Regulation of Human B Cell Activation by Prostaglandin E_2

Suppression of the Generation of Immunoglobulin-secreting Cells

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Abstract

The role of prostaglandin E_2 (PGE_2) in the generation of immunoglobulin-secreting cells (ISC) from human peripheral blood B cells was examined. Initial studies demonstrated that monocyte (Mϕ)-mediated suppression of the generation of ISC in *Staphylococcus aureus* (SA)-stimulated cultures was mitigated by indomethacin, and thus suggested that the cyclooxygenase pathway products of arachidonic acid played a role in the regulation of B cell activation. The possibility that PGE_2, one of the major products of this pathway generated by Mϕ-affected human B cell responses, was therefore investigated. PGE_2 was found to cause concentration-dependent inhibition of the generation of ISC in pokeweed mitogen- or SA-stimulated B cell cultures supported by T cells. Studies were therefore carried out to determine whether PGE_2 inhibited the production of necessary T cell factors or directly altered B cell responsiveness. Initially, the effect of PGE_2 on the capacity of mitogen-stimulated cells to secrete a factor that supported the differentiation of B cells into ISC was investigated. Excessive numbers of Mϕ or PGE_2 inhibited the production of B cell differentiation factor from mitogen-stimulated T cells. The effect of PGE_2 on the capacity of B cells to differentiate into ISC was more complex. PGE_2 inhibited the generation of ISC when B cells were stimulated with SA and B cell differentiation factor-containing T cell supernatants. PGE_2-mediated inhibition of ISC generation was observed even when addition of PGE_2 was delayed until after ISC first were detected in culture. By contrast, PGE_2 caused only minimal inhibition of the generation of ISC cultures stimulated by T cell supernatants alone or protein A-free SA and T cell supernatants. These results suggested that SA-responsive B cells were particularly sensitive to inhibition by PGE_2. Additional experiments supported the conclusion that B cell sensitivity to inhibition by PGE_2 is augmented by the immunoglobulin cross-linking effects of protein A-containing SA. Overall, the results support the conclusion that PGE_2 at physiologically relevant concentrations can influence human antibody responses by means of a direct inhibitory action on the responding B cell or an indirect one on the production of necessary T cell factors.

Introduction

Mononuclear phagocytes (Mϕ)[1] exert a number of regulatory influences on lymphocyte function. Not only are Mϕ or their products necessary for the induction of various immunologic responses (1–8), but they may also suppress the activation and proliferation of T and B lymphocytes (9–12). One mechanism whereby Mϕ regulate immune responses is by producing arachidonic acid metabolites such as prostaglandin E_2 (PGE_2) (13–16). PGE_2 has been shown to inhibit T cell proliferation (17, 18) and the production of lymphokines such as interleukin-2 (IL-2) and γ-interferon (19–21). In addition, PGE_2 can have variable effects on the function of various suppressor T cell populations. Thus, in humans, PGE_2 has been shown to facilitate the activity of the suppressor T cells that inhibit IL-2 production (19) while inhibiting the suppressive capability of those that regulate immunoglobulin production (22, 23).

A number of aspects of B cell function have also been shown to be inhibited by PGE_2. The generation of B cell colonies from murine spleen and lymph node (24), as well as protein A- and phytohemagglutinin-stimulated growth of B cell colonies from human peripheral blood have been shown to be inhibited by PGE_2 (25, 26). In addition, we have recently found that *Staphylococcus aureus* (SA)- but not pokeweed mitogen (PWM)-stimulated human B cell proliferation is significantly inhibited by physiologic concentrations of PGE_2 (27).

In contrast to the inhibitory action of PGE_2 on B cell proliferation, its effects on antibody formation have been less clearly defined. In vivo studies in mice showing that antibody responses could be significantly enhanced by cyclooxygenase inhibitors suggested a role for prostaglandins in regulating the generation of immunoglobulin-secreting cells (ISC) (28). In addition, in vivo studies in man indicate that administration of cyclooxygenase inhibitors may augment secondary responses to immunization with viral antigens while having no effect on primary responses (29). However, in vitro studies have not clearly defined a role for prostaglandins in the regulation of B cell responsiveness. For example, a number of disparate effects of PGE_2 on PWM-stimulated immunoglobulin (Ig) production have been reported. Thus, inhibition of cyclooxygenase activity with indomethacin was found to have no effect on PWM-induced Ig secretion, whereas large concentrations of PGE_2 ($>10^{-7}$ M) were reported to be inhibitory (22, 30). By contrast, it has been shown that indomethacin may inhibit PWM-stimulated Ig secretion and that low concentrations ($\sim 10^{-8}$ M) of PGE_2 could reverse this inhibition (23). These latter results have been interpreted as indicating that PGE_2 can

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1. *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BCGF, B cell growth factor; FBS, fetal bovine serum; IL-2, interleukin-2; ISC, immunoglobulin-secreting cell; LME, L-leucine methyl ester; Mϕ, mononuclear phagocyte; N-SRBC, neuraminidase-treated sheep erythrocyte; PBM, peripheral blood mononuclear cells; PGE_2, prostaglandin E_2; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; PWM, pokewood mitogen; SA, *Staphylococcus aureus* (formalinized); SPA, staphylococcal protein A.

inhibit the function of a suppressor T cell involved in the regulation of Ig production, but that B cell function is not directly effected by these low concentrations of prostaglandin. Larger concentrations of PGE_2 ($>3 \times 10^{-7}$ M) did appear to have a negative effect on B cell function in these studies. Thus, it appears that physiologic concentrations of PGE_2 ($\sim 10^{-8}$ M; 22, 23, 29, 31) have little direct effect on B cell responsiveness although the regulatory action of suppressor T cells may be inhibited under some circumstances.

The studies described in this communication represent a continuation of experiments undertaken to examine in greater detail the effect of PGE_2 on a number of aspects of human B cell responsiveness. Initially, we found that PGE_2 directly inhibited B cell DNA synthesis and growth stimulated by SA but had a negligible effect on the production of B cell growth factor from mitogen-stimulated T cells (27). In the studies reported in this communication, the effect of PGE_2 on the generation of ISC from human peripheral blood B cells was examined. The results indicate that PGE_2 exerts a number of specific inhibitory influences on the cells involved in antibody formation in man. Thus, PGE_2 directly inhibited the generation of Ig-secreting cells from B cells stimulated with SA and T cell supernatants and also inhibited the capacity of T cells to secrete a factor(s) that supported B cell differentiation. These results indicate that PGE_2 produced by Mϕ, and possibly other cells, may have a profound effect on antibody production in humans.

Methods

Cell preparation. Peripheral blood mononuclear cells (PBM) were obtained from healthy adult volunteers by centrifugation of heparinized venous blood on sodium diatrizoate/Ficoll gradients (Isolymph; Gallard-Schlesinger Chemical Manufacturing Corp., Carle Place, NY). Cells were washed three times in Hanks' balanced salt solution (HBSS) before further processing.

