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CORRESPONDENCE

## A novel mechanism of antibiotic resistance in plague?

The specific therapy for plague, as suggested by the WHO Expert Committee on Plague (1970), involves the use of antibiotics such as tetracycline, streptomycin, and chloramphenicol. It is accepted that *Yersinia pestis,* the causative agent of plague, in contrast to the majority of other pathogenic bacteria, lacks antibiotic resistance *in vitro* and *in vivo.* The absence of natural drug-resistant strains may be explained by the rarity of cases of human plague at the present time and the acute character of the disease, and the flea transmission mechanism of the infection which limits contact between *Y. pestis* and natural reservoirs of R factors.

However, it has been shown that the treatment of plague caused by non-capsulate natural or experimental *Y. pestis* strains was less effective when antibiotics such as doxycycline, ampicillin and cefoperazone were used. The non-capsulate strains were also resistant to tetracycline, β-lactam agents and quinolones when inside macrophages cultivated *in vitro* [1-3]. In some cases, the loss of ability to produce capsules did not lead to an increase of LD50 absolute values in the infected mice and guinea-pigs [4]. Microbial pathogenesis is complex and mutifactorial, and several virulence factors may act individually or in concert to produce infection. Removal of any one of these components may or may not render the organism avirulent. Moreover, non-capsulate strains may be isolated from fatal cases of human [5] and rodent plague. Interestingly, when the *Y. pestis* cultures were grown on nutrient media all the non-capsulate strains were as sensitive to antibiotics as capsulate wild-type ones [1-3]. No attempts have been made to explain this phenomenon. Here we propose factors that might determine this novel mechanism of antibiotic resistance.

The pathogenicity of *Y. pestis* is related to its intracellular survival and multiplication in macrophages. Released bacteria are resistant to capture by neutrophils, inside which *Y. pestis* cells can be killed, but those phagocytosed by mononuclear phagocytes begin a new cycle of intracellular propagation [6]. It is also known that some antibiotics are unable to enter macrophages and their phagolysosomes in active form. On the other hand, the extracellular life cycle of *Y. pestis* cell may be too short for a bactericidal affect, while the other, bacteriostatic antibiotic action can only prevent the growth of *Y. pestis* outside macrophage. Phagocytosed non-capsulate bacteria are protected from contact with the antibiotic and are able to multiply. It seems likely that the capsule of *Y. pestis* may influence macrophage-membrane permeability for a range of antibiotics.

Capsular antigen "fraction I" (FI) forming the *Y pestis* capsule is specific to *Y. pestis* and induces a pronounced antiphagocytic effect in a non-immune host [6]. It has been suggested that the FI antigen, a mature polypeptide, inhibits the activity of phagocytic cells by forming large aqueous pores in the membrane of these target cells [7]. Also, it has been shown that channel formation leads to the leakage of $K^+$, *o*-nitrophenylgalactoside, and other low-mol. wt molecules through cell membranes after channel-forming toxin treatment [8]. All things considered, we can speculate *a priori* that, *in vivo,* the FI antigen-induced aqueous pores may be the main route of antibiotic penetration into macrophages and phagolysosomes resulting in antibiotic susceptibility in *Y. pestis* wild-type strains. Schematic representation of events leading to the killing of the intracellular $FI^+$ bacteria may be as shown in the Figure. Step 1 corresponds to capsular antigen secretion into the media surrounding *Y. pestis* [9], the extramacrophage and intraphagolysosomal spaces. The dissolved FI antigen makes contact with the extracytoplasmic surface of the macrophage and phagolysosome membranes (step 2), and in inserted into the lipid bilayer plasma membranes forming the aqueous channels (step 3), facilitating the penetration of more FI into the cytoplasm and FI-pore-forming insertion into the Golgi-apparatus and endoplasmic-reticulum membranes (step 4). The damaged macrophage is unable to prevent antibiotic contact with the capsule-producing bacteria (step 5).

If this hypothesis is correct, the ability of the wild-type *Y. pestis* to enter the macrophage seems to be something of a paradox. It is hoped that further work in this area will clarify the molecular mechanisms by which *Y. pestis* capsular antigen affects antibiotic susceptibility of this microbe.

