Differential Expression of Leukotriene B_4 Receptor Subtypes (BLT1 and BLT2) in Human Synovial Tissues and Synovial Fluid Leukocytes of Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective***. To evaluate the role of leukotriene B_4 (LTB_4) receptors in inflammatory arthritis, we investigated the expression of BLT1 and BLT2 mRNA in synovial tissues of patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Methods. BLT1 and BLT2 mRNA were detected by reverse transcription polymerase chain reaction (RT-PCR) and *in situ* hybridization in synovial tissues from 40 patients with RA and 10 patients with OA.

Results. BLT2 (the low-affinity receptor for LTB_4) showed stronger expression than BLT1 (the high-affinity receptor) in actively inflamed synovial tissue from patients with RA. Synovial macrophages, fibroblast-like cells, and lymphocytes expressed BLT2 mRNA in RA synovial tissues showing active inflammation. BLT2 mRNA was strongly expressed in the synovial lining cells, which also expressed 5-lipoxygenase, an enzyme that synthesizes LTB_4. BLT1 and BLT2 mRNA expression in synovial tissues was stronger in RA than in OA by real-time quantitative PCR. In contrast, leukocytes infiltrating synovial fluid predominantly expressed BLT1 mRNA in patients with RA. It was recently reported that these 2 receptors for LTB_4 have quite different pharmacological effects and a different tissue distribution.

Conclusion. BLT2 is the main receptor mediating the effects of LTB_4 in the synovial tissues of patients with RA; this suggests the possibility of developing a new therapy to block LTB_4 in inflammatory arthritis. (J Rheumatol 2003;30:1712–8)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
LEUKOTRIENE B_4
LEUKOTRIENE B_4 RECEPTORS
IN SITU HYBRIDIZATION

Leukotriene B_4 [LTB_4 (5S,12R)-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid] is a metabolite of arachidonic acid and is one of the most potent chemotactic factors and activators of neutrophils and macrophages[1]. LTB_4 plays an important role in the allergic and inflammatory responses[2]. Overproduction of LTB_4 occurs in various forms of inflammatory arthritis. In particular, synovial fluid (SF) in patients with rheumatoid arthritis (RA) has high levels of arachidonic acid metabolites, especially LTB_4. Levels of SF leukocytes, immune complexes, and rheumatoid factor have been correlated with the LTB_4 level in RA patients[3]. Neutrophils from SF and peripheral blood of RA patients produce more LTB_4 than the corresponding cells from healthy individuals[4]. In patients with RA, peripheral blood mononuclear cells and synovial macrophages are more involved in the synthesis of LTB_4 than neutrophils[5]. In animal models of RA, the presence of LTB_4 is required for the development of arthritis[6].

LTB_4 acts by binding to a specific cell surface receptor. Recently, 2 G-protein-coupled receptors for LTB_4 (BLT1 and BLT2) were cloned and characterized[7,8]. These receptors are from a gene cluster in both the human and the mouse genome and show 45% homology at the amino acid level, suggesting that they were generated by gene duplication.

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Both receptors recognize LTB_4 and activate multiple G-proteins to transduce intracellular signals. BLT1 is only activated by LTB_4, while BLT2 is also activated by various hydroxyeicosatetraenoic acids (HETE), including 12 (s)-HETE and 15 (s)-HETE[9]. The tissue distribution of the 2 receptors is also quite different; BLT1 is expressed by neutrophils and macrophages, while BLT2 is expressed in a wide variety of tissues with the highest levels of expression in the spleen and lymphocytes, suggesting some role of LTB_4 in immunity[8]. Classical leukotriene B4 receptor antagonists have been evaluated for their potency in antagonizing BLT1 and BLT2, and the 2 receptors have been suggested as possible targets for novel antiinflammatory drugs. However, no information has been published about the distribution of these 2 LTB_4 receptors in RA joint tissues.

We evaluated differences in the distribution and expression of the 2 leukotriene receptor subtypes in the synovial tissues of patients with RA and osteoarthritis (OA).

MATERIALS AND METHODS

Reagents. Mouse anti-5-lipoxygenase (5-LOX) monoclonal antibody was purchased from Research Diagnostic Inc. (Flanders, NJ, USA). Anti-CD3 antibody, anti-CD4 antibody, anti-CD8 antibody, anti-CD68 antibody, and von Willebrand factor antibody were purchased from Dako (Carpinteria, CA, USA). A DIG-High Prime DNA Labeling and Detection Kit was purchased from Roche Diagnostics GmbH (Mannheim, Germany), a Vectorstain ABC kit was obtained from Vector Laboratories, Inc. (Burlingame, CA, USA), and 3,3'-diaminobenzidine (DAB) was purchased from Dojindo (Kumamoto, Japan). Real-time quantitative polymerase chain reaction (PCR) kit, qPCR Mastermix for Sybr Green I was purchased from Eurogentec (Seraing, Belgium).

