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Detection of Major Iron Proteins of *Legionella pneumophila*

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Iron availability is a key determinant of *Legionella pneumophila* growth both extracellularly and intracellularly. On solid culture medium, *L. pneumophila* is unable to multiply in the absence of iron supplementation (6), and in broth culture medium, *L. pneumophila* is unable to multiply when iron chelators are present (7). In human mononuclear phagocytes, *L. pneumophila* multiplication is dependent on the availability of iron in the intermediate labile iron pool of the cell, which derives its iron from three major iron-binding proteins: transferrin, lactoferrin, and ferritin (2, 3, 5).

Agents that reduce iron availability inhibit *L. pneumophila* intracellular multiplication. Three types of agents inhibit *L. pneumophila* intracellular multiplication in this way. First, iron chelators, such as the nonphysiologic iron chelator deferoxamine and the physiologic iron chelator apolactoferrin, bind iron in the intracellular iron pool (2, 5). Second, the weak bases chloroquine and ammonium chloride raise endocytic and lysosomal pH and consequently inhibit the pH-dependent release of iron from endocytized transferrin as well as the pH-dependent proteolysis of ferritin and subsequent release of iron from this molecule (4). Third, gamma interferon decreases iron availability by down-regulating transferrin receptor expression on the cell surface and consequently reducing iron uptake into the cell and by down-regulating the intracellular concentration of ferritin (2, 3). The inhibitory effect of gamma interferon on *L. pneumophila* multiplication can be reversed by adding high concentrations of iron compounds to the extracellular medium of the cell (2, 5). *L. pneumophila* thus relies on host cell iron transport systems to deliver iron to it in the replicative phagosome.

Iron is required for the synthesis of a large variety of bacterial iron enzymes and proteins, including heme proteins, iron-sulfur proteins, and iron-binding proteins. These proteins are involved in such diverse functions as electron transfer, oxygen metabolism, peroxide and superoxide metabolism, regulation of gene expression, iron storage, and oxygen binding. While much has been learned about the provision of iron to *L. pneumophila* by host cell iron transport systems, little is known about the role of iron in the physiology of *L. pneumophila*, nor is it known why this organism appears to have such a high metabolic requirement for iron. To learn more about this matter, we have developed methods for detecting major iron proteins of *L. pneumophila*, taking advantage of the very high sensitivity of iron detection allowed by the use of the radioisotope ^{59}Fe as a label.

We were able to detect iron proteins by growing bacteria in the presence of radiolabeled iron, subjecting bacterial proteins to polyacrylamide gel electrophoresis under nondenaturing conditions, and visualizing the iron proteins by autoradiography. When grown on charcoal-yeast extract agar medium, *L. pneumophila* incorporated iron into seven major proteins with apparent molecular masses of approximately 500, 450, 250, 210, 150, 130, and 85 kDa under the conditions studied. The 210-kDa protein was the major iron protein. The 150-kDa protein comigrated with superoxide dismutase activity when analyzed by the method of Beauchamp and Fridovich (1). In cellular fractionation studies, all of the iron proteins were detected in the cytoplasmic and periplasmic fractions; none were detected in the membrane fraction. When grown in yeast extract broth, *L. pneumophila* incorporated iron into the same seven proteins, but their relative amounts differed. Iron proteins were not detected in culture supernatants.

This study demonstrates that ^{59}Fe can be used as a label to visualize the major iron proteins of *L. pneumophila*, and it shows that *L. pneumophila* incorporates iron into seven major proteins. One of the *L. pneumophila* iron proteins may be an iron-containing superoxide dismutase. The role of the other major iron proteins remains to be determined.

This methodology for detecting iron proteins of

L. pneumophila can be used to trace *L. pneumophila* iron proteins during different steps in their purification. Additional studies are required to characterize the iron proteins, determine their precise subcellular localization, and determine their role in iron metabolism and virulence.

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