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Mast cells, cytokines, and metalloproteinases at the rheumatoid lesion: dual immunolocalisation studies

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Abstract

***Objectives*—To examine the distribution and activation of mast cells (MCs) in the rheumatoid lesion (cartilage-pannus junctions demonstrating cartilage erosion), and to determine whether or not their tissue distribution is related to that for tumour necrosis factor α (TNFα), interleukin-1 (IL-1), stromelysin-1, and collagenase.**

***Methods*—Immunolocalisation of MC-tryptase was used to identify MCs and their states of degranulation in 35 specimens of cartilage-pannus junctions. Dual immunolocalisation techniques using alkaline phosphatase and peroxidase conjugated antibodies were used to compare the distributions of MCs with the pro-inflammatory cytokines TNFα and IL-1, and the cartilage or matrix degrading enzymes stromelysin-1 and collagenase.**

***Results*—Stromelysin-1, TNFα, and IL-1β were especially prominent at the cartilage-pannus junctions, albeit with patchy distributions. Extracellular MC tryptase, indicative of MC activation/degranulation, was commonly observed at sites of cartilage erosion, and was often associated with the microenvironmental expression of TNFα, IL-1β, stromelysin-1, and collagenase. Such observations were often associated with localised sites of tissue oedema and stromal disruption.**

***Conclusion*—MC activation was frequently associated with proinflammatory cytokine and metalloproteinase expression by neighbouring cells, thereby suggesting an important contributory role for the MC in mediating matrix degradation and oedematous changes within microfoci of the rheumatoid lesion.**

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Rheumatoid arthritis (RA) is a disease characterised by chronic inflammation, cartilage destruction, and severe joint deformity. The proinflammatory cytokines, tumour necrosis factor (TNFα) and interleukin-1 (IL-1), are purported to play a pivotal role in the pathophysiological processes of joint destruction.[1 2] Both cytokines, mainly produced by synovial macrophages,[3–6] are known to induce the production of matrix degrading enzymes from synoviocytes and articular chondrocytes,[7–9] especially the matrix metalloproteinases stromelysin-1 and collagenase.[10 11] Stromelysin-1 is known to degrade various components of the cartilage matrix such as aggrecan and collagen types II, IX, X, and XI,[12–14] whereas collagenase is the rate limiting enzyme for the degradation of fibrillar collagens.[10 11] Cell–cell interactions, mediated via TNF and IL-1, have formed the basis of numerous reviews that focus attention on the activated macrophage and T cell as the prime instigators of a sequence of events resulting in cartilage damage.[1 2 15] However, the precise factors which activate macrophages to produce TNF and IL-1, and those that control metalloproteinase expression in the rheumatoid lesion, remain uncertain.

Immunolocalisation studies of cytokines and proteinases in rheumatoid synovial tissues have generally supported the concept of microenvironmental, rather than widespread, production of these proteins. For example, IL-1α and IL-1β were shown to be present in macrophages at the cartilage-pannus interface,[4 6] and similar observations were reported for TNFα.[3 5] Enzyme protein and mRNA for stromelysin-1 and collagenase have also been demonstrated in rheumatoid synovial tissue,[16–20] but, as with the cytokines, they often reflect discrete, microfocal expression. Such findings probably relate to the wide spectrum of histological observations reported for the rheumatoid lesion where local concentrations of fibroblasts, macrophages, neutrophils or mast cells have all been recognised at cartilage erosion sites, even within the same specimen.[21 22] Synovial fibroblasts and chondrocytes are recognised as effective producers of stromelysin-1 and collagenase, whereas granulocytes such as neutrophils and mast cells predominantly produce serine proteinases. By contrast, monocyte-macrophages have a modest capacity to elaborate similar matrix degrading enzymes,[23–25] but their ability to stimulate proteinase expression by neighbouring cells is well recognised.[7 9]

Over the past decade, an increasing number of papers have drawn attention to the mast cell (MC) and its potential contributions to the pathophysiological processes of RA.[26–28] Increased numbers of MCs are found in the synovial tissue and fluid of patients with RA,[28–30] and especially at sites of cartilage erosion.[21 31] MC activation or degranulation, as judged by extracellular tryptase, has recently been demonstrated in microfocal locations along the cartilage-pannus junctions of a significant proportion of rheumatoid specimens.[32] As the MC contains a variety of potent mediators such as histamine, heparin, proteinases,

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leukotrienes, and multifunctional cytokines,[33-35] its potential contribution to the processes of inflammation and matrix degradation seem evident. However, it remains a neglected cell in the pathogenesis of RA, and specific functional roles for the MC at the rheumatoid lesion have yet to be demonstrated. We use the term 'rheumatoid lesion' to describe cartilage-pannus junctions associated with cartilage loss and damage.