Culture medium. All cultures were carried out in medium RPMI 1640 (Inland Laboratories, Austin, TX), supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (FBS; Microbiological Associates Inc., Bethesda, MD).

Cell separation. Cell populations were prepared as previously described (5). PBM were separated into adherent cells and nonadherent cells by incubation on glass petri dishes for 1 h at 37°C. The adherent cell population, which contained 85–90% Mϕ by esterase staining, was treated with 40 μg/ml mitomycin C (Sigma Chemical Company, St. Louis, MO) for 45 min at 37°C and washed three to four times before use. In some experiments, PBM were treated with L-leucine methyl ester (LME) as previously described (32) before further processing to deplete Mϕ more completely. T and B cells were prepared from the nonadherent cells by rosetting with neuraminidase-treated sheep erythrocytes (N-SRBC) and separating the T cell-enriched rosetting population and B cell-enriched nonrosetting population by centrifugation on diatrizoate/Ficoll gradients. The nonrosetting cells were incubated a second time with N-SRBC and centrifuged to insure removal of remaining T cells. The resulting population of "B cells" contained ~20–30% esterase-positive Mϕ and <1% T cells as determined by staining with OKT3 and OKT11 (Ortho Diagnostic Systems Inc., Westwood, MA) pan T cell monoclonal antibodies followed by analysis on the fluorescence-activated cell sorter. B cells prepared from LME-treated PBM contained >90% B1-(Coulter Immunology, Hialeah, FL) positive B cells, <1% OKT3- or OKT11-positive T cells, no Leu 11b-(Becton, Dickinson & Co., Cockeysville, MD) positive cells, and <2% esterase-positive Mϕ. The rosette-positive population was treated with NH_4Cl to lyse the N-SRBC and then passed over a nylon-wool column. The resultant T cell population contained <1–2% esterase-staining cells.

Reagents. PWM (Grand Island Biological Company, Grand Island, NY) was used at a final concentration of 10 μg/ml. Formalinized Cowan I strain SA (Calbiochem-Behring Corp., La Jolla, CA) was suspended in HBSS and routinely used at a previously determined optimum final concentration of 1:10,000 to 1:15,000 vol/vol. In some experiments, formalinized protein A-free SA (Calbiochem-Behring Corp.) was similarly employed. Phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co.) was dissolved in ethanol and used at a final concentration of 1 ng/ml. Indomethacin (Merck Sharp & Dohme Research Laboratories, Rahway, NJ) was dissolved in ethanol and used at a final concentration of 1 μg/ml. This concentration of indomethacin completely prevented the production of PGE_2 from PBM or purified Mϕ cultured at 37°C for 48 h. PGE_2 (Upjohn Company, Kalamazoo, MI) was dissolved in 95% ethanol at a concentration of 0.1 mg/ml (2.8×10^{-4} M) and used at final concentrations of 10^{-10} M to 10^{-6} M. In each experiment, ethanol was added to control cultures at the same final concentrations used as a vehicle for either indomethacin or PGE_2 (<0.1%). Ethanol concentrations of 0.05–0.5% had no effect on the generation of ISC in control cultures.

Culture conditions for the generation of ISC. Cells were cultured in microtiter plates with U-bottom wells (Costar, Cambridge, MA). Cultures were carried out in triplicate with each microwell containing 2.5×10^4 B cells in 0.2 ml of culture medium. B cells were cultured alone or supplemented with various numbers of T cells or T cell supernatants at a final dilution of 50%. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At the end of a 5–6-d incubation, cells from triplicate wells were pooled, washed, and suspended in HBSS for enumeration of ISC. In some experiments, B cells were preincubated in 17 × 100 mm polypropylene tubes (1×10^6 B cells/tube in 2 ml of culture medium) for 48 h, washed extensively, and recultured in microtiter plates as indicated above.

Detection of ISC. ISC were detected by using a reverse hemolytic plaque assay that has been described in detail (5, 33). For this assay, the indicator cells were SA protein A-coupled SRBC, the developing antiserum was rabbit anti-human Ig (IgA + IgM + IgG; Cappel Laboratories, Cochranville, PA) diluted 1:100, and the complement source was a 1:20 dilution of guinea pig serum (Pel-Freeze Biologicals, Inc., Rogers, AR) that had previously been absorbed with SRBC. The mean number of ISC for each experimental point was calculated from replicate determinations in which the variability was 10% or less. All data were expressed as the mean number of ISC per 10^6 responding cells initially cultured.

Cultures for the generation of T cell supernatants. T cells were suspended in medium with 10% FBS at a concentration of 5×10^6/ml and incubated with PWM (10 μg/ml) for 2 h at 37°C. The cells were then washed three times with HBSS and resuspended in fresh culture medium at a concentration of 5×10^6/ml. 2 ml of cell suspension was cultured in 17 × 100-mm round-bottom polypropylene tubes for 48 h at 37°C. Supernatants were then harvested, centrifuged to remove cells, filtered free of cellular debris, and stored frozen until used. In some experiments, T cells were cultured in an identical fashion with the exception that they were stimulated with the mixture of phytohemagglutinin (PHA) (1 μg/ml) and PMA (1 ng/ml) instead of PWM. After the 2-h incubation at 37°C, they were washed and cultured as described above. Where indicated, indomethacin (1 μg/ml), PGE_2 (10^{-7} M), or appropriate ethanol controls were added to the T cell cultures at the initiation of the 48-h incubation. In other experiments, T cells (5×10^6/ml) were co-cultured for 48 h with additional Mϕ (1×10^6/ml). Finally, some of the T cell supernatants were harvested after the 48-h culture, depleted of cells, and dialyzed twice against phosphate-buffered saline and once against medium with 10% FBS before use. Supernatants were assayed for B cell differentiation factor (BCDF) activity by testing the capacity of each supernatant to support the generation of ISC in SA-stimulated cultures of T cell-depleted B cells.

Table I. Suppression of SA-induced Generation of ISC by Monocytes: Reversal by Indomethacin

Exp.	Indo-methacin	Number of monocytes added to B and T cell cultures			
		0	5×10^3	1×10^4	2.5×10^4
	1 μg/ml	*SA-induced ISC per 10^6 B cells × 10^{-3}*			
1	0	46.8	54.4	34.4	16.8
	+	77.6	72.4	59.2	50.0
2	0	40.8	22.4	14.0	7.2
	+	65.2	54.0	46.4	37.6
3	0	60.8	67.2	39.2	37.6
	+	80.0	90.8	88.4	75.2
4	0	32.8	24.4	16.0	16.8
	+	53.2	48.4	48.4	32.6
5	0	11.2	—	4.0	2.4
	+	18.0	—	17.2	12.8
6	0	23.6	—	18.8	12.4
	+	25.6	—	31.2	21.2
7	0	43.2	37.6	28.4	7.2
	+	44.4	42.8	51.2	44.0
8	0	96.8	92.8	70.8	46.4
	+	95.6	97.6	86.0	74.0

B cells (2.5×10^4 per microwell) and T cells (5×10^4 per microwell) were incubated alone or with varying numbers of monocytes in the presence or absence of indomethacin as indicated. Cultures were stimulated with SA and ISC were determined after a 6-d incubation.

