RA Pathophysiology

Note: More up to date information regarding RA pathogenesis may be found in lectures given by the author on this website.

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Immune Mediated Inflammatory Disease

In the last decade we have significantly increased our knowledge of the underlying pathobiology of rheumatoid arthritis. The introduction of targeted biological therapy has provided experiential rather than experimental evidence that multiple different immunological and inflammatory pathways are operative. Each year there are descriptions of new cytokines, mediators, and pathways that show additional promise in unraveling the complex pathobiological pathways.

Rheumatoid arthritis is best characterized as **an immune mediated inflammatory disease (IMID).** Within a framework that recognizes both immunological activation and inflammatory pathways, we can begin to evaluate the multiple components of disease initiation and propagation. This framework highlights that once initiated and even after a putative trigger may be eliminated, there are feed forward pathways that result in an auto-perpetuating process.

Histopathology

Synovium

The synovium, in normal joints, is a thin delicate lining that serves several important functions. The synovium serves as an important source of nutrients for cartilage since cartilage itself is avascular. In addition, synovial cells synthesize joint lubricants such as hyaluronic acid, as well as collagens and fibronectin that constitute the structural framework of the synovial interstitium.

1. *Synovial lining or intimal layer:* Normally, this layer is only 1-3 cells thick. In RA, this lining is greatly hypertrophied (8-10 cells thick). Primary cell populations in this layer are fibroblasts and macrophages.

2. *Subintimal area of synovium:* This is where the synovial blood vessels are located; this area normally has very few cells. In RA, however, the subintimal area is heavily infiltrated with inflammatory cells, including T and B lymphocytes, macrophages, mast cells, and mononuclear cells that differentiate into multinucleated osteoclasts. The intense cellular infiltrate is accompanied by new blood vessel growth (angiogenesis). In RA, the hypertrophied synovium (also called pannus) invades and erodes contiguous cartilage and bone. As such, it can be thought of as a tumor-like tissue, although mitotic figures are rare and, of course, metastasis does not occur.

Cartilage

Composed primarily of type II collagen and proteoglycans, this is normally a very resilient tissue that absorbs considerable impact and stress. In RA, its integrity, resilience and water content are all impaired. This appears to be due to elaboration of proteolytic enzymes (collagenase, stromelysin) both by synovial lining cells and by chondrocytes themselves. Cytokines including IL1 and TNF drive the generation of reactive oxygen and nitrogen species and while increasing chondrocyte catabolic pathways and matrix destruction, also inhibit new cartilage formation. Polymorphonuclear leukocytes in the synovial fluid may also contribute to this degradative process.

Bone

Composed primarily of type I collagen, bony destruction is a characteristic of RA. This process is primarily driven by the activation of osteoclasts. Osteoclasts differentiate under the influence of cytokines especially the interaction of RANK with its ligand. The expression of these are driven by cytokines including TNF and IL1, as well as other cytokines including IL-17. There may also be a contribution to bony destruction from mediators derived from activated synovial cells.

Synovial Cavity

The synovial cavity is normally only a "potential" space with 1-2ml of highly viscous (due to hyaluronic acid) fluid with few cells. In RA, large collections of fluid ("effusions") occur which are, in effect, filtrates of plasma (and, therefore, exudative – i.e., high protein content). The synovial fluid is highly inflammatory. However, unlike the rheumatoid synovial tissue in which the infiltrating cells are lymphocytes and macrophages but not neutrophils, in synovial fluid the predominant cell is the neutrophil.

Disease Initiation

The search for an elusive single trigger for RA has been ongoing for many years. Multiple studies have failed to conclusively demonstrate that any organism or exposure is singly responsible for the disease. However, a number of well done epidemiological studies and genetic studies have provided valuable information to inform our genera, albeit still incomplete, understanding of the dynamic process of disease initiation.

