

The aetiology and pathogenesis of RHEUMATOID ARTHRITIS

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Rheumatoid arthritis affects up to 1 per cent of the world's population and is associated with significant morbidity and mortality. This article discusses possible mechanisms by which the disease becomes established

Rheumatoid arthritis (RA) is one of the commonest autoimmune diseases. It is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis. If left untreated, it leads to joint destruction, which is responsible for the deformity and disability seen in this disease. The consequent morbidity and mortality has a substantial socio-economic impact. Research has led to a greater understanding of the disease, and major therapeutic advances have been made.

— EPIDEMIOLOGY

The prevalence of RA is consistent worldwide, affecting about 0.5–1 per cent of the population. Although the disease affects people all over the world, certain populations demonstrate particularly low or high

prevalence (inhabitants of sub-Saharan Africa and native Americans respectively). As with other autoimmune conditions, women are affected more than men at a ratio of 3 to 1. The disease occurs at any age, with the peak age of onset being around the fourth and fifth decade of life. The prevalence increases with age but there is a lower differential between the sexes in older patients.¹

High standardised mortality rates have been observed in the RA population compared with the general population.^{2–4} In particular, recent studies suggest that the increase in life expectancy observed in the general population is not mirrored in RA patients.^{4,5}

The increased mortality in RA patients is mostly associated with cardiovascular disease.⁵ Accelerated atherosclerosis is of growing concern in these patients.⁶ There is increasing evidence that atherosclerotic disease is driven by inflammatory mechanisms similar to those in RA. Cardiovascular morbidity correlates with inflammatory activity in RA.⁶ Oxidised low density lipoprotein is observed in both atherosclerotic disease and RA, and alteration in apolipoprotein and lipoprotein patterns may represent a predisposing factor.^{7,8} In addition, use of certain

drugs in RA, for example, glucocorticoids, cyclo-oxygenase (COX)-2 inhibitors and ciclosporin may affect the risk of cardiovascular morbidity and mortality.

— AETIOLOGY

The aetiology of RA is not clear. It is postulated that a genetically susceptible host is exposed to an unknown pathogen (antigen), and that this interaction gives rise to a persistent immunological response.

Genetic predisposition Studies in families show that first-degree relatives of RA patients develop the disease at a higher rate than the general population. This implies a genetic predisposition to RA. Failure to demonstrate a Mendelian inheritance pattern indicates multiple genetic factors.⁹ Studies in twins illustrate a higher concordance rate of RA in monozygotic twins (12–15 per cent) when compared with dizygotic twins (4 per cent),^{10,11} with genetic factors accounting for up to 60 per cent of disease susceptibility.¹²

The most definite genetic association with RA is with human leucocyte antigen (HLA) alleles of the major histocompatibili-

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ty complex (MHC). The MHC is a large group of genes encoding for MHC class I and II molecules. HLA is found in the MHC and is divided into eight main regions or loci, including "DR", "DP", and "DQ". These three loci encode class II molecules. The HLA-DR4 allele, which is found at the HLA-DRB1 locus is associated with the development and severity of RA. Analysis of genetic markers has demonstrated an association between the development of RA and a number of HLA-DRB1 alleles, including HLA-DR4. All these alleles share a highly conserved amino acid sequence in the third hypervariable region of their DRB1 chain. This is referred to as the shared epitope hypothesis. Studies also indicate that certain HLA alleles correlate with disease severity albeit with a less significant association.¹³

Several other genetic factors have been shown to influence predisposition to RA. Studies have revealed variation (or polymorphisms) of the genes for various proteins (see "The role of cytokines" below), which are known to be heavily implicated in driving the inflammatory process in RA.¹⁴⁻¹⁶ These genetic factors can differ between populations. It is therefore likely that in addition to HLA, the development of RA is influenced by several genetic factors.

Gender The greater prevalence of RA among women suggests that sex hormones are implicated in the development of the disease. This is further endorsed by the observation that pregnancy has an ameliorating effect on RA and patients with RA are more likely to be nulliparous before disease onset when compared with controls.¹⁷ A possible explanation may be the presence of alloantibodies in the maternal circulation developed against paternal HLA.¹⁸ Data have revealed the alloantibodies to be

directed against class II (DR) HLA. Blocking the function of the HLA-DR epitopes associated with RA could downregulate the disease.¹⁹

