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Author

Horwitz, Marcus

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STATE OF THE ART LECTURE

Toward an Understanding of Host and Bacterial Molecules Mediating *Legionella pneumophila* Pathogenesis

MARCUS A. HORWITZ

Division of Infectious Diseases, Department of Medicine, University of California, Los Angeles, Los Angeles, California 90024

By the time of the 1983 International Symposium on *Legionella*, a good general picture of the immunobiology of *Legionella pneumophila* in the mammalian host had been obtained. It was known that the bacterium is an intracellular pathogen of the mononuclear phagocyte, chiefly monocytes and alveolar macrophages (41, 57), that the organism is phagocytized by host cells and resides intracellularly in a specialized phagosome that does not fuse with lysosomes or become highly acidified (35, 36, 38, 40), and that cell-mediated immunity rather than humoral immunity plays a central role in host defense against *L. pneumophila* as it does against other intracellular pathogens (37, 42–44). However, most of the knowledge about the immunobiology of *L. pneumophila* by 1983 was descriptive. What was lacking was an understanding of the molecular basis for the bacterium's interaction with its host cells and the immune system, i.e., of the key host and bacterial molecules that mediate *L. pneumophila* pathogenesis.

Since 1983, substantial progress has been made in understanding the molecular basis for *L. pneumophila* pathogenesis. This review summarizes these advances.

PHAGOCYTOSIS

L. pneumophila is phagocytized frequently but not exclusively by coiling phagocytosis, in which long phagocyte pseudopods coil around the organism as it is internalized (38). Phagocytosis by human monocytes is mediated by a three-component phagocytic system consisting of monocyte complement receptors CR1 and CR3, fragments of complement component C3, and the major outer membrane protein (MOMP) on the surface of *L. pneumophila* (3, 54, 62) (Fig. 1). C3 fixes selectively to MOMP by the alternative pathway of complement activation.

INTRACELLULAR PATHWAY

Inside mononuclear phagocytes, *L. pneumophila* resides in a phagosome that interacts sequentially with host cell smooth vesicles, mitochondria, and ribosomes until a ribosome-lined replicative vacuole is formed (Fig. 2) (35). As already noted, *L. pneumophila* inhibits phagosome-lysosome fusion and phagosome acidification (36, 40).

A mutant *L. pneumophila* that does not inhibit phagosome-lysosome fusion is avirulent for monocytes (39). Complementation of this mutant with wild-type DNA restores its capacity to inhibit phagosome-lysosome fusion, multiply intracellularly in human mononuclear phagocytes, and cause lethal pneumonia in guinea pigs (53).

ROLE OF IRON IN INTRACELLULAR MULTIPLICATION

Virtually all pathogens require iron, but *L. pneumophila* has a relatively high metabolic requirement for this metal ion. *L. pneumophila* acquires iron from the intermediate labile iron pool of the monocyte (17). The iron in this pool is derived from iron-transferrin via transferrin receptors, iron-lactoferrin via lactoferrin receptors, and the iron storage protein ferritin (17, 18, 20).

Agents that reduce the size of the intermediate labile iron pool of the monocyte inhibit *L. pneu-*

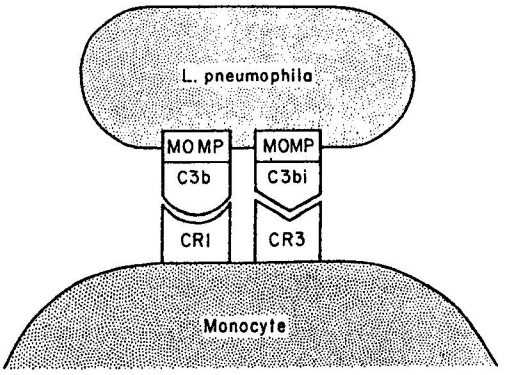

FIG. 1. Diagram illustrating a three-component phagocytic system that mediates phagocytosis of *L. pneumophila* by human monocytes.

