Review

Mesenchymal stromal cells

Nurse-like cells reside in the synovial tissue and bone marrow in rheumatoid arthritis

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Abstract

A major question concerning the immunopathology of rheumatoid arthritis is why the disease is localized to particular joints. A possible explanation could be the presence within the synovium of cells that foster inflammation or easy accessibility of the synovium to migratory disease enhancing cells. Within both the bone marrow and the synovium, fibroblastic stromal cells play an important role in supporting the differentiation and survival of normal cells, and also contribute to the pathologic processes. Among fibroblastic stromal cells in synovial tissue and bone marrow, nurse-like cells are a unique population having the specific capacity to promote pseudoemperipolesis (adhesion and holding beneath) of lymphocytes, and also the ability to promote the growth and function of some populations of lymphocytes and monocytes. Nurse-like cells could therefore contribute to the immunopathogenesis of rheumatoid arthritis, and may contribute to the localization of inflammation within specific joints. The present review considers the evidence that supports these possibilities.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by immunologically enhanced inflammation and damage to articular structures [1,2]. Rheumatoid synovium is a site of intense inflammation, with active involvement by various populations of infiltrating lymphocytes, myeloid cells, and resident synovial fibroblasts or synoviocytes [1]. One question that has not been addressed is why RA preferentially affects certain joints. Although the explanation for the localization of rheumatoid inflammation to particular joints is not clear, one possibility relates to the presence within the synovium of resident cells that can promote inflammation. In addition, cells that can be induced to migrate from adjacent bone marrow structures may contribute to the local facilitation and propagation of inflammation and bone damage. The present review will focus on one such population, the nurse-like cells (NLCs) that populate the rheumatoid synovium and bone marrow.

Fibroblastic stromal cells in bone marrow and synovial tissue

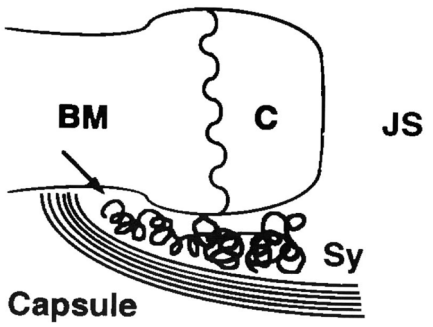
Initially, to examine the relationship between the epiphyseal bone marrow and synovial tissue, we employed the animal model of collagen-induced arthritis [3]. Fibroblastic stromal cells (FSCs) in the bone marrow of Lewis rats were labeled with a fluorescent probe or ^{3}HTdr and were examined for their migration at the onset of arthritis [4]. Accompanying the induction of polyarthritis, a large number of labeled FSCs in bone marrow were found to migrate into the joint cavity through canals observed in the bare zone of the joint (Figure 1), and then to proliferate in the synovial tissue. This observation suggested the hypothesis that pathophysiological cells of RA could be produced in bone marrow, from which some of these cells could migrate into the joint space and potentially play roles in inflammation or tissue damage in and around articular structures. Based on these findings, we have studied FSCs of RA patients, comparing the characteristics of FSCs from bone marrow and FSCs from synovial tissue [5-7].

Nurse-like cells found in bone marrow and synovial tissue

Among the FSCs derived from the bone marrow and synovium of RA patients, a population of NLCs was identified by the capacity to carry out pseudoemperipolesis. The

BST-1 = bone marrow fibroblastic stromal cell antigen 1; FSC = fibroblastic stromal cell; GM-CSF = granulocyte/macrophage colony-stimulating factor; HLA = human major histocompatibility antigen; IFN = interferon; IL = interleukin; mAb = monoclonal antibody; NLC = nurse-like cell; RA = rheumatoid arthritis; RANKL = receptor activator of NF-κB ligand; TNF = tumor necrosis factor; TRAP = tartarate-resistant acid phosphatase.

