LYME NEUROBORRELIOSIS. PATHOPHYSIOLOGICAL AND DIAGNOSIS ASPECTS

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ABSTRACT

Lyme neuroborreliosis is the neurological complication of *Borrelia burgdorferi* infection. The main mechanism involved is acute inflammation (direct infectious or indirect autoimmune) leading to degeneration of the central and peripheral nervous system structures. Several cell populations are involved in mediating the inflammatory response: endothelial cells modify the function of the blood-brain barrier; the microglia and the astrocytes act as immune cells as they present antigens to T cells and secrete prostaglandins, and the dendritic cells which can determine disease relapse or transformation into a chronic condition. All these cells secrete cytokines (interleukins, interferons, TNF), prostaglandins and complement components.

The permeabilization of the blood-brain barrier through inflammation leads to cerebral edema, associated with characteristic CSF modifications which can be detected by specific laboratory tests. Serologic analysis and CSF analysis are the most useful tests for Lyme disease diagnosis. CSF analysis is mandatory in patients with neurological symptoms in the absence of skin manifestations and involves detection of intrathecal antibodies and pro-inflammatory cytokines by ELISA, FIA and Western Blotting. PCR for detecting *Borrelia burgdorferi,* DNA and *Flow Cytometry* are important in the later, chronic stages of the disease.

Lyme neuroborreliosis is a disease with complex manifestations which can induce difficulties in diagnosis, especially in the early stages. False positive diagnosis can occur in patients considered to be at high risk for this disease (endemic zones, certain professions). Therefore collaboration between neurologist, infectionist and laboratory doctor is essential for an early and correct diagnosis; it allows for effective treatment of the disease, with a favourable prognostic.

Key words: inflammation, CNS, Lyme disease, CSF, serologic analysis

Abreviations

BAT	flow cytometric borreliacidal antibody test
Bb	Borrelia burgdorferi
CAP	cationic anti-microbial protein
CRASPs	complement regulator acquiring surface proteins
CSF	cerebrospinal fluid
DC	dendritic cells
ELISA	enzyme linked immunosorbent assay
FIA	focal immunofluorescence assay
IFN	interferon
Ig	immunoglobulins
IL	interleukins
Ly	lymphocites
MHC	major histocompatibility complex class
MIP	macrophage inflammatory protein
NBL	neuroborreliosis Lyme
NGF	neuronal growth factor
PAF	platelet activating factor
PCR	lyme multiplex polymerase chain reaction
PGE	prostaglandine
PLA	phospholipase
SNC	sistem nervos central
SNP	sistem nervos periferic
TLRs	Toll-like receptors
TNF	tumoral necrosis factor
WB	western blotting

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Borreliosis (Lyme disease) is an infectious disease caused by Borrelia burgdorferi (Borrelia spirochaete). The neurological complications of this disease are known as neuroborreliosis.

Lyme disease is the most frequent disease transmitted by arthropods and evolves in successive steps, involving the skin, the joints, the cardiovascular system and the nervous system. The disease is curable at all stages, but persistent sequelae may appear in the advances stages (8,24,25,26,27, 29).

The most frequent neurological manifestations of Lyme disease are mononeuritis, radiculoneuritis of the peripheral and/or cranial nerves, meningitis and inflammation at the CNS (2,26,37,38,53,60).

The majority of B burgdorferi infections occur during the warm season (spring-summer), mostly affecting the 5-14 and 30-70 age categories, with no difference between sex or race. The disease most frequently involves the nervous and the musculoskeletal systems, but up to 10% of the patients in the endemic areas can be serologically positive yet asymptomatic (3,8,15,53,66).

The disease is transmitted by tick bites and it is presumed that a 1-3 days contact with the host is required. A direct invasion of the neurons by B burgdorferi occurs, with destructive consequences. The pathogenic mechanisms are not fully clarified. The parasite triggers a direct, infective mechanism as well as an autoimmune mechanism which determine a chronic inflammatory neurodegenerative transformation of the central and peripheral nervous systems (13,17,20). There is still controversy concerning the advanced stages of the disease, as it is not known whether it involves reinfection, an extension of the primary infection or an autoimmune phenomenon (23,41,42).

The B burgdorferi infection ascends the neuronal circuits through the hippocampus up to the superior cortical centers by transsynaptic transmission from nerve to nerve, mostly affecting cholinergic transmission. There are 3 possible ways for the clinical evolution of the disease: 1) absence of clinical signs with seropositivity; 2) localized clinical signs and immune response (migratory erithema); 3) generalized clinical signs and immune response (affecting the skin, heart, joints and nervous system) (14,24,40,44,46,49,62).

The inflammation is the most important defense mechanism against the tissular aggression and is an essential component of the acute and chronic affections of the nervous system. The leukocyte antigens HLA-DR4 and HLA-DR2, found on proinflammatory immune cells, seem to play an important role in the process (5,17,22,23). Prostaglandins, free radicals and complement accumulate at the lesion site and induce production of chemokines and adhesion molecules which, in turn, stimulate other immune cells and glial cells (28,30).

