Chronic inflammation is characterized by the accumulation of leucocytes within tissues. In rheumatoid arthritis the inflammatory infiltrate shares many architectural features with lymphoid tissue. For example, CD4 T cells and B cells accumulate in perivascular lymphoid structures within synovial tissue. CD8 T cells and neutrophils are found predominantly within synovial fluid. What drives these distinctive lymphoid microstructures and the relative contribution of lymphocytes and stromal cells such as fibroblasts to this process is the subject of this review. Cellular interactions between leucocytes and stromal cells such as macrophages and fibroblasts are important in generating tumour necrosis factor-α within the inflamed synovium. Therefore understanding how leucocytes accumulate within the inflamed synovium is likely to provide new therapeutic approaches to modify the inflammatory process. We have found that fibroblasts play a dominant role in defining the disordered synovial microenvironment in rheumatoid arthritis. Through their production of a variety of cytokines (interferon-β, transforming growth factor-β) and constitutive chemokines (stromal cell-derived factor-1, CXCL12) they directly alter the behaviour of lymphocytes that accumulate within chronically inflamed joints leading to their inappropriate survival and retention. We have extended these observations to another chronic persistent rheumatic disease, Sjögren’s syndrome, and found that ectopic production of the constitutive B cell-attracting chemokine BCA-1 (CXCL13) is associated with lymphocyte accumulation and lymphoid tissue formation. 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:** Leucocyte, Inflammation, Cell adhesion, Chemokines, Rheumatoid arthritis, Fibroblast.
the accumulation of lymphocytes into distinctive micro-anatomical structures with architectural features that strongly resemble lymphoid tissue [4]. Why this occurs has remained unclear, but the studies described here have attempted to address the molecular basis for this and focus on the relative contribution 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 will lead to a clearer understanding of why chronic synovitis persists. The spectacular success of anti-tumour necrosis factor-α (anti-TNF-α) therapy, 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 [5].

**The dynamics of an inflammatory infiltrate**

Little is known about the way leucocyte numbers are regulated during inflammatory responses within tissues. Host responses to tissue injury involve a complex interplay of diverse cellular, humoral and connective tissue elements, which prevent tissue invasion and which, 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 [6]. 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. In chronic inflammation the resolution phase becomes prolonged and disordered leading to persistence of the inflammatory infiltrate, tissue hyperplasia and ultimately tissue scarring.

The maintenance of a persistent 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). While the mechanisms responsible for the recruitment of leucocytes into tissues and their proliferation within them have been well studied, those responsible for their survival, retention and emigration have attracted much less attention.

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 by which the post office delivers 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 [7]. To do this they use a variety of adhesion molecules and chemokines expressed on endothelial cells. Chemokines and their receptors are important molecular ‘signposts’ for leucocytes [8]. 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 and are effectively excluded from peripheral tissues because of lack of appropriate chemokine receptors [9]. In contrast, effector CD45RO T cells, which express a wide range of chemokine receptors, are found predominantly within peripheral tissues and are excluded from lymphoid tissue unless they also express the lymphoid-homing receptor CCR7 [7]. In fact the presence or absence of CCR7 on CD45RO T cells describes an important functional split in T-cell function into effector and memory cells [10, 11]. Recent studies have extended this model and shown that within the CD45RO T-cell population distinct T-cell homing subsets exist with a predilection for homing to skin (CLA+, CCR4+) and intestine (α4β7+, CCR9+) [12]. Interestingly even in the presence of inflammation, only CLA+, CCR4+ CD45RO T cells are found in the skin and only α4β7+, CCR9+ CD45RO T cells are found in the gut, implying that despite ongoing inflammation, site-specific trafficking is faithfully maintained [7].

**Fig. 1.** The dynamic balance of cell accumulation in any tissue compartment depends on the balance of cell recruitment, emigration, division and death. (a) Homeostasis is maintained during normal inflammatory responses leading to resolution. (b) In chronic inflammation inappropriate accumulation of leucocytes is caused by the inappropriate production of pro-survival (type I interferon, IFN-β) and pro-retentive (SDF-1) factors by fibroblasts. Reprinted from Buckley et al. [62] © 2001, with permission from Elsevier. (See Supplementary Fig. 1 available in colour at Rheumatology Online.)
This prevailing paradigm to account for the selective accumulation of distinct leucocyte subsets at sites of inflammation has derived from the supposition that all such selectivity occurs at the point of entry (endothelial selection). Selection within 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 [13, 14]. After crossing post-capillary venules into the subendothelial compartment, leucocytes encounter a stromal microenvironment that is quite distinct from that found 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 [15, 16]. In addition, the interaction of leucocyte integrins with extracellular matrix components in tissues provides co-stimulatory signals that enhance lymphocyte activation and proliferation at the site of inflammation. Therefore we postulated that the stromal microenvironment might directly affect the behaviour of T cells that accumulate within 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 during the resolution phase of viral infections, the initial increase in T-cell numbers in peripheral blood, seen within the first few days, is followed by a wave of apoptosis occurring in the activated T cells [2]. This situation is mirrored within 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].

