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Involvement of Prostaglandin E_1 in Delayed-Type Hypersensitivity Suppression Induced with Live *Mycobacterium bovis* BCG

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We previously showed the suppression of delayed-type hypersensitivity produced by live BCG-induced, plastic-adhering cells. The present study was undertaken to determine whether prostaglandin was one of the pharmacological mediators involved in this suppression. A high level of prostaglandin E_1 was detected in the culture supernatant fluid of the live BCG-induced, adherent cells. Moreover, prostaglandin E_1 could suppress the macrophage migration inhibition activity of peritoneal exudate cells from BCG cell wall-sensitized mice.

An earlier report of ours (13) showed that delayed-type hypersensitivity (DTH) induced in mice with oil-treated BCG cell walls (BCGCW) is suppressed by a previous massive injection of live BCG. Using the macrophage migration inhibition (MI) test to analyze this suppression, we found that the suppression is generated by plastic-adhering cells induced by live BCG injection. However, up to now, the chemical or pharmacological mediators involved in this suppression have remained unknown.

Recent reports have shown that high levels of prostaglandin E (PGE) are produced by murine, guinea pig, and rabbit macrophages and human monocytes with phagocytic and nonphagocytic stimuli (2, 3, 6, 9, 15, 20). On the other hand, several studies have demonstrated that PGE induces inhibitory effects on the immune response in vitro: PGE_1 and PGE_2 produce inhibition of antigen-induced lymphokine secretion in guinea pigs (5) and also produce inhibition of leukocyte inhibitory factor production with phytohemagglutinin-stimulated human lymphocytes (16). Furthermore, prostaglandin suppresses direct cytolysis (7), hemolytic plaque formation by murine leukocytes (17), and mitogen-induced stimulation of murine (18, 26) or human leukocytes (4, 24).

Basing our study on these reports, we tried to determine whether PGE was produced by suppressor peritoneal cavity macrophage-like cells that had been induced by live BCG injection. Our findings suggest that PGE is a probable mediator of DTH suppression.

Male C3H/HeMs (C3H) mice were used at 4 to 8 weeks of age. The MI test was performed as described in our previous report (13). MI activity was measured by the following formula: MI activity (percent migration inhibition) = [area of migration of peritoneal exudate cells (PEC) with purified protein derivative (50 μg/ml)/area of migration of PEC without purified protein derivative] × 100. Indomethacin and prostaglandins were dissolved in 99.5% (vol/vol) ethyl alcohol at 10 mg/ml and diluted with Eagle' minimum essential medium, which resulted in a final concentration of less than 0.05% ethyl alcohol in the cultures of the MI test. This concentration had no effect on the MI test. PEC from C3H mice injected intravenously with live BCG 3 weeks earlier (L-BCG-PEC) or PEC from normal C3H mice (N-PEC) were cultured in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 100 U of penicillin, 100 μg of streptomycin, and 10% fetal calf serum (GIBCO) for 24 h at 37°C in a 5% CO_2-humidified incubator. The culture supernatant fluids were obtained by centrifugation. PGE_1 and $PGF_{2\alpha}$ in cultured supernatant fluids from L-BCG-PEC or N-PEC were measured. The antisera to PGE_1 and $PGF_{2\alpha}$ were raised in rabbits by the method of Jaffe et al. (12). The PGE_1 antibody exhibited 15.0 and 0.6% cross-reactivities with PGE_2 and $PGF_{2\alpha}$, respectively. The $PGF_{2\alpha}$ antibody exhibited 1.4 and 4.7% cross-reactivities with PGE_1 and $PGF_{1\alpha}$, respectively. These cross-reactivities were determined by radioimmunoassay and by an established method in which dextran-coated charcoal was used to separate the bound antibody from free prostaglandins (11). Extraction of prostaglandins from the sample and their separation were carried out as previously reported by Inagawa et al. (10).

