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Intracellular Survival and Replication of *Erysipelothrix rhusiopathiae* within Murine Macrophages: Failure of Induction of the Oxidative Burst of Macrophages

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We investigated the ability of a virulent wild-type parent strain and acapsular avirulent transposon mutants to enter and survive intracellularly within murine peritoneal macrophages. In the presence of normal or immune serum, the parent and mutant strains were both ingested; however, the number of ingested bacteria was three- to fourfold greater in the case of the mutant strains than in the case of the parent strain. The parent strain, but not the mutant strains, survived and replicated intracellularly when ingested in the presence of normal serum, whereas both the parent and the mutant strains were readily killed when ingested in the presence of immune serum. To further investigate the mechanism by which the parent strain can survive and replicate within macrophages, we studied the oxidative burst response of macrophages to these strains by measuring chemiluminescence and intracellular reduction of Nitro Blue Tetrazolium dye. Challenge exposure of macrophages with either the parent strain preopsonized with immune serum or the mutant strains preopsonized with normal or immune serum induced a strong oxidative burst, whereas the level was very low when the parent strain was preopsonized with normal serum. Phagocytosis of either the parent strain, in the presence of immune serum, or the mutant strains, in the presence of normal or immune serum, by macrophages reduced large amounts of intracellular Nitro Blue Tetrazolium, whereas minimal amounts were reduced by the parent strain in the presence of normal serum. These results suggest that virulent *E. rhusiopathiae* can survive and subsequently replicate within murine macrophages when ingested in the presence of normal serum and that the reduced production of reactive oxidative metabolites by macrophages may, in part, be responsible for this occurrence.

Erysipelothrix rhusiopathiae is the causative agent of erysipelas in animals and erysipeloid in humans. Swine erysipelas, a severe disease causing great economic losses in the swine industry, may occur as acute septicemia or chronic disease characterized by polyarthritis and endocarditis (32). The mechanisms of natural resistance and acquired immunity against *E. rhusiopathiae* are poorly understood (31). To understand the pathogenesis of the infection, knowledge of the host response against *E. rhusiopathiae* is required.

The response of phagocytic cells to a bacterial pathogen is the first line of defense against infection. It has been reported that *E. rhusiopathiae* may have a capsule which is important in the pathogenicity of the disease (19). Previously, we reported that the virulence of the organism is associated, in part, with resistance of phagocytosis by murine polymorphonuclear leukocytes (PMNs) and that this property is a function of a capsule (26). It has also been suggested that the organism can survive within murine macrophages (27) and swine PMNs (29). In *E. rhusiopathiae* infection, antibodies are known to play an important role in protection (32, 34), and immunization with *E. rhusiopathiae* bacterins or attenuated vaccines or treatment with antiserum is widely used for disease control (32). Thus, the interaction of this bacterium with phagocytic cells and the mechanisms of host immunity are complicated. To better understand the host response to the organism, we examined phagocytosis and intracellular killing of a virulent wild-type parent strain and acapsular avirulent transposon mutants by murine macrophages in vitro. Furthermore, we investigated the oxidative burst, an important microbicidal activity of phagocytic cells, during phagocytosis of the strains by murine macrophages.

In this study, we found that murine macrophages phagocytose but do not kill virulent *E. rhusiopathiae* following ingestion in the presence of normal serum and that phagocytosis of the virulent *E. rhusiopathiae* by macrophages is not accompanied by an adequate oxidative burst response by macrophages.

MATERIALS AND METHODS

Animals. Seven- to nine-week-old female BALB/c mice were used throughout this study. They were purchased from Oriental Bioservice Kanto Inc., Tsukuba, Japan.

Bacterial strains. The *E. rhusiopathiae* strains used in this study were Fujisawa-SmR, a streptomycin-resistant spontaneous mutant of a highly virulent strain Fujisawa, and its mutant derivatives 33H6, 28G12, and 28G5, which are deficient in capsule production (26). Bacterial strains were usually grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) containing 0.1% Tween 80 (pH 7.6) (BHI-T80) (26).

Sera. Normal mouse sera were obtained from normal mice and pooled. The sera were confirmed to be devoid of antibodies to *E. rhusiopathiae* by an enzyme-linked immunosorbent assay that used intact, formalin-killed *E. rhusiopathiae* as the antigen. Immune sera were obtained as previously described (26).

