Title

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Macrophage Antibacterial Defense

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ROLES OF CELL-MEDIATED AND HUMORAL IMMUNITY IN HOST DEFENSE AGAINST INTRACELLULAR AND EXTRACELLULAR BACTERIA

Mononuclear phagocytes, activated by cellular immune mechanisms, are central to host defense against intracellular bacterial pathogens (*Mycobacterium tuberculosis, M. leprae, Listeria monocytogenes, Brucella* sp., *Salmonella* sp., *Francisella tularensis, Legionella pneumophila*), whereas polymorphonuclear leukocytes, in conjunction with antibody and complement, are central to host defense against extracellular bacterial pathogens. In vivo, mononuclear cells often dominate sites of infection with intracellular pathogens, whereas polymorphonuclear leukocytes often dominate the sites of infection with extracellular pathogens. In vitro, polymorphonuclear leukocytes phagocytize extracellular bacteria more efficiently than do monocytes (3). Humoral immunity is important for host defense against extracellular bacterial pathogens because most of these pathogens are encapsulated, and phagocytes cannot ingest and kill encapsulated extracellular bacteria unless these bacteria are coated with antibody and complement (2, 3).

MILESTONES IN UNDERSTANDING ACQUIRED CELL-MEDIATED IMMUNITY TO INFECTION

Many of the major milestones in understanding the role of cell-mediated immunity and macrophage activation in host defense were made by investigators studying bacterial pathogens, particularly *M. tuberculosis*. These investigations followed upon Koch's discovery of the etiological agent of tuberculosis in 1882, 100 years ago (8). In 1884, Metchnikoff pointed out the importance of mononuclear phagocytes in chronic infection (13). In 1891, Koch described cutaneous delayed-type hypersensitivity to tuberculin (9). A half century later, in 1945, Chase demonstrated that cutaneous delayed-type hypersensitivity to tuberculin was transferable by specifically sensitized lymphoid cells and not by serum (1). About the same time, in 1942, Lurie demonstrated the importance of the macrophage in immunity by demonstrating that macrophages harvested from vaccinated animals display an enhanced capacity to inhibit the multiplication of tubercle bacilli in vitro (11). Following Lurie's discovery, others showed that macrophages harvested from animals immunized with other intracellular pathogens, specifically *Brucella, Salmonella*, and *L. monocytogenes*, were similarly capable of expressing increased antimicrobial activity in vitro. Finally, Mackaness, in the 1960s, explained the functional link between Lurie's and Chase's results. He demonstrated that immunity to *L. monocytogenes* could be passively transferred to mice with sensitized lymphoid cells and that these cells, in the presence of the sensitizing organism or its antigens, conferred on macrophages the capacity to inhibit the multiplication of intracellular bacteria nonspecifically (12).

Soon after, in 1971, Simon and Sheagren reproduced Mackaness's result in vitro by demonstrating that sensitized lymphocytes in cell culture, in the presence of specific antigen, conferred on mononuclear phagocytes the capacity to inhibit intracellular bacteria nonspecifically (15). About this time, Lane and Unanue (10) and North (14) showed that the lymphoid cells responsible for transferring immunity to listeriosis and tuberculosis were T cells. Other investigators demonstrated that lymphocyte alteration of macrophage function is mediated by molecules called lymphokines.

L. PNEUMOPHILA MULTIPLIES INTRACELLULARLY IN MONOCYTES

I have studied the interaction of *L. pneumophila*, the agent of Legionnaires disease, with human leukocytes, and the roles of humoral and cell-mediated immunity in host defense against *L. pneumophila* (4–7). These studies have demonstrated that *L. pneumophila* is an intracellular bacterial pathogen (4). Virulent egg yolk-grown *L. pneumophila*, Philadelphia 1 strain, multiplies intracellularly in human blood monocytes and only intracellularly under tissue culture conditions. Neither polymorphonuclear leukocytes nor lymphocytes support *L. pneumophila* multiplication. Since the bacterium can multiply extracellularly on complex medium, *L. pneumophila* is a facultative intracellular parasite.

