IMMUNOLOGY OF LYME BORRELIOSIS

N. Satz

ABSTRACT

Borrelia burgdorferi shows both a variety of outer surface proteins with molecular weights between 14 and 100 kilo-Dalton and a 41 kilo-Dalton flagellar protein which induce the immunologic response in the infected host. Lipopolysaccharides are responsible for inflammatory reactions and for constitutional symptoms. A vigorous T-cell immune response first develops followed by a more slowly evolving B-cell immune response later on. The humoral response shows the usual pattern of IgM antibodies appearing first, followed by IgG and IgA antibodies. The IgM antibody titre may normalize after recovery while the IgG titre may persist over years or decades. Antibodies to flagellin or to the 60 and 100 kilo-Dalton surface proteins first appear in the course of the disease, later on antibodies to OspA, OspB and OspC can be found. However, elevated ELISA, immunofluorescence titres or the immunoblot do not confirm the diagnosis of Lyme Borreliosis. The diagnosis has to be made clinically and by excluding other diagnoses.

KEY WORDS

Lyme borreliosis, immunology, Borrelia burgdorferi, T-cell immune response, B-cell immune response, immunoblot

THE IMMUNOGENIC SURFACE OF *BORRELIA BURGDORFERI*

The surface of *Borrelia burgdorferi* (*Bb*) shows both a variety of outer surface proteins (Osp) with molecular weights between 14 and 100 kilo-Dalton (kD) and a 41 kD flagellar protein; these immunogenic epitopes induce an immune response in the infected host. Lipopolysaccharides, also constituents of the bacterial capsule, are responsible for the inflammatory reaction, constitutional symptoms and the Jarisch-Herxheimer reaction (1).

A number of different American and European strains of *Bb* have been differentiated on the basis of their proteins. Analysis has shown that all strains have two components of constant molecular weight in common: the 60 kD („common antigen") and the 41 kD flagellar antigen („flagellin"). The common antigen is not specific for *Bb* and can be seen in a wide range of remotely related bacteria. The flagellin

also is unspecific and is similar to the flagellar antigens of other spirochetes (1). The strains vary in several Osp, such as the 21 to 22 kD protein (OspC), the 31 to 32 kD protein (OspA) and the 33 to 36 kD protein (OspB). These antigens differ in their amount, slightly in their molecular size and in their antigenicity. Testing with monoclonal and polyclonal antibodies in general strains of North America has shown a close homogeneity of the Osp and differences mainly in their OspB. European isolates demonstrate a greater variety of their antigenicity and differ in their OspA and OspC. For example, in isolates of the skin from German patients with erythema migrans or acrodermatitis chronica atrophicans, 11 different strains were found and 3 different serotypes were also identified in valley of Valais (Switzerland), thus suggesting the surface varies greatly depending on the patient, the illness pattern and the geographical region (2). Furthermore, gene analysis of *ospA* and *ospB* has shown another large variation of DNA sequences, therefore leading to the identification of three molecular subtypes of *Bb* responsible for the different clinical manifestations of the disease: *Bb* sensu stricto, *B. afzelii* and *B. garinii* (1,3,4).

THE IMMUNOPATHOLOGIC MECHANISMS OF LYME BORRELIOSIS

In the human host, *Bb* first proliferates locally in the skin at the site of a tick bite where it may cause erythema migrans within days to weeks. As implied by the mouse model, *Bb* is first taken up by the tissue-derived macrophages and by the Langerhans' dendritic cells. In the lymph nodes the T-cell epitopes are presented to the lymphocytes where specific T- and B-lymphocytes are recruited, thus stimulating the production of plasma cells and finally the secretion of antibodies. Subsequently, the *Bb* specific lymphocytes recirculate into the inflammatory tissue (5).

