Why does chronic inflammatory joint disease persist?

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ABSTRACT – Inflammation is a beneficial host response to tissue damage. Most episodes of inflammation resolve spontaneously and do not persist. However, in rheumatoid arthritis (RA), as in a number of other chronic inflammatory diseases, the inflammatory response persists and a stable inflammatory infiltrate accumulates in the joint. What drives this persistence and the relative contribution of infiltrating leucocytes and stromal cells such as fibroblasts to the stability of the inflammatory process are the subject of this article.

Fibroblasts play an important role in defining the disordered synovial microenvironment in RA. Through their production of a variety of cytokines and constitutive chemokines they directly alter the behaviour of infiltrating leucocytes, leading to their inappropriate survival and retention. These findings suggest that stromal cells such as fibroblasts play an important role in the switch from acute resolving to chronic persistent arthritis by allowing lymphocytes to accumulate in the wrong place at the wrong time.

KEY WORDS: chemokine, chronic inflammation, fibroblast, lymphocyte, rheumatoid arthritis

Inflammatory responses occur within tissue microenvironments. Such environments are complex and composed of many different cell types, often at different stages of activation and differentiation. Current models of inflammatory synovitis stress the role of antigen-specific lymphocyte responses and attempt to address the causative agent. Recent studies have challenged the primacy of the lymphocyte and have begun to focus on an extended immune system in which stromal cells such as macrophages and fibroblasts also play a role.[1,2]

Rheumatoid arthritis (RA), the most common inflammatory arthritis, is characterised by a disordered synovial microenvironment in which there is hyperplasia of resident stromal cells and a heavy infiltration of haematopoietic cells such as T and B lymphocytes.[3] A characteristic feature of the inflammatory infiltrate is the accumulation of lymphocytes into distinctive micro-anatomical structures with architectural features that strongly resemble lymphoid tissue. Why this occurs has remained unclear. The studies described here attempted to address the molecular basis for this feature and to focus on the relative contribution to the persistence of the inflammatory lesion of both haematopoietic and stromal cells.

The rationale for attempting to solve this long-standing puzzle lies in the assumption that unravelling the molecular basis of this process would lead to a clearer understanding of why chronic inflammation persists. The spectacular success of therapy with antitumour necrosis α (TNFα), an agent with potent effects on leucocyte migration and accumulation within tissues, has provided an excellent precedent for this and strong reason to suspect that such an assumption is valid.[4]

The dynamics of an inflammatory infiltrate

Little is known about how leucocyte numbers are regulated during inflammatory responses within tissues.[2] Host responses to tissue injury involve a complex interplay of diverse cellular, humoral and connective tissue elements which prevent tissue invasion and ultimately re-establish normal tissue integrity. During the early stages of an inflammatory response, large numbers of leucocytes are recruited from the peripheral blood in response to injury or infection. Clearance of unwanted effector cells at the end of an inflammatory response appears to be due to loss of survival signals derived from interactions with stromal cells, leading to apoptosis and subsequent phagocytosis of dead cells.[2] In chronic inflammation, the resolution phase becomes prolonged and disordered, leading to persistence of the inflammatory infiltrate, tissue hyperplasia and ultimately tissue scarring.

Key Points

Chronic inflammation shares molecular features with lymphoid neogenesis

A persistent leucocyte infiltrate at sites of chronic inflammation reflects a distorted homeostatic balance between factors that enhance cellularity (recruitment and proliferation) and those that decrease cellularity (cell death and emigration)

Defining the relative contribution of infiltrating leucocytes and resident stromal cells to the persistence of the inflammatory process is an important therapeutic challenge

Persistence of a leucocyte infiltrate at sites of chronic inflammation reflects a distorted homeostatic balance between factors that enhance cellularity (leucocyte recruitment, proliferation, and retention) and those that decrease cellularity (cell death and emigration) (Fig 1). The mechanisms responsible for the recruitment of leucocytes into and their proliferation within tissues have been well studied, but those responsible for their survival, retention and emigration have attracted much less attention.

