The Immune Response at Onset and During Recovery From Borrelia burgdorferi Meningoradiculitis

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Background: Borrelia burgdorferi causes a wide range of neurologic syndromes. In Europe, acute meningoradiculitis is the most common manifestation.

Objective: To address the nature of the immune response during the course of B burgdorferi meningoradiculitis, with special respect to the early and late changes in cerebrospinal fluid (CSF).

Methods: Serial immunophenotyping was performed and cytokine measurements were obtained in the peripheral blood and CSF of 12 European patients with definite B burgdorferi meningoradiculitis.

Results: Early during infection and before initiation of treatment, we observed high levels of interleukin (IL) 10, IL-6, and IL-8, and large numbers of B cells and plasma cells in the CSF of most patients. At the same time, we found a mainly unspecific intrathecal antibody synthesis. During resolution of the infection, cytokine levels normalized rapidly and plasma cells disappeared from the CSF. In parallel, the percentage of B cells in the CSF increased over several months, accompanied by rising levels of intrathecally produced B burgdorferi–specific antibodies.

Conclusions: Our findings demonstrate that the early phase of B burgdorferi meningoradiculitis is characterized by a well-coordinated immune response involving specific cytokine release and plasma cell recruitment, followed by a long-lasting, antigen-specific B-cell response in the central nervous system.

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BORRELIA BURGDORFERI is the pathogenetic agent of Lyme disease in humans. The spirochete can cause a wide range of diseases, among them, acute and chronic inflammation of the nervous system. In Europe, acute painful meningoradiculitis is most frequently observed. Although the pathologic mechanism by which B burgdorferi causes meningoradiculitis is not completely understood, it is well established that the spirochete infects the brain and meninges. Following bacterial infection, a vigorous immune response is observed at the site of infection, which probably contributes to the neurologic symptoms. In most cases, the immune response in the central nervous system (CNS) clears the bacterial infection, resulting in remission of symptoms. However, in rare cases, B burgdorferi infection is associated with chronic neurologic diseases, such as encephalomyelitis. Although the cause of chronic neuroborreliosis is unknown, it has been hypothesized that it is caused by B burgdorferi–specific T cells cross-reacting with self-antigens.

Borrelia burgdorferi meningoradiculitis is a clinically and microbiologically well-defined infectious disease of the CNS, although little is known about the immune response to this disorder. Experimental studies are impaired by the difficulties in achieving CNS infection in animals. Only in immunosuppressed monkeys is B burgdorferi infection of the CNS found, but these animals do not develop clinical signs of meningoradiculitis. Therefore, studies in humans are required to decrypt the immune mechanisms in acute neuroborreliosis. Although systemic immune responses are mounted after infection with B burgdorferi, the immune response is highly focused to the CNS, involving pleocytosis, disruption of the blood-brain barrier, and intrathecal antibody synthesis. Both T cells and B cells are present in the CSF of patients with neuroborreliosis. Furthermore, neopterin, interleukin (IL) 6, and matrix-metalloproteinases are found in the CSF after B burgdorferi infection, suggesting a role of these molecules in the host response against the spirochete.
Here, we investigated the immune response in a group of European patients with acute *B. burgdorferi* meningoencephalitis. We analyzed the immune cells in the CNS, the local humoral immune response, and the cytokine repertoire before and during resolution of the infection. Our results demonstrate a well-orchestrated immune response during the course of *B. burgdorferi* meningoencephalitis.

**METHODS**

We recruited all patients at the department of neurology at Philipps University (Marburg, Germany). The 12 patients with neuroborreliosis fulfilled at least 4 of the following criteria: (1) acute neurologic symptoms compatible with meningoencephalitis; (2) pleocytosis and disruption of the blood-brain barrier; (3) serum IgG or IgM antibody reactivity to *B. burgdorferi* determined by enzyme-linked immunosorbent assay and Western blot; (4) intrathecal *B. burgdorferi*-specific antibody synthesis; and (5) positive treatment response to antibiotics. All except patient 2 fulfilled all 5 criteria. The control groups consisted of patients with noninflammatory neurologic diseases (NIND) without evidence of intrathecal immune response (normal white blood cell count and IgG, IgA, and IgM levels) or patients with viral meningitis.

**SPECIMENS**

Cerebrospinal fluid (CSF) and serum were examined for protein, albumin, and IgG, IgA, and IgM levels by nephelometry (BN II, Behring, Marburg, Germany) and for the occurrence of oligoclonal bands (Tian Gel; Rolf Greiner Biochemica, Flacht, Germany). The Reiber formula was also used to determine the intrathecal produced fraction of Ig (IgT) and *B. burgdorferi*-specific immunoglobulin ([IgT = 1 − Qlim(IgG) / Qref] × 100; Qlim = upper limit of the reference range, Qref = IgCSF/serum), blood preserved with EDTA and pelleted CSF cells were immediately used for antibody stainings.

