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Cyclic AMP and Immune Responses: Identification of Prostaglandin E-Producing Cells in Mouse Spleens

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Previous work has shown that normal mice respond to the intravenous injection of sheep erythrocytes (SRBC) with a transient increase in splenic cyclic AMP peaking after 2 minutes, and this cyclic AMP response has been shown to be due to prostaglandins (PGs) released from antigen-specific cells. In the present study, the cell type responsible for PG production in response to antigen was determined using a fluorescence-activated cell sorter (FACS) and an electron microscope. C57B1/6 mice injected with SRBC showed a 2% increase of PGE-producing spleen cells relative to controls. In control mice injected with phosphate-buffered saline (PBS), 19% of the spleen cells produced PGE. An electron microscopic study of PGE-producing cells sorted on FACS showed that they were small lymphocytes and plasma cells. In addition, nude mice also increased the number of PGE-producing spleen cells in response to antigen. These findings suggest that lymphocytes involving at least B-cells respond to antigen with an increase in PGE production.

(Key Words: Prostaglandin E, B-Lymphocyte, Fluorescence-Activated Cell Sorter, Nude Mouse)

INTRODUCTION

It has been established that an intravenous injection of sheep erythrocytes (SRBC) into mice increases the level of endogenous cyclic adenosine monophosphate (cAMP) in the spleen at an early stage of the immune response (12, 21). This increase in splenic cAMP has been shown to be antigen-specific and to result from a two-step reaction (13). First, antigen reacts with antigen-specific cells to release mediator(s) and, second, the mediator (s) reacts with mediator-sensitive cells to activate adenylate cyclase (AC) and cause an increase in cAMP. The mediator(s) is thought to be prostaglandin (PG) because PGs mimic the cAMP response to antigen (17) and pretreatment of mice with indomethacin, an inhibitor of PG synthetases, suppresses the cAMP response to antigen (21).

We have recently shown that AC responding cells in antigen (SRBC) -stimulated mouse spleen are lymphocytes and macrophages (16). In the present study, we examined the cells producing PG by means of flow microfluorometry and electron microscopy. Our results strongly suggest that

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lymphocytes involving at least B-cells respond to antigen with an increase in PGE production.

MATERIALS AND METHODS

Mice

Male C57Bl/6 mice, obtained from Charles River (Japan) and female normal and nude (nu/nu) BALB/c mice obtained from CLEA Japan Inc. were used at 6 to 8 weeks of age.

Antigen

SRBC were obtained from Nippon Bio-Test Laboratories and stored in Alsever's solution at 4°C. Before use, the SRBC were washed four times with sterile phosphate-buffered saline (PBS).

Antisera

Rabbit anti-prostaglandin E serum (RAPGE) and fluorescein isothiocyanate conjugated goat anti-rabbit IgG serum (FITC-GARIgG) were obtained from Miles Research Products, Elkhart, Ind., USA and Hyland Laboratories, Cal., USA respectively.

Preparation of spleen cells

Mice were injected intravenously with either 5×10^8 SRBC or 0.1 ml of PBS. The mice were sacrificed by cervical dislocation after two minutes when the cAMP level of SRBC-stimulated mouse spleen reached a maximum (12, 21). Their spleens were immediately removed and squeezed with two glass slides in cold PBS. After washing, spleen cells were depleted of erythrocytes by ammonium chloride lysis, and then washed and resuspended at 1×10^7 cells/ml in RPMI 1640 containing 10% fetal calf serum (RPMI-FCS).

Fluorescein labeling of spleen cells

Labeling was performed as previously described (18). 1×10^7 spleen cells were incubated with 0.1 ml of RAPGE (dissolved in 5 ml of PBS) for 10 min at room temperature. After washing, the cells were further incubated with 0.1 ml of FITC-GARIgG (0.5 mg of protein/ml) for 10 min at room temperature. The cells were then washed and resuspended at a concentration of 5×10^6 cells/ml in RPMI-FCS.