'Area codes' for lymphocytes

The recruitment of leucocytes to tissues is not a random process but depends on an overlapping sequence of molecular interactions between leucocytes and endothelial cells. There is now overwhelming evidence in support of the 'area-code' model of lymphocyte homing. This model, based on the analogy of the post office delivering letters using a post or zip code, proposes a sequential, combinatorial series of steps by which appropriate leucocyte subsets leave the peripheral circulation and enter various tissues via post-capillary venules.[5] A variety of adhesion molecules and chemokines expressed on endothelial cells are used for this. Chemokines and their receptors are important molecular 'signposts' for leucocytes.[6] They support the navigation of lymphocyte subsets across endothelium and into tissue, and play a key role in positioning and retaining lymphocytes within tissues.
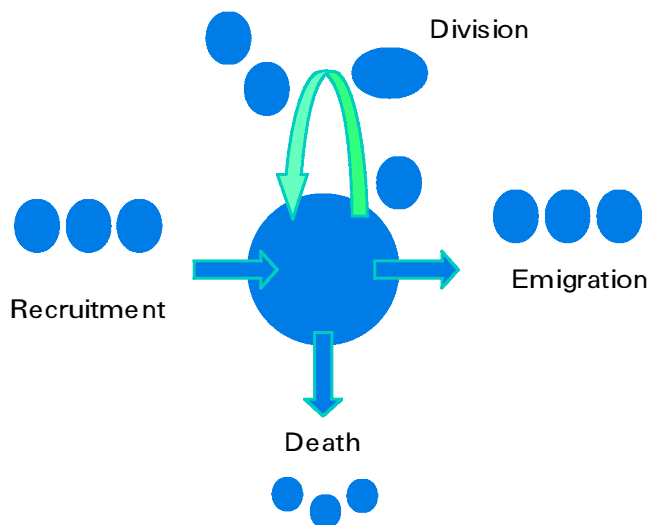
This area-code model provides an elegant molecular explanation for the differential distribution of naïve, memory and effector cells within distinct regions of the body. For example, naïve CD45RA T cells migrate predominantly to lymphoid tissue using the adhesion receptor L-selectin and the chemokine

receptor CCR7; they are effectively excluded from peripheral tissues due to lack of appropriate chemokine receptors. In contrast, effector CD45RO T cells, which express a wide range of chemokine receptors, are predominantly found in peripheral tissues and are excluded from lymphoid tissue unless they also express the lymphoid homing receptor CCR7.

Recent studies have extended this model and shown that distinct T cell homing subsets exist within the CD45RO T cell population with a predilection for homing to skin (CLA$^+$, CCR4$^+$) and intestine ($\alpha 4\beta 7^+$, CCR9$^+$).[7] Even in the presence of inflammation, only CLA$^+$, CCR4$^+$ CD45RO T cells are found in the skin and only $\alpha 4\beta 7^+$, CCR9$^+$ CD45RO T cells in the gut; this implies that, despite ongoing inflammation, site-specific trafficking is faithfully maintained.[5] Interestingly, such fidelity seems to be lost in RA, in that CCR4$^+$, $\alpha 4\beta 7^+$ expressing T cells are found enriched in the synovial compartment although these T cells do not express CCR9 or CLA (Fig 2). This might provide an explanation why skin diseases such as psoriasis and also in inflammatory bowel disease are often associated with inflammatory arthritis.

This prevailing paradigm to account for the selective accumulation of distinct leucocyte subsets at sites of inflammation derives from the supposition that selectivity occurs at the point of entry (endothelial selection). Selection in the tissue (stromal selection) has received little attention, despite the well-defined role for stromal elements in the bone marrow and thymus during lymphocyte development. After crossing post-capillary venules into the subendothelial compartment, leucocytes encounter a stromal microenvironment quite distinct from that in the vascular compartment. In order to interact with this local microenvironment, leucocytes need to adhere to stromal elements or matrix. The transition from a migratory to a stationary phenotype occurs as a consequence of changes in adhesion molecules and chemokine receptors in response to local activating signals such as cytokines, chemokines, growth factors and, in the case of T lymphocytes, engagement of the T cell receptor by antigen. Therefore, we postulate that the stromal microenvironment might directly affect the behaviour of T cells that accumulate in inflamed joints, leading to changes in T cell survival and retention.