Genetic Susceptibilities

In the early 1980's an association was described for the association of RA with class II major histocompatability (MHC) antigens, specifically the shared epitope found in HLA-DR4.   Class II MHC on the surface of an antigen presenting cell interacts with a T cell receptor in the context of a specific antigen, usually a small peptide sequence from a protein. A sequence of amino acid residues with highly conserved sequence and charge characteristics within the hypervariable region of HLA-DR4 remains the largest genetic risk factor described for RA, estimated to contribute approximately 30% of the genetic risk for the disease. It is hypothesized that a triggering peptide (or peptides) with a tight conformational fit for the pocket formed by these residues is an early event leading to the activation of T lymphocytes.   More recently, it has been found that modified citrullinated peptides may have significant binding specificity for shared epitope alleles, with some data now suggesting that citrullinated sequences from different proteins are associated with allelic restriction. (A more detailed discussion of citrullination is below).

Other genetic susceptibilities have been described in RA, but their relative contributions to the disease are still not well defined. These include peptidyl arginine deiminase-4 (PAD-4) which may lead to increased citrullination, PTNP22, STAT4, and CTLA4 which may be involved in T cell activation, TNF receptors, and others.

That RA has a genetic component is also borne out through a number of studies of monozygotic (from the same embryo, thus nearly identical DNA) and dizygotic (from different embryos) twins. In these studies the concordance rates between twins was higher in monozygotic twins ranging from 15-35% compared with dizygotic twins in which the concordance was in the 5% range. Even the dizygotic RA prevalence was higher than the general population estimates of approximately 1%. It is important to emphasize however that even in twins with nearly identical DNA, there was far from perfect correlation of the development of RA, implicating many other factors related to the development of disease than genetic factors.

Triggers of Disease

The fact that there is not perfect genetic concordance implicates other factors in disease development. A search for these elusive triggers has been largely unrevealing. A number of well performed studies have demonstrated that cigarette smoking is a significant risk factor for the development of disease and also with disease severity. Interestingly this relationship is especially strong in individuals who carry the shared epitope, and even more in patients who have RA autoantibodies.

The search for bacterial or viral infections as causes of RA have often been hypothesized, and many patients will relate the onset of their symptoms to an antecedent infection; however, the recovery of organisms or their DNA from blood or joint tissue have been unfruitful in discovering "the" elusive infection responsible for RA. Nonetheless, the ability of an infection to activate a number of immunological and inflammatory pathways may "prime the pump" in combination with other factors.

Perhaps the most exciting developments in the last few years in terms of RA initiation has been the growing research to evaluate the possible role of oral bacteria as a trigger for RA. There has been a longstanding association described between periodontal disease with RA, however cause and effect has been far from proven. Periodontal disease is characterized by significant inflammation of the gums that leads to bone destruction and collagen matrix destruction. Both are inflammatory diseases with many of the same mediators and pathways involved, thus this could simply be an association between two inflammatory processes. However, it is now recognized that a specific species of bacteria, *Porphyromonas gingivalis*, which colonizes patients with periodontal disease and marks the progression from gingivitis to more aggressive periodontitis has an enzyme that can cause citrullination of proteins. With the growing recognition that protein citrullination is an early event leading to an immune response against these in RA, these data suggest that periodontal infection may precede the development of RA in some patients serving as a disease initiation factor. A number of groups worldwide, including our own, are now investigating these pathways to better understand these processes.

Citrullination

The recognition of antibodies directed against citrullinated peptides in RA has been a major development to improve disease identification and provide prognostic information. Citrulline is a post-translational modification that occurs on arginine residues contained within proteins and peptides. There are a number of enzymes that can cause citrullination to occur, present in various cell types and tissues known as peptidylarginine deiminases (PADs). Citrullination is a normal process, required for normal skin formation and other physiologic functions. However, in rheumatoid arthritis an autoimmune response develops against citrullinated peptides detected as anti-citrullinated peptide antibodies (ACPA). One of tests to detect these antibodies detects anti-cyclic citrullinated peptides (anti-CCP), currently the most

commonly used diagnostic test for them. The presence of anti-CCP are >98% specific for the diagnosis of rheumatoid arthritis; however, not all patients with RA will develop anti-CCP antibodies.

