield after 4 days of cytokine treatment: rIL-6, rIL-2, rIL-6+rIL-2: 0.8×10^7, 1.2×10^7, 1.3×10^7 respectively. Liver MNC: Hanks balanced salt solution, rIL-6, rIL-2, rIL-6+rIL-2: 1.5×10^7, 1.0×10^7, 2.7×10^7, 5.6×10^7 respectively

Table 4. Effect of rIL-6 on day –3 MC38 lung metastases[a]

Expt.	rIL-6 (µg)	Mean number of metastases (SEM)	*P*
1	HBSS	229 (25.2)	
	50	17 (6.2)	<0.05[b]
2	HBSS	183 (1.3)	
	50	12 (4.7)	<0.05
	10	41 (14.6)	<0.05
	2	52 (27.5)	<0.05
3	HBSS	42 (4.6)	
	25	8 (3.3)	<0.05
	5	16 (6.4)	<0.05
	1	30 (9.2)	NS[c]

[a] C57Bl/6 mice (five or six mice in each group) were injected with 3×10^5 freshly prepared MC38 tumor cells. After 3 days, mice were injected i.p. three times daily, for 6 consecutive days, with either Hanks balanced salt solution (HBSS) or rIL-6, at various concentrations. On day 14 mice were sacrificed, the lungs removed and the metastases enumerated

[b] P compared with the HBSS control group

[c] NS, statistically not significant

Table 5. Effect of rIL-6 on established MC38 liver metastases[a]

Expt.	Treatment: rIL-6 (μg)	Mean number of metastases (SEM)	*P*
1	HBSS	>250 (0)	
	50	< 1 (0.9)	<0.01
2	HBSS	226 (44.5)	
	5	150 (54.5)	NS
	25	20 (12.3)	<0.05

[a] C57Bl/6 mice were injected intrasplenically with 3×10^5 freshly prepared B16 tumor cells. After 3 days mice were injected i.p. three times daily for 5 consecutive days with either HBSS or various doses of rIL-6. On day 14, mice were sacrificed and the metastases enumerated

Table 6. rIL-6 does not affect day –3 MCA106 lung metastases[a]

Expt.	rIL-6 (μg)	Mean number of metastases (SEM)	*P*
1	HBSS (control)	>250	
	30	>250	NS[b]
2	HBSS	>250	
	50	>250	NS
	5	>250	NS

[a] C57BL/6 mice (five or six mice in each group) were injected with 5×10^5 freshly prepared MCA106 tumor cells. After 3 days, mice were injected i.p. three times daily for 6 consecutive days, with either HBSS or rIL-6. On day 14, mice were sacrificed, the lungs removed and the matastases enumerated

[b] NS, statistically not significant

rIL-6 reduces the number of established MC38 liver metastases

The effect of rIL-6 on established liver metastases was also studied. Mice were injected intrasplenically with 5×10^5 freshly prepared MC38 tumor cells to induce liver metastases. On day 3 after tumor injection, mice were injected i.p. with rIL-6 at different doses, given three times daily for 5 consecutive days. On day 14, mice were sacrificed, the liver removed and the number of metastases enumerated. As demonstrated in Table 5, injection of rIL-6 at 25–50 μg/injection reduced the number of metastases by more than 90%, whereas 5 μg rIL-6 had no effect.

rIL-6 does not affect MCA106 lung metastases

As shown in Table 1, the incubation of MCA106 cells together with rIL-6 inhibited tumor cell growth by 28% after 6 days in culture, in contrast to the effect seen on MC38 cells. It was thus expected that mice bearing established MCA106 tumors would respond to a greater extent to rIL-6 than mice that bore the MC38 tumors. To study this possibility C57BL/6 mice were injected with 5×10^5 tumor cells; this was followed by i.p. injection of 30–50 μg/injection rIL-6, given three times daily for 5 consecutive days. On day 14, mice were sacrificed, the lungs removed and the metastases enumerated. As shown in Table 6, rIL-6 at doses of 30–50 μg/injection had no effect on the number of MCA106 lung metastases.

Table 7. Effect of rIL-6 on the expression of major histocompatibility complex molecules on MC38 and MC106 tumor cells[a]

Tumor		Positive cells (%)	Mean fluorescent channel
MC38	Medium	87.1	132.5
	rIL-6	96.1	126.6
MCA106	Medium	37.8	130.5
	rIL-6	31.8	129.8

[a] MC38 and MCA106 tumor cells, cultured in vitro in the presence or absence of 5000 units/ml rIL-6, were harvested by trypsinization and incubated with 1 : 10 dilution of the anti-$H2^b$ serum. A second fluorescein-isothiocynate-labeled goat anti-[mouse $F(ab)_2'$] antibody was then added for 45 min before washing and analysis on a flow cytometer (FACSCAN 440)