Preparation of murine peritoneal macrophages. Murine exudate macrophages were isolated as previously described (5). Briefly, mice were killed with chloroform 48 h after intraperitoneal injection of 1.0 ml of sterile 10% Proteose Peptone (Difco). The peritoneal exudate cells were obtained by peritoneal lavage with 5.0 ml of Hanks' balanced saline solution (HBSS; Sigma Chemical Co., St. Louis, Mo.) containing 0.01 M EDTA. Macrophages were separated with Ficoll-Paque (Pharmacia, Uppsala, Sweden), washed once, and resuspended in RPMI 1640 medium (Sigma) containing 0.25% bovine serum albumin (RPMI-BSA). Macrophages were further purified as follows (18). Plastic flasks (25 cm^2; Nunc, Inc., Naperville, Ill.) were coated overnight at 4°C with heat-inactivated fetal bovine serum (FBS). After removing FBS, a macrophage cell suspension was introduced into the serum-coated flask and then incubated for 15 min at 37°C in a CO_2 incubator. Nonadherent cells were discarded and rinsed three times with prewarmed RPMI-BSA to remove the nonadherent cells. After incubating with ice-cold Dulbecco's phosphate-buffered saline (PBS; Nissui Pharmaceutical Co., Ltd. Tokyo, Japan) containing 0.2% EDTA and 5% FBS (EDTA-PBS) for 15

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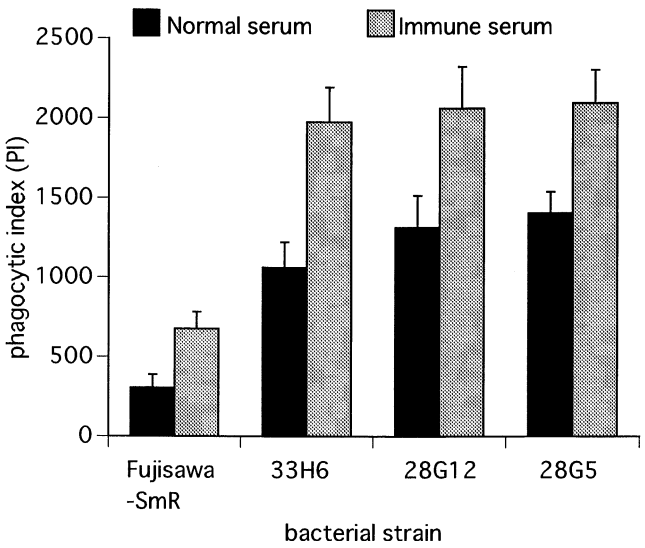

FIG. 1. Phagocytosis of *E. rhusiopathiae* strains by macrophages. Macrophages (10^6) were incubated with bacterial strains (10^7) in the presence of 10% normal or immune serum for 60 min at 37°C. Results were expressed as the mean phagocytic index of three independent experiments ± standard deviation.

min, adherent cells were collected by pipetting with EDTA-PBS, washed with RPMI-BSA twice, and then resuspended in RPMI-BSA at a cell concentration of 10^6/ml.

Phagocytosis assay. Phagocytosis assays were performed as previously reported (26). Bacteria (10^7/ml) were incubated with macrophages (10^6/ml) in a total volume of 1.0 ml of RPMI-BSA containing 10% mouse serum under rotation (8 rpm) for 60 min at 37°C. Phagocytosis was stopped by shaking the tubes in ice, and the uningested bacteria were removed by washing with cold PBS containing 5% FBS and resuspended in 2.0 ml of cold PBS containing 5% FBS. Cytospin smears were prepared, stained with Giemsa's solution, and then examined by light microscopy under oil immersion. The results were expressed as the phagocytic index (5), which is defined as the percentage of macrophages that contain bacteria × the average number of bacteria per macrophage × 100.

Intracellular killing assay. Intracellular killing of microorganisms by murine peritoneal macrophages was measured as described elsewhere (8, 20, 21). Bacteria (10^7/ml) were incubated with macrophages (10^6/ml) in a total volume of 1.0 ml of RPMI-BSA containing 10% normal or immune serum under rotation (8 rpm) for 20 min at 37°C. Noningested bacteria were removed by differential centrifugation (10 min, 900 rpm) at 4°C and two washes with ice-cold RPMI-BSA. The cells containing ingested bacteria were resuspended in RPMI-BSA containing 10% normal or immune serum and then reincubated at 37°C at 8 rpm. After various intervals, a 0.1-ml sample was removed, serially diluted in sterile distilled water containing 0.5% Tween 20 and 5% FBS, and plated on BHI-T80 agar to determine the number of viable intracellular bacteria. Intracellular killing was expressed as the percentage decrease in the initial number of viable intracellular bacteria (8, 20, 21).

