

A long journey to understand Borrelia burgdorferi in Brazil

At the end of my fellowship at TUFTS School of Medicine in 1989, my sponsor Dr Allen C Steere, suggested me to look for Lyme disease (LD) in Brazil. I remember his words "It is like to search for a needle in a pottle of hay". Certainly, he didn't know how hard could be this task.

He offered me some reagents and a tube containing culture of *Borrelia burgdorferi* G 39/40 to start my research in Brazil. During all this long research period I received financial support by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

My first step was to contact an entomologist and fortunately I met Dr Domingos Baggio, a kind man who tried to teach me about ticks. In this sense, we travelled to suspected geographical regions, looking for suspected cases of LD in Brazil and also collecting ticks for analysis.

At that time, nobody knew about LD in Brazil. So, we started to publish review article and to inform Brazilian physicians about possibility of existence of this emerging tick borne disease in the country.

Fortunately, we identified first cases in 1992, in brothers, who became ill after tick bite episode, in a wooded region of Atlantic Forest at Itapevi city, São Paulo State. The boys had fever, erythema migrans (EM) and arthritis and serology for *Borrelia burgdorferi* (ELISA and Western-blotting) done at our Laboratory was positive in both.

After the description of these cases many others were identified. However, careful analysis of the patients revealed that LD in Brazil was different from that described in North hemisphere. Despite of presence of all clinical manifestations of LD in our country, we observed that symptoms were relapsing and serology for *Borrelia burgdorferi* (ELISA) showed low and transient positivity. Furthermore, western blotting results revealed occurrence of few bands and never fulfilled Center for Disease Control and Prevention (CDC) criteria for LD. We never obtained positive culture for *B. burgdorferi* in BSK medium, even when skin biopsies from

LD patients were tested. Additionally, PCR results always gave negative results when specific or conserved primers to identify *B. burgdorferi* sensu stricto or genus *Borrelia* were employed. Another important feature observed with Brazilian LD patients was the appearance of strong autoimmunity as antibodies directed to extract of human brain cells membrane or extract of human skin (commercial products from Sigma Co.).

At this point, we understood that Brazilian LD was completely different from classical LD, and we found many difficulties to report our results. The manuscripts from our group were always refuted to be published by International Journals, because revisers never accepted the clinical and laboratorial findings described in our patients.

Despite of many difficulties, we had published papers in Brazilian Journals, even with criticism of part of Brazilian researchers, in order to inform our physicians about existence of a severe and strange emerging tick borne disease in our country. Also, we had proposed a preliminary diagnostic guideline to help them to identify Brazilian Lyme disease.

In a new attempt to identify etiological agent of Brazilian LD we started to look for microorganisms in peripheral blood of suspected patients. Surprisingly, we identified spirochete-like structures by dark field microscope analysis and electron microscopy study suggested that these organisms could be a mixture of latent microorganisms composed of Mycoplasma, Chlamydia and spirochetes. However PCR and serologic tests done to identify these latent microorganisms were negative.

So, with the aim to understand the meaning of such interesting structures viewed on electron microscopy we performed a careful Medical Literature review. We discovered that spirochetes at its L form, also known as cell wall deficient bacteria, can change its morphology when the survival conditions are not suitable, originating the appearance of the same structures described in Brazilian LD patients.

At this point, we understood the shrewdness of spirochetes responsible for Brazilian LD. Possibly they adopted a survival strategy, deleting or suppressing unnecessary genetic

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Footnote: The references were omitted because they are cited in the articles published in this issue of BJR.

contents to express minor amount of proteins to the host immunological system. So, L form bacteria could represent atypical morphologies of spirochetes that had lost part or total out membrane lipoproteins (Osps) and periplasmic flagella.

However, we considered that they had to keep essential genes and proteins to their survival at its many passages through mammals and ticks hosts. In this sense, we decided to test new primers like those belonging to complex cp 32 and *flgE*. The first is important because it synthesizes factor H which inhibits host immune response against spirochetes.

The selection of *flgE* happened after interesting observation. We knew that spirochete at its L form couldn't express periplasmic flagella, but Brazilian LD patients, in general, exhibit presence of 41 KDa, a flagellin protein, at western blotting analysis. We discovered that flagella is composed of three components, the basal body, a hook (gene *flgE*) and a filament. Since *B. burgdorferi* mutant for synthesis of periplasmic flagella are rod shaped, we assumed that *flgE* could be preserved in Brazilian spirochetes.

Data of these experiments are very preliminary, but the first assays showed interesting results. PCR with cp 32 -2/7 demonstrated positive results but the sequence procedure revealed existence of strong cross-reactivity with human chromosome. Otherwise, use of *flgE* primer seems to be promising, since first results gave positive PCR in some patient and tick samples, and negative results in normal controls. Surprisingly, the sequence analysis revealed homology with *Borrelia burgdorferi* sensu lato spirochetes. Certainly, these results must be repeated and interpreted carefully, but these unbelievable results open new fascinating perspectives to understand many problems related to LD and Brazilian LD.

Due to many particularities, Brazilian LD has been called Baggio-Yoshinari Syndrome (BYS) since 2005. It is

thought to be a new tick borne disease defined as " Peculiar Brazilian tick borne disease caused possibly by *Borrelia burgdorferi* sensu lato spirochete at its L form (mutant), transmitted by ticks not belonging to *Ixodes ricinus* complex (*Amblyomma cajennense*, *Rhipicephalus (Boophilus) microplus*, *Rhipicephalus sanguineus*), which reproduces all the clinical symptoms of LD, except for occurrence of relapsing and autoimmune features"

At the moment, it is not our aim to discuss the relationship between L form bacteria and human infection. However this amazing proposal, explains most of controversies found in BYS as the spirochete persistence for long period in the host, difficulties to culture spirochetes in BSK medium, low immune response against the bacteria, development of autoimmunity and resistance to eliminate this bacteria from the hosts.

I believe that the etiological agent of BYS is a mutant spirochete belonging to *B. burgdorferi* sensu lato complex, as the consequence of existence of enormous geographical differences and biodiversity. So, we assume that an unique spirochete of this complex can cause two different diseases respectively LD and BYS.

Finally, I would like to say to my friend Dr Steere that these unlikely results are the conclusions of a long journey of 20 years of hard research done in Brazil. During all this period of time, my mind was always turning back to lessons and advices given by my Professor. If I had insisted in this long journey, it is a consequence of a promise expressed 20 years ago.

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