Analysis of spleen cells with FACS

Analysis with a fluorescence-activated cell sorter (FACS-II, Becton Dickinson Electronics Laboratory, Mountain View, Cal., USA) was performed essentially as described by Loken and Herzenberg (10). The cells were passed through the FACS at a rate of 2,000—3,000 cells/sec and the intensity of fluorescence was recorded for each individual cell on the pulse height analyzer. The level of background fluorescence was determined by analyzing spleen cells treated with FITC-GARIgG as a negative control. Thus, the percentage of fluorescein-labeled (PGE^+) cells was determined by counting the number of cells emitting fluorescent signals above the background and by dividing it by the total number of viable cells examined. Light-scattering signals distinguished live from dead cells. The calculation by the FACS analyzer was based on the analysis of 10^4 individual viable cells.

Cell sorting

Cell sorting was performed as detailed by Jones et al (8). Spleen cells were passed through the FACS at a rate of 4,000—5,000 cells/sec and sorted into fluorescein-labeled (PGE^+) and unlabeled (PGE^-) populations. The two populations were collected in separate tubes on ice and used in subsequent electron microscopy.

Electron microscopy

Sorted cells in both populations were centrifuged at 800 rpm for 3 min. The cells were resuspended in 1 ml of 0.01 M PBS and placed in polyethylene capsules (Beem Capsules, L.K.B. Instrument Co., Rockville, Md., USA). Following centrifugation, the cells were fixed for 30 min in 1 ml of 1.5% glutaraldehyde buffered with phosphate. The fixed cells were washed overnight in PBS with a gentle rotating motion and fixed for 30 min in 1 ml of PBS containing 1% OsO_4. After washing in PBS, the cells were dehydrated in graded ethanol solutions for 10 min. Finally, the ethanol was drained off and epoxy resin (Epon 812) was added to the capsules and polymerized at 60°C. Ultrathin sections were stained with uranyl acetate and lead citrate. Electron micrographs were taken with a JEOL 100C electron microscope using an accelerated voltage of 80 KV.

RESULTS

Analysis of PGE^+ cells using the FACS

Fluorescein-labeled spleen cells from mice injected with SRBC or PBS were analyzed on the FACS. Table 1 shows the results of five experiments on the PGE^+ cells in C57Bl/6 mouse spleens. The mice injected with SRBC showed about a 2% increase of PGE^+ spleen cells compared with control mice injected with PBS. Fig. 1 shows a typical fluorescence profile of PGE^+ spleen cells. While the PGE^+ cells covered a wide range of fluorescence intensities, the population of PGE^+ cells of relative low to medium fluorescence increased after SRBC stimulation. This finding was also confirmed by a contour display method on FACS (data not shown).

Electron microscopy of sorted cells

A PGE^+ cell population and a population of PGE^- cells of negative fluorescence were sorted from spleen cells of mice injected with SRBC or PBS and collected on the FACS. Sorted PGE^+ and PGE^- cells were then morphologically observed using an electron microscope. Sorted PGE^+ cells from SRBC-injected mice were small lymphocytes (Fig. 2-a) and plasma cells (Fig. 2-b), in which the former was predominant. In PBS-injected mice, the PGE^+ cells were lymphocytes and no plasma cells were detected. Sorted PGE^- cells from SRBC- or PBS-injected mice contained lymphocytes, macrophages, granulocytes, etc.

Analysis of PGE^+ cells from nude mouse spleens

To determine the type of PGE^+ cells, a similar experiment was performed using nude mice which lack functional T-lymphocytes. As shown in Table 2, nude mice had PGE^+ cells to the same extent as normal mice (data not shown) of the same genetic background. The relative number of PGE^+ cells in nude mice significantly increased in response to SRBC.