CL assay. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione)-enhanced chemiluminescence (CL) was measured with a Multi-Biolumat LB9505C (EG&G Berthold, Wildbad, Germany) as previously described (4), with modifications. Murine peritoneal macrophages were prepared as described above and suspended in phenol red-free HBSS (Sigma) containing 10% FBS. Bacteria were opsonized with 10% normal or immune serum at 37°C for 30 min, washed twice, and then suspended in phenol red-free HBSS containing 10% FBS. Then 100 μl of macrophage suspension (10^6/ml), 100 μl of bacterial suspension (10^8/ml), and 10 μl of luminol (10 mM; Sigma) were mixed in a tube, and CL activity was measured.

Intracellular reduction of NBT. To study the reduction of Nitro Blue Tetrazolium dye (NBT) within macrophages, accurate determination of whether cell-associated bacteria are intracellular or bound to the extracellular surface of the macrophages was required. To overcome this problem, we used a modification of a method which differentiated between extracellular and intracellular bacteria (9, 10). Live bacteria were labeled with 0.1 mg of fluorescein isothiocyanate (Organon Teknika N.V., Tuonhout, Belgium) per ml in PBS with 5% FBS for 2 h at 37°C under rotation (10). Bacteria were washed with RPMI-BSA twice and used for further experiments. Macrophage monolayers were incubated with fluorescein isothiocyanate-labeled bacteria as described below and washed three times to remove extracellular bacteria. Ethidium bromide (50 μg/ml) was added to the slides, which were observed with the aid of a fluorescence microscope, under oil immersion, to discriminate between extracellular and intracellular bacteria. These experiments were carried out in parallel with the experiments described below.

Reduction of NBT within macrophages was studied as previously described (30). Briefly, macrophage monolayers were prepared in a Lab-Tek eight-chamber slide (Nunc). Bacteria were exposed to macrophage monolayers (ratio, 10:1) in the presence of NBT at a final concentration of 0.25 mg/ml of RPMI-BSA medium containing 10% normal or immune serum for 2 h at 37°C. After incubation, slides were washed with prewarmed RPMI-BSA three times, fixed with methanol, and then counterstained with 0.2% safranin. The percentage of bacteria that were stained deeply blue with heavy formazan precipitate was determined microscopically. Calculations were based on 100 to 200 cell-associated bacteria.

Statistics. Differences were analyzed for significance by using Student's *t* test.

RESULTS

Phagocytosis of *E. rhusiopathiae*. Virulent and acapsular avirulent mutant strains of *E. rhusiopathiae* were incubated with murine peritoneal macrophages for 60 min in the presence of normal or immune serum (Fig. 1). In the presence of normal serum, bacteria were phagocytosed, but the number of ingested bacteria was three- to fourfold greater in the case of mutant strains than in the case of the parent strain (Fujisawa-SmR). In the presence of immune serum, the parent and the mutant strains were both efficiently phagocytosed, with enhanced phagocytosis seen in the cases of the mutant strains.

Intracellular survival of *E. rhusiopathiae*. Murine peritoneal macrophages were incubated up to 3 h after cell-associated bacteria were removed by washing. Figure 2 shows the time course of intracellular survival of *E. rhusiopathiae* within murine macrophages. When ingested in the presence of normal serum, the number of the viable virulent parent strain (Fujisawa-SmR) decreased only minimally during the first 2 h and then increased significantly by 3 h ($P < 0.001$), whereas the number of an acapsular avirulent mutant, 33H6, decreased significantly during the observation period ($P < 0.001$ at 3 h of incubation). When ingested in the presence of immune serum, the numbers of viable parent and 33H6 both decreased significantly ($P < 0.001$ at 3 h of incubation). Similar to the results obtained for strain 33H6, the numbers of mutants 28G12 and

