The purpose of this study was to evaluate the potential role of LTB4 and cysteinyl leukotrienes in Lyme disease (LD). Therefore, a total number of 34 patients divided into four groups was studied. The patients were classified as having Lyme arthritis ($n = 7$) or Lyme meningitis ($n = 10$), and as control groups patients with a non-inflammatory arthropathy (NIA) ($n = 7$) and healthy subjects ($n = 10$). LTB4 as well as LTC4 secretion from stimulated polymorphonuclear leukocytes (PMNL) from all groups of patients showed no statistical differences. LTB4 levels in synovial fluid were significantly increased in patients with Lyme arthritis (median 142 ng/ml, range 88–296) when compared to the control subjects with NIA (median 46 ng/ml, range 28–72) ($p < 0.05$). No statistical difference of urinary LTE4 levels between all the different groups of patients was observed. These results show that cysteinyl leukotrienes do not play an important role in the pathogenesis of LD. In contrast to previous findings in rheumatoid arthritis, LTB4 production from stimulated PMNL was not found to be increased in LD. However, the significantly elevated levels of LTB4 in synovial fluid of patients with Lyme arthritis underline the involvement of LTB4 in the pathogenesis of this disease.

Key words: Arthritis, Cysteinyl leukotrienes, LTB4, Lyme disease

Introduction

Lyme disease (LD) is a multisystem infection caused by the spirochete *Borrelia burgdorferi* which is transmitted by *Ixodes* ticks.1-3 The disease is associated with a variety of clinical manifestations which include erythema migrans, lymphocytic meningitis, motor or sensory radiculitis and, particularly in later stages, involvement of the joints in the form of asymmetrical mono- or oligoarthritis.4,5

Cytokines, such as interleukin-1, interleukin-6 or tumour necrosis factor, have been implicated in the pathophysiology of LD.6,7 However, the potential role of other mediators of inflammation such as leukotrienes in LD has not received a great deal of attention. Leukotriene B4 (LTB4), one of the most powerful chemotactic and chemokinetic agents,8 has been found to exert strong leukoerytrotropic activities and can cause neutrophil degranulation.9 It is readily synthesized by phagocytic cells, principally neutrophils10 and macrophages11 on challenge with a variety of stimuli. The cysteinyl leukotrienes (LTC4, LTD4, LTE4), however, increase microvascular permeability, induce symptoms of smooth muscle contraction and cause oedema.12-15

Increased LTB4 production by stimulated polymorphonuclear leukocytes (PMNL) from patients with rheumatoid arthritis as well as elevated LTB4 in synovial tissues in rheumatoid arthritis and spondyloarthritis has already been demonstrated.16-18 Elevated urinary LTE4 levels have been reported in patients with active systemic lupus erythematosus, a connective tissue disease, characterized by marked immunological abnormalities leading to inflammation and tissue injury.19 These data also suggest an involvement of leukotrienes in human inflammatory disease such as LD.

The aim of this study was therefore to evaluate the potential role of LTB4 and cysteinyl leukotrienes in patients with Lyme arthritis and Lyme meningitis in comparison to patients with noninflammatory arthropathy and healthy subjects. LTB4 as well as LTC4 production was quantified in stimulated PMNL; moreover, LTB4 levels were determined in synovial fluid and additionally LTE4 was measured in the urine of all patients.

Materials and Methods

Patients: A total of 34 patients was entered into this study after informed consent was obtained.
The patients were classified as having either (i) meningitis due to an infection with *B. burgdorferi* with a characteristic immunoblot pattern and intrathecally produced antibodies (*n* = 10); (ii) mono- or oligoarthritis serologically positive to *B. burgdorferi* by characteristic immunoblot pattern (*n* = 7); or (iii) patients with a noninflammatory arthropathy (NIA) such as degenerative or traumatic joint disease (*n* = 7) as a control group to Lyme arthritis; and (iv) a healthy control group (*n* = 10). None of the patients received any medication before the study and all had a normal liver and renal function. In all patients other bacterial, viral or rheumatological diseases could be excluded.

**Serological assays for diagnosis of Lyme disease:** The indirect immunofluorescence assay, quantitative enzyme immunoassay (EIA) and immunoblot were performed as described previously. Cut-off titres were 256 (IgG) or 32 (IgM), respectively, for IFA and 200 standard units (IgG) for EIA. CSF antibody concentrations were measured by means of the quantitative EIA using a cut-off value of 2.0 standard units. Diagnosis of LD was established in each patient by characteristic immunoblot according to the criteria described previously.

