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Indomethacin up-regulates the generation of lymphokine-activated killer-cell activity and antibody-dependent cellular cytotoxicity mediated by interleukin-2

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Summary. Prostaglandins can inhibit the generation of lymphokine-activated killer (LAK) cells by interleukin-2 (IL-2) whereas indomethacin augmented the induction of LAK cells by inhibiting prostaglandin synthesis. In the present study we demonstrate that prostaglandin E_2 substantially inhibited the generation of both LAK and antibody-dependent cellular cytotoxicity (ADCC) activity by IL-2. In addition, indomethacin enhanced the induction of LAK activity and ADCC in splenocytes exposed to IL-2 in vitro. The effect of indomethacin was dose-dependent, reaching an optimal effect at 1 μM when 100–1000 units/ml IL-2 were employed. The effect of indomethacin on the generation of ADCC was seen in cells taken from both tumor-bearing mice and normal mice. ADCC induced by IL-2 was augmented by culturing cells from the spleen, liver and lungs, in the presence of indomethacin. ADCC induced in the presence of IL-2 and indomethacin was mediated by cells that were mainly plastic non-adherent cells and expressed the asialo-GM1 glycolipid. The potential of indomethacin in combined therapy with cytokines and specific anti-tumor monoclonal antibodies is discussed.

Introduction

Prostaglandins, which are secreted by macrophages [10, 20], can modulate several immune responses including the inhibition of antibody formation, the proliferative response to mitogens, tumor-induced myelopoiesis, granulocyte-macrophage colony-forming unit proliferation and the production of interleukin-2 (IL-2) and IL-2 receptors [7, 9, 31, 25, 23, 18, 34, 22, 16, 14]. In addition, prostaglandins modulate the function of allospecific killer cells, macrophages and lymphocytes as well as natural killer (NK) activity [17, 8, 15, 29, 1]. Indomethacin, a cyclo-oxygenase inhibitor, may augment several immune responses by decreasing the level of prostaglandin production [6]. In addition, tumor-bearing mice, that exhibited reduced NK activity due to the secretion of prostaglandins by macrophages, increased their NK activity following the injection of indomethacin [6, 19]. Furthermore, treatment of mice bearing B16 melanoma lung metastases with IL-2 and indomethacin augmented the generation of LAK activity [13, 19].

We have previously demonstrated that the incubation of murine cells in the presence of IL-2 induced antibody-dependent cellular cytotoxicity, (ADCC) activity [28] mediated by non-adherent FcR^+, $ASGM1^+$, $Thy1^-$ cell populations, and were thus closely related to NK/lymphokine-activated killer (LAK) cells [5]. In the present study we tested the effect of indomethacin on the generation of LAK and ADCC activities in the presence of IL-2. We found that indomethacin augmented the induction of LAK and ADCC activities mediated by IL-2 and that this effect was mediated by asialo-$GM1^+$ ($ASGM1^+$) cells that were closely related to the NK/LAK cell population.

Materials and methods

Animals. Female (C57BL/6 × C3H/Hen) F_1 mice were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Md, and were used when they were 8–16 weeks old.

Tumors. Weakly immunogenic [3] B16 melanoma tumor cells syngeneic to C57BL/6 mice were obtained from Dr. A. Ovejera of Frederick Cancer Research Center, Frederick, Md, and were serially transplated s.c. in syngeneic mice. The EL4 lymphoma syngeneic to C57BL/6 mice were obtained in vivo as ascites tumor.

Antibodies. (B10.A × A/J) F_1 anti-B10 (anti-$H2^b$) alloserum was produced as described elsewhere [27] and was kindly supplied by Dr. David Sachs (Immunology Branch, National Cancer Institute). Rabbit antisera against asialo-GM1 was purchased from Wako Chemicals (Dallas, Tex) and screened to select antisera capable of in vitro elimination of NK splenic activity without affecting the ability to generate allocytotoxic T lymphocytes from splenocytes [5].