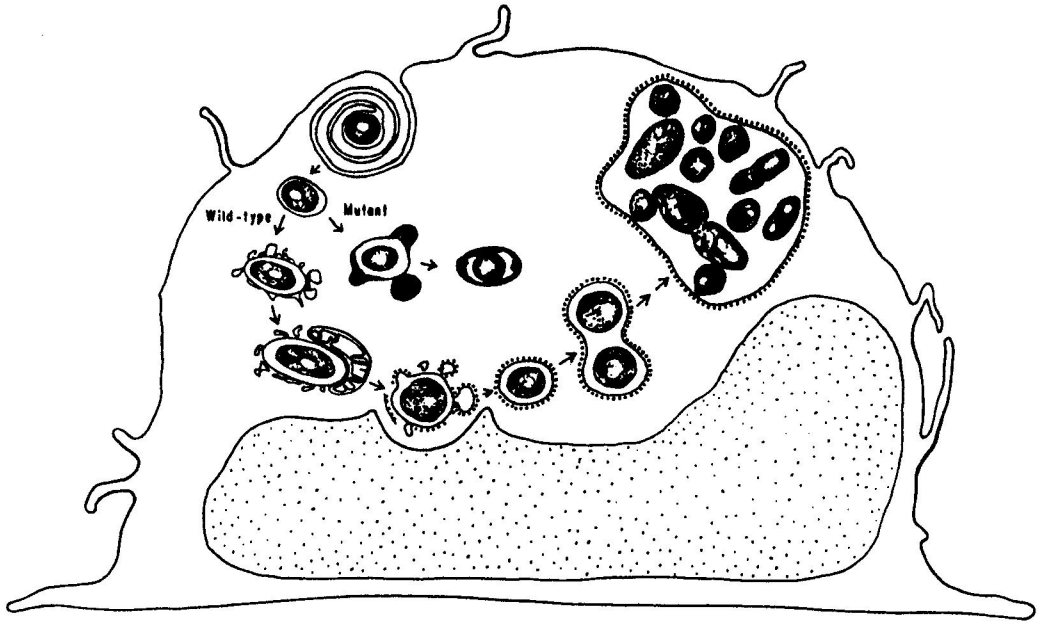

FIG. 2. Intracellular pathways of virulent and avirulent *L. pneumophila* in human monocytes. Both virulent wild-type *L. pneumophila* Philadelphia 1 strain and avirulent mutant 25D derived from it enter phagocytes by coiling phagocytosis. Thereafter, their pathways diverge. Wild-type *L. pneumophila* follows an intraphagosomal pathway in which the phagosome interacts sequentially with host cell smooth vesicles, mitochondria, and ribosomes but does not fuse with lysosomes. Avirulent *L. pneumophila* mutant 25D enters an intraphagolysosomal pathway in which the phagosome does not intereact with the various organelles that surround wild-type phagosomes but does fuse with lysosomes. Wild-type *L. pneumophila* multiplies in the ribosome-lined phagosome until its destroys the monocyte. Mutant 25D remains alive but unable to multiply in the phagolysosome.

mophila intracellular multiplication. Three different types of agents inhibit *L. pneumophila* multiplication in this way. First, the iron chelators, including the nonphysiologic iron chelator deferoxamine and the physiologic iron chelator apolactoferrin, reduce the iron pool by chelating iron within it (17, 20). Second, the weak bases chloroquine and ammonium chloride reduce the iron pool by blocking the pH-dependent release of iron from endocytized iron-transferrin and the pH-dependent proteolysis and release of iron from iron-lactoferrin and ferritin (19). Third, gamma interferon (IFN-γ) reduces iron availability by down-regulating transferrin receptor expression and intracellular ferritin concentration (17, 18).

How *L. pneumophila* internalizes iron remains unknown; possibly, its iron reductase plays a role (48). The iron incorporated into *L. pneumophila* is found in seven major iron-containing proteins, one of which is an iron superoxide dismutase (55). The major iron-containing protein (MICP) of *L. pneumophila* grown on agar has an apparent molecular mass of 210 kDa under nondenaturing conditions and 85 to 90 kDa under denaturing conditions (55). MICP retains iron under mild denaturing conditions (55). MICP is homologous with *Escherichia coli* aconitase and the human iron responsive element binding protein (54a).

CELL-MEDIATED IMMUNITY

As noted above, the host defends itself against *L. pneumophila* by cell-mediated immune mechanisms. Three different types of cell-mediated immune mechanisms have been studied. First, activated human monocytes and alveolar macrophages, including those activated by IFN-γ, have been shown to inhibit *L. pneumophila* intracellular multiplication (4, 5, 44, 47, 56, 57). Second, polymorphonuclear leukocytes (PMN) activated by IFN-γ and tumor necrosis factor have been found to have an enhanced capacity to kill *L. pneumophila* (7). However, killing was modest and required several days, raising some question as to the significance of this immune mechanism. Third, interleukin-2-activated killer cells from nonimmune subjects have been studied by two groups for their capacity to kill *L. pneumophila*. One group reported positive results, and the other reported negative results (8, 75). Whether anti-

gen-specific cytotoxic lymphocytes capable of lysing infected macrophages are generated in Legionnaires disease remains to be determined.

MECHANISMS OF MACROPHAGE ACTIVATION

Activated mononuclear phagocytes inhibit *L. pneumophila* multiplication in two ways. First, they phagocytize about 50% fewer *L. pneumophila*, thereby restricting access of the bacteria to the intracellular milieu that they require for multiplication (44). The mechanism for this process likely involves IFN-γ-mediated down-regulation of the function of complement receptors that mediate phagocytosis of *L. pneumophila* (62, 69). Second, activated monocytes and macrophages markedly slow the multiplication rate of bacteria that are internalized (44). As noted above, IFN-γ-activated monocytes do so by limiting the availability of iron to intracellular *L. pneumophila*, which occurs as a consequence of IFN-γ-induced coordinate down-regulation of transferrin receptor expression and intracellular ferritin concentration (17, 18, 20a).