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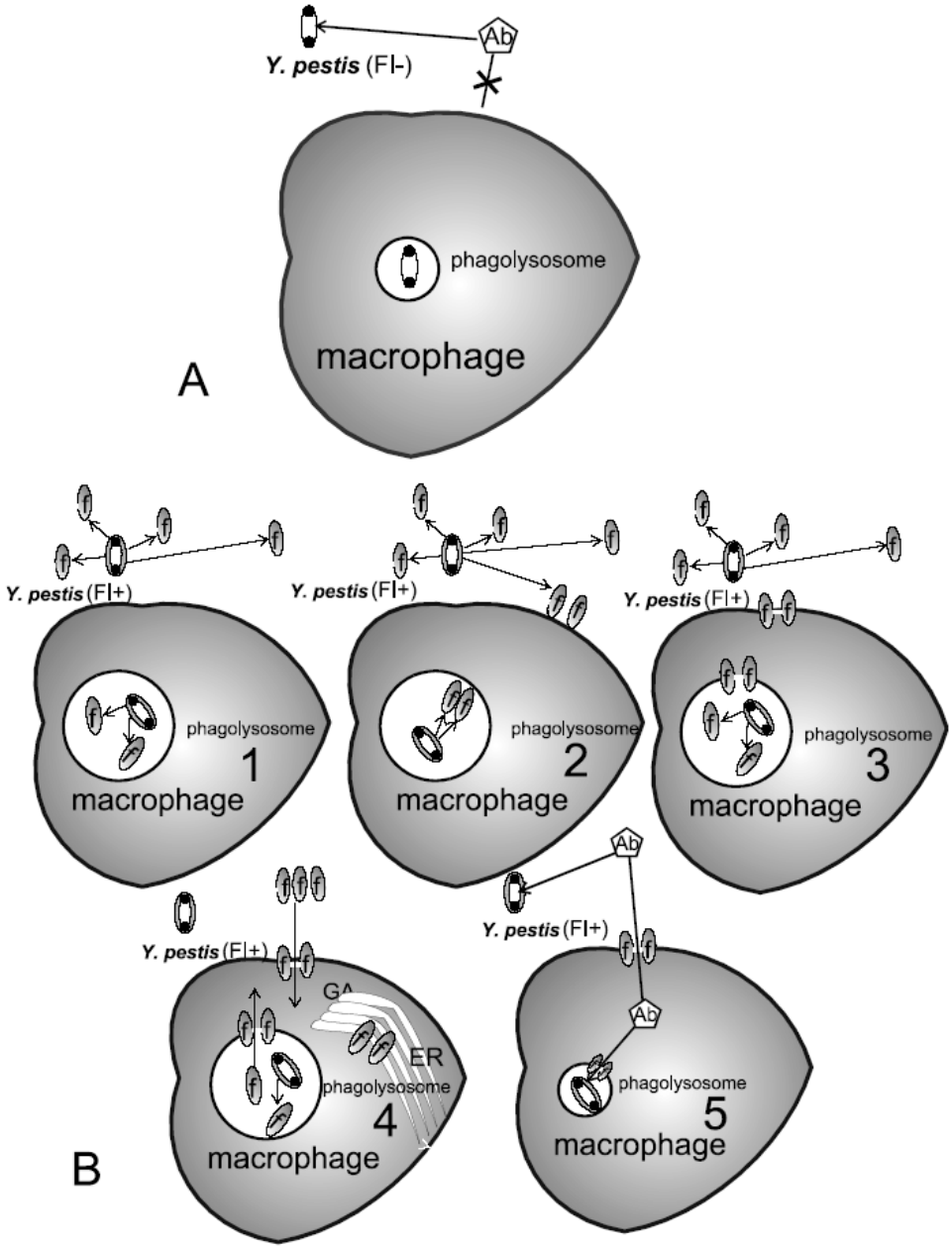


**Fig. 1**. Possible effect on non-capsulate ($FI^-$) and wild-type ($FI^+$) *Y. pestis* cells when an antibiotic (Ab) is added to a cell culture of macrophages mixed with the bacteria. The antibiotic expresses its antimicrobial effect (⟶) when in contact with extracellular microbes (A, B). It is unable to affect (✱) the intracellular capsule-deficient microbial cells (A). The wild-type bacteria secrete the "fraction I" capsular antigen into surrounding milieu. [9] The dissolved antigen FI forms the aqueous channels in macrophage membranes ( ), enabling contact between the antibiotic and the capsule-producing microbes (B).

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