The endothelial cells play an important role, as they express adhesion molecules on their surface and facilitate leukocyte recruitment during the inflammatory process. They are also involved in a process of transcytosis, which modifies the function of the blood-brain barrier (43,47,48).

The microglia and the astrocytes act as immune cells in the inflamed cerebral tissue. The microglia contributes to the inflammatory process through secretion of prostaglandins, inflammatory derivatives of arachidonic acid *under the action of COX-1, COX-2* (51). G prostaglandins also have a neuroprotective action by inducing an increase of AMPc at neuronal level and reduction of nitric oxide (NO) and pro-inflammatory cytokine production. Production of trophic factors and neurotoxin clearance by the astroglia are other protective mechanisms in CNS infections (6,58,61,63).

In Lyme disease the spirochaetes persist in the cerebral tissue and can be associated with amyloid deposits, triggering cortical atrophy and consecutive dementia (12,63). The microglia (equivalent of the monocyte in peripheral blood) acts on the neurons through a cationic anti-microbial protein, CAP37. The microglia-CAP37 complex expresses MHC II (major histocompatibility comples class II) antigens and induces secretion of pro-inflammatory cytokines and chemokines (5,64). Interleukin 8 (IL-8) and the macrophage-secreted cytokines MIP-1 alpha and MIP-1 beta increase during the disease and decrease after 2 weeks of treatment. Recent studies have demonstrated the importance of dendritic cells (DCs) for the intra-thecal immune response. The DCs normally reside in meninges, choroid plexus and the cerebrospinal fluid (CSF) and are absent in the cerebral parenchyma. During the development of neuroborreliosis these cells accumulate in the affected tissue and contribute to disease relapse or chronic inflammation of the CNS (7,9,17). Two distinct phenotypes of DCs seem to be preferentially involved: the myeloid $CD11c^{+}$ $CD123^{dim}$ and the plasmocytic $CD11c^{-}CD123^{high}$. The number of these cells is increased in the CSF. MIP-1 beta, produced by macrophages, and MCP-1 and MCP-3, produced by monocytes, are potent DC chemoattractants. The SDF-1 alpha factor, derived from the stromal cells, also seems to play a role as a DC chemoattractant. Regulation of DC passage in the CSF and from the CSF to the

secondary lymphoid organs may represent a control mechanism for CNS inflammation in Lyme disease (1,5,17). The CXCL13 cytokine is produced by the DCs in the initial stages of the disease and is a useful diagnostic element (22,52). The persistence of CXCL13 and IgG in the tissues certifies the presence of the infection. B cell activation, lymphocyte infiltration and IgG production in the infected tissues are also characteristic for the disease (32). Nevertheless, it is still unclear how B cells and plasmocytes invade the affected tissues (20,35). The Toll-like receptors (TLRs) are well represented in the CNS and play a key role in the pathogenesis of the disease. They react rapidly to the microbial aggression and intervene in post-disease reparatory processes (11). Borrelia burgdorferi stimulates IL-6, TNF-alpha and PGE-2 production in the microglia, triggering NF-kappaB, TLR-2 and CD14 expression (28,31,33,64).

The immune mediators, eicosanoids, pro-inflammatory cytokines, chemokines, complement components, NO and free oxygen radicals have neurotoxic potential. The strongly reactive free radicals and NO trigger neurodegenerative processes by direct oxidation and consecutive destruction of DNA, lipids and proteins. The neurons are very susceptible to direct oxidation and indirect activation of stress-reactive genes. Irrespective of mechanism, the result of these processes is local inflammation mediated by glial cells (astrocytes, microglia) (4,30,45,52,54). Arachidonic acid oxidation produces prostaglandins, leukotriens and tromboxane. The activation of the A2 phospholipase (PLA2) leads to the production of glycerophospholipids, the substrate for platelet activating factor (PAF) synthesis. The docosahexaenoic acid (DHA) is metabolized in resolvins and neroprotectins, lipidic mediators which inhibit the production of prostaglandins, leukotriens and troboxane and NF-kappaB transcription. Thus, the synthesis of pro-inflammatory mediators and the recruitment of leukocytes are inhibited, protecting the healthy neurons against the tissue repairing processes (34,56,63).

The result of the inflammatory process at CNS level is cerebral edema, determined by hyper-permeabilization of the blood-brain barrier. The pro-inflammatory mediators act directly on endothelial cells leading to increased permeability and subsequent vasogenic edema (37,38).

The immune cells also have the ability to produce nerotrophic factors which suport neural survival and can have anti-inflammatory effects. The interleukins IL-18, IL-1beta, sIL-1RII (secreted IL-1R II) and IL-12 are increased in the CSF but not in the serum of Lyme disease patients. Recent studies have shown that auto-reactive IgM antibodies can function as an endogenous mechanism for tissular repair. The IFN-gamma levels increase in acute infection and lead to IL-4 secretion. The persistence of IFN-gamma expression is characteristic for chronic neuroborreliosis (4,50,65). The intrathecal antibody production is very abundant in Lyme disease. CD4+ T cells infiltrate the CSF in the early stages of the disease. The tissular injury in neuroborreliosis can also be mediated by cytolitic CD8 or gamma-delta T cells, which are present at the infection site. There are significant differences between the proportions of different T cell populations locally present during disease development. In the initial stages the Ly HLA-DR$^+$ lymphocytes predominate, while the involvement of CD25+ T cells is relatively decreased (31,39).

