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Effect of Indomethacin In Vivo on Humoral and Cellular Immunity in Humans

JAMES S. GOODWIN,* DANIEL S. SELINGER, RONALD P. MESSNER, AND WILLIAM P. REED

Department of Medicine, Bernalillo County Medical Center, and Department of Medicine, Albuquerque Veterans Administration Hospital, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131

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We studied the effect of indomethacin on intradermal skin testing and antibody responses in humans. Since we and others have shown that prostaglandins are suppressor cell mediators, it was probable that in vivo inhibition of prostaglandin synthesis might enhance the humoral and/or cellular immune response. Administration of indomethacin (Indocin) in a dosage of 100 mg/day to 15 normal men and women resulted in a significantly increased antibody titer to A-Victoria ($P < 0.025$) as compared with age- and sex-matched controls. There was no difference in titer to A-New Jersey. Since 90% of the subjects had antibody titers to A-Victoria before inoculation, whereas none had detectable titers to A-New Jersey, we interpret this data as suggesting that indomethacin enhances the secondary but not the primary humoral immune response. Indomethacin administration did not alter the intradermal skin test responses.

Prostaglandins (PG) have been implicated as endogenous inhibitors of both humoral and cellular immunity in experimental animals (1). Webb and Osheroff have demonstrated a 20- to 80-fold increase in $PGF_{2\alpha}$ in mouse spleens within 2 min of intravenous injection of sheep erythrocytes (SRBC) (14). Blockade of $PGF_{2\alpha}$ synthesis by pretreatment of the animals with indomethacin, a PG synthetase inhibitor, resulted in an increase in the number of splenic plaque-forming cells subsequently formed against SRBC. Thus $PGF_{2\alpha}$ appears to be an endogenously produced inhibitor of B cell activation. Additional work by Webb's group (15) and others (6-8, 16) implicates PGE_2 as well as $PGF_{2\alpha}$ as an inhibitor of B cell function.

We have recently described a prostaglandin-producing suppressor cell that inhibits human lymphocyte activation by T cell mitogens in vitro (1a). This cell secreted PGE_2 and inhibited phytohemagglutinin (PHA) or concanavalin A induced [^{3}H]thymidine incorporation in normal human lymphocytes. Addition of prostaglandin synthetase inhibitors to the mitogen-containing cultures decreased PGE_2 production to 10% of normal and led to an approximate 50% increase in [^{3}H]thymidine incorporation (J. S. Goodwin, D. S. Selinger, and R. P. Messner, Clin. Res. 25:358A, 1977). When the endogenously inhibited PGE_2 was replaced by comparable amounts of exogenous PGE_2 (ca. 10^{-8} M), [^{3}H] thymidine incorporation returned to normal. This PG-producing suppressor cell appears to be responsible for the defect in cellular immunity seen in Hodgkin's Disease. PHA cultures of lymphocytes from Hodgkin's Disease patients produced fourfold more PGE_2 than cultures of normal lymphocytes (2). Addition of PG synthetase inhibitors eliminated this production and restored the depressed mitogen response of these lymphocytes to normal. Thus, PGE_2 clearly has a role in the modulation of cellular immunity in humans.

The present investigation was undertaken to determine the effect of in vivo PG synthetase blockade on humoral and cellular immunity in normal humans. We hypothesized that inhibition of prostaglandin production in vivo might result in enhanced humoral and cellular immunity.

MATERIALS AND METHODS

Studies of humoral immunity. Thirty healthy males and females aged 26 to 32 served as subjects. Fifteen subjects were administered indomethacin (Indocin, Merck Sharp & Dohme, West Point, Pa.), 25 mg orally four times a day for 12 days. On day 2, all subjects were given bivalent influenza vaccine (Merck, Sharp and Dohme, lot 4895 G, ≥200 CCA units each of A/New Jersey/8/76 Hsw 1 Nsw 1 and A/Victoria/3/75 H3N2 prototype strains). On days 1 and 26, blood was drawn for antibody titers to A-Victoria and A-New Jersey. The titers of the treated group were compared to 15 age- and sex-matched controls. Antibody titers were performed by the hemagglutination-inhibition test (12). All studies involving human subjects were approved by the University

of New Mexico Human Research Committee. Informed consent was obtained from all subjects.

Studies of cellular immunity. In the second study, 10 subjects were given a 5-day course of indomethacin, 25 mg, four times a day. On day 3, intradermal skin tests were placed for mumps, candida, and trychophyton. The area of induration was estimated on day 5 (at 48 h) by measuring the vertical and transverse diameters of induration and multiplying these figures. Each patient served as his own control by also having skin tests while not receiving indomethacin. To minimize any possible booster effect, five of the subjects had control tests 2 weeks before and five 2 weeks after the testing while receiving indomethacin. In this study, blood was drawn for in vitro mitogen studies before the course of indomethacin and again on day 3 of indomethacin administration. The dosage of indomethacin employed in these studies (25 mg, three or four times a day) has been shown to produce plasma levels that vary between 0.5 and 3.0 μg/ml (4). These levels cause 90% inhibition of PG production when added in vitro (2).

Peripheral blood mononuclear cells were separated and cultured with three concentrations of PHA with and without the addition of indomethacin (1 μg/ml, final concentration). The details of this assay have been previously reported (1a). All cultures were done in sextuplicate.

