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Impaired Chemiluminescence During Phagocytosis of Opsonized Bacteria

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A simple technique is presented for rapid screening of leukocytes of patients suspected of having a defective intracellular mechanism for the bactericidal and digestive disposal of bacteria.

Children with chronic granulomatous disease are highly susceptible to bacterial infection, and it has been determined that neutrophils and monocytes of these patients ingest bacteria but fail to kill catalase-positive microorganisms (2, 4). The bactericidal ability of normal leukocytes is probably linked to the reduction of O_2 which occurs in a sequence of events involving respiration of the hexosemonophosphate shunt.

Evidence for the appearance of an electronic excitation state during phagocytosis by normal human neutrophils has been presented by Allen et al. (1). These workers showed that chemiluminescence was evoked in neutrophils upon a phagocytic challenge, but not in lymphocytes. They proposed that the chemiluminescence reflected the generation of singlet oxygen ($^1O_2$) which could act per se as a bactericidal agent.

The present study shows that leukocytes isolated from the blood of patients with chronic granulomatous disease fail to elicit chemiluminescence during phagocytosis of opsonized bacteria.

Blood was obtained by venipuncture and mixed with a 5% dextran (molecular weight 250,000) in 0.9% NaCl solution containing heparin as an anticoagulant. The red cells were allowed to settle for 45 min at 37 C. The leukocyte-rich plasma was centrifuged for 4 min at 120 × *g*. The supernatant fluid was decanted, and the sedimented leukocytes were washed by suspension in phosphate-buffered saline (*p*H 7.4) and then centrifuged for 5 min at 100 × *g*. The leukocytes were resuspended in Krebs-Ringer bicarbonate buffer fortified with glucose (80 mg/100 ml) to give a final cell suspension of 10^6/ml. Phagocytosis was induced by addition of 0.6 mg of heat-killed, washed *Propionibacterium sherminii* suspended in 0.5 ml of fresh normal human serum. Chemiluminescence was measured with a Packard Scintillation spectrometer model 3320 operated in the out-of-coincidence mode as described by Stanley and Williams (5) at the ambient temperature of 22 C. For continuous recording, the summation signal was fed into a Packard Ratemeter model 280A and then to a Honeywell Elektronik 19 strip chart recorder (5).

Other workers may find it difficult to monitor this low a level of chemiluminescence. Packard Instrument Co. on request carefully selected low dark current phototubes for our instrument. Operating in the out-of-coincidence mode and in summation at 22 C with the windows set 0-∝ and at 100% gain, the empty well (elevator down) gives 3,000 to 4,000 counts/min. Empty, dark-adapted counting vials give 4,000 to 5,000 counts/min.

Most model 3320 spectrometers require refrigeration to reduce background noise to the levels obtained using our instrument at 22 C; however, the standard model can be used provided the following precautions are taken.

First, counting vials should be kept in the dark, and all manipulations involving these vials should be performed under red (actinic) light. Second, the leukocyte preparations to be tested should be added to the vial and monitored before the phagocytic challenge in order to obtain background activity. This activity should be 10,000 counts/min or less. Third, the leukocytes should be challenged with opsonized bacteria, and the activity should be monitored at 5 min and again at 30 min after addition of bacteria.

Since leukocyte metabolism is temperature dependent, only short 1-minute recordings should be made, and the vials should be removed from the refrigerated chamber and kept at ambient (20-25 C) temperature before and after counting. Photomultiplier tubes in different instruments vary in sensitivity;

therefore, each laboratory should establish a range of values for normal leukocyte preparations.

Temporal traces of chemiluminescence (expressed as counts per minute) are shown in Fig. 1. A base-line reading was recorded for about 10 min. Phagocytosis was then initiated by adding the opsonized bacteria. Chemiluminescence of magnitudes up to 30,000 counts/min was observed within 2 min for leukocytes from normal healthy individuals. A decline in activity to about 20,000 counts/min was noticed for the next 5 min. After this period, a slow but steady increase in chemiluminescence was observed which approached values of 55,000 and 60,000 counts/min within 40 to 50 min. By contrast, the leukocytes from children with chronic granulomatous disease exhibited an insignificant initial chemiluminescence which rapidly declined to resting values. The patients selected for this study showed the clinical signs of chronic granulomatous disease: their leukocytes were incapable of reducing nitroblue tetrazolium to insoluble blue formazan, and there was no respiratory burst or increase in the activity of the hexosemonophosphate shunt upon addition of bacteria. Since the leukocytes of the patients in this study exhibited a normal increase in hexosemonophosphate shunt activity in response to methylene blue the patients did not suffer from severe glucose-6-phosphate dehydrogenase deficiency.

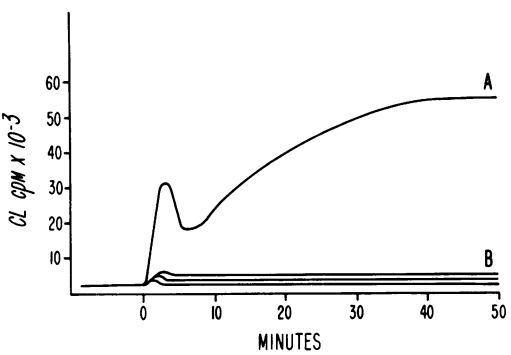

FIG. 1. *Continuous temporal traces of chemiluminescence counts per minute elicited by phagocytizing leukocytes. Curve A represents 5 × 10⁶ normal human leukocytes (66% neutrophils) in 7 ml of Krebs-Ringer bicarbonate buffer supplemented with glucose (80 mg/100 ml). Curves B represent three cases of chronic granulomatous disease. Each vial contained 5 × 10⁶ leukocytes (40% neutrophils) in 7 ml of Krebs-Ringer bicarbonate. At zero time 0.6 mg (dry weight) of heat-killed opsonized bacteria were added.*

The method as outlined above is simple and requires less than 5×10^6 leukocytes. The time required for the entire test is less than 2 hr. It circumvents the tedious measurement of the pentose cycle activity with glucose-*1*-^{14}C and glucose-*6*-^{14}C, and it avoids interpretation of questionable appearance of formazan.

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