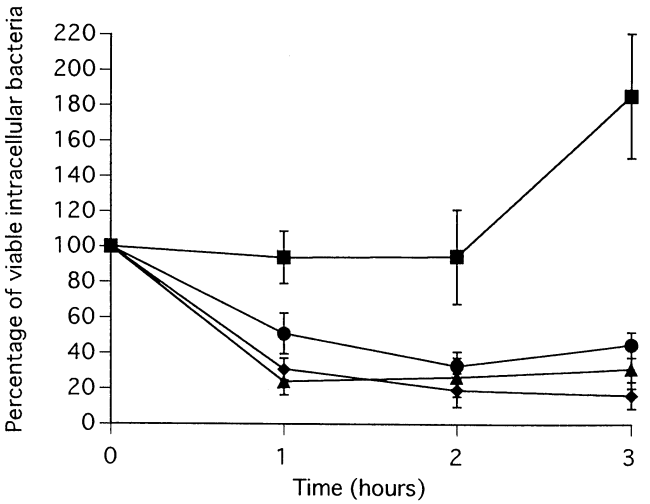

FIG. 2. Time course of intracellular survival of *E. rhusiopathiae* strains within macrophages after ingestion of bacteria in the presence of normal or immune serum. Macrophages (10^6) were allowed to ingest bacteria (10^7) for 20 min in the presence of 10% serum, washed, and then resuspended in RPMI-BSA containing 10% normal or immune serum. Bars represent standard deviations ($n = 6$ for Fujisawa-SmR; $n = 3$ for 33H6). ■, Fujisawa-SmR, normal serum; ●, Fujisawa-SmR, immune serum; ▲, 33H6, normal serum; ◆, 33H6, immune serum.

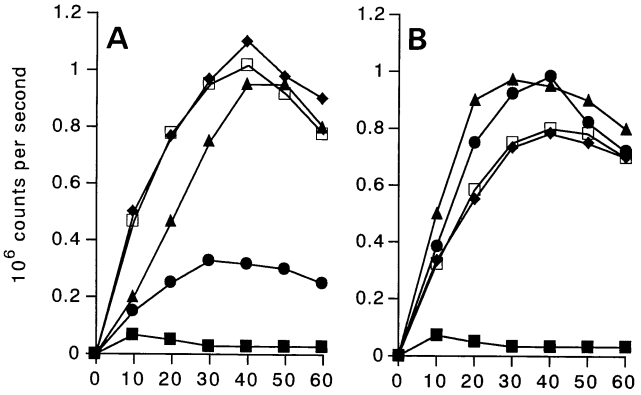

FIG. 3. CL responses of macrophages to *E. rhusiopathiae* strains preopsonized with 10% normal serum (A) or immune serum (B). The reaction mixture consisted of 100 μl of macrophage suspension (10^6/ml), 100 μl of bacterial suspension (10^8/ml), and 10 μl of luminol (10 mM). ■, Bacterium-free control; ◆, 28G12; ●, Fujisawa-SmR; □, 28G5; ▲, 33H6.

28G5 decreased significantly when ingested in the presence of normal or immune serum (data not shown).

CL response of murine macrophages to *E. rhusiopathiae*. Bacteria have developed various strategies for evading the bactericidal activities of phagocytes (7). Some bacteria fail to stimulate an oxidative burst when they are phagocytosed (4, 23, 30). To test the potential role of this strategy in the survival of *E. rhusiopathiae* within murine macrophages, we measured the oxidative burst, which is important in bactericidal activity of macrophages, by monitoring the luminol-enhanced CL responses of murine macrophages to the parent and mutant strains. Typical CL patterns of the parent and mutant strains are shown in Fig. 3. When macrophages were incubated with the bacteria preopsonized with normal serum, the CL response was much lower for the parent strain (Fujisawa-SmR) than for the mutant strains (Fig. 3A), suggesting that the lower level of oxidative response may account, in part, for the intracellular survival of the parent strain. When macrophages were incubated with the bacteria preopsonized with immune serum, both the parent and the mutant strains induced a strong CL response (Fig. 3B).

Intracellular reduction of NBT within murine macrophages. Since CL responses give no information with regard to the responses of individual cells to the bacteria, we used the intracellular NBT reduction assay, in which NBT is reduced by reactive oxidative metabolites to a visible intracellular formazan precipitate. In this assay, it is necessary to discriminate accurately between extracellular and intracellular bacteria. For this purpose, we used a fluorescence quenching method using ethidium bromide, fluorescein isothiocyanate-labeled *E. rhusiopathiae*, and fluorescence microscopy. In three independent experiments, we found that irrespective of the serum used, 85.0 to 87.8% and 75.9 to 95.0% of cell-associated bacteria were internalized for the parent and mutant strains, respectively. These results indicate that macrophages internalized most cell-associated bacteria or that extracellular bacteria were efficiently removed by three washings.

Intracellular reduction of NBT within macrophages was investigated in the presence of normal or immune serum (Table 1). In the presence of normal serum, 26 and 84% of the cell-associated bacteria were formazan stained for the parent and mutant strains, respectively. In the presence of immune serum, the parent and the mutant strains were both mostly formazan stained. Figure 4 shows the differences between the parent and mutant strains in the presence of normal serum.

