Rheumatoid arthritis, cytokines and hypoxia

What is the link?

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that affects approximately 1% of the population, in a female to male ratio of 3:1. The disease can occur at any age, but it is most common among those aged 40-70 years. Despite many years of study, the etiology of RA is still undefined. However, with increased understanding of the immune system the pathogenesis of RA has become clearer. A large bulk of data suggests that T lymphocytes and macrophages play a critical role in the initiation and perpetuation of synovial inflammation. Recently, the cytokine profile of T helper cells has been associated with the disease, the cytokine repertoire of inflamed synovia is categorized as that of T helper 1 response. Moreover, in RA elevated levels of pro-inflammatory or inflammatory cytokines such as Tumor Necrosis Factor - alpha (TNF-α) and Interleukin -1 beta (IL-1ß) have been detected. Hypoxia up-regulates TNF-α and IL-1ß; therefore, considerable research interest has been focused on the biological consequences of the hypoxic nature of the rheumatoid synovium. Hypoxia might underlie the functional polarization of the T cells and cytokine production, and thus may contribute to the progression and persistence of the disease. In this short review, we discuss our current knowledge of the link between cytokines and RA and the role of hypoxia in the pathogenesis of the disease.

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Rheumatoid arthritis (RA) is a chronic recurrent, systemic inflammatory disease primarily involving the synovial membranes of multiple joints, followed by cartilage and bone destruction, which eventually leads to joint deformities and loss of articular functions. The clinical course of the disorder is extremely variable, ranging from mild, self-limiting arthritis to rapidly progressive multi-system inflammation with profound morbidity and mortality. The predominant symptoms of RA are pain, stiffness, and swelling of peripheral joints. These symptoms are accompanied by signs of articular inflammation, including swelling, warmth, erythema, and tenderness on palpation. At the onset of the disease small joints of the hand and feet are involved. Large joints such as knees, hips, elbows, ankles and shoulders commonly become involved later in the course of the disease. In the most severe cases, symptoms can include lesions of tendons, skeletal muscle, peripheral nerves and arteries.

Etiology of RA. The cause of RA remains obscure over the years, a number of potential etiologic agents have been proposed. Some of these agents are illustrated in Table 1. Although many of these suggested pathogens could cause RA, to date no etiologic agent has been conclusively confirmed for RA. The mechanism by which any microorganism may cause RA also remains unclear. However, there are some proposed mechanisms such as (i) the direct infection into the synovium or retention of microbial...
The initial stage in the expression of certain class II MHC gene products. Degradation is mediated by a family of proteolytic enzymes Matrix Metalloproteinase (MMPs), produced by both macrophages and activated fibroblasts in response to proinflammatory cytokines such as interleukin-1-β (IL-1β) and tumor necrosis factor-α (TNF-α). Important enzymes in this category include collagenase (MMP-1), gelatinase (MMP-2) and stromelysin-1 (MMP-3). These enzymes are secreted as proenzymes, which are later activated by proteolytic activity of other enzymes, such as trypsin and plasmin, and these activated enzymes are regulated by inhibitory proteins such as tissue inhibitors of metalloproteinases (TIMPs). The role may predispose to disease by positively selecting for auto-reactive clones of T cells or by negatively selecting against immuno-regulatory clones.

Pathogenesis of RA. The initial stage in the chronic synovial inflammation involves some replication of cells in the synovial membrane to form a multi-cellular layer, which thickens primarily due to the simultaneous recruitment to this area of large numbers of macrophages, and mature T cells. In the chronic phase of RA, infiltration of lymphocytes and macrophages becomes more abundant. Moreover, the chronic synovial inflammation in RA results in the formation of the pannus. This highly vascularized granulation tissue is composed of a variety of cell types, including not only lymphocytes and macrophages but also fibroblast synoviocytes and mast cells. Pannus is found at the marginal areas of diarthrodial joints, where it impinges upon articular cartilage and subchondral bone. It is likely that mature T cells (CD4+ CD45RO+) are capable of triggering several factors responsible for rheumatoid symptoms, including cytokine release and immunoglobulin (Ig) production by B cells. Immunoglobulin M (the most prominent isotype) and IgG rheumatoid factors (RFs), together with anti-type-II-collagen activity and antibodies to collagen types V, VI and IX have been detected in the synovium of RA patients. The binding of such immune complexes to cartilage could exacerbate the immune response by activating complement and triggering further cellular inflammatory response.