We have applied immunohistological techniques to 35 different cartilage-pannus junctions to examine the distribution of MCs, their states of activation, and whether or not their tissue distribution bears any relation to that for TNFα, IL-1α and β, stromelysin-1, and collagenase.

Materials and methods

TISSUE SPECIMENS

Specimens of cartilage-pannus junction and rheumatoid synovial tissue were obtained from 35 different patients with late stage classic RA undergoing arthroplasty of the knee (27 women and eight men; age range 46–79 years). Joint tissues were transferred to the laboratory in Hanks' balanced salt solution (HBSS) and junctional specimens of cartilage with overlying synovial tissue were carefully sampled. In addition, rheumatoid synovial tissues not immediately associated with cartilage or bone were obtained from 26 remedial synovectomies and processed similarly. All specimens were fixed in Carnoy's fixative for one hour, dehydrated and conventionally embedded in paraffin wax. Sections 5 μm thick were cut, dewaxed and rehydrated, and examined for the presence of mast cell tryptase, collagenase, and stromelysin, and the proinflammatory cytokines TNFα and IL-1α or β, using immunolocalisation techniques.

IMMUNOLOCALISATION OF MC TRYPTASE

Mouse monoclonal antibody to human MC tryptase (Chemicon International Inc. London, UK) was diluted 1:300 in Tris-buffered saline (TBS) pH 7·6, and applied to tissue sections for two hours at 20°C. After three 10 minute washes in TBS, alkaline phosphatase (AP) conjugated goat antimouse IgG (Dako Ltd, UK), diluted 1:50 in TBS, was applied for one hour at 20°C. After further washing the AP was developed using New Fuchsin. Tissue sections were lightly counterstained with Harris's haematoxylin.

The application of TBS or normal mouse IgG (Dako) in concentrations of IgG similar to those of the primary antibody was substituted for the primary antibody on control tissue sections and consistently produced negative observations.

IMMUNOLOCALISATION OF STROMELYSIN-1, COLLAGENASE, TNFα, IL-1α, AND IL-1β

For all antibody treatments, tissue sections were first pretreated with normal swine serum (NSS) diluted 1:10 for 30 minutes. Affinity purified rabbit polyclonal antibodies to either collagenase or stromelysin-1, shown to be monospecific by rigorous analysis as described previously,[25] were diluted 1:10 and 1:20 in TBS, respectively. Rabbit polyclonal antisera to the cytokines TNFα (generously provided by Dr S Foster, Zeneca Pharmaceuticals, UK), IL-1α and IL-1β (Genzyme, UK) were diluted 1:50, 1:25 and 1:50 in TBS, respectively. Each primary antibody was applied to tissue sections for two hours at 20°C. After three 10 minute washes in TBS, horseradish peroxidase (HRP) conjugated swine antirabbit IgG (Dako) diluted 1:50 was applied for one hour at 20°C. After further washing the peroxidase was developed using the substrate 1,3-diaminobenzidine (DAB) and the sections were counterstained with Harris's haematoxylin.

For controls, TBS or normal rabbit serum or IgG at dilutions similar to the primary antibodies were applied to tissue sections; these consistently gave negative results. Similar negative results were obtained when each primary antibody was immunoadsorbed with its specific antigen.

DUAL IMMUNOLOCALISATION OF MC TRYPTASE WITH STROMELYSIN-1, COLLAGENASE, TNFα, IL-1α, OR IL-1β

Tissue sections were pretreated for 30 minutes with a combination of normal goat serum and NSS, both at 10% (v/v) final concentration in TBS. After draining the sections were incubated at 20°C for two hours with a combination of primary antibodies: mouse anti-tryptase with any one of the rabbit polyclonal antibodies in TBS containing a final concentration of 5% (v/v) goat and swine sera. After three 10 minute washes in TBS, secondary antibodies (AP conjugated goat antimouse IgG, and HRP conjugated swine antirabbit IgG) were applied together at final concentrations as described, for one hour at 20°C, followed by three 10 minute washes in TBS. Peroxidase was developed first using DAB (brown colour), the sections were washed in tap water, followed by development of AP using New Fuchsin (red colour). The sections were lightly counterstained with Harris's haematoxylin to demonstrate nuclei, dehydrated, mounted in Histomount (Mensura Technology Ltd, Wigan, UK), and examined and photographed using a Zeiss Photomicroscope III and Kodak Ektachrome 160 Tungsten film.