Assay of PGE_2 in cell supernatants. The amount of PGE_2 present in cell supernatants was measured by radioimmunoassay as previously described (34).

Results

Indomethacin mitigates Mϕ-mediated suppression of SA-induced generation of ISC. Initial experiments examined the capacity of Mϕ to inhibit SA-induced generation of ISC. For these experiments, B cells that had been prepared in standard fashion were employed. Such B cells contained 20–30% esterase-positive cells. When these B cells were co-cultured with T cells and stimulated with SA, ISC were routinely generated. The addition of Mϕ to these cultures decreased the number of ISC found (Table I). The possibility that the inhibition of responsiveness observed was the result of the cyclooxygenase pathway products released by Mϕ was examined by adding indomethacin to these cultures. As can be seen in Table I, indomethacin augmented the generation of ISC in most of the control cultures and, in each experiment, substantially decreased Mϕ-mediated suppression of responsiveness. These results suggested that cyclooxygenase pathway products of arachidonic acid metabolism produced by Mϕ could inhibit the generation of ISC. In order to examine this possibility, the effect of PGE_2 on the generation of ISC was explored. This was predicated on the observation that PGE_2 is one of the major cyclooxygenase pathway metabolites produced by human Mϕ in culture (35–37).

PGE_2 suppresses the generation of ISC. PGE_2 suppressed the generation of ISC in mitogen-stimulated cultures containing B and T cells (Table II). In SA-stimulated cultures, significant suppression was observed with 10^{-10} M PGE_2 (mean percentage of inhibition±SEM: 35.2±6.7%), whereas 10^{-9} M PGE_2 suppressed responsiveness by a mean of 50.4±3.7%. Although larger concentrations of PGE_2 caused increased inhibition, the generation of ISC could not be completely suppressed even by the largest concentration tested (10^{-6} M). When the effect of PGE_2 on PWM-stimulated ISC was examined, somewhat different effects were observed. Although suppression was routinely observed, less inhibition of PWM-induced ISC was noted with each concentration of PGE_2 than was observed with the SA response. Thus, a mean of 50% inhibition of PWM responsiveness (52.0±4.5%) required 10^{-7} M PGE_2 compared with 10^{-9} M for the SA response. Moreover, low concentrations of PGE_2 were not always suppressive and in some experiments (exp. 4, Table II), 10^{-10} and 10^{-9} M PGE_2 increased PWM-induced generation of ISC. With higher concentrations ($>10^{-7}$ M), PGE_2 suppressed PWM-induced ISC in all experiments. However, the maximum degree of inhibition noted was less than that observed in SA-stimulated cultures (percentage of inhibition 57.5±5.0% for PWM vs. 76.4±3.5% for SA).

Table II. Suppression of the Generation of ISC by PGE_2

Addition	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	PWM	SA	PWM	SA	PWM	SA	PWM	SA
mol/liter	*Mitogen-induced ISC per 10^6 B cells × 10^{-3}*							
Nil	19.2	33.6	12.4	32.8	21.2	67.6	—	—
Ethanol	19.2	33.6	12.4	31.2	22.4	62.8	7.6	12.0
PGE_2 (10^{-10})	12.8	19.2	9.2	20.4	19.6	52.4	12.4	6.4
PGE_2 (10^{-9})	10.4	16.4	6.4	12.4	13.2	35.6	10.0	6.4
PGE_2 (10^{-8})	9.2	12.4	7.2	13.2	12.8	36.4	6.4	5.6
PGE_2 (10^{-7})	8.4	10.0	7.2	11.2	8.0	24.0	4.0	3.6
PGE_2 (10^{-6})	8.8	7.6	7.2	6.8	8.4	20.8	2.8	2.0

B cells (2.5×10^4 per microwell) and T cells (5.0×10^4 per microwell) were co-cultured and incubated with either PWM or SA. ISC were determined after a 6-d incubation. The cultures also contained various concentrations of PGE_2 dissolved in ethanol as shown. Ethanol alone at a comparable concentration was added to culture where indicated.

These experiments indicated that PGE_2 suppressed the generation of ISC. In that the generation of ISC in these cultures requires both B cell activation and the production of necessary T cell factors (38–40), it was possible that PGE_2 inhibited helper T cell function, B cell responsiveness, or both. The next series of experiments was undertaken therefore to examine the effect of PGE_2 on the various cells involved in the generation of ISC.

Inhibition of the generation of BCDF activity by Mϕ and PGE_2. Initially, experiments were carried out to determine whether Mϕ and PGE_2 suppressed the release of BCDF by activated T cells. As shown in Table III, PWM-stimulated T cells were cultured in the presence of either Mϕ or PGE_2. In both circumstances, depressed BCDF activity was found in the T cell supernatants. The presence of PGE_2 or another low molecular weight inhibitor that might be present in these supernatants could not explain the apparent lack of BCDF activity in that dialysis of the supernatants did not lead to augmented generation of ISC. It should be noted that Mϕ-mediated suppression of the generation of BCDF was largely reversed by indomethacin (suppression of BCDF production by 20% Mϕ, 92%; suppression by 20% Mϕ in the presence of indomethacin, 16%—mean of three experiments). Moreover, after dialysis, T cell supernatants generated in the presence of Mϕ did not suppress the generation of ISC supported by control T cell supernatants (data not shown).

The inhibitory effect of PGE_2 of BCDF production was not unique for PWM-stimulated T cells. PGE_2 also inhibited the generation of BCDF activity from PHA + PMA-stimulated T cells. This combination of stimuli induced the elaboration of much more potent T cell supernatants than PWM. Despite this, PGE_2 inhibited production of BCDF (mean inhibition, 45.6±7.6%, n = 4), albeit somewhat less effectively than was noted with PWM-stimulated T cells. Moreover, after dialysis, supernatants generated from PHA + PMA-stimulated T cells in the presence of PGE_2 did not suppress the generation of ISC supported by control T cell supernatants (data not shown). These results support the conclusion that Mϕ or PGE_2 inhibited the release of BCDF from mitogen-stimulated T cells.

