Commentary

Cytokines and direct cell contact in synovitis: relevance to therapeutic intervention

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Introduction

In chronic inflammation, which leads to tissue destruction and fibrosis, immunocompetent cells migrate through the vascular endothelium to the target tissue. A prototype of these events is synovitis, which occurs in diseases such as rheumatoid arthritis. The hypothesis that cells from the bone marrow could also migrate directly to the synovium through channels interconnecting the two compartments is still under debate. Also, there is no definitive answer regarding the number of cells that result from infiltration of the synovium after migration, or from proliferation at the local site. Furthermore, the survival of the cells in synovitis is being subjected to some scrutiny, because there is some evidence for a lack of apoptosis in pathological conditions.

The interaction between lymphocytes of different subsets and monocyte/macrophages (type A synovial cells) results in the production of proinflammatory cytokines. These include interleukin (IL)-1 and tumour necrosis factor (TNF)-α, which induce connective tissue cells (type B synovial cells or synoviocytes) to produce large amounts of matrix metalloproteinases (MMPs), which in turn degrade extracellular matrix components (eg collagens and proteoglycans).

Simultaneously, counter-regulatory mechanisms (cytokine inhibitors, anti-inflammatory cytokines and protease inhibitors) are triggered in an attempt to block inflammation and tissue destruction. During, and shortly after the onset of synovitis chondrocytes and bone-derived cells (osteoblasts and osteoclasts) are activated by the same cytokines, together with prostanoids [mainly prostaglandin E_2 (PGE_2)], to degrade the extracellular matrix via MMPs and to remove the mineral phase of the bone. The inflammatory and destructive process is often followed by attempts at repair which, unfortunately, result mostly in fibrosis and nonfunctional tissue. The role of cytokines (eg TNF-α and IL-1), growth factors and tissue destruction has been extensively reviewed, and, owing in particular to the concept of inhibition of TNF-α, crucial advances in therapeutic intervention have been made [1,2].

Proinflammatory and anti-inflammatory cytokines

The research of the past few years has mostly focused on soluble factors [mainly proinflammatory and anti-inflammatory cytokines derived from T helper (Th)1, Th2 or Th3] as well as on growth factors and angiogenic factors, and more recently cytokines such as IL-15, IL-16, IL-17 and IL-18 were analyzed in depth in the context of synovitis. IL-15 plays a proinflammatory role in rheumatoid arthritis by inducing cell migration and the production of TNF-α [3]. IL-16 released by tissue-infiltrating $CD8^+$ T cells in rheumatoid synovitis influences the anti-inflammatory activity by inhibiting the production of interferon-γ, IL-1β and TNF-α in synovium [4]. IL-17 secreted by $CD4^+$-activated memory T cells induces nuclear factor-κB, IL-6, IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF) and PGE_2 production by human fibroblasts and acts synergistically with TNF-α and IL-1 [5,6]. IL-18, together with IL-12 or IL-15, induces significant interferon-γ production by synovial tissue *in vitro*, TNF-α synthesis by $CD14^+$ macrophages in synovial culture, and promotes GM-CSF and nitric oxide production. IL-18 is upregulated by TNF-α and IL-1β and promotes Th1 cell development in synovial membrane. In collagen-induced arthritis in a murine model [7], IL-18 facilitates the devel-

CCR = chemokine receptor; GM-CSF = granulocyte–macrophage colony-stimulating factor; IL = interleukin; LFA = lymphocyte function associated antigen; MMP = matrix metalloproteinase; PGE_2 = prostaglandin E_2; Th = T helper; TIMP = tissue inhibitor of metalloproteinase; TNF = tumour necrosis factor.

opment of erosive, inflammatory arthritis. The role of IL-18 is complex, however, and it can also act as an inhibitor of osteoclast formation; this process is contact dependent [8]. IL-18 produced by osteoblastic stromal cells inhibits osteoclast formation in murine haematopoietic and primary osteoblast stromal cells. This action is mediated via GM-CSF production, and not interferon-γ, because neutralizing antibodies to GM-CSF were able to rescue IL-18-induced inhibition of osteoclastogenesis. The elevated levels of IL-18 production in osteoblastic cells appear to correlate with cells at a more differentiated stage. Thus, IL-18 production by mature osteoblasts may be one of the mechanisms that limit osteoclast formation by these cells. By counteracting IL-1, IL-18 may regulate bone homeostasis.

