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Failure of Phorbol Myristate Acetate to Damage DNA in Leukocytes from Patients with Chronic Granulomatous Disease

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Unlike leukocytes from normal donors, leukocytes from patients with chronic granulomatous disease do not suffer DNA damage on exposure to the tumor promoter, phorbol myristate acetate.

Chronic granulomatous disease (CGD) is a rare, genetically determined disorder in which peripheral blood neutrophils fail to kill certain bacteria after phagocytosis. Neutrophils from CGD patients fail to undergo a normal respiratory burst dependent on oxidative metabolism when exposed to particulate or soluble stimuli (4, 7). The term respiratory burst describes the production of reactive oxygen species such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, which are thought to play an important role in microbial killing by neutrophils. The molecular defect in this disease, although not completely understood, is thought to be related to an abnormality of NADPH or NADH oxidase or both (1, 5, 6).

We have recently shown that exposure of normal leukocytes to phorbol myristate acetate (PMA), a tumor promoter and a stimulator of the respiratory burst, causes considerable damage to their DNA (2). Indirect evidence was presented to suggest that an oxidase enzyme was involved, since O_2^- and hydrogen peroxide were implicated in the damaging process (2). More compelling support for this hypothesis would be gained if PMA failed to damage DNA in CGD leukocytes, which have an abnormality in oxidative metabolism and the generation of reactive species of oxygen. We have now examined leukocytes from three patients with CGD to determine whether PMA-induced DNA damage occurs. The diagnosis of CGD was established by the clinical history, by finding a markedly impaired killing capacity for *Staphylococcus aureus* 502A by blood neutrophils, and by the failure of neutrophils to reduce nitroblue tetrazolium dye. All CGD patients were taking trimethoprim (2 mg/kg per day) and sulfamethoxazole at the time of the study, but none had an active infection. Control neutrophils were examined in parallel. Blood was collected in Vacutainer tubes containing EDTA as anticoagulant and coded so that CGD patients could not be distinguished from controls. The samples were sent by air from Toronto to Chalk River; the temperature in transit was maintained at 2 to 4°C. The time from collection in Toronto to analysis for response to PMA in Chalk River was less than 20 h in all cases.

Leukocytes were isolated after lysis of erythrocytes by treatment with ammonium chloride (2). Leukocytes were suspended in a balanced salt solution at a concentration of 1×10^6 to 2×10^6 per ml. Ten milliliters of cell suspension was incubated in a siliconized glass tube in the presence or absence of the indicated concentration of PMA (Sigma Chemical Co.). After 40 min of incubation at 37°C, cells were collected by centrifugation and analyzed for DNA strand breaks by the FADU method (3). In this procedure, DNA strand break damage is detected as an increase in the rate of DNA unwinding in alkali compared to DNA from controls cells. A fluorescent dye is used to determine the rate of DNA unwinding. DNA damage is expressed as the number of induced strand breaks per cell produced by the experimental treatment.

The results of experiments utilizing coded samples from six donors (three normal and three CGD patients) are shown in Fig. 1. The amount of DNA damage which resulted from exposure of leukocytes to increasing concentrations of PMA fell into two distinct groups, one exhibiting the "normal" amount of damage (2) and a second exhibiting little or no damage. As predicted, all cases exhibiting no DNA damage involved CGD leukocytes and all with marked DNA damage involved control leukocytes. These findings therefore provide strong support for the hypothesis that an oxidase-linked reaction is involved in the production of leukocyte DNA strand breaks by PMA. It is likely that the

