Kallikreins, kininogens and kinin receptors on circulating and synovial fluid neutrophils: role in kinin generation in rheumatoid arthritis

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Objectives. Neutrophils traffic into and have the capacity to generate kinins in SF of RA patients. The aim of this study was to assess the expression of kallikreins, kininogens and kinin receptors in circulating and SF neutrophils, as well as synovial tissue of RA patients, and to assess kinin generation in SF.

Methods. Neutrophils were isolated from blood and SF of RA patients and blood of healthy volunteers. Expression of kallikreins, kininogens and kinin receptors in neutrophils and synovial tissue was assessed by immunocytochemistry using specific antibodies, with visualization by brightfield and confocal microscopy. Levels of basal and generated kinins in SF of RA patients were measured by ELISA.

Results. Kinin labelling was significantly reduced, indicating the loss of the kinin moiety from kininogen on circulating (P < 0.001) and SF neutrophils (P < 0.05) of RA patients. Immunolabelling of tissue kallikrein was also decreased, whereas kinin B1 and B2 receptor expression was increased in circulating and SF neutrophils of RA patients. Immunolabelling of kallikreins and kinin receptor proteins was similar in RA and normal synovial tissues. The basal kinin level in SF of RA patients was 5.7 ± 6.1 ng/ml and the mean concentration of kinins generated in vitro was 80.6 ± 56.3 ng/ml. The capacity for kinin generation was positively correlated with measures of disease activity.

Conclusions. Kallikrein–kinin proteins on neutrophils play an important role in kinin generation and the pathophysiology of RA. Specific kallikrein and kinin receptor antagonists may be useful as IA therapies for inflamed joints.

KEY WORDS: RA, Neutrophils, Synovium, Kallikreins, Kinins, Kinin receptors, Kininogen, Tender and swollen joints.

Introduction

RA is an autoimmune disease characterized by chronic synovitis, which manifests as joint pain and swelling, and often progresses to bone and joint destruction [1, 2]. Inflammation in RA may result from a number of different mechanisms, including antibody-mediated complement activation and cellular injury, T-cell-mediated mechanisms and generation of pro-inflammatory mediators [1, 3]. Early changes in the inflamed joint include endothelial cell damage, synovial oedema, fibrin deposition, leucocyte invasion and hyperplasia of the synovial lining cells. This is followed by infiltration of neutrophils, monocytes, T- and B lymphocytes, macrophages and plasma cells, overgrowth of fibrovascular granulation tissue known as the pannus and striking angiogenesis and proliferation of bone vessels. The inflammatory milieu of the rheumatoid arthritic joint is therefore a consequence of immune responses and persistent inflammation, arising from the activation of leucocytes and complex interactions among kinin peptides, cytokines and chemokines that leads to progressive destruction of bone and cartilage and ultimately to joint deformities [1, 4].

The serine proteases, plasma kallikrein (PK, hKBI) and tissue kallikrein (TK, hK1), release kinins from endogenous kininogen substrates and are therefore called kininogenases. As a prelude to inflammation in the joint, the contact system comprising Hageman factor (factor XII), plasma pre-kallikrein (PPK) and high molecular weight kininogen (H-kininogen) is activated by contact with negatively charged surfaces, including monosodium urate and calcium dihydrate crystals, vascular basement membranes and glycosaminoglycans [4–6]. PPK is bound to domain 6 of H-kininogen on the outer surface of the neutrophil membrane [7, 8]. TK is sequestered within cytoplasmic granules and also bound to the membrane of neutrophils [9, 10], and cleaves both low molecular weight kininogen (L-kininogen) and H-kininogen to form bradykinin (BK) and lys-BK.

There is considerable evidence supporting a role for the kallikrein–kinin cascade in inflamed joints [4, 11]. Thus PK is present in SF [12], and TK has been identified in SF and SF neutrophils of patients with RA [13, 14]. Kinins released into SF by the proteolytic action of kallikreins on kininogens on the surface of neutrophils are likely to cause vasodilatation and pain, increase vascular permeability, promote leucocyte margination and stimulate cytokine release from monocytes [4, 15]. The capacity of kinins to induce the release of IL-1β, TNF-α [16] and other mediators of inflammation [6] is also crucial to the persistence of inflammation. In addition, kinins enhance bone resorption through prostaglandin formation [17], and open junctions between endothelial cells, thus promoting local diapedesis of neutrophils and plasma extravasation [6, 9].