PGE_2 inhibits B cell responsiveness to SA. To determine whether PGE_2 had a direct inhibitory effect on the capacity of B cells to generate ISC, B cells were purified from LME-treated PBM. Such B cells were utilized because they were markedly depleted of Mϕ, containing <2% esterase-positive cells. In culture, these B cells produced undetectable concentrations of PGE_2. As can be seen in Table IV, culturing these cells with no stimulus or in the presence of SA alone resulted in the generation of no ISC. By contrast, when these cells were stimulated with T cell supernatants alone, small numbers of ISC were generated in each experiment. However, when the B cells were stimulated with both SA and T cell supernatant, much larger numbers of ISC were generated. When PGE_2 was added to cultures of B cells stimulated with T cell supernatant alone, no inhibition of the generation of ISC was seen. In fact, in many experiments a modest augmentation in the number of ISC found at the end of the 5-d incubation was noted. By contrast, when PGE_2 was added to B cells stimulated with SA plus T cell supernatant, marked inhibition in the generation of ISC was observed in each experiment. The addition of 10^{-9} M PGE_2 caused inhibition in six of the seven experiments, with inhibition ranging from 30 to 68%, whereas 10^{-7} M PGE_2 caused marked inhibition in each of the experiments (range: 60–98%). Although PGE_2 caused marked inhibition in the generation of ISC, a complete suppression of responsiveness was never observed. The magnitude of the response stimulated by SA plus T supernatant in the presence of PGE_2 was usually comparable to the response generated by T cell supernatant alone.

In the aforementioned experiments, PHA + PMA-stimu-

Table III. Effect of PGE_2 on the Production of BCDF

T cell supernatants			SA-induced generation of ISC		
PGE_2	Mϕ	Dialysis	Exp. 1	Exp. 2	Exp. 3
			ISC per 10^6 B cells × 10^{-3} (PGE_2, nM/liter)		
No supernatant			0	0	0
0	0	0	6.8 (0.2)	9.6 (0.3)	7.6 (0.3)
0	0	+	4.4 (0)	10.0 (0.1)	7.6 (0)
+	0	0	1.6 (28.1)	2.0 (17.1)	0.4 (21.1)
+	0	+	1.2 (0.2)	1.6 (0)	0.4 (0.1)
0	+	0	0 (178.7)	0 (158.2)	0 (70.5)
0	+	+	0 (0.2)	0 (0.1)	0 (0)

Supernatants were generated from cultures of PWM-stimulated T cells (5.0×10^6 per ml), T cells plus PGE_2 (10^{-7} M), or T cells plus 20% Mϕ (1.0×10^6 per ml). Supernatants were harvested after a 48-h incubation and dialyzed as indicated before analysis for BCDF activity. Supernatants were assayed for BCDF activity by adding them at a 50% final dilution to B cell cultures (2.5×10^4 per microwell) stimulated with SA. The generation of ISC was assessed after a 6-d incubation.

Table IV. Effect of PGE_2 on the Generation of ISC

Exp.	Addition to B cell cultures	Generation of ISC: No stimulus	SA	T cell supernatant	SA + T supernatant
		ISC per 10^6 B cells × 10^{-3}			
1	Nil	0	0.2	17.8	109.8
	Ethanol	0	0	14.4	120.4
	PGE_2—10^{-9} M	0	0	16.9	53.3
	PGE_2—10^{-7} M	0	0	18.9	24.9
2	Nil	0	0.6	11.6	76.8
	Ethanol	0	0.6	16.2	68.4
	PGE_2—10^{-9} M	0.2	0.8	20.2	44.8
	PGE_2—10^{-7} M	0	0.2	14.2	14.8
3	Ethanol	0.4	0.8	20.6	61.8
	PGE_2—10^{-9} M	0.6	0.8	25.4	41.6
	PGE_2—10^{-7} M	0	0.6	22.6	19.0
4	Ethanol	0	0	5.4	13.6
	PGE_2—10^{-9} M	0	0	5.4	8.8
	PGE_2—10^{-7} M	0	0.2	5.2	5.4
5	Ethanol	0.2	0	2.8	26.0
	PGE_2—10^{-9} M	0	0	4.0	26.6
	PGE_2—10^{-7} M	0	0	2.4	4.4
6	Ethanol	0	0.8	32.2	129.2
	PGE_2—10^{-10} M	0	0.2	25.2	113.6
	PGE_2—10^{-9} M	0	0	24.4	89.8
	PGE_2—10^{-8} M	0	0	33.8	47.0
	PGE_2—10^{-7} M	0	0.2	29.6	24.2

B cells prepared from LME-treated PBM were cultured for 5 d in the presence of the various stimuli and additions as noted and assayed for the generation of ISC.

lated T cell supernatants were utilized. In additional experiments, similar results were observed when PWM-stimulated T cell supernatants were utilized to support the generation of ISC. Moreover, similar results were noted when control B cells were used instead of LME-treated B cells as responders using either PWM- or PHA + PMA-stimulated T cell supernatants (data not shown). These data thus support the conclusion that PGE_2 directly inhibits B cells stimulated by SA and BCDF with a resultant diminution in the number of ISC generated.

Kinetics of the inhibitory effect of PGE_2 on B cell differentiation into ISC. The next series of experiments was undertaken to determine whether PGE_2 needed to be present from the initiation of culture in order to suppress the generation of ISC. Fig. 1 shows two experiments carried out to examine this issue. B cells prepared from LME-treated PBM were incubated with T cell supernatant or SA plus T cell supernatant for various lengths of time and assayed for the number of ISC generated. As can be seen, ISC were detected after a 3-d incubation. At this time, there was no substantial difference between the number of ISC observed in B cell cultures supported by T cell supernatant alone or in those supported by SA and T cell supernatant. However, as the length of incubation was increased, markedly increased numbers of ISC were found in the cultures stimulated by SA and T cell supernatant compared with those found in cultures stimulated by T cell supernatant alone. As noted previously, the addition of PGE_2 did not inhibit the number of ISC found in cultures supported with T cell supernatant alone. On the contrary, in both experiments the addition of PGE_2 at the initiation of culture, but not thereafter, resulted in an increase in the number of ISC found, which became more pronounced as the length of the culture was increased.

