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Research Article

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Suppression of Immune Complex

Vasculitis in Rats by Prostaglandin

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A B S T R A C T Immune complex-induced vascular damage can be markedly suppressed by treatment of rats with either prostaglandin (PG)E_1 or its stable derivative, 15-(S)-15-methyl PGE_1, but not with $PGF_{2\alpha}$. In addition, PGD_2 and PGE_2 also show suppressive effects. The PGE_1 derivative is considerably more effective than PGE_1 and shows potent anti-inflammatory activity even after oral administration. Suppression of the vasculitis reaction is reflected by a greatly diminished increase in vasopermeability, indicating little or no vascular damage. In suppressed animals, the infiltration of neutrophils is greatly reduced, and those leukocytes that have appeared at tissue sites fail to show phagocytic uptake of immune complexes. In suppressed animals, the skin sites nevertheless show deposits of immune complexes and C3 fixation in vascular walls. Neutrophils harvested from the blood of rats treated with PGE_1 show depressed responsiveness in chemotaxis and in enzyme secretion after incubation with chemotactic peptide. These studies indicate that certain PG have potent anti-inflammatory activity, which may be related to their effects on leukocytes.

INTRODUCTION

Abundant experimental evidence supports the view that prostaglandins influence the development and modulation of immune responses and inflammatory reactions (1, 2). Although prostaglandin (PG)E compounds clearly are local mediators of inflammation,

they also suppress diverse effector systems of inflammation. Thus PGE compounds, probably by virtue of their ability to increase cyclic AMP, reduce endocytosis-induced extrusion of lysosomal enzymes from polymorphonuclear leucocytes (PMN) (3), prevent immunoglobulin (Ig)E-induced release of histamine from basophiles and lung fragments (4, 5), and inhibit lymphocyte-mediated cytotoxicity (6). In addition, pharmacological doses of PGE_1 and PGE_2 suppress acute and chronic inflammation in several experimental animal models (7, 8).

PGE compounds can both suppress and enhance cell-mediated and humoral immunity in vitro and appear to regulate the character and intensity of immune response in vivo (2). Long-term treatment of experimental animals with PGE_1 has proven feasible: treatment of NZB/NZW mice (a murine model for human systemic lupus erythematosus) with pharmacological doses of PGE_1 prevents development and retards progression of immune complex-induced glomerulonephritis and prolongs survival markedly (9).

