AD-A058 664   NEW ENGLAND MEDICAL CENTER HOSPITAL BOSTON MASS   F/G 6/16
MECHANISM OF BACTERIAL KILLING BY THE PERITONEAL AND HEPATIC MA--ETC(U)
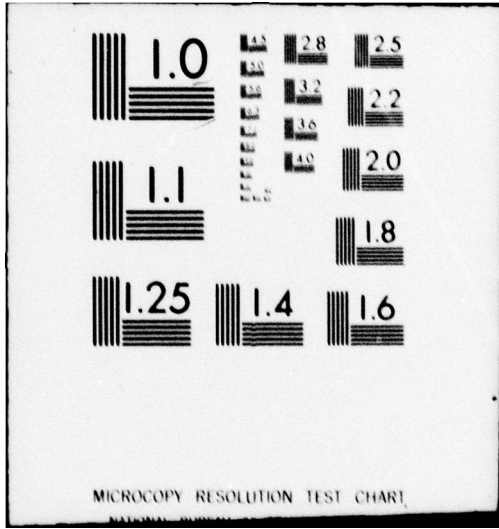NOV 76   B M BABIOR   DAMD17-74-C-4055

UNCLASSIFIED   NL

AD
A058 664

END
DATE
FILMED
-11--78

DDC

1.0

1.1

1.25 1.4 1.6

2.8 2.5
3.2 2.2
3.6
4.0 2.0

1.8

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU

Contract No. DAMD 17-74-C-4055

LEVEL

(6) MECHANISM OF BACTERIAL KILLING BY THE PERITONEAL
AND HEPATIC MACROPHAGE POPULATION: OXIDATIVE
BACTERICIDAL MECHANISMS OF PHAGOCYTIC CELLS.

(9) Final Progress Report. 1 Jan 74—31 Dec 76,

(20) Bernard M. Babior, M.D.

New England Medical Center Hospital

171 Harrison Avenue

Boston, Massachusetts 02111

(16) 3A161102B71Q (17) ΦΦ

(12) 10 p.

D D C
RECEIVED
SEP 15 1978
A

Submitted to the

U.S. Army Medical Research and Development Command

Washington, D.C. 20314

(11) 28 Nov � 76

Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mechanism of Bacterial Killing by the Peritoneal and Hepatic Macrophage Population: Oxidative Bactericidal Mechanisms of Phagocytic Cells AD-A058663		5. TYPE OF REPORT & PERIOD COVERED FINAL REPORT 1 Jan 1974 - 31 Dec 1976
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Bernard M. Babior, M. D.		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-74-C-4055
9. PERFORMING ORGANIZATION NAME AND ADDRESS Tufts-New England Medical Center 171 Harrison Avenue Boston, Massachusetts 02111		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A 3A161102B71Q.00.087
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Washington, D. C. 20314		12. REPORT DATE 25 November 1976
		13. NUMBER OF PAGES 11 pages
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE
1 JAN 73

ABSTRACT

The overall purpose of this project is to understand the means by which phagocytes kill bacteria. The particular goal since the beginning of the report period has been to develop a system to examine the effect of oxygen tension on the respiratory burst of neutrophils.

Previous studies showed that the methods we had used to provide a hypoxic or anoxic environment for neutrophils to work in had led to damage to the cells. During the project period, we were able to devise a technique for incubating neutrophils under hypoxic conditions, and examined the relationship between oxygen tension and O_2^- production by these cells.

SUMMARY

The overall purpose of the project is to understand the means by which phagocytes kill bacteria. The goal set for this project period was to devise a method by which the effect of oxygen tension on neutrophil function could be examined. We were able to accomplish this task through the use of a gassing technique based on submersion of the reaction vessel, and were able to examine phagocytosis and O_2^- production as a function of oxygen tension. The experiments showed that phagocytosis was as efficient under N_2 as under air, a result confirming previous studies. O_2^- production rates by whole neutrophils were constant at oxygen tension levels between room air and 1% O_2, then fell in a linear manner with further declines in oxygen tension.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

TABLE OF CONTENTS

BODY OF REPORT

Background

Upon exposure to opsonized bacterial, normal granulocytes undergo
changes in their oxidative metabolism (the "respiratory burst") which
result in a marked increase in oxygen uptake, a rise in H_2O_2 and super-
oxdie (O_2^-) production and a tenfold increase in the amount of glucose
metabolized via the hexosemonophosphate shunt (1-8). These changes in
metabolism are thought to be related to the activation of oxygen-dependent
bactericidal mechanisms, since patients whose granulocytes cannot undergo
these metabolic alterations (e.g., patients with chronic granulomatous
disease or severe glucose-6-phosphate dehydrogenase deficiency) are
unusually susceptible to bacterial infections (9,10). Hydrogen peroxide
in particular has been implicated in a bactericidal process, since Klebanoff
has shown that bacteria are readily killed when incubated with hydrogen
peroxide and halide ion in the presence of myeloperoxidase, an enzyme present
in large amounts in the primary granules of polymorphonuclear leukocytes (11).

