RELATIONSHIP BETWEEN LEUKOTRIENE B₄ AND IMMUNOLOGICAL PARAMETERS IN RHEUMATOID SYNOVIAL FLUIDS

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Abstract—Leukotriene B₄ (LTB₄) was measured in synovial fluid from 20 patients with rheumatoid arthritis and 15 patients with osteoarthritis. The level of LTB₄ was significantly higher in synovial fluid from rheumatoid arthritis patients as compared with synovial fluid from osteoarthritis patients. LTB₄ levels also significantly correlated with cell numbers, rheumatoid factor, and immune complexes in synovial fluid from rheumatoid arthritis patients. There was an inverse correlation between LTB₄ levels and complement components. The high-pressure liquid chromatography peak of immunoreactivity extracted from the synovial fluid occurred at a retention volume identical to that of authentic LTB₄. These results suggest that the increased level of this mediator in synovial fluid may contribute to perpetuation of inflammation and tissue destruction in rheumatoid arthritis.

INTRODUCTION

Joint inflammation in rheumatoid arthritis (RA) is characterized by the presence of macrophages and lymphocytes, hyperproliferation of synovial lining cells, and an increased accumulation of synovial fluid (SF) that contains a number of inflammatory cells and mediators. All of these may lead to the eventual joint destruction that is characteristic of the disease (1, 2). There has been much speculation about the role of immune mediators in the pathogenesis of RA. Leukotriene B₄ (LTB₄) appears to have an inflammatory role in various inflammatory processes. Its major effects are to enhance the ability of inflammatory leukocytes to penetrate the vascular endothelium (3), to stimulate their move-
ment towards inflammatory sites (4), and to increase the production of lysosomal enzymes (5). These activities suggest that LTB₄ could be involved in the inflammation and destruction occurring in rheumatoid joints. Analysis of the constituents of immune complexes (IC) detected in the early stages of RA could indicate the nature of the initial trigger for this disease. However, an immune response to the putative trigger and the onset of autoimmunity probably occur very early in most RA patients, either before or at the first evidence of clinical symptoms (6). It has been suggested that prostaglandin E₂ (PGE₂) and other products of arachidonic acid metabolism modulate some characteristic symptoms of inflammation (edema, vasodilation, and hyperalgesia) by amplifying the actions of mediators such as bradykinin and 5-hydroxytryptamine (7). Thus it is possible that LTB₄ regulates the production of mediators involved in inflammation and tissue damage.

The present study was undertaken: (1) to measure the levels of LTB₄ in SF from RA in comparison with SF from osteoarthritis (OA) patients, and (2) to determine the clinical significance of this mediator with immunological parameters found in RA.

**MATERIALS AND METHODS**

**Synovial Fluid.** SF samples were collected from the knee joints of 20 patients with RA (13 female, seven male; mean age 57, range 36–77 years) and 15 patients with OA (10 female, five male; mean age 55, range 44–71 years). All patients satisfied the ARA criteria for classical or definite RA (8), and none of the patients had received treatment with gold compounds, D-penicillamine, or steroids within the preceding six weeks. Samples were collected in plastic tubes and centrifuged at 1800g for 5 min at 5°C. The cell-free supernatant of SF was stored at -70°C until used.

**Measurement of Leukotriene B₄.** LTB₄ was separated and characterized from the supernatant by passage through an octadecylsilyl silica column (Walter Associates, Inc., Massachusetts) followed by reverse-phase high-pressure liquid chromatography (HPLC), and was assayed physicochemically by measurement of ultraviolet absorbance at 260, 270 and 281 nm (9) (Figure 1).

**Measurement of Rheumatoid Factor, Immune Complexes, and Complements.** The rheumatoid factor (RF) was measured by laser nephelometry, and IC were measured by solid phase C1q binding assay (10). Total hemolytic complement (CH50) and alternative pathway hemolytic complement (AH50) were measured by hemolysis of sheep erythrocytes and rabbit erythrocytes, respectively (11, 12). C₃, C₄, and C₅ complement proteins were measured by radial immunodiffusion using agarose plates (Hoechst).

**Disease Activity.** RA patients were clinically assessed for disease activity and a clinical score was recorded as the total number of swollen joints, duration of morning stiffness, fatigue, strength of hand grip, and erythrocyte sedimentation rate (ESR). The methods for measurement and scoring have been described in detail elsewhere (13). Routine measurement of ESR and C-reactive protein (CRP) were performed as part of the assessment in the research clinic.