In our previous study (13), DTH suppression by pretreatment with live BCG was analyzed by the MI test. This test revealed the suppressive

TABLE 1. Effect of indomethacin on the MI activity of the mixture of E-PEC and L-BCG-PEC or N-PEC[a]

E-PEC mixed with[b]	Indomethacin concn (μg/ml)	Presence of PPD[c] (50 μg/ml)	Area (cm^2) of migration[d]	MI activity
N-PEC	0	−	2.22 ± 0.09	69.8[e]
		+	1.55 ± 0.38	
N-PEC	10^{-2}	−	2.17 ± 0.22	66.8[e]
		+	1.45 ± 0.19	
L-BCG-PEC	0	−	2.12 ± 0.43	93.3
		+	2.0 ± 0.45	
L-BCG-PEC	10^{-2}	−	2.85 ± 0.17	59.6[f]
		+	1.7 ± 0.18	
L-BCG-PEC	10^{-3}	−	3.1 ± 0.2	51.6[f]
		+	1.6 ± 0.24	

[a] Mice were immunized subcutaneously with 300 μg of oil-treated BCGCW. Four weeks later, their PEC (E-PEC) were obtained.

[b] The inhibitory effects of L-BCG-PEC or N-PEC were tested with E-PEC (1:1 ratio) in medium with or without indomethacin.

[c] PPD, Purified protein derivative.

[d] Significance between the area of migration of PEC with purified protein derivative and that without purified protein derivative was calculated by the Student's t test.

[e] $0.001 < P < 0.01$.

[f] $P < 0.001$.

effect of L-BCG-PEC on the MI activity of PEC from C3H mice immunized with BCGCW (E-PEC). In the present experiments, the two PEC populations were mixed in a ratio of 1:1 and packed into capillary tubes; the cells then migrated in the presence of purified protein derivatives. To examine the involvement of PGE_1 in the MI suppression with L-BCG-PEC, we evaluated the MI activity of the mixture of E-PEC and L-BCG-PEC in the presence of indomethacin, a potent prostaglandin synthesis inhibitor (25). MI activity was observed in a medium containing 10^{-2} or 10^{-3} μg of indomethacin per ml, whereas in the mixture in the control medium without indomethacin, MI activity was not observed (Table 1). These findings suggest that suppressor macrophage-like cells induced by live BCG injection produce prostaglandins.

We next measured the amounts of PGE_1 and $PGF_{2\alpha}$ in a culture supernatant fluid in which L-BCG-PEC had been cultured at 37°C for 24 h in RPMI 1640 medium supplemented with 10% fetal calf serum. The culture supernatant fluid of L-BCG-PEC showed high levels of PGE_1 whereas that of N-PEC did not (Table 2). In addition, a high concentration of PGE_1 appeared in the culture supernatant fluid of plastic-adhering cells in L-BCG-PEC, suggesting that macrophages in L-BCG-PEC may produce PGE_1. The concentrations of $PGF_{2\alpha}$ in the culture supernatant fluids of both L-BCG-PEC and N-PEC were not high.

Finally, we attempted to determine whether PGE has a suppressive effect on MI activity. E-PEC were measured for their MI activity in the presence of PGE_1, PGE_2, or $PGF_{2\alpha}$ (kindly donated by Ono Pharmaceutical Co., Ltd., Osaka, Japan). MI activity was inhibited in the presence of PGE_1 and PGE_2 but not $PGF_{2\alpha}$ (Table 3).

Our findings are consistent with those of Gordon et al. (5), who also found that PGE has a suppressive effect on MI activity. Scott (23) and Kirchner et al. (14) demonstrated that the *C. parvum*-activated macrophages inhibit the mitogen response of mouse spleen T cells and the generation of T cells. Metzger et al. (18) reported that concanavalin A-induced proliferation of murine splenic lymphocytes is partially suppressed by *C. parvum*- or thioglycolate-activated PEC at a PEC-to-lymphocyte ratio of 1:10 to 1.5:10 and that indomethacin reverses this partial suppression. In addition, Grimm et al. (6) reported that spleen macrophages from *C. parvum*-injected mice produce high levels of PGE. Taking these findings into consideration, we conclude that the function of *C. parvum*-induced