Cytokines. Human IL-2 was kindly supplied by Cetus Corp. (Emeryville, Calif). The biological and biochemical activities of IL-2 have been de-

Table 1. Prostaglandin E_2 (PGE_2) inhibits the generation of LAK activity and ADCC mediated by IL-2[a]

Expt.	PGE_2 (M)	IL-2	Cytotoxicity (LU/10^7 effectors)			
			No Ab	(LAK)	+AB	(ADCC)
1	–	+	23.2		106.3	
	10^{-5}	+	10.0	(57)	20.0	(81)[b]
	10^{-7}	+	20.0	(13)	34.4	(68)
	10^{-9}	+	23.0	(0)	74.0	(30)
2	–	+	62.5		185.1	
	10^{-5}	+	11.6	(82)	58.8	(69)
	10^{-7}	+	27.7	(56)	142.8	(24)
	10^{-9}	+	50.0	(19)	250.0	(–35)

[a] A sample of 2×10^6 splenocytes from mice bearing day – 16–20 B16 melanoma lung metastases was cultured for 3 days in the presence of 1000 U/ml IL-2 with or without various concentrations of PGE_2

[b] The percentage reduction was calculated from $100 \times$ [1–(cytotoxicity in presence of PGE_2)/(cytotoxicity in absence of PGE_2]

scribed [26]. Purified material had a specific activity of 3.6×10^6 U/mg. In our proliferation assays, 1 U Cetus rIL-2 is equivalent to 2 U Biological Response Modifier Program standard. The endotoxin level in the purified preparation was less than 0.1 ng/10^6 U IL-2, measured by a standard *Limulus* assay.

Indomethacin. (Sigma cat. no. I-7378). A 20 mM solution in absolute ethanol was prepared and kept in the dark at 4° C. Before use, the indomethacin solution was diluted at least 200-fold (100 µM) in complete medium.

PGE_2. (Sigma cat. no. P-5640). A 2 mM solution of prostaglandin E_2 (PGE_2) in absolute ethanol was prepared and kept in the dark at 4° C until use.

Isolation of cells from various organs. Liver and lungs were excised, minced into 1–3-mm fragments and stirred in the triple-enzyme mixture described elsewhere [28] for 2–3 h. The cells were then transferred to a lympholyte M gradient, centrifuged at room temperature for 20 min and the interphase was collected; the erythrocytes were then lysed by resuspending the cell pellet in 10% buffered ammonium chloride solution (NIH Media Section). The cells were then washed three times in Hanks balanced salts solution and resuspended in complete medium. Spleen and thymus were excised, crushed with the hub of a syringe, passed through 100-gauge nylon mesh (Nitex), and washed three times after the erythrocytes had been lysed as described above.

^{51}Cr-release assay of cytotoxicity. Fresh EL4 tumor cells were labeled in ^{51}Cr (New England Nuclear, Boston, Mass) for 1 h at 37° C, washed three times with complete medium, and recounted before dilution and incubation in triplicate with effector cells at varying ratios in 96-well round-bottom plates (Costar, Cambridge, Mass), as previously described [5]. To test for ADCC 10 µl/well of the alloantisera at the appropriate dilution was incubated with the target cells for 20–30 min in 37° C before the addition of effector cells. The optimal concentrations of alloantisera to achieve maximal target lysis were determined in titration experiments (between 1/50 and 1/100 for all antisera) [5].

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. Plates were harvested as previously described [5] and radioactivity was counted. Spontaneous release was less than 35% (usually ranging from 15% to 25%) of maximal release in all data presented. The percentage cytotoxicity was determined by:

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

where ^{51}Cr release was measured in cpm.

Cytotoxicity was expressed in LU/10^7 effector cells, 1 LU being defined as the number of effector cells that caused 30% lysis of 10^4 Cr-labeled target cells.

Generation of LAK cells. LAK cells were prepared as previously described [5]. In brief, 2×10^6 splenocytes/ml were placed in either a 175-cm^2 flask (Falcon) or a 24-well tissue-culture plate (Costar, Cambridge, Mass; 2 ml/well) in complete medium together with 1000 U/ml IL-2. The plates were incubated for 3 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 a 4-h chromium-release assay.

Induction of subcutaneous (s.c.) and lung tumors. Subcutaneous tumor was induced by injecting $(1-5) \times 10^6$ tumor cells subcutaneously into the hind limb of F1 mice. After approximately 14 days the tumor reached 1 cm in diameter. Lung metastases were induced by injecting $(1-2) \times 10^5$ B16 melanoma cells into the tail vein. After 14–20 days there were more than 250 macrometastases in the lungs.

Enrichment for non-adherent cells. A sample of 2×10^7 splenocytes in 10 ml complete medium was placed in a 100-mm petri dish (Falcon 3003) and incubated for 45 min at 37° C in a humidified incubator containing 5% CO_2. The non-adherent cell were then collected, washed and resuspended in complete medium.