Table 1 Percentages of PGE^+ spleen cells from C57B1/6 mice

Experiment	% Labeled cells	
	SRBC[a)]	PBS
1	22	20
2	17	17
3	16	15
4	34	30
5	18	14
	21.4[b)]	19.2

a) Three mice per group
b) Arithmetical mean

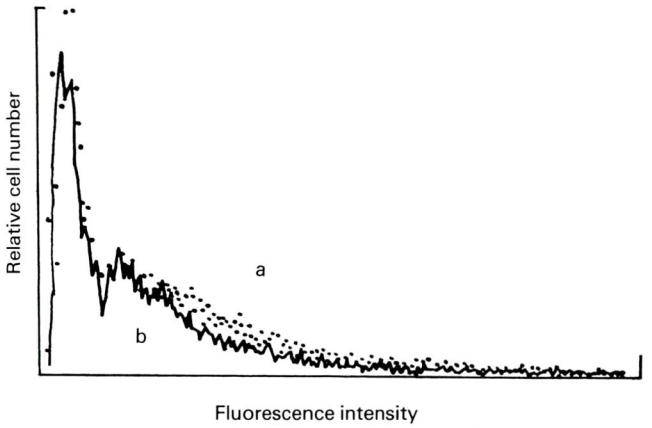

Fig. 1 Fluorescence profiles of PGE^+ spleen cells from SRBC(a)- or PBS(b)-injected C57B1/6 mice. The sharp peak on the left represents the background fluorescence.