The kinin B1 and B2 receptors are G-protein-coupled rhodopsin-like transmembrane receptors, with three extracellular and four intracellular loops [18]. Activation of these receptors results in the recruitment of second messengers that mediate the biological actions of kinins [6, 18]. We have previously reported the immunolocalization of B2 receptors, but not B1 receptors, in SF neutrophils of RA patients [19] and also in synovial tissue [20]. The acute nociceptive, inflammatory and vasoactive properties of kinins appear to be mediated by the B2 receptor.

The aim of this study was to investigate the expression of the kallikreins, kininogens and kinin receptors on circulating and synovial neutrophils of RA patients; on circulating neutrophils of healthy volunteers; and in normal and RA synovial tissue. The loss of the kinin moiety from domain 4 of the kinogen molecule on the external membrane of circulating and SF neutrophils...
was assessed. In addition, basal kinin levels and the capacity for kinin generation in SF of RA patients were assessed and correlated with measures of disease activity.

Materials and methods

Patients

Patients with a diagnosis of RA, who attended the outpatient clinics at the Department of Medicine, Nelson Mandela School of Medicine, Durban (Centre A) and the Department of Rheumatology, Sir Charles Gairdner and Royal Perth Hospitals, Perth (Centre B) were enrolled in the study. This research study was approved by the institutional ethics committees and informed consent was obtained from all subjects.

Patients were included in the study if they met the revised 1987 ACR criteria for RA, and had active synovitis of at least one knee joint, as indicated by the presence of pain and a palpable warm effusion. Exclusion criteria included IA steroid injections within the last 3 months, a change in DMARD therapy within the last 3 months and oral steroid therapy at >10 mg/day.

The demographic and other characteristics of the patients recruited at the two centres are presented in Table 1. Among the Centre B patients, 10 were treated with MTX, five with LEF and three with TNF-α inhibitors. For 20 Centre A patients and all Centre B patients, swollen and tender 28-joint counts (SJC28 and TJC28, respectively) were performed [21], and ESR and CRP were measured in 20 patients. ESR and CRP were measured in 12 patients. CRP was measured in 13 patients.

Collection of SF and blood

Using sterile techniques, 10–15 ml of SF was aspirated from the knee joints of RA patients requiring diagnostic or therapeutic arthrocentesis. Blood samples collected in 3.8% sodium citrate were obtained from RA patients and healthy volunteers who did not have any illness, and were not taking regular medication. All blood samples were centrifuged (1000 g, 10 min, 4°C) within 3 hours of collection.

Neutrophil isolation

The SF and anti-coagulated blood samples were mixed with an equal volume of phosphate buffered saline (PBS). Neutrophils were isolated by centrifugation on Percoll (density 1.088) at 1000 g for 30 min. Erythrocytes were lysed with ice-cold water and neutrophils were pelleted by centrifugation, washed twice, re-suspended in PBS and pipetted onto glass slides. After air-drying overnight, slides were fixed in acetone-methanol (1:1) for 5 min and stored at room temperature.

Tissue specimens

Archival SM tissue sections were obtained from the Department of Tissue Pathology, PathWest, Perth, Australia. The normal tissue was obtained from a left knee SM biopsy (male, 50 years). The RA tissue was obtained from a right foot SM biopsy (male, 54 years). The immunological parameters of this patient were anti-cyclic citrullinated peptide autoantibody >100 U (normal <5 U), RF 57 kU/l (normal <21 kU/l), ESR 20 mm/h (normal 1–15 mm/h). Tissues were fixed in formaldehyde, embedded in paraffin wax, sectioned and adhered to poly-l-lysine coated slides before dewaxing in xylene and rehydration in absolute ethanol. Antigen retrieval was performed by boiling the tissue sections in 0.1 M EDTA-Tris buffer, pH 8.0, followed by rehydration in PBS. Synovial tissue sections were also stained with haematoxylin and eosin.

