ENHANCED INTRATHECAL NEOPTERIN PRODUCTION AND TRYPTOPHAN DEGRADATION IN PATIENTS WITH LYME NEUROBORRELIOSIS

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ABSTRACT

Various immune system compartments respond to *Borrelia burgdorferi* infection. TH2-type and TH1-type immune responses seem to be important. It recently was found that surface proteins of *Borrelia burgdorferi* induce the formation of interferon-γ *in vitro*.

Upon stimulation with interferon-γ, monocytes/macrophages release large amounts of neopterin and degradation of tryptophan is induced in a variety of cells. In humans increased neopterin concentrations and tryptophan degradation in body fluids indirectly indicate the endogenous formation of interferon-γ and TH1-type immune response.

Using serum and cerebrospinal fluid from patients with Lyme neuroborreliosis and with late Lyme encephalopathy, we investigated neopterin concentrations by radioimmunoassay and tryptophan and kynurenine by HPLC.

Increased neopterin and kynurenine concentrations were found in cerebrospinal fluid but not in the serum of patients with Lyme neuroborreliosis, whereas tryptophan was decreased in cerebrospinal fluid. Abnormalities were related to the activity of the disease, during antibiotic treatment concentrations of neopterin and kynurenine tended to normalize. In patients with late Lyme encephalopathy the deviations of the analytes'concentrations from normal were only marginal compared to patients with acute Lyme neuroborreliosis.

Increased formation of neopterin and degradation of tryptophan suggest a pathogenetic role of intrathecally released interferon-γ and activated monocytic cells in patients with Lyme neuroborreliosis.

KEY WORDS

Borrelia burgdorferi infection, Lyme neuroborreliosis, neopterin, tryptophan, kynurenine, interferon-γ

INTRODUCTION

Infections with common bacteria lead to a cascade of immunological reactions. The front line of immune reaction to bacteria includes the immunological processing of pathogens by monocytes, the exchange of signals between monocytes and lymphocytes and the formation of antibodies by activated B lymphocytes. The cytokines primarily involved in this so-called TH2-type immune response comprise mainly interleukins-4, -5 and -10. The situation is even more complex concerning intracellular bacteria or viruses.

In this case the type of immune response is more on the side of the T-cell/macrophage axis and TH1-type cytokines, interferon-γ and interleukin-2, play a more important role. In the end, certain antigens or toxins of preferentially Gram-negative bacteria are able to directly activate T-lymphocytes for cytokine production; this kind of immune reaction mainly involves TH1-type cytokines such as interferon-γ. There is a cross-regulation of TH1-type and TH2-type immune responses (1). Cytokines involved in TH1- or TH2-type immune response down-regulate each other.

Various immune system compartments respond to *Borrelia burgdorferi* (*Bb*) infection. TH2-type immune response is easily documented in patients by determining the antibody seroconversion after the acute infection (2). Apart from TH2-type immunity, TH1-type immune response especially seems to play a major role in the later course of infection when the primary immune response has been unable to clear the organism from the invading spirochetes. Moreover, it was discovered recently that *Bb* itself is able to induce formation of interferon-γ (3,4) and it seems that interferon-γ could play a role in the pathogenesis of Lyme neuroborreliosis (LNB). However, only little is known about the interferon-γ system in *Bb* infection. This is partly due to the fact that direct measurement of interferon-γ concentrations is complicated because of the short biological half-life of this cytokine *in vivo* and the test systems generally applied also don't appear to be sensitive enough for studies of patients (5).

Upon stimulation with interferon-γ, human monocytes/macrophages release large amounts of neopterin (6) and in parallel degradation of tryptophan is induced (Fig. 1) in a variety of cells (7,8). Increased neopterin concentrations and tryptophan degradation - estimated from tryptophan and kynurenine measurements - in body fluids in patients indirectly indicate endogenous formation of interferon-γ and TH1-type immune response. Therefore, monitoring neopterin concentrations turned out to be useful for follow-up and for predicting the further clinical course in patients with infectious diseases including human immunodeficiency virus (HIV) infection, in autoimmune diseases and various types of malignancies (6,9).

In this study we compared the results of three earlier investigations (10-12) in which we measured concentrations of neopterin, tryptophan and kynurenine in serum and cerebrospinal fluid (CSF) in patients with LNB and late Lyme encephalopathy.