PMN-MONOCYTE COOPERATION

PMN are prominent in histological specimens from the lungs of patients with Legionnaires disease, and studies of PMN-depleted guinea pigs challenged with *L. pneumophila* indicate that PMN play an important role in host defense; such guinea pigs have greater susceptibility to infection, higher numbers of *L. pneumophila* in their lungs, and higher mortality than do control animals (31). Yet in in vitro studies, human PMN lack the capacity to kill appreciable numbers of *L. pneumophila*, even in the presence of anti-*L. pneumophila* antibody and complement (42) or when activated with IFN-γ and tumor necrosis factor (7). The finding that apolactoferrin inhibits *L. pneumophila* intracellular multiplication in human monocytes has raised the possibility that PMN play a role in host defense by cooperating with monocytes (20). Apolactoferrin is a major protein in the specific granules of PMN that is released at sites of inflammation, such as occurs in the *L. pneumophila*-infected lung. By providing infected mononuclear phagocytes with apolactoferrin and thereby allowing them to inhibit *L. pneumophila* intracellular multiplication, PMN may play an important indirect role in host defense against *L. pneumophila* (20) (Fig. 3).

IMMUNOPROTECTION

Four different antigenic preparations have been shown to induce strong cell-mediated immune responses, manifest by cutaneous delayed-type hypersensitivity and splenic lymphocyte proliferation, and strong protective immunity in the guinea pig model of Legionnaires disease: the avirulent mutant described above that fails to inhibit phagosome-lysosome fusion; *L. pneumophila* membranes; the 39-kDa major secretory protein (MSP) of *L. pneumophila*; and the major cytoplasmic membrane protein (MCMP) of *L. pneumophila*, a genus-common antigen and member of the Hsp60 family of heat shock proteins (9–12, 12a, 14). The MSP is able to induce protective immunity across serogroups of *L. pneumophila* and in some cases across species of *Legionella* (11). Interestingly, although MSP is a highly potent immunoprotec-

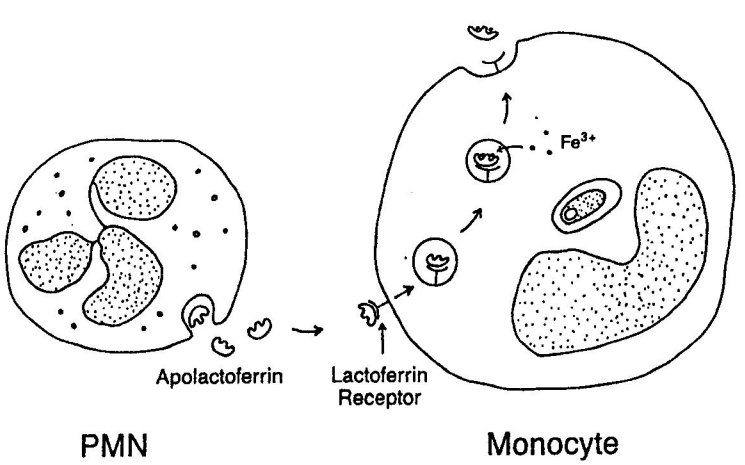

FIG. 3. Potential PMN-monocyte cooperation in host defense against *L. pneumophila*. Apolactoferrin is released by PMN at sites of inflammation. Apolactoferrin is endocytized by lactoferrin receptors on the surface of monocytes. By chelating iron in the intracellular labile iron pool of the cell, apolactoferrin inhibits *L. pneumophila* intracellular multiplication. Thus, PMN may play an indirect role in host defense by providing monocytes with apolactoferrin in the *L. pneumophila*-infected lung.

TABLE 1. Characteristics of *L. pneumophila* MSP

Characteristic	Reference
Biologic	
Major secretory protein	39
Zinc metalloprotease	27
Weak hemolytic activity	49
Cytotoxic for CHO cells	49
Genetic, immunologic, and cytoxic differences among species	63
Structurally and functionally homologous to *Pseudomonas aeruginosa* elastase	6
Produced intracellularly in monocytes	25
Immunologic	
Protective immunogen	10
Cross-serogroup and variable cross-species protection	12
Virulence	
Not virulence determinant in human mononuclear phagocytes	72
Not virulence determinant in guinea pigs	13
Homologous with zinc metalloprotease and potential virulence determinant of fish pathogen *Vibrio anguillarum*	59

tive molecule, it is not a virulence determinant in the guinea pig model of Legionnaires disease (13). Isogenic MSP^+ and MSP^- strains of *L. pneumophila* have the same 50% and 100% lethal doses for guinea pigs, multiply at the same rate in the guinea pig lung, and cause indistinguishable pathologic lesions in the lung.

