INDUCTION OF T-CELL DEPENDENT SPLENIC PROSTAGLANDIN $F_{2\alpha}$ BY T-CELL DEPENDENT ANTIGEN

Phyllis Liu Osheroff*, David R. Webb* and John Paulsrud+

*Roche Institute of Molecular Biology

+Roche Research Laboratories
Huffmann-LaRoche
Nutley, New Jersey 07110

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Summary The intravenous injection of mice with an immunogenic dose of sheep erythrocytes results in a nearly 100 fold increase in the level of prostaglandin $F_{2\alpha}$ in the spleen within 2 min after injection. The increase in prostaglandin is dependent on the presence of thymus derived T cells since injection of sRBC into athymic mice results in only a limited (10 fold) increase in the level of prostaglandin $F_{2\alpha}$.

Introduction

Lymphocytes stimulated by antigens or mitogens have been shown to produce a number of soluble mediators which function to modulate the activity of other lymphocytes as well as other types of immunocompetent cells (1,2). In addition to such studies, other workers have noted that a number of pharmacologically active substances such as biogenic amines (3), cholinergic agents (4), insulin (5) and the prostaglandins (6) may affect the development of immune responses as well as affecting events induced by phytomitogens. In addition to the well known effects of prostaglandins on inflamation (7), Smith et al. (8) have demonstrated the capacity of PGE_1 to block phytohemmaglutinin-stimulated transformation in human peripheral blood lymphocytes (presumably thymus derived T-cells). Also, Melmon et al. (9) have demonstrated the capacity of bone-marrow derived (B cells) lymphocytes to bind PGE_1 by removing up to 50% of the antibody-forming-cells to sheep erythrocytes following the exposure of the spleen cells to Sepharose-bound PGE_1. Many

of the effects of the prostaglandins in lymphocytes have been ascribed to their ability to alter the intracellular levels of cyclic nucleotides (10). In relation to this, Yamamoto and Webb (11) recently reported that sheep erythrocyte antigens could induce an early, transient elevation of cAMP levels in whole mouse spleens, and a smaller but longer lasting increase in cGMP levels. They also establish that this increase in cyclic nucleotide levels was antigen-dependent, T-cell dependent, not the result of autonomic nervous system involvement, and most importantly that it was blocked by an inhibitor of prostaglandin synthesis, indomethacin. These facts, coupled with the report of Ferraris and DeRubertis (12) that mouse spleen cultures could be stimulated by mitogens and antigens to release PGE, led us to investigate whether a significant accumulation of prostaglandin occurred in whole spleens, *in vivo*, following the intravenous administration of an immunogenic dose of sheep erythrocytes (sRBC).

Materials and Methods

Male and/or female C57Bl/6J mice 6-10 weeks old were purchased from Jackson Laboratories, Inc. (Bar Harbor, Maine). The Balb/Bom athymic mice were kindly provided by NIH. Sheep erythrocytes from a single sheep were obtained from the Colorado Serum Co. (Denver, Colorado). Radioimmunoassay of $PGF_{2\alpha}$ was performed with aliquots of 1% perchloric acid splenic extracts neutralized with potassium bicarbonate. Preparation of anti-$PGF_{2\alpha}$ antiserum and the assay procedure were essentially as those described by Caldwell *et al.* (13) except that zirconyl phosphate gel (z-gel) (15) at pH 4 was used to separate the antibody-bound from free $[^3H]PGF_{2\alpha}$. $[^3H]PGF_{2\alpha}$ equivalent to 8-12,000 cpm in 0.2 ml of 10 mM potassium phosphate, 1 mM EDTA, 0.1 g/l thimerosal, 1 g/l gelatin (buffer A) was added to all samples and standard $PGF_{2\alpha}$ in a concentration range of 0.01 to 1 mg. Antibody at a dilution of 1/1300 in 0.2 ml buffer A which gives 50% binding was then added and the mixture incubated for 1 hr at room temperature. 0.6 ml of a mixture of 1 part of z-gel and 2 parts of buffer B which differs from buffer A in gelatin

concentration (5 g/l) were added and the mixture centrifuged. A 0.75 ml aliquot of the supernatant containing the non-bound $PGF_{2\alpha}$, dissolved in aquasol was assayed by scintillation spectrometry.