**Quantification of LTB₄ and LTC₄ in stimulated PMNL:** Heparinized venous blood was obtained from all patients studied. After removal of mononuclear cells by Ficoll–Hypaque density gradient centrifugation, the neutrophil-rich pellet was sedimented by dextran. Residual erythrocytes were lysed by hypotonic saline (0.45%). Purity and viability of the neutrophil suspension as assessed by Trypan blue exclusion was consistently more than 95%. Activation of isolated PMNL with calcium ionophore A23187 (10 μM; Sigma Chemical Co., St Louis, MO) and 0.5 mM Heparinized venous blood was aspirated in all patients with Lyme arthritis and noninflammatory arthropathy as part of diagnostic procedures from knee joints and centrifuged at 2000 × g for 10 min to remove cells and particulate material; the supernatant fluid was stored at −80°C until LTB₄ was extracted. After ⁢²H-labelled LTB₄ was added to each 2–3 ml sample of synovial fluid, the samples were titrated to pH 4.0 with 2 M citric acid. Each sample was extracted three times with 4 ml of chloroform:methanol (2:1 vol/vol), the organic phases were pooled and dried under nitrogen. LTB₄ was resolved and purified by Sep-Pak C18 extraction and RP-HPLC as described above.

**Urine LTE₄ analysis:** Urine was obtained from spontaneous micturition and mixed with two volumes of 90% aqueous methanol of pH 8.5 containing 0.5 mM EDTA, 1 mM HTMP, and 20 mM KHCO₃, and stored at −80°C until later use. Aliquots of each urine sample were screened to exclude the presence of pathological constituents. Urinary LTE₄ was measured essentially as described elsewhere. Urine samples were tested in duplicate. Samples were allowed to thaw immediately before the assay. ⁢²H-labelled LTE₄ (Du Pont–New England Nuclear, Boston, MA) was added as an internal standard. Samples were then acidified to pH 4.5 by addition of 0.1 M HCl, homogenized, and pumped through activated Sep-Pak cartridges as already described. Fractions having the same elution time as the synthetic LTE₄ were separated by RP-HPLC as described above for LTC₄. The immunoreactive LTE₄ content was determined by EIA (Cayman, Ann Arbor, MA).

**Statistical analysis:** The Wilcoxon–Mann–Whitney test for the one-sided problem was used for statistical comparison between the different groups of patients. Differences were considered significant when *p* was less than 0.05.

**Results**

LTB₄ and LTC₄ secretion from stimulated PMNL: LTB₄ as well as LTC₄ secretion from stimulated PMNL from all groups of patients is shown in...
Table 1. LTB4 and LTC4 production from stimulated polymorphonuclear leukocytes from patients with Lyme arthritis, noninflammatory arthropathy (NIA), Lyme meningitis, and healthy subjects. Values are given as the median with the range in brackets.

<table>
<thead>
<tr>
<th>Leukotriene</th>
<th>Lyme arthritis (n = 7)</th>
<th>NIA (n = 7)</th>
<th>Lyme meningitis (n = 10)</th>
<th>Healthy subjects (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTB4 (ng per 10^6 cells)</td>
<td>40.0 (34.2-47.1)</td>
<td>38.4 (33.4-44.8)</td>
<td>39.3 (32.0-45.9)</td>
<td>36.2 (33.4-44.6)</td>
</tr>
<tr>
<td>LTC4 (ng per 10^6 cells)</td>
<td>4.8 (4.1-5.6)</td>
<td>4.6 (4.0-5.3)</td>
<td>5.3 (4.4-5.8)</td>
<td>5.0 (3.9-5.5)</td>
</tr>
</tbody>
</table>

Table 1. No statistical difference of LTB4 as well as LTC4 production between all groups of patients was observed.

**LTB4 in synovial fluid:** LTB4 was detected in all synovial fluid samples tested. LTB4 levels in synovial fluid were significantly increased in patients with Lyme arthritis (median 142 ng/ml, range 88-296) when compared to the control subjects with NIA (median 46 ng/ml, range 28-72) (p < 0.05) (Fig. 1). Although the total white blood cell counts in synovial fluid were higher in the patients with Lyme arthritis than in NIA, no significant correlation was found between the white blood cell count and the LTB4 levels in synovial fluid of patients with Lyme arthritis (r = 0.14) or with NIA (r = 0.15).