Proteases and prodestructive cytokines

It was not until recently that the role of direct contact between cells was studied more systematically [9]. Even in severe diseases, in which the activation and interaction of circulating blood cells such as monocytes and lymphocytes might be expected, it is very difficult to demonstrate that cell–cell contact is direct and that it leads to the production of cytokines or MMPs in the blood stream. This is illustrated by the difficulty in measuring circulating pro-inflammatory cytokines such as IL-1 and TNF-α, even if their abundance is clearly established at the local inflammatory site. However, as soon as the inflammatory cells have migrated to the tissue, it is likely that, in addition to the role of soluble products, direct cell contact prompts the release of inflammatory mediators and proteolytic enzymes. This suggests that many molecules, mostly large macromolecules, present in the plasma prevent cell–cell contact. These molecules may occur in much lesser concentrations, or be absent in the interstitial tissue, thus permitting cell–cell contact.

Many investigators have advanced sound arguments for T lymphocytes playing a pivotal role in the pathogenesis of synovitis, at least at some stage of the disease. In rheumatoid arthritis, T lymphocytes that display a mature helper phenotype are the main infiltrating cells in the synovium, accounting for 16% of total cells in 'transitional areas' and for 75% in lymphocyte-rich areas. Extravasation of T lymphocytes occurs at the level of high endothelial venules.

In the perivascular space, activated T lymphocytes bind to matrix proteins. They are in close contact with monocytes, and also with synoviocytes at a more advanced stage of the disease. The T-cell population in inflamed synovial tissue belongs predominantly to the Th1 subset [10]. Interestingly, these T cells show a marked staining for the chemokine receptors CCR5 and CXCR3, and are only occasionally positive for CCR3. It appears that CCR5 is highly expressed on Th1 cells and is rarely present in Th2 cells, whereas CCR3 is found in Th2 cells but not in Th1 cells and CXCR3 is highly expressed in both T-cell subsets. MIP-1β appears to be a selective ligand for CCR5, eotaxin is a ligand for CCR3, and IP-10 is a ligand for CXCR3.

Importance of cell contact

The importance of cell contact, and not only soluble factors, has been emphasized in transgenic mice expressing T cell-targeted membrane-associated human mutant TNF-α, which displayed proliferative synovitis and chronic inflammatory arthritis [11]. This suggests that at least part of the pathogenic activity of T cells *in vivo* may be due to the expression of the membrane-associated form of TNF-α by T lymphocytes. In addition to T cells, macrophage-derived cells play a crucial part, and indeed a positive correlation was established between CD14 cell counts of both lining and sublining CD68 cells and articular destruction [12]. Thus, many observations suggest that both T cells and macrophages are important and that contact between T cells and macrophages, or even synoviocytes of the fibroblast lineage, in the pannus may be involved in the pathogenesis of inflammatory destructive arthritis. Other cells may play an important role in the onset of the inflammatory process, such as mast cells, which are often associated with the production of TNF-α and IL-1β by adjacent cells, especially at sites of cartilage erosion [13].

The activation of effector cells mediated by T lymphocytes has been well documented by the induction of B-cell production and antibody secretion, both requiring direct cell–cell contact and soluble factors. The claim that autoantibodies induce arthritis has recently been challenged [14].