The neurological clinical manifestations are accompanied by modifications of CSF composition and accumulation of activated T cells. Antigen presentation and T cell activation continue in chronic disease and in the disease forms which are resistant to treatment. The increased concentration of soluble ICAM-1, ICAM-2 and ICAM-3 in the CSF demonstrates their important role in the inflammatory process in Lyme disease (5,48,67). The complement system intervenes in the initial stages of the inflammation. The C1q, C3b and iC3b opsonins interact with the complement receptors and initiate phagocytosis, while the C3a and the C5a anaphylatoxins initiate local inflammation. Increased C1q, C3, C3a and C4 levels were found in the CSF but not in the plasma of neuroborreliosis patients (33,34,57).

The excessive production of pro-inflammatory cytokines (IL-6, TNF-alpha can trigger neuronal destruction. B burgdorferi can avoid the destructive effects of the immune response by blocking the activation of the complement system through the complement regulator acquiring surface proteins (CRASPs). The epithelial destruction stimulates the expression of IL-1, TNF-alpha, IFN-gamma, adhesion molecules and acute phase proteins. The cytolitic CD8 lymphocytes also induce chronic tissular injury by IFN-gamma production. Recent data indicates that the glucocorticoid hormones may also have a pathogenic role by stimulation of cellular migration, cytokine production and activation of transcription factors at cerebral level (5,33,50,65).

The cerebral inflammation plays a dual role as it determines tissular injury through the destructive activity of inflammatory mediators but it can also initiate tissue repair and re-establishment of homeostasis. The inflammatory mediators produces by astrocytes and the cytokines produced by the immune cells stimulate the expression of neurotrophic factors, as the neuronal growth factor (NGF). Increased expression of NGF and other trophic factors in the CNS can suppress the inflammatory processes. The trophic factors of the nervous system protect the axons and the myelin sheath but also contribute to maintaining the immune privilege of the brain (5,6,33,34).

Serological and CSF laboratory investigations are useful to confirm the diagnosis. The most important serological tests are ELISA (Enzyme Linked Immunosorbent Assay), FIA (Focal Immunofluorescence Assay) and Western Blotting (WB). The initial screening is done by ELISA, which detects anti-*Borrelia* antibodies using plated purified antigen. Ninety percent of patients have a positive IgM ELISA test during the acute phase as well as during convalescence. After a few weeks the majority of patients have increased IgG antibodies, reflecting previous exposure. Although ELISA and FIA have over 80% sensibility, their specificity is only around 72% and false positive reactions for syphilis and lupus can occur. The Western Blotting test has superior specificity and a positive Western Blot is a clear indication for treatment. Positive ELISA and FIA associated with a negative Western Blot are considered to be false positive (8,14,19,35).

Other serological methods are PCR – Lyme multiplex polymerase chain reaction for detection of *Borrelia burgdorferi* DNA and BAT – Flow Cytometric Borreliacidal Antibody Test. PCR can be positive in up to 50% of the cases, mainly during persistent or recurrent disease. Flow cytometry is used in the late stages of the disease, having a 99% specificity compared to the standard methods (ELISA) (4,8,15,21).

CSF examination is not absolutely necessary if IgM and IgG antibodies are present in serum, but is mandatory for patients with neurological disease in the absence of skin involvement. CSF analysis can detect intra-thecal anti-*Borrelia* IgG and IgM antibodies and a moderate lymphocytic pleocytosis (over 100/cube mm); however the antibodies cannot be detected in the CSF during the initial stages of the disease (35). In certain atypical cases of Lyme meningitis leukocytosis, increased protein levels and reduced glucose levels can be detected in the CSF. Increased concentration of ICAM-1, ICAM-2, ICAM-3 in the CSF stands proof of their important role in the inflammatory process (67). Measurement of the pro-inflammatory cytokines IL-1beta, IL-6, TNF-alpha and IFN-gamma in the CSF can be very useful for monitoring the evolution of local inflammation and the efficacy of the treatment (18,33,52).

In conclusion, Lyme disease is triggered by *Borrelia burgdorferi* both through a direct infectious, and an indirect inflammatory mechanism, resulting in cerebral edema. The cerebral inflammation initiates homeostasis and repair but can also determine acute and chronic injury of the nervous tissue through inflammatory mediators, leading to degeneration of the central and peripheral nervous systems. A very important role is played by the astrocytes, the microglia and the cellular and humoral mediators (cytotokic T cells, interleukins, TNF-alpha, chemokines and complement components) (22,23,50,65).

Lyme disease has complex manifestations which can lead to difficulties in diagnosis, especially during the initial stages. False positive diagnosis can also occur in patients living in endemic zones who have increased professional risk for this disease. Collaboration between the neurologist, infectionist and the laboratory specialist is essential in order to establish a correct diagnosis. If the disease is diagnosed in its early stages and is rigorously treated under the supervision of the infectious disease specialist the prognostic is favorable (10,14,15,16,67).

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