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Endothelial Cells and the Pathogenesis of Rheumatoid Arthritis in Humans and Streptococcal Cell Wall Arthritis in Lewis Rats

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Abstract Endothelial cells play a fundamental role in the pathogenesis of chronic inflammatory arthritis in humans such as rheumatoid arthritis (RA), as well as experimental animal models such as streptococcal cell wall (SCW) arthritis in Lewis (LEW/N) rats. This review summarizes data in support of this concept. The earliest apparent abnormalities in synovial tissues of patients with RA and Lewis rats with SCW arthritis appear to reflect microvascular endothelial cell activation or injury. At the molecular level, the abnormalities include enhanced expression by endothelial cells of activation markers such as class II major histocompatibility complex antigens, phosphotyrosine, leukocyte adhesion molecules, oncoproteins such as c-Fos and c-Myc, and metalloproteinases such as collagenase and transin/stromelysin. The development of severe, chronic, destructive arthritis is dependent upon thymic-derived lymphocytes and is accompanied by tumorlike proliferation of cells in the synovial connective tissue stroma (blood vessels and fibroblastlike cells), which results in resorptive destruction of bone and cartilage. Multiple criteria support the analogy to a neoplastic process. Paracrine and autocrine factors such as interleukin-1 (IL-1), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), and heparin-binding fibroblast growth factors (HBGF, FGF) appear to play important roles in the generation of these lesions. Finally, in addition to the autocrine and paracrine regulatory factors, neuroendocrine factors, particularly the hypothalamic-pituitary-adrenal axis, appear to be involved in the counterregulation of the inflammatory process. The counterregulatory effects are mediated, in part, by inhibition of endothelial cell activation by corticosteroids.

Key words: neuroendocrine, cytokines, adhesion, transin, stromelysin, collagenase

In recent years there has been an increasing appreciation of the critical role played by vascular endothelial cells in inflammatory processes. Recognition of this fact is particularly true in the case of inflammatory arthritides, such as rheumatoid arthritis (RA) in humans [1–6], and experimental models such as streptococcal cell wall (SCW)-induced arthritis in Lewis rats, which closely mimics many features of RA [4–7]. The objective of this presentation is to provide a brief overview of data that support this concept.

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ENDOTHELIAL CELLS AND EARLY EVENTS IN RHEUMATOID ARTHRITIS AND STREPTOCOCCAL CELL WALL ARTHRITIS IN LEWIS RATS

Although an etiologic agent(s) has not been defined, abnormalities of microvasculature of the synovium, the delicate tissue that lines joint cavities, is a consistent feature of all stages of RA. Microscopically apparent abnormalities include: a) gaps between the microvascular endothelial cells facilitating exudation of plasma proteins from the intravascular space, b) swelling of the endothelial cells resulting in a narrowing of the intraluminal space, c) platelet aggregation and fibrin thrombus formation, and d) perivascular accumulation of mononuclear inflammatory cells [2,8, and Wilder RL, unpublished data].

Similar microvascular abnormalities develop rapidly in female Lewis rats injected with cell wall fragments (peptidoglycan-polysaccharides) from group A streptococci and a variety of other bacteria [9,10].

SCW arthritis is a valuable model because the etiologic agent (i.e., bacterial cell walls) is well characterized, and the timing of development of the disease is experimentally determined. The earliest phase of SCW arthritis is characterized by local cell wall deposition. Within 24 hours of a single intraperitoneal injection of cell wall fragments to female Lewis rats, arthritis begins to develop in peripheral joints. This rapid onset of disease is due to the dissemination of cell walls and localization in the joints within several hours of their injection. Particularly relevant, cell wall antigens can be detected in the cytoplasm of synovial microvascular endothelial cells. Later, they are also detectable in inflammatory phagocytic cells that infiltrate the synovial tissues. The cell walls also localize to phagocytic cells in the liver, spleen, and bone marrow [9]. Arthritogenic cell walls are highly resistant to biodegradation [10] and, therefore, persist in these sites for periods of months. Coincident with the localization of the cell walls to the joints, abnormalities in the synovial microvasculature develop. Prominent synovial microscopic changes include platelet aggregation and fibrin thrombus formation, the development of edema fluid secondary to plasma protein exudation, swelling of synovial endothelial cells, and infiltration of phagocytic inflammatory cells, primarily around blood vessels with swollen endothelial cells [9].