The synovial-stromal microenvironment prevents T cell death

The successful resolution of an inflammatory response requires the removal of the vast majority of immune cells that were recruited and expanded during the active phase of the response. A number of studies have shown that the initial increase in T cell numbers in peripheral blood in the first few days of the resolution phase of viral infections is followed by a wave of apoptosis occurring in the activated T cells.[2] This situation is mirrored in tissues, where Fas-induced apoptosis occurs at the peak of the inflammatory response and may be responsible for limiting the extent of the immune response. In contrast, the resolution phase appears to be principally triggered by cytokine deprivation-induced apoptosis.[2]

Fig 1. The dynamic balance of cell accumulation in any tissue compartment. This depends on the balance of cell recruitment, emigration, division and death. Homeostasis is maintained during normal inflammatory responses, leading to resolution.[1]

We have now identified that factors such as type I interferons (IFNs), produced by synovial fibroblasts and macrophages, are responsible for the prolonged and inappropriate survival of T cells in the rheumatoid joint.[8] This stromal survival mechanism leads to T cell survival in the absence of cell proliferation and is fully consistent with the phenotype of T cells found in vivo within inflamed joints (quiescent, noncycling). It is likely that such a mechanism occurs in many chronic inflammatory conditions where T cells accumulate with many of the phenotypic characteristics seen in the rheumatoid joint.

The synovial microenvironment promotes T cell retention

The inhibition of T cell apoptosis by IFNβ at sites of chronic inflammation is clearly a crucial factor in T cell accumulation, but does not explain the long-term stability of inflammatory infiltrates. If the recruitment of cells is episodic, mostly occurring during periods of systemic immune activation, why do the cells not simply leave the joint during periods of quiescence?

Are synovial T cells actively retained within the synovial microenvironment? We have found that the synovial microenvironment directly contributes to the inappropriate retention of T cells by an active chemokine-dependent process.[9] Thus, the

inflammatory infiltrate in RA appears to persist as a direct result of the sustained recruitment, inappropriate retention and enhanced survival of cells mediated by stromal factors associated with the local microenvironment itself. These observations provide compelling evidence that the synovial microenvironment directly contributes to the inflammatory process by modulating the behaviour of leucocyte subsets that accumulate in the rheumatoid joint.

Fibroblasts define the microenvironment in the rheumatoid synovium

Fibroblasts are ubiquitous cells that give mechanical strength to tissues by providing a supporting framework of extracellular matrix. They are extremely versatile cells and display a remarkable capacity to differentiate into other members of the connective tissue family, including cartilage, bone, adipocyte and smooth muscle cells. Fibroblasts from different anatomic regions display characteristic phenotypes that are maintained even after prolonged culture *in vitro*, suggesting that many fibroblasts have a remarkably stable imprinted phenotype.[10,11] Even within a single tissue there is growing evidence that fibroblasts are not homogeneous but exist as subsets of cells, much like tissue macrophages and dendritic cells. It is likely that connective tissue contains a mixture of distinct fibroblast lineages with mature fibroblasts coexisting with immature fibroblasts (often
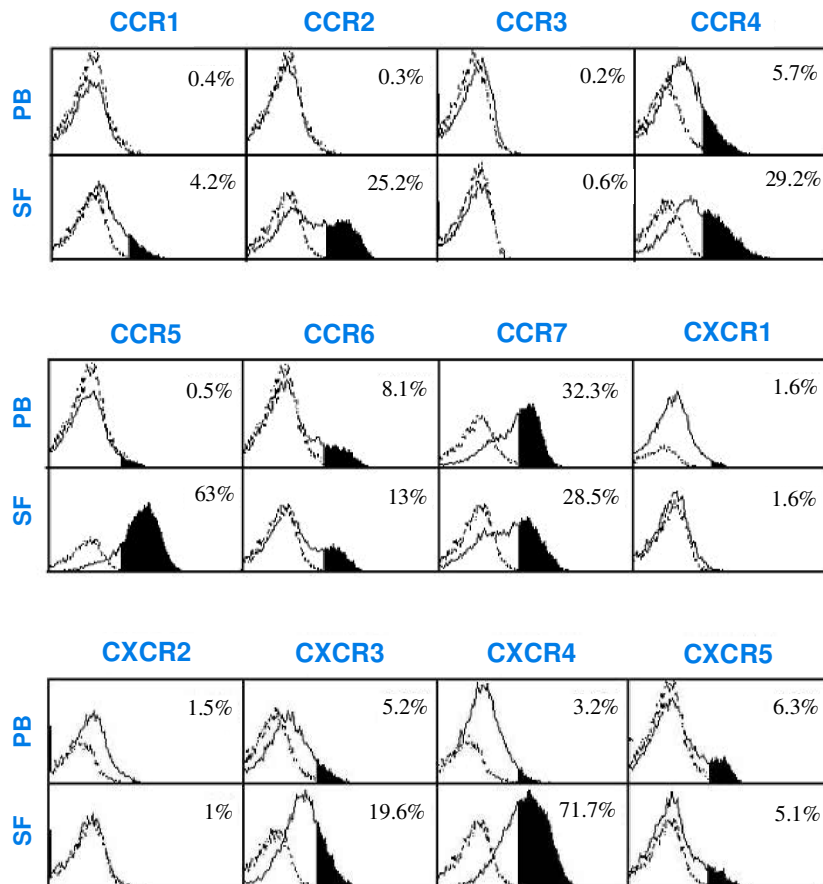
Fig 2. Expression of a range of chemokine receptors on peripheral blood (PB) and synovial fluid (SF) CD3+ T cells.
CD3+ CD45RO+ T cells were analysed for the expression of chemokine receptors using three-colour flow cytometry (full line). The percentage of positive cells compared with the irrelevant isotype matched control (dotted line) is shown in the top right-hand of each box (shaded black).

