

C3a and C4a: Complement Split Products Identify Patients with Hyperacute Lyme Disease

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Summary: Lyme disease, discovered more than 30 years ago, is the most prevalent arthropod-borne illness in the United States and Europe. Lyme disease is caused by a spirochete, *Borrelia burgdorferi*, and spread by the bite of ticks of the *Ixodes ricinus* complex [1]. Laboratory diagnosis of Lyme disease within the first few weeks of infection is inadequate because it requires the presence of antibody to *B burgdorferi*, confirmed by Western blot showing antibody reactivity to several proteins of the organism [2]. After infection, 2 to 3 weeks are required for antibody production and Western blot reactivity. Not all patients are positive by antibody measurement. Lyme disease generally manifests with flu-like symptoms of fever, malaise, headaches, arthralgia, and myalgia. A typical skin rash, erythema migrans chronicum (EMC), appears in < 50% of Lyme disease patients [3]. Both the innate and the adaptive immune responses are needed to control *B burgdorferi* infection [1]. To explore early innate immune responses in acute Lyme disease, we studied complement components and activation products in patients seen soon after tick bites. Patients presenting with typical Lyme symptoms 2 to 4 days after tick bite, with or without EM, were included. Control subjects were healthy individuals and patients with tick bites but no illness. Complement components and complexes, including C2, C3, C4, Factor B, C4d, and immune complexes (C1q binding and C3d containing), were similar in Lyme disease patients and control subjects. However, Lyme disease patients had significantly higher levels of C4a and C3a (split products of C4 and C3) than did control subjects ($p < .05$). All Lyme disease patients but only 3 control subjects had elevated levels of C3a, C4a, or both. Thus, testing for C3a and C4a should allow clinicians to detect Lyme disease in the acute stage and begin appropriate treatment.

Keywords: Lyme disease, complement diagnostic markers, C4a, C3a

Introduction

Lyme disease, an increasingly common infectious disease, is caused by infection with the tick-borne spirochete *Borrelia burgdorferi*. Currently, no test is available to diagnose acute Lyme disease. Innate and acquired immune-mediated responses are important for eradicating the spirochete (Figure 1). Innate immune responses, especially complement, could serve

as a marker of illness in patients seen shortly after a tick bite.

Rationale for Use of Complement Split Product Assays to Aid in Diagnosis of Lyme Disease