The maintenance of the integrity of cartilage and other extracellular matrix (ECM) tissue is a dynamic process. In the normal state, the synthesis of ECM components by chondrocytes and fibroblasts is counterbalanced by a specific degradation of these molecules. Degradation is mediated by a family of proteolytic enzymes Matrix Metalloproteinase (MMPs), produced by both macrophages and activated fibroblasts in response to proinflammatory cytokines such as interleukin-1-β (IL-1β) and tumor necrosis factor-α (TNF-α). Important enzymes in this category include collagenase (MMP-1), gelatinase (MMP-2) and stromelysin-1 (MMP-3). These enzymes are secreted as proenzymes, which are later activated by proteolytic activity of other enzymes, such as trypsin and plasmin, and these activated enzymes are regulated by inhibitory proteins such as tissue inhibitors of metalloproteinases (TIMPs).
inhibitor of Metalloproteinase (TIMPs). These are also released by macrophages and fibroblasts forming the pannus.\textsuperscript{19} In addition, there are other proteases that probably play an important role in tissue damage in RA include elastase and cathepsin G, secreted by macrophages and other inflammatory cells.\textsuperscript{20}

\textbf{The link between cytokines and rheumatoid arthritis.} Cytokines play a crucial role in mediating tissue damage. Interleukins (IL-1, IL-6) and TNF-\(\alpha\) may directly activate osteoclasts, stimulate cyclooxygenase-2 (COX-2) resulting in increased prostaglandin \(E_2\) (PGE\(_2\)) production and may cause ECM degradation via their ability to stimulate collagenase and stromelysin production by synoviocytes and chondrocytes.\textsuperscript{21}

The increase in intercellular adhesion molecules-1 (ICAM-1) expression, which accompanies the thickening of the pannus, is also enhanced by the action of IL-1\(\beta\), IL-6, and interferon-\(\gamma\) (IFN-\(\gamma\)).\textsuperscript{22,23} The pathogenesis of RA is illustrated diagrammatically in Figure 1. Rheumatoid arthritis is associated with an increased production of a wide range of cytokines.\textsuperscript{24} Cytokines such as IL-1 and TNF-\(\alpha\) produced by activated synoviocytes, mononuclear cells or by articular cartilage significantly up-regulate MMP gene expression. Matrix Metalloproteinase form a family of enzymes capable of degrading various extracellular matrix components.\textsuperscript{20,21} High levels of IL-1, TNF-\(\alpha\), IL-6, IL-8, IL-15, IL-18, transforming growth factor-beta (TGF-\(\beta\)) granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein (MIP-1), and monocyte-chemoattractant protein (MCP-1) are detected in RA synovial tissue. Tumor necrosis factor-\(\alpha\) may be the major cytokine involved in the inflammatory process, whereas IL-1\(\beta\) is a key mediator with regard to cartilage and bone destruction.\textsuperscript{21-24} Analysis of the synovial cell infiltrate in early RA revealed a positive correlation between the disease activity, the number of macrophages, IL-6, and TNF-\(\alpha\) expression in the synovium, suggesting that macrophage-derived cytokines play an important role in progression of the disease.\textsuperscript{16} Successful drug treatment of patients with RA resulted in a decrease in inflammatory cytokine levels such as TNF-\(\alpha\) and IL-1\(\beta\).\textsuperscript{16,25}