Results

Stromelysin-1 was observed in most specimens, but was especially prominent in microfoci of the cartilage-pannus junctions (table). Its patchy distribution along these junctions included intracellular staining of synoviocytes with fibroblastic morphology and strong extracellular staining of the stromal matrix and cartilage interface (fig 1). Stromelysin-1 was often associated with tissue locations showing matrix disruption or localised oedema, both at cartilage erosion sites and in synovial stromal

Summary of immunolocalisation data

Antigen	*Cartilage-pannus junctions (n = 35)*	*Rheumatoid synovial tissue (n = 26)*	*Key to biological functions*
MC tryptase	Approx. 50% of specimens showed local accumulations of MCs, most with extracellular enzyme indicative of activation/degranulation.	All specimens contained MCs, but with variable distributions and states of activation both between and within individual specimens.	Tryptase: an enzyme unique to all human MC granules, but with poorly defined biological functions.[34 35]
Stromelysin-1	Approx. 90% of specimens showed some intra- and extracellular staining; most with a patchy distribution	Most specimens (approx. 75%) showed intracellular staining for some cells with fibroblastic morphology; occasionally extensive staining of stromal tissue observed.	Stromelysin-1: a matrix metalloproteinase known to degrade a variety of matrix components, including proteoglycans and most cartilage collagen species.[12 13]
Collagenase	Approx. 40% showed patchy staining; less obvious than that for stromelysin-1.	Approx. 40% of specimens stained; usually confined to fibroblastic cells and a patchy distribution on stromal tissue.	Collagenase: the matrix metalloproteinase specific and rate limiting for the degradation of the fibrillar collagens types I, II, and III.[10 11]
TNFα	Approx. 80% of specimens stained; occasional extracellular staining of cartilage matrix and intracellular staining of chondrocytes	Most specimens showed only intracellular staining; usually macrophages of the synovial lining layer.	TNFα, IL-1α, and IL-1β: proinflammatory cytokines known to induce the production of matrix metalloproteinases by synoviocytes and chondrocytes.[1 2]
IL-1β	Approx. 80% of specimens positive; extracellular distribution often reflecting that for TNFα.	Commonly observed for a proportion of macrophages, especially those in the lining layer.	
IL-1α	Staining limited to about 30% of specimens and usually associated with macrophages.	Mainly restricted to macrophages of the synovial lining layer.	

MC = Mast Cell.

tissue. In comparison, interstitial collagenase was less frequently demonstrated (table); approximately 40% of all specimens examined showed immunoreactive collagenase production, in accord with an earlier report.[16] However, the expression of both metalloproteinases was more marked at sites of cartilage erosion, occasionally showing diffuse staining within the cartilage matrix in addition to strong staining of the cartilage interface.

Immunolocalisation of the proinflammatory cytokines TNFα, IL-1α and IL-1β showed marked regional differences regarding their intra- or extracellular distributions. A patchy, extracellular demonstration of TNFα and IL-1β was observed for approximately 80% of cartilage-pannus junctions examined, whereas predominantly intracellular staining was observed for macrophages of the synovial lining and deeper layers (table). By contrast, IL-1α was less frequently observed—usually in cells of macrophagic morphology (table).

Immunolocalisation of MC tryptase proved to be a sensitive marker for synovial MCs, with extracellular staining providing some indication of MC activation/degranulation. Although MCs were observed in all synovial specimens, there were marked variations in regional distributions for each specimen. For example, local MC accumulations were often