Of significant importance is the recognition that these anti-CCP antibodies may be detected up to 15 years before the onset of clinical symptoms of RA indicating a preclinical phase of disease in which immunologic activation is already ongoing. Moreover, it has recently been demonstrated that specific citrullinated peptide sequences bind to shared epitope alleles with high affinity and can lead to T cell activation.

The mechanisms to citrullination that lead to RA remain unclear. A polymorphism in the PAD4 gene which may lead to increased citrullination has been described populations. In RA patients, autoantibody responses also develop against the PAD4 protein, associated with a more aggressive disease course. One species of oral bacteria *Porphyromonas gingivalis* has a PAD enzyme. Given the relationships described with periodontal disease and RA, it has been hypothesized that this bacteria may also serve to initiate citrullination in the preclinical phases of RA.

Propagation of Disease

T cell activation

Upon encounter with antigen in the context of MHC on an antigen presenting cell, a T lymphocyte is positioned for 3 possible fates: activation, anergy/tolerance, or apoptosis (death). T cell activation is only possible if the T cell receives a "second signal" through engaging additional cellular receptors. One of the most important of these second signals is delivered through the CD28 molecule on the surface of the T cell but many other second signals are involved in this process of "costimulation". Upon engagement of these receptors, a T cell usually becomes activated. Failure to engage the stimulatory receptors, or engagement of a down-regulator receptor will cause the cell to become tolerant to the antigen (eg does not activate when exposed to the antigen) or to undergo programmed cell death through apoptosis. The process of T-cell costimulation is interrupted by abatacept, a biological therapy used to treat RA.

When T cells become activated, they will in turn proliferate and begin to secrete additional cytokines including IL-2 which furthers their proliferation, and depending on other exposures, cytokines such as IFN-γ, TNF, and IL-4. It is the effect of these T-cell derived cytokines that additional cells become activated. T cells also directly interact through surface receptors with other cells to generate additional activation signals.

B Cell Activation and Autoantibodies

B cells become activated through interactions with T cells and through soluble cytokines that enhance their proliferation and differentiation. B cells express a number of receptors on their surfaces during their differentiation, including the molecule CD20, which is lost upon terminal differentiation to antibody-forming plasma cells. B cells and plasma cells can be found in rheumatoid synovium sometimes as lymphoid aggregates in the subsynovium. The effects of B cells extend beyond their roles in forming plasma cells including cytokine production, direct cellular interactions, and they themselves serve as antigen-presenting cells to T lymphocytes. The role of B cells in RA has been clearly demonstrated with the efficacy of rituximab which eliminates circulating B cells, though with limited impact on autoantibody formation.

One of the features of most autoimmune diseases is the presence of disease-specific autoantibodies that help to define disease phenotypes. Antibodies are made by plasma cells, which represent the terminal stage of differentiation for B lymphocytes. Rheumatoid arthritis is characterized by the presence of autoantibodies known as rheumatoid factors (RF) and anti-citrullinated peptide antibodies (ACPA, which includes the anti-cyclic citrullinated peptide antibody or anti-CCP). Rheumatoid factors have been long recognized as a feature of many patients with RA. These are autoantibodies in the classical sense; they are antibodies directed against native antibodies, most classically described as IgM antibodies that recognize the Fc portion of IgG molecules, but RF may also be of the IgG or IgA isotypes. Rheumatoid factors are not specific for the diagnosis of RA, but are seen in many other inflammatory and autoimmune conditions. These include Sjogren's syndrome, chronic infections including tuberculosis and endocarditis, hepatitis C, chronic kidney or liver disease, lymphoproliferative diseases including myeloma, and other conditions. While the rheumatoid factor may be seen in other inflammatory conditions, ACPA are highly specific for rheumatoid arthritis and define a more aggressive disease phenotype (discussed more above).