Patients and samples. Synovial specimens were obtained from 40 patients with RA and 10 patients with OA who were treated at our institution. All the RA patients fulfilled the criteria of the American College of Rheumatology[10,11]. OA was diagnosed according to clinical and radiological criteria. Synovial membrane samples were obtained during surgery for the treatment of joint disease and all patients gave informed consent for the use of their samples in research. In each disease group, the clinical characteristics of the patients (e.g., sex, age, erythrocyte sedimentation rate, and C-reactive protein) were consistent with the diagnoses (Table 1). The antirheumatic drugs used by these patients before surgery were methotrexate (16 patients), corticosteroids (10), salazosulfapyridine (8), and gold (6). In some patients, these drugs were used in combination.

Table 1. Clinical features of the 50 patients with RA and OA.

Feature	Diagnostic Group RA	OA
Patients, n	40	10
Female: male	34:6	8:2
Age, yrs	60.7 ± 11.8	68 ± 4.3
Disease duration, yrs	17.3 ± 13.9	7.2 ± 4.1
Laboratory data		
ESR, mm/h	59.6 ± 29.7	22.3 ± 20.7
CRP, mg/ml	2.14 ± 1.98	0.24 ± 0.45
Histologic class of synovitis, n*		
1. Serofibrous	10	6
2. Lymphoplasmacytic	8	4
3. Ulcerous	9	0
4. Fibroblast-transformed	13	0

* Histological classification of synovitis (Fritz *et al*, 1989) class 3 (ulcerous), class 4 (fibroblast-transformed) are often associated with active RA.

Samples were fixed in 4% paraformaldehyde within 6 h of resection and were embedded in OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) after cooling by liquid nitrogen. Each sample was evaluated by a pathologist and synovitis was classified into the following 4 histologic classes: (1) serofibrous, (2) lymphoplasmacytic, (3) ulcerous, and (4) fibroblast-transformed synovitis[12,13]. Classes 3 and 4 correspond to inflammatory synovitis in RA patients.

SF leukocytes were obtained from 10 patients with RA and 3 patients with OA by arthrocentesis. SF samples were centrifuged, and then cells were fixed on silane-coated slide glasses by cytospin and were stored at –70°C until use. Neutrophils and mononuclear cells in the SF were detected by Wright-Giemsa staining.

Assessment of LTB_4 receptor subtype mRNA expression in synovial tissues by RT-PCR, real-time quantitative PCR, and Southern hybridization. Total RNA was isolated from synovial tissues and SF leukocytes by the guanidium thiocyanate/phenol/chloroform method (Isogen Reagent Kit; Nippon Gene Co. Ltd., Toyama, Japan); cDNA was synthesized from 2 µg of the total RNA using RAV2 reverse transcriptase and Oligo (dT) primers (Takara Shuzo Co. Ltd., Shiga, Japan), as described[14]. The primers for human BLT1 (GenBank accession number: BC004545) were 5'-TATGTCTGCGGAGTCAGCATGTACGC-3' (sense: 624-649 bp) and 5'-CCTGTAGCCGACGCCCTATGTCCG-3' (antisense: 946-969 bp), and the PCR product obtained with these primers was 346 bp in size[7]. The primers for human BLT2 (GenBank accession number: AB029892) were 5'-AGCCTGGAGACTCTGACCGCTTTCG-3' (sense: 547-570 bp) and 5'-GACGTAGAGCACCGGGTTGACGCTA-3' (antisense: 843-867 bp), and the PCR product was 321 bp in size[8]. The primers for human 5-LOX (GenBank accession number: J03571) were 5'-CCGGCACTGACGACTACATCTA-3' (sense: 81-102 bp) and 5'-CACGGGGTAAATCCTTGTGG-3' (antisense: 514-533 bp), and the PCR product was 453 bp in size[15]. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank accession number: M33197) was used as the internal control, with the 2 primers (5'-CATCATCTCTGCCCCCTCTG-3' and 5'-CCTGCTTCACCACCTTCTTG-3') yielding an expected PCR product of 437 bp[16]. PCR was performed using a reaction mixture comprising 25 µM of each primer, 2.5 mM of each dNTP, and 2.5 U of Taq DNA polymerase (Takara Shuzo). It was carried out in an automated DNA cycler (Takara Shuzo) and involved 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 3 min. The resulting RT-PCR fragments were separated by electrophoresis on 2% (w/v) agarose gel, and the gel was stained with ethidium bromide. PCR products were subjected to electrophoresis on 2% agarose gel, transferred to nylon membranes, and hybridized with the following digoxigenin (DIG) end-labeled oligonucleotide probes: 5'-TGTCCCAGAAGCTACGCACCAA-3' for human BLT1, 5'-CTCTGACCGCTTTCGTGCTTCCTT-3' for human BLT2, and 5'-CTCCGGGAAACTGTGGCGTGAT-3' for human GAPDH. Three-primed end-labeling of the probes and detection after hybridization were performed using a DIG-High Prime DNA labeling and detection kit (Roche Diagnostics GmbH). The membrane was subsequently exposed to Fuji RX-R film.