Figure 1



Migration of fibroblastic stromal cells from epiphyseal bone marrow (BM) into the joint space (JS) forming synovial (Sy) tissue in collagen-induced arthritis. C, cartilage.

function of the NLCs was reminiscent of thymic nurse cells [8,9], which have the capacity to interact with populations of thymic cells and gather them beneath their cell bodies in a process known as pseudoemperipolesis (adhesion and holding beneath). *In vivo*, such thymic nurse cells were thought to support the development and expansion of thymocytes and to also play a role in positive/negative selection of T cells in mouse and rat thymus. A very similar capacity to interact and support the maturation of some population of lymphocytes and monocytes was noted for FSCs of bone marrow [5,7] and for FSCs of synovial tissue [6,7] of RA patients, suggesting that the NLC function of FSCs could contribute to the pathophysiology of RA [7].

We established RA-NLC clones with the ability to promote pseudoemperipolesis from bone marrow [5] and synovial tissue [6] of RA patients. These RA-NLC clones were determined to be of mesenchymal origin, given that they expressed vimentin but not cytokeratin. They did not exhibit desmosomes or classical junctional complexes, both of which are characteristic features of epithelial cells. Elongated and branching mitochondria were present in the cytoplasm of the clones, and caveolae, which are unique to cells of mesenchymal origin, were present on the surface [5,6].

NLCs have a number of unique functional activities that could contribute to rheumatoid inflammation. Among these activities are their ability to promote antibody production by B cells, the capacity to protect lymphocytes from apoptosis, the ability to secrete large amounts of cytokines and chemokines that could promote the accumulation and activation of lymphocytes and monocytes, and their unique capacity to promote the differentiation of osteoclasts from myeloid precursors in a receptor activator of NF-κB/receptor activator of NF-κB ligand (RANKL)-independent manner [10].

Multipotent mesenchymal stem cells from bone marrow were also found to exist in the synovial membrane [11-14]. Those cells were shown to have multipotency to develop into various cells such as cartilage, bone, fat, and muscle. Although it is currently unknown whether these cells can differentiate into NLCs, RA-NLCs are a more differentiated population. Multipotential mesenchymal stem cells from the synovial fluid and bone marrow of patients with inflammatory and degenerative arthritis were reported to be negative for CD45 and to be positive for D7-FIB, CD13, CD105, CD55, and CD10 [13]; these mesenchymal stem cells therefore have a very different phenotype from that of RA-NLCs mentioned in the following.

Surface phenotype of rheumatoid arthritis nurse-like cells

RA-NLC clones from bone marrow and synovial tissue [5-7] expressed CD29, CD44, CD49c, CD54, CD106, and HLA-A, HLA-B, and HLA-C (class I major histocompatibility complex), but did not express CD1a, CD18 (LFA-1), CD35, CD40, CD154, or CD56. RA-NLCs constitutively expressed CD106 after long-term culture in the absence of cytokine stimulation. Constitutive expression of CD106 appears to be a characteristic appearance of nurse cell lines, permitting them to be distinguished from fibroblasts [7]. Human dermal fibroblast also expressed CD29, CD49c, CD54, and class I major histocompatibility complex, whereas constitutive expression of CD106 was minimal. IFNγ (100 U/ml) stimulation of RA-NLCs induced expression of CD40 and HLA-DR (class II major histocompatibility complex), but not expression of CD35 or CD154. The surface phenotype of RA-NLCs was therefore similar to that of FSCs derived from synovial tissue and bone marrow cells from non-RA controls. Namely, the phenotype of NLCs derived from osteoarthritis patients and human skin nurse cells was similar to that of RA-NLCs. Enhanced expression of CD106 and CD157 by IFNγ (mentioned below) was the characteristic observation in RA-NLCs and was different from human dermal fibroblasts [7].

Expression of CD106 by RA-NLCs was modestly enhanced by culture with normal peripheral B cells, and was markedly enhanced by IFNγ. In contrast, expression of CD106 by human dermal fibroblasts was much less marked after stimulation with IFNγ or by culture with peripheral B cells. One of the features of NLCs is their capacity to promote the survival of B lymphocytes [5-7]. Such B-cell survival was reduced by a blocking anti-CD106 mAb to the same level as B cells cultured in medium alone.