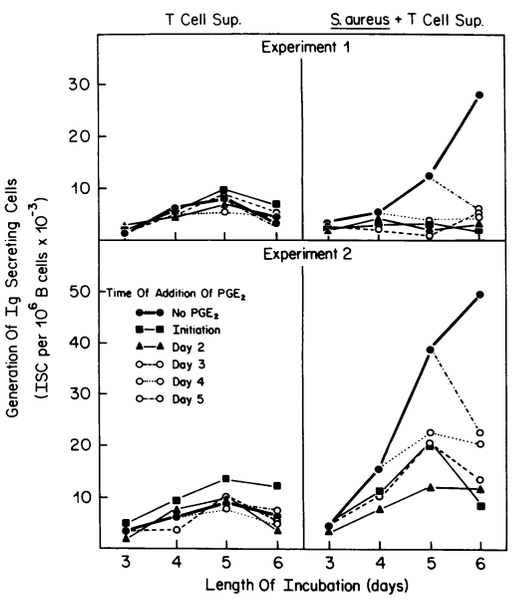

Figure 1. Effect of the delayed addition of PGE_2 on the generation of ISC. B cells prepared from LME-treated PBM were cultured with either supernatant obtained from PHA + PMA-stimulated T cells alone or SA and the T cell supernatant. The number of ISC was assayed on a daily basis after a 3–6-d incubation. PGE_2 (10^{-7} M) was added at the initiation of culture or various times thereafter. The control curve (no PGE_2) indicates the mean number of ISC in cultures that received ethanol instead of PGE_2 at the indicated times. The addition of ethanol had no substantial effect on the generation of ISC.

The effect of PGE_2 in cultures stimulated by SA and T cell supernatant was markedly different. PGE_2 did not alter the number of ISC detected after a 3-d incubation, independent of its time of addition. As the culture was prolonged, however, PGE_2 became progressively more inhibitory. The degree of inhibition was comparable when PGE_2 was added at the initiation of culture or 48 h later. Moreover, even if the addition of PGE_2 was delayed until day 3 of the incubation, at which time ISC were already present, a similar degree of subsequent inhibition was observed. Even if PGE_2 was added as late as the 4th or 5th d of culture, when substantial numbers of ISC were present, marked inhibition of responsiveness became apparent within a 24-h incubation. These results indicate that PGE_2 did not inhibit the initial generation of ISC but appeared to inhibit the subsequent increase in the number of ISC found in SA and T cell supernatant-stimulated cultures. Moreover, B cells remained sensitive to the inhibitory effect of PGE_2 throughout the length of the incubation.

One possible explanation for these results was that PGE_2 interfered with the synthesis and/or secretion of Ig as has previously been suggested in the murine system (41). However, the results shown in Table V indicate that this is unlikely to be the explanation. As can be seen, the addition of PGE_2 did not inhibit the generation of ISC in cultures supported by T cell supernatant only. By contrast, PGE_2 substantially inhibited responsiveness in SA and T cell supernatant-stimulated cultures. Marked inhibition of responsiveness was observed when PGE_2 was added as late as the 4th d of the 5-d culture. However, when PGE_2 was added 2 h before assaying the number of ISC, no inhibition was noted. This can be contrasted with the effect of adding cycloheximide, a protein synthesis inhibitor, 2 h before harvesting. Cycloheximide totally inhibited detection of ISC in cultures stimulated either by T cell supernatant alone or by SA and T cell supernatant. Thus, it appears unlikely that PGE_2 affects either the synthesis or secretion of Ig directly.

Role of SA in PGE_2-mediated inhibition of ISC. The data presented heretofore indicated that ISC generation stimulated by SA and T cell supernatant was inhibited by PGE_2, whereas

Table V. PGE_2 Does Not Inhibit Ig Synthesis

Addition	Time of addition	Generation of ISC: T cell supernatant	Generation of ISC: SA + T cell supernatant
		ISC per 10^6 B cells × 10^{-3}	
Ethanol	—	10.7±2.9	43.3±2.9
PGE_2	Initiation	18.2	17.0
PGE_2	Day 2	9.6	14.0
PGE_2	Day 4	8.6	23.8
PGE_2	2 h before assay	10.0	52.2
Cycloheximide	2 h before assay	0	0.2

B cells prepared from LME-treated PBM were cultured with T cell supernatant or SA + T cell supernatant and assayed for the number of ISC generated after a 5-d incubation. PGE_2 (10^{-7} M) was added at the beginning of culture or at various times thereafter. Control cultures (ethanol) received ethanol instead of PGE_2 at the indicated times. Data shown for control cultures are mean±SEM of cultures receiving ethanol at the various times noted. In addition, cycloheximide (100 μg/ml) was added to culture where indicated.

Table VI. Inhibition of the Generation of ISC by PGE_2

Exp.	Addition	Generation of ISC: T sup	T sup + SA (+SPA)	T sup + SA (−SPA)
		ISC per 10^6 cells × 10^{-3}		
1	Ethanol	16.8±1.2	137.0±3.0	98.6±2.4
	PGE_2	21.2±3.6	30.4±2.8	69.8±0.2
		(+26)	(−78)	(−28)
2	Ethanol	10.8±1.6	134.4±2.8	46.0±1.2
	PGE_2	16.4±1.6	13.2±0.8	52.6±3.8
		(+52)	(−90)	(+14)
3	Ethanol	11.8±1.0	133.0±3.0	53.4±1.8
	PGE_2	14.2±1.4	18.6±1.0	35.0±1.0
		(+20)	(−86)	(−35)

B cells prepared from LME-treated PBM were cultured for 5 d with T supernatant alone or with T supernatant and SA that contained or lacked staphylococcal protein A (SPA) each at a final concentration of 1:15,000. In addition, cultures contained PGE_2 (10^{-7} M) or ethanol as control. After a 5-d incubation, the number of ISC was determined. Values in parentheses indicate percentage change from ethanol control.

the smaller numbers of ISC stimulated by T cell supernatants alone was not. Experiments were therefore undertaken to examine the role of SA in facilitating the inhibition caused by PGE_2. In the first series of experiments, B cells were stimulated with T cell supernatant in the presence of SA that contained protein A or SA that lacked this Ig-binding protein and the effect of PGE_2 was determined. As can be seen in Table VI, both protein A-containing SA and SA-lacking protein A were capable of stimulating the generation of large numbers of ISC in the presence of T cell supernatant. The generation of ISC stimulated by protein A-containing SA and T supernatant was markedly inhibited by PGE_2. By contrast, the generation of ISC stimulated by SA that lacked protein A was much less affected by 10^{-7} M PGE_2. As noted previously, the generation of ISC in cultures stimulated by T cell supernatant alone was increased by PGE_2. These results indicate that inhibition of responsiveness is augmented by protein A-containing SA and suggest the possibility that the capacity of this Ig-binding protein to interact with surface Ig on B cells may enhance the inhibitory capacity of PGE_2.