Real-time quantitative PCR was performed to compare the amounts of BLT1 and BLT2 mRNA expression in synovial tissues of patients with RA and OA. The cDNA of 40 patients with RA and 10 patients with OA was used for real-time quantitative PCR. The primers for real-time PCR are as follows. The primers for human BLT1 were 5'-GCACCGGGCCTTCCATCTAATCT-3' (sense: 869-891 bp) and 5'-CCAGGAAGGGCAGCAGGAAGC-3' (antisense: 907-927 bp), and the PCR product was 59 bp in size. The primers for human BLT2 were 5'-AGGCGGGCTGCAAGGCGGTGTA-3' (sense: 272-293 bp) and 5'-AGGAAGGGGCGGGTGACTG-3' (antisense: 365-383 bp), and the PCR product was 112 bp in size. The primers for human GAPDH was 5'-ACCAGCCCCAGCAAGAGCACAAG-3' (sense: 1081-1103 bp) and 5'-TTCAAGGGGTCTACATG-

GCAACTG-3' (antisense: 1180-1203 bp), and the PCR product was 123 bp in size, used as control. The real time PCR was carried out using a Real-time quantitative PCR kit (Eurogentec) according to the manufacturer's protocol[17]. The detection was performed by fluorescence using SYBR Green fluorescent dye (Eurogentec). Amplification was performed according to a standard protocol recommended by the manufacturer with an annealing temperature 58°C (2 min at 50°C, 10 min at 95°C, 40 cycles at 95°C for 15 s, and 58°C for 1 min). All samples were measured in duplicate. Data analysis was carried out in the ABI PRISM 7700 Sequence Detection System (Applied Biosystems Japan Ltd., Tokyo, Japan), and the calculated cycle threshold values (Ct) were exported to Microsoft Excel. The amounts of BLT1 and BLT2 mRNA in RA and OA synovial tissues were compared and expressed with that of an arbitrary unit of 1 as control (non-inflammatory synovial tissue).

In situ hybridization of BLT1 and BLT2 in synovial tissues. PCR products of human BLT1 and BLT2 fragments on the agarose gels were purified using a StrataPrep PCR purification kit (Stratagene Cloning Systems, La Jolla, CA, USA) and were cloned using a Qiagen PCR cloning Kit (Qiagen, Tokyo, Japan). Then the cDNA of BLT1 and BLT2 clones were sequenced using an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems).

Cryosections of synovium were mounted on silane-coated glass slides and fixed with 4% (w/v) paraformaldehyde. DIG-labelled antisense riboprobes for human BLT1 and BLT2 were prepared by *in vitro* transcription of pDrive Cloning Vector (Qiagen) which contained human BLT1 and BLT2 cDNA as described[18] and sense riboprobe prepared likewise. The sections of synovium were treated with 10 µg/ml proteinase K and hybridized with the labeled riboprobes in hybridization solution (Novagen, Madison, WI, USA) for 18 h at 50°C in moistened plastic boxes. After hybridization, the sections were treated with 20 µg/ml Rnase A. After extensive washing, probe binding was visualized with an alkaline-phosphate conjugated anti-DIG antibody in 5-bromo-4-chloro-3 indolyl-phosphate and 4-nitroblue tetrazolium chloride solution (Roche Diagnostic GmbH). Then all the slides were counterstained with hematoxylin before examination.

Immunohistochemistry of human 5-LOX and surface markers. Immunostaining was performed with a modification of the avidin-biotin-peroxidase complex technique. Immunoperoxidase staining was carried out using a Vectorstain kit according to the manufacturer's protocol[19]. Sections prepared from frozen samples were incubated in methanol containing 3% (v/v) H_2O_2 for 20 min to enhance endogenous peroxidase activity. The sections were preincubated in 0.3% (v/v) bovine serum albumin (Sigma) in phosphate buffered saline (PBS) for 1 h, and then incubated with diluted goat serum for 20 min. This was followed by incubation in a humid chamber with an anti-5-LOX antibody diluted 1:100 (Research Diagnostics Inc.), anti-CD68 antibody, anti-CD4 antibody, von Willebrand factor antibody (Dako), or mouse purified normal IgG, respectively, for 1 hour. Then, the sections were washed with PBS, incubated with biotinylated goat anti-mouse IgG for 30 min, and washed again in PBS. Color was developed by treatment with 3,3'-diaminobenzidine (DBA) (Dojindo, Kumamoto, Japan) and sections were counterstained with hematoxylin.