Synovial T cells in rheumatoid arthritis, like those isolated from many sites of inflammation, are highly differentiated CD45RO+ CD45RB dull T cells and represent cells at the extreme limit of differentiation. This finding immediately presented a conundrum to Mike Salmon and colleagues in Birmingham, since such cells are exquisitely susceptible to apoptosis and would not normally be expected to survive within inflamed joints unless their death was actively inhibited [17, 18].

In a series of elegant experiments they showed that the persistence of T cells within the rheumatoid synovium could be at least in part explained by the inappropriate inhibition of T-cell apoptosis [18]. This survival pathway shares all the essential hallmarks of a stromal cell-mediated mechanism (high Bcl-XL, low Bcl-2 and lack of cell proliferation) and fibroblasts isolated from the rheumatoid synovium were able to keep T cells alive. Type I interferons, produced by synovial fibroblasts and macrophages, were identified as the principal factor responsible for prolonged T-cell survival in the rheumatoid joint [19]. 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, non-cycling). It is likely that such a mechanism occurs in many chronic inflammatory conditions where T cells accumulate, with many of the phenotypic characteristics seen within the rheumatoid joint.

The synovial microenvironment promotes T-cell retention

While the inhibition of T-cell apoptosis by interferon-β (IFN-β) at sites of chronic inflammation is clearly an important factor in T-cell accumulation, it does not fully explain the long-term stability of inflammatory infiltrates. The problem is that the recruitment of cells is episodic, mostly occurring during periods of infection or other immune activity. Therefore why do the cells not simply leave the joint during periods of quiescence? We proposed that synovial T cells, in addition to their enhanced survival, might be actively retained within the synovial microenvironment. We have now found that the synovial microenvironment does directly contribute to the inappropriate retention of T cells by a chemokine-dependent process [20].

The expression of chemokine receptors on T lymphocytes depends on both their state of activation as well as their stage of differentiation. We therefore postulated that the pattern of chemokine receptors expressed by T cells in rheumatoid synovial fluid would reflect features of their microenvironment and give us a clue as to whether specific chemokines were involved in their accumulation and retention. We therefore compared paired peripheral blood and synovial fluid samples from a number of patients with rheumatoid arthritis. As T cells in the rheumatoid synovium are exclusively of the CD45RO phenotype, we matched cells for CD45 isoform expression so that a direct comparison to the peripheral blood compartment could be made. To our surprise we found that there was marked discordance between the expression of a number of chemokine receptors on CD45RO T cells in synovial fluid and peripheral blood matched for CD45RO expression (Fig. 2). In particular, there was very high expression of the chemokine receptor CXCR4 on T cells within synovial membrane and fluid compared with peripheral blood (Fig. 3).

Conventional explanations for this suggest that enrichment of these cells within the joint occurs because of the selective recruitment from peripheral blood of these cells into the inflamed microenvironment. However, this explanation ignores the potential role of the microenvironment in altering the expression of
Fig. 2. Expression of a range of chemokine receptors on peripheral blood (PB) and synovial fluid (SF) CD3+ T cells. CD3+CD45RO+ cells were analysed for the expression of chemokine receptors using three-colour flow cytometry. The percentage of positive cells compared with the irrelevant isotype-matched control is shown in the top right hand of each box.

Fig. 3. Synovial fluid and membrane T cells express high levels of CXCR4. The expression of CXCR4 is shown by enzymatic (A) and fluorescence (B) methods. Not all CXCR4 cells are positive for CD3. (C) Flow cytometry of CD3+CD45RO+CXCR4+ T cells from peripheral blood, synovial membrane and synovial fluid. (See Supplementary Fig. 3 available in colour at Rheumatology Online.)
chemokine receptors on cells that are resident, often for prolonged periods, within inflamed tissues. In a series of withdrawal, depletion and replacement studies we found that factors produced within the synovial microenvironment were responsible for up-regulating CXCR4 on synovial T cells. An extensive screen of potential candidates identified transforming growth factor-β (TGF-β) as the factor. In the same study we also found high expression of the ligand for CXCR4 stromal cell-derived factor-1 (SDF-1) CXCL12 on synovial endothelial cells. The high level of CXCR4 on synovial T cells was functional since exposure of these cells to SDF-1 led to rapid, sustained adhesion to both intercell adhesion molecule-1 (ICAM-1) and fibronectin [20]. These findings have subsequently been confirmed independently by Nanki et al. [21]. Others have also observed up-regulation of CXCR4 on infiltrating T cells and expression of its ligand SDF-1 on stromal cells in inflamed intestine and skin [22–23]. Furthermore, inhibiting SDF-1/CXCR4 interactions has been shown to ameliorate arthritis in collagen-induced arthritis models in mice [24].