Expression of MHC molecules on MC38 and MCA106 tumor cells

As shown in previous experiments, injections of rIL-6 affected established MC38 tumors but had no effect on MCA106 lung metastases. To study whether rIL-6 affected MC38 tumors by increasing the expression of MHC antigens on these cells, thus inducing MHC-restricted cytotoxic cells with antitumor activity, we incubated the MC38 and MCA106 tumor cells with 5000 units/ml rIL-6 for 5 days. The cells were then washed twice in complete medium, incubated for 45 min in ice with anti-($H2^b$) allosera and then incubated under the same conditions with fluorescein-isothiocyanate-labeled goat anti-[mouse $F(ab)_2'$] antibody. As illustrated in Table 7 and Fig. 1, the initial number of cells expressing MHC (class I+II) antigens was substantially lower in MCA106 than in MC38 tumor cells. After incubation with rIL-6, there was no effect on the density of MHC antigens (mean fluorescent channel) on both types of tumor cell, whereas the number of cells expressing MHC antigens increased slightly in the MC38 but not in the MCA106 tumor cell population.

Discussion

In the present study we demonstrate findings on the antitumor activity of recombinant IL-6 (rIL-6), expressed in eukaryotic cells [24]. In mice bearing day-3 MC38 lung metastases, the injection of 50 μg/injection rIL-6 caused a significant reduction of more than 90% in the number of lung metastases (Table 4). The antitumor effect of rIL-6 on established MC38 lung metastases was observed even at 2 μg/injection of the cytokine, and caused a 72% reduction in the number of metastases. Similarly, rIL-6 at 50 μg and 25 μg/injection caused a significant reduction of 99% and 88% in the number of MC38 liver metastases respectively (Table 5). It should be emphasized that, in the immunotherapy experiments, the survival of tumor-bearing mice that were treated with IL-6 was not scored since we

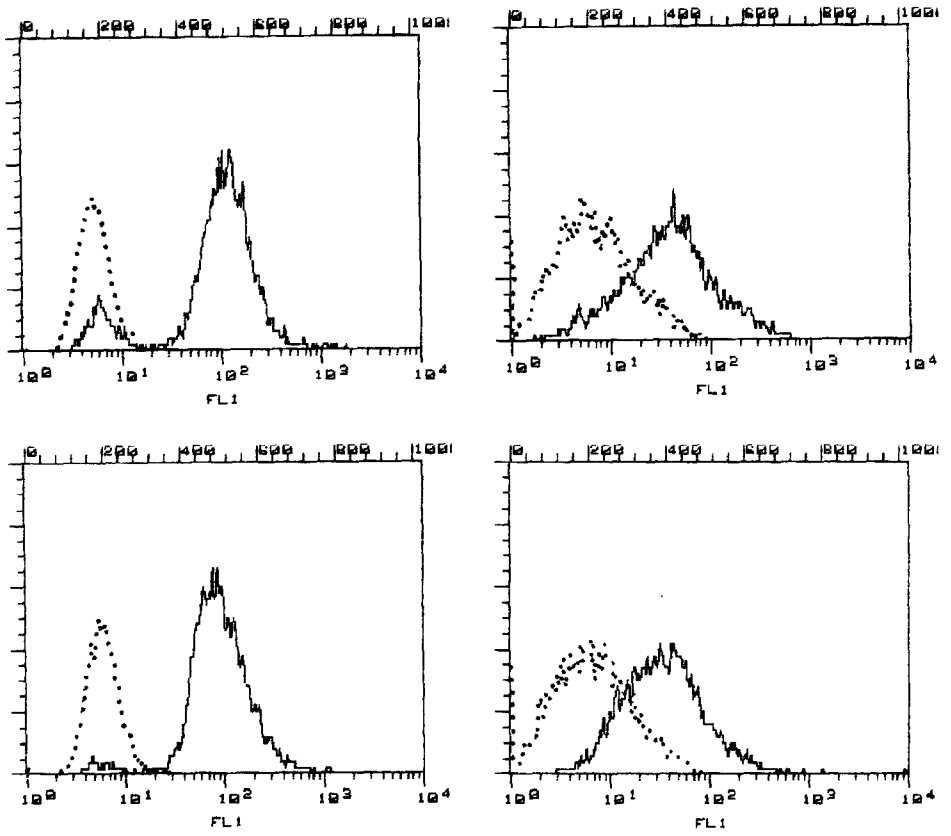

Fig. 1. Expression of major histocompatibility complex (MHC) molecules on MC38 and MCA106 tumor cells incubated with or without interleukin-6 (IL-6). MC38, MCA106 tumor cells, incubated for 5 days with or without 5000 units IL-6, were tested for the expression of MHC molecules. *Upper left:* MC38 in complete medium; *bottom left:* MC38 with IL-6; *upper right:* MCA106 in complete medium only; *bottom right:* MCA106 with IL-6. ..., Cells labeled with second fluoroscein-isothiocyanate-labeled goat anti-[mouse $F(ab)_2'$] only

have rarely seen mice free of metastases, and thus no effect on the survival could have been expected. In all the experiments, no toxicity was observed in mice receiving rIL-6 even at the highest doses (50 μg). Similar results were obtained by others by employing rIL-6 expressed in *E. coli* when 10–50 μg/injection of the cytokine was employed for solid tumors and Friend-virus-induced erythroleukemia in mice [14, 23]. In contrast to the effect on MC38 established metastases, no effect was seen when rIL-6 was injected into mice bearing day-3 MCA106 lung metastases (Table 6), even at doses of 50 μg.

