EFFECT OF EXOGENOUS PROSTAGLANDIN E_2 ON DIFFERENT STAGES OF FORMATION OF THE HUMORAL IMMUNE RESPONSE

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Intensive studies of both biologically active substances known as prostaglandins (PG), one of the producers of which is the mononuclear phagocytes [10], have recently been undertaken. Exposure to various factors (antigens, mitogens, endotoxin, and so on) has been shown to stimulate the formation of PG of the E group (PGE) by peripheral blood monocytes and peritoneal macrophages [7, 10, 13]. There is reason to suppose that these factors play a definite role in the control of the primary immune response [14]. However, the mechanisms of the effect of PGE on the various stages of the antibody formation process, especially *in vivo*, remain unexplained.

The writers showed previously that "antigenic blockade" of the mononuclear phagocytic system, caused by injection of sheep's red blood cells (SRBC) into mice 24 h before sublethal irradiation, results in a dose-dependent increase in the number of endogenous splenic colonies (ESC) of hematopoietic cells, formed by hematopoietic stem cells [2].

Meanwhile we know from the literature that injections of PGE_2 increased the proportion of polypotent hematopoietic stem cells (CFU-S) in the S phase of the cell cycle [8], and that ESC are formed by stem cells which, at that given moment of irradiation, are in the stage of DNA synthesis [3].

This paper gives the results of a study of the effect of exogenous PGE_2 on the different stages of immunogenesis [1], including the action of the factor on the number of ESC formed by the CFU-S in the spleen of sublethally irradiated mice, and also the effect of PGE_2 on the stages of differentiation of B lymphocytes and antibody-forming cells (AFC).

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 mice aged 2-3 months were obtained from the Stolbovaya nursery of Laboratory Animals, Academy of Medical Sciences of the USSR. SRBC, used as the antigen, were injected intravenously into the mice in a dose of $2 \cdot 10^8$. PGE_2, supplied by the experimental sector of the Experimental-Technical Base, Academy of Sciences of the Estonian SSR, was used in doses of 10^{-6}, 10^{-5}, and $5 \cdot 10^{-4}$ M subcutaneously 24 h and 2 h before immunization of the animals. Indomethacin, which inhibits PG synthesis [12], was generously provided by Dr. T. Y. Shen (New Jersey, USA), and was injected intraperitoneally into the mice in doses of 10^{-6}, 10^{-5}, and $5 \cdot 10^{-4}$ M at the same time. The number of AFC in the spleen of the mice was determined on the 4th day after injection of antigen by a modified method [4]. To study the effect of PGE_2 and indomethacin on the formation of ESC of hematopoietic cells, treatment with the preparations was given 24 and 2 h before irradiation of the mice in a dose of 500 R. The mice were killed 9 days after irradiation and the number of ESC in their spleens was counted. Irradiation was given on the RUP 150/30-1 apparatus (dose rate 50 R/min, tube voltage 180 kV, current 10 mA, filter Al-3).

The significance of differences between arithmetic mean values of the parameters studied was assessed by Student's t test.

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TABLE 1. Number of AFC in Spleen and Number of Splenic Colonies of Hematopoietic Cells in (CBA × C57BL/6)F_1 Mice after Two Injections of PGE_2 and Indomethacin (M ± m)

Experimental conditions	Dose of preparation, M	Number of ESC	Number of nucleated spleen cells × 10^6	Relative No. of AFC/10^6 nucleated spleen cells	Absolute number of AFC in spleen
Control	—	6,1±1	195±18	152±15	28507±2750
PGE_2	10^{-6}	7,3±1	224±17	257±33*	48662±4900*
	10^{-5}	13,5±3*	157±11	238±28**	35756±3820*
	$5\cdot10^{-4}$	10,8±4	212±10	344±53*	49640±8500*
Indomethacin	10^{-6}	7,1±1	240±20	197±20	45321±4550*

Legend. PGE_2 and indomethacin injected twice, 24 and 2 h, respectively, before immunization of mice with SRBC or before irradiation in a dose of 500 R. Here and in Table 2 asterist indicates differences significant at $P < 0.05$.