\begin{figure}[h]
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\caption{Pathogenetic pathways of joint destruction in rheumatoid arthritis patients.}
\end{figure}
Pro-inflammatory cytokines. Interleukin-1 beta and TNF-α are thought to play a key role in the inflammation and joint damage that occur in RA. The pathologic effects caused by these cytokines include leukocytic infiltration leading to synovial hyperplasia, cell activation, cartilage breakdown, and inhibition of cartilage matrix synthesis. Moreover, IL-1 and TNF-α increase the expression of adhesion molecules, such as ICAM-1, PGE₂, MMPs, chemokines (IL-8), and other pro-inflammatory cytokines (IL-6). Interleukin-1 beta shares many biological functions with TNF-α and is a potent factor in inducing damage to cartilage and bone, because it induces proteoglycan degradation and inhibits proteoglycan synthesis. Proteoglycans are important structural constituents of connective tissue, particularly of cartilage. Two distinct TNF-α receptor (TNF-αR) types have been demonstrated, 55-kDa (TNF-R1) and 75-kDa (TNF-R2). Macrophages are the principal cells producing IL-1β and TNF-α. It is not clear yet which mechanism controls this production, however at least 2 major pathways exist: 1) induction of cytokine release by T and B lymphocytes, mast cells, or soluble factors (such as immune complex or other cytokines) and 2) induction by direct contact between the macrophages and activated T lymphocytes. Interleukin-1 and TNF-α are usually synthesized and secreted simultaneously, although their production is controlled by different mechanisms. Tumor necrosis factor-α is one of the proinflammatory cytokines that plays a pivotal role in RA. It is produced intracellularly (by macrophages, monocytes, lymphocytes and transformed cell lines of hemopoietic and non-hemopoietic origin) as a 26-kDa pro-peptide and is also present in the cell membrane in an active form. The active form is a trimer with a subunit of 17-kDa. Like IL-1β, lipopolysaccharide (LPS) induces the production of TNF-α and TNF-β, TNF-α/β can also be stimulated by other cytokines such as IFN-γ, IL-1, CSF and IL-17. Tumor necrosis factor-α has a wide range of functions; it induces IL-1β and other proinflammatory cytokines such as GM-CSF, IL-6 and IL-8 production. Moreover, TNF-α up regulates expression of HLA-DR and IL-2R. Interleukin-18 is a strong inducer of IFN-α production by T cells and enhances the cytotoxicity in acquired and innate immunity. It also induces the production of the CC and CXC chemokines, TNF-α, IL-1β, GM-CSF, IL-2, IL-4, IL-5, IL-10, IL-13, PGE₂, and adhesion molecules such as ICAM-1. Interleukin-18 works in synergy with IL-12 in the induction of Th1 cells because of the reciprocal regulation of the receptors.
been associated with Th2 response, angiogenesis and neutrophil activation, suggesting a broader role for IL-18. Moreover, administration of IL-18 promoted the development of collagen induced arthritis. In addition to that, IL-18-binding protein (IL-18BP) has been described as a novel modulator of the Th1 cytokine response. In an animal model, IL-18 blockade through polyclonal antibodies or recombinant human IL-18BP significantly reduced disease activity. Finally, neutralizing IL-18 monoclonal antibodies have the potential to become valuable tools for therapeutic approaches in vivo.

**Anti-inflammatory cytokines.** Raised levels of plasma interleukin-1 receptor antagonist (IL-1ra) have been detected in patients with juvenile arthritis, RA, polymyositis, and systemic lupus erythematosus (SLE). High levels of IL-1ra have also been detected in synovial fluid of patients with RA. However, RA patients exhibit a lower IL-1ra to IL-1β ratio than patients suffering from osteoarthritis. This indicated that a high level of IL-1ra is essential to reduce the effect of IL-1. In vitro experiments have revealed that an excess of 100 times the amount of IL-1ra is required to inhibit IL-1β activity, whereas in vivo, 100-2000 times more IL-1ra is needed. However, IL-1ra production by RA synovial cells is deficient relative to total IL-1 production. The capacity of IL-1ra to reduce in vitro and in vivo cartilage degradation and the progression of inflammatory diseases such as RA has elicited much attention in therapeutic approaches. Treatment of RA with IL-1ra resulted in reduced mononuclear infiltration of synovial membrane, which may represent the in vivo inhibition of biologically relevant IL-1-mediated pathogenic.