Taken together these findings suggest that the SDF-1/CXCR4 chemokine/chemokine receptor pair may play an important role in the inflammatory process, enabling the switch from a migratory to stationary phenotype to occur for leucocytes once they enter tissue microenvironments. Thus the inflammatory infiltrate in rheumatoid arthritis 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 within the rheumatoid joint. Why this occurs and the contribution played by fibroblasts is addressed in the next section.

Fibroblasts help define the microenvironment in the rheumatoid synovium

Fibroblasts are ubiquitous cells that provide mechanical strength to tissues by providing a supporting framework of extracellular matrix [25]. 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 anatomical regions display characteristic phenotypes that are maintained even after prolonged culture in vitro, suggesting that many fibroblasts have an imprinted phenotype that is remarkably stable [26–29]. Even within a single tissue there is growing evidence that fibroblasts are not a homogeneous population, but exist as subsets of cells, much like tissue macrophages and dendritic cells [30]. It is likely that connective tissue contains a mixture of distinct fibroblast lineages with mature fibroblasts existing side by side with immature fibroblasts (often called mesenchymal fibroblasts) that are capable of differentiating into other connective tissue cells. Tantalizing evidence now suggests that fibroblast precursors circulate in peripheral blood and precondition the synovium prior to the development of lymphocyte aggregates and the inflammatory response in mouse models of arthritis [31]. These cells share many properties of bone marrow stromal stem cells and are capable of differentiating into several cell lineages [32, 33].

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 co-stimulatory molecules such as CD40, suggesting a fundamental role in immune responses and disease processes [26]. This diversity in phenotype and function that characterizes 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.

Cultured rheumatoid fibroblasts display a stellate morphology, enhanced growth, altered migratory capacity and constitutively overexpress certain pro-inflammatory genes, metalloproteinases and matrix proteins that facilitate the localization of immune cells in the joint [34, 35]. They look very different to fibroblasts derived from other tissues such as skin and lung and their pattern of gene expression as assessed by gene expression profiling with microarrays is very different (Fig. 4). Rheumatoid fibroblasts share some of the intrinsic properties of follicular dendritic cells, i.e. binding of germinal centre B cells and protection from apoptosis [36]. Transplantation of enzymatically dispersed rheumatoid fibroblasts with cartilage matrix into SCID mice leads to the invasion of the cartilage matrix and the production of pannus-like tissue. Fibroblasts from patients with osteoarthritis and normal dermal fibroblasts did not invade the cartilage suggesting that this phenotype was intrinsic to rheumatoid fibroblast [37]. Rheumatoid fibroblasts express a number of bone marrow stromal cell markers such as VCAM-1 and BST-1 (CD157) and constitutively express cytokines that support haematopoietic progenitor cells such as granulocyte-macrophage colony-stimulating factor (GM-CSF), SDF-1 and interleukin 6 (IL-6). Rheumatoid fibroblasts also express receptors for bone morphogenetic proteins (BMP) and proteins involved in developmental patterning (the Wnt protein receptors frizzled and wingless) that are found in mesenchymal progenitor cells. This suggests that the unusual phenotypic properties of rheumatoid fibroblasts may be partly attributable to expression of developmentally restricted genes [37].

It has been assumed that the predominant interaction of T lymphocytes in the synovial microenvironment is with antigen-presenting cells such as monocytes–macrophages, dendritic cells and B cells. However, interactions between infiltrating bone marrow-derived
haematopoietic cells such as lymphocytes, and endogenous stromal cells such as fibroblasts, have been shown to contribute directly to the intensity and persistence of chronic inflammation [38]. For example, T cell–fibroblast interactions within the synovium induce the expression of adhesion molecules, cytokines and chemokines by synovial fibroblasts [39, 40].

