CELLULAR IMMUNOLOGY 90, 190–195 (1985)

Inhibition of Interleukin 2 Production by Prostaglandin E_2 Is Not Absolute but Depends on the Strength of the Stimulating Signal

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Received June 12, 1984; accepted August 5, 1984

In view of the eminently important role of interleukin-2 (IL-2) in T-cell responses, and in view of reports about immune stimulatory effects of PGE_2, we reinvestigated the question whether PGE_2 inhibits IL-2 production. It was found that PGE_2 does not inhibit IL-2 production in murine spleen cell cultures after optimal stimulation (5 μg/ml concanavalin A) but does inhibit at suboptimal stimulation conditions. The failure of PGE_2 to inhibit IL-2 production at optimal concanavalin A concentration was demonstrated by two independent IL-2 assays namely by the co-stimulator assay and by the proliferation of IL-2-dependent T-cell clone W-2. Our observations indicated that the inhibitory effect of PGE_2 depends on the strength of the stimulating signal. IL-2 production in cultures with 5 μg/ml concanavalin A was also *not* suppressed by PGE_1, by prostaglandin D_2, thromboxane B_2 (T × B_2), and prostaglandin F_2.

INTRODUCTION

Prostaglandins of the E series and the lymphokine interleukin 2 (IL-2)[1] play altogether an eminently important role as intercellular mediators in the regulation of T lymphocyte responses. Prostaglandins are produced by macrophages in the course of immunogenical reactions (reviewed in (1, 2)) and are also produced by various types of tumor cells (reviewed in (3)). Patients with Hodgkin's disease have been found to contain prostaglandin-producing suppressor cells which are believed to be responsible for the depressed cellular immunity in these patients (4). It has also been reported that prostaglandins inhibit the rejection of tumors (3, 5); and prostaglandin of the E series, PGE_1 and PGE_2, have been shown to inhibit the activation of cytotoxic T lymphocytes (CTL) (6, 7). Prostaglandins have been shown to inhibit the proliferative response of lymphoid cells including interleukin-2-dependent cytolytic T-cell lines (8–12, reviewed in (1, 13)); and indomethacin and other inhibitors of prostaglandin synthesis have been shown to enhance the induction of cytotoxic responses against allogeneic cells (10). Recently, prostaglandins of the E type have also been reported to inhibit the production of IL-2 in human lymphocyte preparations (12, 14, 15). IL-2 of course, is of central importance for T-cell proliferation and for the generation of CTL responses as reviewed in (16).

[1] Abbreviations: CSCS, concanavalin A-activated spleen cell supernatant; CTL, cytotoxic T Lymphocyte; IL-2, interleukin-2; PG, prostaglandin; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; TCF2, T cell cytotoxicity inducing factor 2; T × B_2, thromboxane B_2.

0008-8749/85 $3.00

Recent reports about PGE_2 induced suppressor factors (17, 18) suggested, however, the possibility that such inhibitory factors might have interfered with the IL-2 assays and might have been responsible for the apparent reduction of the IL-2 titers by PGE_2 in the reported experiments (12, 14, 15). Moreover, in spite of the numerous reports on the suppressive effects of prostaglandins, there are also reports that prostaglandins may augment T-cell reactivity under certain experimental situations. Anti-PGE antibodies have been reported to inhibit the *in vivo* development of several types of T-cell-mediated immune reactions (19). A PGE_2 derivative (diM-PGE_2) was found to increase T-cell-mediated immune repsonses in B16 melanoma-bearing C57BL/6 mice and delayed the development of the tumor whereas indomethacin was found to enhance tumor development (20). And finally, PGE_2 has also been implicated as possible mediator of the biological activity of thymic factor (21). In view of these stimulatory effects of PGE_2, and in view of the central role of IL-2 in T-cell responses, we reinvestigated the question of whether PGE_2 inhibits IL-2 production. Using two different IL-2 assays we found that PGE_2 does *not* inhibit the production of IL-2 in murine spleen cell cultures with standard (optimal) concentrations of concanavalin A.

MATERIALS AND METHODS

Animals. The mice were obtained from Bomholtgard, Ry, Denmark. Most of the experiments were performed with 8- to 19-week-old male animals.