Therefore, similar to the direct contact between T and B cells, the T cell–monocyte interaction occurs as shown in experimental systems. Surface molecules involved in the T-cell signalling of monocyte/macrophages by direct contact is being investigated and has resulted in the observation that this contact leads to the production of IL-1 and TNF-α by monocytes, and more markedly after differentiation into macrophages by 1,25-dihydroxyvitamin D_3 [15,16]. This has been further illustrated in terms of specificity, because IL-10 is not produced in a similar system [17].

Membrane-associated cytokines such as TNF and IL-1, and other surface molecules, could activate monocyte/macrophages upon contact with stimulated T cells. The cooperation between activated monocyte/macrophages and interferon-γ-secreting CD4 helper (Th1) cells is controlled by two categories of molecules: cell-surface molecules including major histocompatibility complex antigen, B7.1/2, lymphocyte-function-associated antigen (LFA3), LFA1, CD40 on macrophage, and T cell receptor, CD28, cytotoxic T-lymphocyte-associated antigen-4, CD2, intercellular adhesion molecule-1, CD40L on Th1 cells. During the course of this interaction, Th1 cells produce IL-2 and IL-17, which act on T cells in an autocrine or paracrine fashion, and

interferon-γ, which acts on the interferon-γ receptor on macrophages. In turn, macrophages produce IL-1 and TNF, which also act in an autocrine fashion but, more important, on other target cells in the synovium. A great deal of attention is being paid to CD40/CD40L, which is involved in the contact activation of both human and murine monocyte/macrophages by T lymphocytes stimulated for a short period [18,19]. Furthermore, peripheral blood T lymphocytes isolated from CD40L-knockout mice and stimulated for a short period failed to induce monocyte activation. In contrast, when stimulated for a longer period, T lymphocytes isolated from both CD40L-knockout and wild-type mice triggered monocyte activation, but to a lower extent [20]. An argument against the predominant role of CD40/CD40L is the fact that the most effective human T-cell line for inducing signalling of monocytes by direct contact (human lymphocytic cell line HUT-78) does not express CD40L messenger RNA, whether in resting or activated conditions. This suggests that CD40/CD40L might be involved in contact-activation of monocyte/macrophages by T lymphocytes stimulated for short periods of time, but not for long periods, the latter cells by then no longer expressing CD40L.

Specific cell-surface molecules involved in IL-1 and TNF production

One study [21] has shown that functional CD40L was expressed by T lymphocytes from the synovial fluid of rheumatoid arthritis patients. Although immunohistochemical analysis of synovial tissue demonstrated CD40L expression in infiltrating cells of the vascular/perivascular area, no staining was observed in infiltrating cells that migrated farther [22]. These results suggest that CD40L may be predominantly involved in the extravasation of T lymphocytes into the pannus through the vascular endothelium, but have less involvement in IL-1/TNF and MMP production. The study also implies that cell-surface factors other than CD40L were involved in T lymphocyte contact-signalling of monocytes. The general conclusion to be drawn is that, depending on the timing and consequently the stage of the immunoinflammatory condition, different molecules could be used for similar functions, and these points have to be taken into consideration for therapeutic intervention. Other studies [23] have shown that cytokine production was induced in monocytes by soluble CD23. Our studies [17] have shown that LFA-1 (CD11a/CD18) and CD69 play a role in the activation of human monocytic cells by stimulated T cells [17]. Antibodies to CD11a, CD11b, CD11c and CD69 partially inhibited the activity of contact-activation factors. The latter data were recently confirmed by a study [24] that showed that IL-15 induced synovial T cells from rheumatoid arthritis patients to activate the production of TNF-α by macrophages. This effect was inhibited by antibodies to CD69, LFA-1 and intercellular adhesion molecule-1. Antibodies to known cell-surface antigens (CD2, CD11a, CD11b, CD11c, CD14, CD18, CD23, CD29, CD40, CD40L, CD54, CD69, cytotoxic T-lymphocyte associated antigen-4, CD95, CD95L) or membrane-associated cytokines (interferon-γ, IL-2, GM-CSF, IL-1, TNF-α, leukotrienes), and cytokine inhibitors (IL-1 receptor antagonist, TNF soluble receptors) failed to abolish the activity of contact-activation factors in monocytes [9]. Thus, it is possible that some already identified surface molecules are involved in T-cell-signalling of monocyte/macrophages. Inhibitors (eg antibodies) to these molecules fail to abolish monocyte activation altogether, however, suggesting that the required factor(s) for T-cell-signalling of human monocytes by direct contact remain(s) to be identified.