To examine this possibility, the experiments described in Table VII were undertaken. In these experiments, B cells were

Table VII. Effect of SA on PGE_2-mediated Inhibition of the Generation of ISC

Addition to B cell cultures			Generation of ISC					
	Terminal 72 h		Exp. 1		Exp. 2		Exp. 3	
Initial 48 h	T cell supernatant	SA	ETOH	PGE_2	ETOH	PGE_2	ETOH	PGE_2
			ISC per 10^6 B cells × 10^{-3}					
Nil	−	—	0	—	0	—	0	—
	+	—	3.2±0.4	5.2±1.6	9.8±1.4	9.0±1.2	7.6±0.8	8.2±1.4
	+	1:15,000	3.8±0.2	2.2±0.2	6.8±1.6	10.4±0.8	3.2±0.4	3.6±0.8
	+	1:45,000	6.0±1.6	3.0±0.6	16.6±0.2	10.0±1.6	9.0±1.0	4.8±1.2
	+	(−SPA), 1:15,000	ND	ND	6.0±0.4	7.4±1.4	6.8±0.8	5.4±0.6
T cell supernatant	−	—	0	—	2.4±0.4	—	0	—
	+	—	8.6±2.2	7.8±0.6	24.3±0.7	23.2±0.4	8.0±0.4	9.6±0.8
	+	1:15,000	13.6±2.8	9.0±0.6	28.2±1.0	33.0±3.0	9.8±1.0	6.4±0.8
	+	1:45,000	31.6±3.2	14.4±0.4	78.0±2.0	43.6±2.0	14.2±0.2	7.4±0.6
	+	(−SPA), 1:15,000	ND	ND	26.8±2.8	24.6±1.4	7.4±0.6	7.0±0.2
SA	−	—	0	—	0	—	0	—
	+	—	251.2±4.8	102.4±3.2	119.8±2.2	58.6±1.0	115.4±5.8	72.6±5.0
	+	1:15,000	149.0±3.0	17.4±0.2	41.6±0.8	19.2±1.2	110.2±3.0	23.6±0.2
	+	1:45,000	350.6±7.8	50.6±1.4	162.4±2.4	23.2±2.6	138.2±2.2	41.4±2.2
	+	(−SPA), 1:15,000	ND	ND	140.6±4.2	63.2±1.6	116.8±3.2	85.4±1.8
T supernatant	−	—	0.2±0.2	—	6.0±0.4	—	0.2±0.2	—
+ SA	+	—	286.8±2.8	110.8±2.8	187.8±5.8	97.4±2.2	148.8±0.8	61.0±5.0
	+	1:15,000	128.6±8.6	20.8±3.6	80.4±2.8	54.4±4.4	72.4±1.6	21.0±4.6
	+	1:45,000	357.2±7.6	52.6±1.8	193.2±2.8	70.4±0.4	125.8±2.2	38.0±2.0
	+	(−SPA), 1:15,000	ND	ND	197.8±2.6	99.0±3.0	142.6±4.6	76.0±4.0

B cells were cultured in tubes (0.5×10^6/ml) either alone or with T cell supernatant (final concentration, 50%), SA (final concentration, 1:15,000 vol/vol), or T cell supernatant and SA for 48 h, washed, and aliquoted into the wells of microtiter plates and cultured alone or with T cell supernatant and SA at the final concentrations indicated. Where indicated, SA that lack SPA [SA(−SPA)] were used instead of SA. After a 72-h incubation, the number of ISC generated was determined. These latter cultures all contained PGE_2 (10^{-7} M) or ethanol as control where indicated. In addition, indomethacin (1 μg/ml) was also present during the terminal 72 h in Exp. 1. ND, not determined.

preincubated for 48 h with the various stimuli indicated. They were then washed extensively and recultured in the presence or absence of T cell supernatant. After an additional 72-h incubation, the number of ISC was determined. As can be seen, minimal numbers of ISC were generated when T cell supernatant was omitted from the terminal 72-h culture. If no stimulus was included in the initial 48-h culture, few ISC were generated regardless of the presence of T cell supernatant in the second incubation. When T cell supernatant was included in the first incubation, small numbers of ISC were generated when T cell supernatant was also included in the second culture. Of note, when cells were stimulated with SA or T cell supernatant plus SA for the first 48 h, washed, and then recultured with T cell supernatant, large numbers of ISC were generated.

The addition of PGE_2 to the terminal 72-h cultures substantially inhibited the generation of ISC only when SA had been included during the initial 48-h incubation. The capacity of SA to enhance the inhibition of ISC generation was examined by adding it into the terminal 72-h incubation. In the situations in which B cells were cultured alone or stimulated with T cell supernatant for the first 48 h, the addition of SA during the terminal 72 h augmented responsiveness. Although this augmentation was inhibited by PGE_2, inhibition below the level seen in the absence of the additional stimuli was not observed.

When B cells that had previously been stimulated with SA or SA plus T cell supernatant were examined, different results were observed. It was found that the addition of SA at the previously determined optimal concentrations to the terminal 72-h incubation frequently caused inhibition of responsiveness. This inhibition did not appear to be related to production of endogenous PGE_2 in that it was not altered by the addition of indomethacin. When PGE_2 and SA were both added during the terminal 72-h incubation, substantially increased inhibition was observed above that seen with either SA or PGE_2 alone. The addition of a lower concentration of SA during the terminal 72 h of culture did not directly inhibit the generation of ISC. However, even these lower concentrations of SA markedly increased the degree of inhibition noted when PGE_2 was also added to these cultures. By contrast to the effect of protein A-containing SA, the addition of SA that lacked protein A during the terminal 72 h of culture did not increase the degree of inhibition caused by PGE_2. Thus, the degree of inhibition of responsiveness caused by PGE_2 was comparable in the presence or absence of SA-lacking protein A.

These results suggested that SA that contained protein A augmented the degree of inhibition caused by PGE_2. In order to confirm that conclusion, one additional experiment was carried out. B cells were stimulated for 48 h in the presence of SA, washed, cultured with T cell supernatant for an additional 3 d, and then assayed for the number of ISC. In the experiment shown in Fig. 2, the addition of various concentrations of PGE_2 during the terminal 72-h incubation with T cell supernatant caused dose-dependent inhibition of responsiveness. The addition of low concentrations of SA to these cultures markedly increased the degree of inhibition caused by all concentrations of PGE_2. In the presence of SA, a comparable degree of inhibition was observed with 10–100-fold less PGE_2. These results support the conclusion that SA increases the sensitivity of activated B cells to the inhibitory action of PGE_2.