called mesenchymal fibroblasts) capable of differentiating into other connective tissue cells. Tantalising evidence now suggests that fibroblast precursors circulate in peripheral blood.[12] These cells share many properties of bone marrow stromal stem cells and are able to differentiate into several cell lineages.[13]

It is becoming increasingly clear that fibroblasts are not passive players in immune responses. Different fibroblasts secrete distinct patterns of cytokines and chemokines and express variable levels of costimulatory molecules such as CD40, suggesting a fundamental role in immune responses and diseases processes.[10] This diversity in phenotype and function that characterises fibroblasts from different anatomical sites may play a significant role in the intrinsic susceptibility of different organs to inflammatory insults. It may also provide the molecular basis for the well described, but as yet poorly understood, clinical finding that relapses in chronic inflammation are often tissue- and site-specific.

Chronically inflamed tissues such as the rheumatoid joint often contain lymphoid aggregates that share many of the structural and functional features of secondary lymphoid tissue.[14] This suggested to us that, as occurs in lymphoid neogenesis, stromal cells within the inflamed synovium might overproduce chemokines that play a role in lymphoid organogenesis. Using standard immunohistochemical analysis, we have found expression of BCA-1 (CXCL13), SDF-1 (CXCL12) and lower levels of ELC (CCL19) in the rheumatoid joint (Fig 3). We are uncertain which cells produce these chemokines, but have found that only rheumatoid fibroblasts can produce extraordinarily high levels of chemokines, as assessed by gene expression profiling with microarrays (Fig 4).

The rheumatoid synovium as a 'foster home' for lymphocytes

The transition from an acute inflammatory response to acquired immunity is a vulnerable time for the immune system. In order to generate an efficient adaptive immune response to antigen, immature dendritic cells must sample antigen in inflamed tissue and then migrate to the draining lymph node where they present antigen to T cells.[15] This process is spatially separated from the site of inflammation and requires careful chemokine mediated choreography for the appropriate immune cells to encounter each other. During this time, stromal elements, largely defined by fibroblasts, attempt to repair damaged tissue. At the resolution of the primary response, a small number of lymphocytes must be retained for immune memory to occur. Therefore, the successful resolution of an immune response is a tightly regulated balancing act between keeping enough antigen-specific T cells alive to allow a memory response and repair of damaged tissue within the inflammatory microenvironment.[16]

Based on this central requirement for a small number of lymphocytes to be rescued from apoptosis and retained in a physiologically supportive niche, it is tempting to suggest that chronic inflammation occurs when these two processes are subverted. In such cases, the wrong cells (lymphocytes/dendritic cells) accumulate in the wrong place (in tissue) at the wrong time (during the resolution phase of inflammation). Thus, chronically inflamed tissue such as the rheumatoid synovium appears to act as a 'foster home' for leucocytes, leading to their inappropriate retention and survival. This mechanism not only provides a molecular explanation for the similarities between lymphoid