The studies documenting the importance of oxygen to bacterial killing
by neutrophils have almost always been conducted in room air. However,
infected sites tend to be regions of diminished oxygen tension. While killing
in the absence of oxygen can be accomplished by neutrophils (12), the aerobic
bactericidal mechanisms are exceptionally powerful--probably more so than the
oxygen-independent bactericidal mechanisms --so it is of interest to know
the extent to which these mechanisms can operate under conditions of hypoxia.

Results

The following method was used to carry out incubations under defined
atmospheres. Siliconized 15 ml Warburg flasks with single sidearms were
used as reaction vessels. The cells (2.5×10^7 in 1.5 ml PBS) were placed
in the main compartment of the flask, and zymosan (6 mg in 0.5 ml PBS) in
the sidearm. The mouth of the flask was stoppered with a syringe port, and
the sidearm stoppered with a venting plug cut sufficiently short so the out-
let would be under water during the incubation. The venting plug was attached
to a source of the desired gas by means of a rubber tube, and the syringe
port was pierced by a 20 gauge needle connected to a short length of plastic
intravenous tubing. The whole assembly was placed on a low rocking platform
designed specifically for this purpose which was placed in the bottom of a
Thelco Model 83 water bath and driven by means of an Ames aliquot mixer to
which it was connected by a long rod. The water level was such that the
needle hub and the venting plug outlet were submerged, and the plastic
tubing was arranged so that its remote end was under water as well. The
rocking motor was turned on, and the flask was gassed for 7
minutes. Gassing was terminated by first closing the venting plug, then
removing the needle from the syringe port; this procedure ensured that the
flask would contain the gas at atmospheric pressure. After incubating
for 3 more minutes to establish equilibrium between the constituents in
the flask and the overlying atmosphere, the contents of the sidearm were
tipped into the main compartment, taking care to keep the reaction vessel
completely submerged. The flask was placed back on the rocking platform
and the incubation continued for the desired length of time. The incubation
was terminated by removing the flask from the water bath and immediately
placing it in melting ice. The syringe port was then removed and the contents

of the flask, now chilled, were transferred to a homogenizer for further work.

To confirm that the atmospheric composition was maintained in these experiments, measurements were made of O_2^- production by neutrophils incubated under N_2 for 60 minutes. With opsonized zymosan as the respiratory burst stimulus, O_2^- production by whole cells was negligible under nitrogen while values obtained under room air were comparable to those previously reported. These results indicate that this technique is suitable for conducting incubations under defined atmospheres.

Phagocytosis was measured using opsonized zymosan as the test particle. The number of particles associated with the neutrophils (4.8 ± 0.2 SE particles/cell after 25 minutes of incubation (50 cells were counted to obtain this value)) as measured in air was statistically identical to the value obtained in incubations conducted under N_2. O_2^- production was determined by a previously reported technique, again using opsonized zymosan as the test particle. The rate of O_2^- production under 1% oxygen was 85% that measured in room air. Below 1% oxygen, O_2^- production fell more precipitously, declining linearly with oxygen tension.

Conclusion

These findings indicate that a highly efficient respiratory burst can be mounted by neutrophils at oxygen tensions less than 10% those of arterial blood, and that even under the very hypoxic conditions found at sites of infection, the oxygen-requiring bactericidal mechanisms of the neutrophil are likely to contribute significantly to bacterial killing.

Bibliography

1. Babior, B.M., Kipnes, R.S. and Curnutte, J.T.: J. Clin. Invest. 52, 741 (1973).

2. Curnutte, J.T. and Babior, B.M.: J. Clin. Invest. 53, 1662 (1974).

3. Curnutte, J.T. and Babior, B.M.: Blood 45, 851 (1975).

4. Johnston, R.B., Jr., Keele, B.B., Jr., Misra, H.P., Lehmeyer, J.R. Webb, L.S., Baehner, R.L. and Rajagopalan, K.V.: J. Clin. Invest. 55, 1357 (1975).

5. Weening, R.S., Wever, R. and Roos, D.: J. Lab. Clin. Med. 84, 245 (1975).

6. Sbarra, A.J. and Karnovsky, M.L.: J. Biol. Chem. 234, 1355 (1959).

7. Iyer, G.Y.N., Islam, M.F. and Quastel, J.H.: Nature 192, 535 (1961).

8. Baehner, R.L., Gilman, N. and Karnovsky, M.L.: J. Clin. Invest. 59, 692 (1970).

9. Nathan, D.G. and Baehner, R.L.: Prog. Hematol. 7, 235 (1971).

10. Cooper, M.R., DeChatelet, L.R., McCall, C.E., LaVia, M.F., Spurr, C.L. and Baehner, R.L.: J. Clin. Invest. 51, 769 (1972).

11. Klebanoff, S.J. and Hamon, C.B.: J. Reticuloendothelial Soc. 12, 170 (1972).

12. Mandell, G.L.: Inf. Immun. 9, 337 (1974).