RESULTS

Effect of indomethacin on antibody response to influenza vaccine. Before vaccination none of the subjects or controls had detectable titers (≥1:10) to A-New Jersey, whereas 90% had measurable antibody titers to A-Victoria, with the mean titer between 1:20 and 1:40. This suggests that the antibody responses to A-New Jersey and A-Victoria would represent primary and secondary immune responses, respectively. The data on antibody titers before and after inoculation is presented in Table 1. Compared to age- and sex-matched controls, the indomethacin-treated group had a larger titer rise to A-Victoria ($P < 0.02$). There was no difference in titer rise to A-New Jersey.

Effect of indomethacin on response to intradermal testing. The results of this experiment are shown in Table 2. There was no significant change in area of induration with any of the skin tests as a result of indomethacin administration. Eight skin tests increased with indomethacin administration, nine decreased and 13 remained the same (were within ±10% of each other).

Effect of indomethacin in vivo on mitogen response of lymphocytes. The results of this experiment are presented in Table 3. Concurrent in vivo indomethacin administration did not influence the response to PHA in vitro. When indomethacin was added to the PHA cultures in vitro, we saw an enhancement of [^{3}H]-thymidine incorporation as previously reported (Goodwin et al., Clin. Res. **25**:358, 1977). This enhancement was similar whether or not the subject was taking indomethacin orally.

TABLE 2. *Effect of indomethacin administration on skin testing*

Indomethacin	Test antigen		
	Candida[a]	Mumps[a]	Trychophyton[a]
+	275 ± 96	450 ± 303	37 ± 26
−	196 ± 107	552 ± 311	57 ± 54

[a] Area of induration in square millimeters; mean and standard error for 10 subjects with and without concurrent administration of indomethacin.

TABLE 3. *Effect of in vivo indomethacin administration on in vitro PHA response*

In vivo indomethacin	Optimum PHA	% Increase in cpm with in vitro indomethacin
+	11,154 ± 3,205[a]	49 ± 8
−	13,530 ± 3,589	44 ± 8

[a] Data given as counts per minute of optimal PHA response for eight subjects, mean + standard error, before and during indomethacin administration.

TABLE 1. *Effect of indomethacin on humoral immune response*

Strain	No. of subjects					Mean increase in tube dilutions	Statistical significance
	Total	Titer rise					
		None	2×	4×	≥8×		
A-New Jersey (primary response)							
No treatment	15	3	3	2	7	2.5 ± 0.5	{Not significant
Indomethacin	15	2	3	4	6	2.2 ± 0.6	
A-Victoria (secondary response)							
No treatment	15	6	7	2	0	0.7 ± 0.2	{$P < 0.025$
Indomethacin	15	3	6	3	3	1.5 ± 0.4	

DISCUSSION

From the above experiments it appears that indomethacin is capable of enhancing the humoral immune response in humans. The enhancement of the humoral immune response in humans parallels the results of Webb and Osheroff, who showed that indomethacin enhanced B cell activation in mice (14). In both cases, indomethacin presumably acts by inhibiting the synthesis of PG, perhaps in a suppressor T cell population (13, 14). The dose given in this current study has been shown to cause significant in vivo inhibition of PG synthesis in humans (3).

The fact that indomethacin enhanced the antibody response to A-Victoria and not to A-New Jersey suggests that endogenously produced PG exerts a negative feedback on the secondary, but not the primary, immune response in man. Smith et al. found no effect of PGE on immunoglobulin production in pokeweed mitogen-stimulated cultures of human lymphocytes (11), whereas several groups have demonstrated that PGE inhibits the murine hemolytic plaque formation response to SRBC in vivo (7, 16) and in vitro (6, 7). In none of these studies was the effect of PGE on the secondary humoral immune response examined. It has been suggested that suppressor cells play a larger role in secondary than in primary humoral immunity (13). Perhaps the dose of indomethacin we used in humans was sufficient to allow detection of changes in only the most sensitive parameter of humoral immunity.

The failure of in vivo indomethacin to cause enhancement of skin test reactivity in normal humans is in marked contrast to its enhancement of cell-mediated immunity in vitro (1a). The enhancing effect of indomethacin seen in vitro in cultures of normal lymphocytes is evidently too subtle to be noted in vivo with the techniques employed here in a relatively small number of subjects. These results also differ from the in vivo effects of indomethacin in subjects with depressed cellular immunity. Other studies in our laboratory indicate that in vivo indomethacin administration to a patient with adult combined immunodeficiency can partially restore the T cell defect (J. S. Goodwin, D. S. Selinger, A. D. Bankhurst, and R. P. Messner, in preparation). This is confirmed by a larger enhancing effect of PG synthetase inhibitors in vitro in mitogen cultures of lymphocytes from patients with this disorder. This observation, along with the data presented above, suggests that PG plays an important role in the regulation of humoral and cellular immunity in humans and that in vivo administration of PG synthetase inhibitors can alter the immune response.

Robinson and his co-workers reported in 1968 that pretreatment of mice with indomethacin enhanced their ability to resist some infections (9), but this result aroused little attention, presumably because the mechanism of action of indomethacin was not known at that time. Similar effects might be found in humans, especially in subjects with immune deficiencies. Further studies of the effects of in vivo indomethacin in human disease states associated with depressed humoral and/or cellular immunity appear warranted. Such studies should proceed with caution, however, for indomethacin treatment has been associated rarely with depression of granulocytes and platelets (5).

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