CONCISE COMMUNICATION

Soluble CD14 Levels in the Serum, Synovial Fluid, and Cerebrospinal Fluid of Patients with Various Stages of Lyme Disease

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Levels of circulating soluble CD14 (sCD14) in patients with various stages of Lyme disease (LD) were examined. Patients with early or untreated late LD had significantly higher levels of sCD14 than did healthy controls (P = .0001 and .0007, respectively); levels returned to normal within 3 months after antibiotic therapy. Patients with persistent posttreatment symptoms of LD had sCD14 levels equivalent to those of healthy controls. Differences in the serum sCD14 levels in patients with various stages of LD are likely to be directly correlated with differences in bacterial burden, suggesting that posttreatment symptoms may not require continued presence of the organism. sCD14 levels in the cerebrospinal fluid (CSF) of patients with any stage of LD were no different from those of control subjects. Levels of synovial fluid sCD14 from patients with Borrelia burgdorferi in their joints were elevated, compared with levels in normal serum, and may play a role in the pathogenesis of arthritis.

Lyme disease (LD) is a multisystem infection caused by the spirochete Borrelia burgdorferi [1]. The early stage of Lyme disease (ELD) is characterized by erythema migrans rash and can be accompanied by polynuropathies, meningitis, or cardiac conduction abnormalities. If left untreated, the disease can progress to a late stage (LLD) characterized by localized symptoms—most typically, intermittent or chronic arthritis, progressive polynuropathy, or encephalopathy. With antibiotic treatment, the majority of patients with either ELD or LLD will improve. A small percentage of patients will continue to have symptoms after treatment. Posttreatment chronic Lyme disease (PTLD) is characterized by symptoms of fatigue, arthralgias, paresthesias, or memory loss. Recovery of B. burgdorferi from tissues of these patients has been rare, and the cause of ongoing symptoms is unknown.

CD14 is a 55-kDa glycoprotein expressed on the surface of various cells, including monocytes, macrophages, neutrophils, and chondrocytes. CD14 can be found in a membrane-bound (mCD14) and a circulating soluble (sCD14) state. Both mCD14 and sCD14 can function as receptors for the lipopolysaccharides (LPSs) of gram-negative bacteria and for various cell-wall products of gram-positive bacteria [2]. Binding of LPS to mCD14 enhances signaling by Toll-like receptor–2, resulting in activation of the cell and release of proinflammatory mediators [3]. LPS-sCD14 complexes can bind to cells that do not normally express mCD14, resulting in cellular activation [4, 5]. Circulating levels of sCD14 have been correlated with LPS exposure experimentally [6].

In humans and experimental models of LD, the presence of B. burgdorferi has been linked to increases in proinflammatory cytokines. Although B. burgdorferi lacks LPS, borrelian lipoproteins can bind to CD14, and monoclonal antibodies that block LPS binding to CD14 also block B. burgdorferi–induced release of proinflammatory cytokines [7–9].

We were interested in examining whether sCD14 could be involved in the inflammatory response to B. burgdorferi in patients with LD and if so, whether this varied by stage or manifestation of the disease. To this end, we performed a retrospective analysis of serum, synovial fluid, and CSF sCD14 levels in patients with ELD, LLD, and PTLD.

Patients and Methods

Patients with ELD. Serum was obtained from 18 patients (age range, 30–73 years) from the northeastern United States who had been given a diagnosis of ELD. All patients met the Centers for Disease Control and Prevention (CDC) surveillance definition for the diagnosis of LD. All 18 patients had erythema migrans rash ≥5 cm in diameter. B. burgdorferi was isolated by culture from skin biopsy specimens from 6 of the 18 patients. Fourteen of the...
18 patients had evidence of LD by serologic testing. All samples were obtained within 1 week of the onset of EM.

**Patients with LLD.** Paired serum and synovial fluid (SF) samples were obtained from 18 patients with Lyme arthritis, acquired in the northeastern United States, who had symptoms for >6 months (mean, 2.2 years; range, 6 months–6 years) after their original erythema migrans lesion. All patients had oligoarticular arthritis involving one or both knees. All patients had positive IgG Western blot serology for LD and positive results from either SF culture or polymerase chain reaction (PCR) for *B. burgdorferi*. No patient had received antibiotic therapy active against *B. burgdorferi* before the specimens were taken. All samples were aliquoted and stored at −70°C until use.

