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Human Peritoneal Macrophage Phagocytic, Killing, and Chemiluminescent Responses to Opsonized *Listeria monocytogenes*

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Opsonization with normal human serum, purified immunoglobulin G, or immunoglobulin G-deficient serum promoted phagocytosis of *Listeria monocytogenes* by human peritoneal macrophages. However, normal human serum was the most effective opsonin in eliciting killing and chemiluminescent responses. Macrophages phagocytized and killed almost as much as polymorphonuclear leukocytes but produced considerably less chemiluminescence.

Although *Listeria monocytogenes* has been extensively studied in a variety of animal systems, the role of serum factors in the phagocytic process has not been completely evaluated. *Listeria* cell wall fractions activate the alternative complement pathway (2), and a heat-labile serum factor increases phagocytosis of *L. monocytogenes* by murine macrophages (8), presumably representing the generation of an opsonically active fragment of the complement system, such as C3b. Heat-stable factors are also involved in *Listeria* uptake by human polymorphonuclear leukocytes (PMN) and monocytes (11). Human PMN and macrophages grown from monocytes in vitro are capable of killing *Listeria* spp. by an oxygen-dependent mechanism (4, 5, 14).

To delineate the relative roles of antibody and complement in the opsonic requirements of *L. monocytogenes*, we opsonized *L. monocytogenes* with normal human serum, purified human immunoglobulin G (IgG), and serum deficient in IgG. Bacterial uptake, killing, and chemiluminescence (CL) assays were performed with human peritoneal macrophages (PMϕ) and blood PMN.

L. monocytogenes serotype 1 was provided by R. Postlethwaite, University of Aberdeen, United Kingdom. This strain was originally isolated from a neonatal autopsy specimen. The 50% lethal dose of this isolate by mouse peritoneal challenge is 2×10^6. For the present investigation, this organism was inoculated and grown in Mueller-Hinton broth containing [^{3}H]thymidine (15). Radiolabeled bacteria were harvested by centrifugation and washed twice in sterile phosphate-buffered saline, pH 7.4, before being adjusted to 5×10^8 CFU/ml. Bacteria were opsonized for 60 min at 37°C with 10% normal serum (pooled from five healthy donors), 2 mg of purified human IgG per ml (a gift from J. A. Hooper, Hyland Laboratories, Inc., Glendale, Calif.), or 10% IgG-deficient serum. Hanks balanced salt solution enriched with 0.1% gelatin (GHBSS) was used to dilute each opsonin to the desired final concentration. The IgG-deficient serum was prepared by treating serum from a patient with Bruton agammaglobulinemia with protein A-coated Sepharose beads at 4°C. The final concentration of IgG was <3 mg/100 ml, IgM was undetectable, and the total hemolytic complement was normal. This IgG-deficient serum was used to study opsonization by the serum complement system in the absence of IgG; purified IgG was used to study opsonization in the absence of complement. The final concentration of opsonized bacteria was 5×10^7 CFU/ml.

PMN were isolated as previously described (11). PMϕ were obtained from peritoneal effluents of uninfected end-stage renal disease patients treated by maintenance intermittent peritoneal dialysis. Three liters of effluent were centrifuged at 1,600 × *g* for 30 min, and pellets were suspended in 20 ml of GHBSS. Cells were washed twice and suspended to a final volume of 5 ml. Total and differential cell counts were performed. Preparations containing >80% PMϕ and <10% PMN were used. Peripheral blood PMN and PMϕ were suspended at a final concentration of 5×10^6 phagocytes per ml of GHBSS. Uptake of radiolabeled bacteria was determined as previously described (11).