To analyze the expression of BLT1 and BLT2 mRNA in synovial tissues of RA or OA, we correctly counted the cells on *in situ* hybridization-stained preparations using an image analysis program, SCION Image (Scion Corp., Fredrick, MD, USA) on a personal computer (Dell Inspiron 3700). Evaluation of the *in situ* hybridization samples was performed as reported[20]. Slides were observed by microscope (CX41; Olympus, Tokyo, Japan) and images were photographed at magnification of ×200 by a digital camera (CAMEDIA C-4040ZOOM; Olympus, Tokyo, Japan). Ten images were obtained from every slide and converted to adequate image forms by Adobe Photoshop (v5.0.2; Adobe Systems Incorporated, CA, USA). The final size of all digital images for cell count was 750 × 750 pixels which corresponds to about 50 × 50 µm of tissue on the slide. The positively stained cells in the images were counted by the SCION Image program according to the manufacturer's directions[20].

Statistical analysis. Results are expressed as the mean ± standard deviation. Mean values were compared by Mann-Whitney test and $p < 0.05$ was considered to indicate a significant difference.

RESULTS

Expression of BLT1 and BLT2 mRNA in synovial tissues of RA and OA patients. We examined the expression of mRNA for the 2 LTB_4 receptors, BLT1 and BLT2, in synovial tissue from 40 patients with RA and 10 patients with OA by RT-PCR, real-time quantitative PCR and Southern hybridization. The BLT2 mRNA signal was strong in RA synovium showing class 4 changes (fibroblast-transformed synovitis), which featured multiple layers of synovial lining cells as well as lymphocyte follicles. In class 3 synovial tissue (ulcerous synovitis) BLT2 mRNA was weakly positive. BLT1 mRNA signals were also observed in the samples, but were weaker than the signals for BLT2. The non-inflamed synovial tissues (class 1 or 2) from RA and OA patients showed very weak expression of the 2 LTB_4 receptor subtypes.

These findings indicate that the main receptor for LTB_4 is BLT2, not BLT1, in inflammatory synovium such as that seen in RA (Figure 1A). On the other hand, 5-LOX mRNA was detected in synovial tissues from patients with RA and OA (Figure 1B).

In situ hybridization of inflamed synovium. To determine the distribution of BLT1 and BLT2 mRNA in synovial tissues from patients with RA and OA, we performed *in situ* hybridization using DIG-labelled riboprobes. Both BLT1 and BLT2 mRNA were expressed in class 4 synovial tissues from 24 patients with RA. Macrophages were identified by positive staining with the anti CD68 antibody (Figure 2H). Fibroblast-like cells were spindle-shaped ones that showed negative staining with anti-CD68 and CD3 antibody. Endothelial cells were identified by factor VIII antibody. Intense signals of BLT1 and BLT2 mRNA were seen in the synovial lining cells. BLT1 was found on synovial macrophages, but not on fibroblast-like cells, lymphocytes, or vascular endothelial cells (Figure 2A, B, C, D). Intense signals for BLT2 were seen in macrophages, fibroblast-like cells of the synovial lining, and in macrophages and lymphocyte follicles in the sublining (Figure 2C, D). The number of BLT2 mRNA-positive cells was significantly higher in RA patients compared with OA patients (Figure 3). In addition, immunostaining for 5-LOX (an enzyme involved in LTB_4 synthesis) was seen in synovial lining cells from patients with RA and OA, with both 5-LOX and BLT2 being strongly expressed in multilayered synovial lining (Figure 2E, F, G).

Leukotriene receptor expression by SF cells. Synovial leukocytes were 84 ± 5% neutrophils (n = 10) and 16 ± 5% mononuclear cells (n = 10) in patients with RA. BLT1 mRNA was predominantly expressed in the synovial leukocytes of RA patients, while BLT2 mRNA was not detected or showed very weak expression (Figure 4A). In addition,