ASGM1 depletion. A total of 4×10^7 fresh splenocytes, prepared as previously described, were incubated with 1 ml anti-ASGM1 at 1:50 dilution. After 45 min on ice, cells were washed once in cold RPMI-1640 (Biofluids Inc., Rockville, Md) supplemented with 0.3% Pentex bovine albumin (Path-O-Cyte 4, Miles Scientific, Naperville, Ill) and 25 mM HEPES (HEPES buffer, 1 M, Biofluids). Freshly prepared 1:10 diluted low-tox-m rabbit complement (Cederlane Laboratories Limited, Hornby, Ontario, Canada) was then added, and the cells were cultured at 37° C for 20 min. The complement treatment was repeated and cells were then washed twice in the above medium and resuspended in complete medium.

Results

PGE_2 reduces the generation of LAK and ADCC activities by IL-2

Prostaglandins have been shown to down-regulate NK activity and the generation of LAK cells [6, 19, 13]. Because we have previously shown that the induction of murine LAK cells by IL-2 is related to the induction of ADCC activity, we tested the effect of prostaglandins on the generation of ADCC. For this purpose we cultured splenocytes taken from C57BL/6 × C3H/Hen F1 mice in the presence of 1000 U/ml IL-2 [2] and various concentrations of PGE_2. After 3 days the cells were washed and tested for ADCC against EL4 targets coated with 1:100 diluted anti-$H2^b$ allosera. As shown in Table 1, reduction in LAK and ADCC activities, induced by PGE_2, ranged from 57% in expt. 1 to 82% in expt. 2, and from 81% in expt. 1 to 69% in expt. 2, at 10 µM concentration respectively.

Indomethacin enhances the induction of LAK and ADCC activities by IL-2

Indomethacin, which is known as an inhibitor of prostaglandin synthesis, can increase the lytic activity of both NK

Table 2. Indomethacin (IM) enhances the generation of both LAK and ADCC activity by IL-2[a]

Expt.	PG (10 μM)	IM (1 μM)	IL-2 (1000 U/ml)	Cytotoxicity LU/10^7 effectors)		Increase (-fold)[b] in	
				No Ab (LAK)	+Ab (ADCC)	LAK	ADCC
1	–	+	–	<1	<1		
	–	–	+	16	200		
	–	+	+	45	667	2.8	3.3
2	–	+	–	<1	<1		
	–	–	+	62	800		
	–	+	+	111	1190	1.8	1.5
3	–	+	–	2	8		
	–	–	+	12	303		
	–	+	+	34	1000	2.8	3.3
4	–	–	+	12	112		
	–	+	+	49	250	4.1	2.1
5	–	–	+	90	185		
	–	+	+	200	555	2.2	2.8
6	–	–	+	<1	61		
	–	+	+	<1	137	–	2.2
7	–	–	+	9	22		
	–	+	+	18	76	2.0	4.2
8	–	–	+	3	161		
	–	+	+	10	454	2.7	2.8
	+	–	+	<1	80		
	+	+	+	<1	78	–	0.9

[a] A sample of 2×10^6 splenocytes from F1 mice was cultured for 3 days in the presence of indomethacin, IL-2 or the combination. The cells were then washed and tested for LAK activity and ADCC

[b] -Fold increase was calculated from (cytotoxicity in the presence of indomethacin and IL-2)/(cytotoxicity in the presence of IL-2 alone)

Table 3. The effect of indomethacin (IM) and IL-2 on the generation of LAK activity and ADCC in F1 splenocytes taken from normal and tumor-bearing (TB) mice[a]

Expt.	Cell origin	IM (1 μM)	Cytotoxicity (LU/10^7 effectors)		(IM+IL-2)/IL-2[b]	
			No Ab (LAK)	+Ab (ADCC)	LAK	ADCC
1	Normal mice	–	5.2	27.0		
		+	9.5	74.0	1.8	2.7
	TB mice (lung)	–	<1.0	3.0		
		+	3.0	41.6	>3.0	13.8
2	Normal mice	–	3.7	161.2		
		+	10.0	454.5	2.7	2.8
	TB (lung)	–	<1.0	23.8		
		+	47.6	192.3	>47.6	8.3
	TB (sc)	–	<1.0	66.6		
		+	29.4	400.0	>29.4	6.0