Antibodies

The following specific antibodies were kindly donated by Prof. W. Müller-Esterl (Institute of Biochemistry, Johann Wolfgang Goethe-University of Frankfurt, Frankfurt, Germany) and have been previously characterized [22, 23]: rabbit anti-tissue prokallikrein (TproK1), rabbit anti-PPK, rabbit anti-plasma kallikrein (PK 11), rabbit anti-B1 receptor, rabbit anti-B2 receptor and a monoclonal i-kininogen antibody. A monoclonal anti-bradykinin antibody (SBKI) used to detect the kinin moiety of kininogen was kindly donated by M. Webb (Sandoz, London, UK). A polyclonal IgG antibody against recombinant TK was raised in goat and rabbit polyclonal TK and H-kininogen antibodies were purchased from Abcam, Cambridge, UK. All antibodies were reconstituted and diluted in 0.01 M PBS containing 1% IgG-free BSA and 0.2% sodium azide.

Immunohistochemistry and brightfield microscopy

Tissue section and neutrophil slides were incubated with peroxidase blocker (Dako, Melbourne, Australia) and thereafter with reagents to block non-specific labelling. Slides were then incubated with specific primary antibodies to kallikreins, kininogens and B1 and B2 receptor at optimized dilutions for 1 h (tissue sections) or 3 h (neutrophils). After washing, slides were incubated with horseradish peroxidase-labelled secondary antibodies (EnVision, Dako) for 30 min, followed by diaminobenzidine (DAB) chromophore. Slides were then counterstained with Mayer’s haematoxylin (30 s) and dehydrated using increasing concentrations of ethanol. The slides were cleared with xylene and mounted using aqueous mounting medium (Immumount, ThermoShandon, Pittsburgh, PA, USA) or DePex (Merck, Melbourne, Australia). Slides were viewed at ×60 magnification and a CCD camera was used to capture the images through a 460 nm blue band pass filter, a neutral density-6 filter and a KB-4 daylight filter. As positive controls, the duct cells of the human sub-mandibular salivary glands were immunolabelled with TproK, TK, PPK, PK11, and B1 and B2 receptor antibodies. As negative controls, primary antibody was replaced with PBS or universal rabbit serum (Dako).

Confocal laser-scanning microscopy

For fluorescence microscopy, tissue sections and neutrophils labelled with the primary antibodies were incubated with FITC (Sigma, St Louis, MO, USA) conjugated goat anti-rabbit secondary antibodies for 40 min in the dark. Slides were mounted (Immumount), and fluorescence immunolabelling was visualized on a BioRad MRC 1000/1024 UV laser scanning confocal microscope at ×600 magnification [24]. Pseudo-colours were applied to the fluorescent images according to specific bands of pixel intensity that ranged from 0 to 255 pixels/μm². Specific immunolabelling was depicted on the images as: red to white, maximum; green to...
yellow, moderate; light blue, low; and purple to blue, none. Replacement of the primary antibody with PBS served as the negative control for each labelling run. The intensity of IF on neutrophils of healthy volunteers ($n = 8$) and RA patients ($n = 8$) was quantified and expressed as pixels per square micrometre per subject. The mean fluorescence intensities for TK, the kinin moiety in kininogen and kinin $B_1$ and $B_2$ receptors, in circulating and SF neutrophils of RA patients were compared with those for circulating neutrophils of healthy volunteers.

**Measurement of basal kinins and kinin generating capacity of SF**

For 20 Centre A patients, both the basal kinin levels in SF and the capacity for kinin generation in SF were measured. For the measurement of basal kinins, SF was immediately mixed with an equal volume of a cocktail containing protease inhibitors (0.4 mg/ml aprotinin, 4 mg/ml soybean trypsin inhibitor) and kininase inhibitors (10 $\mu$M captopril, 10 $\mu$M phosphoramidon and 60 mM EDTA). For the measurement of generated kinins, SF was incubated at 37°C for 60 min with an equal volume of the kininase inhibitor cocktail only. During this incubation kinins are generated by the enzymic action of kallikreins on the substrate kininogens, both of which are present in SF [25]. The aliquots of SF for measurement of basal and generated kinins were stored at −20°C.