**WESTERN BLOT AND ENZYME-LINKED IMMUNOSORBENT ASSAY**

We measured immunoreactivity to *B. burgdorferi* by enzyme-linked immunosorbent assay using an assay based on bacterial lystate from strain pKo (Enzygnost Borreliosis IgG and IgM; Dade-Behring, Germany) and recombinant antigens (RecomWell Borrelia IgG and IgM; Mikrogen, Germany) and determined the intrathecal antibody index ([B. burgdorferi]-specific Ig in CSF/B. burgdorferi-specific Ig in serum/[total Ig in CSF/total Ig in serum] >2). Western blots were performed and analyzed according to the manufacturer’s protocol (RecomBlot IgG and IgM; Mikrogen). The Western blot includes the following antigens: p100 (*B. afzelii*), p41 (flagellin *B. burgdorferi* sensu strictu), BmpA (*B. afzelii*), OspA (*B. afzelii*), OspC (*B. afzelii*), B. garinii, B. burgdorferi sensu stricto, p41lim (B. garinii), p41lim (B. afzelii), and p18 (B. afzelii). The results of serologic tests for *Treponema pallidum* were negative for all patients.

**FLOW CYTOMETRY**

Flow cytometry on peripheral blood and CSF cells was performed as described previously. Monoclonal antibodies were used for the following markers: CD3 (T cells), CD4 (CD4+ T cells), CD8 (CD8+ T cells), a/b (T-cell receptor a/b), g/d (T-cell receptor g/d T cells), CD36/CD16 (natural killer cells), CD56/CD16/CD3 (natural killer–like T cells), CD14 (monocytes), CD19 (B cells), and CD19/CD38 (plasma cells). We used an isotype control stain to exclude unspecific antibody binding.

**CYTOKINE ANALYSIS**

Cytokine analysis, including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor (TNF) α, and interferon (IFN) γ was performed by cytometric bead arrays (Th1/Th2 cytokine and human inflammatory CBA kit; BD Pharmingen, San Diego, Calif) following the manufacturer’s instruction. For each staining, 30 µL of undiluted CSF was used. The range of the assays was 10 to 5000 pg/mL for TNF-α, IFN-γ, IL-1B, and 5 to 5000 pg/mL for all other cytokines.
We performed immunophenotyping of CSF and blood cells in patients with meningitis (roborreliosis but not in the CSF of controls, and to a lesser extent in patients affected by NIND or viral meningitis. At the onset of disease, high percentages of plasma cells (CD19+) and plasma cells (CD19+/CD138+) from both patients. The numbers represent the relative percentage of B cells and plasma cells. SSC indicates side scatter.

RESULTS

PLASMA CELLS AND B CELLS IN THE CSF OF PATIENTS WITH NEUROBORRELIOSIS

We used the paired t test to compare the mean percentages of expression of each subpopulation between peripheral blood and CSF in each patient. The t test was used to compare CSF cell populations between patients with neuroborreliosis and controls. The Spearman rank correlation was performed to analyze the relationship between antibody synthesis and CSF immune cells and to compare CSF cytokine concentration between patients and controls.

CSF CYTOKINES AND CELLS DURING RECOVERY

To investigate the dynamics of the immune response, we analyzed blood and CSF immune cells during the course of the disease. In all patients, blood-brain barrier function, intrathecal antibody synthesis, and pleocytosis normalized within 4 weeks (Figure 4). Plasma cell numbers dropped immediately and were below 0.5% after 30 days. In contrast, B cells persisted and even relatively increased in CSF during the first weeks of recovery. High B cell numbers were found even after more than 100 days in the CSF. T cell numbers slightly decreased over time for all other cytokines, no difference between patients with neuroborreliosis and those with NIND was found. In patients with meningitis, CSF concentrations of IL-6 and IL-8 were similar to those in patients with neuroborreliosis. In contrast, IL-10 was found at significantly higher concentrations in patients with neuroborreliosis. The high cytokine levels in patients with neuroborreliosis did not correlate with the cellular composition in CSF (data not shown). Cytokine levels in the serum of neuroborreliosis patients are produced locally.