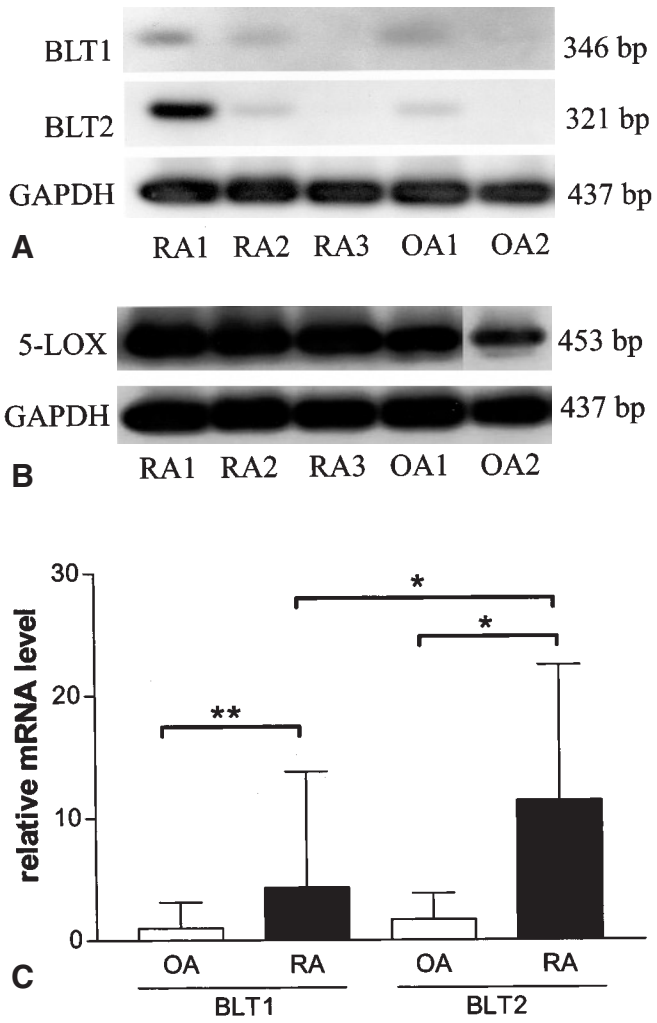


Figure 1. A. BLT1 and BLT2 mRNA expression in synovial tissues from patients with RA or OA detected by RT-PCR and Southern hybridization. Expression of BLT1 was detected in synovial tissue from some patients with RA or OA (RA1, RA2, and OA1), but not in other samples (RA3 and OA2). Expression of BLT2 was similar to that of BLT1. In synovial tissue from OA patients, the expression of BLT2 was weak. The pathological classification of synovitis[12]: RA1: fibroblast-transformed synovitis (class 4), RA2: ulcerous synovitis (class 3); RA3 and OA1: lymphoplasmacytic synovitis (class 2), and OA2: serofibrous synovitis (class 1). B. Expression of human 5-LOX mRNA in synovial tissues from patients with RA or OA detected by RT-PCR and Southern hybridization. 5-LOX was detected in all synovial tissue samples. C. Comparison of the expression of BLT1 and BLT2 mRNA in synovial tissues from 40 patients with RA and 10 patients with OA by real-time quantitative PCR methods. BLT2 signals expressed significantly higher than BLT1 signals in synovial tissues of patients with RA. Moreover BLT1 and BLT2 signals in RA synovial tissues expressed higher than that of OA. *$p < 0.05$; **$p < 0.01$, by 2-tailed Mann-Whitney test.

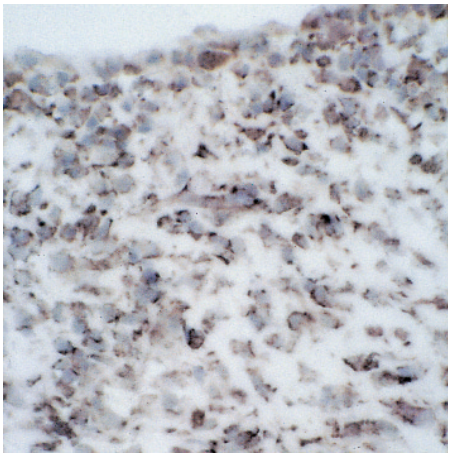
the expression of BLT1 mRNA and BLT2 mRNA was investigated by *in situ* hybridization. BLT1 mRNA was highly expressed in neutrophils and macrophages from the SF of RA patients (Figure 4B), while SF leukocytes from RA patients did not show BLT2 mRNA expression (Figure 4C).

DISCUSSION

We detected strong expression of the BLT2 receptor for LTB_4 in synovial tissues with histopathological inflammatory changes, in contrast to weaker expression of BLT1. These 2 receptors are co-expressed in lymphocytes and macrophages. Northern blotting has shown that human peripheral leukocytes express both BLT1 and BLT2, which agrees well with the reported co-existence of high- and low-affinity LTB_4 receptors in these cells[21]. Some investigators have speculated that the high-affinity receptor mediates chemotaxis for LTB_4 and the low-affinity receptor mediates LTB_4-induced degranulation and superoxide anion generation[22]. BLT2 was not inhibited by most of the previously developed LTB_4 receptor antagonists.