DISCUSSION

We have examined the interaction of the bacterium *E. rhusiopathiae* with murine macrophages. The results of this study demonstrate that virulent *E. rhusiopathiae* is ingested by murine macrophages in the presence of normal serum and that the ingested organism can survive and replicate within murine macrophages. Our results also show that in the presence of normal serum, the virulent *E. rhusiopathiae* fails to induce a strong oxidative burst during the ingestion.

Oxygen metabolic burst following phagocytosis is one of the essential events in bactericidal activity of phagocytes (7). Some bacteria, such as *Salmonella typhi* (23), *Haemophilus somnus* (4), and *Brucella abortus* (17), fail to trigger the oxidative burst or elicit a weak and short-lived oxidative burst, resulting in successful intracellular survival. The data presented in this report show that the virulent parent strain fails to induce an oxidative burst or elicits a weak oxidative burst when ingested in the presence of normal serum, whereas acapsular mutant strains stimulate a strong oxidative burst in the presence of normal serum. These results suggest that the impaired production of reactive oxidative metabolites by macrophages may account, in part, for the intracellular survival of the parent strain. At least two possible hypotheses could explain the reduced production of reactive oxidative metabolites when the parent strain enters macrophages. First, the organism quenches oxidative metabolites by enzymes such as superoxide dismutase (SOD) and catalase. These enzymes have been reported to play an important role in protection against killing by phagocytes (1, 12, 22). *E. rhusiopathiae* produces SOD (unpublished results) but not catalase (16). We could not demonstrate that addition of exogenous SOD does prevent the intracellular killing of the mutant strains by macrophages (data not shown). In addition, both the mutant strains and the parent strain produced the enzyme (data not shown). Taken together, these results suggest that the role of this enzyme in the pathogenesis of erysipelas infection may be limited. However, it is possible that once the mutants are ingested and, as described below, the macrophage becomes activated, the effect of SOD may not be detected. To clarify the role of this enzyme in the pathogenicity, further experiments using isogenic strains deficient in enzyme production are required. A second hypothesis is that receptors involved in phagocytosis of the virulent parent strain, in the presence of normal serum, do not stimulate the oxidative burst of macrophages, whereas those involved in phagocytosis of the mutant strains do. This hypothesis is supported by the observation that the number of ingested bacteria differs between the parent and mutant strains. It has been reported that the receptor used for phagocytosis may influence the intracel-

TABLE 1. Intracellular reduction of NBT within murine macrophages[a]

Strain	% of formazan-stained bacteria[b]	
	Normal serum	Immune serum
Fujisawa-SmR	25.8 ± 4.0	82.6 ± 5.1
33H6	85.3 ± 1.8[c]	89.8 ± 2.4
28G12	83.7 ± 3.2[c]	87.1 ± 8.5
28G5	84.7 ± 3.7[c]	92.0 ± 1.5

[a] Macrophage monolayers were incubated with bacteria (ratio, 1:10) in the presence of NBT at a concentration of 0.25 mg/ml of RPMI-BSA medium containing 10% normal or immune serum for 2 h at 37°C (see text).
[b] Mean of three independent experiments ± standard deviation.
[c] $P < 0.001$ compared with Fujisawa-SmR in normal serum.

FIG. 4. Intracellular reduction of NBT within macrophages. Macrophage monolayers were exposed to bacteria for 2 h in the presence of 10% normal serum. (A) Bacterium-free NBT control; (B) Fujisawa-SmR; (C) 33H6; (D) 28G12. In panel B, the bacteria are not stained or partially stained (arrowheads), but the majority of the bacteria are stained deeply blue by formazan precipitate (arrows) in panels C and D.

lular fate of bacteria, such as *Salmonella* spp. (15) and *Listeria monocytogenes* (11). It has also been reported that killing of organisms by phagocytes is closely related to the cell surface properties of the organism (6, 13, 30). These findings suggest that the cell surface properties of the organism may affect the intracellular fate of the organism by which such receptor-ligand interactions are transduced, resulting in stimulation of the bactericidal activities of macrophages. We cannot exclude the possibility that the survival and replication of the organism within macrophages result from its resistance to oxygen-independent killing mechanisms by macrophages and are merely coincidental with the reduced production of reactive oxidative metabolites by macrophages. Assuming that the organism has the strategy of, using certain receptor(s), being ingested without stimulating the bactericidal activities of macrophages, it is possible that neither oxygen-dependent nor oxygen-independent killing mechanisms are stimulated. Our studies do not address the identity of the cell surface antigen(s) involved in ingestion of the organism. To fully understand the pathogenicity of the organism, further studies of bacterial antigen(s), macrophage receptor(s) for the organism, and receptor-related mechanisms for the activation of the macrophages are needed.