**Discussion**

The present data indicate that the synovial levels of the lipoxygenase product LTB4 is elevated in Lyme arthritis compared with the levels in synovia of subjects with NIA (Fig. 1). Similar results of increased synovial LTB4 levels have been reported only in rheumatoid arthritis.17,18 LTB4 is chemotactic for neutrophils and eosinophils in vitro at a concentration as low as 3 ng/ml and evokes a maximal chemotactic response at 30 ng/ml.25 Thus, the concentration of LTB4 in synovial fluid may be sufficient to contribute to the local inflammatory reaction. Because the involvement of LTB4 in several T-cell activation stages, namely proliferation, induction of helper and suppressor function and production of interleukin-2, interferon-Î³ and interleukin-1,27 LTB4 might contribute to the pathogenesis of Lyme arthritis.

In contrast to synovial fluid and previous findings of elevated LTB4 production from PMNL in rheumatoid arthritis,16 the LTB4 secretion from stimulated PMNL was detected at similar levels in patients with Lyme arthritis, NIA, Lyme meningitis, and healthy controls (Table 1). This demonstrates that peripheral blood leukocytes do not produce increased quantities of LTB4 in LD. These results suggest that local but not systemic

Table 2. LTE4 in urine of patients with arthritis or meningitis due to an infection with *B. burgdorferi* (Lyme disease), noninflammatory arthropathy (NIA) and healthy subjects. Values are given as the median with the range in brackets.

<table>
<thead>
<tr>
<th>LTE4 (concentration)</th>
<th>Lyme arthritis (n = 7)</th>
<th>NIA (n = 7)</th>
<th>Lyme meningitis (n = 10)</th>
<th>Healthy subjects (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol/l</td>
<td>279 (89-476)</td>
<td>262 (72-435)</td>
<td>273 (95-465)</td>
<td>266 (70-430)</td>
</tr>
<tr>
<td>nmol/mol creatinine</td>
<td>25 (18-62)</td>
<td>20 (15-54)</td>
<td>23 (16-56)</td>
<td>19 (11-48)</td>
</tr>
</tbody>
</table>
production of LTB₄ could account for symptoms of arthritis in late LD.

Although isolation of B. burgdorferi is very difficult in Lyme arthritis it has been achieved in some cases from synovial fluid years after onset.²⁸ Up to now, it is not clear whether autoreactivity as well as increased synovial LTB₄ is an epiphenomenon of persistent infection or is an important factor in tissue damage. The mechanisms underlying this process are a prospect for future research. An exact knowledge of the role of LTB₄ in the pathogenic contribution in Lyme arthritis may yield new therapeutic approaches, e.g. in the (local) application of selective inhibitors or antagonists.

The results also show that cysteinyl leukotrienes are not enhanced when generated in LD. LTC₄ production from PMNL in patients with LD did not differ from those measured in NIA and healthy subjects (Table 1). Urinary LTE₄ has been proposed and used as the index metabolite for the systemic generation of cysteinyl leukotrienes in humans.²⁹⁻³¹ Urine LTE₄ levels in patients with Lyme arthritis or Lyme meningitis, however, were not significantly different from patients with NIA or healthy controls (Table 2). Recent data indicate that increased synthesis of leukotrienes as measured by a rise in urinary LTE₄ levels is associated with active systemic lupus erythematosus and scleroderma and suggest that cysteinyl leukotrienes may mediate certain symptoms associated with these diseases.²⁹ It seems possible that elevated LTE₄ levels were not seen in our patients with Lyme arthritis due to the more localized nature of the inflammatory process. As in rheumatoid arthritis where LTE₄ levels were also found to be normal,²⁹ most inflammation is localized to the synovium.

In summary, the results show that cysteinyl leukotrienes do not play an important role in the pathogenesis of LD. LTB₄ production from stimulated PMNL was not found to be increased in LD. However, the significantly elevated levels of LTB₄ in synovial fluid in patients with Lyme arthritis underline the involvement of LTB₄ in the pathogenesis of this disease.

References


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