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 [41–43]. This suggested to us that, as occurs in lymphoid neogenesis, stromal cells within the inflamed synovium might overproduce chemokines that have been shown to play a role in lymphoid organogenesis. Thus the consequences of interactions between cells of the synovial membrane and infiltrating lymphocytes might be to induce the formation of lymphoid structures in the synovial microenvironment. Using standard immunohistochemical analysis we found expression of B cell-attracting chemokines BCA-1 (CXCL13), SDF-1 (CXCL12) and lower levels of ELC (CCL19) within the rheumatoid joint (Fig. 5). While the cells producing these chemokines remain unclear, we found that rheumatoid but not other fibroblasts were capable of producing extraordinarily high levels of SDF-1 as measured by real-time PCR analysis, even when cultured under resting conditions in isolation in vitro. This suggested that the rheumatoid microenvironment in general, and rheumatoid fibroblasts in particular, are capable of producing constitutive chemokines known to play a role in regulating lymphoid organogenesis.

A striking feature of the rheumatoid synovium is the distribution of T-cell subsets within the rheumatoid synovial compartment. CD4 T cells preferentially accumulate in a perivascular distribution, whereas CD8 T cells are sparsely distributed throughout the synovial tissue [4]. Furthermore, the ratio of CD8 to CD4 cells within the synovial tissue is much lower than synovial fluid. The molecular basis for this high degree of cellular organization within distinct micro-domains remains unclear. We have found that CD4 and CD8 T-cell subsets demonstrate distinct patterns and rates of migration into rheumatoid synovial fibroblast monolayers when co-cultured using an in vitro model of T-cell accumulation. This model relies on the ability of some fibroblasts to support the accumulation of lymphocytes within and under a monolayer by a process termed pseudoemperipolesis [44]. This is easily quantified as lymphocytes become phase dark when they accumulate within the fibroblast monolayer. We found that RA synovial fibroblasts influence T-cell distribution and migration in a distinct manner compared with fibroblasts isolated from patients with self-limiting arthritis or from other sites such as skin or lung (Fig. 6). Interestingly, this phenotype persists through many passages in cultures and is inhibited by antibodies to SDF-1 and CXCR4, suggesting that this distinctive phenotype of
cultured rheumatoid fibroblasts is stable, disease specific and depends on CXCR4/SDF-1 interactions. We have therefore been able to complete the biological cycle from our initial observation of high levels of CXCR4 expression on synovial T cells, to differential SDF-1 production by rheumatoid fibroblasts and suggest that rheumatoid fibroblasts play a dominant role in the retention of T cells within the inflamed synovium.

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 within inflamed tissue and then
migrate to the draining lymph node where they present antigen to T cells [45]. This process is spatially separated from the site of inflammation and requires careful chemokine-mediated choreography in order 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 in order 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 [2, 46].

Based on this central requirement for a small number of lymphocytes to be rescued from apoptosis and retained within 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 (within 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 (Fig. 7). This mechanism not only provides a molecular explanation for the similarities between lymphoid aggregates in the rheumatoid synovium and lymphoid tissue, but it also provides a potential functional explanation for the success of anti-TNF therapy in rheumatoid arthritis, since TNF family members are needed for efficient lymph node development [42, 43].

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

The findings described above for rheumatoid arthritis provide an explanation for 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 rheumatoid arthritis, or represents a universal feature in many chronic rheumatic diseases, we examined another chronic rheumatic disease. Sjögren’s syndrome (SS) is characterized by the accumulation of lymphocytes within salivary, lachrymal and other exocrine glands in the respiratory tract, gastrointestinal tract and vagina [47]. The inflammatory infiltrate consists predominantly of CD4+ T cells with 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 centers. 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 [48]. Germinal centres classically arise in primary B-cell follicles of secondary lymphoid organs and provide a unique microenvironment for B-cell maturation into plasma cells. Lymphoid aggregates with ectopic germinal centre-like structures (sometimes called tertiary lymphoid tissue) are found in a number of chronic inflammatory diseases including the rheumatoid synovial membrane and the thyroid gland in Hashimoto’s thyroiditis [49–52]. Whether these ectopic structures contain all the necessary components required for dendritic cells to enter lymph nodes and present antigen to naive T cells: CD45RA+. These cells gain entry to lymph nodes via high endothelial venules (HEVs) using the chemokine receptor CCR7. Once activated, antigen-experienced cells (CD45RO+) are released into the blood and express chemokine receptors that allow them entry to acute inflamed tissue (CCR5, CXCR3), but unless they also express CCR7 they are barred from entry to lymph nodes via HEVs. In chronic inflammatory sites cells are prevented from dying and leaving inflamed tissue by the inappropriate expression of pro-survival (type I interferon, IFN-β) and pro-retention signals (SDF-1) by stromal elements, such as fibroblasts. Reproduced from Buckley et al. [62]; © 2001, with permission from Elsevier. (See Supplementary Fig. 7 available in colour at Rheumatology Online.)
to generate a local B-cell driven response, or simply represent a focus point for the collection of autoantibody-producing B cells that migrate into inflamed tissue from nearby secondary lymphoid tissue, remains unclear [53–55].