Another explanation is the change in the hormonal profile. Placental corticotropin-releasing hormone directly stimulates the secretion of dehydroepiandrosterone, the major androgen in women, by foetal adrenal cells.²⁰ Androgens exert an immunosuppressive role on both cellular and humoral immune responses.²¹ Dehydroepiandrosterone is the principal substrate for placental oestrogen synthesis. Oestrogens and progesterones have been shown to stimulate humoral immune (Th2) and inhibit cellular immune (Th1) responses.²² Since RA is driven predominantly by Th1 responses, oestrogens and progesterones would be expected to oppose the development of RA. It has been reported that oral contraceptives protect against the development of RA or are associated with a reduction in the incidence of more severe disease.^{23,24}

Infectious agents Many viruses and bacteria have been implicated as causative agents. These include the Epstein-Barr virus, parvovirus, lentiviruses and bacterial organisms such as Mycoplasma, Mycobacteria and Yersinia. It has been suggested that these organisms infect the host cell and alter the reactivity or responsiveness of the T-cells of the immune system sufficiently to trigger the disease. However, no agent has been conclusively shown to be causative.

Heat shock proteins Heat shock proteins (HSPs) are a family of proteins produced by cells of all species in response to stress. These proteins have conserved amino acid sequences, that is, there is a sequence homol-

ogy. Certain human HSPs and *Mycobacterium tuberculosis* HSPs have 65 per cent sequence homology.²⁵ A potential hypothesis is that antibodies and T-cells exist that recognise epitopes shared by the HSP of both the infectious agent and host cells. This would facilitate cross-reactivity of lymphocytes with host cells, triggering an immunological reaction. This is referred to as "molecular mimicry".

HISTOLOGICAL CHANGES

Rheumatoid arthritis can affect any joint where cartilage overlies bone and with a joint cavity lined by synovial membrane that contains synovial fluid. The changes to the synovium, the primary site of pathology, is crucial to the inflammatory process that occurs in RA.

Normal synovial tissue consists of an intimal lining (comprising one to three cell layers that are loosely associated without a definite underlying basement membrane) and the synovial sublining, which merges with the joint capsule. The intimal lining consists of two major cell types: macrophages and fibroblast-like cells. Usually, the synovial sublining is relatively acellular, containing scattered blood vessels, fat cells and fibroblasts.

In the early stages of the disease, the most noticeable feature is tissue oedema. Clinically, this is manifested by joint swelling and pain. Vessel proliferation and new vessel formation (angiogenesis) is also observed on arthroscopy. Further, synovial lining hyperplasia begins to develop.

As the disease enters a more chronic phase, synovial lining hyperplasia becomes more pronounced, sometimes extending to a depth of over 10 cells. The cells consist of type A (macrophage-like) and also type B (fibroblast-like) synoviocytes. The sublining also evolves with disease progression. Most noticeably, there is an exuberant infiltration with mononuclear cells comprising T-cells (predominantly CD4+ helper T-cells), B-cells, macrophages and plasma cells. New blood vessel formation continues and both the degree and content of the cellular infiltrate changes. Many of the cell types are activated, with HLA class II expression consequently increased.

Generally, all inflammatory arthropathies exhibit infiltration similar to that described above. Another feature includes the ability of the hypertrophied synovium of RA to become locally invasive at the synovial interface with cartilage and bone. This results in the formation of a mass of tissue called "pannus". It is this tissue that serves as the origin of joint erosions. In RA, the intensity of such changes is characteristic. The histological features of pannus are distinct from that of other areas of the synovium. In particular, the cells express large amounts of messenger RNA encoding for destructive proteins

called matrix metalloproteinases.

CELLULAR INFILTRATION

The cells which infiltrate the synovium in RA are described below.

Lymphocytes Lymphocytes are the key cells that control the immune response by specifically recognising foreign material and distinguishing it from the body's own cells. There are two main types of lymphocyte: B-cells, which produce antibodies, and T-cells, which have a number of functions.

B-cells and rheumatoid factor B-cells carry surface immunoglobulin that acts as their antigen receptor. These cells contribute to a relatively small fraction of synovial cells (approximately 1–5 per cent). There is strong evidence, however, that they make a significant contribution to the inflammatory process. This is by way of the autoantibodies that they produce, known as rheumatoid factor, which form immune complexes. These complexes result in complement fixation, neutrophil activation and inflammation.

T-cells There are two subsets of T-cells — CD8+ cytotoxic lymphocytes and CD4+ helper lymphocytes. Cytotoxic T-cells are capable of destroying virally infected target cells. On the other hand, T-helper cells help B-cells to divide and produce antibodies, activate phagocytes to destroy pathogens and control the level and quality of the immune response. T-cells comprise approximately 40 per cent of synovial tissue cells. The predominant T-cell subset in the synovial sublining in RA is the CD4+ cell.

