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Neutrophils Are Critical for Host Defense against Primary Infection with the Facultative Intracellular Bacterium *Francisella tularensis* in Mice and Participate in Defense against Reinfection

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It is generally believed that immunity to experimental infection with the facultative intracellular bacterium *Francisella tularensis* is an example of T-cell-mediated immunity that is expressed by activated macrophages and mediated by *Francisella*-specific T cells. According to the results presented herein, neutrophils are also essential for defense against primary infection with this organism. It is shown that mice depleted of neutrophils by treatment with the granulocyte-specific monoclonal antibody RB6-8C5 are rendered defenseless against otherwise sublethal doses of *F. tularensis* LVS inoculated intravenously or intradermally. In neutrophil-depleted mice, the organism grew progressively in the livers, spleens, and lungs to reach lethal numbers, whereas infection was resolved in normal mice. Although neutrophils were found to contribute to resistance to reinfection, their participation was less important. The results suggest that neutrophils are needed for defense against primary infection because they serve to restrict the growth of *F. tularensis* before it reaches numbers capable of overwhelming a developing specific immune response. The exact way that neutrophils achieve this is not clear at this time, although it is probable that they contribute in ways other than by ingesting and killing the bacterium.

Francisella tularensis, the etiological agent of the zoonotic disease tularemia is a highly virulent, facultative intracellular bacterium capable of parasitizing both professional phagocytes and nonphagocytic host cells (reviewed in reference 20). The live vaccine strain (LVS) of *F. tularensis*, although attenuated for humans, is virulent for mice, and murine tularemia caused by LVS is considered a model of human tularemia caused by virulent strains of the pathogen (3, 7, 9). *F. tularensis* LVS survives and grows inside macrophages (1, 8), and there is evidence that host defense against tularemia is expressed by these phagocytic cells following their activation by lymphokines secreted by *Francisella*-specific T cells (2, 8, 9, 13). On the other hand, the role of neutrophils in murine host defense against tularemia has received little attention, in spite of the fact that these leukocytes are the predominant cells recruited to infectious foci during the early stages of infection (4, 5, 16, 19). A recent report from this laboratory (5) suggested that, during the first day of infection with *F. tularensis* LVS, neutrophils recruited to infectious foci in the liver function to cause the lysis of parenchymal cells parasitized by this organism. It was proposed that deliberate lysis of infected hepatocytes serves to abort infection in these otherwise permissive target cells, thereby releasing the organism into the extracellular space where it can be killed by macrophages. The existence of this defense mechanism was revealed by treating mice with a CD11b-specific monoclonal antibody (MAb) that prevents the extravasation of myelomonocytic cells into foci of *F. tularensis* infection. In mice so treated, the failure of neutrophils to accumulate at foci of liver infection was associated with the failure of infected hepatocytes to undergo lysis and with rapid growth of *F. tularensis* within them. Because neutrophils were the predominant leukocytes to accumulate at foci of *F. tularensis* infection in the liver during the first 24 h, it was assumed that any effect of the anti-CD11b MAb during this time was likely due to its action on these cells. However, because anti-CD11b MAbs can also bind to and prevent the subsequent recruitment of macrophages and other leukocytes to infectious foci (18), a role for cells in addition to neutrophils in early defense against *F. tularensis* infection could not be ruled out.

The purpose of this study was to further examine the role of neutrophils in defense against tularemia by selectively depleting mice of these leukocytes by using the granulocyte-specific MAb RB6-8C5 (6, 17, 21). The results show that neutrophil-depleted normal mice failed to control an otherwise sublethal primary *F. tularensis* infection and that neutrophil-depleted immune mice showed a decreased ability to resolve reinfection.

MATERIALS AND METHODS

Mice. Female B6D2F1 (C57BL/6 × DBA/2) mice were used when they were 7 to 9 weeks old. All mice were obtained from the Trudeau Institute Animal Breeding Facility and were free of common viral pathogens on the basis of results of routine screening by the Research Animal Diagnostic Laboratory, University of Missouri, Columbia. Five mice per group per time point were used in the experiments except where stated otherwise.

