

The Regulatory Effect of Macrophages on the *in Vitro* Synthesis of IgE

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The effects of indomethacin or macrophage-depletion on the *in vitro* unstimulated and pokeweed mitogen (PWM)-induced synthesis of IgG and IgE were compared. The effect on the unstimulated synthesis of IgG and IgE was similar, namely, no effect due to indomethacin and enhancement due to macrophage depletion. In contrast, the effect on PWM-induced synthesis was dissimilar. Macrophage depletion enhanced IgG but inhibited IgE production. The effect of indomethacin paralleled that of macrophage depletion suggesting the involvement of prostaglandins. It seems that the regulatory effect of macrophages is different in the IgE system compared with the IgG system.

Key words: IgE synthesis; indomethacin; macrophages.

Accepted for publication 27 October 1984

Increased *in vivo* IgE synthesis is the hallmark of allergy. Mechanisms involved in the enhanced production of IgE are still obscure. Recently, *in vitro* cultures of peripheral blood mononuclear cells have elucidated some of the mechanisms which play a role in the synthesis of IgG, IgM and IgA. Several of these studies, and many others, have established the major regulatory role of macrophages in the production of these immunoglobulins (1, 2, 4, 5, 9).

The aim of the present study was to find out whether macrophages play a similar role in the synthesis of IgE.

PATIENTS AND METHODS

The study group included eight healthy non-atopic individuals. Their mean serum IgE was 30 IU/ml.

Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradients. Following washing, the cells were incubated in RPMI-1640 (GIBCO) supplemented with 20% fetal calf serum (FCS: Flow Laboratories), 2

2 mM L-glutamine, 100 μ /ml streptomycin, and 2.5 mM N-2-hydroxyethylpiperazine-N-2 ethanesulfonic acid (HEPES) buffer. Cultures, 2 ml each, were performed in 16 mm flat-bottomed tissue culture wells (Linbro 24 wells, Flow Laboratories) with a cell concentration of 1.0×10^6 /ml in the absence or presence of pokeweed mitogen (PWM) (GIBCO), final dilution 1:100. Cell viability was tested by the trypan blue method. Cultures were run in duplicate and harvested on day 7. Supernatants were collected and each tested for both IgE and IgG.

Three sets of cultures from each individual were run in parallel. The first as described above. The second set included indomethacin, whereas the third was depleted of macrophages. Indomethacin was added on day -0, in a final concentration of 3×10^{-6} M. Macrophages were removed prior to culture by the plastic adherence technique (12). 5×10^6 PBMC in RPMI + 10% FCS were incubated in 60 mm petri dishes (Falcon) at 37°C. The dishes were pre-coated with autologous serum. The non-adherent macrophage-depleted cells

were removed with cold medium after 1 h of incubation. Macrophages were identified by non-specific esterase staining and phagocytosis of latex particles. The number of macrophages was decreased following adherence from 11–17% to 4–5%.

IgE was determined by a radioimmunoassay as described by Saxon & Stevens (11). Anti-IgE antibody was obtained from Meloy (Springfield, VA). Standard curves were established by IgE obtained from Pharmacia (Prist kits). IgE levels as low as 50 pg/ml could be detected by this assay. IgM, IgG or light chains were not detected by this assay even when used in mg quantities. The binding of ^{125}I -anti-human IgE to the wells during the second incubation could be inhibited by purified human IgE but not by IgG, IgM or light chains. IgG was determined by a radioimmunoassay as described by Ohta et al. (8).

Statistical analysis was done by the paired t-test.

RESULTS

Effect of PWM, indomethacin and macrophage depletion on IgG synthesis (Fig. 1)

(The given numbers refer to mean $\pm$ SD)

PWM induced a marked increase in IgG synthesis (1223 ± 894 vs. 211 ± 92 ng/ml). Indomethacin had no effect on the unstimulated synthesis of IgG; however, it significantly enhanced the synthesis induced by PWM (2080 ± 1091 vs. 1223 ± 894). Depletion of macrophages enhanced both the unstimulated and the PWM-induced production of IgG (1577 ± 715 vs. 211 ± 92 and 4139 ± 2464 vs. 1223 ± 894).

Effect of PWM, indomethacin and macrophage depletion on IgE synthesis (Fig. 2)

(The given numbers refer to mean $\pm$ SD)

PWM induced a marked increase in IgE synthesis (2204 ± 2655 vs. 249 ± 145 pg/ 2×10^5 cells). Indomethacin had no effect on either the unstimulated or the PWM-induced synthesis of IgE. Depletion of macrophages enhanced the

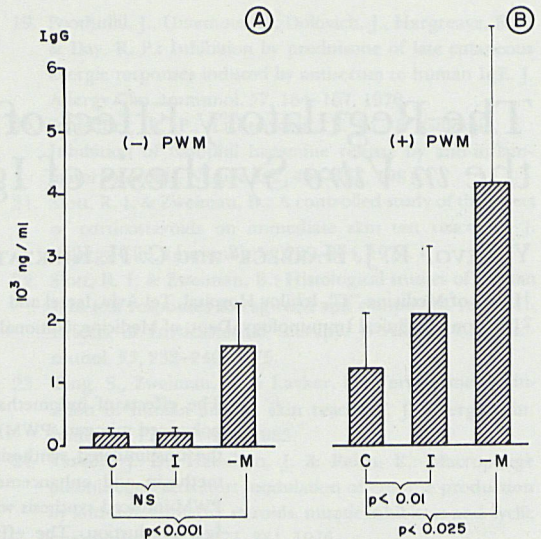


Fig. 1. Effect of indomethacin (I) and macrophage depletion (-M) on IgG synthesis.