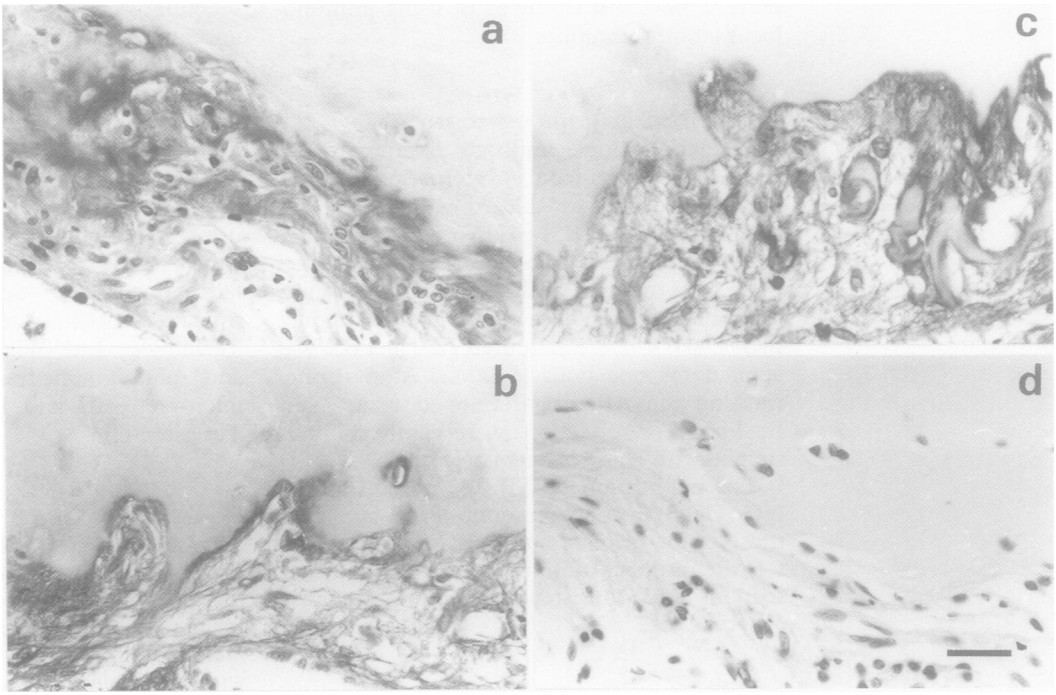

*Figure 1 Immunolocalisation of stromelysin-1 at the cartilage-pannus junction, visualised by peroxidase (dark staining). Note the patchy extracellular staining within the pannus (**a**), along the cartilage-pannus interface (**b**), and associated with cartilage fragments (**c**). Whilst most of the staining is extracellular, some synoviocytes in **a** and the chondrocytes in **b** show intracellular staining. Such observations were commonly restricted to microenvironments and seldom extended along the whole length of each cartilage-pannus junction. **d:** Control tissue section that received non-immune rabbit serum in place of the primary antibody, and counterstained with haematoxylin. Bar = 40 μm for each micrograph.*

observed in microfoci along cartilage-pannus junctions or in peripheral locations around lymphocytic infiltrations. Some specimens showed only intracellular tryptase demonstrating 'intact' MCs, these usually being surrounded by well ordered stromal tissue (fig 2a). In contrast, other specimens showed numerous MCs with extracellular 'halos' of tryptase, or diffuse staining of the stroma or cartilage matrix. Such observations, indicative of MC activation/degranulation, were commonly associated with histological evidence of stromal disruption and local oedema (fig 2). As these sites of activated MCs and matrix breakdown bore some resemblance to the tissue distributions for TNFα, IL-1β, stromelysin-1, and collagenase, dual immunolocalisation studies were used to examine such MC:cytokine:metalloproteinase associations.

The codistribution of extracellular MC tryptase and TNFα was commonly observed in microenvironmental sites along the cartilage-pannus junctions of most specimens (fig 3a, b). TNFα was seldom seen in tissue locations containing 'intact' MCs (those demonstrating only intracellular tryptase). Of special note was the TNFα expression by chondrocytes or cartilage invading cells at erosion sites associated with MC activation (fig 3b). Although TNFα is reportedly produced by MCs, neighbouring cells were more prominently stained for the cytokine. The tissue distribution of IL-1β was also observed, like TNFα, in tissue sites where MC degranulation was evident. Although IL-1β was often observed intracellularly within macrophage-like cells, in a few specimens its extracellular distribution was prominently observed at cartilage-pannus junctions associated with MC activation (fig 3d, e). In contrast, the localisation of IL-1α was less frequently observed and was only occasionally related to the distribution of extracellular MC tryptase (fig 3f). While most of the observations supported the co-distribution of TNFα and IL-1β in areas of MC activation, a few specimens showed tissue locations in which only one of these three antigens was localised.

As TNFα, IL-1, and soluble MC products are all reported to stimulate metalloproteinase production in vitro,[7-9 36] the distributions of stromelysin-1 and collagenase were examined for their relationship with MC activation. Stromelysin-1 was often observed in and around tissue sites containing extracellular tryptase (fig 4a, b), but was seldom observed in synovial tissue showing no evidence for MC activation (fig 4c). As with stromelysin-1, the distribution of collagenase was often related to areas of MC activation in most of the specimens (fig 4d, e, f), but we again emphasise the patchy, microenvironmental nature of these dual observations.

There were exceptions to these observations of codistribution, as one might expect; indeed, a few specimens showed no correlations for extracellular tryptase and the four antigens examined, whilst others showed variable