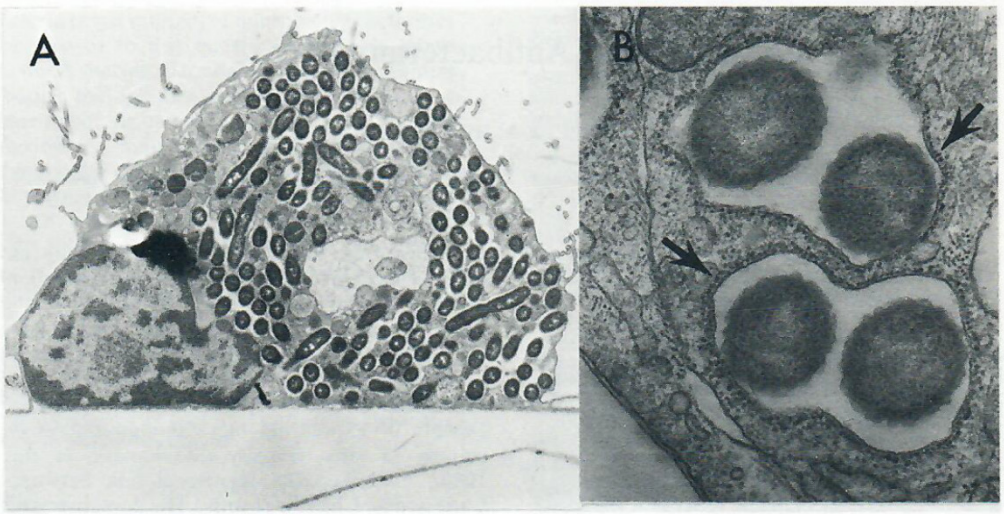

FIG. 1. Electron micrographs of human monocytes infected with *L. pneumophila*. (A) Monocyte heavily infected with *L. pneumophila*. ×5,400. (B) *L. pneumophila* inside a monocyte in membrane-bound vacuoles studded with ribosome-like structures. Each vacuole contains two bacilli. The ribosome-like structures (arrows) appear to be separated from the cytoplasmic face of the vacuolar membrane by a gap of ~10 nm. ×32,400. (Reprinted from reference 4.)

L. pneumophila multiplies in monocytes with a mid-log-phase doubling time of 2 h, a rate of growth that is faster than that observed in specialized media used to grow the organism. In monocyte cultures, the bacteria multiply until all the monocytes are destroyed.

Inside monocytes, all bacteria are found in membrane-bound cytoplasmic vacuoles (Fig. 1). Strikingly, the cytoplasmic sides of the membrane-bound vacuoles surrounding the *L. pneumophila* are studded with structures resembling monocyte ribosomes (Fig. 1B). This morphological feature has also been observed in infected alveolar macrophages in human lung tissue specimens obtained from patients with Legionnaires disease. *L. pneumophila* is evidently unique among bacterial pathogens in promoting the formation of these vacuoles, the origin and role of which are unknown.

ROLE OF HUMORAL IMMUNITY IN HOST DEFENSE AGAINST *L. PNEUMOPHILA*

Patients with Legionnaires disease respond to the infection by producing antibody against *L. pneumophila*. To assess the role of humoral immunity in host defense against *L. pneumophila*, I have studied the influence of antibody on the interactions between virulent *L. pneumophila* and human polymorphonuclear leukocytes, monocytes, and complement, in vitro, under antibiotic-free conditions (5, 6).

L. pneumophila in concentrations ranging from 10^3 to 10^6 colony-forming units per ml is completely resistant to the bactericidal effects of 0 to 50% fresh normal human serum, even in the presence of high concentrations of rabbit or human anti-*L. pneumophila* antibody. The resistance to complement is not due to a failure of the bacteria to fix complement. In the presence of antibody, the bacteria fix complement to their surface, but this does not result in the death of the bacteria.

Polymorphonuclear leukocytes efficiently phagocytize *L. pneumophila* only in the presence of both antibody and complement. Polymorphonuclear leukocytes also require both antibody and complement to kill any *L. pneumophila*; even then, polymorphonuclear leukocytes reduce colony-forming units of *L. pneumophila* by only 0.5 log under conditions in which they reduce colony-forming units of a serum-resistant strain of *Escherichia coli* by 2.5 logs.

Monocytes also require both antibody and complement to efficiently phagocytize *L. pneumophila* and to kill any *L. pneumophila* cells; even then, monocytes kill only a limited proportion (0.25 to 0.5 log) of an inoculum (6). The surviving bacteria multiply several logs in the monocytes and multiply as rapidly as when the bacteria enter monocytes in the absence of antibody.

These findings suggest that humoral immunity

may not be an effective host defense against *L. pneumophila*. Consequently, a vaccine that resulted only in antibody production against *L. pneumophila* may not be efficacious.

ROLE OF CELL-MEDIATED IMMUNITY IN HOST DEFENSE AGAINST *L. PNEUMOPHILA*

To investigate the role of cell-mediated immunity in host defense against *L. pneumophila*, I have examined the interaction between in vitro-activated human monocytes and virulent egg yolk-grown *L. pneumophila* (7).