TABLE 2. In vitro prostaglandin production in L-BCG-PEC and N-PEC cultures

Expt	No. of cultured cells	Prostaglandin type	Prostaglandin production (ng/ml) in cultures of[a]: L-BCG-PEC	N-PEC
1	10^7/ml (whole PEC)	E_1	16.7 (4.7 × 10^{-8})	2.6 (7.3 × 10^{-9})
		$F_{2\alpha}$	1.6 (4.5 × 10^{-9})	2.3 (6.5 × 10^{-9})
	10^7/ml (adherent PEC)	E_1	21.2 (6.0 × 10^{-8})	3.5 (1.0 × 10^{-8})
		$F_{2\alpha}$	1.3 (3.7 × 10^{-9})	6.7 (1.8 × 10^{-8})
2	10^7/ml (whole PEC)	E_1	19.1 (5.3 × 10^{-8})	0.2 (5.6 × 10^{-10})
		$F_{2\alpha}$	2.5 (7.1 × 10^{-9})	3.8 (1.1 × 10^{-8})

[a] Approximate molar concentrations are given in the parentheses. Results are given as the means of duplicate samples.

suppressor macrophages is closely associated with PGE synthesis. Furthermore, there have been reports that PGE may be a mediator molecule which is produced by suppressor cells (21, 26).

In the present investigation, the MI activity of the mixture of E-PEC and L-BCG-PEC was restored with indomethacin, a prostaglandin synthesis inhibitor, and high levels of PGE_1 were detected in the culture supernatant fluids of plastic-adhering cells in the L-BCG-PEC. These results suggest that suppressor adherent cells (macrophage-like cells) in the PEC that have been induced with live BCG may release the mediator molecule PGE_1, which is involved in the MI suppression of the macrophage-like cells. Thus, evidence points to the possibility that PGE_1 plays an essential role in the negative expression observed in the MI test: live BCG-induced macrophages prevented E-PEC from releasing macrophage MI factor. However, whether such an inhibitory action of PGE_1 in vitro is actually generated in vivo in DTH suppression induced by suppressor macrophages is not clear from the present study.

Many studies have shown that treatment with PGE_1 in vivo for several days results in the inhibition of B- and T-cell functions in vivo or in vitro (1, 7, 22, 24, 27, 28). We previously showed that suppressor adherent cells appear in the peritoneal cavity from at least the 12th day after intravenous live BCG injection on. Therefore, in our experimental system, if PGE_1 produced from L-BCG-PEC is a mediator of this suppressor adherent cell function, we believe that it acts for many days to suppress the DTH, as evidenced by the observation that DTH was not induced by subcutaneous BCGCW injections which had been given as late as 21 days after live BCG treatment. Zurier and Quagliata (29) and Herrin (8), however, reported that PGE_1 treatment of rats or guinea pigs sensitized with Freund complete adjuvant or BCG vaccine markedly enhances the DTH response to purified protein derivatives. In addition, Parnham et al. (19) showed that preincubation of sensitized lymph node cells with PGE_1 leads to enhancement of the response to purified protein derivatives after transfer of these cells into syngeneic recipient rats. The reason that our findings differ from those of the above researchers is unclear. One explanation is that in our experiments, the continuous supply of PGE_1 derived from PEC of mice immunized with live BCG probably resulted in the DTH suppression, contrary to the enhanced responses obtained by temporary treatment or single injection with exogenous PGE_1, as mentioned above. Further studies are necessary to clarify the modulation of DTH with PGE in vivo.

TABLE 3. Inhibitory effect of prostaglandins on MI activity of E-PEC

Expt	Prostaglandin type	Concn (μg/ml)	MI activity (%)[a]
1	E_1	5	97.7[b]
	$F_{2\alpha}$	5	72.0[c]
	Control		75.2[d]
2	E_1	0.1	64.2[c]
	E_1	1	94.7[b]
	E_1	5	91.2[b]
	Control		76.8[d]
3	E_2	0.1	70.6[d]
	E_2	1	110.7[b]
	Control		53.4[c]

[a] MI activity of E-PEC was measured in a medium with PGE_1, PGE_2, or $PGF_{2\alpha}$.

[b] $P > 0.05$.

[c] $P < 0.001$.

[d] $0.05 > P > 0.02$.

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