Prior to the measurement of the kinins, the viscosity of the SF samples was reduced by incubation with hyaluronidase, and $\alpha_2$-M and RF were removed [26]. Briefly, SF aliquots were mixed with an equal volume of 0.003% HCl in absolute ethanol, followed by vortexing and incubation at −20°C for 90 min. After centrifugation (7000 $g$, 4°C, 10 min) and removal of the supernatant, the precipitate was washed with one volume of acid alcohol (diluted 1:1 with water). The supernatants were pooled, evaporated to dryness at 55°C, 10 min) and removal of the supernatant, the precipitate was washed with one volume of acid alcohol (diluted 1:1 with water). The supernatants were pooled, evaporated to dryness at 55°C, the residue was reconstituted in assay buffer and the kinins measured by competitive ELISA.

**Kinin ELISA**

BK was conjugated to cytochrome C with the linker molecule, N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP, Sigma) [27]. Before use, each new batch of conjugate was tested to determine the optimum concentration for coating the ELISA plate. Immulon Maxisorp microtitre plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 3–4 $\mu$g/ml of BK-cytochrome C conjugate (100 $\mu$l/well) in sodium carbonate buffer, pH 9.6. The samples (acid–alcohol extracts reconstituted to the original volume of SF in PBS) and BK standard were pre-incubated overnight at 4°C with an equal volume of anti-BK monoclonal antibody (SBK1, 9.4 $\mu$g/ml). The plate was blocked with 1% BSA for 30 min at room temperature and pre-incubated samples and standards (100 $\mu$l/well) were then added to the plates and incubated at 37°C for 3 h. Alkaline phosphatase-labelled anti-mouse IgG (Sigma) was added and incubated at 37°C for 2 h. Finally, disodium p-nitrophenyl substrate (Sigma) was added and after incubation for 1 h, the absorbance was measured at 405 nm. Non-specific binding was determined by incubating anti-BK antibody (pre-incubated with buffer only) in wells that had been treated with coating buffer alone, and the values were subtracted from all absorbance readings. Concentrations of basal and generated kinin in SF samples were determined from the BK standard curve.

**Statistical analysis**

Results are presented as mean values with 95% CI or range. Mean fluorescence intensities for circulating neutrophils of healthy subjects and RA patients and SF neutrophils of RA patients were compared by one-way analysis of variance with Bonferroni’s multiple comparison test. Correlations were assessed using Pearson’s correlation coefficient. $P$-values <0.05 were considered statistically significant.

**Results**

**Immunolabelling of kallikrein–kinin cascade proteins in SF neutrophils**

The immunolabelling of TK, PK, H- and L-kininogen in SF neutrophils of RA patients, as visualized by brightfield microscopy, is shown in Fig. 1.

**Tissue kallikrein**

Fluorescence immunolabelling of TK in circulating and SF neutrophils of RA patients and circulating neutrophils from healthy volunteers is shown in Fig. 2. There was a clear decrease in IF in circulating and SF neutrophils from RA patients (Fig. 2E and F) compared with circulating neutrophils from healthy volunteers (Fig. 2D). The mean fluorescence intensity for TK on circulating neutrophils from healthy volunteers was 1975 (95% CI 156, 2294) pixels/$\mu$m$^2$, while for circulating and SF neutrophils from RA patients it was 1600 (95% CI 516, 2684) and 1225 (95% CI 156, 2294) pixels/$\mu$m$^2$, respectively.

**Immunolabelling of the kinin moiety of kininogen on the neutrophil membrane**

Immunolabelling of kininogen on neutrophils was observed as a peripheral ring (Fig. 3). IF was clearly decreased on circulating and SF neutrophils from RA patients (Fig. 3E and F) compared with circulating neutrophils from healthy volunteers (Fig. 3D), indicating loss of the kinin moiety from kininogen. The mean fluorescence intensity was 2225 (95% CI 1337, 3113) pixels/$\mu$m$^2$ in circulating neutrophils from healthy volunteers, whereas it decreased to 1100 (95% CI 631, 1569) in SF neutrophils ($P < 0.05$) and 375 (95% CI 138, 888) in circulating neutrophils ($P < 0.001$) from RA patients.