The uptake of unopsonized and opsonized radiolabeled bacteria was followed for 60 min

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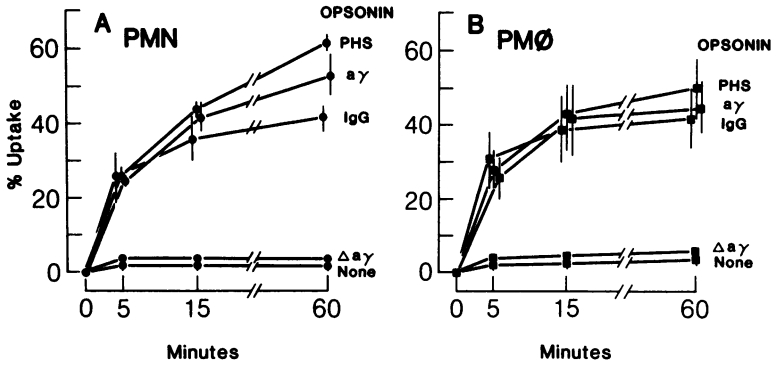

FIG. 1. Uptake of *L. monocytogenes* by (A) PMN and (B) PMϕ. The rate of uptake of [^{3}H]thymidine-labeled bacteria was followed for 60 min after opsonization with 10% normal (pooled) human serum (PHS), 10% IgG-deficient serum (aγ), 2 mg of purified IgG per ml (IgG), heated IgG-deficient serum (Δaγ), or buffer GHBSS (None). Points represent the average of three experiments ± the standard error.

(Fig. 1). *L. monocytogenes* opsonized with normal (pooled) human serum, IgG, or IgG-deficient serum were effectively taken up by both PMN and PMϕ. The rates of uptake of bacteria opsonized with each of these three opsonins were similar for PMϕ (no significant difference between normal serum and IgG-depleted serum; $P > 0.05$). Uptake by PMN was somewhat greater when normal serum was used as an opsonin than when IgG was so used ($P < 0.05$). When IgG-deficient serum was heated (56°C, 30 min) to inactivate complement, its opsonic activity was abolished. Heat inactivation of normal serum did not affect its opsonic activity (data not shown), indicating that this serum source contains a high titer of a heat-stable opsonin, most likely IgG, for this *Listeria* strain. Electron micrographs (prepared by standard techniques) of PMϕ incubated with *L. monocytogenes* revealed that few unopsonized bacteria were internalized. Bacteria opsonized with IgG, IgG-deficient serum, or normal serum were readily detected within the phagocytic vacuoles of PMϕ.

Bacterial killing was measured in duplicate by using mixtures constituted as for uptake experiments. CFU of *L. monocytogenes* were determined immediately after constituting phagocytosis mixtures (zero time) and after 60 min of incubation. Phagocytes were lysed in ice-cold sterile water and serially diluted before plating out on nutrient agar.

Bacterial killing was measured in tandem with uptake (Fig. 2). Normal (pooled) human serum mediated the greatest bacterial killing; opsonization by IgG alone and serum deficient in IgG produced less killing by both PMN and PMϕ. Of the *Listeria* organisms, 89 ± 7% survived when incubated at 37°C in the absence of phagocytes.

A CL assay was used to measure the generation of reactive oxygen species (1). For CL studies, *L. monocytogenes* was opsonized in 20% normal (pooled) human serum, 4 mg of IgG per ml, or 20% IgG-deficient serum for 60 min at 37°C with rotation. Opsonized bacteria were washed and suspended in GHBSS to a concentration of 1.5×10^7 CFU/ml. PMN and PMϕ were prepared as before and adjusted to a concentration of 5×10^4 phagocytes per ml. Next, 1 ml of bacterial suspension, 3.5 ml of GHBSS, and 20 μl of luminol (275 μM in dimethyl sulfoxide) were added to dark-adapted glass vials. After recording background levels in a liquid scintillation counter (model LS-100C; Beckman Instruments, Irvine, Calif.), 1 ml of phagocytes (5×10^4 cells) was then added sequentially to the appropriate vials, giving a *L. monocytogenes*-to-phagocyte ratio of 300:1. The CL response was observed for 120 min. Figure 3 shows an average of two representative experiments. Phagocytes incubated by themselves never produced CL of $>12 \times 10^3$ CPM/10^4 cells. All three opsonins proved capable of producing CL above the level of unopsonized bacteria. However, bacteria opsonized with normal serum generated an earlier CL response than did bacteria opsonized with IgG or IgG-deficient serum. PMN produced approximately four times the CL of PMϕ.