One notable product of NLCs is human bone marrow fibroblastic stromal cell antigen 1 (BST-1). This product was originally cloned from a human bone marrow FSC cell line by surveying for any unknown factors [15], supporting the FSC-dependent growth of the murine pre-B-cell line DW34. A new growth factor was identified, having the ability to enhance DW34 cell growth, and it was designated BST-1 [16]. Human BST-1 is expressed in various tissues and cell

lines, such as umbilical vein endothelial cells, myeloid cells, as well as FSCs of bone marrow and also synovial cells in RA, but is not expressed in lymphoid cell lines. Notably, serum levels of BST-1 were higher (30-fold to 50-fold) in 7% of RA patients than in non-RA samples [17]. Human BST-1 was later designed as CD157, and the human *Bst-1* gene was assigned to chromosome 4q15, regulating humoral immune responses *in vivo* [18]. Expression of CD157 (BST-1) was detected on all RA-NLCs, as well as on human dermal fibroblasts. Expression of CD157 by RA-NLCs, but not by dermal fibroblasts, was enhanced by IFNγ. This enhancement was much more marked with bone marrow-derived RA-NLCs compared with synovium-derived RA-NLCs. It should be noted that expression of CD106 and CD157 mRNA was found in all RA-NLC clones. Soluble CD157 together with RA-NLCs further increased the survival of B cells, which was reduced by a blocking anti-CD157 polyclonal antibody [7].

Cytokine production by nurse-like cells of RA patients

RA-NLCs produced numerous cytokines [5-7]. RA-NLCs from both bone marrow and synovial tissue produced detectable levels of IL-6, IL-8, and granulocyte/macrophage colony-stimulating factor (GM-CSF), and the production of IL-6 and IL-8 was quite robust. RA-NLCs from bone marrow but not synovial tissue produced IL-7, whereas RA-NLCs from synovial tissue produced granulocyte colony-stimulating factor and a greater amount of IL-6. Regulation of the production of cytokines was examined by co-culture of RA-NLCs from synovial tissue in direct contact with B cells. Secretion of IL-6, IL-8, granulocyte colony-stimulating factor, and GM-CSF was markedly increased by co-culture with B cells. IL-1β and TNF were only detected in the culture supernatants after co-culture with B cells. The effect of co-culture with B lymphocytes on the secretion of cytokines and immunoglobulin production by the B cells were examined under various culture conditions [5-7] (Table 1). After co-culture with B cells, the levels of IL-6, IL-8, granulocyte colony-stimulating factor, GM-CSF, and the levels of IgM were increased, and IL-1β and TNF were detected. Direct contact with the B-cell clone was required for RA-NLCs to produce IL-1β and TNF and higher levels of the other cytokines.

Inhibition of spontaneous apoptosis of lymphocytes and the effect of adhesion molecules

RA-NLCs were found to promote lymphocyte viability. Although peripheral blood B cells cultured in medium alone rapidly died, culture of B cells with RA-NLCs markedly increased the B-cell viability. The loss of viability of B cells cultured alone related to the induction of apoptosis, whereas co-culture of B cells with RA-NLCs substantially blocked their apoptosis. The mechanism of the prevention of apoptosis of B cells involved the contact-dependent upregulation of Bcl-x_L by RA-NLCs [19].

The regulation of pseudoemperipolesis (adhesion and holding beneath) by RA-NLCs was examined using MC/car cells and a cloned RA-NLC line from synovial tissue [20]. Pretreatment with anti-CD29 (integrin β_1 chain) or anti-CD49d (integrin α_4 chain) reduced adhesion by MC/car cells by approximately 50%. This result indicated that integrin $\alpha_4\beta_1$ (very late antigen 4) on MC/car cells was involved, at least in part, in the cells' ability to participate in pseudoemperipolesis with RA-NLCs, although such interactions were not involved in IL-6 and IL-8 production by RA-NLCs. Pretreatment of MC/car cells with the Rho-specific inhibitor C3 transferase significantly inhibited the migration of MC/car cells underneath RA-NLCs in a concentration-dependent manner, whereas the same treatment did not inhibit the adhesion of the MC/car cells to RA-NLCs. In addition, RA-NLCs produced comparable levels of IL-6 and IL-8 when co-cultured with C3-treated transmigration-defective MC/car cells. The processes of pseudoemperipolesis, adhesion and holding beneath were therefore thought to be independent events [20]. Moreover, very late antigen 4 ($\alpha_4\beta_1$)-independent lymphocyte adhesion and not holding beneath induced the enhanced proinflammatory cytokine production by the RA-NLCs [20].