BLT2 is expressed ubiquitously, while BLT1 is expressed predominantly in leukocytes. The highest BLT2 mRNA level is in spleen, followed by the liver, ovary, and peripheral blood leukocytes in mice[8]. In synovial lining cells, macrophages, and lymphocytes of RA patients, expression of BLT2 was stronger than that of BLT1. Human granulocytes, eosinophils, and mononuclear cells express both BLT1 and BLT2. BLT1 is expressed almost exclusively in peripheral leukocytes, whereas BLT2 is expressed ubiquitously with strongest expression in inflammatory synovium. Accordingly, it seems that LTB_4 mainly acts via BLT2, not BLT1, in the active inflammatory synovial tissues of patients with RA. On the other hand, BLT1 is strongly expressed by leukocytes in SF. This differential distribution of LTB_4 receptor subtypes may suggest a new role of LTB_4 in RA joint disease. Because patients were treated therapeutically, we could not exclude an influence of treatment. We could not find an association between treatment for RA and the expression of BLT1 and BLT2 signals in synovial tissues.

The roles of BLT1 and BLT2 are not clearly understood at present. LTB_4 seems to be the best ligand for BLT2. Recently, Yokomizo, *et al* also reported that hydroxyeicosanoids, 12-HETE and 15-HETE, can bind and activate BLT2, but not BLT1. The binding of LTB_4 to BLT2 is not inhibited by most of the previously reported LTB_4 receptor antagonists[9]. The 5-LOX pathway is inhibited by 15 (S)-HETE[23]. BLT1 may induce neutrophil chemotaxis and activation in the joint cavity, while BLT2 in synovial tissues may inhibit 5-LOX after binding by 15-HETE or 12-HETE. Although LTB_4 was reported to be the strongest ligand among several tested for BLT2, we should keep in mind the possible existence of more selective ligands for BLT2 than LTB_4. Further studies will be needed to clarify the possible role of LTB_4 in RA joints mediated through BLT2. 5-LOX inhibitor (MK886, L-656,224) blocks production of the inflammatory cytokine interleukin 1 in synovial explants from RA patients[24]. In a 4-week, placebo controlled trial of zileuton, a 5-LOX specific inhibitor, the amount of LTB_4 synthesis was reduced by 70% in whole blood of patients



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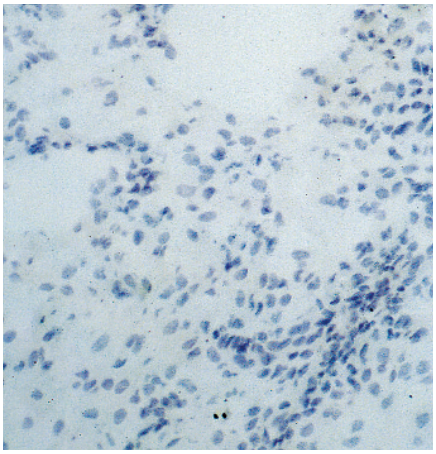

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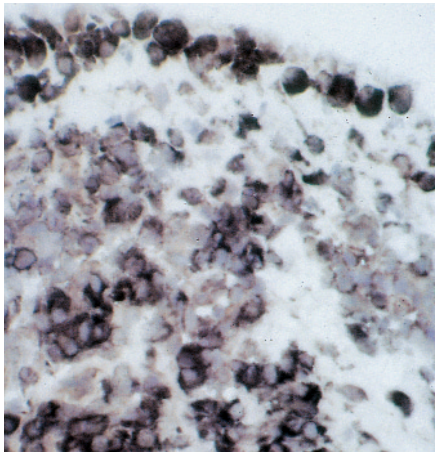

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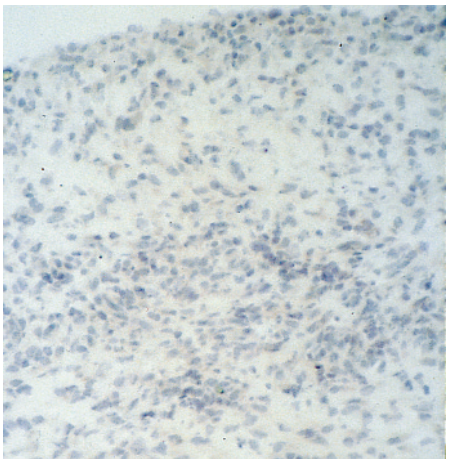