[a] F1 mice were injected i. v. with 2×10^5 freshly prepared B16 melanoma cells. After 17 days the splenocytes from the tumor-bearing (B16 lung metastases) as well as from normal F1 mice were isolated and cultured for 3 days in the presence of 1000 U/ml IL-2 and 1 μM indomethacin. The cells were then tested for ADCC in a 4-h ^{51}Cr-release assay against EL4 targets coated with anti-H2[b] allosera. Cell recovery at the end of the culture before assaying for ADCC (expt. 1): cells from normal mice + IL-2, 42% of the initial cell number; IL-2 + IM, 48%; cells from TB mice + IL-2, 46%; IL-2 + IM, 40%

[b] [Cytotoxicity (LU) IM + IL-2]/[cytotoxicity (LU)IL-2]

and LAK cells in splenocytes taken from normal as well as tumor-bearing mice [6, 19]. In addition, indomethacin significantly increases in vivo NK activity in tumor-bearing mice [6]. As PGE_2 substantially inhibited ADCC induced by IL-2 we tested the effect of indomethacin on the generation of ADCC in cells taken from tumor-bearing mice. F1 mice bearing day-16–20 B16 lung metastases were sacrificed, the spleens removed and the splenocytes were cultured in either 1 μM indomethacin, 1000 U/ml IL-2 or the combination. After 3 days the cells were washed and tested for LAK and ADCC activities. As shown in Table 2, which summarizes eight separate experiments, IL-2 as previously shown, induced LAK and ADCC activities in splenocytes. The addition of indomethacin further increased by 1.5–4.2-fold the ADCC generated by IL-2 and also increased lysis in the absence of antibody (LAK activity) in seven out of eight experiments. Moreover, the addition of 10 μM PGE_2 to cultures containing 1 μM indomethacin and IL-2 completely abrogated the effect of indomethacin on both LAK and ADCC activity and even reduced these activities when compared to cultures with IL-2 alone. In addition, as shown in Table 3, indomethacin increased ADCC in

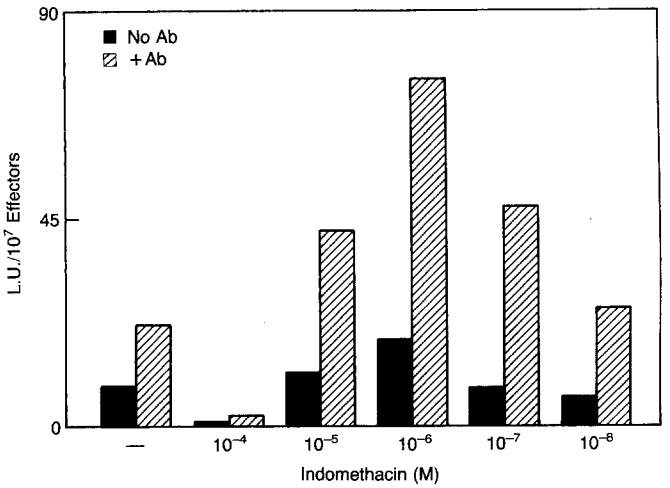

Fig. 1. Dose/response effect of indomethacin on the induction of lymphokine-activated killer (LAK) cells and antibody-dependent cellular cytotoxicity (ADCC) activities in the presence of interleukin-2 (IL-2). Splenocytes isolated from F1 mice bearing day-20 B16 melanoma lung metastases were cultured in the presence of 1000 U/ml IL-2 and different concentrations of indomethacin. After 3 days in culture, the cells were washed and tested for ADCC against anti-$H2^b$-coated EL4 targets in a 4-h ^{51}Cr-release assay. *No Ab*, LAK activity; *+ Ab*, ADCC