Bacteria. *F. tularensis* LVS (ATCC 29684), obtained from the American Type Culture Collection, Rockville, Md., was grown to the log phase in modified Mueller-Hinton broth as described previously (5), harvested, and frozen at −70°C in 1-ml aliquots (8×10^8 CFU/ml) in the presence of 10% (wt/vol) sucrose. *Salmonella typhimurium* C5R was prepared as described elsewhere (5). For each experiment, a frozen vial was thawed and the bacteria were washed once in 0.9% (wt/vol) sterile saline and diluted to the required concentration in saline for intravenous (i.v.) inoculation in 200 μl or intra-

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dermal (i.d.) inoculation in 50 μl. Inoculations i.d. were performed in the shaved skin of the upper abdomen, whereas i.v. injections were given in a lateral tail vein. The number of bacteria in the thawed inocula was always determined by plating. The $\log_{10}$ 50% lethal dose (LD_{50}) of *F. tularensis* LVS given i.v., determined over a period of 28 days, was approximately 5.2, whereas the $\log_{10}$ LD_{50} via the i.d. route was 7.0. In some experiments, mice immunized by i.v. inoculation of a sublethal dose (5×10^3 CFU) of *F. tularensis* LVS 6 weeks earlier were used. The i.v. $\log_{10}$ LD_{50} for these immune mice was 7.5. *F. tularensis* was enumerated in the livers, spleens, and lungs by plating 10-fold serial dilutions of homogenates of these organs on cystine heart agar (Difco Laboratories, Detroit, Mich.) supplemented with 1% (wt/vol) hemoglobin. Colonies were counted after 48 h of incubation at 37°C. To determine bacterial growth in the skin, a 1-cm^2 piece of skin (with the inoculum site at its center) together with the underlying body wall was excised, homogenized, and plated as described above. *S. typhimurium* was enumerated by plating on Trypticase soy agar.

MAb. MAb RB6-8C5 is a rat immunoglobulin G2b antibody that selectively binds to and depletes mature mouse neutrophils and eosinophils (6, 17, 21). The hybridoma secreting RB6-8C5 was a kind gift from R. Coffman, DNAX Research Institute, Palo Alto, Calif. An isotype-matched MAb directed against keyhole limpet hemocyanin (anti-KLH MAb) was used as the control immunoglobulin G. This MAb was produced at the Trudeau Institute by L. L. Johnson. Both MAbs were purified from ascites by ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-Sephacel (Pharmacia LKB, Piscataway, N.J.). The immunoglobulin concentrations of the purified MAbs were determined from their peak heights during elution from a high-performance liquid chromatography size exclusion column. MAbs were given in a dose of 0.25 mg intraperitoneally at 2 days and again at 4 h before inoculation of bacteria. This regimen of RB6-8C5 treatment routinely depletes >95% of the circulating neutrophils (6).

RESULTS

Treatment with neutrophil-depleting MAb RB6-8C5 renders mice highly susceptible to an i.d. inoculum of *F. tularensis* LVS. To mimic a natural route of infection, *F. tularensis* LVS was inoculated i.d. in initial studies. The infection-enhancing effect of MAb RB6-8C5 is strikingly illustrated by a preliminary experiment that measured the ability of RB6-8C5-treated mice to survive a normally sublethal i.d. inoculum of *F. tularensis* LVS. Figure 1 shows that all RB6-8C5-treated mice died within 8 days after i.d. inoculation of 10^2 CFU of *F. tularensis*, whereas all mice treated with an isotype-matched control MAb survived an i.d. inoculum of 10^6 CFU. At 14 days postinoculation, no bacteria were found in the livers, spleens, or lungs of control mice.

In view of the preceding result, a more detailed experiment that monitored the effects of RB6-8C5 treatment on the growth of *F. tularensis* LVS at the site of its inoculation in the skin as well as systemically in the liver, spleen, and lungs was performed. Figure 2 shows that in mice treated with control MAb and inoculated i.d. with 4×10^4 CFU of *F. tularensis*, the organism grew progressively in the skin for 2 days and then began to decline in number. In these mice, bacteria were detected by 24 h of infection in the livers where they grew progressively until day 3, after which their numbers declined. In these same mice, *F. tularensis* grew progressively in the spleen throughout the 4-day period of the experiment but was present only transiently in the lungs. Despite the short duration of this experiment, it seems reasonable to expect that control mice would have gone on to resolve infection given that in the experiment illustrated by Fig. 1 all control mice survived a 25-fold-larger inoculum.