Table 2 Percentages of PGE^+ spleen cells from BALB/c nude mice

Experiment	% Labeled cells	
	SRBC[a)]	PBS
1	19	12
2	26	17
	22.5[b)]	14.5

a) Two mice per group
b) Arithmetical mean

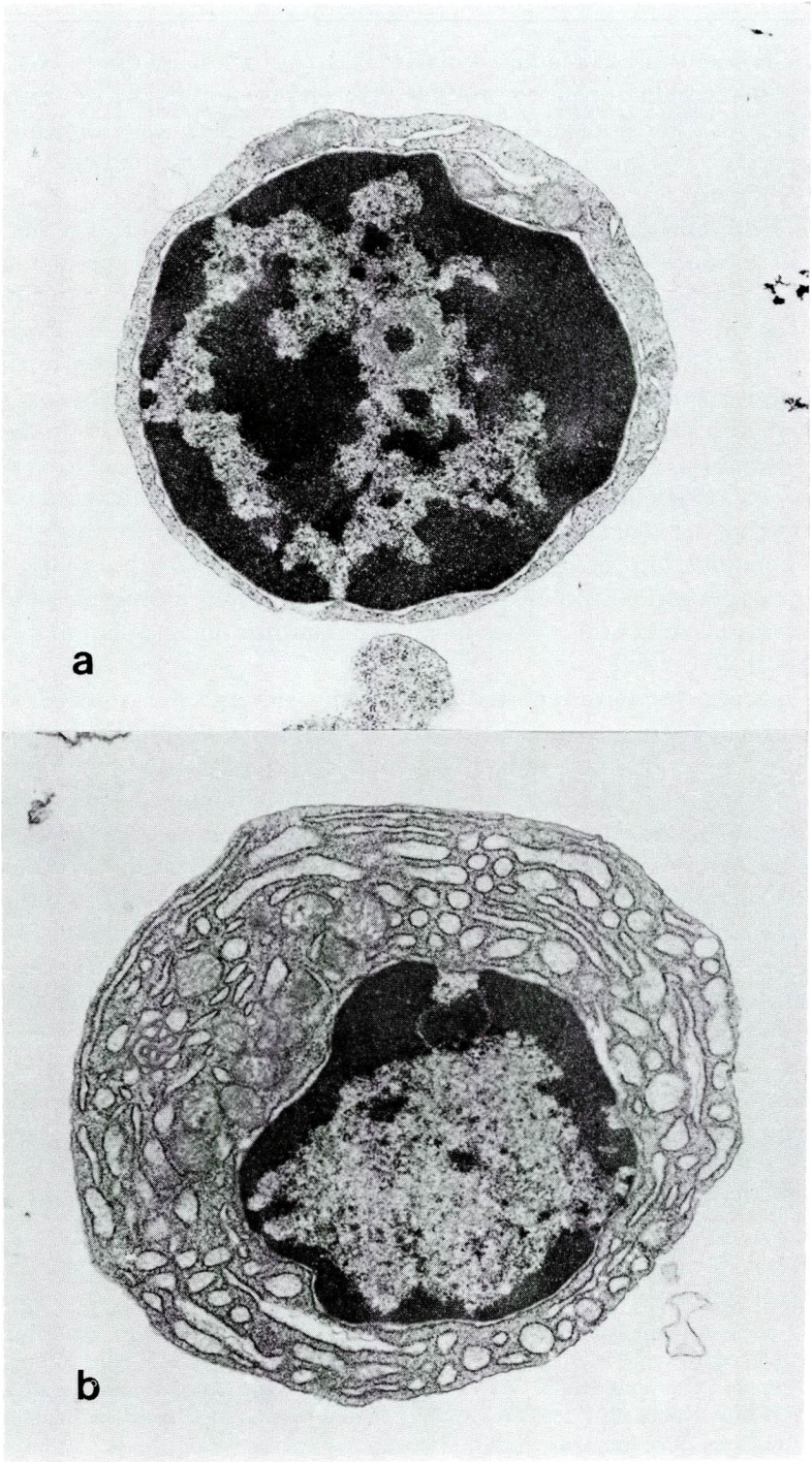

Fig. 2 Lymphocyte (a, ×18,000) and plasma cell (b, ×14,000) in sorted PGE^+ cells from SRBC-injected C57B1/6 mouse spleen

DISCUSSION

The results presented here indicate that normal mouse spleen contains PGE^+ cells which appear to produce PGE and these cells slightly increase in response to antigen (SRBC). The PGE-producing cells were shown to be lymphocytes and plasma cells. The predominant PGE-producing cell is the lymphocyte.

There is much evidence that macrophages, and T- and B-lymphocytes can all produce PGs (5). In human peripheral blood, the predominant PGE-producing cell has been thought to be the monocyte (9, 11, 20). In mice, on the other hand, peritoneal macrophages (7, 9), splenic macrophages (6) and splenic lymphocytes (predominant T-cells) (19) have been reported to produce PGE. Indirect evidence that mouse splenic B-cells also may synthesize PG has been reported (22). In the present study, the PGE-producing cells were also observed in nude mice. Thus, at least one of the PGE-producing cells appears to be B-cells. In this paper we have not examined whether or not T-cells produce PGE. Macrophages were not sorted into PGE^+ populations of spleen cells. There is, however, a possibility that macrophages as PGE-producing cells may be removed during preparation of spleen cells for FACS. At this time, we do not rule out T-cells and macrophages as PGE^- cells.

Recently, we have reported that lymphocytes and macrophages show activation of AC in response to antigen (SRBC) (16). Our findings have been supported by studies indicating that lymphocytes (1, 2, 4) and macrophages (3, 14) respond to PGE with a cAMP increase. Although the relationship between PGE-producing cells and AC responding cells is not clear at present, there are two possible explanations. One is that AC is activated in the same cell in which PGE is produced. The other is that AC is activated in a different cell which has the receptor for PGE released from the cell in which PGE is produced. In our system, the AC responding cells are lymphocytes and macrophages. The PGE-producing cells are lymphocytes (involving B-cells) and plasma cells, but not macrophages. In recent immunocytochemical studies using an electron microscopic indirect peroxidase-labeled antibody method, PGE has been shown to be localized on the cell membrane of lymphocytes, but not macrophages, in SRBC-stimulated mouse spleen (15).

The function of the PGE-producing cells in our system is also unclear. Studies concerning this are currently underway.

