Influence of Type of Ingested Particle on Human Leukocyte Metabolism (35761)

GERALD L. MANDELL

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Polymorphonuclear neutrophils (PMN) exhibit a marked increase in oxygen consumption and hydrogen peroxide production following phagocytosis (1). Ingestion of a wide variety of substances (*i.e.*, polystyrene, starch, clay, bacteria, fungi) will stimulate PMN postphagocytic metabolism. How the ingested particle or microbe stimulates leukocyte metabolism is unknown. It is also unclear if the particle is passive, and stimulation is due to a phenomenon such as internalization of the PMN membrane or if there are other PMN–particle interactions. PMN metabolic response to the ingestion of three different types of particles was studied by quantitating oxygen consumption and hydrogen peroxide production.

Methods. Bacteria. Staphylococcus aureus or Micrococcus sp. were grown overnight in trypticase soy broth, washed $\times$ 2, and boiled for 10 min. The heat-killed bacteria were then rewashed and resuspended in Hanks' balanced salt solution (HBSS). The bacteria were diluted in HBSS to achieve the desired bacteria:PMN ratio.

Polystyrene latex particles. Particles (Dow Chemical Company, Midland, Michigan) 1.08-μ diameter were washed $\times$ 2, and suspended in HBSS to achieve the desired particle:PMN ratio.

Polymorphonuclear neutrophils. Blood obtained from healthy donors was heparinized and leukocytes were separated by dextran sedimentation and differential centrifugation. Most of the erythrocytes remaining with the leukocytes were lysed with iced distilled water (2).

Oxygen consumption. Suspensions of 1–3 $\times$ 10^7 PMN in 3 ml of HBSS with 10% autologous serum plus heat-killed bacteria or polystyrene latex spheres were placed in the chambers of a polarographic oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio, Model 53). At the end of the determination of oxygen consumption, samples of leukocytes were removed, smears were made, and the numbers of intracellular particles or bacteria were enumerated.

Hydrogen peroxide production. ^{14}C-Formate oxidation was measured to quantitate hydrogen peroxide production (1). The PMN obtained from peripheral blood were washed and resuspended in Krebs–Ringer phosphate medium containing 5 mmoles of glucose/liter. A suspension of 10^7 PMN and 0.5 μCi of ^{14}C-formate (total formate = 0.7 μmoles) in Krebs–Ringer phosphate medium with 25% autologous serum was placed in 10-ml siliconized Erlenmyer flasks. 0.2 ml of 10% KOH was placed in a plastic well and this was suspended through an airtight rubber stopper over the flask (Kontes Glass Co., Vineland, New Jersey). 10^9 heat-killed staphylococci or 10^9 latex particles were added to the flask to make the final volume of the suspension 1.0 ml. After incubation, with shaking, at 37° for 30 min, 0.5 ml of 1 *N* HCl was added to stop the reaction and liberate CO_2. The flask was incubated for 15 min more; and the plastic well containing KOH and absorbed CO_2 was put into a counting vial with 10 ml of scintillation counting fluid (toluene with 0.4% Liquifluor, and 30% methanol); and radioactivity was assayed in a Nuclear-Chicago liquid scintillation counter. Samples of the cell–particle suspensions were removed just prior to the addition of HCl; and stained smears were prepared to enumerate the bacteria or latex particles ingested per PMN. Experiments were performed in triplicate.