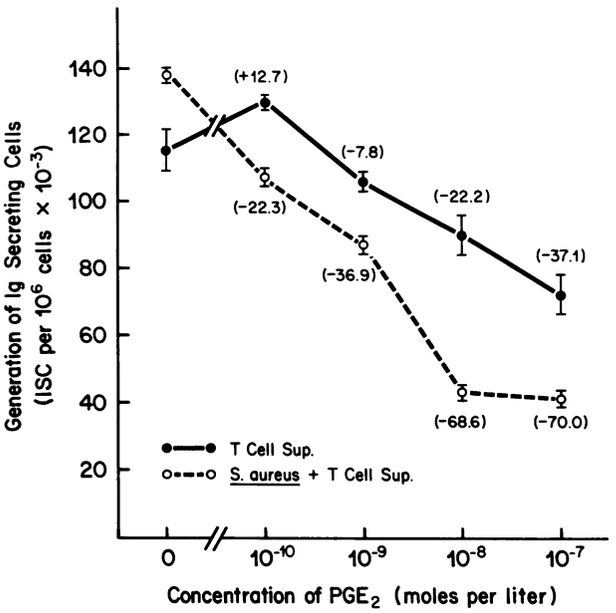

Figure 2. SA increases PGE_2-mediated inhibition of ISC generation. B cells obtained from LME-treated PBM were incubated for 48 h with SA in 17 × 100-mm polypropylene tubes, washed extensively, cultured in microwells with T cell supernatant, and assayed for the number of ISC after an additional 72-h incubation. In addition, the latter cultures contained various concentrations of PGE_2 with or without additional SA (1:45,000 vol/vol). Numbers in parentheses indicate the PGE_2-mediated percentage change in the number of ISC generated compared with the response noted without PGE_2 (ethanol control).

Discussion

The experiments described herein were undertaken to examine the regulatory role of PGE_2 on the generation of ISC from human peripheral blood B cells. This line of investigation was stimulated by the observation that a portion of Mϕ-mediated suppression of the generation of ISC in response to stimulation by SA could be accounted for by cyclooxygenase pathway products of arachidonic acid, as indicated by its partial reversal by indomethacin. A number of previous reports have failed to show consistent reversal of Mϕ-mediated suppression of the generation of ISC by indomethacin (10, 11), and thus have concluded that prostaglandins play little role in regulating B cell responses. However, most of these studies used PWM as the stimulus. In our own unpublished studies, we also found that Mϕ-mediated suppression of PWM-stimulated generation of ISC was much less consistently altered by indomethacin. In the current studies, SA was used as the polyclonal B cell activator. A number of features of SA appear to be important in the demonstration of indomethacin-reversible inhibition of the generation of ISC. First, SA increased Mϕ production by PGE_2 by two- to threefold, whereas other polyclonal B cell activators such as PWM caused no increase in Mϕ PGE_2 synthesis in the presence or absence of T cells (27). Secondly, SA itself appeared to increase the sensitivity of B cells to inhibition by PGE_2. The use of SA as the polyclonal B cell activator, therefore, made it possible routinely to observe indomethacin-reversible inhibition of the generation of ISC. When the effect of prostaglandin was examined directly by

adding it to culture rather than indirectly by inhibiting its synthesis with indomethacin, PGE_2 was found to suppress the generation of ISC in cultures of T and B cells stimulated by either SA or PWM, although the PWM response was always less sensitive to inhibition by PGE_2, as would be anticipated.

It should be noted that Mϕ appear to be able to suppress the generation of ISC by mechanisms other than the production of prostaglandins, as has been noted in other studies. Thus, Mϕ have been shown to suppress B cell responses by the generation of noncyclooxygenase-derived inhibitory molecules with direct effects on B cells (10), through the facilitation of suppressor T cell activity (42), or by absorbing necessary helper T cell factors (43). Although these mechanisms were not examined in detail in the current study, it was found, for example, that Mϕ-mediated suppression of the generation of BCDF was not completely reversed by indomethacin, suggesting that another suppressive mechanism might be playing a role in limiting the amount of BCDF found in mitogen-stimulated supernatants of T cells co-cultured with Mϕ (data not shown).

One mechanism whereby Mϕ and PGE_2 altered the generation of ISC was by inhibiting the production of BCDF from mitogen-stimulated T cells. It should be noted that this inhibition was selective in that PGE_2 had no effect on mitogen-induced production of B cell growth factor (BCGF) as asayed by the capacity of the supernatants to augment proliferation of SA-stimulated B cells. Thus, the supernatants of mitogen-stimulated T cells cultured in the presence of PGE_2, which were markedly deficient in BCDF activity as shown in the current study, were found to have comparable levels of BCGF activity as supernatants of control T cells (27). Although PGE_2 had little effect on the production of BCGF, it has been shown to inhibit secretion of a variety of other T cell lymphokines, including IL-2 (19, 20), γ-interferon (21), and leukocyte migration inhibitory factor (44). PGE_2 appears to inhibit the production of IL-2 not by a direct effect on the IL-2 producing cells, but by facilitating the induction of suppressor T cells (45). The current studies did not examine the mechanism by which PGE_2 altered BCDF production. Thus, it is possible that PGE_2 directly inhibited the function of the BCDF-producing cell, induced the generation of regulatory suppressor T cells, or, alternatively, inhibited the production of a needed helper factor, such as IL-2, that might be involved in the generation of BCDF by mitogen-stimulated T cells. Preliminary evidence suggests that Mϕ might inhibit the production of BCDF from purified T4 cells less effectively than from mixtures of T4 and T8 cells, thus suggesting a role for suppressor T cells in limiting BCDF production. The finding that release of BCGF and BCDF is differentially affected by PGE_2 suggests that these products may be secreted by different T cell populations or that their production is differentially altered by PGE_2-responsive regulatory mechanisms.

Besides its capacity to inhibit the production of BCDF, PGE_2 was also found to inhibit B cell function directly, altering the capacity to generate ISC upon stimulation with SA plus BCDF-containing T cell supernatants. This inhibitory effect was predominantly observed when SA was used as the polyclonal activator; B cells stimulated to differentiate into ISC by T cell supernatant alone or by T cell supernatant and SA that lacked protein A were inhibited to a far less degree. In addition, the results of experiments not reported here in detail, indicated that B cells stimulated by the combination of T cell supernatant plus PWM were also not inhibited by PGE_2. This latter observation is in agreement with the results of Ceuppens and Goodwin (22), who noted that Ig production by B cells stimulated by PWM-induced T cell factors was not inhibited by concentrations of PGE_2 below 3×10^{-7} M. Moreover, the dichotomy between PWM- and SA-stimulated B cells is consistent with our previous findings that B cells stimulated to proliferate by SA plus BCGF-containing T cell supernatants were inhibited profoundly by PGE_2, whereas those undergoing DNA synthesis in response to PWM and T cell help were not affected by PGE_2 (27). These results support the conclusion that subsets of B cells may be defined functionally by their sensitivity to inhibition by PGE_2.

