"What Can One Learn That is Clinically Relevant from the Results of *in vitro* Studies on Persisters?"

The results of five clinical trials indicate that extended antibiotic therapy offers no clear and lasting benefit in relieving post-treatment syndromes of Lyme disease (PTLDS), a condition often referred to as "chronic Lyme disease" (1-4); note that no evidence of active infection was found in any of these studies by culture or molecular methods. Despite such findings, as well as the fact that evidence of harm was unambiguous (2,3), some continue to claim that these syndromes are the result of a persistent *Borrelia* infection that can be eliminated only by several months --or more-- of treatment with still different kinds of antibiotics, given either singly or in combination. This unproven view is bolstered by the results of *in vitro* experiments demonstrating the presence of viable *Borrelia*, in cultures treated with antibiotics (5, 6). Such "persisters", after isolation and re-cultivation, have not been found to be antibiotic-resistant mutants (5, 7). How does one then explain the existence of "persisters", and what is their clinical relevance with respect to Lyme disease?

The work of Abel zur Wiesch et al. (8) describes a testable model in which the presence of "persisters" *in vitro* can be explained solely by classic chemical kinetics involving the interaction between antibiotic and its target molecule. In the case of doxycycline, the bacteriostatic antibiotic of choice for the treatment of Lyme disease (9), this involves the competitive binding of doxycycline to the 30S subunit of the ribosome; this binding of antibiotic interferes with -- or displaces -- the binding of aminoacyl tRNA to the 30S ribosome subunit. The end result is reversible suppression of bacterial protein synthesis and decreased growth (bacteriostasis), not irreversible killing or sterilization (10).

The binding of doxycycline and aminoacyl tRNA to the same target site (the 30S ribosomal subunit) is both competitive and reversible. As is the case for the kinetics of most chemical reactions, there is a forward reaction that involves association (binding), as well as a reverse reaction that involves dissociation (unbinding or release) of doxycycline (or aminoacyl tRNA), from the target molecule (the 30S ribosomal subunit) until equilibrium is achieved. The net result is either suppression or enhancement of protein synthesis and increased or decreased bacterial growth, depending on the concentration or density of each reactant. For example, if one increases the concentration of doxycycline without changing the density of bacterial cells and their 30S ribosomal subunits, then there is inhibition of protein synthesis and decreased bacterial growth (bacteriostasis). Alternatively, if one decreases the concentration of doxycycline without changing the density of the population of bacteria, e.g., by washing away antibiotic and then transferring bacterial cells to fresh medium without antibiotics, then inhibition of protein synthesis is reversed, thereby resulting in increased bacterial growth. Such manipulations have been conducted and the results documented for *in vitro* studies

involving different bacterial species and various antibiotics (11). All of these observations are consistent with the model proposed by Abel zur Wiesch et al. (8).

Although these events surely occur when antibiotics are given *in vivo*, there are major differences that can greatly influence the final outcome. First, the *in vivo* environment represents an open system in which the concentration of antibiotics as well as the density of the bacterial population are continually changing, thereby influencing the pharmacokinetics (i.e., the concentration, diffusion, elimination, and dissemination of reactants throughout the body); obviously, establishing and controlling the chemical equilibrium described above in a closed *in vitro* environment is much easier than in an open *in vivo* environment. Second, and perhaps of greater importance, is the inability to approximate the humoral and cellular protective effects of the host immune system *in vitro.* Since the protective effects of the host immune system play a decisive role in curing or limiting infections *in vivo*, evaluating the clinical significance of "persisters" simply by conducting *in vitro* experiments alone is impossible.

Some investigators have reported the presence of intact bacterial cells in the tissues of animals treated with what appears to be adequate amounts of antibiotics after infection with *Borrelia burgdorferi.* However, these may just be intact non-viable cells; unlike the "persisters" found in *in vitro* studies, these intact cells -- which appear to have pharmacological properties-- have not yet been isolated, re-cultured, and then shown to produce disease (12-14).

Although there is no evidence to indicate that "persisters" play a significant role in the pathogenesis of Lyme disease in humans, the complete elimination of infection is seldom used as the benchmark for success in the treatment of other infectious diseases; with the exception of tuberculosis, the resolution of symptoms and the lack of relapse, rather than the detection of viable bacteria, are of primary concern. However, in addition to the well-known bactericidal and bacteriostatic effects of antibiotics, they also have many other biological, physiological, and immunomodulatory properties that could have a significant impact on various host defense mechanisms. These include their ability to: (a) suppress the expression of virulence factors (e.g., quorum sensing mechanisms, as well as the production of exotoxins, exopolysacccharides, pili, flagellin, and lipopolysaccharides; (b) accumulate in inflammatory cells in high concentration, thereby providing more efficient delivery of antibiotic to sites of infection; (c) downregulate the molecular expression of integrins known to influence leucocyte adhesion and the accumulation of macrophages and neutrophils at sites of infection; (d) inhibit the maturation and proliferation of subsets of T lymphocytes, as well as to influence immunoglobulin secretion and isotype class switching by B lymphocytes; (e) protect the respiratory ciliated epithelium from bacterial injury by interfering with bacterial adherence and colonization; (f) inhibit neutrophil migration; (g) modulate the expression of adhesion molecules and to reduce the production of chemotactic factors at the site of inflammation; (h) increase the production of various inflammatory cytokines (e.g., IL-8, IL-1β, and TNF-α) that are potent activators of neutrophils; (g) increase

the production of IL-2 colony stimulating factor, and other cytokines that modulate the induction of TH1 and TH2 lymphocyte activity; and, (h) to cause significant reductions in the numbers of lymphocytes and the ratio of CD4+CD8+T lymphocytes (15). The implications of these findings with respect to extended antibiotic therapy remains to be fully assessed. If one considers the fact that as many as 15 different β-lactam antibiotics, including penicillin and its derivatives, exert profound neuroprotective effects (16), it often may be very difficult to attribute the beneficial effects of antibiotic therapy solely to the elimination of an active infection.

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