Results. Oxygen consumption. PMN that

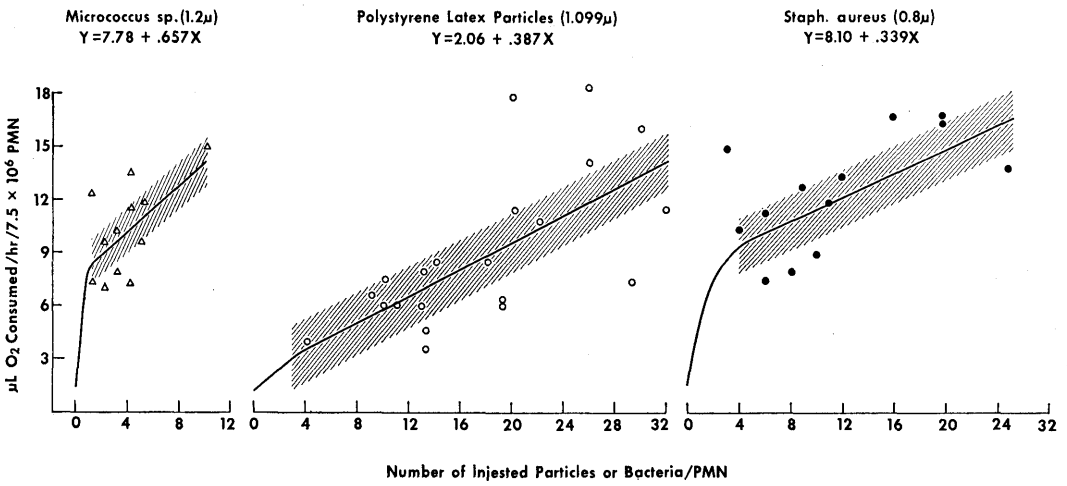

FIG. 1. Oxygen consumption of PMN after ingestion of polystyrene latex particles, *S. aureus* or Micrococcus sp. Simple linear regression curves are shown and one standard deviation is indicated.

had ingested heat-killed Micrococcus sp. showed the greatest increase in postphagocytic oxygen consumption. The somewhat smaller *S. aureus* 502A was the second most effective stimulator of oxygen consumption and latex particles were least effective (Fig. 1). Expressing the data in another way, PMN that have ingested 8 particles/cell show the following oxygen consumption: The Micrococcus-containing cells consumed 13.3 μl of O_2/hr/7.5 $\times$ 10^6 PMN; the *S. aureus*-containing cells, 10.8 μl of O_2/hr/7.5 $\times$ 10^6 PMN; and the polystyrene latex sphere-containing cell, 5.2 μl of O_2/hr/7.5 $\times$ 10^6 PMN.

Hydrogen peroxide production. Formate oxidation (as an index of hydrogen peroxide production) was more pronounced in leukocytes that had ingested *S. aureus* when compared to leukocytes that had ingested latex spheres (Table I). Precise enumeration of the number of particles ingested was not possible because of the high particle:PMN ratio necessary for measurable formate oxidation, but PMN engulfed equivalent numbers of polystyrene latex spheres and bacteria (60–100/cell).

Discussion. Krenis and Strauss (3) demonstrated that the oxygen consumption of human blood leukocytes was directly proportional to the size and numbers of latex particles ingested. As the size and number of particles increased, the oxygen consumption increased. Roberts and Quastel (4) observed the same phenomenon with guinea pig PMN. In a series of experiments performed with mouse peritoneal mononuclear phagocytes, Axline and Cohn (5) showed that *in vitro* induction of lysosomal enzymes was altered by the nature of the particle ingested. When erythrocytes and nondigestable particles were compared, it was found that phagocytosis of

TABLE I. ^{14}C-1-Formate Oxidation by 10^7 PMN After 30-min Incubation (counts/min).[a]

PMN	PMN + latex spheres	PMN + *S. aureus*
1693 ± 221 (6)	2577 ± 388 (6)	3795 ± 497 (6)

[a] Numbers of experiments performed are shown in parentheses and each experiment is the mean of triplicate runs. The SEM is indicated. PMN that ingested *S. aureus* or polystyrene latex particles showed significantly greater formate oxidation than "resting" PMN ($p < 0.002$ and $p < 0.01$, respectively). PMN that ingested *S. aureus* oxidized more formate than did PMN that ingested polystyrene latex spheres ($p < 0.01$).

erythrocytes induced a marked increase in levels of lysosomal enzymes, but in contrast to this, phagocytosis of polyvinyl toluene, polystyrene, and insoluble starch particles, produced no increase in macrophage lysosomal enzymes.

Our studies demonstrate that heat-killed bacteria are more effective as stimulators of postphagocytic metabolism than are latex particles of a similar or even somewhat greater size. This suggests that the metabolic changes noted postphagocytosis, are not due simply to internalization of PMN cellular membrane, but may reflect an interaction between the ingested particle and the PMN.

Summary. The metabolic activity of human polymorphonuclear neutrophils (PMN) was studied following the ingestion of polystyrene latex spheres (1.08 μ), heat-killed *S. aureus* (0.8 μ) or heat-killed Micrococcus sp. (1.2 μ). Phagocytsois of heat-killed bacteria resulted in a greater postphagocytic increase in oxygen consumption and hydrogen peroxide production than did phagocytosis of latex particles. These data suggest that the nature of the ingested particles has an influence on postphagocytic metabolic events.

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