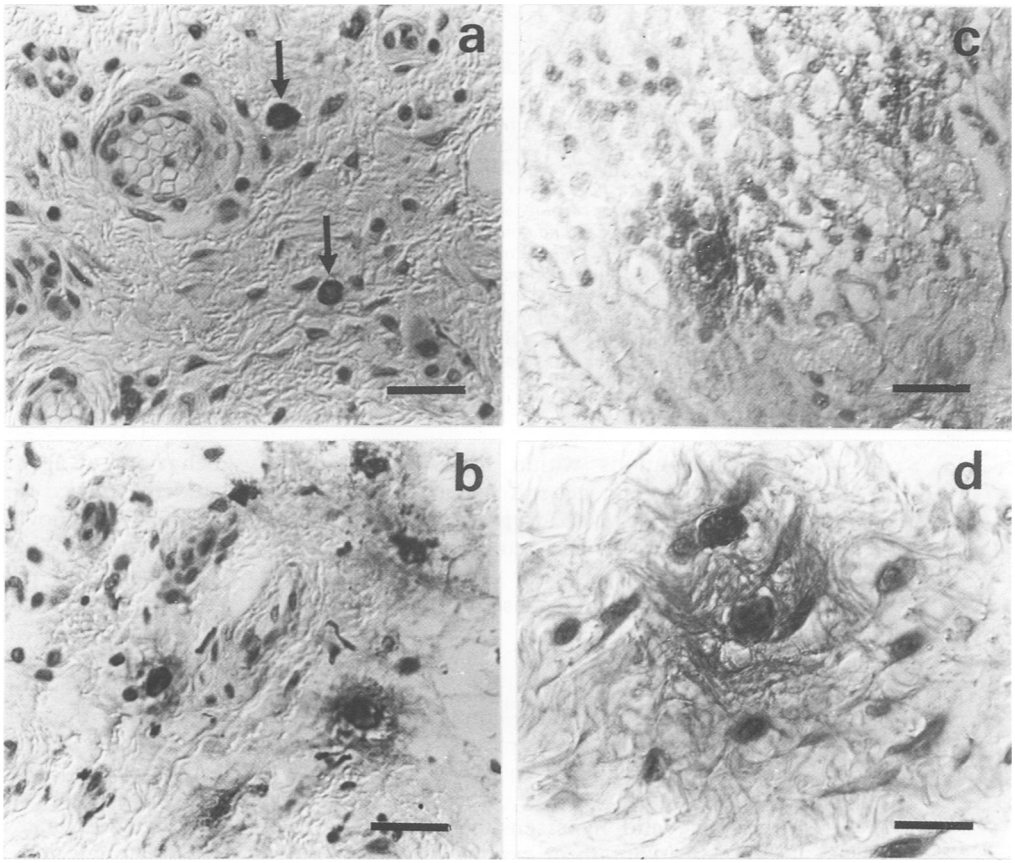

Figure 2 Immunolocalisation of mast cell (MC) tryptase in rheumatoid synovium viewed using Nomarski interference microscopy to illustrate the association of MC activation with stromal disruption/oedema. **a:** *Photomicrograph showing intact MCs (arrows) with no extracellular tryptase and well ordered stromal tissue.* **b–d:** *Photomicrographs showing extracellular tryptase indicative of MC activation and its common association with observations of local stromal disruption and oedema. All lightly counterstained with haematoxylin. a, b, c: bar = 40 μm;* **d:** *bar = 25 μm.*