To elucidate the reasons for the differences observed in the response of these tumors to rIL-6, we tested the direct antitumor effect of rIL-6 in vitro, since in several studies this effect was observed on selected tumor cells [1, 2, 18, 26, 38]. Our results demonstrate that the incubation of tumor cells in the presence of high concentrations of rIL-6 (50000 units/ml), for up to 6 days, had a different effect on MC38 and MCA106 tumors. While IL-6 increased the number of viable MNC in cultures by 80% and 30% on days 3 and 6 respectively, a reduction of 28% in the number of MNC was seen in MCA106 tumor cells when compared to cells not incubated with rIL-6 (Table 1). These results exclude the possibility that a direct antitumor effect caused the regression of established metastases. Since IL-6 was shown to cause the differentiation of MHC-restricted cytotoxic cells [11, 14, 17, 25, 37, 40], we assumed that the distinct responsiveness of the two tumors to rIL-6 related to differences in the expression of MHC molecules. Our results confirmed this possibility in experiments where cultured tumor cells, from which normal infiltrate cells were excluded in multiple in vitro passages, were employed. We show that the majority of MC38 tumor cells (87%) expressed MHC molecules (Table 7, Fig. 1), while only 37% of the MCA106 cells expressed these molecules. Since there was a difference in the number of cells expressing MHC molecules on the two murine tumors, and rIL-6 was shown to up-regulate specific surface markers on tumor cells, including MHC molecules [2, 21], we further analyzed the effect of IL-6 on these molecules expressed on both types of tumor cell. Our results demonstrate that rIL-6 did not alter the density of MHC molecules, revealed by the mean fluorescent channel on both MC38 and MCA106 tumors, and had a minor effect on the number of cells expressing these molecules (Table 7). Although not completely excluded, it seems unlikely that IL-6 in vivo acted by increasing the expression of MHC antigens on tumor cells. This assumption is based on the inability of IL-6 in vitro to cause such an effect on tumor cells even at concentrations (5000 units/ml for 5 days, Table 7) higher than those expected in the serum of mice treated with IL-6, at doses that caused antitumor responses [23]. These results suggest that the antitumor effect of rIL-6 on MC38 tumor is not mediated via the up-regulation of MHC antigens on these cells and confirms previous observations that rIL-6 may have different effects on various tumor cells. Further studies to reveal the mechanism by which rIL-6 causes the regression of established MC38 tumors are currently being conducted. These studies include histological examination of tumors as well as analysis of phenotypic markers and cytotoxic activity of cells isolated from mice treated with IL-6.

Another question that was addressed in the present study was the effect of rIL-6 on the induction of cells mediating ADCC. It has been previously shown that IL-2 generated both MHC-restricted CD3+ cells [31] isolated from the tumor (TIL), and non-MHC-restricted LAK cells and cells mediating ADCC [3, 32].

The induction of ADCC by IL-2 could be further augmented, both in vitro [3] and in vivo [6], by the addition of other cytokines including tumor necrosis factor, IL-1 and IFNα and IFNγ. When rIL-6 was tested for the ability to induce LAK and ADCC activities no effect was observed either in culture (Table 2) or when it was administered to mice (Table 3). Furthermore, the addition of rIL-6 to IL-2 did not increase the induction of LAK and ADCC acitvities mediated by IL-2, either in vitro or in vivo (Tables 2, 3). Since LAK and cells mediating ADCC act in a non-MHC-restricted fashion [35], our results, similar to these of others [8], indicate that IL-6 does not generate non-MHC-restricted cells.

Finally, from the effects of rIL-6 on two murine tumors, it can be concluded that rIL-6 may act differently on various tumors. The reasons for these differences are not conclusive but it is suggested, at least from our findings, that the responsiveness to rIL-6 might correlate with the number of tumor cells expressing MHC antigens. If such a correlation is found for a larger number of tumors it may serve as an indicator to predict the antitumor efficacy of rIL-6 for a given tumor. Studies to reveal the correlation between IL-6 responsiveness and the expression of MHC molecules on other tumors are also currently being conducted.

Acknowledgements. The collaboration of Prof. Michel Revel, Department of Virology, The Weizman Institute of Science, Rehovot, Israel and of Yael Koren, Interpharm Ltd, Nes-Ziona, Israel, is gratefully acknowledged.

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