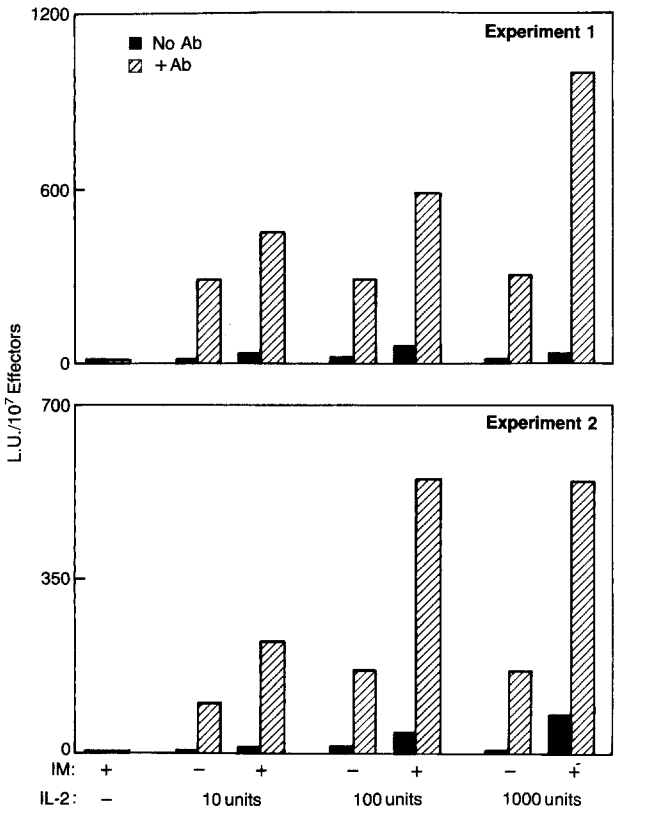

Fig. 2. Dose/response effect of IL-2 on the generation of LAK and ADCC activities in the presence of 1 μM indomethacin. Splenocytes taken from F1 mice with established B16 melanoma lung metastases were cultured in different concentrations of IL-2 in the presence or absence of 1 μM indomethacin. After 3 days in culture the cells were washed and tested for ADCC against EL4 targets coated with anti-$H2^b$ allosera

splenocytes taken from both normal F1 mice and mice with day-17 B16 lung metastases. As the relative effect of indomethacin on ADCC was more prominent in cells isolated from tumor-bearing mice, we selected splenocytes harvested from these mice for our next experiments.

Dose/response effect of indomethacin on the induction of LAK and ADCC activities in the presence of IL-2

Indomethacin was shown to exert its immunoregulatory effect on NK activity at a concentration of 1 μM [18]. To optimize the conditions for the generation of ADCC in the presence of indomethacin, splenocytes taken from F1 mice bearing day-20 B16 melanoma lung metastases were cultured in the presence of 1000 U/ml IL-2 and 0.01 μM–100 μM indomethacin. After 3 days in culture the cells were tested for ADCC against EL4 targets. As illustrated in Fig. 1, cells cultured in IL-2 alone exhibited 9 LU and 20 LU lytic activity in the LAK and ADCC assay, respectively. When indomethacin was added to the cultures, 100 μM completely abrogated ADCC, 0.01 μM had virtually no effect on ADCC, whereas indomethacin at 1 μM caused the highest effect on LAK and ADCC activities.

Dose/response effect of IL-2 on the generation of LAK and ADCC activities in the presence of 1 μM indomethacin

We next studied the effect of indomethacin on the induction of LAK and ADCC activities in the presence of various IL-2 concentrations. Splenocytes from mice with established day-19 B16 melanoma lung metastases were cultured in 10, 100 and 1000 U IL-2 in the presence or absence of 1 μM indomethacin. After 3 days the cells were tested for LAK and ADCC activities against EL4 target cells. As shown in Fig. 2, the highest increase in ADCC in the presence of indomethacin was seen in cells that were cultured in 1000 U/ml IL-2 in experiment 1 and 100, and 1000 U in experiment 2. Indomethacin also increased LAK activity in the presence of 10–1000 U IL-2. We thus selected for our next experiment the combination of indomethacin at 1 μM and 1000 U/ml IL-2, to achieve maximal effect on ADCC.

Induction of LAK and ADCC activities in the presence of indomethacin and IL-2 in mononuclear cells isolated from various organs

We have previously shown that IL-2 generated LAK cells and ADCC in mononuclear cells isolated from various organs. We have also shown that indomethacin increased the induction of LAK and ADCC activities mediated by IL-2 in splenocytes. To test the effect of indomethacin and IL-2 on cells taken from other organs, mononuclear cells were isolated from the lungs, liver, thymus and spleen of F1 mice bearing 1-cm-diameter subcutaneous B16 melanoma tumors. The mononuclear cells were then cultured for 3 days in the presence of 1000 U/ml IL-2 and 1 μM indomethacin and tested for LAK and ADCC activities against EL4 targets. As shown in Table 4, indomethacin alone had no effect in generating LAK or ADCC activity.