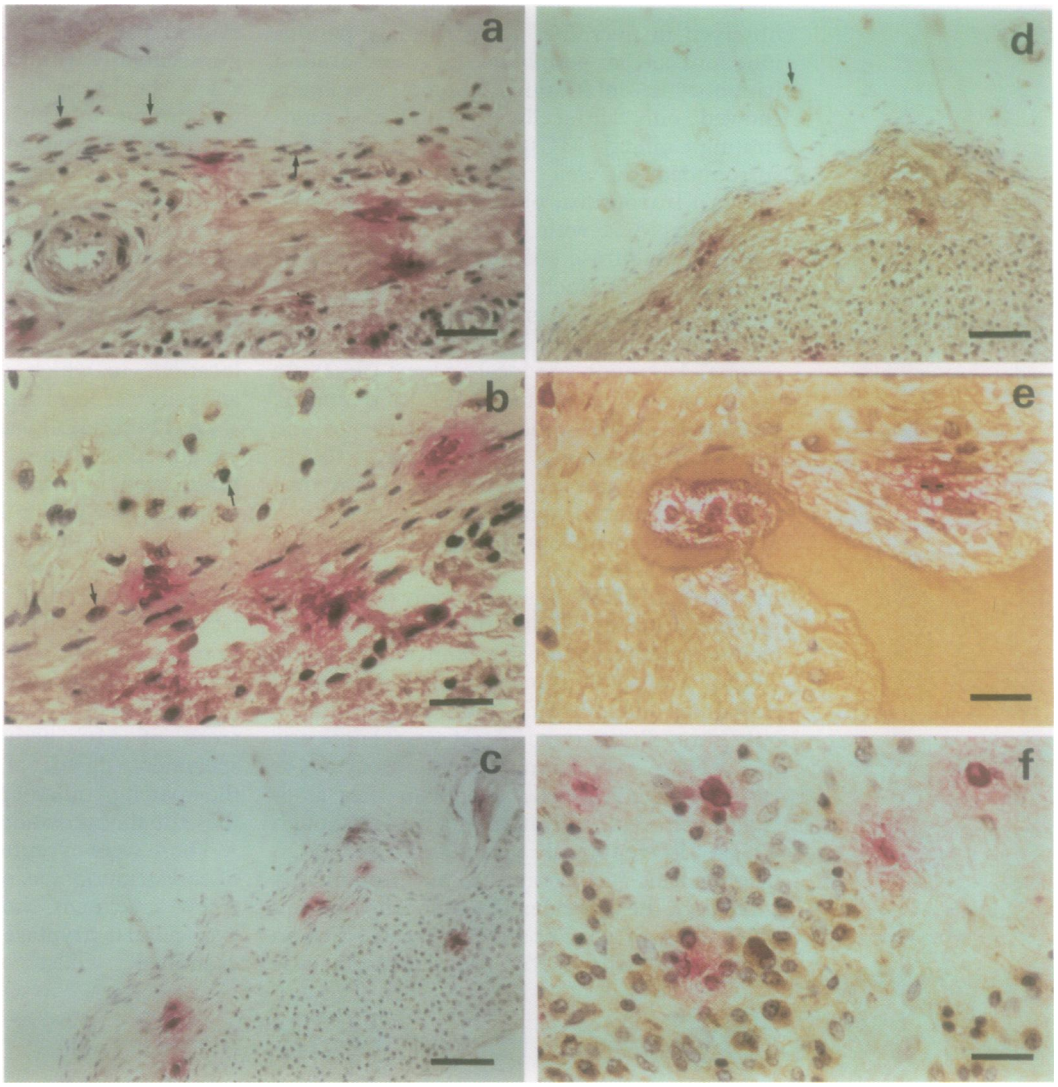

Figure 3 Dual immunolocalisation of mast cell tryptase with TNFα, or IL-1 at sites of cartilage erosion. **a:** *Low power photomicrograph showing extracellular staining of mast cell (MC) tryptase (red) and TNFα (brown) at the cartilage-pannus junction. Note the TNFα staining of synoviocytes and chondrocytes close to the interface (arrows). Bar = 40 μm.* **b:** *Photomicrograph showing MC tryptase and TNFα at cartilage-pannus junction. Note the close proximity of activated MCs with the cartilage matrix, and evidence of stromal disruption and lysis. Note also the strong TNFα staining for chondrocytes and/or cartilage invading cells (arrows). Bar = 25 μm.* **c:** *Photomicrograph of control tissue section stained for MC tryptase (red) and non-immune rabbit serum in place of the primary antibody for TNFα. Note total absence of peroxidase staining. Lightly counterstained with haematoxylin. Bar = 65 μm.* **d:** *Low power photomicrograph of a cartilage-pannus junction immunostained for MC tryptase and IL-1β. Note the extracellular staining of IL-1β especially pronounced on stroma close to the cartilage and also around chondrocyte lacunae (arrow). Bar = 65 μm.* **e:** *Photomicrograph showing MCs and their activation in close apposition with cartilage matrix and diffuse intra- and extracellular staining of IL-1β in this junctional microenvironment. Bar = 25 μm.* **f:** *Photomicrograph of rheumatoid synovium stained for MC tryptase (red) and IL-1α (brown). Note intracellular staining of IL-1α and the extracellular distribution of tryptase. Bar = 25 μm.*

codistributions within the same specimen. However, our general observation was that extracellular MC tryptase, TNFα, IL-1β, stromelysin-1, and collagenase were often associated with sites of matrix disruption, degradation, and localised oedema.

Discussion

Mast cells have been implicated in the expression of a variety of biological responses, and are considered to play a pivotal role in inflammation, tissue remodelling, angiogenesis, and hypersensitivity reactions.[33 35 37] As MCs contain or express numerous potent mediators including histamine, heparin, leukotrienes, cytokines, and growth factors,[33 34] it seems likely that MC secretion or activation could potentially bring about changes in the behaviour of all neighbouring cells. Previous studies showed that soluble MC products stimulated synoviocytes or chondrocytes in vitro to produce increased amounts of metalloproteinase and prostaglandin E,[36 38] and induced monocyte-macrophages to increase IL-1 production.[39] These effects are probably explained by the recent realisation that MCs can express several multifunctional cytokines, including the proinflammatory mediators TNF-α and IL-1β,[40-42] and the 'repair' cytokines such as IL-3, IL-4, IL-5, and IL-6,[43 44] all of which opens up numerous possibilities for the functional importance of MCs in the rheumatoid lesion. In addition, the potent MC mediator, histamine, has the capacity to transform the behaviour of synovial cells and chondrocytes via histamine H_1 and H_2 receptors,[26 45] and its effect on endothelial cells

