Interferon-γ-induced protein 10 in Lyme disease

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Abstract

Lyme disease is an infectious disease caused by bacteria of the Borrelia type, that affects about 300,000 people a year in the USA and 65,000 people a year in Europe. Borrelia infection, and Lyme disease, following occupational exposure has been reported in USA, Europe and Asia.

The manifestations of Lyme disease include erythema migrans (EM), arthritis, neuroborreliosis (NB), and others.

Cytokines and chemokines primarily orchestrate leukocyte recruitment to the areas of Borrelia infection, and they are critical mediators of immune and inflammatory responses, in particular of the induction of interferon (IFN)-γ and IFN-γ dependent chemokines.

In EM high levels of T helper (Th) 1 cells chemoattrantants [monokine induced by IFN-γ (MIG), IFN-γ-induced protein 10 (IP-10), and IFN-inducible T cell alpha chemoattractant (I-TAC)] have been shown.

Synovial tissues and fluids of patients with Lyme Arthritis (LA) (overall with antibiotic-refractory LA) contained exceptionally high levels of Th1 chemoattractants and cytokines, particularly MIG and IFN-γ.

In NB concentrations of IP-10 and I-TAC in the cerebrospinal fluid (CSF) were significantly higher, suggesting that IP-10 and I-TAC create a chemokine gradient between the CSF and serum and recruit C-X-C chemokine receptor 3-expressing memory CD4+ T-cells into the CSF of these patients.

A positive association between the disseminating capacity of B. burgdorferi and early type 1 IFN induction has also been shown.

These results suggest that IFN-γ dependent chemokines are important biomarkers to monitor the progression and diffusion of the disease in patients with Borrelia infection; further larger studies are needed.


Key words: borrelia, interferon, IP-10, Lyme disease, occupational Lyme disease

State of the Art

Lyme disease

Lyme disease is an infectious disease caused by bacteria of the Borrelia type (1). Lyme disease is transmitted to humans by the bite of infected ticks. It is estimated to affect 300,000 people a year in the USA and 65,000 people a year in Europe (1, 2). Infections are most common in the spring and early summer (1). In North America, B. burgdorferi sensu stricto and B. mayonii are the main cause (1, 3); while in Europe and Asia, the bacteria B. afzelii and B. garinii (1). Approximately 25–50% of infected people do not develop a disease. The most common sign of infection is an expanding area of redness, known as erythema migrans (EM), that begins at the site of a tick bite about a week after it has occurred. Other early symptoms may include fever, headache and feeling tired. If untreated, symptoms may include loss of the ability to move one or both sides of the face, joint pains, severe headaches with neck stiffness, or heart palpitations, among others. Despite appropriate treatment, about 10 to 20% of people develop joint pains, memory problems, and feel tired for at least six months (4, 5).

Diagnosis is based upon a combination of symptoms, history of tick exposure, and possibly testing for specific antibodies in the blood (6, 7). Blood tests are often negative in the early stages of the disease (1).

Prevention includes efforts to prevent tick bites such as by wearing long pants and using N,N-diethyl-meta-toluamide (1). Using pesticides to reduce tick numbers may also be effective (1). Ticks can be removed using tweezers (8). If the removed tick was full of blood, a single dose of doxycycline may be used to prevent development of infection (1). If an infection develops, a number of antibiotics are effective, including doxycycline, amoxicillin, and cefuroxime, for 2-3 weeks (1).

Borrelia infection, and Lyme disease, following occupational exposure has been reported in USA, Europe and Asia, as it occurs for other occupational diseases (9-23).

Cytokines and chemokines primarily orchestrate leukocyte recruitment to the areas of Borrelia infection, and they are critical mediators of immune and inflammatory responses, in particular of the induction of Interferon (IFN)-γ and IFN-γ dependent chemokines.

The objective of this narrative review is to evaluate studies about Borrelia infection and cytokines, IFN, and IFN-γ dependent chemokines. The presentation of data has been reported according to the International Narrative Systematic Assessment (INSA) tool (24).

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**IP-10 in Lyme disease**

IP-10 is a chemokine potentially able to regulate inflammation at several levels. It can induce integrin activation and mediate chemotaxis of multiple cell types (for example, activated T cells, monocytes, and natural killer cells) (25). IP-10 can also induce apoptosis of pancreatic beta cells, inhibit the proliferation of both epithelial and endothelial cells (26, 27), induce molecules such as interleukin (IL)-8 and chemokine (C-X-C motif) ligand (CXCL)-5, and up-regulate costimulatory cell surface molecules (as CD54, CD80, and CD86) on monocytes.

The secretion of IP-10 depends on IFN-γ, that is itself mediated by the IL-12 cytokine family (28). Under the influence of cytokines, IP-10 is secreted by several cell types, like T lymphocytes, monocytes, splenocytes, fibroblasts, keratinocytes, thyocytes, preadipocytes, etc. The presence of high level of IP-10 in peripheral liquids is so a marker of host immune response, particularly T helper (Th)1 oriented T-cells.