**Kinin B$_1$ receptor**

Immunolabelling of kinin $B_1$ receptor on neutrophils is shown in Fig. 4. IF was clearly increased on circulating and SF neutrophils from RA patients (Fig. 4E and F) compared with circulating neutrophils from healthy volunteers (Fig. 4D). Mean fluorescence intensity increased from 813 (95% CI 234, 1391) pixels/$\mu$m$^2$ in
circulating neutrophils from healthy volunteers to 1449 (95% CI 632, 2266) and 1734 (95% CI 305, 3163) pixels/$\mu$m$^2$, respectively, in circulating and SF neutrophils from RA patients.

**Kinin B$_2$ receptor**

Immunolabeling of kinin B$_2$ receptor on neutrophils is shown in Fig. 5. IF was clearly increased on circulating and SF neutrophils from RA patients (Fig. 5E and F) compared with circulating neutrophils from healthy volunteers to 1449 (95% CI 632, 2266) and 1734 (95% CI 305, 3163) pixels/$\mu$m$^2$, respectively, in circulating and SF neutrophils from RA patients.

![Phase contrast images of neutrophils](https://academic.oup.com/rheumatology/article-abstract/48/5/490/1786494/1645901786694)

**Fig. 2.** Phase contrast (A–C) and confocal (D–F) images showing immunolabelling of tissue kallikrein in neutrophils. (A and D) Circulating neutrophils from healthy volunteers. (B and E) Circulating neutrophils from RA patients. (C and F) SF neutrophils from RA patients. (G) Positive control, confocal image showing TK in the duct cells of the human submandibular salivary gland. The intensity of IF is indicated by the colour strip: red to white, maximum; green to yellow, moderate; light blue, low; and purple to blue, none. Scale bar indicates 10 $\mu$m.

**Fig. 3.** Phase contrast (A–C) and confocal (D–F) images showing immunolabelling of kininogen (kinin moiety) in neutrophils. (A and D) Circulating neutrophils from healthy volunteers. (B and E) Circulating neutrophils from RA patients showing decreased IF due to partial loss of the kinin moiety. (C and F) SF neutrophils from RA patients showing weak IF due to almost complete loss of the kinin moiety. The intensity of IF is indicated by the colour strip: red to white, maximum; green to yellow, moderate; light blue, low; and purple to blue, none. Scale bar indicates 10 $\mu$m.

**Fig. 4.** Phase contrast (A–C) and confocal (D–F) images showing immunolabelling of the kinin B$_1$ receptor in neutrophils. (A and D) Circulating neutrophils from healthy volunteers. (B and E) Circulating neutrophils from RA patients showing a moderate increase in IF. (C and F) SF neutrophils from RA patients showing a marked increase in IF, indicating up-regulation of the B$_1$ receptor. (G) Positive control, confocal image showing kinin B$_1$ receptor in neurons of the substantia gelatinosa in the spinal cord. The intensity of IF is indicated by the colour strip: red to white, maximum; green to yellow, moderate; light blue, low; and purple to blue, none. Scale bar indicates 10 $\mu$m.

**Fig. 5.** Phase contrast (A–C) and confocal (D–F) images showing immunolabelling of the kinin B$_2$ receptor in neutrophils. (A and D) Circulating neutrophils from healthy volunteers. (B and E) Circulating neutrophils from RA patients showing a moderate increase in IF. (C and F) SF neutrophils from RA patients showing a marked increase in IF, indicating up-regulation of the B$_2$ receptor. (G) Positive control, confocal image showing kinin B$_2$ receptor in the collecting ducts and tubules of normal kidney. The intensity of IF is shown on the colour strip: red to white, maximum; green to yellow, moderate; light blue, low; and purple to blue, none. Scale bar indicates 10 $\mu$m.
neutrophils from healthy volunteers (Fig. 5D). Mean fluorescence intensity increased from 463 pixels/$C^2m^2$ (95% CI 249, 677) in circulating neutrophils from healthy volunteers to 2238 (95% CI 442, 4033) and 1638 pixels/$C^2m^2$ (95% CI 211, 3064), respectively, in circulating and SF neutrophils from RA patients.