Macrophages grown from blood monocytes in vitro rapidly phagocytize *Listeria* spp. opsonized with human sera (4, 13, 14). The present study shows that human PMϕ from chronic peritoneal dialysis patients are also capable of phagocytizing *L. monocytogenes* if opsonized with normal serum containing both IgG and complement, or with IgG alone, or with a heat-labile factor present in IgG-deificient serum, most probably representing production of an opsonically active fragment of C3 such as C3b. In contrast, sheep erythrocytes are only internalized if coated wtih IgG (9). The difference between our findings with *L. monocytogenes*

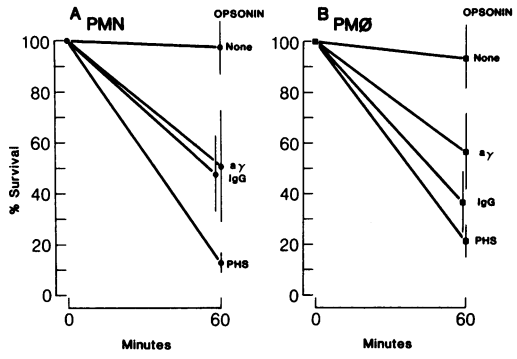

FIG. 2. Killing of *L. monocytogenes* by (A) PMN and (B) PMϕ after opsonization with 10% normal (pooled) human serum (PHS), 10% IgG-deficient serum (aγ), 2 mg of purified IgG per ml (IgG), or buffer GHBSS (none). Points represent an average of three experiments ± the standard error.

and the studies with opsonized sheep erythrocytes is probably a reflection of the different particles used in the two systems.

Human macrophages grown in vitro can kill 70% of opsonized *Listeria* spp. (4), whereas alveolar macrophages kill approximately 60% of *Listeria* spp. in 1.5 h (6). In this study we found that PMϕ could kill 78 ± 6% of *L. monocytogenes* opsonized with human sera. The finding that killing was somewhat greater than would have been expected from the uptake experiments may indicate that there is some extracellular killing of *Listeria* spp., as reported in a different assay system (3). Our findings also imply that killing and CL can occur through either a complement or an Fc receptor mechanism. However, the observation that the killing and CL responses were significantly delayed when *L. monocytogenes* was opsonized with IgG or IgG-deficient serum in contrast to normal serum, which contains both IgG and an intact complement system, suggests the possibility that maximal killing and stimulation of oxidative metabolism may require opsonization by both IgG and C3b.

PMN generate significantly greater CL than PMϕ. This, however, is not reflected in the killing of *Listeria* spp. by these cells, suggesting either the presence of an efficient oxygen-independent mechanism of killing (12) in macrophages or a high level of redundancy in oxygen radicals produced by PMN. Since the measurement of CL in the presence of the chemiluminogenic substrate luminol is absolutely dependent upon a myeloperoxidase-mediated reaction (7), it is also possible that the relatively poor CL response observed with PMϕ reflects a deficiency of myeloperoxidase of PMϕ when compared with PMN.

It is not yet known whether the phagocytic, killing, and CL responses of the dialysate-elicited PMϕ used in this study are representative of resident human PMϕ or reflect an activated monocyte-derived exudate PMϕ population. Nevertheless, the efficient killing of *Listeria* spp. by human PMN, monocytes, alveolar macrophages, and now PMϕ may partly explain why only 13% of adult listeriosis occurs in otherwise healthy individuals (10).

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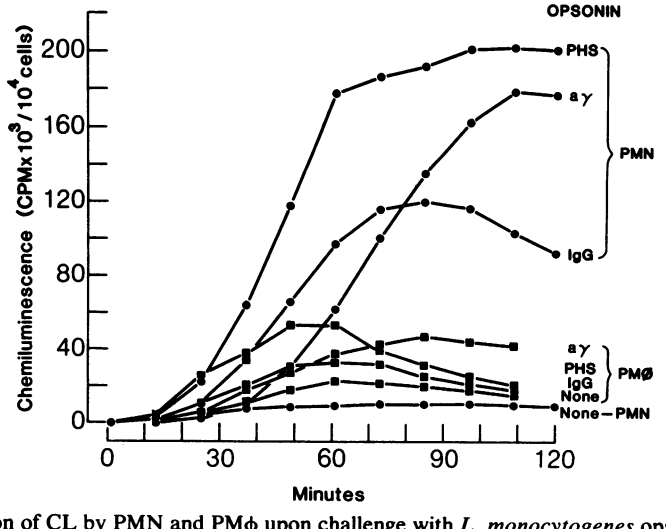