Thus the role of PG in immune/inflammatory reactions is complex and not well understood, but these ubiquitous compounds do appear important in regulating cell functions and host defenses. We therefore investigated the effect of several PG on a model of immune complex-mediated acute vasculitis, the reversed passive Arthus reaction (RPA). The RPA is induced by intravenous administration of antigen and intradermal injection of antibody (10). Results of these studies indicate that treatment of rats with PGE_1 or its more stable analog 15-(S)-15-methyl PGE_1 inhibits
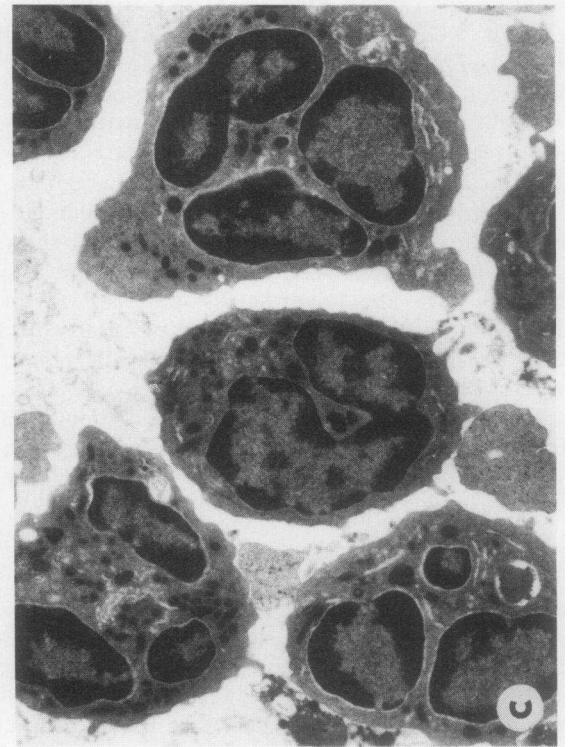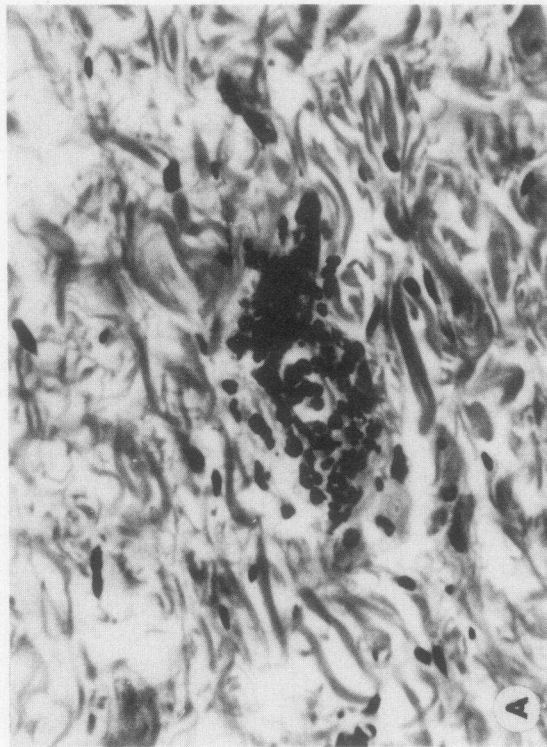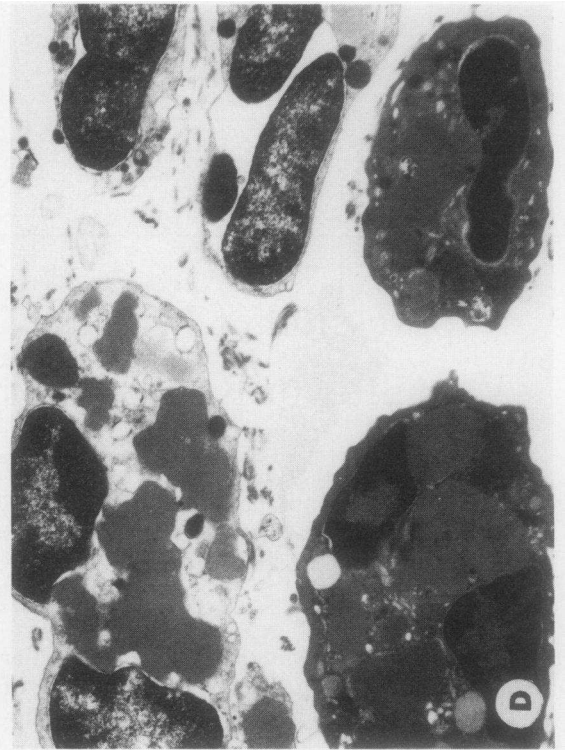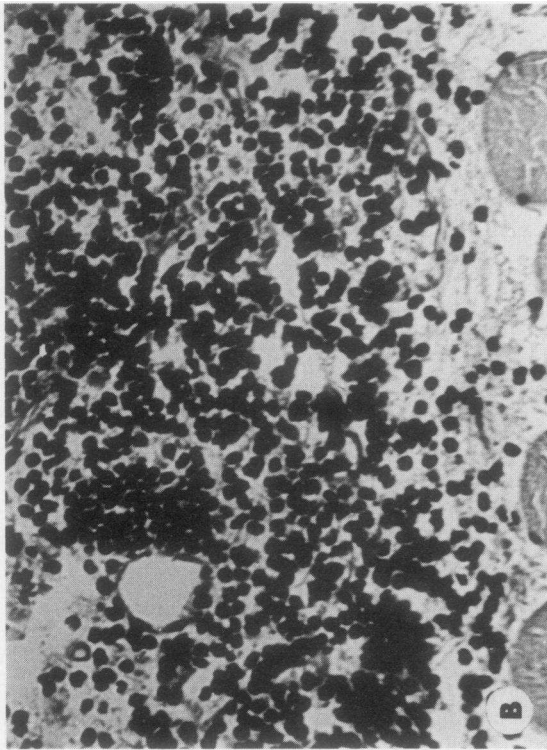
tissue injury despite the fact that intra- and perivascular deposition of antigen and complement is not prevented.