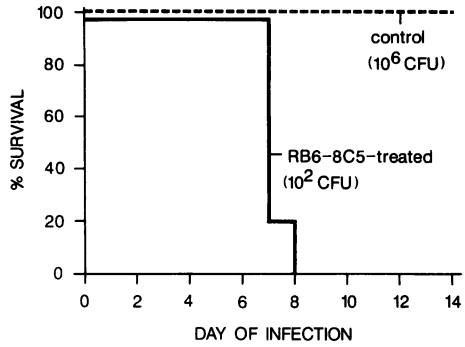

FIG. 1. Mice treated with the neutrophil-depleting MAb RB6-8C5 (solid line) were rapidly killed by an i.d. inoculum of 10^2 CFU of *F. tularensis* LVS, whereas control mice (broken line) treated with an irrelevant anti-KLH MAb survived a 10,000-fold-larger inoculum. MAbs were administered intraperitoneally in a dose of 0.25 mg at 2 days and again at 4 h before infection. Each group consisted of five mice.

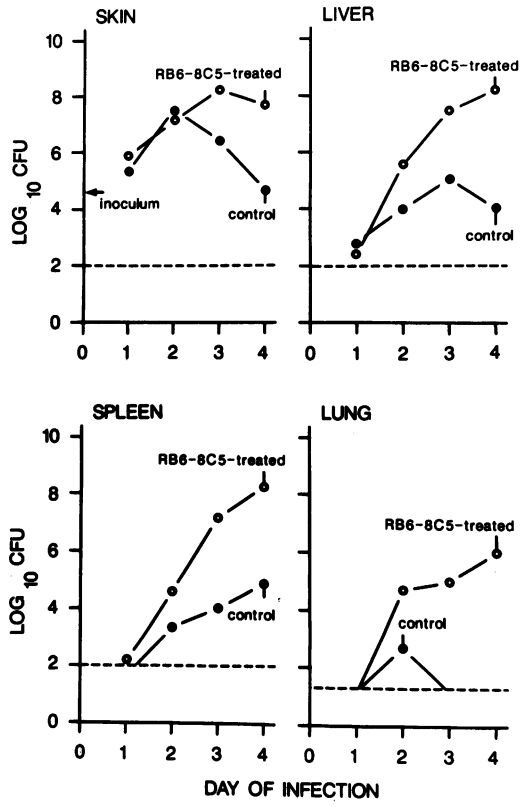

FIG. 2. Growth curves of *F. tularensis* LVS in the skin, livers, spleens, and lungs of RB6-8C5-treated mice (open circles) and of anti-KLH-treated control mice (solid circles). MAbs were administered as described in the legend to Fig. 1. Mice were inoculated i.d. with 4×10^4 CFU of *F. tularensis*, and bacterial growth was monitored for 4 days. The broken line represents the detection limit of the assay. The means for five mice per group per time point are shown. The standard errors of the means were <0.5 $\log_{10}$ units.

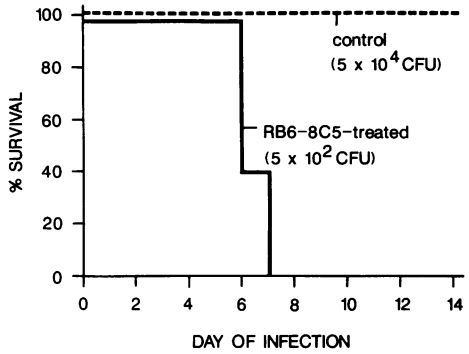

FIG. 3. Evidence that mice treated with neutrophil-depleting MAb RB6-8C5 were rendered highly susceptible to an i.v. inoculum of *F. tularensis* LVS. Mice treated with MAb RB6-8C5 (solid line) died following i.v. inoculation of 5×10^2 CFU of *F. tularensis*, whereas mice treated with control anti-KLH MAb (broken line) survived a 100-fold-larger inoculum. Five mice per group were used.