We previously demonstrated that virulent *E. rhusiopathiae* resisted phagocytosis by murine PMNs in the presence of normal serum (26). The phagocytic index of PMNs for the parent strain in the presence of normal serum was 8.3 ± 1.2 (26). This value is much lower than the value of 303.3 ± 83.0 for macrophages (this study) (Fig. 1). The results are consistent with other studies of swine, in which phagocytosis of erysipelas bacteria is carried out primarily by macrophages, not PMNs (2, 3). We cannot explain the difference between PMNs and macrophages in the ability to phagocytose the organism. This difference may be due to differences of expression of the receptors which are involved in phagocytosis of the bacteria. The involvement of complement receptors in phagocytosis of intracellular pathogens by phagocytic cells has been documented (14, 24, 25). These receptors are known to mediate phagocytosis but not elicit the reactive oxidative metabolites from the phagocytes (24, 33). Thus, entry via these receptors may allow intracellular pathogens to avoid oxygen-dependent killing by phagocytes. Although in erysipelas infection, complement is thought to play a key role in host defense (28), further investigations are required to examine whether complement receptors are involved in successful intracellular infection of *E. rhusiopathiae.*

In conclusion, the present results demonstrate that *E. rhusiopathiae* can survive and replicate within macrophages when ingested in the presence of normal serum and that phagocytosis of the virulent *E. rhusiopathiae* by macrophages is not accompanied by an adequate oxidative burst response by macrophages. We have hypothesized that to enter macrophages, virulent *E. rhusiopathiae* uses a receptor which does not trigger the oxidative burst by macrophages. In addition, the cell surface properties of the organism may influence the intracellular fate of the organism. We are currently investigating whether

the mechanisms of ingestion by macrophages differ between the parent and mutant strains.

