JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 531-532

Vol. 100, No. 1
Printed in U.S.A.

Leukocyte Bactericidal Activity in Chronic Granulomatous Disease: Correlation of Bacterial Hydrogen Peroxide Production and Susceptibility to Intracellular Killing

GERALD L. MANDELL[1] AND EDWARD W. HOOK[1]

Department of Medicine, New York Hospital-Cornell Medical Center, New York, New York 10021

Received for publication 25 June 1969

Susceptibility of bacteria to intracellular killing by polymorphonuclear leukocytes from a patient with chronic granulomatous disease could be correlated with bacterial hydrogen peroxide production.

Patients with chronic granulomatous disease of childhood (CGD) suffer from severe and recurrent bacterial infections. Polymorphonuclear neutrophils (PMN) from these patients phagocytize bacteria normally but are unable to kill certain organisms after ingestion. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella-Aerobacter*, *Salmonella*, *Serratia marcescens* and several other gram-negative enteric bacilli are not killed normally within PMN of CGD patients. These bacteria are by far the most common infecting organisms in patients with CGD (7). In contrast, *Diplococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus fecalis* are killed readily within PMN of patients with CGD and have not been reported to play a role in the recurrent infections in these patients (3). The reasons for the variation in bactericidal activity of CGD leukocytes for different species of bacteria are not known.

Studies of the metabolic abnormalities of PMN from patients with CGD have shown that these cells do not demonstrate the normal postphagocytic increase in hydrogen peroxide production (2). Hydrogen peroxide, in combination with myeloperoxidase and an appropriate oxidizable substance such as iodide, has been shown to be a potent bactericidal system in PMN (4). The lack of hydrogen peroxide production by PMN from patients with CGD is the most likely explanation for the defective bactericidal capacity of these cells. Klebanoff and White (5) have postulated that intraleukocytic formation of hydrogen peroxide by bacteria could make up for the defect in hydrogen peroxide generation by leukocytes and account for the ability of CGD leukocytes to kill certain bacteria.

The present study was undertaken to determine if there is a correlation between the capacity of bacteria to generate hydrogen peroxide and the susceptibility of these bacteria to the lethal action of CGD leukocytes.

The bactericidal capacity of PMN from normal individuals and a patient with CGD was tested against 10 different microorganisms by utilizing a membrane filter (Millipore Corp., Bedford, Mass.) technique to separate free bacteria from cell-associated bacteria (G. L. Mandell, and E. W. Hook, Amer. J. Med., *in press*). In this test bacteria and leukocytes in a 1:1 ratio were incubated for 2.5 hr in a shaker-incubator at 37 C. At the end of the incubation period, the suspension of cells and bacteria was diluted and filtered through a 3 μm membrane filter (Millipore). The filter sheet was placed on a Trypticase Soy Agar (BBL) plate and incubated at 37 C for 18 hr. The colonies on the filter sheet were counted to quantitate the number of viable cell-associated bacteria. Under these conditions more than 99.9% of the bacteria were phagocytized by leukocytes from the patient and from normal controls. Results obtained with this test were confirmed by standard differential centrifugation tests (6) for *D. pneumoniae*, *S. faecalis*, *S. aureus*, and *S. marcescens*.

Hydrogen peroxide formation by bacteria was determined by the method of Krause et al. (6) by utilizing a benzidine-blood agar medium in which peroxide-producing bacteria grow in black colonies. Catalase was quantitated by the method of Amin and Olson (1).

[1] Present address: University of Virginia School of Medicine, Charlottesville, Va. 22901.

TABLE 1. *Relationship of hydrogen peroxide and catalase production by bacteria to susceptibility to intracellular killing by CGD leukocytes*

Organism	Susceptibility to intracellular killing by CGD leukocytes[a]	H_2O_2 production	Catalase activity (H_2O_2 destroyed after 15 min of incubation)
			%
Diplococcus pneumoniae (type 19)	0.49:1	Pos	0
Streptococcus pyogenes	0.92:1	Pos	0
Streptococcus fecalis	1.7:1	Pos	0
Salmonella typhimurium[b]	3.3:1	Neg	100
Pseudomona aeruginosa[b]	3.4:1	Neg	100
Klebsiella-Aerobacter	5.5:1	Neg	100
Staphylococcus aureus (502A)[b]	8.1:1	Neg	100
Proteus mirabilis	35:1	Neg	100
Escherichia coli (0111B4)	50:1	Neg	100
Serratia marcescens[b]	85:1	Neg	100

[a] Expressed as the ratio of organisms surviving intracellularly in PMN from the patient with CGD to the number of organisms surving intracellularly in simultaneously tested control PMN. Ratios greater than 2.5:1 were considered indicative of abnormal intracellular killing.

[b] Species that caused severe infection in this patient with CGD.

The results are shown in the Table 1. The bacteria that produced catalase and had no detectable hydrogen peroxide formation were not killed normally by leukocytes from the one patient with CGD tested. In contrast, organisms that did not produce catalase but showed hydrogen peroxide production were killed normally by CGD leukocytes.

These data are compatible with the hypothesis that formation of hydrogen peroxide by bacteria within leukocytes could compensate for the defect in hydrogen peroxide production by CGD leukocytes. The hydrogen peroxide produced by bacteria, acting in concert with myeloperoxidase within the phagocytic vacuole could account for the ability of CGD leukocytes to kill certain bacteria.

This investigation was supported by Public Health Service grants TO1 AI00255 and AI05940 from the National Institute of Allergy and Infectious Diseases and contract U-1107 with the Health Research Council of the City of New York.

LITERATURE CITED

1. Amin, W. M., and N. F. Olson. 1968. Influence of catalase activity on resistance of coagulase-positive staphylococci to hydrogen peroxide. Appl. Microbiol. 16:267–270.
2. Holmes, B., A. R. Page, and R. A. Good. 1967. Studies of the metabolic activity of leukocytes from patients with genetic abnormality of phagocytic function. J. Clin. Invest. 46: 1422–1432.
3. Kaplan, E. L., T. Laxdal, and P. G. Quie. 1968. Studies of polymorphonuclear leukocytes from patients with chronic granulomatous disease of childhood: bactericidal capacity for streptococci. Pediatrics 41:591–599.
4. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism J. Exp. Med. 126:1063–1079.
5. Klebanoff, S. J., and L. R. White. 1969. Iodination defect in the leukocytes of a patient with chronic granulomatous disease of childhood. New Engl. J. Med. 280:460–466.
6. Kraus, F. W., J. F. Nickerson, W. I. Perry, and A. P. Walker. 1957. Peroxide and peroxidogenic bacteria in human saliva. J. Bacteriol. 73:727–735.
7. Quie, P. G., J. G. White, B. Holmes, and R. A. Good. 1967. *In vitro* bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. J. Clin. Invest. 46:668–679.