In RB6-8C5-treated mice, in contrast, infection was greatly exacerbated at all sites examined. At the inoculation site in the skin, *F. tularensis* grew no more quickly than it did at this site in control mice during the first 2 days of infection. However, between days 2 and 3, the organism continued to grow in the skin of RB6-8C5-treated mice, whereas it was being inactivated at this site in control mice. RB6-8C5-treated and control mice were similar with regards to the numbers of bacteria in their livers, spleens, and lungs up to 24 h of infection. However, after this time, *F. tularensis* grew progressively and more rapidly in these organs in RB6-8C5-treated mice, and there was no evidence that infection was being controlled in any internal organ by day 4. Moreover, bacteremia (>10^4 CFU/ml of blood) was evident by day 4 in RB6-8C5-treated mice, whereas bacterial numbers were below the detection limit (<10^2 CFU/ml) in the blood of control mice (data not shown).

Mice treated with MAb RB6-8C5 are highly susceptible to an i.v. inoculum of *F. tularensis* LVS. Because *F. tularensis* LVS is not toxigenic (11), it seemed reasonable to propose that, in the foregoing experiment, RB6-8C5-treated mice inoculated i.d. with this organism did not die of uncontrolled bacterial growth in the skin but rather from unrestricted growth in internal organs. Therefore, the latter aspect of the infectious process became the focus of all further experiments, in which for convenience infection was initiated directly by the i.v. route. The results of one such experiment show (Fig. 3) that an i.v. inoculum of 5×10^2 CFU of *F. tularensis* LVS killed all RB6-8C5-treated mice but not control mice, which survived a 100-fold-larger inoculum.

TABLE 1. Effect of depleting neutrophils with MAb RB6-8C5 on the growth of *F. tularensis* and *S. typhimurium* in the liver and the spleen during the first day of infection

Mice	Log_{10} CFU ± SD[a]			
	F. tularensis		*S. typhimurium*	
	Liver	Spleen	Liver	Spleen
Control	5.54 ± 0.14	4.48 ± 0.28	4.38 ± 0.19	3.96 ± 0.26
RB6-8C5 treated[b]	5.87 ± 0.08	5.56 ± 0.18	6.87 ± 0.23	6.25 ± 0.17

[a] Untreated control and RB6-8C5-treated mice were inoculated i.v. with 10^4 CFU of *F. tularensis* or 10^5 CFU of *S. typhimurium*, and the bacterial numbers in the organs were determined 24 h later. Four mice per group were used.

[b] RB6-8C5-treated mice received 0.25 mg of MAb intraperitoneally at 2 days and again at 4 h before i.v. inoculation of bacteria.

This prompted an investigation of the effect of RB6-8C5 treatment on the growth of *F. tularensis* in the liver and spleen during the first day following i.v. inoculation. For comparison, additional mice were inoculated with *S. typhimurium* to determine the effect of RB6-8C5 on a pathogen highly susceptible to the microbicidal actions of neutrophils (12). Table 1 shows that RB6-8C5 treatment had little effect on the growth of *F. tularensis* in the liver and spleen during the first 24 h of infection. By contrast, by 24 h after i.v. inoculation of *S. typhimurium*, approximately 200-fold more of these bacteria were recovered from the livers and spleens of RB6-8C5-treated mice than from these organs of control mice. This suggests that neutrophils recruited to foci of infection during the first day are crucial for controlling the early growth of *S. typhimurium* but relatively unimportant in restricting the growth of *F. tularensis*.

To determine the stage of tularemia at which the infection-exacerbating actions of MAb RB6-8C5 occur, the growth of *F. tularensis* was monitored over a 10-day period in the livers, spleens, and lungs of RB6-8C5-treated and control mice