Effector Cell Activation

While T cells and B cells represent the immunological aspects of RA, most of the damage from the disease is driven through effector cells and their products including cytokines and other mediators. The synovial lining in RA represents an expansion of fibroblast like cells and macrophages. It is the **macrophage** that has been seen as one of the master orchestrators of the effector damage in RA. Macrophages are rich sources and major producers of proinflammatory cytokines including TNF, IL-1, IL-6, IL-8, and GMCSF. These cytokines further stimulate the macrophage, as well as other cells in the microenvironment in a including fibroblasts and osteoclasts, and finally at distant sites in the body through cell surface receptors including the hepatocyte which is responsible for the generation of acute phase response proteins (such as C-reactive protein). Macrophages are also producers of prostaglandins and leukotrienes, nitric oxide, and other pro-inflammatory mediators with local and systemic effects. The **synovial fibroblast**, also secretes cytokines including IL-6, IL-8 and GM-CSF, and other mediators including destructive proteases and collagenases.

Neutrophils are recruited in very large numbers to the rheumatoid cavity where they can be aspirated in the synovial fluid. The recruitment of neutrophils to the joint is likely driven by IL-8, leukotriene B4, and possibly localized complement activation through C5a. Neutrophils in the synovial fluid are in an activated state, releasing oxygen-derived free radicals that depolymerize hyaluronic acid and inactivate endogenous inhibitors of proteases, thus promoting damage to the joint.

Chondrocytes, like synovial fibroblasts, are activated by IL1 and TNF to secrete proteolytic enzymes. They may, therefore, contribute to the dissolution of their own cartilage matrix, thus explaining the progressive narrowing of joint spaces seen radiographically in this disease.

Inflammatory Mediators in RA

Cytokines

One of the most important group of mediators in RA are cytokines. The most prominent of these are **TNF, IL-1, and IL-6**. These cytokines, released in the synovial microenvironment have autocrine (activating the same cell), paracrine (activating nearby cells), and endocrine (acting at distant sites) effects and accounting for many systemic manifestations of disease. There are many shared functions of TNF, IL-1, and IL-6, and these cytokines in turn upregulate the expression of the others. Among the important effects of these cytokines are:

- Induction of cytokine synthesis

- Upregulation of adhesion molecules

- Activation of osteoclasts

- Induction of other inflammatory mediators including prostaglandins, nitric oxide, mtrix metalloproteinases

- Induction of the acute phase response (e.g. C-reactive protein, increased ESR)

- Systemic features (e.g., fatigue, fever, cachexia)

- Activation of B cells (IL-6)

Other cytokines are increasingly described in RA. These include **IL-8** which is involved in cellular recruitment, **GM-CSF** involved in macrophage development, **IL-15** involved in T cell proliferation, **IL-17** which has pleiotropic effects on multiple cell types including osteoblast expression of RANK leading to osteoclast activation, and **IL-23** involved in increasing TH17 cell differentiation.

Soluble mediators of inflammation that may diffuse in from blood and/or be formed locally within the joint cavity include **prostaglandins, leukotrienes, matrix metalloproteinases**. Prostaglandins are involved in pain senstitization localized inflammation, and some effects on bone, and leukotrienes play roles in vascular permeability and chemotaxis. Matrix metalloproteinases (MMPs) are potent in their ability to enzymatically degrade the collagen matrix of cartilage. Kinins cause release of prostaglandins from synovial fibroblasts, and are also potent algesic (pain-producing) agents. Complement may be available for interaction with immune complexes to generate additional chemotactic stimuli. The neuropeptide substance P is a potent vasoactive, proinflammatory peptide that has also been implicated in RA.

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