The application of indomethacin and prostaglandins. Indomethacin is a nonsteroidal anti-inflamatory drug and a potent irreversible inhibitor of prostaglandin synthesis (22–24). A stock solution of indomethacin (Sigma) was prepared by dissolving 10 mg/ml) in ethanol. The cell cultures contained usually 0.5 μg indomethacin per ml, i.e., 1:20,000 dilution of the stock solution in culture medium. The various types of prostaglandins were obtained from Sigma. Stock solutions (10^{-2} *M* in ethanol) were stored at −20°C. The prostaglandins were applied to cultures at concentrations of 10^{-6} *M* if not indicated otherwise. Control cultures with corresponding concentrations of ethanol were also tested routinely.

The preparation of Con A-activated spleen cell supernatant (CSCS). The CSCS preparations were obtained by culturing spleen cells (1×10^7 cells/ml) with concanavalin A (GIBCO) (5 μg/ml if not indicated otherwise) in culture medium with fetal calf serum (GIBCO) and 3×10^{-5} *M* 2-mercaptoethanol usually for 24 hr. (The CSCS preparations in Fig. 2 have been obtained after 48 hr incubation.) Some of the cultures contained also prostaglandins (10^{-6} *M* if not indicated otherwise) and/or indomethacin (usually 0.5 μg/ml). the supernatants were isolated by centrifugation at 300*g* for 10 min and stored frozen. Some of the CSCS preparations were dialyzed 3 times against 10× the volume of culture medium in order to remove the prostaglandins. α-Methylmannoside at a final concentration of 1 m*M* (0.194 mg/ml) and HEPES (5×10^{-3} *M*) were added to the supernatant in order to neutralize the contaminating concanavalin A.

Assay for interleukin 2 with an IL-2-dependent T-cell line. Our interleukin 2 data are given as IL-2 activity units, based on the method described by Farrar *et al.* (25). The amount of IL-2 in supernatants was measured by their ability to support the growth of the T-cell clone W-2 as described in detail elsewhere (26).

Costimulator assay (27). The determination of IL-2 by the costimulator assay was performed as described by Shaw *et al.* (27). Briefly, 1×10^5 CBA thymocytes

were cultured with 0.6 μg concanavalin A and twofold dilutions of the CSCS preparation under test in 0.2 ml microcultures for 3 days. [^{3}H]Thymidine (1 μCi) was then added and the cultures were harvested on a Skatron cell harvester 4 hr later.

RESULTS AND DISCUSSION

We observed that the IL-2 production in spleen cell cultures with a standard concentration of 5 μg/ml of concanavalin A was not inhibited by PGE_2 in concentrations up to 10^{-6} *M* as determined by the proliferation of the IL-2-dependent T-cell clone W-2 (Table 1) and by the costimulator assay (27) (Fig. 1). We found also that the presence of 10^{-6} *M* PGE_2 in Con A-stimulated spleen cell cultures did not alter the capacity of the resulting spleen cell blasts to absorb IL-2

TABLE I

The Effect of Prostaglandins on the Production of Interleukin 2 by Con A-Activated Spleen Cells[a]

Group	Indomethacin added	Prostaglandin added		IL-2 units/ml Exp. I	Exp. II	Exp. III
1	−	None		43	100	148
2	+	None		66	163	148
3	+	PGE_2	10^{-6} *M*	57	146	n.t.
4	+		10^{-7} *M*	66		135
5	+	PGE_1	10^{-6} *M*	61		
6	+	PGI_2	10^{-6} *M*	44	64	104
7	+		10^{-7} *M*		111	146
8	+	6-Keto-$PGF_{1\alpha}$	10^{-6} *M*			114
9	+		10^{-7} *M*			124
10		$PGF_{2\alpha}$	10^{-6} *M*	69		
11	+	PGD_2	10^{-6} *M*	71		
12	+	$T \times B_2$	10^{-6} *M*		142	
13	+	PGI_2 was added to CSCS prep. (Gr. 2) after Con A activation and dialysis	10^{-6} *M*			176
14	+	None				145
15	+	PGI_2 was added to CSCS prep. (Gr. 2) after Con A activation and dialysis but was removed again by a second dialysis procedure	10^{-6} *M*			140

[a] CBA spleen cells (3×10^8) were incubated in 30 ml culture medium with 0.15 mg concanavalin A plus the indicated materials. After 24 hr the supernatants were collected by centrifugation and then subjected to dialysis for 24 hr to remove the prostaglandins. The supernatants of groups 13 and 15 received the prostaglandin only after this dialysis procedure; and the supernatant in group 15 was then dialyzed a second time. These controls show that the IL-2-dependent proliferation of W-2 cells is not inhibited by PGI_2. All supernatants were finally mixed with α-methylmannoside (1 m*M*) and tested for IL-2 activity.