A: Unstimulated production (-PWM).

B: PWM-induced production (+PWM).

unstimulated synthesis (396 ± 151 vs. 249 ± 145) but decreased the PWM-induced (695 ± 467 vs. 2204 ± 2655).

DISCUSSION

The development of culture systems in which the *in vitro* synthesis of immunoglobulins (Ig) can be induced and studied have elucidated some of the mechanisms and cell-cell interactions involved. Up to very recently, the only Ig classes studied were IgM, IgG and IgA. Some of the findings pointed to the enhancing effect of various mitogens and to the regulatory role of T-helpers, T-suppressors and macrophages.

The mitogen most commonly used has been the PWM. It is a T-dependent mitogen which reproducibly enhances the *in vitro* synthesis of IgM, IgG and IgA (2, 4, 9). Its effect on IgE is somewhat controversial. However, several groups of investigators have presented data supporting the enhancing effect of PWM on the production of IgE (7, 11, 15).

The present study confirms that PWM promotes the synthesis of both IgG and IgE (Figs. 1 and 2).

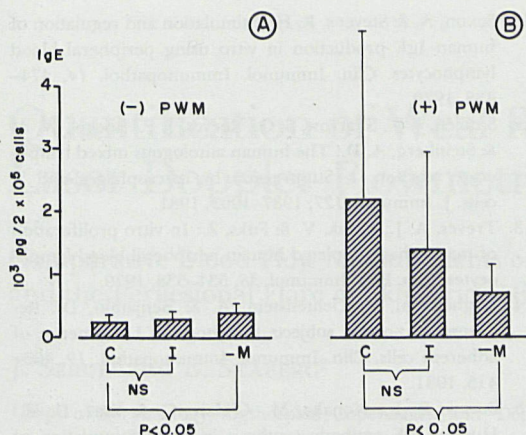


Fig. 2. Effect of indomethacin (I) and macrophage depletion (-M) on IgE synthesis.

A: Unstimulated production (- PWM).

B: PWM-induced production (+ PWM).

Macrophages were found to have a dual effect on the synthesis of IgM, IgG and IgA. Partial removal resulted in increased synthesis, whereas complete removal had a severe inhibitory effect (1). Similar results were obtained in another *in vitro* system measuring primary anti-DNP antibody production in man (6). Macrophages seem to have the same dual effect on the proliferative response of lymphocytes to various mitogens (13, 14). The suppressive effect of macrophages has been attributed to the secretion of inhibitory factors in which prostaglandins seem to play a major role. In fact, the enhancing effect of "partial depletion of macrophages" could be reproduced by the addition of indomethacin to the culture media (10, 14).

The regulation of IgE seems to be dissociated from that of IgG. Previous findings suggested that the IgE antibody response is regulated not only by antigen-specific mechanisms but also by isotype-specific mechanisms. Some of these depend on IgE-specific suppressor or helper, T-cells, or T-derived factors (3). Possibly, macrophages also take part in the isotype-specific regulation. Therefore, we wanted in the present study to investigate the effects of macrophage depletion and indomethacin on the *in vitro* synthesis of IgE in comparison with that of IgG. We have con-

firmed that in the IgG system macrophage removal increases both the unstimulated and the PWM-induced IgG synthesis. Indomethacin increased only the PWM-induced production.

The effects on the IgE system were very different. Macrophage depletion resulted in a minimal but significant enhancement of the unstimulated production of IgE, but had a marked inhibitory effect on the PWM-induced synthesis. Indomethacin had no effect on the unstimulated production and an inhibitory, though not significant, effect on the PWM system.

Since IgE production in the absence of PWM was minimal to start with, the most striking difference between the IgE and the IgG system relates to the effect of macrophage depletion on the PWM-induced synthesis. This procedure resulted in a marked inhibitory effect on IgE in the presence of a marked enhancing effect on IgG.

These results suggest that the two systems, IgG and IgE, have different requirements with regard to the accessory function of the macrophage. Since macrophages have a dual effect on Ig synthesis, it is possible that IgE synthesis as compared with that of IgG is more dependent on macrophage function, i.e., optimal IgE synthesis requires a higher macrophage: lymphocyte ratio.

In our study the effect of indomethacin always paralleled that of macrophage removal, though to a lesser extent. Therefore, it is possible that the regulatory effects of the macrophages on both IgG and IgE synthesis are mediated by prostaglandins.

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