Table 4. Generation of LAK activity and ADCC in cells isolated from various organs after culturing in indomethacin and IL-2[a]

Expt.	Organ	Treatment	Cytotoxicity (LU/10^7 effectors)	
			No Ab (LAK)	+Ab (ADCC)
1	Lungs	IM	<1.0	<1.0
		IL-2	75.1	384.6
		IM+IL-2	138.8	689.6
	Spleen	IM	<1.0	<1.0
		IL-2	43.4	571.4
		IM+IL-2	84.0	1000.0
2	Thymus	IL-2	<1.0	20.8
		IM+IL-2	<1.0	23.0
	Lungs	IL-2	<1.0	17.2
		IM+IL-2	7.7	38.5
	Liver	IL-2	40.8	256.4
		IM+IL-2	149.2	632.9
	Spleen	IL-2	10.0	166.7
		IM+IL-2	80.0	550.0
3	Thymus	IL-2	<1	18.5
		IM+IL-2	<1	6.2
	Spleen	IL-2	<1	66.6
		IM+IL-2	29.4	400.0

[a] F1 mice bearing a 1-cm B16 melanoma s. c. tumor were sacrificed, the thymus, lungs, liver and spleen were removed and mononuclear cell were isolated and cultured in 1 μM indomethacin (IM) and 1000 U/ml IL-2. After 3 days in culture the cells were tested for ADCC in a 4-h ^{51}Cr-release assay against EL4 targets coated with anti-H2^b allosera. There was no difference in the number of viable cells recovered between the various cultures after 3 days of incubation

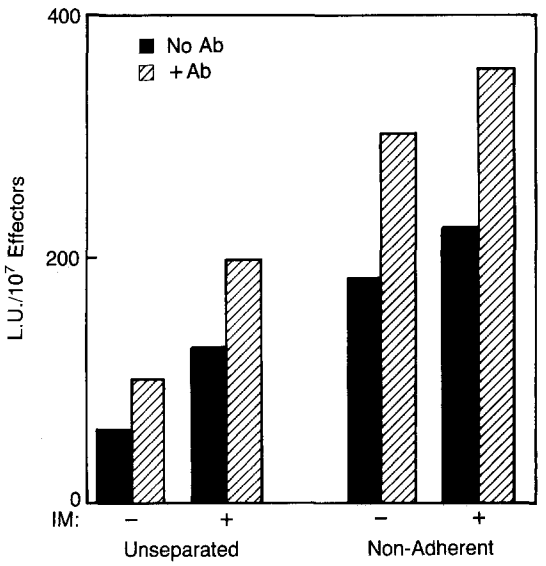

Fig. 3. The precursor of cells mediating LAK and ADCC activities generated in the presence of IL-2 and indomethacin is a non-adherent cell. Splenocytes isolated from F1 mice with established B16 melanoma lung metastases, were incubated at 2×10^7 cells/10 ml complete medium in a 100-mm plastic petri dish. After 45 min the non-adherent cells were collcted and cultured in medium containing 1000 U/ml IL-2 and 1 μM indomethacin. After 3 days the cells were washed and tested for ADCC and LAK activities in the presence or absence of anti-H2^b allosera. Unseparated splenocytes were cultured under the same conditions and served as control cells