Results and Discussion

The data presented in Fig. 1a depicts the results obtained when 2 x 10^8 sRBC are injected through the tail vein of C57Bl/6J mice. Whole spleens were removed at short intervals after injection and quickly frozen between two precooled (-70°C) aluminum bars, when extracted and assayed for $PGF_{2\alpha}$. These results show that a very large, approximately 70-fold, increase occurs in $PGF_{2\alpha}$ levels within 2 min after antigen injection. The levels then drop substantially so that by 10 min post-injection the levels are approximately 35 fold above control levels, and by 60 min the concentration of $PGF_{2\alpha}$ is roughly 15 fold above control values. It is noteworthy that the early rapid increase and decline of $PGF_{2\alpha}$ is remarkably similar to the increase and decline of whole splenic cAMP levels alluded to earlier (11). Because of results obtained by us in nude mice in which no change in cyclic nucleotide levels was obtained following sRBC injection (11), we performed an experiment in

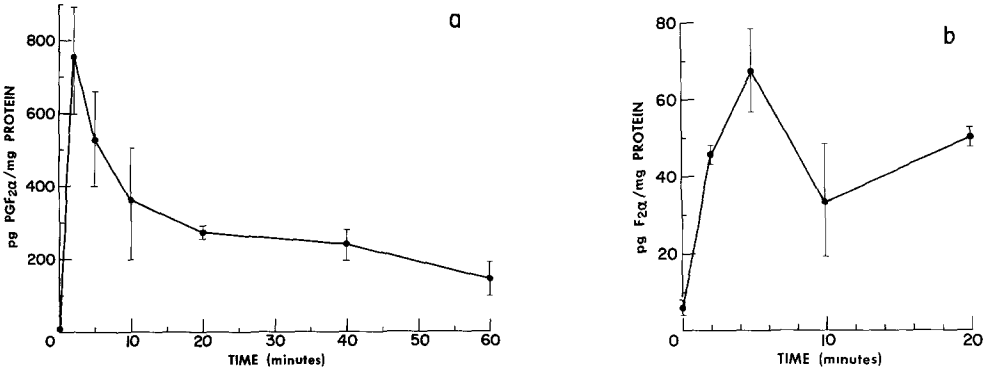

Figure 1a-1b. Levels of $PGF_{2\alpha}$ in whole mouse spleens following intravenous injection of sRBC.

Splenic levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a) C57Bl/6J and b) Balb/Bom athymic nude mice at different time intervals following intravenous injection of sRBC.

which splenic $PGF_{2\alpha}$ levels were assayed in athymic nude mice following the intravenous injection of sRBC. The results shown in Fig. 1b indicate that while the $PGF_{2\alpha}$ levels are in fact elevated, the increase is 10 fold lower than that obtained in the normal mice. It should be noted that the basal levels of $PGF_{2\alpha}$ in both C57 and the nude mice are nearly identical, 7.48 picograms/mg protein in C57 and 5.86 picograms/mg of protein in the nude mice. The fact that a small but significant change in $PGF_{2\alpha}$ levels occur in the athymic nude mice may reflect the presence of a small antigen sensitive T and/or B cell population. Alternatively, the limited increase may be due to a non-immunocompetent cell responding to sympathetic nerve excitation, epinephrine or norephinephrine. We regard this latter alternative as being less likely since the correlative increases in cyclic nucleotide levels in normal, antigen-stimulated spleens are not sensitive to α,β adrenergic or cholinergic blocking agents (11), whereas they are sensitive to indomethacin. However, the possibility of a non-antigen specific minimal stimulation of non-immunocompetent cells cannot be ruled out. In addition, no detectable increase in cyclic nucleotide levels occur in the nude mice regardless of the dose of sRBC used (11). Therefore these data are supportive of the notion that increases in the level of $PGF_{2\alpha}$ in response to a T-cell dependent antigen (sRBC) is in large part, dependent on the presence of T cells.

We have been able to demonstrate that a T-cell dependent antigen will induce the production of a pharmacologically active substance by a putative immunocompetent T-cell; and that this substance, prostaglandin, appears to be involved in the induction of alterations in intracellular cyclic nucleotide levels in other, probably similar, immunocompetent cells (11). It is worthy of note that in comparison to others (12) working in vitro, the changes we see occur very rapidly after antigen exposure and persist for at least one hour after the injection of the antigen. Ferraris and DeRubertis (12) reported an increase in prostaglandin after 16 hr exposure to staphococcal enterotoxin B and 48 hr after exposure to PHA in mouse spleen cell cultures.

Thus, the change reported here is all the more remarkable in that it may represent a rapid new synthesis of prostaglandin and subsequent breakdown since Ferreira et al. (14) have shown in hormone-stimulated dog spleens that the release of prostaglandin is much greater than can be extracted from the tissue. This suggests that changes in prostaglandin levels in the spleen represent new synthesis rather than release of stored-material. Lastly, in experiments to be reported elsewhere[+] it has been possible to show that blockage of prostaglandin synthesis has a number of ramifications for the subsequent development of the immune response. This suggests that in addition to their role in the control of inflamation, the prostaglandins play an active part in the regulation of critical early events which occur following the interaction of antigen with immunocompetent cells.

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