Although data available on the early synovitis of RA are somewhat limited, it is apparent that infiltration of inflammatory cells is accompanied by a number of biochemical alterations in the synovial microvascular endothelial cells [1–3]. To the degree they have been compared, similar molecular events are noted during the acute, rapid-onset phases of SCW arthritis in Lewis rats. For example, a pronounced increase in the expression of class II major histocompatibility complex (MHC) antigens is notable. Normal synovial microvascular endothelial, as well as other types of synovial cells, express little or no class II MHC antigen. It appears likely that this enhanced antigen expression by endothelial cells is involved in the process of "antigen presentation" to immune cells [9]. In addition, enhanced expression of other activation markers such as c-fos [4] and phosphotyrosine [11] have been noted in the synovial endothelium. Other documented changes include a marked increase in the production of metalloproteinases, such as transin/stromelysin [7,12], by synovial endothelial cells, as well as fibroblastlike cells and macrophages. Transin/stromelysin is a proteoglycanase, as well as a major activator of procollagenase, and, thus, appears to play an important role in mediating the destruction of extracellular matrix that is characteristic of diseases like RA and SCW arthritis.

Although the in vivo data are more preliminary, it appears that the process of endothelial cell activation/injury during the development of the inflammatory process involves the expression of various adhesion molecules specific for different types of inflammatory cells, e.g., ELAM-1, ICAM-1, and others. A rapidly enlarging body of in vitro data, however, provides evidence in support of the concept that expression of specific leukocyte adhesion molecules by endothelial cells is involved in regulating the type and number of inflammatory cells that infiltrate the synovial membrane [1–3,13].

ENDOTHELIAL CELLS AND CHRONIC SYNOVITIS IN RHEUMATOID ARTHRITIS AND SCW ARTHRITIS IN LEWIS RATS

The development of severe, chronic, destructive arthritis in patients with RA and LEW/N rats with SCW-induced disease is clearly dependent upon thymic-derived lymphocytes, particularly cells expressing the cell surface CD4 marker [9,14]. For example, drugs that block the activation of CD4-positive T-cells, such as cyclosporin A, are potent inhibitors of RA in humans and SCW arthritis in Lewis rats [15,16]. Available data suggest that the accumulation of T-cells in the synovium is stimulated and maintained, to a large extent, by nonspecific actions of various chemotactic agents [3], by enhanced binding of T-cells to post-capillary venules [2,3,8,17,18], and by binding of T-cells to synovial fibroblastlike cells [19]. Similar, but likely distinct, pathways appear to be involved in the recruitment of other types of leukocytes [20,21].

The T-cell dependent chronic disease in RA and SCW arthritis is accompanied by and drives tumorlike proliferation of the synovial connective tissue stroma that is comprised primarily of fibroblastlike cells and new blood vessels [4,5,7,12,22]. Bone and cartilage destruction develop at sites where they join the highly proliferative and invasive synovial tissue. Angiogenesis

is clearly a prominent feature of the invasive process [4,22]. Moreover, a large body of data, both in vivo and in vitro, support the tumorlike concept. For example, in vivo, these tissues express high levels of oncoproteins such as c-myc and c-fos, of metalloproteinases which facilitate extracellular matrix resorption such as collagenase and transin/stromelysin, and of cytoskeletal markers such as vimentin which characterize poorly differentiated, mesenchymal cells [4,5,7,12,22]. In vitro, early-passage cultures of synovial fibroblastlike cells from diseased joints grow rapidly, do not contact inhibit, form foci, and can be grown under anchorage-independent conditions in soft agarose [22,23]. All of these features are markers of an immature, transformed-like phenotype by the proliferative and invasive synovial mesenchymal cell population. In addition, both rheumatoid and SCW arthritis-derived synovial tissues form short-lived tumorlike nodules when implanted in athymic nude mice [22,24]. However, although the inflammatory process in RA and SCW arthritis behaves like a highly vascular, invasive tumor, it is important to note that it is not malignant, in that it is only locally invasive and that its development is clearly dependent upon the immune system and factors generated in the inflammatory milieu of the arthritic joint [4–6,22,23,25]. For example, athymic nude rats do not develop the severe, proliferative, and invasive disease characteristic of euthymic rats given SCW [9].

CYTOKINES

A wide variety of cytokines, such as interleukin-1 [23,25–27] platelet-derived growth factor-like factors [23,25–28], transforming growth factor-beta 1 and 2 [23,25,26,29–31], and heparin-binding fibroblast growth factor [4,28], have been implicated in the pathogenesis of rheumatoid arthritis and SCW arthritis by their presence in synovial tissues or synovial fluids. Interleukin-1 has received particular attention. Intraarticular injection of IL-1 produces both acute and chronic synovitis in experimental animals [1]. Many of these effects may be mediated, in part, by direct effects of IL-1 on endothelial cells [1–3]. For example, endothelial cell changes induced by IL-1 include stimulation of procoagulant activity, plasminogen activator inhibitor and platelet activating factor, the secretion of colony stimulating factors, as well as the expression of leukocyte adhesion molecules. Collectively, these changes promote thrombosis, leukocyte chemotaxis, and adhesion. In addition, IL-1 has important effects on a variety of other cell types that participate in the inflammatory process in the joints [26,27]. The multitude of proinflammatory effects of IL-1 justify attempts to interrupt its activity in inflammatory arthritides [26].