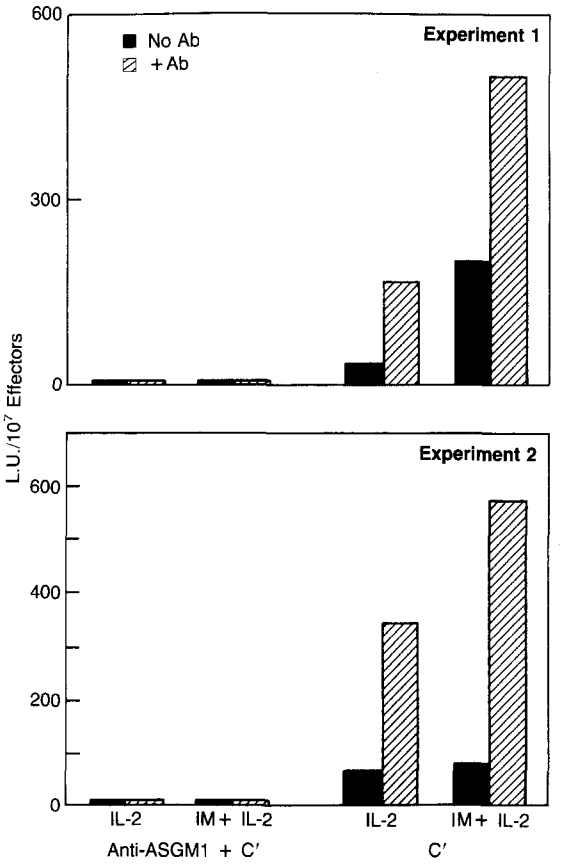

Fig. 4. The precursor of cells mediating LAK and ADCC activities in the presence of IL-2 and indomethacin express the ASGM1 glycolipid. Splenocytes isolated from F1 mice with established B16 lung metastases were incubated with anti-ASGM1 serum followed by two cycles of complement treatment. Control cells were treated with complement (C') alone. The cells were then incubated for 3 days in complete medium containing 1000 U/ml IL-2 and 1 μM indomethacin, washed and tested for LAK and ADCC activities against EL4 targets

Its addition to IL-2, however, showed a relative increase in LAK activity and ADCC in cultures of mononuclear cells taken from the lung, liver and spleen when compared to cells cultured in IL-2 alone. Mononuclear cells isolated from the thymus mediated ADCC after exposure to IL-2, but the addition of indomethacin had no effect (expt. 2) or even reduced (expt. 3) ADCC in thymocytes.

The precursor of the cell mediating LAK activity and ADCC after culture in IL-2 and indomethacin is a non-adherent cell

We have previously shown that the precursor of the cell that mediated ADCC after exposure to IL-2 was a non-adherent cell. To test the nature of cells involved in the generation of ADCC mediated by the combination of IL-2 and indomethacin we enriched the non-adherent spleen cell population after removing the plastic-adherent cells. The non-adherent cells were then cultured in the presence of 1000 U/ml IL-2 and 1 μM indomethacin. After 3 days in culture the cells were tested for ADCC against EL4 targets. As demonstrated in a representative experiment in Fig. 3, 20% of the initial number of cells that were harvested after

plastic adherence exhibited a threefold increase in LAK activity and ADCC when cultured in IL-2 alone. The addition of indomethacin to these cultures further increased LAK activity and ADCC twofold in the unseparated cells but had a relatively limited effect on the non-adherent cells.

The precursor of cell-mediating LAK and ADCC activities after exposure to IL-2 and indomethacin expresses the ASGM1 glycolipid

We have previously shown that the cells mediating LAK and ADCC activities after exposure to IL-2 resided in the ASGM1+ cells [5]. To test the nature of cells mediating ADCC after culturing in IL-2 and indomethacin we treated splenocytes, with anti-ASGM1 serum + complement. The cells were then cultured in the presence of IL-2 and indomethacin for 3 days and tested for ADCC. As shown in Fig. 4, pretreatment with anti-ASGM1 and complement completely abrogated the generation of both LAK and ADCC activity in cells cultured in either IL-2 alone or IL-2 + indomethacin when compared to cells that were pretreated with complement alone.

Discussion

We have previously shown that the incubation of murine cells in the presence of IL-2 generated cell capable of mediating ADCC [28, 5]. In the present study, we obtained similar results to those reported by others for the generation of LAK cells [11], by substantially suppressing LAK activity and ADCC by adding 0.1–10 μM PGE_2 to splenocytes cultured in IL-2. The addition of indomethacin, an inhibitor of prostaglandin synthesis, enhanced IL-2-induced LAK and ADCC activities in murine splenocytes. The effect of indomethacin was optimal when 100–1000 U/ml IL-2 were employed together with 1 μM indomethacin. These concentrations of indomethacin were similar to those reported by others to be optimal in inducing NK activity [6]. Indomethacin at 100 μM had a negative effect on LAK activity and ADCC as it substantially reduced the IL-2-induced ADCC activity (Fig. 1). In addition, indomethacin alone had no effect on the generation of both LAK and ADCC activity, in contrast to its effect on NK activity [33, 24, 21].