A number of pieces of evidence support the conclusion that cross-linking of surface Ig facilitates PGE_2-mediated inhibition of B cell function. Thus, for example, PGE_2 inhibited generation of ISC stimulated by SA that contained staphylococcal protein A (SPA) but altered responses stimulated by SA that lacked SPA to a much lesser degree. Moreover, B cells stimulated by T cell supernatant alone in the absence of SA were not inhibited at all by PGE_2. Finally, the degree of inhibition caused by PGE_2 was markedly increased by the late addition of SPA-containing SA. In addition, SPA-containing SA resulted in a much greater sensitivity to inhibition by PGE_2, as evidenced by the decreased concentration of this prostaglandin that inhibited responsiveness. SPA has been shown to bind human B cell surface Ig (46) and thus could facilitate cross-linking of surface Ig. It is reasonable, therefore, to speculate that this cross-linking of surface Ig by SPA-containing SA may sensitize B cells to inhibition by PGE_2. This contention was substantiated in preliminary experiments by the finding that the addition of anti-μ antibody to cultures stimulated by SA and T cell supernatant also increased the degree of inhibition caused by PGE_2. Thus, the presence of an additional surface Ig cross-linker appeared to increase the sensitivity of responding B cells to PGE_2.

B cells that differentiate into ISC in response to T cell supernatant alone were not inhibited by PGE_2. These cells appear to be a distinct population that can differentiate into ISC in response to factors present in the T cell supernatants without the requirement for additional signals conveyed by SA. Moreover, they appear to be completely resistant to the inhibitory effects of PGE_2 or to PGE_2 in the presence of SA. Furthermore, anti-μ antibody did not facilitate inhibition of these cells by PGE_2. T supernatant-stimulated B cells were not only resistant to inhibition by PGE_2, but the generation of ISC from them was frequently augmented by the presence of PGE_2. The explanation for this finding is unclear but it appears to be mediated by an effect of PGE_2 directly on the responding B cell, inasmuch as it was observed with cells prepared from LME-treated PBM that had thus been rigorously depleted of Mϕ. Moreover, since the B cells were not significantly contaminated with T cells, modulation of suppressor T cell function as has been suggested to occur in PWM-stimulated cultures of T and B cells (13) did not appear to explain this observation. PGE_1 and other cyclic AMP (cAMP)-elevating agents have been shown to cause differentiation of a murine Abelson virus-induced pre-B cell lymphoma as evidenced by increased Fc-receptor expression (47). Whether a similar prostaglandin-mediated cAMP-dependent maturation of this subpopulation of human B cells to ISC explains this finding is currently under study. The importance of alterations in cyclic nucleotide levels in the activation of human B cells is suggested

by the finding that PWM induces the increase of both cyclic guanosine monophosphate and cAMP in human B cells with the former but not the latter inhibited by anti-μ (48). Additional confirmation of the potential complexity of the effect of PGE_2 on B cell function is the finding that further differentiation of murine B cells as detected by the expression of a surface plasma cell antigen appears to be inhibited by inducers of cAMP (49). The differing sensitivities of various stages of B cell activation to modulation by PGE or changes in cAMP is emphasized by the finding that PGE_1 or dibutyryl cAMP enhanced antibody production by primed rabbit B cells when added at the initiation of culture, but decreased antibody production when added >24 h later (50). Thus, the various effects of PGE_2 observed on the differentiation of human B cells in response to T cell supernatants or SA and T cell supernatants may reflect differences in the stages of maturation of the respective B cell precursors.

The mechanism of PGE_2-mediated inhibition of the generation of ISC from SA- and BCDF-stimulated B cells has not been clearly delineated. It is important to note, however, that PGE_2 did not need to be present from the initiation of culture to cause inhibition. Thus, PGE_2 could be added at any time during the culture and cause inhibition of the number of ISC observed within 24 h of subsequent culture. Indeed, the initial ISC generated and observed within 3 d of the start of culture appeared to be resistant to the effects of PGE_2. Two features of this system are useful in interpreting these results. First, the ISC generated between days 3 and 7 of culture are not stable terminally differentiated plasma cells, but rather rapidly dividing Ig-secreting lymphoblasts (51). In addition, PGE_2 has been shown to cause significant inhibition of proliferation of B cells stimulated by SA plus BCGF-containing T cell supernatants although it has no effect on initial activation (27). In this system, inhibition of B cell proliferation was noted even when PGE_2 was added to culture after initial B cell division. These results suggest the possibility that the mechanism of action of PGE_2 may involve inhibition of growth of SA-activated and rapidly proliferating ISC. Thus, PGE_2 produced at inflammatory sites may play an important role in regulating on-going antibody responses.

Previous reports have suggested that one additional action of PGE_2 is to inhibit the regulatory influence of suppressor T cells (22), although this has not been uniformly found. Thus, in different systems it has been claimed that PGE_2 can either inhibit suppressor T cell function (52) or stimulate suppressor T cells that limit IL-2 production (43), antibody formation induced by trinitrophenyl-Ficoll in cultures of cells from elderly subjects (53), or concanavalin A-induced suppressors with nonspecific inhibitory effects (54). In the experiments reported here, effects of PGE_2 on suppressor cell function were not examined in detail. Although it was possible that the induction of suppressor cells explained the limitation of BCDF production caused by PGE_2, a contrasuppressive effect of PGE_2 was not routinely observed. However, it is clear that in other systems an inhibitory effect on suppressor cell function may also be an important immunomodulatory effect of PGE_2 on antibody formation (22, 55).

As a result of in vitro studies, it has become apparent that the effect of PGE_2 on antibody responses may be complex, determined by the interplay of a number of distinct, often opposing physiologic processes. The relationship of these various influences may contribute to the disparate influence of inhibitors of cyclooxygenase activity observed on various specific antibody responses in intact animals or humans. Thus, inhibitors of prostaglandin biosynthesis may augment the antibody response to sheep erythrocytes in mice (28). In humans, however, inhibitors of cyclooxygenase activity do not uniformly cause an increase in all antibody levels. Administration of these agents has, for example, been reported to augment secondary antibody responses to viral antigens while having no effect on primary responses (29). During various pathologic conditions, the relationship between the regulatory mechanisms controlling the magnitude of antibody responses may be altered and thus lead to differential effects of locally produced PGE_2. For example, the local production of large concentrations of PGE_2 by rheumatoid synovium (56, 57) has been suggested to lead to tonic inhibition of suppressor T cell function (22) with resultant local production of Ig and rheumatoid factor. In this circumstance, administration of cyclooxygenase inhibitors decreases titers of circulating rheumatoid factor, apparently by a permissive effect on suppressor T cell activity (58). Thus, PGE_2 may augment or inhibit antibody production in humans depending on the nature of the response, the regulatory influences involved in its control, and the derangements in control mechanisms caused by various pathologic influences.

In summary, Mϕ-derived PGE_2 at physiologically relevant concentrations has been shown to exert a number of inhibitory influences on the generation of ISC in humans. PGE_2 inhibited the production of BCDF from mitogen-stimulated T cells and suppressed the generation of ISC induced by SA- and BCDF-containing T cell supernatants. These results support the conclusion that PGE_2 may play an important role in regulating the magnitude of antibody responses in vivo.

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