**Lyme neuroborreliosis.** CSF was obtained from 9 patients with neurologic symptoms and evidence of central nervous system invasion by *B. burgdorferi*, as documented by either a positive PCR for *B. burgdorferi* or evidence of intrathecal antibody production (CSF : serum IgG antibody ratio of ≥1.2). All but 2 of these patients had received at least one course of antibiotic therapy appropriate for LD. Mean duration of symptoms was 3.2 years (range, 2 months–17 years). Six of the 9 patients had encephalopathies, consisting mostly of memory deficits, and 3 of 9 patients had paresthesias. Only 2 patients, both with relatively early LD (2 months), had significant pleocytosis (59 and 72 white blood cells [WBCs]/μL, respectively). Both of these patients had been treated for LD at the time when their CSF samples were obtained.

**Patients with PTLD.** Serum and CSF samples were obtained from patients enrolled in a study of the efficacy of ceftriaxone and doxycycline in the treatment of PTLD. Enrollment criteria for the study included a history of an acute syndrome consistent with LD, a positive IgG Western blot for LD, prior treatment with at least one course of antibiotics active against *B. burgdorferi*, and persistent symptoms, which could include arthralgias, memory loss, or paresthesias. All patients tested negative for evidence of detectable infection in serum and CSF, determined by culture and PCR for *B. burgdorferi*. The median duration of symptoms was 3.2 years (range, 6 months–9 years). Eighteen of 20 patients had ongoing arthralgias, 15 reported memory deficits, and 13 had paresthesias. Patients had received an average of 2.6 courses of antibiotic treatment appropriate for LD (range, 1–6).

**Control patients.** Control serum was obtained from 20 healthy volunteers (age range, 26–55 years) with no symptoms of LD. Control CSF was obtained by lumbar puncture from 10 patients undergoing spinal anesthesia for nonneurologic conditions. All serum and CSF samples were aliquoted and stored at −70°C until use.

**Diagnostic testing for LD.** The antibody response to *B. burgdorferi* in serum was determined by indirect ELISA [10] and Western blotting, by use of a commercially available kit (MarDx, Carlsbad, CA). Positive results were interpreted according to the criteria established by the CDC and the Association of State and Territorial Public Health Laboratory Directors [11]. Concomitant serum and CSF samples were tested for intrathecal production of IgG antibody to *B. burgdorferi* by antibody-capture enzyme immunoassay, as described elsewhere [12]. *B. burgdorferi* DNA was detected in joint fluid or CSF by PCR, using 2 different primer sets that target different regions of the plasmid DNA encoding outer-surface protein A of the spirochete, as described elsewhere [13, 14].

**sCD14 ELISA.** ELISA kits for measuring sCD14 (Quantikine; R&D Systems, Minneapolis) were used according to the manufacturer’s instructions. Human serum and SF samples were diluted 1 : 800 prior to use. CSF samples were diluted 1 : 50. All samples were run in duplicate. Intra- and interexperiment variability was <10%.

**Results**

*Serum levels of sCD14 in patients with ELD, LLD, and PTLD.* We measured sCD14 levels in serum from healthy donors and from patients with ELD, LLD, and PTLD (figure 1A). Patients with ELD had significantly higher levels of sCD14 in their serum than did healthy control subjects (*P* = .0001). The mean concentration of sCD14 in patients with ELD was 3058 ng/mL (95% confidence interval [CI], 2733–3383 ng/mL), compared with a mean of 2104 ng/mL (95% CI, 1965–2245 ng/mL) in healthy control subjects.

Untreated patients with LLD manifested as Lyme arthritis also had significantly elevated sCD14 levels compared with those of controls (*P* < .0007; see figure 1). The mean sCD14 level for patients with LLD was 2684 ng/mL (95% CI, 2408–2959 ng/mL). The sCD14 level in patients with LLD was lower than that in patients with ELD, with values that approached but did not reach significance (*P* = .078).

Posttreatment serum samples (obtained ~3 months from the initial infection) were available for 6 of these patients after therapy for ELD. All patients received a 3–4-week course of oral doxycycline (100 mg twice per day). sCD14 levels decreased to the normal range in all 6 patients and were significantly different from the pretreatment values (*P* = .0003).