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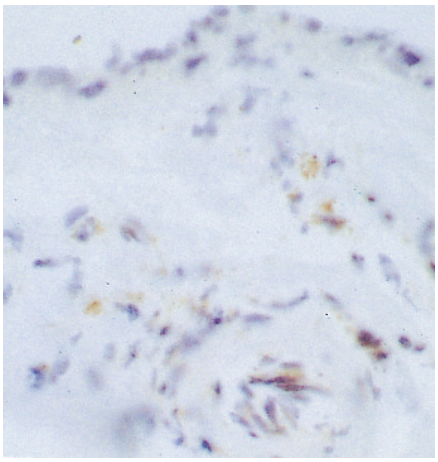

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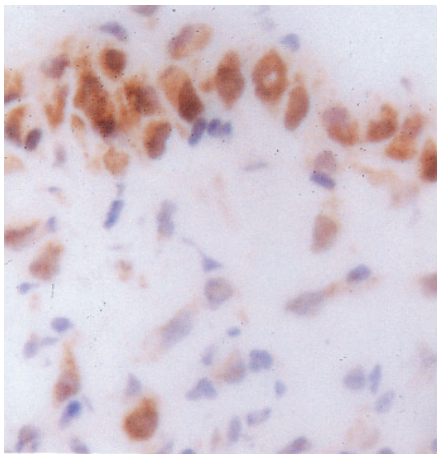

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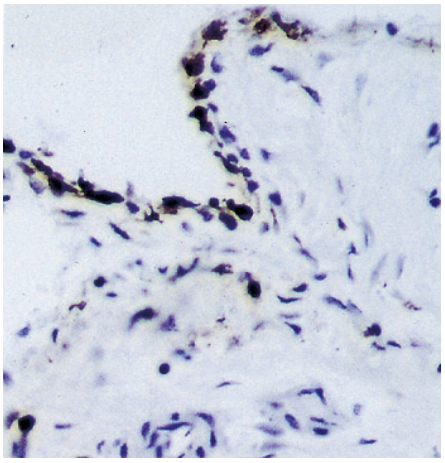

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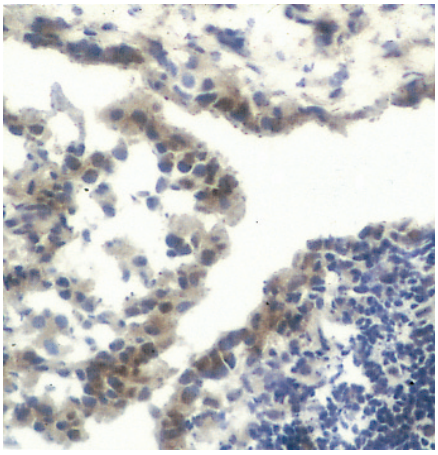

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Figure 2. In situ hybridization of human BLT1 and BLT2 mRNA, and immunohistochemical analysis of 5-LOX, in synovial tissues from patients with RA or OA. A. BLT1 mRNA expression in synovial tissue from a patient with RA. Expression of BLT1 mRNA is seen in synovial lining cells and monocytes (original magnification ×200). B. Negative control, BLT1 sense probe staining of synovial tissue from the same patient (original magnification ×100). C. Expression of BLT2 mRNA in synovial tissue from a patient with RA. Macrophages infiltrating the synovial tissue and synovial lining cells express BLT2 mRNA; expression is stronger than that of BLT1 (original magnification ×200). D. Negative control, BLT2 sense probe staining of synovial tissue from the same patient (original magnification ×100). E. Expression of BLT2 mRNA in synovial tissue from a patient with OA. The expression of BLT2 mRNA is weak (original magnification ×200). F. Immunohistochemical detection of 5-LOX using a specific 5-LOX antibody in synovial tissue from a patient with RA. Positive staining of synovial lining cells is seen (original magnification ×200). G. Detection of 5-LOX in synovial tissue from a patient with OA. Strong positive staining of synovial lining cells is seen; distribution is the same as that in RA (original magnification ×200). H. Distribution of CD68 positive cells in RA synovial tissue. Macrophages were identified by positive staining with an anti-CD68 antibody.(original magnification ×100).