Regarding NLCs, another group reported that CD14(+) monocytes could differentiate into NLCs and support the viability of chronic lymphocytic leukemia B cells [21-23], and also support the viability of primary B cells in RA [24,25]. These effects were dependent on interactions between RA-NLC-expressed CD106 and B-cell-expressed very late antigen 4 [24], which were quite similar to the interactions between RA-NLCs and B cells we had previously reported [7]. Although the other group's NLCs were identified to be derived from CD14 myelomonocytic cells [22,23,25] we have not yet clarified the stem cell of our RA-NLCs, but it clearly appears to be of mesenchymal origin [5,6].

RANKL-independent differentiation of osteoclast-like cells supported by RA nurse-like cells

RA-NLCs also promoted a specific pathway of the differentiation of CD14(+) monocytes. After 3–4 weeks of co-culture, CD14(+) monocytes differentiated into tartarate-resistant acid phosphatase (TRAP)(+) mononuclear cells with abundant cytoplasm and an off-center nucleus without the involvement of RANKL. It was noted that RA-NLCs supported such differentiation of peripheral blood CD14(+) monocytes not only from RA patients, but also from normal control subjects [10]. The second step of differentiation from such TRAP(+) mononuclear cells into multinucleated bone-resorbing giant cells (osteoclast-like cells) could also be induced without RANKL in the presence of IL-3, IL-5, IL-7, or GM-CSF, and was inhibited by mAb to each cytokine [10]. Differentiation of these TRAP(+) mononuclear cells into multinucleated bone-resorbing giant cells could also be promoted by macrophage colony-stimulating factor and RANKL [26].

Table 1

Effects of co-culture on production of cytokines from rheumatoid arthritis nurse-like cells (RA-NLCs)

	Cytokines in cell culture supernatant (pg/ml)[a]									IgM (μg/ml)[a]	
	IL-1α	IL-1β	IL-6	IL-7	IL-8	G-CSF	GM-CSF	TNFα	TNFβ	Experiment 2	Experiment 3
Cytokine production from RA-NLCs derived from synovium and immunoglobulin from B cells[b] [6]											
RA-SNCs	<5.0	<10.0	2,200		4,300	460	40	<5.0	<5.0	<1.5	<1.5
B cells	<5.0	<10.0	<10.0		<10.0	<10.0	<2.5	<5.0	<5.0	1.8	2.7
B cells + RA-SNCs (separated)[c]	<5.0	<10.0	1,800		3,900	510	30	<5.0	<5.0	<1.5	<1.5
B cells + RA-SNCs	<5.0	153	15,900		34,500	2,400	740	690	<5.0	5.6	8.6
Cytokine production from RA-NLCs derived from bone marrow cells[d] [5]											
RA-BMNC-1 cell line	–	–	38,250	–	1,480	–	150	–			
+ MC/car cell line	–	320	89,015	–	33,510	755	915	275			
+ Molt-17 cell line	–	235	78,750	–	10,615	540	355	255			

RA-BMNCs, cytokine production from RA-NLCs derived from bone marrow cells; RA-SNCs, cytokine production from RA-NLCs derived from synovium; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; –, not detectable. [a]The amount of each cytokine and IgM in the culture supernatant was measured with an enzyme-linked immunosorbent assay kit. [b]B-cell clones (1×10^5) and RA-SNC3 (5×10^4) were cultured under the indicated conditions for 3 days in 24-well plates. [c]B-cell clones were cultured in a Millicell culture insert. [d]RA-BMNC cells (3×10^4 cells/well) were inoculated and cultured overnight, and 1×10^6 cells MC/car cells or Molt-17 cells were added to the culture. After 5 days of incubation, the culture supernatants were collected and the amount of each cytokine in the culture supernatant was measured with an enzyme-linked immunosorbent assay kit.