In addition to IL-1ra, there are several inhibitory cytokines that play a critical role in modulation of proinflammatory cytokines. Transforming growth factor-beta is a multi functional cytokine that exhibits both stimulatory and inhibitory effects upon a range of cell types. Elevated levels of TGF-α have been found in the synovial fluid of RA patients. The inhibitory effect of TGF-α results in inhibition of T and B cell proliferation and cytokine production; as well as inhibition of MMPs. Also it is capable of inducing TIMP secretion.

Interleukin-4 induces IL-1ra production and upregulates the expression of the 'decoy receptor' thus exerting an anti-inflammatory response. Interleukin-4 also suppresses biosynthesis of MMPs in alveolar macrophages. Interleukin-4 can also inhibit the production of superoxide in macrophages. Substantial amounts of IL-10 have been detected in RA synovium and synovial fluid but insufficient to reduce the effect of IL-1. Neutralization of endogenously produced IL-10 in RA synovial membrane cultures resulted in a 2 to 3-fold increase in the protein levels of TNF-α and IL-1β. Similar effect has been observed when exogenous recombinant IL-10 was added to the RA synovial membrane cultures.

Interleukin-11 has been detected in the serum, synovial fluid, and synovial membrane of patients with RA. Treatment with recombinant human IL-11 (rH-IL-11) decreased the production of TNF-α and IL-1β and nuclear factor-κ B (NF-κB)-binding activity (a transcription factor that mediates expression of certain cytokines genes such as TNF-α and IL-1) in RA synovium in vitro. Blockade of endogenous IL-11 resulted in a 2-fold increase in TNF-α levels and the addition of IL-11 inhibited TNF-α, MMP-1 and MMP-3 production. In addition, IL-11 stimulates MMP inhibitor levels in RA synovial tissue.

**Hypoxia, oxidative stress and RA.** The rheumatoid synovial cavity has a positive intra-articular pressure, in contrast to a normal joint, which has negative pressure. In RA synovium, the cavity pressure is raised and upon movement this pressure exceeds the capillary perfusion pressure, causing collapse of the blood vessels. This leads to the production of multiple episodes of hypoxic reperfusion injury, which consequently generates reactive oxygen species (ROS).

The hydroxyl radicals are very highly reactive, attacking a wide range of targets including hyaluronic acid in the synovial fluid, causing loss of lubricant properties and consequently mechanical damage to the joint. The hydroxyl radicals break single and double stranded DNA and attack amino acids, thus may be involved in the formation of rheumatoid factors (IgG, IgM and IgA). Proteins modified by hydroxyl radicals, could induce autoimmune responses and release of autoantibodies by B cells.

Oxidative stress also has an effect on T cells; it has been observed that depletion of an antioxidant resulted in reduction of T cell proliferation, IL-2R expression and IL-2 production. Hypoxia transcriptionally upregulates TNF-α and IL-1, but down regulates IL-2, therefore hypoxia might underlie the functional polarization of Th1/Th2 cells and so favor proinflammatory cytokines such as IL-1 and TNF-α. Moreover, low concentration of H₂O₂ caused activation of T cells and down-regulation of CD3 expression.

As protection against oxidative stress, cells possess anti-oxidants that maintain the intracellular redox environment. Glutathione (GSH) is a major cellular anti-oxidant found in all eukaryotic cells. Cellular H₂O₂ is eliminated in the cytoplasm by GSH-peroxidase-catalyzed reduction, with GSH as a substrate. As a result of this reaction, oxidized GSH (GSSG) is formed, and then the GSSG is restored.

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to GSH by GSH reductase. Thioredoxin (TRX) is a small multifunctional cellular protein that is also capable of reducing some ROS including H$_2$O$_2$. Moreover, H$_2$O$_2$ can be eliminated by the enzyme catalase in the peroxisomes.