Th1 lymphocytes that are recruited may be responsible for the enhancement of the IFN-γ and tumor necrosis factor (TNF)-α production; this enhanced production in turn stimulates IP-10 secretion from a variety of the above mentioned cells, thus creating an amplification feedback loop.

Serum IP-10 are increasing with age. Furthermore, recent reports have shown an increasing of serum and/or the tissue expressions of IP-10 in organ specific autoimmune diseases (29), such as type 1 diabetes (TID) (30-33), Graves’ disease (GD), or Graves’ ophthalmopathy (GO) (34-39), autoimmune thyroiditis (40-43), or systemic rheumatological disorders like rheumatoid arthritis (RA) (44), systemic sclerosis (SSc) (45-47), psoriasis or psoriatic arthritis (48-51), sarcoidosis (52), HCV-related cryoglobulinemia (53-55), other HCV immune mediated disorders (56-59), Lupus (60), and also in cancers (61-68).

**Borrelia infection, cytokines, and IFN-γ dependent chemokines**

A first study aimed to evaluate the contribution of chemokine (C-X-C motif) receptor (CXCR)3 and the corresponding ligands IP-10 and IFN-induced T cell alpha chemoattractant (I-TAC); to the recruitment of peripheral blood (PB) memory CD4+ T-cells into the cerebrospinal fluid (CSF) of patients with acute neuroborreliosis (NB). Percentages of memory CD45RO+CD4+ T-cells expressing CXCR3 were significantly enriched in the CSF compared to the PB. Concentrations of IP-10 and I-TAC in the CSF of NB patients were significantly higher. These results suggested that IP-10 and I-TAC create a chemokine gradient between the CSF and serum and recruit CXCR3-expressing memory CD4+ T-cells into the CSF of NB patients (69).

*B. burgdorferi*, the agent of Lyme disease, promotes proinflammatory changes in the endothelium that lead to the recruitment of leukocytes. The host immune response to infection results in increased levels of IFN-γ in the serum and lesions of Lyme disease patients that correlate with greater severity of disease. *B. burgdorferi* and IFN-γ synergistically augmented the expression of chemokines, that [chemokine (C-C motif) ligand (CCL)7, CCL8, chemokine (C-X3-C motif) ligand (CX3CL1), CXCL9/monokine induced by IFN-γ (MIG), IP-10, and I-TAC] attract T lymphocytes, or (CXCL2) neutrophils. IL-β, TNF-α, and lipopolysaccharides also cooperated with IFN-γ to induce synergistic production of IP-10 by the endothelium. IFN-γ thus alters gene expression by endothelia exposed to *B. burgdorferi* in a manner that promotes recruitment of T cells, facilitating the development of chronic inflammatory lesions in Lyme disease (70).

Another study aimed to investigate the possible role of chemokines and cytokines in the pathogenesis of Lyme Arthritis (LA) in 65 synovial fluid (SF) samples and 7 synovial tissue (ST) samples from 17 patients with antibiotic-responsive LA and 35 patients with antibiotic-refractory LA seen during the past 18 years (71). SF from patients with antibiotic-refractory arthritis contained exceptionally high levels of Th1 chemoattractants and cytokines, particularly MIG and IFN-γ. Compared with the patients whose arthritis was responsive to antibiotic treatment, those with antibiotic-refractory arthritis had significantly higher levels of MIG and IP-10, IFN-γ, TNF-α, IL-1β. During the post-antibiotic period, patients with antibiotic-refractory arthritis continued to exhibit high SF and ST levels of these chemokines and cytokines (71).

The above mentioned results were confirmed in C57BL/6 mice deficient in toll-like receptor (TLR) 2 (that develop more severe arthritis following infection with *B. burgdorferi* than wild-type C57BL/6 mice). Transcripts for the IFN-inducible T cell chemokines, MIG and IP-10, were greatly enhanced in joint tissue from TLR2(-/-) mice, as were transcripts for a prototypical IFN-inducible gene IFN-γ-activated GTPase (72).

The clinical pictures and chemokine and cytokine mRNA levels in lesional skin EM were compared in the 19 *B. burgdorferi*-infected USA patients and the 37 *B. afzelii*-infected Austrian patients. Those of *B. burgdorferi*-infected USA patients had significantly higher mRNA levels of chemokines associated with activation of macrophages, including chemoattractants for neutrophils (CXCL1), macrophages (CCL3 and CCL4), and Th1 cells (MIG, IP-10, and I-TAC), such as IL-1β and TNF-α (73).

In patients with LA, *B. burgdorferi* but not IFN-γ induced peripheral blood mononuclear cells (PBMCs) to secrete CCL4 and CCL2, and *B. burgdorferi* and IFN-γ each stimulated the production of MIG and IP-10. The percentage of T cells expressing CXC3 or C-C chemokine receptor type 5 was significantly greater in SF mononuclear cells (SFMC) than PBMC, confirming that Th1 effector cells were recruited to inflamed joints. These results suggested that *B. burgdorferi* stimulates monocytes/macrophages directly and indirectly to guide innate and adaptive immune responses in patients with LA (74).