METHODS

Our studies comprised 18 patients (11 females, 7 males, age: 28 - 70 years) with LNB and 10 patients (5 females, 5 males, age: 29 - 66 years) with late Lyme encephalopathy. The diagnosis LNB was defined as a) neurologic symptoms compatible with neuroborreliosis and a positive antibody test, and signs of intrathecal antibody production in CSF, b) neurologic signs and symptoms within three months after onset of erythema migrans, c) bilateral facial palsy in a child with CSF pleocytosis and no other known disease. In serum and CSF of patients neopterin concentrations were measured by radioimmunoassay (BRAHMS-Henning, Berlin, Germany). In addition, tryptophan and kynurenine concentrations were measured by high pressure liquid chromatography (HPLC) on reversed phase C18 material (Chromosorb, Merck, Darmstadt, Germany) as previously described (12). Compounds were quantified using fluorescence measurements in the case of tryptophan (285 nm excitation wavelength and 360 nm emission) and by detection of UV absorption in the case of kynurenine (360 nm wavelength; detection limit: < 0.1 µmol/l). The concentrations found in our patients were compared to previously established normal ranges obtained by implementing the same methodology.

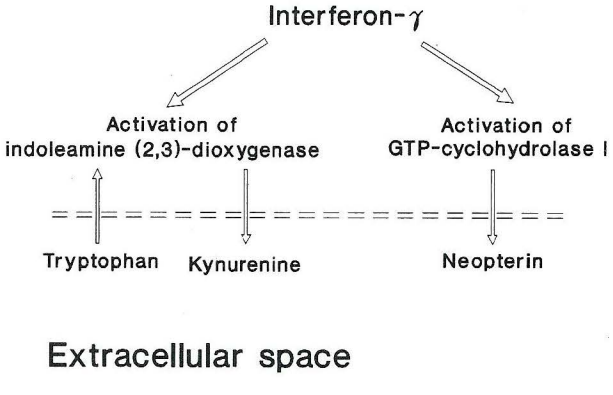

Fig. 1. Interferon-γ activates guanosine triphosphate-(GTP)-cyclohydrolase I in human monocytes/macrophages which leads to the formation of large amounts of neopterin. At the same time it induces indoleamine (2,3)-dioxygenase in a variety of cells which degrades tryptophan via the kynurenine pathway, with kynurenine being one of its first products.

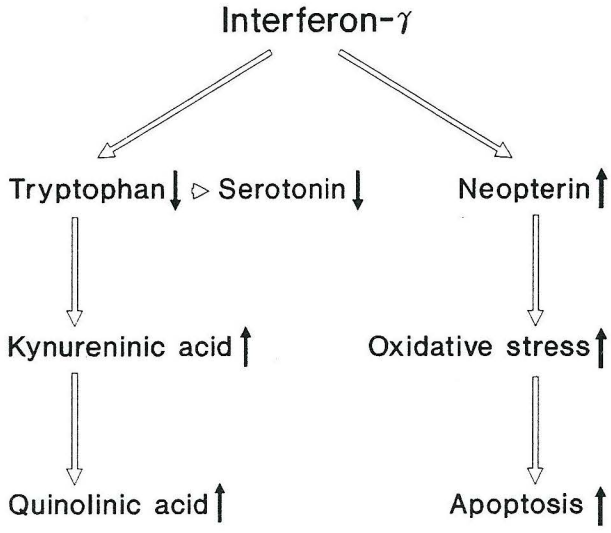

Fig. 2. Interferon-γ mediated neopterin formation and tryptophan degradation could insult neuroendocrine tissue. On the one hand, degradation of tryptophan may reduce its availability for the biosynthesis of serotonin (left part); on the other hand, it may lead to the accumulation of degradation products, some of which like kynurenic acid and quinolinic acid, are potent neurotoxins interfering with the NMDA receptor. Increased concentrations of neopterin derivatives are able to enhance oxygen free-radical mediated processes such as apoptosis (right part).

Matched serum and CSF specimens were collected, samples were kept frozen at -20°C until measurements were performed.

Statistical evaluations of the data included the non-parametric Wilcoxon test for comparisons of grouped data and the Spearmen rank correlation analyses.