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REFERENCES

1. **Beaman, B. L., C. M. Black, F. Doughty, and L. Beaman.** 1990. Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of *Nocardia asteroides* within the intact host. Infect. Immun. **58:**3122–3128.
2. **Böhm, K. H., R. Soliman, and W. Leibold.** 1982. Interactions between erysipelas bacteria and swine macrophages with special reference to virulence, p. 146. *In* R. R. Necoechea, C. Pijoan, A. Cesarin, and M. Guzman (ed.), Proceedings of the International Pig Veterinary Society Congress, Mexico City.
3. **Böhm, K. H., and V. Suphasindhu.** 1980. Investigations on phagocytosis of erysipelas bacteria by neutrophile leucocytes with special reference to virulence, p. 197. *In* N. C. Nielsen, P. Hogh, and N. Bille (ed.), Proceedings of the International Pig Veterinary Society Congress. Reproset, Copenhagen.
4. **Czuprynski, C. J., and H. L. Hamilton.** 1985. Bovine neutrophils ingest but do not kill *Haemophilus somnus* in vitro. Infect. Immun. **50:**431–436.
5. **Czuprynski, C. J., and P. M. Henson, and P. A. Campbell.** 1984. Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. J. Leukocyte Biol. **35:**193–208.
6. **Densen, P., and G. L. Mandell.** 1978. Gonococcal interactions with polymorphonuclear neutrophils: importance of the phagosome for bactericidal activity. J. Clin. Invest. **62:**1161–1171.
7. **Densen, P., and G. L. Mandell.** 1980. Phagocyte strategy vs. microbial tactics. Rev. Infect. Dis. **2:**817–838.
8. **Dijkmans, B. A., P. C. J. Leijh, A. G. P. Braat, and R. van Furth.** 1985. Effect of bacterial competition on the opsonization, phagocytosis, and intracellular killing of microorganisms by granulocytes. Infect. Immun. **49:**219–224.
9. **Drevets, D. A., and P. A. Campbell.** 1991. Macrophage phagocytosis: use of fluorescence microscopy to distinguish between extracellular and intracellular bacteria. J. Immunol. Methods **142:**31–38.
10. **Drevets, D. A., and P. A. Campbell.** 1991. Roles of complement and complement receptor type 3 in phagocytosis of *Listeria monocytogenes* by inflammatory mouse peritoneal macrophages. Infect. Immun. **59:**2645–2652.
11. **Drevets, D. A., B. P. Canono, and P. A. Campbell.** 1992. Listericidal and nonlistericidal mouse macrophages differ in complement receptor type 3-mediated phagocytosis of *Listeria monocytogenes* and in preventing escape of the bacteria into the cytoplasm. J. Leukocyte Biol. **52:**70–79.
12. **Franzon, V. L., J. Arondel, and P. J. Sansonetti.** 1990. Contribution of superoxide dismutase and catalase activities to *Shigella flexneri* pathogenesis. Infect. Immun. **58:**529–535.
13. **Harmon, B. G., L. G. Adams, and M. Frey.** 1988. Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. Am. J. Vet. Res. **49:**1092–1097.
14. **Hondalus, M. K., M. S. Diamond, L. A. Rosenthal, T. A. Springer, and D. M. Mosser.** 1993. The intracellular bacterium *Rhodococcus equi* requires Mac-1 to bind mammalian cells. Infect. Immun. **61:**2919–2929.
15. **Ishibashi, Y., and T. Arai.** 1990. Roles of the complement receptor type 1 (CR1) and type 3 (CR3) on phagocytosis and subsequent phagosome-lysosome fusion in *Salmonella*-infected murine macrophages. FEMS Microbiol. Immunol. **64:**89–96.
16. **Jones, D.** 1986. Genus *Erysipelothrix* Rosenbach 1909, 367^{AL}, p. 1245–1249. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams & Wilkins, Baltimore.
17. **Kreutzer, D. L., L. A. Dreyfus, and D. C. Robertson.** 1979. Interaction of polymorphonuclear leukocytes with smooth and rough strains of *Brucella abortus*. Infect. Immun. **23:**737–742.
18. **Kumagai, K., K. Itou, S. Hinuma, and M. Tada.** 1979. Pretreatment of plastic petri dishes with fetal calf serum. A simple method for macrophage isolation. J. Immunol. Methods **29:**17–25.
19. **Lachmann, P. G., and H. Deicher.** 1986. Solubilization and characterization of surface antigenic components of *Erysipelothrix rhusiopathiae* T28. Infect. Immun. **52:**818–822.
20. **Leijh, P. C. J., M. T. van den Barselaar, M. R. Daha, and R. van Furth.** 1982. Stimulation of the intracellular killing of *Staphylococcus aureus* by monocytes: regulation by immunoglobulin G and complement components C3/C3b and B/Bb. J. Immunol. **129:**332–337.
21. **Leijh, P. C. J., M. T. van den Barselaar, I. Dubbeldeman-Rempt, and R. van Furth.** 1980. Kinetics of intracellular killing of *Staphylococcus aureus* and *Escherichia coli* by human granulocytes. Eur. J. Immunol. **10:**750–757.
22. **Mandell, G. L.** 1975. Catalase, superoxide dismutase, and virulence of *Staphylococcus aureus*. In vitro and in vivo studies with emphasis on staphylococcal-leukocyte interaction. J. Clin. Invest. **55:**561–566.
23. **Miller, R. M., J. Carbus, and R. B. Hornick.** 1972. Lack of enhanced oxygen consumption by polymorphonuclear leukocytes on phagocytosis of virulent *Salmonella typhi*. Science **175:**1010–1011.
24. **Mosser, D. M., and P. J. Edelson.** 1984. The third component of complement (C3) is responsible for the intracellular survival of *Leishmania major*. Nature (London) **327:**329–331.
25. **Payne, N. R., and M. A. Horwitz.** 1987. Phagocytosis of *Legionella pneumophila* is mediated by human monocyte complement receptors. J. Exp. Med. **166:**1377–1389.
26. **Shimoji, Y., Y. Yokomizo, T. Sekizaki, Y. Mori, and M. Kubo.** 1994. Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. Infect. Immun. **62:**2806–2810.
27. **Timoney, J.** 1969. The inactivation of *Erysipelothrix rhusiopathiae* in macrophages from normal and immune mice. Res. Vet. Sci. **10:**301–302.
28. **Timoney, J.** 1970. The effect of decomplementation on *Erysipelothrix rhusiopathiae* infection in the mouse. Immunology **19:**561–567.
29. **Timoney, J.** 1970. The inactivation of *Erysipelothrix rhusiopathiae* in pig buffy-coat leucocytes. Res. Vet. Sci. **11:**189–190.
30. **Wilson, C. B., V. Tsai, and J. S. Remington.** 1980. Failure to trigger the oxidative metabolic burst by normal macrophages. J. Exp. Med. **151:**328–346.
31. **Wood, R. L.** 1984. Swine erysipelas—a review of prevalence and research. J. Am. Vet. Med. Assoc. **184:**944–949.
32. **Wood, R. L.** 1992. Erysipelas, p. 475–486. *In* A. D. Leman et al. (ed.), Diseases of swine. Iowa State University Press, Ames, Iowa.
33. **Wright, S., and S. C. Silverstein.** 1983. Receptors for C3b and C3bi promote phagocytosis but not release of toxic oxygen from human phagocytes. J. Exp. Med. **158:**2016–2023.
34. **Yokomizo, Y., and Y. Isayama.** 1972. Antibody activity of IgM and IgG fractions from rabbit anti-*Erysipelothrix rhusiopathiae* sera. Res. Vet. Sci. **13:**294–296.