Immunolabelling of kallikrein–kinin cascade proteins in synovial tissue

In the normal synovial and the underlying stromal cells, TproK, TK, PPK, PK11, B1 and B2 receptor showed intensity 3+ staining in the cytoplasm of >80% of cells. The pattern of immunolabeling for all six antibodies, both with regard to cytoplasmic intensity of staining and the number of cells labelled, was similar in RA and normal synovial tissue (Fig. 6). Histologically, in RA tissue the surface layer synovial cells were hyperplastic, and the architecture showed villous finger-like projections. The stromal connective tissue was increased, was infiltrated with lymphocytes and plasma cells and contained lymphoid follicles. Capillary vascularization of the stroma was prominent.

Kinin levels in SF of patients with RA

The mean basal kinin level was 5.7 ± 6.1 ng/ml (range 0.43–26.17, $n = 20$). The mean concentration of kinins generated after in vitro incubation of SF at 37°C for 60 min was 80.6 ± 56.3 ng/ml (range 12.5–216.02, $n = 20$), indicating that SF of RA patients had the capacity for generating large amounts of kinins. There was a significant inverse correlation between basal kinin and generated kinin levels ($r = –0.454; P < 0.05$). The capacity for kinin generation in SF correlated significantly with measures of disease activity, including SJC28 ($r = 0.509, P < 0.05$), TJC28 ($r = 0.536, P < 0.05$), ESR ($r = 0.598, P < 0.05$) and CRP ($r = 0.725, P < 0.01$) (Fig. 7).

Discussion

Both tissue and plasma kallikrein and H- and L-kininogens enter the inflammatory milieu of rheumatoid joints from the capillary circulation, on neutrophils and from desquamated microvascular endothelial cells of pannus vessels [6, 7, 9, 19, 28]. The activation of the pro-kininogenases by TK is likely to promote re-modeling of synovial tissue. In the present study a significant loss of TK labelling was demonstrated in SF neutrophils from RA patients. This finding confirms previous reports identifying both tissue and plasma kallikreins in SF of RA patients [12–14, 29]. When neutrophils are activated in SF, TK is released both by secretion and degranulation. This would result in decreased immunolabelling of TK on the neutrophils, and TK released into SF would then interact with kininogen on the neutrophil surface to form kinins.

The kininogen molecule has been previously localized on the surface of normal human neutrophils [7, 8]. The kinin moiety, which is cleaved by the kallikreins, is located in domain 4, between the polypeptide domains 3 and 5 of the kininogen molecule. The presence of kininogen on SF neutrophils of RA patients therefore provides the substrate for kallikrein-mediated generation of kinins [5]. Loss of the kinin moiety from kininogen on neutrophils has been previously reported in sepsis [30]. In the present study, the intensity of labelling for the kinin moiety on kininogen, and also for tissue kallikrein, was clearly decreased in circulating and SF neutrophils from RA patients compared with circulating
neutrophils from healthy volunteers. The significant loss of labeling for the kinin moiety on kininogen and for the kininogenase, TK, suggests that these proteins are actively involved in the generation of kinins by circulating and SF neutrophils of RA patients, and are therefore implicated in both the local and systemic pathophysiology of RA.

The kinin B2 receptor is ubiquitous and mediates most of the physiological effects of BK and lys-BK [18]. Kinin B2 receptors are located on the plasma membrane of normal human neutrophils [31] and were previously immunolocalized on SF neutrophils of RA patients [19]. Since there is an influx of neutrophils into the inflamed joint, the intensity of labeling for B2 receptors on circulating neutrophils from healthy volunteers was compared with that on circulating and SF neutrophils from RA patients. There was a clear increase in the intensity of B2 receptor labelling in neutrophils from RA patients compared with those from healthy volunteers, providing further evidence that kinins may be responsible for systemic and local effects in RA. The acute nociceptive, inflammatory and vasoactive properties of kinins, as well as the enhanced influx of neutrophils into the inflamed joint, are likely to be mediated by the B2 receptor [4, 6]. In addition, tissue kallikrein in SF may also directly activate the kinin B2 receptor, independently of kinin generation [32].