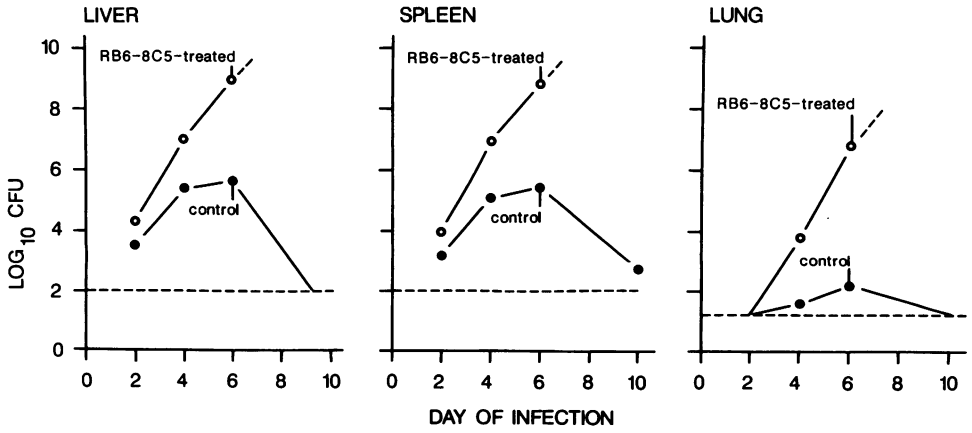

FIG. 4. Growth curves of *F. tularensis* LVS in the livers, spleens, and lungs of MAb RB6-8C5-treated mice (open circles) and of anti-KLH-treated control mice (solid circles) inoculated i.v. with 10^2 CFU. The means for five mice per group are shown. The standard errors of the means were <0.6 $\log_{10}$ units. Broken lines show the detection limits for *F. tularensis* LVS in the organs.

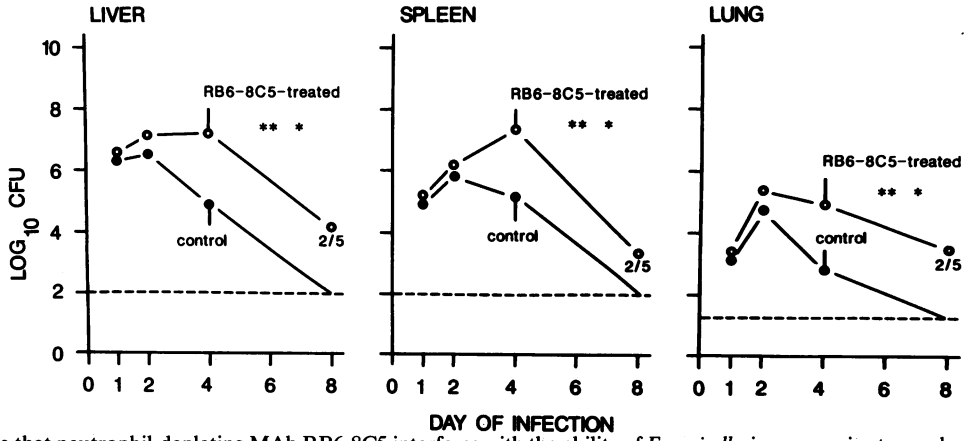

FIG. 5. Evidence that neutrophil-depleting MAb RB6-8C5 interferes with the ability of *Francisella*-immune mice to resolve reinfection initiated by a large i.v. inoculum of bacteria. Mice inoculated i.v. 6 weeks previously with 5×10^3 CFU of *F. tularensis* LVS were treated with MAb RB6-8C5 (open circles) or control MAb (solid circles) before being reinoculated i.v. with 10^6 CFU. Bacterial growth was monitored in their livers, spleens, and lungs for 8 days. The means for five mice per group are shown. Standard errors of the means were <0.7 $\log_{10}$ units. An asterisk indicates the death of individual mice.

inoculated i.v. As shown by the results in Fig. 4, an i.v. inoculum of 10^2 CFU of *F. tularensis* grew progressively in the organs of control mice for 6 days before being brought under control. By contrast, in RB6-8C5-treated mice, *F. tularensis* grew unrestrictedly in all three organs until the mice died on day 7 or 8 of infection. It will be noted that in this experiment there was relatively little exacerbation of infection in any organ in RB6-8C5-treated mice during the first 2 days. However, from day 2 on, *F. tularensis* grew more rapidly in all three organs in mice treated with MAb RB6-8C5.