Fig 3. Expression of constitutive chemokines in rheumatoid synovial tissue. Rheumatoid synovial tissue expresses the constitutive chemokines ELC (CCL19), SDF-1 (CXCL12) and BCA-1 (CXCL13). Comparison of sections from tonsil and rheumatoid synovial tissue using immunoperoxidase (brown) are shown. For rheumatoid synovial tissue, alkaline phosphatase (red) was used (RA = rheumatoid arthritis).
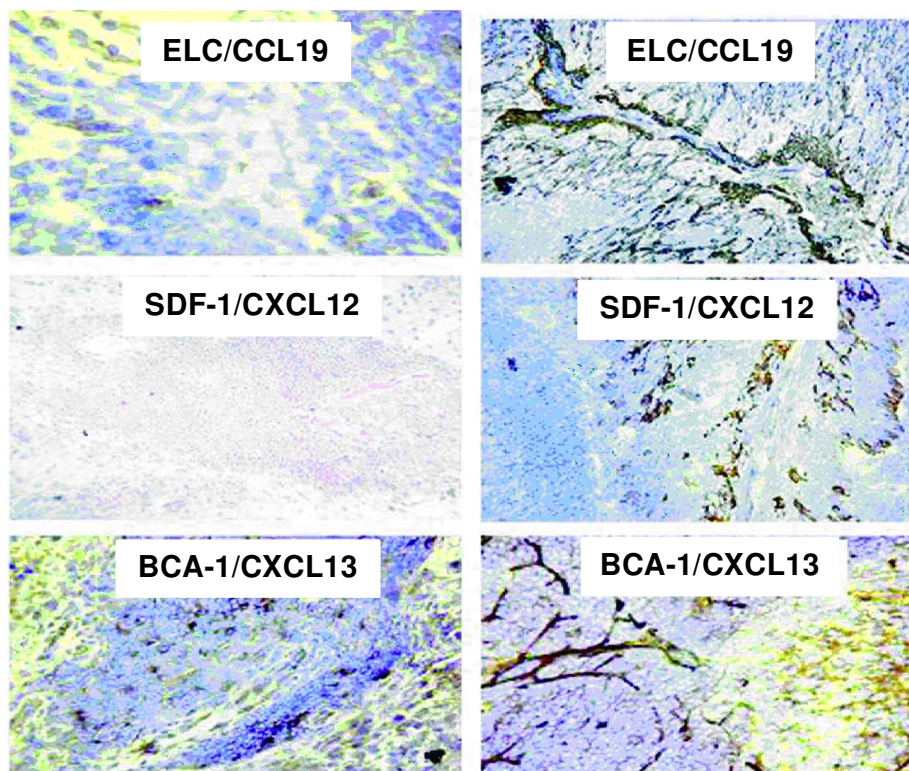
aggregates in the rheumatoid synovium and lymphoid tissue, but also provides a potential functional explanation for the success of anti-TNF therapy in RA, since TNF family members are needed for efficient lymph node development.

Chemokines: the link between lymphoid neogenesis and chronic inflammatory arthritis?

The findings described for RA explain why rheumatoid tissues mimic many of the structural features of stable supportive stromal cell niches such as the bone marrow and lymphoid tissue. To determine whether this is a unique feature of RA or represents a universal feature in many chronic rheumatic diseases we examined another chronic rheumatic disease, Sjögren's syndrome (SS). This disease is characterised by the accumulation of lymphocytes in salivary, lachrymal and other exocrine glands in the respiratory and gastrointestinal tracts and the vagina. The inflammatory infiltrate consists predominantly of CD4+ T cells and fewer CD8+ T cells, B cells and plasma cells that first appear as small clusters around ductal tissue, later enlarging to form structures resembling ectopic germinal centres (GCs). The development of such ectopic lymphoid follicles has been implicated in the pathogenesis of SS since large amounts of autoantibodies, including rheumatoid factor and anti-Ro and anti-La, are a characteristic feature of the syndrome.

TNF and lymphotoxin play a critical role in the development and maintenance of lymphoid tissue.[17] The lack of normal lymphoid organs in mice deficient in the lymphoid homing chemokine receptors CXCR4 and CXCR5 has also implicated chemokines such as SDF-1 (CXCL12) and BCA-1 (CXCL13) in

lymphoid neogenesis.[18] Elegant studies in transgenic mice overexpressing the constitutive lymphoid chemokine BCA-1 have confirmed that lymphotoxin, TNF, and BCA-1 act in a common pathway of lymphoid neogenesis.[19] Moreover, these studies have suggested that the molecular mechanisms responsible for lymphoid development during embryogenesis share many features with lymphoid aggregates in chronic inflammation, particularly with regard to their production of chemokines.