METHODS

Animals. Adult male, Fisher 344 rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 250–300 g were employed.

RPA. Rabbit IgG containing precipitating antibody to bovine serum albumin (BSA) was isolated and employed as described (11). Usually, 0.15 ml IgG containing 100 μg antibody nitrogen was injected intradermally followed by the intravenous injection of 10 μg BSA and 1 μCi ^{125}I-rat serum albumin (5 μg), which served as a permeability marker. Negative control sites were injected with phosphate-buffered saline. 3 h after challenge, animals were sacrificed and blood and skin sites obtained for measurements of radioactivity (12).

PG treatment. PG were a gift of Dr. John E. Pike, Upjohn Co., Kalamazoo, Mich. Stock solutions containing 2 mg/ml were prepared in ethanol and sterile saline and injected either *per os* or subcutaneously. Between five and eight animals were used for each PG studied.

Morphological analyses. After assessment for radioactivity, all biopsy specimens were processed for frozen section analysis. Portions from each biopsy site were fixed in buffered (pH 7.0) 10% formaldehyde. In selected cases, fresh tissues were fixed in glutaraldehyde and processed for transmission electron microscopy.

Isolation and testing of neutrophils. Blood was drawn from the aorta of normal and PG-treated (500 μg subcutaneously) rats. The cells were isolated by Ficoll-Hypaque (Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Hypaque, Winthrop Laboratories, New York) methods (13), resulting in a suspension with 95% neutrophils. The cells were tested in vitro for chemotactic responsiveness and for lysosomal enzyme release according to methodologies described in detail elsewhere (14, 15).

Immunofluorescence studies. Frozen sections were obtained from skin sites and processed in the routine manner (12). Sites were examined for fixation of BSA (which had been injected intravenously) and for C3.

Statistical analysis. The unpaired Student's *t* test was used to analyze the data. The probability (*P* value) in Table I was computed by comparing raw data from experimental and control animals.

RESULTS

Suppression of dermal vascular reactions. In four different experiments, animals were treated with

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; PG, prostaglandin(s); PMN, polymorphonuclear leukocyte(s); RPA, reversed passive Arthus reaction.

various dose schedules of PG (Table I). Vascular permeability changes were always related (as a ratio) to changes found in saline-injected sites of five separate negative control animals. Animals serving as positive controls received intradermal injections of antibody and intravenous injections of BSA plus labeled rat albumin. For each of the experiments (Table I), five animals had three negative (saline-injected) and three positive (antibody-injected) skin sites. The mean permeability change for each site was then calculated.

PGE$_1$ treatment (5 d) resulted in an average suppression in vascular damage (as measured by permeability changes) of 66%, whereas PGF$_{2\alpha}$ increased permeability slightly (Table I). In experiment B, PGE$_1$ inhibited the inflammatory reaction in a dose-response fashion when given 30 min before induction of the skin reaction. A plateau in the anti-inflammatory effect of PGE$_1$ was noted at the 500-μg dose. In experiment C, a single dose (500 μg) of PGE$_1$ inhibited the vasculitis reaction by 59%. When 250 μg of the more stable analog 15-(S)-15-methyl PGE$_1$ was given subcutaneously 12 h before RPA induction, it inhibited vascular permeability by 69%. Oral administration of 500 μg of this derivative 12 h earlier caused similar suppression of tissue injury (62%). In experiment D, PGE$_2$ and PGD$_2$ were given in a single subcutaneous dose of 500 μg 30 min before skin testing. PGE$_2$ had a mean suppressive effect of 58%, whereas PGD$_2$ suppressed permeability by 37%.