MSP has been extensively studied. Its major characteristics are summarized in Table 1.

ANTIGEN PROCESSING AND PRESENTATION

The finding that MSP is not a virulence determinant demonstrates that an immunoprotective molecule need not be a virulence determinant. What it presumably must be is a molecule that allows the immune system, especially lymphocytes, to recognize infected host cells and mount an effective antimicrobial defense against them. This assumption lead us to postulate that MSP is released by *L. pneumophila* in infected monocytes and subsequently processed and presented on the surface of the monocytes in association with major histocompatibility complex (MHC) molecules. Consistent with this hypothesis, immunohistochemical and immunoelectron microscopy studies using affinity-purified anti-MSP antibody have demonstrated that *L. pneumophila* produces MSP and releases it into its phagosome in infected human monocytes (25). It is not released by *L. pneumophila* in the presence of erythromycin, which blocks bacterial protein synthesis and inhibits *L. pneumophila* intracellular multiplication (4, 45).

Interestingly, immunoelectron microscopy studies have demonstrated that MHC class I and II molecules are scarce on the membrane of phagosomes containing *L. pneumophila* (26a). Such molecules are excluded from the phagosome during coiling phagocytosis of *L. pneumophila* (26). This finding suggests that immunogenic epitopes of MSP may not bind to MHC molecules in the phagosome but may bind elsewhere in the cell in an extraphagosomal compartment.

VIRULENCE DETERMINANTS

Only one *L. pneumophila* molecule, the Mip protein, has been rigorously shown to be a virulence determinant. This 24-kDa protein is required for the full expression of virulence of *L. pneumophila* in mononuclear phagocytes and guinea pigs (22, 23). Interestingly, Mip recently has been shown to inhibit protein kinase C activity (46).

Several other molecules of *L. pneumophila* that are potentially important to pathogenesis have been isolated. Biologic and immunologic characteristics of these molecules are summarized in Table 2. In addition, two molecules from *Legionella micdadei* are of potential significance. First, a protein kinase of apparent molecular mass 35 kDa catalyzes phosphorylation of PMN proteins, including tubulin, and phosphatidylinositol (66). Second, an acid phosphatase of apparent molecular mass 68 kDa inhibits superoxide production by human PMN and dephosphorylates phosphatidylinositol biphosphate (64, 65).

CONCLUSION

Substantial strides have been made in understanding key host and bacterial molecules that mediate *L. pneumophila* pathogenesis. However,

TABLE 2. *L. pneumophila* molecules of potential importance to pathogenesis

Molecule	Subunit apparent molecular mass (kDa)	Characteristic(s)	Reference(s)
Flagellin	47	Common antigen in serogroups 1-3	28
Legiolysin	39	Hemolytic activity	73
		Tyrosine-dependent browning and yellow-green fluorescence	73
Lipopolysaccharide	Variable	Weak endotoxin activity in vivo	74
		Predominant molecule recognized by human antiserum	32
		Serogroup-specific antigen	24, 60
		Complex and unusual structure that lacks lipid A moieties essential for maximal endotoxic effects	71
MCMP	58/60/65	Major cytoplasmic membrane protein	33
		Predominant protein recognized by human antiserum	33, 68
		Genus-common antigen	61, 68
		Heat shock protein	50, 51
		Member Hsp60/65 family	70
		Protective immunogen	12a
		Gene cloned and sequenced	34, 67
MICP	85/90	Major iron-containing protein on solid medium	55
		Aconitase activity	54a
		Homologous with *E. coli* aconitase and human iron responsive element binding protein	54a
		Gene cloned and sequenced	54a
Mip	24	Potentiates infection of human mononuclear phagocytes	23
		Virulence determinant in guinea pigs	22
		Protein kinase C-inhibitory activity	46
		Conserved throughout genus	21
		L. pneumophila and *L. micdadei* gene sequenced	2, 29
MOMP	25/29	Cation-selective porin	32
		Genus-specific epitope	16
		Species-specific epitope	58
		C3 acceptor molecule	3
MSP	38/39	See Table 1	
PBP	31	Peptidoglycan-bound protein	15
PAL	19	Peptidoglycan-associated lipoprotein	30, 52
Phospholipase C	50/54	Hydrolyzes phosphatidylcholine	1

large gaps in our knowledge remain. For example, molecules that mediate the selection of the intraphagosomal pathway, inhibition of phagosome-lysosome fusion, and inhibition of phagosome acidification; molecules that mediate iron uptake; iron-containing molecules; immunoprotective molecules in addition to MSP; and virulence determinants in addition to Mip remain to be identified and characterized.

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