Platelet-Derived Growth Factor Can Activate Purified Primate, Phorbol Myristate Acetate-Primed Eosinophils

M.K.	Michael K.	Bach
J.R.	John R.	Brashler
B.K.	Barbara K.	Stout
H.G.	Herbert G.	Johnson
M.E.	Martin E.	Sanders

Hypersensitivity Diseases and Clinical Pharmacology, The Upjohn Company, Kalamazoo, Mich., USA

Abstract

We are interested in the physiologic mechanisms of eosinophil activation because of the presumed participation of activated eosinophils in the inflammatory sequelae of asthma. Suspecting that other formed elements of the blood may contribute to such an activation, we examined the capacity of platelet-derived growth factor (PDGF), a product of activated platelets, to activate eosinophils. We found that highly purified monkey and human eosinophils, but not guinea pig eosinophils, were activated by PDGF (superoxide anion production) in a dose-dependent fashion. Moreover, this activation was further dependent on a prior 'priming' of the cells by a brief exposure to subthreshold concentrations of phorbol ester. The response was specific for the BB homodimer of PDGF suggesting it is receptor-dependent.

Correspondence to: Dr. Michael K. Bach, Hypersensitivity Diseases and Clinical Pharmacology, The Upjohn Company, Kalamazoo, MI 49001 (USA)

Our current understanding of asthma is that its chronic aspects are best considered as representing a pulmonary inflammatory disease. It is often speculated that eosinophils, which are consistently found in the lungs and sputa of asthmatics, may be responsible for much of the inflammatory damage to lung tissue by way of the secretion of their stored products and/or the production of inflammatory mediators. It remains unclear how resting eosinophils become 'activated' within the asthmatic lung, an essential prerequisite to their participation in the reaction, but it is interesting to speculate that interaction of eosinophils with other formed elements with which they come into contact during their travel to the lung (e.g. platelets and endothelial cells) may play a role in this process. Accordingly, we examined the effect of one product of activated platelets, platelet-derived growth factor (PDGF) on eosinophil activation employing the generation of superoxide anion as an indicator of activation.

Eosinophils were purified from leukapheresis packs obtained from normal volunteers by a combination of isopycnic centrifugation on discontinuous Percoll gradients and negative selection with a monoclonal antibody to CD 16 [1]. Rhesus monkey and guinea pig eosinophils were elicited with horse serum and purified using Percoll. Formation of superoxide anion (O_2^-) was measured spectrophotometrically as the superoxide dismutase-inhibitable reduction of fer-ricytochrome C to ferrocycytochrome C using microtiter equipment [2]. The linear rate of O_2^- production, which was computed from serial readings of the plates using a computer-linked program, was used for the evaluation of eosinophil activation.

Both human and monkey eosinophils, but not guinea pig eosinophils, could be induced to produce small amounts of O_2^- with PDGF in a dose-related manner (0.1–5 nM PDGF). The response to PDGF was furthermore dependent, in dose-response fashion, on preincubation of the cells with priming concentrations of phorbol myristate acetate (PMA; 0.2–0.5 nM) which caused little if any O_2^- production by itself [3]. The maximum net rate of O_2^- production due to PDGF after priming was 0.15 nmol/min/10⁵ cells which is approximately 12% of the rate achievable with a maximally stimulating concentration of PMA (5 nM). This compares favorably with O_2^- production by the same cells when they are stimulated (with or without priming) by other receptor-dependent activators.

168

Bach/Brashler/Stout/Johnson/Sanders

The response to PDGF was specific in that the c-sis recombinant product, which is a B chain homodimer, was active while the recombinant AA homodimer was inactive. Furthermore, other growth factors, such as basic fibroblast growth factor and epidermal growth factor, were inactive over a wide range of concentrations. These results suggest that the stimulation is dependent on a receptor for the B chain of PDGF on these cells. Efforts to demonstrate the presence of these receptors either on the cell membrane [4], or in the cytoplasm [5], have been unsuccessful suggesting that the receptor density must be very low.

Though the chemotactic activation of neutrophils by PDGF has been previously reported [6], to our knowledge, this is the first demonstration that normal 'resting' human eosinophils can be activated with this factor. The requirement for a priming reaction for the expression of the response to PDGF supports the notion that eosinophil activation may, under certain situations, represent a two signal event with PDGF supplying the second signal in this case. These results lend support to speculations that PDGF may be a physiologically important activation signal for these cells and about the possible existence of feedback loops involving eosinophils and platelets or endothelial cells. But it is premature to express these in any detail.

References

- Bach MK, Brashler JR, Sanders ME: Preparation of large numbers of highly purified normodense human eosinophils from leukapheresis. *J Immunol Methods* 1990;130:277–281.
- Sedgwick JB, Vitis RF, Gourley MF, Busse WW: Stimulus-dependent differences in superoxide anion generation by normal human eosinophils and neutrophils. *J Allergy Clin Immunol* 1988;81:876–883.
- Bass DA, McPhail LC, Schmitt JD, Norris-Natschke S, McCall CE, Wykle RL: Selective priming of rate and duration of the respiratory burst of neutrophils by 1,2-diacyl- and 1-O-alkyl-2-acyl-glycerides. Possible relation to effects of protein kinase C. *J Biol Chem* 1988;264:19610–19617.
- Williams LT, Tremble PM, Lavin MF, Sunday ME: Platelet-derived growth factor receptors form a high affinity state in membrane preparations. Kinetics and affinity cross-linking studies. *J Biol Chem* 1984;259:5287–5294.
- Rakowicz-Szulczynska EM, Linnenbach A, Kaprowski H: Rapid and sensitive method to detect expression of growth factor receptors. *J Immunol Methods* 1989;116:167–173.
- Deuel TF, Senior RM, Huang JS, Griffin GL: Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. *J Clin Invest* 1982;69:1046–1049.