Morphological studies. When vascular damage was suppressed by PG treatment (Table I), skin reactions were markedly inhibited and exhibited little edema, induration, and erythema. Histologically, the reference positive sites showed diffuse intraluminal, perivascular, and interstitial infiltrates of neutrophils (Fig. 1B). In contrast, sites from rats treated with a suppressive dose of PGE$_1$ (Table I, experiment C) showed markedly diminished infiltrates of neutrophils. When found, these infiltrates were confined to vascular walls and perivascular locations (Fig. 1A). Similar histological changes were found in animals treated with 15-(S)-15-methyl PGE$_1$ subcutaneously and by mouth (not shown).

Transmission electron microscopy of the reference positive control sites revealed the presence of electron-dense immune complexes within phagocytic vacuoles

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FIGURE 1 RPA in normal and in PGE$_1$-treated (as described in Table I, experiment C) rats. In A, taken from a PGE$_1$-treated rat, there is an early intraluminal infiltrate of neutrophils but few cells are in the interstitium. B reveals diffuse perivascular and interstitial infiltrates of neutrophils in the deep dermis, in a positive control animal (hematoxylin and eosin, ×65). C and D represent transmission electron microscopy of leukocytes from infiltrates described in A and B In C, interstitial leukocytes present in the skin site of a PGE$_1$-treated animal reveal no phagocytic vacuoles and demonstrate intact lysosomal granules. In D, several neutrophils are present in the interstitium with large phagocytic vacuoles filled with electron-dense material (consisting of immune complexes). Degranulation of lysosomal granules has occurred (glutaraldehyde fixation, ×9,500).

TABLE I
TABLE I
Suppression of Vasculitis by Prostaglandins

Treatment of animals*	Ratio‡	P value	Suppression of damage§
			%
Experiment A			
None, positive control	4.1±.75	<0.001	
PGE₁	1.4±.50	<0.2	66
PGF₂α	4.5±1.0	<0.001	0
Experiment B			
None, positive control	4.5±.90	<0.001	
PGE₁, 125 μg	3.5±.86	<0.005	22
PGE₁, 250 μg	2.6±.69	<0.005	42
PGE₁, 500 μg	1.6±.40	<0.2	64
PGE₁, 1,000 μg	1.4±.35	<0.2	69
Experiment C			
None, positive control	3.9±.50	<0.001	
PGE₁	1.6±.62	<0.2	59
15-(S)-15-methyl PGE₁, subcut.	1.2±.31	<0.2	69
15-(S)-15-methyl PGE₁, orally	1.5±.29	<0.2	62
Experiment D			
None, positive control	5.7±1.1	<0.001	
PGE₂	2.4±.63	<0.005	58
PGD₂	3.6±.52	<0.05	37

* In experiment A animals received 250 μg PG twice daily, subcutaneously, for 5 d and then 500 μg before induction of skin reactions. In experiment B animals received a single injection of PGE, 30 min before induction of the skin reactions. In experiment C animals received 500 μg 15-(S)-15methyl PGE₁ subcutaneously or orally 12 h earlier, or 500 μg PGE₁ 30 min before reactions. In experiment D animals received 500 μg PGE₂ or PGD₂ subcutaneously 30 min before skin testing.

‡ Ratio represents permeability increase in Arthus sites relative to permeability changes in saline sites.

§ Calculated on the basis of the positive control sites in each experiment.

of neutrophils and degranulation of these cells (Fig. 1D). In those animals treated with PGE₁, the occasional neutrophil seen in the tissue space had no phagocytic vacuoles and cytoplasmic granules were intact (Fig. 1C). In animals treated with 15-(S)-methyl PGE₁, similar electron microscopic observations were made (not shown).