***Francisella*-immune mice treated with MAb RB6-8C5 have a reduced capacity to resolve reinfection.** Others have shown (3, 9, 13) that mice that resolve primary sublethal *F. tularensis* LVS infection acquire a state of predominantly T-cell-mediated specific immunity that allows them to resolve subsequent reinfection initiated by bacterial inocula that are lethal for nonimmune mice. To determine whether neutrophils participate in the expression of this immunity, mice that received a sublethal (5×10^3 CFU) i.v. inoculum of *F. tularensis* LVS 6 weeks earlier were treated with MAb RB6-8C5 or control anti-KLH MAb and rechallenged i.v. with 10^6 CFU, an inoculum that was lethal for all nonimmune animals. Bacterial growth in control immune mice and in RB6-8C5-treated immune mice was monitored in the liver, spleen, and lungs. The results (Fig. 5) show that in control immune mice bacteria grew progressively in all three organs during the first 2 days of infection, after which infection was brought under control and resolved in all three organs by day 8. Infection in RB6-8C5-treated immune mice, by contrast, was greatly exacerbated in all of these organs by day 4, and some mice (three of five) died by day 7. However, two of five mice went on to resolve the infection. When a similar experiment was performed with a 100-fold-smaller challenge inoculum, it was again found that infection was severely exacerbated in the livers and spleens of immune RB6-8C5-treated mice by day 4 (data not shown). However, with this dose of *F. tularensis* LVS, all RB6-8C5-treated mice were able to resolve the infection. No bacteria were detected in the lungs of control immune mice or RB6-8C5-treated immune mice in this experiment. Taken together, these results indicate that although neutrophils play a role in host defense against reinfection with *F. tularensis*, they are less important than they are in primary infection.

DISCUSSION

On the basis of the results presented here, neutrophil function is essential for defense of mice against primary infection with the facultative intracellular bacterium *F. tularensis*. Our results show that mice selectively depleted of neutrophils and eosinophils by treatment with a granulocyte-specific MAb, designated RB6-8C5 (21), are incapable of controlling infection with a sublethal inoculum of the live vaccine strain of *F. tularensis* given i.v. or i.d. This was the case, moreover, even though the mouse strain employed is otherwise highly resistant to *F. tularensis*. Because there is no histological or other evidence in the literature implicating a role for eosinophils in anti-*Francisella* defense, it seems reasonable to assume that the infection exacerbating the effect of MAb RB6-8C5 was due to the depletion of neutrophils that are normally present at infectious foci.

The exact roles of neutrophils in anti-*Francisella* defense were not determined herein, but on the basis of a previous study from this laboratory (5), an important early function of neutrophils in defense against *F. tularensis*, *Listeria monocytogenes* (5, 6), or *S. typhimurium* (5) infection in the liver is to destroy infected hepatocytes that otherwise permit the unrestricted intracellular growth of these microorganisms. In this way, neutrophils cause these pathogens to be exposed to attack by macrophages that are presumed to be the cells that ultimately express specific immunity to infection with facultative intracellular pathogens (10). However, whereas this neutrophil-mediated defense strategy severely restricts the growth of *L. monocytogenes* and *S. typhimurium* at infectious foci during the first 24 h of infection, depletion of neutrophils had relatively little effect on the growth of *F. tularensis* during the first 2 days of infection. This suggests that *F. tularensis* is more resistant to the antimicrobial actions of neutrophils recruited early into infectious foci. The demonstration (15) that the LVS strain of *F. tularensis* is killed by human neutrophils might explain why this strain is attenuated for humans.

Even so, given the array of immunoregulatory cytokines that neutrophils might produce at sites of inflammation (reviewed in reference 14), it is obvious that they almost certainly are recruited to foci of infection to perform functions in addition to ingesting and killing microorganisms. For example, neutro-

phils recruited early to infectious foci represent a potential source of cytokines and chemokines capable of attracting macrophages and T cells into these sites, where they may cooperate to resolve infection. In particular, neutrophils can secrete tumor necrosis factor alpha, a cytokine that has been shown by others to be critical for controlling tularemia (13). The precise function of tumor necrosis factor alpha in this situation is unknown, but it might induce secretion of gamma interferon (22), another cytokine of critical importance in anti-*Francisella* defense (2, 13). Whatever their roles in defense against primary murine tularemia, it is clear that neutrophils have a less important role in resistance to reinfection. The reason for the more limited role of these cells in defending against reinfection is not known but may result from the possession by immunized mice, but not naive mice, of an acquired mechanism of T-cell-mediated immunity that may operate rapidly enough to preempt the need for significant neutrophil involvement.

