Antibiotics and Polyelectrolytes Modulate Bacteriolysis and the Capacity of Bacteria to Trigger an Oxygen Burst in Neutrophils*

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Introduction

The invasions of tissues by pathogenic microorganisms is followed by a sequence of events which culminate in phagocytosis and the intracellular killing of the ingested agents, by “professional” phagocytes [19]. It is also expected that the rich arsenal of hydrolases present in neutrophils and macrophages, including the muralectylic enzyme lysozyme is adequate to degrade the complex architecture of the bacterial cells. Surprisingly, however, most pathogenic bacteria are extremely resistant to lysozyme action [14,21] and the fate of phagocytosed bacteria in vivo is not fully known [7,8,16,23]. The sequelae of the lack of bacterial degradation by leukocytes may be the “storage” of peptidoglycan-polysaccharide or peptidoglycan-lipopolysaccharide complexes within macrophages leading to the generation of granulomas and to the initiation of prolonged immune responses. This is pivotal to the initiation of immunopathological reactions [7,8,16,23]. We have recently proposed [10,11,12,13,15,29] that the biodegradation of certain microorganisms can be mediated through the activation, by cationic agents and phospholipases, of the bacterial own autolytic wall enzymes (suicidal phenomenon) which leads to the breakdown of the rigid cell walls. On the other hand, a variety of sulfonated anionic polyelectrolytes [11–13, 15] likely to be present in inflamed issues, may inhibit the biodegradation of the walls by the autolytic enzymes. One of the ways by which antibiotics, of the beta lactam series, could be involved in the killing and biodegradation of bacteria by leukocytes is through their capacity to change the cell walls via the release of lipoteichoic acid and the reduction of peptidoglycan cross linking [17,18,24,25].

It has been recently demonstrated, however, that the same anionic polyelectrolytes which inhibited autolysis in staphylococci [11,12] also markedly interfered the penicillin-induced lytic phenomenon [28]. Thus, a delicate balance between activators and inhibitors of the autolytic wall enzymes may determine whether microbial cell wall components may persist in tissues and serve as phlogistic agents [see 11,13]. Since antibiotics may also modify the bacterial surfaces and also

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interfere with the biosynthesis of certain virulence factors [1, 3] it is conceivable that such modified cell surfaces may also result in the induction of an altered “oxygen burst” in phagocytic cells. Such a burst usually takes place during membrane perturbation by opsonized bacteria during phagocytosis [19, 22]. The purpose of the present communication is a): to further describe the role played by beta lactam antibiotics in the biodegradation of staphylococci and micrococci by leukocytes and some of their factors in vitro and in vivo, and b) to describe the altered chemiluminescence patterns which occur when antibiotic-grown streptococci are employed to stimulate human neutrophils [9].

Material and Methods

Bacterial strains

Staphylococcus aureus (strain Peltzer), Micrococcus lysodeikticus and group A streptococci type 3 (M-protein negative) were cultivated in brain heart infusion broth (Difco) either in the absence or presence of added penicillin G and 14C-N-acetylglucosamine 0.2–0.5 μC/ml (specific activity 30–50 mci/mmol) New England Nuclear Boston, Mass., USA. Both the labeled and unlabeled cells were harvested from different phases of growth. The cells were washed several times in saline and resuspended either in 0.1M acetate buffer pH 5.0, saline buffered with 0.01M phosphate pH 7.4, 0.1M phosphate buffer pH 6.5 or in Hanks balanced salt solution (HBSS) containing 1 mM HEPES buffer pH 7.33. The bacterial suspension were adjusted to different cell densities (see below).

Lysis of staphylococci

14C-NAGA staphylococci, which had been cultivated either in regular brain heart infusion broth or in broth containing sub-inhibitory concentrations of penicillin G (0.01–0.02 μg/ml) were washed in saline and suspended in 0.1M acetate buffer pH 5.0 to a cell density of 0.5 OD at 550 nm. The labeled cells were exposed for 2–4 hours at 37°C to
1. buffer alone,
2. crystalline egg with lysozyme (50 μg/ml),
3. leukocyte cationic proteins (LCP) (50 μg/ml). Following incubation the reaction mixtures were centrifuged at 2,000 g for 15 minutes, and the solubilized radioactivity was determined in the supernatant fluid as described [11].

Lysis of Micrococcus lysodeikticus (ML) by lysozyme

ML was inoculated into brain heart infusion broth in the absence or presence of penicillin G (0.03–0.5 μg/ml). The cultures were harvested after 16 hrs of incubation at 37°C. The cells were washed in saline and resuspended in 0.1M phosphate buffer pH 6.5 and adjusted to an O.D. of 0.7 at 540 nm. Egg white lysozyme (5 μg/ml) was