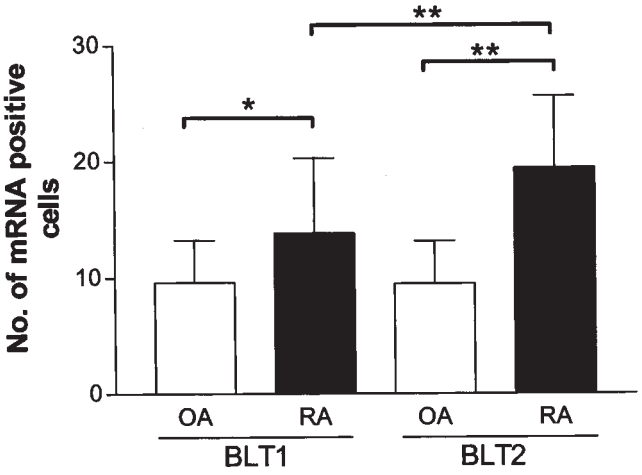

Figure 3. Comparison of BLT2 mRNA-positive cells in synovial tissues from patients with RA and OA. The number of BLT2 mRNA-positive cells was determined by capturing the microscope image in 10 areas per tissue and counting the positive-stained cells in the image on a personal computer. Results were expressed as the mean ± standard deviation. The number of BLT2 mRNA-positive cells in pathological active inflamed RA synovial tissue was significantly higher than in OA synovial tissue. Moreover, in RA synovial tissues, the number of BLT2 mRNA positive cells was significantly higher than that of BLT1 mRNA positive cells. *$p < 0.05$, **$p < 0.01$ by 2-tailed Mann-Whitney test.

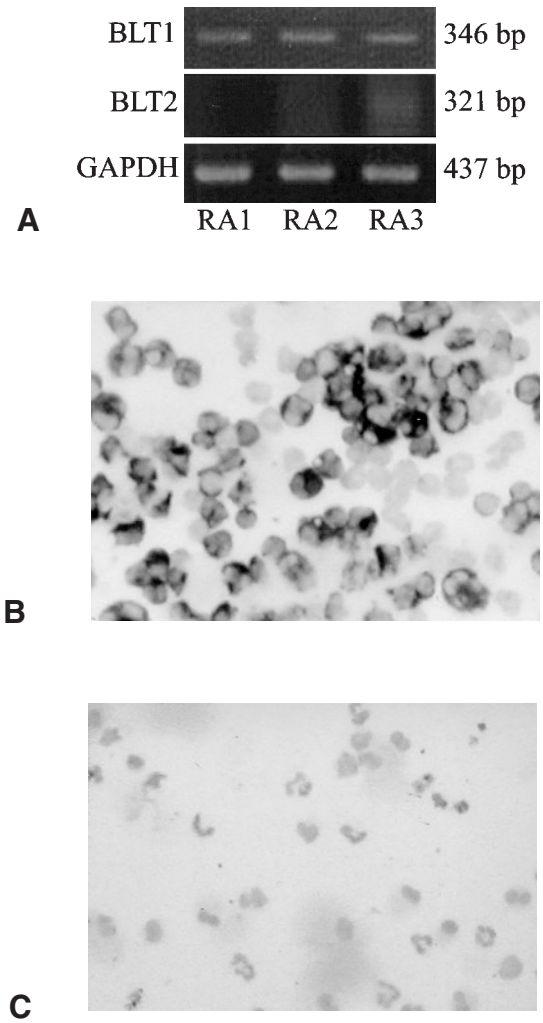

Figure 4. A. Expression of BLT1 and BLT2 mRNA by SF leukocytes from patients with RA. BLT1 mRNA was detected in SF leukocytes from 3 patients with RA, while BLT2 mRNA was not detected in the same samples. B. Expression of BLT1 and BLT2 mRNA in SF leukocytes from patients with RA by *in situ* hybridization. BLT1 signals are strong in RA SF leukocytes, especially neutrophils. C. In contrast, BLT2 mRNA shows very weak signals.

with RA. The improvement of clinical variables (the number of painful joints and swollen joints) did not reach statistical significance comparing zileuton and placebo[24].

5-LOX was most strongly expressed in the synovial lining layer of RA patients, but lining cells of OA patients also expressed 5-LOX. The actions of LTB_4 may be regulated by changes in expression of its receptors. Our data suggest that the main receptor for LTB_4 in RA synovium is BLT2, while leukocytes (mainly neutrophils) in the SF express BLT1. Neutrophil infiltration into joint cavities is induced by several chemotactic factors, including LTB_4, which induces chemotaxis via BLT1 expressed on neutrophils. However, the role of BLT2 expressed on synovial lining cells, sublining macrophages, and lymphocytes is still unclear. BLT2-selective agonists and antagonists would be needed to assess the effect of LTB_4 mediated via BLT2 in RA joints.

In conclusion, we found that BLT2 was the main LTB_4 receptor subtype expressed by synovial lining cells, macrophages, and lymphocytes in actively inflamed synovial tissue from patients with RA. BLT2 may provide a

novel target for antiinflammatory therapy and these findings expand our information about leukotrienes in RA. No LTB_4 receptor antagonists are yet available for clinical use and further work is needed to understand the role of the lipoxygenase system in inflammatory arthritis such as RA.

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