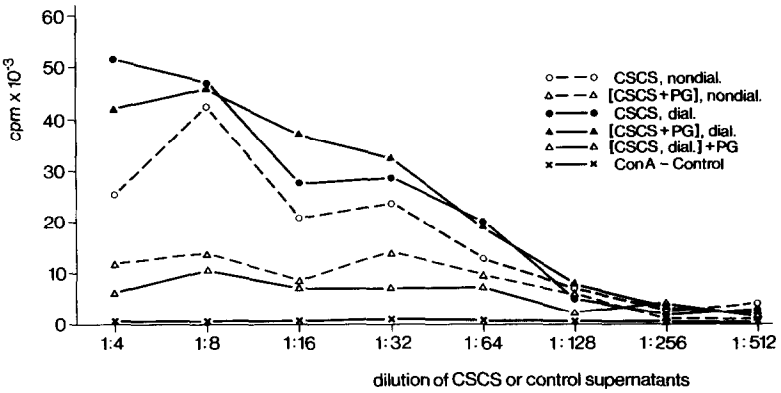

FIG. 1. Effect of PGE_2 on the activity of CSCS in the costimulator assay (27). Concanavalin A-stimulated thymocyte cultures received the indicated concentrations of a standard CSCS preparation without (O - - - O) or with (● —— ●) subsequent dialysis. A portion of the dialyzed CSCS preparation was mixed with PGE_2 (10^{-6} *M*) and then tested in the costimulator assay (Δ —— Δ). Another type of CSCS preparation was obtained by culturing the spleen cells with concanavalin A in the presence of 10^{-6} *M* PGE_2. This preparation was tested either without (Δ - - - Δ) or with (▲ —— ▲) subsequent dialysis. A control preparation contained no CSCS but only the corresponding amounts of concanavalin A (× —— ×). A series of CSCS preparations which were obtained as described above but with the only difference that 0.5 μg/ml indomethacin was added to the Con A-stimulated spleen cell cultures gave the same pattern of results (data not shown).

(data not shown). It was noted, however, that the IL-2 titers of CSCS preparations were (partly) inhibited when prostaglandin (PGI_2) or its degradation product 6-keto-prostaglandin F_1 was added to the Con A-stimulated spleen cell cultures (Table 1). The CSCS preparations had been subjected routinely (except for group 13 in Table 1 and the indicated groups in Fig. 1) to dialysis before the IL-2 assay in order to remove the prostaglandins. The control group 13 in Table 1 showed that PGI_2 did not suppress the proliferation of the Il-2-dependent W-2 T-cell line directly. Prostaglandin E_2 was found to suppress the proliferation of the W-2 cell line (Table 2), but it was obviously completely removed by dialysis as indicated by the data of Tables 1 and 2. The inhibitory effect of PGE_2 on the proliferation of an IL-2-dependent T-cell line has been described previously (11).

However, PGE_2 failed to suppress the production of IL-2 only in spleen cell cultures with 5 μg/lml concanavalin A but not with 1 μg/ml concanavalin A (Fig. 2). (The concentration of 5 μg/ml was in earlier experiments found to be the optimal concentration for the stimulation of IL-2 production and therefore routinely used as a standard concentration.)