REFERENCES

1) Bach MA: Differences in Cyclic AMP Changes after Stimulation by Prostaglandins and Isoproterenol in Lymphocyte Subpopulations. J Clin Invest 55: 1074, 1975
2) Droller MJ, Lindgren JA, Claessen HE, et al: Production of Prostaglandin E_2 by Bladder Tumor Cells in Tissue Culture and a Possible Mechanism of Lymphocyte Inhibition. Cell Immunol 47: 261, 1979
3) Gemsa D, Steggemann L, Menzel J, et al: Release of Cyclic AMP from Macrophages by Stimulation with Prostaglandins. J Immunol 114: 1422, 1975
4) Goodwin JS, Kaszubowski PA, William Jr RC: Cyclic adenosine monophosphate response to prostaglandin E_2 on subpopulations of human lymphocytes. J Exp Med

150: 1260, 1979

5) Goodwin JS, Webb DR: Review Regulation of the Immune Response by Prostaglandins. Clin Immunol Immunopathol 15: 106, 1080

6) Grimm W, Seitz M, Kirchner H, et al: Prostaglandin Synthsis in Spleen Cell Cultures of Mice Injected with Corynebacterium parvum. Cell Immunol 40: 419, 1978

7) Humes JL, Bonney RJ, Pelus L, et al: Macrophages synthesize and release prostaglandins in response to inflammatory stimuli. Nature 269: 149, 1977

8) Jones PP, Cebra JJ, Herzenberg LA: Immunoglobulin (Ig) allotype markers on rabbit lymphocytes: Separation of cells bearing different allotypes and demonstration of the binding of Ig to lymphoid cell membranes. J Immunol 111: 1334, 1973

9) Kurland JI, Bockman R: Prostaglandin E produced by human blood monocytes and mouse peritoneal macrophages. J Exp Med 147: 952, 1978

10) Loken MR, Herzenberg LA: Analysis of cell populations with a fluorescence-activated cell sorter. Ann N Y Acad Sci 254: 163, 1975

11) Passwell JH, Dayer JM, Merler E: Increased prostaglandin production by human monocytes after membrane receptor activation. J Immunol 123: 115, 1979

12) Plescia OJ, Yamamoto I, Shimamura T: Cyclic AMP and immune response: Changes in the splenic level of cyclic AMP during the response of mice to antigen. Proc Nat Acad Sci USA 72: 888, 1975

13) Plescia OJ, Skelly R: Specificity and control of the cyclic AMP response of mice to antigen. Fed Proc 35: 532, 1976

14) Remold-O'Donnell E, Alpert HR: Alteration of Hormone-Stimulated Cyclic AMP Synthesis in Guinea Pig Peritoneal Macrophages. Cell Immunol 45: 221, 1979

15) Sasadaira H, Shimamura T: Immunocytochemical identification of prostaglandin E on the surface of mouse spleen cells. Int J Immunopharmac 2: 246, 1980

16) Shimamura T, Sasadaira H, Hasegawa H, et al: Cyclic AMP and Immune Responses: Cytochemical Identification and Localization of Adenylate Cyclase Responding Cells in Antigen-Stimulated Mouse Spleen. Tokai J Exp Clin Med 4: 171, 1979

17) Skelly RR, Steinberg AD, Plescia OJ: Regulation of Antigen Induced Changes in Cyclic Nucleotide Level in NZB/WF1 Mice. Cell Immunol 36: 283, 1978

18) Tsuru S, Zinnaka Y, Nomoto K: Decrease in cholera toxin-binding T cells in aged mice and human volunteers. Int Archs Allergy Appl Immun (in press)

19) Webb DR, Nowowiejski I: Mitogen-Induced Changes in Lymphocyte Prostaglandin Level: A Signal for the Induction of Suppressor Cell Activity. Cell Immunol 41: 72, 1978

20) Yamamoto M, Takai NA, Rapoport B, et al: Modulation by thymus-derived (T) cells of thyroid cell-stimulated prostaglandin E release by human peripheral blood mononuclear cells. Proc Nat Acad Sci USA 76: 6627, 1979

21) Yamamoto I, Webb DR: Antigen-Stimulated Changes in Cyclic Nucleotide Levels in the Mouse. Proc Nat Acad Sci USA 72: 2320, 1975

22) Zimecki M, Webb DR: The regulation of the immune response to T-independent antigens by prostaglandins and B cells. J Immunol 117: 2158, 1976