**Glutathione and redox balance.** Glutathione (L-γ-Glutamyl-L-cysteinylglycine, GSH) is a major intracellular non-protein sulfhydryl anti-oxidant, and plays a central role in defending cells against free radicals and electrophiles and maintaining cellular oxidation-reduction (redox) equilibrium. The protective role of GSH consists of 4 components: (i) chemical reaction with intracellular targets (in most cases DNA); (ii) enzymatic reduction of peroxides to prevent their decomposition; (iii) enzymatic detoxification of electrophiles, as a coenzyme for formaldehyde dehydrogenase and glyoxalase and as a substrate for the GSH S-transferase, and (iv) maintenance of the redox state of cellular thiol. Most cell types can metabolize extracellular GSH by an ectoenzyme, γ-glutamyl transpeptidase (GGT), which transfers glutamate to amino acid acceptors. The rate of transport and the intracellular availability of cysteine appear to control the rate of GSH synthesis. The balance between reduced and oxidized glutathione is an important mechanism for balancing the effect of reactive oxygen species and alkylating agents. Oxidized glutathione can modify selected proteins in a process referred to as S-glutathiolation and is used as a means of minimizing oxidative damage. These reversible disulfide bonds are an effective means of protecting modified proteins against irreversible oxidative damage. Redox signaling involves modification of cellular proteins, and it leads to a decrease in the cellular glutathione levels, (glutathione depletion has an effect on gene expression). On one hand, GSH depletion is associated with augmentation of an oxidative stress-mediated production of proinflammatory cytokines. It has been shown that pre-treatment with L-buthionine (S-R) sulfoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase, prior to exposure to LPS, augmented in a dose-dependent manner LPS-induced TNF-α and IL-6 biosynthesis in alveolar epithelial cells, an effect associated with the induction of intracellular accumulation of ROS, and reduced IL-12 production by human alveolar macrophages. Moreover, BSO treatment of bronchial epithelial cells (BECs) facilitated TNF-α-induced mitogen-activated protein kinase (MAPK), p38 activation and RANTES production.

On the other hand, antioxidant drugs can in turn act as modulators of the immune response. In particular, N-acetylcysteine (NAC), through interfering with or counteracting ROS-induced subcellular changes can modulate several cell functions. N-acetylcysteine treatment induced a significant upregulation of IL-1β, IL-2, IL-15, and IL-12 production. Furthermore, in contrast to BSO, which activates NF-κB, antioxidants such as NAC inhibit the activation of NF-κB and AP-1, and consequently block transcription of cytokine genes. Lymphocyte function is particularly dependent on GSH levels. Glutathione is known to influence lymphocyte growth and activation as well as their responsiveness to cytokines. Rheumatoid arthritis synovial fluid T cells contain decreased GSH levels compared to cells isolated from peripheral blood of RA patients or healthy people. However, synovial T cells show features of hyporesponsiveness. This hyporesponsiveness is reflected by low proliferative responses as well as low calcium responses upon stimulation. It has been shown that depletion of intracellular GSH levels in T lymphocytes through treatment with BSO results in hyporesponsiveness due to the abrogation of the proximal TCR-mediated signaling and displacement of the adaptor protein Linker for Activation of T cells (LAT) from the plasma membrane. This has been also observed in Synovial fluid T cells in RA, may be due to subjection to chronic oxidative stress and decreased GSH levels in these cells. Antigen presenting cells (APC), macrophages, dendritic cells and B cells are central to the development of either Th1 or Th2 immunity. GSH depletion in APC inhibits Th1-associated cytokine producing IFN-γ. Furthermore, decreased intracellular GSH levels in macrophages resulted in upregulation of the production IL-6 and IL-10 (critical effector cytokine of Th2), and down regulated IL-12 (Th1 cytokine), thus GSH plays an important role in the polarization of Th1/Th2 balance. However, increasing intracellular thiol pools with NAC in splenocytes resulted in upregulation of IL-4 production (Th2 cytokine) after anti-CD3 stimulation.

In conclusion, we have touched on the involvement of some important cytokines in the pathogenesis of RA and also we have shown clearly the involvement of hypoxia and oxidative stress in the pathogenesis of the disease. Cytokines and hypoxia are all linked to the pathogenesis of RA. We did not, however, touch in details on the current treatment for RA. The future may reveal more important scientific data using our current and future knowledge in developing more effective approaches for the treatment of RA.

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References

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