TABLE 2. Number of AFC in Spleen of Immune (CBA × C57BL/6)F_1 Mice after a Single Injection of PGE_2 and Indomethacin 24 h before Estimation of Number of Antibody Producers (M ± m)

Experimental conditions	Dose of preparation, M	Number of nucleated spleen cells × 10^6	Relative number of AFC per 10^6 nucleated spleen cells	Absolute number of AFC in spleen
Control	—	214±19	100±17	25 223±2200
PGE_2	10^{-6}	273±25	150±19	38 175±3720*
	10^{-5}	240±17	118±12	29 959±3400
	$5\cdot10^{-4}$	247±18	82±9	21 221±2040
Indomethacin	10^{-6}	215±20	112±10	25 899±1510
	10^{-5}	255±20	109±10	29 862±1950
	$5\cdot10^{-4}$	328±29*	120±18	31 623±4300

EXPERIMENTAL RESULTS

It will be clear from Table 1 that two injections of PGE_2 24 and 2 h before immunization of (CBA × C57BL/6)F_1 mice with SRBC led to a twofold increase in the relative and absolute numbers of AFC in the spleen compared with the control values. After two injections of indomethacin, an increase in only the absolute number of AFC per spleen was observed at this same period.

A single injection of PGE_2 into mice in a dose of 10^{-6} M on the 3rd day after immunization, i.e., in the productive phase of antibody formation, caused an increase in the absolute number of AFC per spleen, whereas injection of PGE_2 in doses of 10^{-5} and $5\cdot10^{-4}$ M had no effect on antibody production (Table 2). Injection of indomethacin at these same times caused no significant changes in antibody formation.

The experimental results showed that injection of PGE_2 in a dose of 10^{-5} M 24 h before sublethal irradiation of mice caused an increase in the number of ESC of hematopoietic cells formed by hematopoietic stem cells (Table 1). Injection of PGE_2 in doses of 10^{-6} and $5\cdot10^{-4}$ M or injection of indomethacin at the same period did not affect the number of ESC.

Data in the literature on the action of PGE_2 on antibody formation *in vivo* and *in vitro* are scanty. It has been shown that inhibition of endogenous PG synthesis by indomethacin in a dose of 10^{-6} M in Mishell–Dutton culture *in vitro* and injection of the drug into mice *in vivo* 24 and 2 h before injection of SRBC stimulate the immune response [14]. This can be taken as evidence of the suppressive effect of PGE on antibody formation. An increase in antibody production also has been found after injection of PGE_2 into mice in doses of $5\cdot10^{-7}$ to $5\cdot10^{-6}$ M simultaneously with SRBC *in vivo* or after addition of PGE_2 in a dose of $5\cdot10^{-9}$ M to the culture *in vitro* [9]. The results of the present experiments indicate that injection of PGE_2, in the concentrations tested, or of indomethacin into animals on two occasions 24 and 2 h before injection of antigen leads to an increase in the number of AFC. In all probability the effect of PG depends on the dose of the exogenous preparation and on the endogenous PGE level in the lymphoid organs responsible for formation of the immune response.

There is evidence that PGE_2 exerts its effect not only when injected into mice before immunization with antigen, but also when injected in the later stages of antibody formation. In particular, antibody production has been shown to be depressed when PGE_2 is injected 2, 4, and 6 h before estimation of the number of AFC [14]. The results now obtained indicate that PGE_2, if injected into animals 24 h before estimation of the number of AFC, has a relatively weak action: An increase in the absolute number of AFC was observed only after injection of PGE_2 in a dose of 10^{-6} M. The effect observed was evidently due to the influence of PGE_2 on the cyclic AMP level in the AFC population [6].

The increase in the number of ESC of hematopoietic cells discovered after injection of PGE_2 into animals in a dose of 10^{-5} M in the present experiments agrees with results obtained by other workers concerning the effect of this factor on proliferation and differentiation of CFU-C *in vivo* and *in vitro* [5, 8, 11].

It can thus be postulated that PGE plays an important role at all stages of immunogenesis: from CFU-S to the AFC population. In all probability, the unequal changes in the relative and absolute numbers of AFC under the influence of PGE_2 and indomethacin, found in the present experiments, reflect the diversity of cells concerned with the process of formation of AFC clones, the normal functioning of which is evidently under endogenous PG control. However, the sensitivity of different cells to PGE may vary quantitatively, so that their contribution to the process of antibody formation will differ.

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