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and monocyte numbers increased (Figure 5). No significant changes were observed for the other immune cells in CSF. We did not find changes of the immune repertoire in blood during the entire disease course (Figure 5). In parallel, the CSF cytokine changes during recovery from infection with B burgdorferi were studied. The levels of IL-8, IL-6, and IL-10 rapidly decreased after initiation of treatment (Figure 4). Levels of IL-10 and IL-6 were found to be below the detection limits of the assay at the end of the observation period. In contrast, low levels of IL-8—comparable with NIND patients—were still detectable in the CSF months after the infection.

RELATIONSHIP OF HUMORAL AND CELLULAR IMMUNE RESPONSES

Next, we determined IgM and IgG antibody titers against B burgdorferi in CSF and serum during the course of neuroborreliosis in 9 patients. To define local intrathecal antibody production, we calculated the overall and the differentiation of the IgG isotype revealed that intrathecally released antibodies were mostly IgG1 (data not shown). The absolute level of intrathecally synthesized IgG (r = 0.69; P < .001) and IgM (r = 0.5; P = .04) strongly correlated with the percentage of plasma cells in the CSF (Figure 6A). In contrast, a correlation was found between the B burgdorferi–specific IgG antibody synthesis in the CSF and the percentage of CSF B cells (r = 0.55; P = .01) but not plasma cells (Figure 6B).

We demonstrated that the immune response in acute B burgdorferi meningoradiculitis follows a defined sequence: in the very early phase, local intrathecal release of IL-6, IL-8, and, most characteristic, IL-10 is observed. At the same time, various lymphomononuclear cells accumulate in the CSF, most typically plasma cells. The initial pleocytosis is accompanied by a strong but largely unspecific antibody release in the CNS. During recovery, cytokine levels rapidly normalize and plasma cells disappear from the CSF. Days to weeks after treatment, the percentage of B cells and monocytes in the CSF gradually increases. This is paralleled by increasingly spe-
cific local *B burgdorferi* antibody production, although the absolute amount of intrathecally secreted Ig decreases. In contrast with the CSF finding, the distribution of immune cells in the peripheral blood does not change during the course of acute neuroborreliosis.

The findings in humans are in line with observations in experimental models. In vitro *Borrelia* lysate induces the release of IL-6, IL-8, and IL-10 from cultured immune, brain, and endothelial cells.23 This effect is mediated by lipidated *B burgdorferi* outer surface proteins, which bind to CD14 or toll-like receptors on the cell surface, leading to nuclear translocation of nuclear factor-kB and cytokine secretion.24-26 The release of IL-6 and IL-10 seems to be particularly important for host defense since IL-6 and IL-10 knockout mice show reduced antibody responses to *B burgdorferi* antigens and develop more frequent and more severe arthritis than their wild-type littermates.27,28

Since none of the cell populations in the CSF correlate with the cytokine levels, it seems most likely that infiltrating immune cells (eg, macrophages, microglia) or CNS cells are the source for the local cytokine production. All of these lymphokines promote the development of a humoral immune response: IL-6 stimulates B-cell proliferation and differentiation and induces the secretion of IgG, IgM, and IgA.29 Interleukin 6 induces the final maturation of B cells into immunoglobulin-secreting plasma cells, favoring the secretion of the IgG1 subclass.

**Figure 4.** A, Levels of interleukin (IL) 8, IL-6, and IL-10 at different time points during the disease course. B, Cerebrospinal fluid cell count, Qalb, and Q immunoglobulin G. The insets show the findings in patient 4. The data represent the findings in 11 patients.
Interleukin 8 is a chemotactic factor for various immune cells and selectively inhibits IgE synthesis of B cells without affecting other immunoglobulins. The combined effect of those cytokines may lead to pleocytosis and support differentiation of B cells. In a timely relationship to cytokine release, high numbers of antibody-producing plasma cells that secrete large amounts of IgG and IgM antibodies locally are found. However, most of the intrathecal antibodies are not B. burgdorferi–specific, which may be explained through unspecific activation of infiltrating B cells by the cytokine milieu and the B-cell mitogens of the spirochete. Since B cells are initially activated in the periphery, but plasma cells were not found in the peripheral blood of our patients during active disease, it is tempting to speculate that the B cells accumulated in the CNS and locally differentiated into IgG- and IgM-producing B cells and plasma cells. Immediately after initiation of antibiotic treatment, the cytokine levels rapidly drop and plasma cells disappear. During this phase, the percentage of B cells in the CSF increases, and this increase is possibly responsible for the long-lasting B. burgdorferi–specific immune response in the CNS.

In summary, we provided insights into the series of events following spirochetal infection of the CNS that contribute to the clearance of B. burgdorferi.
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