Although it does not directly stimulate endothelial cells, platelet-derived growth factor, at least the A chain polypeptide, is produced by activated endothelial cells [31]. Since PDGF is a potent fibroblast mitogen [23,25,27,28], endothelial cell-derived PDGF-A may play a role in stimulating the proliferation of fibroblastlike cells in the synovium of arthritic joints. Transforming growth factor-beta types 1 and 2 have been implicated in rheumatoid arthritis and SCW arthritis [23,29,30]. In vitro, TGF-beta 1 is a potent direct inhibitor of endothelial cell proliferation, but local injections of TGF-beta into the skin of mice induce marked angiogenesis, presumably indirectly, through the production of endothelial cell growth factors [31].

Heparin-binding fibroblast growth factor-1 (HBGF-1, the precursor of acidic FGF) has also been implicated in the pathogenesis of rheumatoid arthritis, as well as SCW and adjuvant arthritis in Lewis rats [4,28]. This growth factor is an extremely potent mitogen for endothelial cells in vitro. It is also a potent angiogenic factor in vivo. By immunohistochemical criteria, little or no HBGF-1 is detected in normal joints. But in the experimentally-induced arthritis models, HBGF-1 appears rapidly, paralleling other markers of the inflammatory disease process, but it requires the thymic-dependent components of the immune system to sustain its expression. Similarly, HBGF-1 is readily immunohistochemically stained in inflamed rheumatoid synovial tissues, is minimally expressed in non-inflammatory synovial tissues, and is not immunohistochemically detected in normal synovial tissues. Thus, the available data support the view that HBGF-1 may play an important role in driving angiogenesis in synovia of patients with RA and Lewis rats with experimentally-induced arthritides. A role for other angiogenic polypeptides, such as basic-FGF/HBGF-2, also seems likely, but is less well established.

NEUROENDOCRINE REGULATORY MECHANISMS

Although local autocrine and paracrine factors have received the most attention as regulators of endothelial cell growth and function in

the inflammatory site, recent data provide compelling evidence that systemic neuroendocrine factors, particularly factors operative through the hypothalamic-pituitary-adrenal (HPA) axis, are equally important [32,33]. It is now well recognized that immune and inflammatory processes bidirectionally communicate with the central nervous system, resulting in a feedback regulatory system for the maintenance of physiological homeostasis [34]. For example, IL-1, a major product of activated macrophages, is a potent stimulator of the HPA axis, i.e., enhanced production of hypothalamic corticotropin releasing factor, pituitary adrenocorticotropin, and adrenal corticosteroids. Corticosteroids, of course, are the most potent endogenous anti-inflammatory substances known, and their production during inflammation serves to restrain and prevent unchecked amplification of the inflammatory process that can produce self-injury, i.e., autoimmune disease. The importance of this neuroendocrine system is highlighted by recent work from our laboratories that shows that the Lewis rat has a profound defect in HPA axis activation [32,33]. For example, in response to SCW, interleukin-1, and a variety of other stressors, LEW/N rats show a minimal response. In contrast, arthritis-resistant rats such as F344/N exhibit robust activation of the HPA axis in response to the same stimuli. In contrast to Lewis rats, this rat strain shows little evidence of the endothelial cell activation and injury that develops in the Lewis rat after injection of SCW. Importantly, treatment of Lewis rats with physiological concentrations of corticosteroids beginning at the same time that the SCW are administered markedly inhibits the development of arthritis. Conversely, blocking the effects of corticosteroids with a corticosteroid receptor antagonist, RU 486, allows the development of severe, systemic inflammatory disease in SCW-injected F344/N rats. These data provide strong support that genetically determined variation in activation of the HPA axis and the production of corticosteroids plays a critical role in the development of the inflammatory process. Endothelial cells are obviously major targets of this regulatory system.

PROSPECTS

Endothelial cells form a selective biological interface between blood and tissue. It is not, therefore, surprising that these cells play a critical role in virtually all phases of destructive inflammatory disease processes such as rheumatoid arthritis. The importance of these cells is further highlighted by parallel data generated in experimental animal models such as SCW arthritis in Lewis rats. It should be apparent from this brief overview that a high probability exists that further delineation of the molecular, cellular, and systemic control of endothelial cell growth and function during inflammatory diseases, such as discussed here, may generate therapeutic opportunities applicable to a wide variety of disease processes.

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