Freshly explanted human monocytes activated by incubation with concanavalin A and human lymphocytes inhibit the intracellular multiplication of *L. pneumophila* (Fig. 2). Both concanavalin A and lymphocytes are required for activation. Concanavalin A is consistently maximally effective at ≥4 μg/ml.

Monocytes activated by incubation with cell-free filtered supernatant from concanavalin A-sensitized mononuclear cell cultures also inhibit the intracellular multiplication of *L. pneumophila*. The most potent supernatant is obtained from mononuclear cell cultures incubated with ≥15 μg of concanavalin A per ml for 48 h. The degree of monocyte inhibition of *L. pneumophila* multiplication is proportional to the length of time monocytes are preincubated with supernatant (48 h > 24 h > 12 h) and to the concentration of supernatant added (40% > 20% > 10% > 5%). Monocytes treated with supernatant daily are more inhibitory than monocytes treated initially only. With time in culture, monocytes progressively lose a limited degree of spontaneous inhibitory capacity and also their capacity to respond to supernatant with inhibition of *L. pneumophila* multiplication.

Supernatant-activated monocytes inhibit *L. pneumophila* multiplication in two ways. They phagocytize fewer bacteria, and they slow the rate of intracellular multiplication of bacteria that are internalized. As was the case with nonactivated monocytes, antibody has no effect on the rate of intracellular multiplication in supernatant-activated monocytes.

These findings show that human monocytes can be activated to inhibit the multiplication of *L. pneumophila*. Thus, inhibition of *L. pneumophila* multiplication is accomplished by activating the monocytes and not by coating the bacteria with antibody and complement. This indicates that cell-mediated immunity likely plays a major role in host defense against *L. pneumophila*, as it does against other intracellular pathogens.

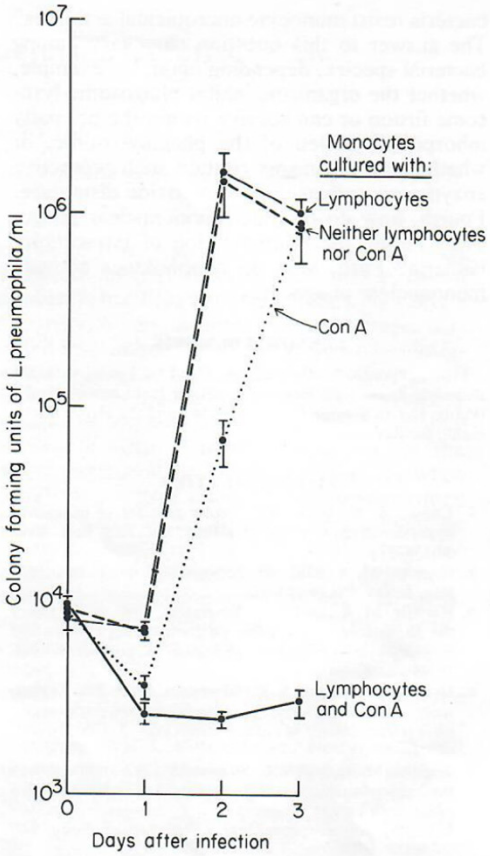

FIG. 2. Inhibition of the intracellular multiplication of *L. pneumophila* by monocytes activated by incubation with concanavalin A (ConA) and by human lymphocytes. Monocytes were cultured with lymphocytes only, ConA only, neither lymphocytes nor ConA, and both lymphocytes and ConA for 24 h, and were then infected with *L. pneumophila*. *L. pneumophila* multiplied, as determined by assaying colony-forming units in cultures daily after infection, in all monocyte cultures except those containing both ConA and lymphocytes, where multiplication was inhibited. (Reprinted from reference 7.)

UNANSWERED QUESTIONS

Much remains to be learned about the interactions between macrophages and intracellular bacteria. Here are but a few of the unanswered questions. First, since antibody and complement are not required, what ligands on the bacteria mediate their ingestion by phagocytes? Second, what are the special features of the phagosome that render it a hospitable environment for bacterial multiplication? Third, how do intracellular

bacteria resist monocyte microbicidal activities? The answer to this question may vary among bacterial species, depending upon, for example, whether the organisms inhibit phagosome-lysosome fusion or can survive within the normally inhospitable milieu of the phagolysosome, or whether the organisms contain such protective enzymes as catalase and superoxide dismutase. Fourth, how do activated mononuclear phagocytes inhibit the multiplication of intracellular bacteria? Fifth, how do lymphokines activate mononuclear phagocytes?

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