There was no significant difference in the sCD14 levels between patients with PTLD and healthy control subjects (*P* = .41). The mean sCD14 level in serum from patients with PTLD was 2196 ng/mL (95% CI, 2040–2353 ng/mL).

**SF levels of sCD14 in patients with LLD.** We studied the SF of the 18 patients with LLD. All 18 patients had evidence of presence of the organism in the SF by either culture or PCR for *B. burgdorferi*. sCD14 was present in the SF of all patients (figure 1B). SF sCD14 levels in patients with LLD were significantly higher than serum levels of healthy control subjects (*P* = .0035; mean sCD14 in SF, 2724 ng/mL; 95% CI, 2357–3092 ng/mL). There was no significant difference between paired serum and SF levels of sCD14 in patients with LLD (*P* = .86). SF levels of sCD14 were greater than serum levels in 6 of the 18 patients, equal in 7 patients, and lower in 5 patients. There was no correlation between WBCs in the SF and sCD14 level, suggesting that at least for some patients, there may be local release of sCD14 in the joints.

**sCD14 levels in the CSF of patients with neuroborreliosis and PTLD.** We examined the CSF of healthy control subjects, patients with evidence of neuroborreliosis, and patients with PTLD for the presence of sCD14. We found minimal sCD14 in the CSF of healthy subjects (figure 1B). Neither neurobor-
Figure 1. Soluble CD14 (sCD14) levels (in ng/mL) in patients with early, late, and posttreatment Lyme disease (ELD, LLD, and PTLD, respectively). Each mark represents the average of duplicate samples from a single patient. The number of patients in each group is indicated in parentheses. Horizontal lines indicate the mean of each group. Solid lines connect paired samples from individual patients. A, Results from serum samples. B, Results from serum, synovial fluid (SF), and cerebrospinal fluid (CSF) samples.

Discussion

Bacterial infections may cause disease manifestations through multiple mechanisms, including, but not limited to, direct effects of bacterial products, effects of the host immune response to the organism, and persistent actions of the host immune response after clearance of the organism. Although there is little question that the symptoms of ELD result from the presence of the organism, the cause of symptoms in patients with “chronic” LD is more controversial. Many studies have added to the confusion by combining patients with different treatment histories under the category of chronic LD.

By using stringently applied criteria to distinguish patients, we have studied a component of the host immune response, sCD14, in patients with different stages of LD. We found that sCD14 levels are greatest in patients with ELD, slightly lower...
in patients with LLD, and similar to those of controls in patients with PTLD. The sCD14 levels of patients treated for ELD decline to normal within 3 months of therapy. This is consistent with experimental data showing that bacterial burden is highest during acute infection and declines over time as the host immune system restricts the organism to certain sites. Both human and animal studies have found that bacteria or bacterial DNA is not typically recovered after antibiotic therapy. One of the hypotheses for the mechanism of disease in patients with PTLD has been incomplete eradication of the organism. Our data provide indirect evidence that the bacterial burden in these patients is likely to be very low or nonexistent and that the mechanism of disease in PTLD may be different from those in ELD and LLD.

There does not appear to be expression of sCD14 in the CSF of patients with evidence of neuroborreliosis or in patients with PTLD with neurologic symptoms. This is not surprising, given that sCD14 is not expressed by astrocytes, glial cells, or other neural tissue. Elevated levels of sCD14 in the CSF of patients with bacterial meningitis are thought to derive from the presence of WBCs. Unfortunately, we did not have samples from patients with acute Lyme meningitis prior to therapy.

Although normal SF levels of sCD14 have not been established, the presence of elevated levels of sCD14 (compared with normal serum levels) in the SF of patients with LLD raises the possibility that sCD14 may be involved in the pathogenesis of Lyme arthritis. For a subset of patients, there even appears to be local production of sCD14, which may arise from stimulation of chondrocytes by *B. burgdorferi*. Given the ability of sCD14-LPS complexes to activate both non–CD14– and CD14–expressing cells, it is tempting to speculate that sCD14 may play a role in chronic activation of inflammatory pathways at localized sites such as the joint. Our data in LLD patients are similar to results reported in patients with rheumatoid arthritis [15]. It will be important to determine whether sCD14 is involved in the pathogenesis of these chronic destructive diseases or whether it is simply an acute-phase marker for cellular activation by lipoproteins.

References