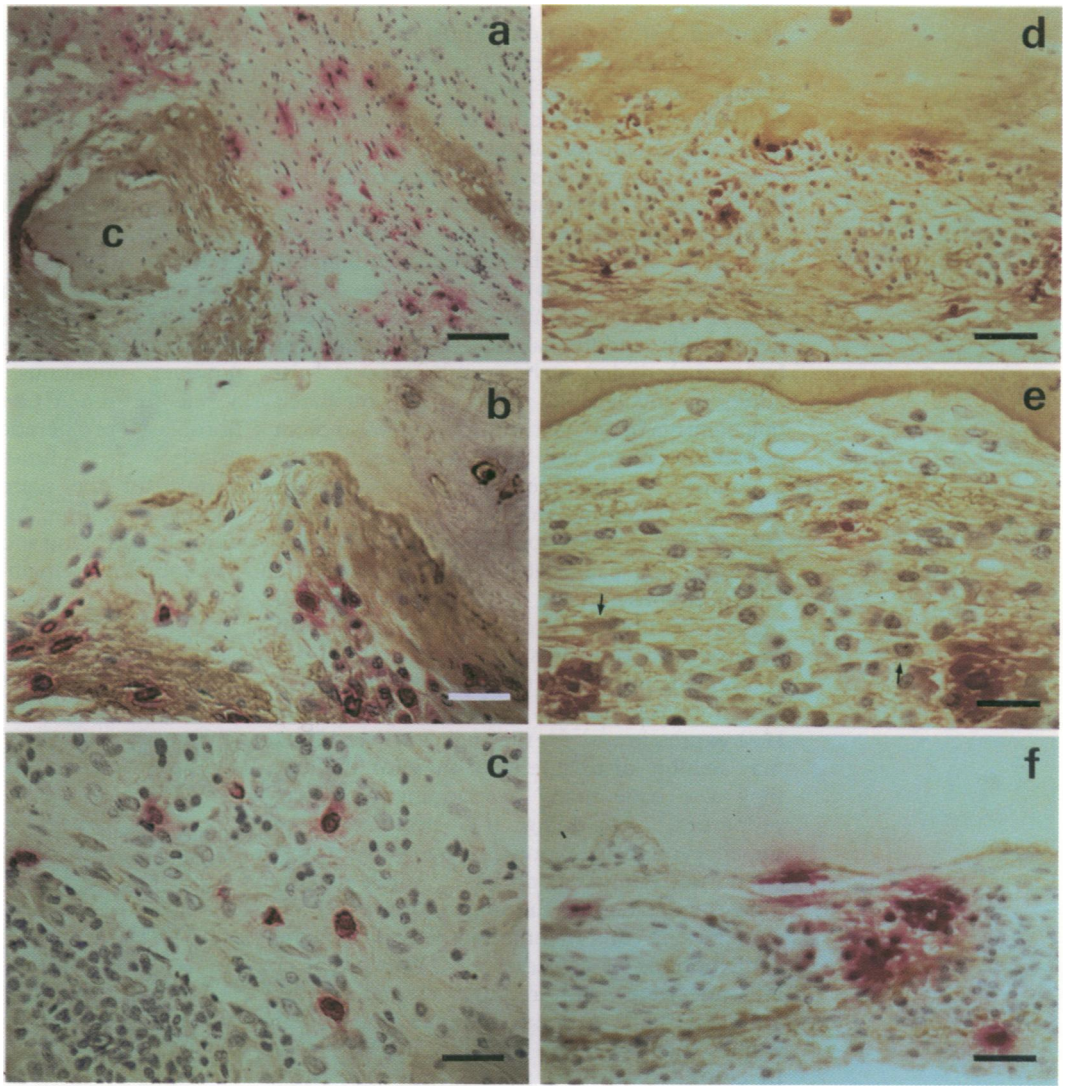

Figure 4 Dual immunolocalisation of mast cell (MC) tryptase with stromelysin-1 or collagenase in the rheumatoid lesion. **a:** *Low power photomicrograph showing MC tryptase (red) and stromelysin-1 (brown). Note extracellular staining of tryptase indicative of MC activation, and the patchy distribution of stromelysin-1, especially around the cartilage fragment (c). Note also the areas negative for both enzymes. Bar = 65 μm.* **b:** *Photomicrograph demonstrating MC tryptase (red) and stromelysin-1 (brown) at the cartilage-pannus-bone junction. Note the intense staining of stromelysin-1 associated with the stroma of the pannus, and the localised concentration of MCs in this microenvironment. Bar = 25 μm.* **c:** *Photomicrograph of rheumatoid synovium stained for MC tryptase and stromelysin-1. Note the presence of intact MCs, but the absence of staining for stromelysin-1 in this region. Bar = 25 μm.* **d:** *Low power photomicrograph showing MC tryptase (red) and collagenase (brown) at the cartilage-pannus junction. Note the extracellular tryptase staining for some MCs and the diffuse staining for collagenase within the cartilage matrix. Bar = 65 μm.* **e:** *Photomicrograph of a cartilage-pannus junction showing halos of extracellular tryptase (red) indicative of MC activation, and the strong collagenase staining (brown) at the edge of the cartilage. Note that some synoviocytes around the MCs are stained for collagenase (arrows). Bar = 25 μm.* **f:** *Photomicrograph showing extracellular tryptase (red), and patchy collagenase staining (brown). Note the stromal disruption associated with the area of MC activation. Bar = 40 μm.*

probably explains changes in vasopermeability[46] and the localised oedema associated with MC degranulation observed in this study.

