Leukotriene $B_4$, a mediator of inflammation present in synovial fluid in rheumatoid arthritis

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SUMMARY Leukotriene $B_4$ (LTB$_4$), generated from arachidonic acid following lipoxygenase activity by a variety of inflammatory leucocytes, has been shown to be present in synovial fluid from patients with active rheumatoid arthritis. It does not persist as such, being converted to less active metabolites. The role of LTB$_4$ as one of the natural mediators of inflammation is discussed.

Polyunsaturated fatty acids, the most important being arachidonic acid, are liberated from phospholipids and further transformed into biologically active compounds that can act as mediators or modulators of inflammatory reactions. Three main groups of derivatives—prostaglandins, thromboxanes, and leukotrienes—are derived from arachidonic acid by oxygenation and further enzymatic actions. The leukotrienes comprise peptidolipids which are the slow-reacting substances of anaphylaxis and a dihydroxy-eicosatetraenoic acid known as leukotriene $B_4$ (LTB$_4$).

The role of LTB$_4$ would appear to be as a local mediator released by resident and newly arriving cells in developing inflammatory exudates in response to a variety of inflammatory stimuli. Its major effects are to enhance the ability of inflammatory leucocytes to penetrate the vascular endothelium, to stimulate their movement towards inflammatory foci, and, after arrival, to cause degranulation and the release of lysosomal enzymes. In addition LTB$_4$ acts in concert with vasodilatory prostaglandins, PGE$_2$ and PGL$_3$, to produce an increased vascular permeability and to cause plasma exudation.

The leukotriene therefore satisfies one important criterion for a putative mediator of inflammation in that it produces effects in vitro and in vivo, which provide a facsimile of characteristic events in inflammatory sequences. Other criteria are whether it may be detected at inflammatory sites and whether it is removed from such sites by further metabolism.

The present work was designed to investigate its formation and presence in synovial fluid of patients with rheumatoid arthritis (RA) and to obtain evidence that there are mechanisms to inactivate LTB$_4$ in the inflammatory exudate.

Materials and methods

We studied 12 patients, 9 male and 3 female, with either classical or definite RA (American Rheumatism Association's criteria) being treated with non-steroidal anti-inflammatory drugs but neither gold nor penicillamine. Two of the patients were also receiving prednisolone (6 mg per day). The mean age of the patients was 49-4 years (range 29 to 61 years) and mean duration of RA was 9-3 years (range 2 to 11 years). Seven patients had a positive Rose-Waaler test.

Synovial fluid specimens aspirated from the knee joint were diluted with an equal volume of 0-9% w/v sterile saline containing heparin (20 units ml$^{-1}$) and the supernatant was stored at $-20^\circ$C after removal of the cells by centrifugation at 500 g for 10 min. The supernatant was analysed for its content of LTB$_4$ and the cells for their ability to both produce and metabolise the leukotriene.

LTB$_4$ was separated and characterised from the supernatant by passage through an octadecysilyl silica column followed by reverse-phase high-pressure liquid chromatography (HPLC), and it was assayed either physicochemically, by measurement of ultraviolet absorbance at 260, 270 and 281 nm, or biologically by aggregation of rat peritoneal polymorphonuclear leucocytes (PMNs). One or both of these assay procedures were used to study both the generation of LTB$_4$ from suspensions of the synovial fluid cells exposed to the calcium ionophore A23187* and also the removal of exogenous LTB$_4$ added either to the suspended synovial cells or to PMNs.
prepared from human blood. Further experiments were performed with tritium-labelled LTB₄ obtained by incubating rat peritoneal PMNs with ³H-ara-chidonic acid (Radiochemical Centre, Amersham, Bucks). The disappearance of the radioactive LTB₄ and the formation of tritiated polar metabolites from human peripheral PMNs were monitored.

### Results

By the ultraviolet absorption method no LTB₄ was detected in the relevant HPLC fraction from any of the synovial fluid specimens. In our hands the lower limit of detection with this technique is 2·5 ng ml⁻¹—that is, 25 to 60 ng per whole specimen. The results of the more sensitive bioassay procedure, given in Table 1, show that detectable amounts of the LTB₄ were present, the mean value (±SEM) was 0·34 ± 0·14 ng ml⁻¹, and the range was 0 to 17 ng per whole specimen.

When the synovial fluid cells were exposed to the calcium ionophore the major lipoygenase-derived products were produced in similar quantities to those reported for human peripheral PMNs. These products comprise 5-monohydroxyeicosatetraenoic acid, LTB₄, the all-trans-isomers of the leukotriene, and its more polar 20-hydroxy and 20-carboxy derivatives.

The results in Fig. 1 show the rate of disappearance of 100 ng of LTB₄ assessed by measurement of its aggregating effect in suspensions containing 10⁷ cells ml⁻¹ of either human synovial or human peripheral leucocytes. The rate of removal of the leukotriene by each cell type was both rapid, the half life being

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Volume (ml)</th>
<th>Total LTB₄ (ng)</th>
<th>Concentration of LTB₄ (ng ml⁻¹)</th>
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<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>1·5</td>
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<td>3</td>
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<td>0·7</td>
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<td>5·2</td>
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<tr>
<td>6</td>
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<td>17·3</td>
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<td>1·8</td>
<td>0·14</td>
</tr>
</tbody>
</table>

### Discussion

The present results confirm the finding of earlier workers that LTB₄ is present in the synovial fluid of
patients with active rheumatoid arthritis. They also suggest that the most likely source of the leukotriene is the inflammatory leucocytes in the synovial fluid since these cells are able to generate LTB₄ when exposed to a calcium ionophore. In addition, the leukotriene was found to be biologically deactivated within minutes by leucocytes, either peripheral or obtained from synovial fluid. A major cause for this deactivation appears to be the conversion of LTB₄ to its more polar 20-hydroxy and 20-carboxy derivatives. These have been detected and characterised in human leucocyte preparations by other workers, who have reported them to be over 50 times less active than LTB₄ in promoting leucocyte adhesion to vascular endothelium. In the present work at least 25% of radiolabelled LTB₄ was converted to these polar derivatives in minutes.

If LTB₄ is generated by leucocytes which enter and accumulate in inflammatory exudates, such as the synovial fluid in patients with rheumatoid arthritis (RA), then it has strong claims as a local mediator of inflammation in man. Its effects resemble and supplement those of the complement-derived peptide, C5a, which is formed in the fluid phase of the exudate. The 2 cytotaxins promote the adherence, diapedesis, and degranulation of leucocytes and interact with vasodilatory prostaglandins to cause increased vascular permeability. Thus prostaglandins, C5a, and LTB₄, all of which occur in the synovial fluid of patients with chronic self-destructive conditions, such as RA, may act sequentially and in combination to produce the local oedema and cellular infiltration which characterise inflammatory responses.

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References

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