One of the major questions concerns the mechanism by which indomethacin augments the IL-2-induced LAK and ADCC activities. It may directly increase the generation of the cells mediating LAK and ADCC activities by inducing IL-2 or IL-2 receptors [19, 30]. This explanation seems less likely since there was no difference in the number of viable cells recovered after incubation in either IL-2 alone or IL-2 plus indomethacin (Table 3). Alternatively as a cyclo-oxygenase inhibitor [6], it could augment the generation of ADCC cells by decreasing the level of prostaglandin production, similar to the results shown by others for the induction of LAK cells [27]. Our results suggest the involvement of a prostaglandin/indomethacin regulation of IL-2-induced LAK activity and ADCC, since indomethacin enhanced both activities (Tables 2, 3) while PGE_2 reduced these activities (Table 1) even in the presence of indomethacin and IL-2 (Table 2). Furthermore, prostaglandins can be secreted by macrophages [20, 10] and these cells could participate in the effect of indomethacin on LAK and ADCC activities. In the presence of indomethacin, increased ADCC was found in cells taken from the spleen (330%) liver (250%) and lungs (220%), all known to contain macrophages [18]. In thymocytes, however, which are relatively poor in macrophages, although IL-2 induced ADCC comparable to lung mononuclear cells, the addition of indomethacin had no further effect on ADCC (Table 4). Furthermore, its effect on ADCC was less prominent in the non-adherent cell population when compared to the results in unseparated splenocytes (Fig. 3), although non-adherent cells exhibited relatively higher ADCC upon exposure to IL-2 (Fig. 3). Similar results were obtained by others [20], who showed that indomethacin augmented human NK activity but had no effect on monocyte-depleted effector cells. These findings suggest that adherent cells, presumably macrophages, may be involved in regulating LAK and ADCC activities by synthesis and secretion of prostaglandins and that indomethacin may negate this effect by inhibiting prostaglandin synthesis. Although indomethacin affected the induction of ADCC in cells from normal, non-tumor-bearing mice (Table 3) and B16 melanoma cells do not secrete prostaglandins [19], these cells may still secrete tumor soluble factors that cause the synthesis of prostaglandins by macrophages [36, 37].

Another question addressed in our study was the nature of cells mediating LAK activity and ADCC after exposure to IL-2 and indomethacin. We, as well as others, have shown that the IL-2-induced cells that mediate ADCC were ASGM1+, non-adherent cells and thus appeared to be related to the NK/LAK cell population [28, 5, 12]. In the present study we showed that the combination of indomethacin and IL-2 generated cells exhibiting ADCC that were non-adherent cells, expressed the ASGM1 glycolipid (Figs. 3, 4) and were closely related to the LAK and ADCC cells induced by IL-2. Although NK cells are closely related to LAK cells and can mediate ADCC, our results suggest that these cells were less likely to participate in the indomethacin-induced ADCC for two reasons. First, indomethacin was shown to augment NK activity without any additional stimulation [33] whereas in our study it had no effect on ADCC when cultured without IL-2 (Table 2). In addition, ADCC in our experiments was tested against NK-resistant EL4 tumor targets, against which no ADCC could be demonstrated using freshly isolated, non-stimulated effector cells [28]. The similarity in the generation of ADCC by IL-2 alone in culture or together with indomethacin, as reflected in IL-2 and indomethacin dose dependence, generation of ADCC activity in various organs and cell characteristics, suggests that these cells, as previously described [5], belong to the FcR+ LAK population. In addition, in most experiments the effect of PGE_2 and indomethacin in the presence of IL-2 was similar in the generation of both LAK and ADCC activity. These results further suggest that the cells mediating ADCC after exposure to indomethacin and IL-2 were closely related to LAK cells. Finally, indomethacin was recently shown to mediate anti-tumor effects against B16 melanoma lung metastases when combined with IL-2 [6, 19] and this effect was medi-

ated by ASGM1+ cells [19, 13]. In our studies, using a different B16 melanoma clone (B16BL6) for the induction of lung metastases, IL-2 given alone had no statistically significant effect on these metastases [4, 35] differing from the results obtained by others [32]. Moreover, even the addition of anti-B16 melanoma mAb to IL-2, which significantly augmented the anti-tumor effect on established B16 liver metastases [3], had no effect on B16 lung metastases [4]. The ability of indomethacin to increase substantially the generation of ADCC induced by IL-2 may be valuable in improving the anti-tumor efficacy of the combined treatment with cytokines and anti-tumor mAb against established lung metastases as well as tumor growing at other anatomical sites.

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