The "Chronic Lyme Disease" / Post Treatment Lyme Disease Symptoms Controversy:

Light at the End of the Tunnel?

The results of five clinical trials indicate that extended antibiotic therapy offers no clear and lasting benefit in relieving post-treatment Lyme disease symptoms (PTLDS), a condition sometimes referred to as "chronic Lyme disease" (1-4). No evidence of active infection was found in any of these studies by culture or molecular methods. Despite these findings, as well as the fact that evidence of harm was unambiguous (2,3), some continue to believe that these symptoms are caused by a persistent *Borrelia burgdorferi* infection that can be eliminated only after several months --or more-- of treatment with different antibiotics, given either singly or in combination. This "anchoring bias" is bolstered by the results of *in vitro* experiments demonstrating the presence of viable *B. burgdorferi*, in cultures treated with antibiotics (5, 6). Such "persisters", after isolation and re-cultivation, are not antibiotic-resistant mutants (5, 7); in studies on two different strains of *B. burgdorferi* (8), "persisters" sub-cultured in the presence of the same antibiotic exhibited killing with a pattern identical to that of the original cell population, i.e., all sub-cultured cells were antibiotic sensitive, except for a small proportion that became "persisters". To date, it has not been possible to deliberately generate antibiotic-resistant mutant strains of *B. burgdorferi* using approaches known to be successful when applied to other bacteria. How does one explain the phenomenon of "persisters"?

The work of Abel zur Wiesch et al. (9) describes a testable model in which the presence of "persisters" *in vitro* can be explained solely by classic biochemical kinetics involving the interaction between an antibiotic and its target molecule. In the case of doxycycline, the bacteriostatic antibiotic of choice for the treatment of Lyme disease (10), this involves the competitive binding of doxycycline to the 30S subunit of the ribosome; such binding interferes with -- or displaces -- the binding of aminoacyl tRNA to the same 30S ribosome subunit, resulting in reversible suppression of bacterial protein synthesis and decreased growth (bacteriostasis), rather than irreversible killing or sterilization (11).

Since the binding of doxycycline and aminoacyl tRNA to the same target site (the 30S ribosomal subunit) is both competitive and reversible, there is a forward rate of reaction that involves association (binding), as well as a reverse rate of 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/or increased or decreased bacterial growth, depending on the concentration or density of each reactant. If one increases the concentration of doxycycline without changing the density of bacterial cells and their 30S ribosomal subunits, 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 bacterial cell population, e.g., by washing away antibiotic and then transferring the same number of bacterial cells to fresh antibiotic free medium, the equilibrium is changed in which the inhibition of protein synthesis is reversed, resulting in increased bacterial growth. Such manipulations have been conducted and the results documented for *in vitro* studies involving different bacterial species and antibiotics (11).

The screening of existing anti-cancer drug libraries to identify candidates more effective than the antibiotics currently used to treat borreliosis indicated that the inhibitory effects of all candidate drugs considered to be most promising were concentration dependent in *in vitro* studies (12). All these observations are consistent with the model proposed by Abel zur Wiesch et al. (9). Clinical studies on the efficacy of using vancomycin, an antibiotic often reserved for use only to treat the most intractable bacterial infections, showed it to be no more advantageous than the recommend oral therapy for treating Lyme disease with doxycycline, amoxicillin, or cefuroxime axetil (13). Since vancomycin requires the placement of an intravenous catheter, its use to treat Lyme disease is not justified and may even result in a variety of potentially serious adverse effects.

Although these events surely occur when antibiotics are given *in vivo*, there are major differences that can greatly influence the 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 *B. burgdorferi.* However, these may just be intact non-viable cells; unlike the "persisters" found in *in vitro* studies, these intact cells --which have pharmacological properties-- have not yet been isolated, re-cultured, and then shown to produce disease (14-17).

Recently, Jutras et al. reported that when *B. burgdorferi* multiply during infection, they shed peptidoglycan (PG), a pharmacologically active cell component that elicits an inflammatory response *via* an interaction with host cells that play an important role in the innate immune response (19). Such an interaction might well contribute to the expression of many of the symptoms associated with PTLDS. Because Borrelia are unable to re-cycle PG back into progeny cells, it accumulates in tissues

at the site of infection, thereby enabling PG to exert its inflammatory effects, long after infection has been cured by antibiotic therapy. The longer an infection progresses without treatment, the more PG will accumulate to exert its effect, even though the initial infection was subsequently cured by adequate antibiotic therapy. Thus, symptoms usually associated with PTLDS may very well be the result of a persistent pharmacologically active PG, rather than a persistent bacterial infection in which case extended antibiotic therapy would be of no value.

To test this hypothesis, Jutras et al., injected isolated, purified PG into the joints of mice and discovered it was able to generate the same type of dramatic joint inflammation observed in a well-characterized animal model of Lyme-induced arthritis (19). In other studies, synovial fluids were collected from patients with confirmed Lyme-induced antibiotic-refractory arthritis, i.e., from patients who were seropositive, unresponsive to extended antibiotic therapy, and with no evidence of active infection; such specimens were found to contain significant amounts of PG. Since synovectomy has been shown to alleviate antibiotic-refractory Lyme-induced arthritis (20), all these observations are consistent with the hypothesis.

The results of other studies showed that the number of arthritis episodes in patients with antibiotic-refractory Lyme-induced arthritis declines slowly but progressively with time and disappears within 9 years with no additional antibiotic treatment (21). Although this indicates that PG is slowly eliminated from synovial fluid with time, physical removal may not be a practical solution to this problem. Perhaps a better strategy might be to devise an approach that involves neutralizing the pharmacological effects of PG as suggested by Jutras et al. (19). Hopefully, an awareness and appreciation of the significance of these observations will encourage others to conduct definitive studies in that regard, instead of treating patients with different antibiotics, given singly or in combination for extended periods of time, to cure an unproven persistent Borrelial infection. In this context, the results of a small clinical trial showed that the symptoms of PTLDS were eliminated in 9 of 10 patients treated with gabapentin or Lyrica (22). It remains to be determined if treatment with other pain-relieving/anti-inflammatory agents would be as or even more effective in that regard.

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 (23). 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 (24), it often may be very difficult to attribute the beneficial effects of antibiotic therapy solely to the elimination of an active infection.

Phillip J. Baker

Executive Director

American Lyme Disease Foundation

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