Immunofluorescence studies. In all positive control reactions, the skin sites revealed extensive deposits of both BSA and C3 in walls of dermal venules and capillaries. In addition, a diffuse interstitial spread of immune complexes was found, extending into the intermuscular capillaries of the deep dermis. This pattern was identical in animals treated with PGF₂α. In rats treated with PGE₁ or 15-(S)-15-methyl PGE₁, the deposits of BSA and C3 were confined mainly to the walls of dermal venules and to the immediate adjacent perivascular connective tissue.

In vitro neutrophil function. Peripheral neutrophils isolated from animals treated with PGE₁ exhibited a 32±6.4% reduction in chemotaxis, when treated with 1 nM f-Met-Leu-Phe, as compared to the response of PMN isolated from untreated animals. Peripheral blood PMN from untreated rats released 54±8.5% of the total cellular β-glucosaminidase after exposure to 10 nM f-Met-Leu-Phe. In contrast, PMN from PGE₁-treated rats released 2.5±0.7% of the total cellular β-glucosaminidase when treated with the same concentration of chemotactic stimuli.

DISCUSSION

The data presented in this paper indicate that with appropriate experimental conditions systemic administration of PGE₁ and its stable derivative 15-(S)-15-methyl PGE₁, as well as PGD₂ and PGE₂ results in potent suppression of an immunologically induced acute inflammatory reaction. In addition, 15-(S)-15-methyl PGE₁ is considerably more effective than PGE₁ and exhibits potent anti-inflammatory action even after oral administration. This compound is more resistant than PGE₁ to the activity of 15-hydroxy-PG-dehydrogenase (16). The activity of this dehydrogenase may be one reason why pharmacological doses of PG are required to achieve physiological effects. The precise

mechanism whereby these compounds inhibit vasculitis and tissue injury are not completely clear. Our current understanding of the pathogenesis of the Arthus reaction includes: formation of immune complexes in vascular walls, activation of complement and local generation of chemotactic peptides derived from C5, influx of neutrophils in response to the chemotactic products, endocytosis of immune complexes by and subsequent release of lysosomal enzymes from neutrophils, and resulting vascular injury. Injury to vessels can be measured readily by the great increase in vascular permeability and by development of hemorrhage. PGE therapy prevents the increased vascular permeability characteristic of RPA. Our immunofluorescence microscopy studies suggest that in PGE-treated animals immune complexes form and the complement sequence is activated. However, egress of leukocytes from the circulation appears to be impaired in these animals. The electron microscopy studies indicate that neutrophils that do leave the circulation and find their way to the reaction site do not ingest immune complexes. Thus, the cytoprotective effects of PGE demonstrated in these experiments appear to be caused, in part, by interference with directed motion of neutrophils and with degranulation of these cells. Clinically, this would necessitate administration of PG before neutrophils could release damaging degradative enzymes.

Platelet aggregation and subsequent release of vasoactive amines is important to initiation and propagation of inflammation (17). Although platelet aggregation is depressed by PGE_1 (18), this observation is probably irrelevant to our studies, because it has been shown that platelet depletion does not alter the intensity of the RPA in rabbits (19). Furthermore, PGD_2, which also suppresses the RPA, does not inhibit aggregation of rat platelets in vitro (20).

Although it is generally accepted that PG and/or their antecedents in the arachidonic acid cascade are somehow involved in inflammation, it is by no means clear what the exact role is of any of these fatty acid derivatives in discrete processes of inflammation. It will therefore be important to determine how these products modulate leukocyte responses during inflammatory reactions.