Balance between IL-1/IL-1 receptor antagonist and MMPs/TIMP

Subcellular fractionation showed that the activation factors are located in the plasma membranes of stimulated T cells. T-cell clones expanded from a single healthy blood donor express surface factors that activate monocyte/macrophages, but to varying extents. Interestingly, the products that are induced in the target cell differ depending on the nature of the stimulating agent and the time of stimulation of T lymphocytes. This could imply that several contact-activation factors, probably acting synergistically, are expressed on the surface of stimulated T lymphocytes in a hierarchy that varies depending on the type and time of activation. The T-cell subsets are important because Th1 clones that preferentially express CCR5 are, because of cell–cell contact, potent inducers of IL-1β and TNF-α on macrophages while inducing virtually no IL-1 receptor antagonist, whereas Th2 clones induce large amounts of IL-1 receptor antagonist and almost no IL-1β [25]. Plasma cell membranes from antigen-activated Th1 and Th2 clones also proved to be potent inducers of MMP-1 production by a human monocytic cell line, whereas tissue inhibitor of metalloproteinase (TIMP)-1 levels were not affected. Using neutralizing reagents, cell membrane-associated TNF was found to be partially involved in this MMP-1 induction by both Th1 and Th2 cells.

During advanced chronic inflammation, stimulated T lymphocytes can also potentially contact cells other than mononuclear phagocytes that are involved in pathogenesis. Such target cells include synoviocytes. Indeed, upon contact with membranes of stimulated T lymphocytes, synoviocytes produce large amounts of MMP-1 and PGE_2, but no TIMP-1 [26]. The surface factors involved in contact activation of synoviocytes have been identified as membrane-associated cytokines, mainly TNF-α and IL-1α. These cytokines are not involved in the activation of monocyte/macrophages by T-cell membranes. It is therefore intriguing that T lymphocytes should have developed different cell-signalling systems adapted to the different target cells.

Conclusion

Current experimental results strongly suggest that by direct cell–cell contact, membranes of stimulated T lymphocytes attracted by specific chemokines potentiate the inflammatory response. They do so by favouring the extravasation of cells from the immune system into the target tissue through the endothelium, and by activating the production of proinflammatory cytokines and MMPs at inflammatory sites (ie by stimulating monocytes and synoviocytes). This mechanism (cell–cell contact with stimulated T lymphocytes) induces an unbalanced production of MMPs and TIMP-1 *in vitro* and may lead to tissue destruction *in vivo*. We thus hypothesize that cell–cell contact between stimulated T lymphocytes and surrounding cells represents an important mechanism that contributes to the pathogenesis of inflammation and tissue destruction in chronic inflammatory diseases such as rheumatoid arthritis.

Despite the impressive clinical results obtained with anti-TNF therapy, approximately 25% of the patients seem to be resistant. This hints at the possibility that, during the course of the disease, other important mechanisms trigger synovitis and tissue destruction. In addition to newly described interleukins (IL-15, IL-17, IL-18), some of the mechanisms could involve direct contact between stimulated T cells and macrophages or synoviocytes. If partly induced by TNF-α, the production of IL-1 can also be triggered by mechanisms independent of TNF, and the production of MMPs is not solely induced by TNF. It is therefore likely that therapeutic intervention will have to aim at additional cytokines and direct cellular contact to block fully the pathogenesis of rheumatoid arthritis.

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