The immunolocalisation of MC tryptase, an enzyme unique to all human MC granules,[34 35] has provided a useful means of identifying not only intact, non-activated MCs, but also those having undergone degranulation.[32] Our concerns as to whether some observations of extracellular tryptase may have been artificially induced by the physical trauma of tissue sampling were alleviated to some extent by the following observations: i) circular 'halos' rather than a unidirectional redistribution of MC granules possibly expected from tissue sectioning; ii) a common association of MC tryptase with sites of oedema and disruption which normally would require both a functional vasculature and time for enzymatic action; and iii) evidence of intracellular tryptase uptake by neighbouring cells as previously reported for fibroblasts and phagocytes.[32 47 48] Moreover, the consistent observations of only intracellular tryptase for a proportion of 'intact' MCs in virtually all specimens provided further reassurance that MC integrity was retained during sampling procedures.

Although MCs were demonstrated in every specimen examined, their increased distribution and apparent activation at cartilage-pannus junctions were evident in a significant proportion of specimens.[32] MC tryptase is a tetrameric enzyme which loses activity upon its dissociation from heparin.[49] As we do not know for how long the inactive tryptase monomers remain in the synovial tissue awaiting processing and elimination, it is difficult to judge precisely when MC degranulation

occurred before fixation. Thus it is possible that the tissue retention of redundant, extracellular tryptase may persist long after the shorter term events such as oedema, and cytokine and metalloproteinase expression have subsided; this provides a possible explanation for the lack of correlations in some specimens.

MC degranulation observed in this study may have several explanations, including the possibilities of stimulation by soluble factors from T lymphocytes, monocyte-macrophages, neutrophils, endothelial cells and platelets, or by neuropeptides such as substance P.[35] In addition, involvement of the IgE mediated mechanisms of MC activation would also seem possible, either via IgE associated antiglobulins[50] or by the IgE sensitisation to cartilage collagens that has recently been reported for a proportion of patients with RA.[51] Whatever the cause of MC degranulation, a common consequence appears to be localised matrix disruption, probably reflecting both oedema and proteolysis.

TNFα is purported to play a pivotal role in RA, not least through its ability to promote IL-1 expression.[2 52 53] Although TNF and IL-1 may exhibit distinct effects in joint disease, they also have many overlapping biological properties,[54] including the stimulation of metalloproteinase expression by synoviocytes, chondrocytes and osteoblasts.[7-9] While macrophages appear to be the major source of both cytokines in the rheumatoid lesion,[3-6 55] the contributions of MC derived TNF and IL-1 are uncertain and require further study, although there is good evidence from other studies that MCs can elaborate TNFα.[40 41 56] In contrast, we have no evidence that MCs elaborate stromelysin-1 or collagenase, these being derived primarily from 'activated' fibroblasts.[24 25] It therefore seems likely that the tissue distribution of the metalloproteinases probably reflects areas of local stimulation, such as that provided by MC degranulation, macrophage activation, or both. While it is tempting to suggest a sequence of events such as MC activation →oedema and macrophage cytokine production →fibroblast/chondrocyte metalloproteinase production, it is also likely that the codistributions observed reflect complex, possibly cyclical cellular interactions. Moreover, since MC-tryptase activates the inactive precursor of stromelysin,[57 58] and MC chymase is capable of activating both prostromelysin and procollagenase,[58-60] it would seem that MCs have the ability not only to stimulate metalloproteinase production directly and indirectly, but also subsequently to activate both precursors.

Our studies support the view that individual cartilage erosion sites reflect transient or intermittent chondrolytic activity, these being spatially and temporally separated with regard to specific cell types, and cytokine and proteinase production.[21 22] Such microenvironmental locations appear to be governed by locally regulated signals such as cytokines, prostanoids, immune complexes, and local degradation products, which may all contribute to the recruitment, activation, and proliferation of specific cells. This study indicates that the MC could make a major contribution to the pro-inflammatory and degradative events within microfoci of the rheumatoid lesion, MC activation often being associated with localised cytokine and proteinase release. Its potential influence on the behaviour of macrophages, synoviocytes and chondrocytes, and its presence at sites of matrix disruption and oedema, suggest it should now be recognised as a serious contributor to the cellular interactions leading to cartilage destruction. There are also, however, numerous publications advocating a role for the MC in fibrosis, angiogenesis, fibroblast proliferation, and tissue homeostasis[33 37 61 62]—functional roles probably of import for the scarring process of synovial pannus formation. As yet, the factors and conditions controlling such a spectrum of potential MC functions remain unresolved.

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