Recent studies have established a critical role for TNF and lymphotoxin (LT) in the development and maintenance of lymphoid tissue [56]. 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 [57–58]. Elegant studies in transgenic mice overexpressing the constitutive lymphoid chemokine BCA-1 have confirmed that LT, TNF and BCA-1 act in a common pathway of lymphoid neogenesis [59]. 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 [41, 42].

We therefore postulated that accumulation of lymphocytes in germinal centre-like structures within 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 we found that lymphoid aggregates in SS share many of the structural features of germinal centres including the expression of BCA-1 by stromal cells within germinal centre-like follicles (Fig. 8).

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 [60]. The lymphocytes accumulating within the germinal centre-like structures expressed the appropriate receptors for BCA-1 (CXCR5) and SDF-1 (CXCR4) (Fig. 9).

Fig. 9. Lymphocytes expressing receptors for SDF-1 and BCA-1 accumulate within lymphoid aggregates (A). CXCR5+B cells (CD22) accumulate around BCA-1 expressing tissue in lymphoid follicles in Sjögren’s syndrome. Serial sections of Sjögren’s tissue (top and bottom left) and tonsil (top and bottom right) were stained with two-colour immunofluorescence for CD22 (green) and CXCR5 (red, top left and top right) or BCA-1 (red, bottom left, bottom right). (B) CXCR4+ T cells (CD3) accumulate around SDF-1 expressing epithelial ducts in Sjögren’s syndrome. Frozen tissue sections of Sjögren’s tissue (left) and tonsil (right) were stained with three-colour immunofluorescence for CD3 (blue), CXCR4 (red) and SDF-1 (green). Epithelial cells in the capsule of the tonsil express SDF-1. Reproduced from Amft et al. [60]; © 2001 American College of Rheumatology, with permission from John Wiley & Sons, Inc. (See Supplementary Fig. 9 available in colour at Rheumatology Online.)

Fig. 8. BCA-1 is highly expressed in Sjögren’s tissue but not normal salivary tissue. Frozen sections were stained with an isotype-matched irrelevant control antibody (control), or antibody to BCA-1 in tissue sections from Sjögren’s salivary gland, normal salivary tissue or tonsil. BCA-1 expression (brown staining) was found only in Sjögren’s tissue in a reticular pattern (similar to that seen in tonsil) and also on tubular structures resembling endothelial cells. BCA-1 was not detected in normal salivary tissue. Reproduced from Amft et al. [60]; © 2001 American College of Rheumatology, with permission from John Wiley & Sons, Inc. (See Supplementary Fig. 8 available in colour at Rheumatology Online.)
Taken together with observations from transgenic mice that overexpress constitutive chemokines such as BLC (CXCL13), SLC (CCL21) and SDF-1 (CXCL12) [61], these findings suggest that inappropriate temporal and spatial expression of chemokines plays an important role in determining the generation of lymphoid aggregates within chronically inflamed tissues. Furthermore, these studies show that the site at which overexpression occurs modifies the nature of the leucocytic infiltrate providing strong support for the hypothesis that the stromal microenvironment regulates leucocyte accumulation in chronic inflammatory diseases.

Conclusions

The immune system is a diverse collection of many cell types that are spatially and temporally separated from each other. In order 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 as well as 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 [27, 28]. 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. In the studies presented here 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 the stromal microenvironment in general, and tissue fibroblasts in particular, are likely to be an important target for future anti-inflammatory therapy.

Acknowledgements

None of this work would have been possible without the support advice and friendship of Mike Salmon. In addition I would like to thank David Adams and my clinical colleagues, Dr Simon Bowman and Mr Andrew Thomas, for help with samples and reagents. Finally I would like to thank the very talented postdoctoral fellows, PhD students and technicians who have worked with me in the last 5 years and in particular Drs P. Bradfield, N. Amft, D. Pilling and Dr D. Scheel-Toellner. The work described here was funded by grants from the Wellcome Trust, Medical Research Council and the Arthritis Research Campaign.

Conflict of interest

The author has declared no conflicts of interest.

<table>
<thead>
<tr>
<th>Rheumatology</th>
<th>Key Messages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Chronic inflammation shares features with lymphoid neogenesis</td>
</tr>
<tr>
<td></td>
<td>• Stromal cells play a role in determining the nature and number of infiltrating leucocytes</td>
</tr>
<tr>
<td></td>
<td>• The stromal microenvironment is an important target for future therapy</td>
</tr>
</tbody>
</table>

References