- Plasma C3a and C4a rise rapidly after activation

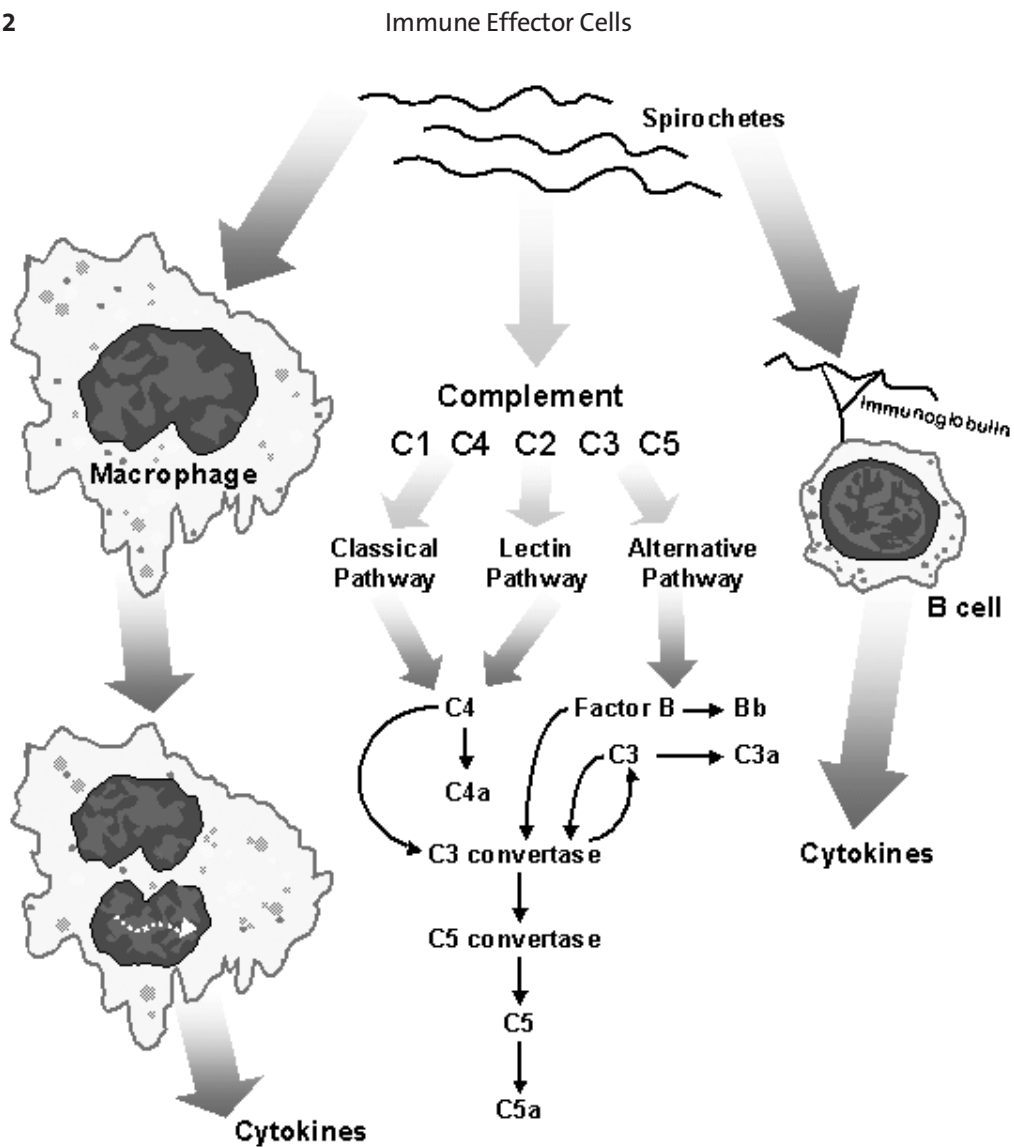


Figure 1. Innate immune response to *Borrelia burgdorferi*. *B. burgdorferi* spirochetes activate complement in the plasma and generate C4a by the classical and lectin pathways. The alternative pathway is activated, possibly by spirochete membrane lipopolysaccharides, and generates the split products Bb and C3a from factor B and C3. C5a is also produced by cleavage of C5. Macrophages and B- and T-lymphocytes are activated and release proinflammatory cytokines.

- Elevated levels of C4a:
 - fall with successful treatment
 - are maintained absent treatment
 - are short-lived; ongoing high levels may reflect ongoing stimulus for production
- Relevance of C3a
 - Chemotactic for eosinophils
 - Smooth muscle constriction
 - Releases proinflammatory compounds from WBCs: oxidants, leukotrienes, enzymes
 - Specific receptor (C3aR) on many cell types, including smooth muscle, adipocytes, endothelial cells in lung, brain, liver, kidney, some T-cells
 - Amplification loop from alternative pathway generates large amounts C3a

Relevance of C4a

- Generated by cleavage of C4
- Formed by activation of classical pathway or lectin pathway, but NOT alternative pathway
- Weak anaphylatoxin
- *Purpose*

In this case-control study we investigated the utility of C3a and C4a measurement for detection of acute Lyme disease.

Method

Patients

Thirty-one consecutive patients with acute Lyme disease, 14 with and 17 without an erythema chronicum migrans (ECM) skin rash seen by a physician within 96 h of a tick bite, were matched with 20 consecutive tick-bite patients (2–4 days after bite) without Lyme disease symptoms or ECM and 37 apparently healthy patients undergoing routine physical examinations. Individuals with any of the following were excluded from the study: antibiotic usage at time of bite; previous tick bite or Lyme disease in the past 30 days; presence of other inflammatory conditions; previously elevated C3a or C4a; or diagnosed lupus or pancreatitis. This study was approved by an institutional review board and all participants provided informed written consent.

Laboratory Methods

Factor B, C4, and C3 complement proteins were determined at Quest Diagnostics by nephelometry using specific anti-sera. Immune complexes binding C1q (Binding site, San Diego, CA) and containing C3d (IBL-Diagnostics, Hamburg, Germany) were tested with ELISA kits. C2 protein was determined by diffusion in antibody-impregnated agar gels. Levels of C3a des Arg (Quidel Labs, San Diego, CA) and C4a des Arg (Pharmingen BD, San Jose, CA) were determined with kits.

In Vitro Testing

Pure cultures of *B burgdorferi* and *B hermsii* were added to normal human serum. C3a, C4a, and split products of factor B and C5 were measured at 60 minutes.

Results

Levels of C2, C3, C4, and factor B did not differ significantly between patients with Lyme disease (with or without ECM) and control subjects (with or without tick bite); none of the subjects had decreased levels of these markers.

Patients with acute Lyme disease had significantly higher levels of C3a and C4a than did tick-bite and healthy control subjects (data not shown). All patients with acute Lyme disease had elevated levels of C3a (>368 ng/mL), C4a (>745 ng/mL), or both. Among ECM-positive patients with acute disease, 10 of 10 had increased C4a and 12 of 12 had increased C3a levels. Among ECM-negative patients with acute disease, 10 of 17 (59%) had elevated C3a and 13 of 15 (87%) had elevated C4a. None of the healthy controls and few of the tick-bite controls had elevated levels of C3a or C4a.

Addition of *B burgdorferi* or *B hermsii* to normal human serum led to *in vitro* activation of complement by both the classical and alternative pathways (data not shown).

Conclusions

- C3a and C4a levels were significantly higher in patients with acute Lyme disease than in tick-bite and healthy control subjects.
- Elevated C3a or C4a levels were present in all patients with acute Lyme disease and absent in almost all control subjects.
 - C3 and C4 are generally decreased in SLE and other immune complex diseases but were normal in all patients with Lyme disease.
- C3a and C4a measurements are the only relevant tests currently available for differentiating patients with acute Lyme disease

from individuals with tick-bite without Lyme disease.

- *B burgdorferi* and *B hermsii* activate both the classical and alternative complement pathways in normal human serum, consistent with our findings of complement activation in patients with Lyme disease.

References

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