The kinin B2 receptor antagonist HOE 140 (Icatibant), which inhibits Gαq-coupled signals, has proved of value in experimental models of inflammatory disease, suppressing pain [33], BK-induced plasma extravasation in ratarthritic joints [34] and knee swelling in adjuvant-induced arthritis in rats [35]. Furthermore, B2 receptor antagonists have been shown to inhibit the release of neutrophil elastase, reduce the lipopolysaccharide-induced increase in vascular permeability, inhibit BK-induced release of arachidonic acid, IL-6 and IL-8, and to have an analgesic effect [36]. A recent clinical trial of HOE 140 for the treatment of hereditary angioedema provides conceptual support for its potential use as an IA therapy in swollen and painful RA joints [37, 38]. Further development of more specific B2 receptor antagonists may not only enhance understanding of the pathogenesis of inflammatory arthritis, but also provide novel therapeutic modalities.

The gene for the kinin B1 receptor is normally repressed, but is induced in experimental models of inflammation and sepsis [39]. Recently, kinin B1 receptor mRNA and protein were shown to be expressed in circulating neutrophils of asthmatic patients [40]. However, the present study is the first to demonstrate the presence of the kinin B1 receptor on circulating and SF neutrophils of RA patients. Furthermore, the kinin B1 receptor was up-regulated on circulating and SF neutrophils of RA patients when compared with circulating neutrophils from healthy volunteers, suggesting induction of the kinin B1 receptor in joint inflammation and a potential functional role for B1 receptors in RA joints. The exact role of the B1 receptor in joint inflammation requires further research, and this will be assisted by the development of specific B1 receptor antagonists.

Kinins are vasoactive peptides that induce the cardinal manifestations of inflammation, including pain, vasodilatation and oedema. In addition, kinins stimulate the release of IL-1β, TNF-α [16] and other secondary mediators of inflammation [6, 11, 18], and also enhance bone resorption by stimulating prostaglandin formation [17]. In this study, basal kinin levels and the capacity for kinin generation in SF were inversely correlated, suggesting that kinin generation may be subject to some degree of feedback inhibition by kinins in the inflammatory milieu. Furthermore, kininase activity is greater in inflamed compared with non-inflamed joints [41, 42], and the endogenous basal kinin levels may therefore have been even greater than those actually measured.

While the capacity for kinin generation in SF of RA patients has been demonstrated previously [25], this is the first report where the capacity for kinin generation in SF was correlated with clinical measures of disease activity. Thus, significant positive correlations were identified between kinin generation and SJC28 and TJC28, as well as ESR and CRP, reflecting an increase in the kinin-generating capacity of SF with increased disease activity. Presumably, more active disease would be associated with more severe joint inflammation, greater numbers of neutrophils in SF and hence greater amounts of kallikreins and kininogen substrate, resulting in an enhanced capacity for kinin generation in SF. The generated kinins are likely to have many pathophysiological effects that enhance and perpetuate inflammation of the rheumatic joint. Therefore, these results suggest a primary involvement of the kallikrein–kinin cascade proteins in the pathophysiology of RA, and provide evidence supporting the potential clinical application of kallikrein inhibitors [43] and kinin receptor antagonists [36, 44] as IA therapies. The results from the present study also have important implications for the development of novel therapeutic modalities that could potentially be used in the treatment of inflamed joints.

Rheumatology key messages

- Neutrophils generate kinins in SF of RA patients and kinin generation correlates with disease activity.
- Specific kallikrein and kinin receptor antagonists may have potential as IA therapies for inflamed joints.

Acknowledgements

We thank Dr Strinivasan Naidoo, Clinical and Experimental Pharmacology, Nelson Mandela School of Medicine, University of Natal, Durban, South Africa, for technical assistance with the kinin generation experiments and Ms Lorella Manso, PathWest, Perth, Western Australia, for technical assistance with immuno-histochemistry of synovial membrane tissue.

Funding: This work was supported by a grant from the Arthritis Foundation of Western Australia.

Disclosure statement: The authors have declared no conflicts of interest.

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