We therefore postulated that accumulation of lymphocytes in GC-like structures in inflamed tissue in SS is associated with the ectopic expression of the lymphoid tissue homing chemokines BCA-1 and SDF-1. Using immunohistochemistry to determine the expression of chemokines and their receptors, lymphoid aggregates in SS were found to share many of the structural features of GCs, including the expression of BCA-1 by stromal cells within GC—like follicles. Inflamed glands from patients with SS, but not control tissue, expressed very high levels of BCA-1 on endothelial like structures that were abundantly expressed throughout the inflamed tissue. In contrast, SDF-1 was expressed on ductal epithelial tissue.[20] Taken together with observations from transgenic mice that overexpress constitutive chemokines such as BLC (CXCL13) and SLC (CCL21), these findings suggest that the inappropriate temporospatial expression of chemokines plays an important role in determining the persistence and patterning of lymphoid aggregates in chronically inflamed tissues. In addition, they provide strong support for the hypothesis that the ectopic expression of chemokines is a general feature that drives leucocyte accumulation in chronic inflammatory rheumatic diseases.
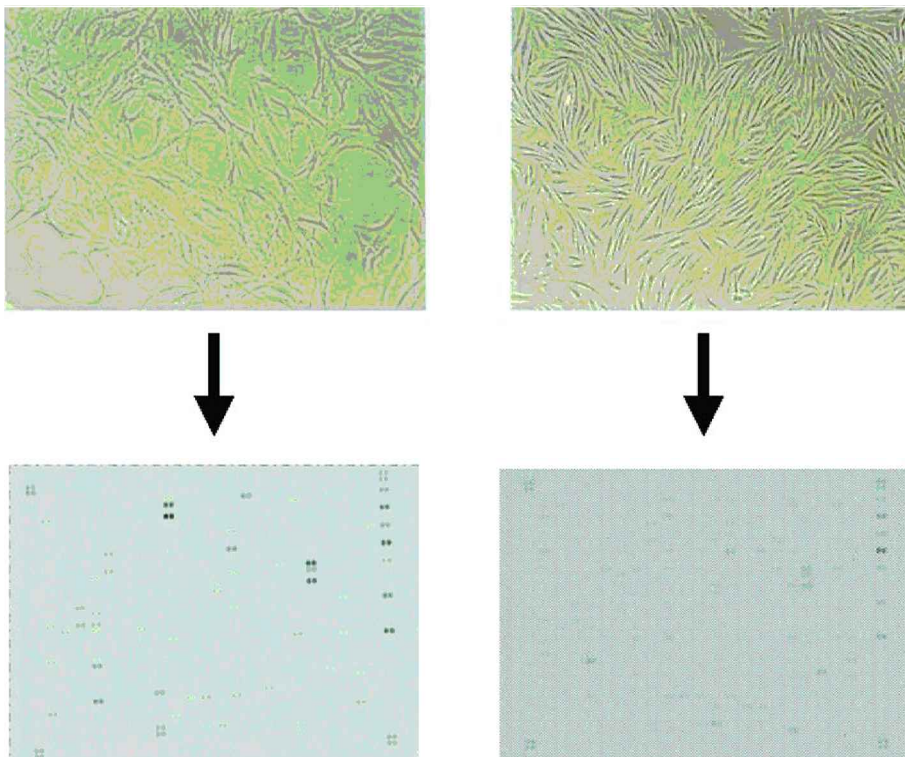

Fig 4. Illustration of the difference between fibroblasts. Fibroblasts are not all the same. Microscopy of rheumatoid synovial and skin fibroblasts shows that rheumatoid fibroblasts are more dendritic in morphology. When stimulated with tumour necrosis factor α (24 hours at 10 µg/ml), the rheumatoid synovial fibroblasts produce different gene expression patterns from those of skin fibroblasts, as analysed by hybridisation to over 400 known genes in this microarray.

Conclusions

The immune system is a diverse collection of many cell types spatially and temporally separated from each other. For efficient immune responses to occur, cells of the innate and acquired immune system must interact with each other. They do this both through release of soluble mediators and through direct cell-cell contact. An emerging theme in recent years is that cells of mesenchymal origin such as fibroblasts play a critical role in modulating leucocyte behaviour and function. These cells have a wide and varied biosynthetic repertoire and, together with tissue macrophages, act as sentinel cells for the immune system. As a result of their production of chemokines and extracellular matrix, fibroblasts actively define tissue microenvironments and play an important role in the transition from acute inflammation to acquired immunity.

We have systematically examined the hypothesis that chronic inflammatory joint diseases persist because of an abnormal stromal microenvironment in which fibroblasts play a dominant role. The inappropriate production of chemokines and matrix components by fibroblasts has dramatic effects on cells of the acquired immune system and may lead to the establishment of chronic inflammation. Therefore, targeting the stromal microenvironment in general, and tissue fibroblasts in particular, is likely to be an important target for future anti-inflammatory therapy.

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