Taken together, our experiments revealed that PGE_2 did not inhibit the IL-2 production in murine spleen cell cultures after optimal stimulation (5 μg/ml concanavalin A) as determined by two independent IL-2 assays, whereas IL-2 production in cultures with 1 μg/ml concanavalin A was markedly reduced. This indicated that the inhibitory effect of PGE_2 depends on the strength of the activating signal. Our experiments are not incompatible with the series of reports that PGE_2 inhibits IL-2 production in cultures of mitogen activated human lymphocytes (12, 14, 15). Possibly, these studies have been dealing with suboptimal stimulation conditions. The possibility that the IL-2 assays might have been sensitive against a PGE_2-induced suppressor factor might also explain the PGE_2-mediated inhibition

TABLE 2

Effect of PGE_2 on the Proliferation of the W-2 Cell Line (on the Apparent IL-2 Titers)[a]

Factor and materials added	Apparent IL-2 units/ml
Exp. I	
Gr. 1 CSCS conc.	130
2 CSCS 1:3 diluted	26
3 CSCS 1:9 diluted	8.5
4 CSCS conc. + PGE_2 (10^{-6} *M*)	51
5 CSCS 1:3 + PGE_2 (10^{-6} *M*)	13
6 CSCS 1:9 + PGE_2 (10^{-6} *M*)	3.4
Exp. II	
Gr. 7 CSCS	160
8 CSCS + PGE_2 (10^{-6} *M*)	110
9 CSCS + PGE_2 (10^{-6} *M*) dialysed	154
Exp. III	
Gr. 10 El-4 sup.	100
11 EL-4 sup. + PGE_2 (10^{-7} *M*)	28

[a] The IL-2 titer of two different CSCS preparations and an IL-2 containing EL-4 supernatant alone or in mixture with PGE_2 was assayed as described under Materials and Methods. In group 9, the mixture of CSCS plus PGE_2 had been dialyzed before the assay. Apparently, the prostaglandin was almost completely removed.

in the previous reports (12, 14, 15) and in our experiment of Fig. 2. Several laboratories have reported that PGE_2 activates inhibitory T cells (28–30), which produce suppressor factors (17, 18).

Our experiments showed also that PGE_2 inhibits the IL-2-dependent cell proliferation. This effect of PGE_2 is probably mediated by increased cyclic AMP levels in

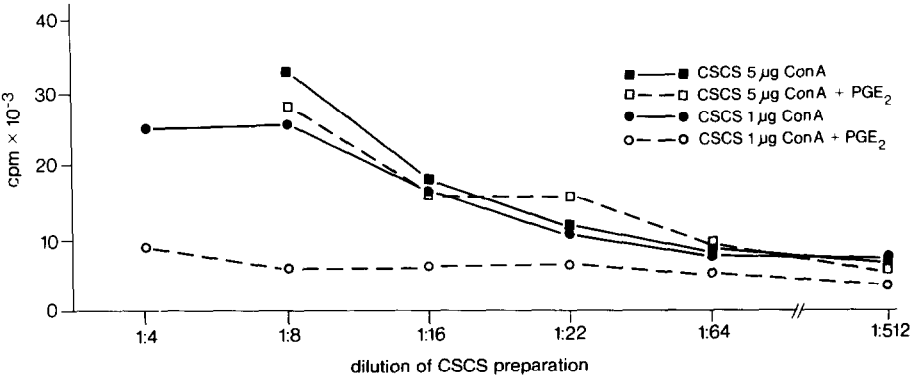

FIG. 2. PGE_2 inhibits the stimulation of IL-2 production by low concentrations of concanavalin A. CSCS was either prepared with 5 μg/ml concanavalin A and without PGE_2 (standard conditions, ■——■), or with 5 μg/ml Con A and with 10^{-6} *M*) PGE_2 (□- - -□), with 1 μg/ml Con A and without PGE_2 (●——●), or with 1 μg/ml Con A and with 10^{-6} *M* PGE_2 (○- - -○). All CSCS preparations were dialyzed and subsequently tested for IL-2 activity in the costimulator assay (27). PGE_2 was found to reduce the apparent IL-2 titer in Con A-activated spleen cell cultures with 1 μg/ml Con A but not with 5 μg/ml Con A.

the target cells (1). The possibility that PGE_2 reduces the expression of IL-2 receptors seems less likely; at least in Con A-activated spleen cell cultures PGE_2 did not alter the capacity to absorb IL-2 (unpublished observation).

ACKNOWLEDGMENTS

We are grateful to Mrs. S. Nick and Mrs. H. Schmidt for expert technical assistance and to Mrs. I. Fryson and Mrs. J. Rami for assistance in the preparation of the manuscript. We thank Dr. D. Gemsa for valuable discussions.

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