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REFERENCES

1. **Anthony, L. S. D., R. D. Burke, and F. E. Nano.** 1991. Growth of *Francisella* spp. in rodent macrophages. Infect. Immun. **59:**3291–3296.
2. **Anthony, L. S. D., E. Ghadirian, F. P. Nestel, and P. A. L. Kongshavn.** 1989. The requirement for gamma interferon in resistance of mice to experimental tularemia. Microb. Pathog. **2:**421–428.
3. **Anthony, L. S. D., and P. A. L. Kongshavn.** 1987. Experimental murine tularemia caused by *Francisella tularensis*, live vaccine strain: a model of acquired cellular resistance. Microb. Pathog. **2:**3–14.
4. **Baskerville, A., and P. Hambleton.** 1976. Pathogenesis and pathology of respiratory tularemia in the rabbit. Br. J. Exp. Pathol. **57:**339–347.
5. **Conlan, J. W., and R. J. North.** 1992. Early pathogenesis of infection in the liver with the facultative intracellular bacteria *Listeria monocytogenes*, *Francisella tularensis*, and *Salmonella typhimurium* involves lysis of infected hepatocytes by leukocytes. Infect. Immun. **60:**5164–5171.
6. **Conlan, J. W., and R. J. North.** 1994. Neutrophils are essential for early anti-*Listeria* defense in the liver, but not in the spleen or the peritoneal cavity as revealed by a granulocyte-depleting monoclonal antibody. J. Exp. Med. **179:**259–268.
7. **Eigelsbach, H. T., and C. M. Downs.** 1961. Prophylactic effectiveness of live and killed tularemia vaccine. I. Production of vaccine and evaluation in the white mouse and guinea pig. J. Immunol. **87:**415–425.
8. **Fortier, A. H., T. Polsinelli, S. J. Green, and C. A. Nacy.** 1992. Activation of macrophages for destruction of *Francisella tularensis*: identification of cytokines, effector cells, and effector molecules. Infect. Immun. **60:**817–825.
9. **Fortier, A. H., M. V. Slayter, R. Ziemba, M. S. Meltzer, and C. A. Nacy.** 1991. Live vaccine strain of *Francisella tularensis*: infection and immunity in mice. Infect. Immun. **59:**2922–2928.
10. **Hahn, H., and S. H. E. Kaufmann.** 1981. The role of cell-mediated immunity in bacterial infections. Rev. Infect. Dis. **3:**1221–1250.
11. **Hood, A. M.** 1977. Virulence factors of *Francisella tularensis*. J. Hyg. Camb. **79:**47–60.
12. **Hsu, H. S.** 1989. Pathogenesis and immunity in murine salmonellosis. Microbiol. Rev. **53:**390–409.
13. **Leiby, D. A., A. H. Fortier, R. M. Crawford, R. D. Schreiber, and C. A. Nacy.** 1992. In vivo modulation of the murine immune response to *Francisella tularensis* LVS by administration of anticytokine antibodies. Infect. Immun. **60:**84–89.
14. **Lloyd, A. R., and J. J. Oppenheim.** 1992. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. Immunol. Today **13:**169–176.
15. **Löfgren, S., A. Tärnvik, M. Thore, and J. Carlsson.** 1984. A wild and an attenuated strain of *Francisella tularensis* differ in their susceptibility to hypochlorous acid: a possible explanation of their different handling by polymorphonuclear leukocytes. Infect. Immun. **43:**730–734.
16. **Moe, J. B., P. G. Canonico, J. L. Stookey, M. C. Powanda, and G. L. Cockerell.** 1975. Pathogenesis of tularemia in immune and nonimmune rats. Am. J. Vet. Res. **36:**1505–1510.
17. **Rogers, H. W., and E. R. Unanue.** 1993. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. Infect. Immun. **61:**5090–5096.
18. **Rosen, H., S. Gordon, and R. J. North.** 1989. Exacerbation of murine listeriosis by a monoclonal antibody specific for the type 3 complement receptor of myelomonocytic cells. J. Exp. Med. **170:**27–37.
19. **Schricker, R. L., H. T. Eigelsbach, J. Q. Mitten, and W. C. Hall.** 1972. Pathogenesis of tularemia in monkeys aerogenically exposed to *Francisella tularensis* 425. Infect. Immun. **5:**734–744.
20. **Tärnvik, A.** 1989. Nature of protective immunity to *Francisella tularensis*. Rev. Infect. Dis. **11:**440–451.
21. **Tepper, R. I., R. L. Coffman, and P. Leder.** 1992. An eosinophil-dependent mechanism for the anti-tumor effect of interleukin-4. Science **257:**548–551.
22. **Tripp, C. S., S. F. Wolf, and E. R. Unanue.** 1993. Interleukin 12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc. Natl. Acad. Sci. USA **90:**3725–3729.