Expression of MMP-2, MMP-9, and MMP-12 was increased in both TRAP(+) mononuclear and multinucleated cells after differentiation by culture with RA-NLCs, and these cells could induce cartilage degeneration *in vitro* by a mechanism that was completely blocked by inhibitors of MMP-2 and MMP-9. Although MMP-2 expression was significantly increased in TRAP(+) mononuclear cells, expression of MMP-9 and MMP12 was also higher in TRAP(+) multinucleated cells [27]. Of note, both TRAP(+) mononuclear and multinucleated cells differentiated by culture with RA-NLCs specifically expressed MMP-12 [27], whereas multinucleated cells expressing MMP-12 were clearly found near the bone erosions (S Yamane, M Maeda-Tanimura, Y Shimaoka, M Yukioka, T Toyosaki-Maeda, S Ishida, N Yamane, Y Tsuruta, T Itoh, N Fukui, *et al.*, unpublished observation). RA-NLCs were therefore found to promote the differentiation of CD14(+) monocytes in a characteristic two-step differentiation process into multinucleated osteoclast-like cells with the capacity to degrade bone and cartilage.

Although TNF [28], IL-1 [29], macrophage colony-stimulating factor, and RANKL [30] are very important factors for developing osteoclasts, the RANKL-independent two-step differentiation of CD14(+) monocyte supported by RA-NLCs [10,26] may be an alternative pathway to develop multinucleated osteoclast-like cells specifically in RA. Beside the destruction of bone tissue by osteoclasts or osteoclast-like cells, we could confirm that FSCs from RA patients inoculated *in vivo* showed aggressive behavior, invading cartilage as reported previously [31-33], although we have not yet confirmed that pure RA-NLC lines have such function.

Comparison of the properties of RA nurse-like cells and fibroblast-like synoviocytes

A considerable amount of work has characterized another population of cells found in the rheumatoid synovium, namely fibroblast-like synoviocytes. The cells are thought to play a role in rheumatoid pathogenesis, especially because of their capacity to contribute to tissue damage [31-33]. RA-NLCs, however, have a number of specific attributes that suggest they may play a unique role in RA pathogenesis (Table 2).

Mechanisms of progressive proliferation of fibroblastic stromal cells specifically found in joint

To explain the remarkable proliferation of synovial tissue in the RA patient, various mechanisms have been reported such as the involvement of protooncogenes [34], inflammatory cytokines [35], and perturbations of Fas-mediated apoptosis [36]. As a mechanism specifically found in the synovial space but not in the bone marrow, we found that the interference with Fas-mediated apoptosis could upregulate specifically the growth of synovial FSCs [37,38]. In this regard, soluble Fas ligand was found to inhibit competitively the Fas–Fas ligand-mediated apoptosis [37] of FSCs bearing Fas. The levels of human soluble Fas ligand in synovial fluid from RA patients were found to be significantly higher than those from osteoarthritis patients.

Table 2

Comparison of the properties of rheumatoid arthritis nurse-like cells and fibroblast-like synoviocytes

Property	Rheumatoid arthritis nurse-like cells	Fibroblast-like synoviocytes
Pseudoemperipolesis	+	–
Constitutive expression of CD106	+	–
Enhanced expression of CD106 and CD157 by IFNγ	+	–
Promote B-cell differentiation	+	–
Promote differentiation of osteoclast-like cells from CD14(+) monocytes	+	–
Inhibit lymphocyte apoptosis	+	–

In contrast, soluble Fas ligand was not detected in the peripheral blood, and also not in bone marrow blood in RA patients [38]. This mechanism, therefore, could at least partially upregulate the FSC growth in synovial tissue, but not in bone marrow.

Conclusion

A specific population of FSCs, RA-NLCs reside in both the bone marrow and synovium of RA patients and have the functional capacity to interact with lymphocyte and monocyte populations, inducing cellular differentiation and biologic activities that mimic pathophysiologic features of rheumatoid inflammation. These findings suggest that RA-NLCs may play an essential role in the development of local immune and inflammatory responses in the synovium and the bone marrow. RA-NLCs could therefore be central elements in the pathologic events in RA and might be appropriate targets for therapeutic intervention in RA.

Competing interests

The authors declare that they have no competing interests.

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