To investigate the contribution of different Th associated cytokine/chemokine responses, the levels of IP-10 (Th1 marker), CCL2 (Th2 marker), IL-17 (Th17 marker) and CXCL8 (general inflammation marker), in serum and in CSF from untreated patients with confirmed NB (n = 133), and non-NB patients (n = 96) were measured. Patients with confirmed NB and possible early NB had significantly higher CSF levels of IL-17, IP-10, CCL2 and CXCL8 compared to both the non-NB group and the control group. These results...
support the notion that early NB is dominated by a Th1-type response, eventually accompanied by a Th2 response (75).

It was also shown the B. burgdorferi ribosomal RNA intergenic spacer type (RST) 1 (OspC type A) genotype, followed by the RST3 (OspC type 1) genotype, causes greater inflammation and more severe disease. In fact, in PBMCs, RST1 strains again stimulated significantly higher levels of IFN, IFN-α, IFN-γ, and IP-10. These results suggested a link between spirochetal virulence and host inflammation (76).

Single-nucleotide polymorphisms (SNPs) in several genes encoding TLR1 (1805GG), TLR2 (2258GA), and TLR5 (1174CT) were evaluated in patients with Lyme disease with EM, joint fluid of patients with LA, and supernatants of B. burgdorferi-stimulated PBMCs from patients with LA. The frequency of the TLR1-1805GG polymorphism was greater in patients with antibiotic-refractory arthritis compared with patients with EM or those with antibiotic-responsive arthritis. Those infected with B. burgdorferi 16S-23S RST1 strains, had higher serum levels of IFN-γ, MIG, and IP-10 and had more severe infection than EM patients carrying the 1805TG/TT polymorphism. These inflammatory responses were amplified in patients with LA, and the highest responses were observed in patients with 1805GG in the antibiotic-refractory group. These results suggested the TLR1-1805GG polymorphism in B. burgdorferi RST1-infected patients was associated with stronger Th1-like inflammatory responses (77).

It was also shown that in LA the infiltration of inflammatory cells, mainly neutrophils (polymorphonuclear cells [PMNs]) and T cells, into the joints, is associated with accumulation of neutrophil-activating protein A (NapA), IFN-γ, IL-17, CCL2, CCL20, and IP-10 in SF from patients with LA (78).

A further study evaluated the levels of 23 cytokines and chemokines, representative of innate and adaptive immune responses, that were assessed in sera from 86 antibiotic-treated European patients with EM, 45 with post-Lyme symptoms and 41 without symptoms, who were evaluated prior to treatment and 2, 6, and 12 months thereafter. High Th1-associated responses (MIG and IP-10) correlated with more effective immune-mediated spirochetal killing, whereas high Th17-associated immune responses, often accompanied by autoantibodies, correlated with post-Lyme symptoms (79).

Lyme borreli genotypes differ in their capacity to cause disseminated disease. B. burgdorferi B515, a clinical isolate that causes disseminated infection in mice induces a significant increase of IFN-β, IP-10, and signal transducer and activator of transcription 2 (Stat2). These results establish a positive association between the disseminating capacity of B. burgdorferi and early type I IFN induction in a murine model of Lyme disease (80).

Discussion

Lyme disease is an infectious disease caused by bacteria of the Borrelia type, affecting about 300,000 people a year in the USA and 65,000 people a year in Europe. Borrelia infection, and Lyme disease, following occupational exposure has been frequently reported in USA, Europe and Asia. The manifestations of Lyme disease include EM, arthritis, NB, and others.

Cytokines and chemokines mainly orchestrate leukocyte recruitment to the areas of Borrelia infection, and are considered critical mediators of immune and inflammatory responses, particularly of the induction of IFN-γ and IFN-γ dependent chemokines.

In EM high levels of Th1 cells chemoattractants (MIG, IP-10, and I-TAC) have been shown.

ST and fluids of patients with LA (in particular with antibiotic-refractory LA) showed extremely high levels of Th1 chemoattractants and cytokines, particularly MIG and IFN-γ.

B. burgdorferi induced the expression chemokines (MIG, IP-10, and I-TAC) that attract T lymphocytes in endothelial cells.

IP-10 and I-TAC levels in the CSF were significantly higher in NB, suggesting that IP-10 and I-TAC are able to create a chemokine gradient between the CSF and serum and recruit CXCR3-expressing memory CD4+ T-cells into the CSF of these patients.

A positive association between the disseminating capacity of B. burgdorferi and early type I IFN induction has also been reported.

Conclusion

These results suggest that IFN-γ dependent chemokines are key biomarkers to monitor the progression and diffusion of the disease in patients with Borrelia infection; further larger studies are needed.

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