Within days to weeks *Bb* may also spread haematogenously into several target tissues. Some spirochetes are destroyed in the blood-stream by antimicrobial substances such as the complement factors. Other spirochetes coated with host-derived proteinases, such as plasminogen, escape and invade the target tissues through endothelial cell layers. The adherence to the endothelial cells is mainly mediated trough OspA and OspB. This process can be inhibited by pretreating the spirochetes with monoclonal antibodies to OspA and OspB. It is postulated that the interaction between the spirochetes and the endothelium also activates cytokines, promoting chemotaxis and invasion of leucocytes through the vessel wall into the inflamed lesion (5).

THE IMMUNE RESPONSE OF THE HOST

A prominent B- and T-cell immune response occurs during dissemination of *Bb*. At first a vigorous T-cell immune response develops, followed later on by a more slowly evolving humoral B-cell immune response. The delayed onset of the humoral immune response may explain why antibodies to *Bb* could not be detected early in the course of the disease (3,4).

The total amount of blood lymphocytes and their subpopulations changes neither in the early nor in late stages of the illness. Only the number of natural killer cells could be raised. However, the lymphocytes are activated. *In vitro* experiments have revealed that lymphocytes derived from each stage of the disease were able to be highly activated by phytohaemagglutinin, by concanavalin A or by the pokeweed mitogen in the presence of *Bb* (6). At first the T-cell immune response is induced by the 41 kD flagellar antigen. Later on the 15, 17 and the 60 kD antigen and finally OspA and OspB play an important role in maintaining the stimulation.

The use of T-cell proliferation tests may be very favorable for clinical purposes, because T-cell activation occurs prior to the humoral immune response. However, clinical studies have shown controversial results concerning sensitivity, specificity and correlation to clinical activity of the disease. Furthermore, there isn't any reliable and standardized test system and no clinical laboratory has greater experience with them (4).

The humoral immune response includes the production of immunoglobulins M, G and A. IgM antibodies could be found 2 to 4 weeks after infection. IgM antibodies typically peak at 6 to 8 weeks after infection and then continuously decline or occasionally persist for years. Unfortunately, in most European cases with early or late LB a measurable amount of IgM antibodies could not be found even when examined by immunoblot. IgG antibodies appear later on after about 6 to 8 weeks. Immunoblot techniques have demonstrated that the first antibodies arise to flagellin, followed by antibodies to antigens with high molecular weights such as the 60 and 100 kD proteins. If the disease

remains active for months or years, the antibody response expands with time to include additional antigens, particularly OspA, OspB and OspC. Serum levels of specific IgA which rise parallel to IgG are not measured routinely (3,4,7). Once the levels of IgG and partly IgM antibodies are elevated, they may remain (at the same level) for years even after successful treatment and clinical remission. Therefore, a positive test result may only indicate a short (presence of antibodies to flagellin, 60 and 100 kD protein) or a long (additional presence of OspA, OspB or OspC) exposure to *Bb*. However, an active infection cannot be distinguished from a past exposure to the organism (4).

The humoral immune response can also be inhibited. Particularly when antibiotics are given early in the course of the disease, the production of immunoglobulins may be blunted for a long time while the T-cell immune response remains unaffected. This leads to the typical dissociation of B- and T-cell immune response as described by Dattwyler and coworkers (6). Moreover, steroids or other immunosuppressive agents delay the rise of antibodies. Immune complexes, typically seen in early Lyme Borreliosis, bind the antibodies. This sequestration may also lead to false negative serological test results. On the other hand, there are false positive test results of the widely used ELISA and immunofluorescence, caused by cross-reactions, e.g. in cases with general abnormal serum proteins, elevated gammaglobulins, positive rheumatoid factor, positive antinuclear antibodies, syphilis etc. Usually, however, they can be differentiated by an immunoblot examination (3,4).

Clinicans must be aware that neither a negative test result rules out an actual Lyme Borreliosis nor can a positive serological test confirm the diagnosis of LB. Therefore, the diagnosis of Lyme Borreliosis has to be made clinically and by excluding other possible differential diagnoses (4).

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AUTHOR'S ADDRESS

Norbert Satz, MD, Praxis am Paradeplatz, Poststrasse 5, CH-8001 Zürich, Switzerland