RESULTS

Increased concentrations of CSF neopterin (> 6 nmol/l) were found in 16/18 patients with early or progressive LNB (mean ± S.D.: 31.8 ± 45.4 nmol/l; controls: 3.2 ± 1.88) and neopterin levels correlated to the cell counts (r_s = 0.775, $p < 0.001$) and protein content (r_s = 0.812, $p < 0.001$) of the CSF (10,12). A subgroup of patients (67%) with LNB had detectable levels of CSF kynurenine (0.1 - 2.0 µmol/l) and there was a positive correlation between the neopterin and kynurenine levels (r_s = 0.791, $p < 0.001$) (11,12). Preferentially those patients who presented with relatively low CSF neopterin concentrations had undetectable levels of CSF kynurenine similar to controls. CSF tryptophan was below 0.5 µmol/l in 3/18 patients, who showed the highest neopterin and kynurenine levels (11,12). The patient with the highest kynurenine (2.0 µmol/l) and neopterin (183 nmol/l) concentrations in their CSF had undetectable levels of tryptophan (< 0.2 nmol/l). Abnormalities were related to the activity of the disease and concentrations of neopterin tended to normalize during antibiotic treatment (10). There were only minor changes in the concentrations of analytes seen in the serum specimens (neopterin: 7.8 ± 3.48 nmol/l) and only 3/18 patients had increased neopterin compared to healthy controls (10-12). Serum tryptophan tended to decrease and kynurenine to increase compared to controls (11,12). However, the absolute changes are much lower than those observed intrathecally.

In patients with late Lyme encephalopathy the deviations of the analytes' concentrations in their CSF from normal were only marginal compared to patients with acute LNB (12). However, the changes took the same direction as was observed in patients with LNB: 1/9 patients had increased CSF neopterin and 2/9 patients had detectable levels of kynurenine in their CSF. It appeared that the serum concentrations of tryptophan decreased over the duration of the disease. In fact, the mean serum tryptophan was decreased (57.9 ± 8.8 µmol/l) compared to the healthy controls (89.2 ± 15.6 µmol/l) (12).

DISCUSSION

In our study we found increased levels of neopterin and kynurenine and decreased tryptophan concentrations in the CSF of patients with LNB. The correlation found between increased neopterin and tryptophan metabolic changes suggests that the decrease in tryptophan is due to degradation rather than a reduced dietary intake of this essential amino acid. The increased formation of neopterin and degradation of tryptophan was located inside the blood-brain-barrier as only minor changes could be observed in the serum of patients. In addition, these results were obtained almost exclusively in patients with LNB but not in patients with late Lyme encephalopathy.

The intrathecal formation of neopterin and degradation of tryptophan demonstrate the TH1-type immune response taking place in patients (Fig. 1) and suggest a pathogenetic role of intrathecally released interferon-γ and activated monocytic cells

in patients with LNB. It seems plausible that interferon-γ is formed in response to certain spirochetes' surface proteins which are known to stimulate the release of this cytokine *in vitro* (2,3). Alternatively, it could also result from a cell-mediated immune response to infected cells. It is also a fact that during other cerebral infections such as toxoplasmosis, measles or HIV infection (13-15) large amounts of neopterin are produced inside the blood-brain-barrier. Thus, an immune response is established locally and monocytic cells like microglia or astrocytes or monocytes/macrophages, which have permeated the blood-brain-barrier, are involved in the formation of increased neopterin. Interestingly a correlation was found between CSF cell counts and neopterin levels (10). However, in other situations like HIV infection, equally high neopterin levels have been found despite of low CSF cell counts (15).

Enhanced formation of neopterin and tryptophan degradation could be involved in the pathogenesis of symptoms in LNB (Fig. 2). Neopterin derivatives have been described to potentially interact with reactive oxygen intermediates released by stimulated immunocompetent cells. In fact, neopterin was recently found to enhance the effects of hydrogen peroxide *in vitro* (16) and one role of neopterin derivatives was found to superinduce apoptosis triggered by tumor necrosis factor-a (17). Thus, loss of neuroendocrine tissue during cerebral *Bb* infection could be enhanced by the overwhelming and continuous production of neopterin derivatives within intrathecal TH1-type immune response (18). Oxidative stress due to chronic activation of immunocompetent cells may be enhanced by the presence of large amounts of neopterin derivatives at local sites (19).

Also tryptophan metabolic changes could play a role in precipitating symptoms in patients with LNB. Degradation of tryptophan leads to the accumulation of toxic products like kynurenic acid or quinolinic acid (18,20) inside the brain and, thus, may interfere with NMDA receptor mediated signaling pathways. Moreover, tryptophan is the precursor molecule of 5-hydroxy-tryptamine (serotonin), an important neurotransmitter (Fig. 2). The availability of tryptophan is crucial for the biosynthesis of serotonin. Thus, decreased tryptophan may limit serotonin production which is especially important for neuroendocrine cells (8).

It should be mentioned that abnormalities of neopterin and tryptophan concentrations have been observed almost exclusively in patients with LNB but not in those with late Lyme encephalopathy. This data favours the view that neurological sequelae are merely caused by the acute infectious process and there is no or only a very limited contribution from a chronic ongoing infection at the time of the sample collection

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