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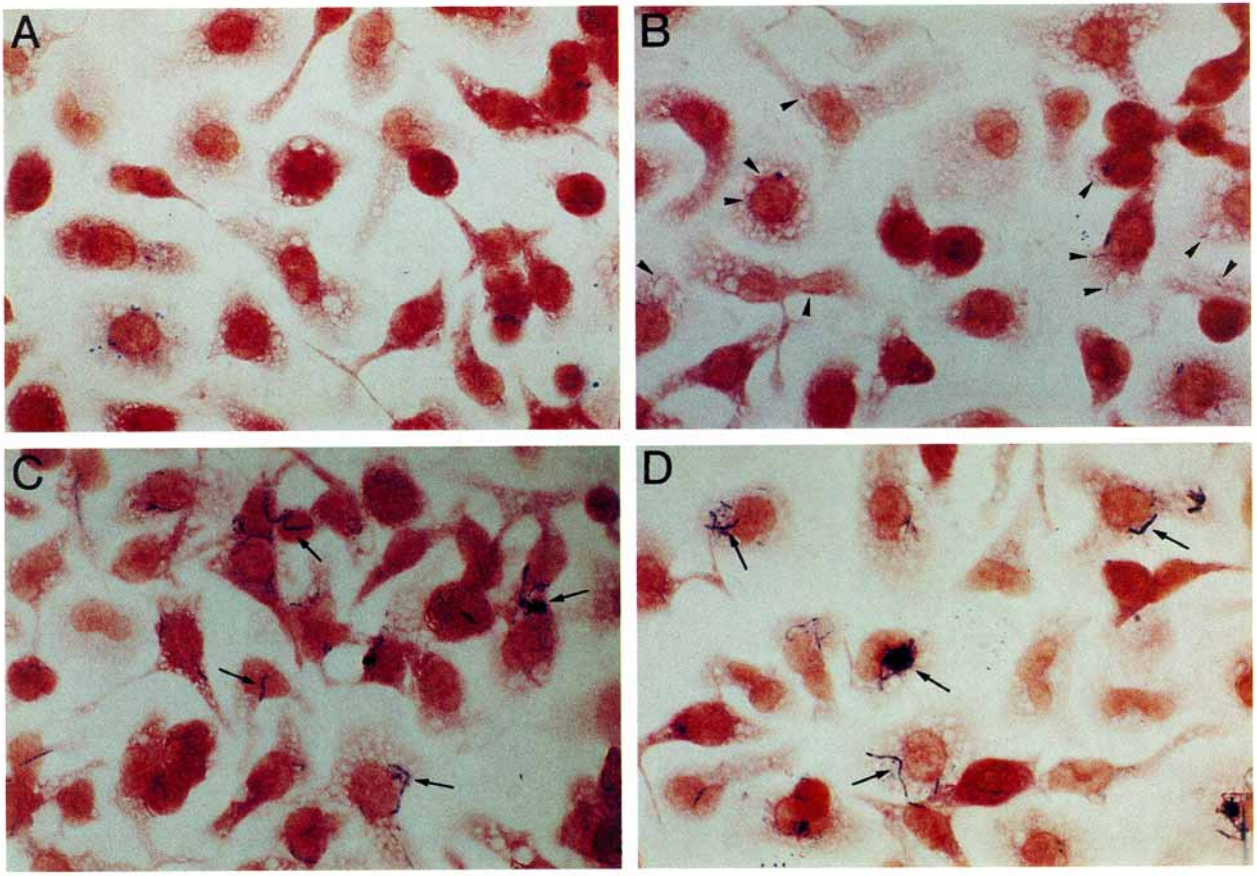
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