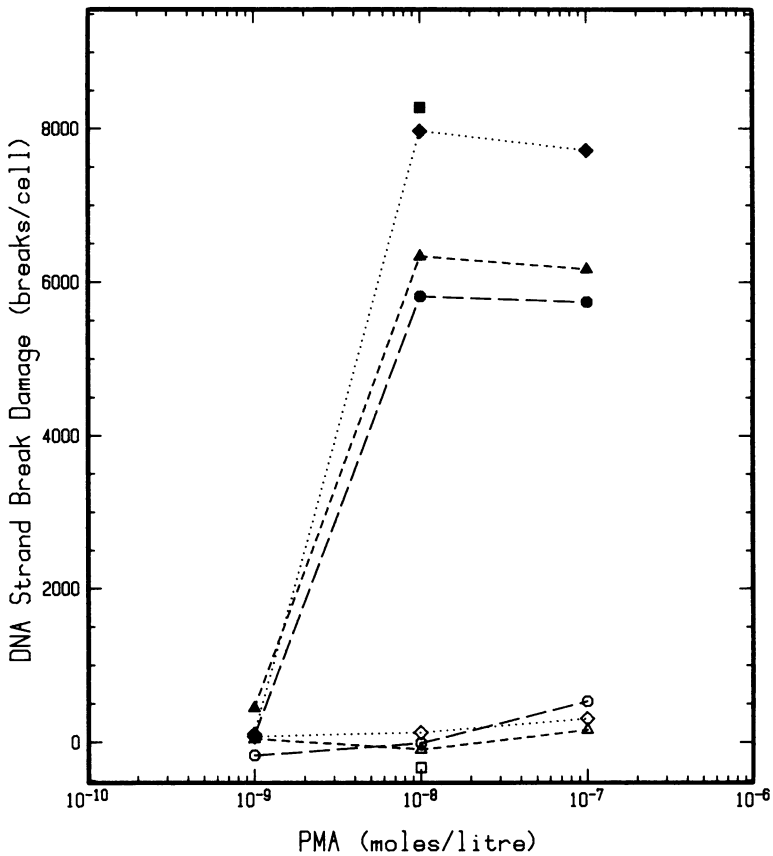

FIG. 1. Damage to DNA in leukocytes after exposure to PMA. Isolated peripheral blood leukocytes from three CGD patients (open symbols) or normal donors (filled symbols) were incubated in a balanced salt solution with PMA for 40 min at 37°C, chilled to 0°C, and analyzed for DNA damage by the FADU procedure (3).

species of activated oxygen produced after stimulation of this pathway with PMA are involved in the damaging process. However, the precise mechanism of damage is not known (H. C. Birnboim, Proceedings of Active Oxygen and Medicine Symposium, Can. J. Physiol. Pharmacol., in press). Although PMA is known to have numerous other effects on cells both in vitro and in vivo (4a), we postulate that it acts as a potent tumor promoter by damaging DNA through pathways dependent on oxidative metabolism and its products, reactive oxygen derivatives.

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LITERATURE CITED

1. **Baehner, R. L., and D. G. Nathan.** 1967. Leukocyte oxidase: defective activity in chronic granulomatous disease. Science **155**:835–836.
2. **Birnboim, H. C.** 1982. DNA strand breakage in human leukocytes exposed to a tumor promoter, phorbol myristate acetate. Science **215**:1247–1249.
3. **Birnboim, H. C., and J. J. Jevcak.** 1981. Fluorometric method for rapid detection of DNA strand breaks in human white blood cells procured by low doses of radiation. Cancer Res. **41**:1889–1892.
4. **Curnutte, J. T., D. M. Whitten, and B. M. Babior.** 1974. Defective superoxide production by granulocytes from patients with chronic granulomatous disease. N. Engl. J. Med. **290**:593–597.

4a. **Hecker, E., N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann (ed.).** 1982. Carcinogenesis—a comprehensive survey, vol. 7. Raven Press, New York.

5. **Hohn, D. C., and R. I. Lehrer.** 1975. NADPH oxidase deficiency in X-linked chronic granulomatous disease. J. Clin. Invest. **55**:707–713.
6. **Holmes, B., and A. R. Page.** 1966. Metabolic abnormalities of leukocytes from children with CGD. J. Cell Biol. **31**:48a–49a.
7. **Roos, D., and R. S. Weening.** 1979. Defects in the oxidative killing of microorganisms by phagocytic leukocytes. Ciba Found. Symp. **65**:225–254.