ACKNOWLEDGMENTS

REFERENCES

1. Vane, J. R. 1976. Prostaglandins as mediators of inflammation. In Advances in Prostaglandin and Thromboxane Research. B. Samuelsson and R. Paoletti, editors. Raven Press, New York. 791–798.
2. Zurier, R. B. 1979. Modulation of inflammation and immune responses by prostaglandins. In Actions of Nonsteroidal Agents in the Alteration of Prostaglandin Synthesis. A. Ryan, editor. Postgraduate Medical Communications, Minneapolis, Minn. 46.
3. Zurier, R. B., G. Weissmann, S. Hoffstein, S. Kammerman, and H. H. Tai. 1974. Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists and agents which affect microtubule function. J. Clin. Invest. 53: 297–309.
4. Lichtenstein, L. M., and R. DiBernardo. 1971. The immediate allergic response: in vitro action of cyclic AMP and other drugs on the two stages of histamine release. J. Immunol. 107: 1131–1136.
5. Orange, R. P., W. G. Austen, and K. F. Austen. 1971. Immunological release of histamine and slow reactive substance of anaphylaxis from the lung. I. Modulation by agents influencing cellular levels of cyclic AMP. J. Exp. Med. 134: 136x–148s.
6. Henney, C. S., H. R. Bourne, and L. M. Lichtenstein. 1971. The role of cyclic AMP in the specific cytolytic activity of lymphocytes. J. Immunol. 108: 1526–1534.
7. Zurier, R. B., and F. Quagliata. 1971. Effects of prostaglandin E_1 on adjuvant arthritis. Nature (Lond.). 234: 304–305.
8. Bonta, I. L., M. J. Parnham, and L. VanVliet. 1978. Combination of theophylline and prostaglandin E_1 as inhibitors of the adjuvant induced arthritis syndrome of rats. Ann. Rheum. Dis. 37: 212–216.
9. Zurier, R. B., I. Damjanov, P. L. Miller, and B. F. Biewer. 1978. Prostaglandin E treatment prevents progression of nephritis in murine lupus erythematosus. J. Clin. Lab. Immunol. 1: 95–98.
10. Cochrane, C. G. 1968. Immunologic tissue injury mediated by neutrophilic leukocytes. Adv. Immunol. 9: 97–163.
11. Kabat, E. A., and M. M. Mayer. 1967. Experimental Immunochemistry, Charles C Thomas, Publisher, Springfield, Ill. 2nd edition. 781–797.
12. Johnson, K. J., and P. A. Ward. 1974. Acute immunologic pulmonary alveolitis. J. Clin. Invest. 54: 349–351.
13. O'Flaherty, J. T., D. L. Kreutzer, and P. A. Ward. 1978. Chemotactic factor influences on aggregation, swelling and foreign adhesiveness of human leukocytes. Am. J. Pathol. 90: 537–550.
14. Showell, H. J., R. J. Freer, S. H. Zigmond, E. Schiffmann, S. Aswanikumar, B. Corcoran, and E. L. Becker. 1976. The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. J. Exp. Med. 143: 1154–1169.
15. Ward, P. A., C. G. Cochrane, and H. J. Müller-Eberhard. 1965. The role of serum complement in chemotaxis of leukocytes in vitro. J. Exp. Med. 122: 327–330.
16. Hansen, H. S. 1976. 15-Hydroxyprostaglandin dehydrogenase: a review. Prostaglandins. 12: 647–679.
17. Zucker, M. B. 1974. Platelets. In The Inflammatory Process. B. W. Zweifach, L. Grant, and R. T. McCluskey, editors. Academic Press, Inc., New York. 511–539.
18. Honstra, G. 1971. Degree and duration of prostaglandin E_1 induced inhibition of platelet aggregation in the rat. Eur. J. Pharmacol. 15: 343–349.
19. Humphrey, J. H. 1955. The mechanism of Arthus reaction. II. The role of polymorphonuclear leukocytes and platelets in reversed passive Arthus reaction in the guinea pig. Br. J. Exp. Pathol. 36: 283–291.
20. Nishizawa, E. E., W. L. Miller, R. R. Gorman, and G. L. Bundy. 1975. Prostaglandin D_2 as a potential antithrombotic agent. Prostaglandins. 9: 109–121.