FIG. 3. Production of CL by PMN and PMϕ upon challenge with *L. monocytogenes* opsonized with normal (pooled) human serum (PHS), IgG-deficient serum (aγ), purified IgG (IgG), or buffer GHBSS (None).

LITERATURE CITED

1. **Allen, R. C., and L. D. Loose.** 1976. Phagocytic activities of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem. Biophys. Res. Commun. **69**:245–252.
2. **Baker, L. A., P. A. Campbell, and J. R. Hollister.** 1977. Chemotaxigenesis and complement fixation by *Listeria monocytogenes* cell wall fractions. J. Immunol. **119**:1723–1726.
3. **Bast, R. C., R. P. Cleveland, B. H. Littman, B. Zbar, and H. J. Rapp.** 1974. Acquired cellular immunity: extracellular killing of *Listeria monocytogenes* by a product of immunologically activated macrophages. Cell. Immunol. **10**:248–259.
4. **Biroum-Noerjasin.** 1977. Listericidal activity of non-stimulated and stimulated human macrophages *in vitro*. Clin. Exp. Immunol. **28**:138–145.
5. **Cline, M. J.** 1970. Bactericidal activity of human macrophages: analysis of factors influencing the killing of *Listeria monocytogenes*. Infect. Immun. **2**:156–161.
6. **Cohen, A. B., and M. J. Cline.** 1971. The human alveolar macrophage: isolation, cultivation in vitro and studies on morphological and functional characteristics. J. Clin. Invest. **50**:1390–1398.
7. **DeChatelet, L. R., G. D. Long, P. S. Shirley, D. A. Bass, M. J. Thomas, F. W. Henderson, and M. S. Cohen.** 1982. Mechanism of the luminol-dependent chemiluminescence of human neutrophils. J. Immunol. **129**:1589–1593.
8. **Harrington-Fowler, L., P. M. Henson, and M. S. Wilder.** 1981. Fate of *Listeria monocytogenes* in resident and activated macrophages. Infect. Immun. **33**:11–16.
9. **Mantovani, B., M. Rabinovitch, and V. Nussenzweig.** 1972. Phagocytosis of immune complexes by macrophages. Different roles of macrophage receptor sites for complement (C3) and immunoglobulin (IgG). J. Exp. Med. **135**:780–792.
10. **Nieman, R. E., and B. Lorber.** 1980. Listeriosis in adults: a changing pattern. Report of eight cases and a review of the literature. 1968–1978. Rev. Infect. Dis. **2**:207–227.
11. **Peterson, P. K., J. Verhoef, D. Schmeling, and P. G. Quie.** 1977. Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leukocytes and monocytes. J. Infect. Dis. **136**:502–509.
12. **Root, R. K., and M. S. Cohen.** 1981. The microbicidal mechanisms of human neutrophils and eosinophils. Rev. Infect. Dis. **3**:565–598.
13. **Steigbigel, R., L. M. Lambert, and J. S. Remington.** 1974. Phagocytic and bactericidal properties of normal human monocytes. J. Clin. Invest. **53**:131–142.
14. **Steigbigel, R. T., L. H. Lambert, and J. S. Remington.** 1976. Polymorphonuclear leukocyte, monocyte and macrophage bactericidal function in patients with Hodgkin's disease. J. Lab. Clin. Med. **88**:54–62.
15. **Verhoef, J., P. K. Peterson, and P. G. Quie.** 1977. Kinetics of staphylococcal opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using [^{3}H]thymidine labeled bacteria. J. Immunol. Methods **14**:303–311.