Phagocytes Phagocytes include blood monocytes, macrophages and neutrophils. These cells engulf pathogens, antigens and cell debris, subsequently destroying them. Antibodies and complement components bound to particles facilitate the process of phagocytosis.

Macrophage-like cells Approximately 20 per cent of rheumatoid synovial cells express macrophage surface markers, including macrophage-like type A synoviocytes and true macrophages. These cells express high levels of surface HLA-DR, which presents antigen to CD4+ T-cells. Synovial macrophages are also the source of several cytokines produced by the synovium responsible for the inflammatory picture.

Neutrophils There is a relative paucity of neutrophils in the rheumatoid synovium compared with synovial fluid. These cells enter the joint space via the circulation and migrate into the tissue. Neutrophils in the synovium may have a role in cartilage dam-

age and synovial inflammation. After ingestion of immune complexes, neutrophils can release lysosomal enzymes and destructive oxygen free radicals that can damage cartilage and supportive joint structures.

Fibroblast-like cells Fibroblast-like cells are major components of rheumatoid synovial cells. They are non-phagocytic and do not express surface HLA-DR. They express adhesion molecules and secrete growth factors. It is thought that these type-B synoviocytes may show loss of growth control that occurs in normal fibroblasts. This might contribute to synovial lining hyperplasia and the locally invasive properties of pannus.

THE IMMUNE RESPONSE

T-cells There is substantial evidence indicating that T-cells, particularly CD4+ helper cells, are crucial in the early immunological response. For example, the mononuclear infiltrate described above includes T-cells. Another feature that adds weight to the suggestion that T-cells are important in the immune response is the increased presence of HLA-DR (MHC class II) molecules on the cell surface, its association with shared epitope representing the most definite genetic susceptibility factor. Helper T-cells recognise antigenic peptides that have become associated with MHC molecules. The T-cell antigen receptor recognises antigenic peptides and binds to the antigen-MHC complex, initiating responses to combat the antigen. The association with particular HLA-DR molecules in RA implies a pathogenic role; either at the level of antigen presentation by the MHC molecule or at the level of MHC plus antigen recognition by CD4+ T-cell receptor.

Despite the apparently integral role of T-cells, it is likely that other cells and their products drive RA synovitis. This is suggested by the fact that there is little proliferation of the synovium-infiltrating T-cells, with evidence of more memory T-cells in RA synovium suggesting previous exposure of antigen followed by a post-activation primed state. In addition, the soluble mediators driving the inflammatory process are mainly non-T-cell in origin. In conclusion, T-cells are likely to be pivotal in the induction of the processes leading to RA but have a less clear role in the mediation of the inflammatory pathways.

THE ROLE OF CYTOKINES

Cytokines are soluble proteins that serve as chemical messengers between cells and are known to be involved in almost all biological processes, including cell growth and differentiation, inflammation, tissue repair and remodelling and regulation of the immune response. Cytokines determine the

balance between cell-mediated and humoral (antibody) mediated immune responses. Broadly, they possess either pro-inflammatory or anti-inflammatory effects.

T-cell products Upon activation, naive T-cells divide and differentiate into effector T-cells. This process is driven primarily by interleukin (IL)-2, which is produced by activated T-cells.

The differentiation of naive CD4+ cells into the two major classes of CD4+ effector T-cells (Th1 or Th2) represents an important stage in the immune response. A naive CD4+ T-cell differentiates into either a Th1 or a Th2 cell, influenced primarily by the cytokines present during the initial proliferative phase. This differentiation determines whether the acquired immune response to the antigen is dominated by macrophage activation and a cell-mediated response (Th1) or by a humoral antibody-mediated, response (Th2). T-cells initially stimulated in the presence of interferon-gamma and IL-12 generally develop along a Th1 pathway. Th2 cell development is promoted by stimulation of naive cells in the presence of IL-4 and IL-10. Each mode of response suppresses the other.

The balance between cytokines is a major determinant of the immune response. Analysis of T-cell clones in rheumatoid synovium indicates that the infiltrating T-cells are biased towards a Th1 phenotype. It is relevant to note that there are few T-cell derived cytokines in inflamed rheumatoid synovium. However, many other cytokines have been observed at significant levels in the rheumatoid synovium. It is clear that the dysregulation of the immune system and an excess production of pro-inflammatory cytokines, for example, IL-1, IL-6, TNF-alpha (tumour necrosis factor-alpha), IL-18 and GM-CSF play a crucial role.²⁶ In particular, TNF-alpha appears to be a critical factor in the inflammatory cascade.²⁶

TNF-alpha and IL-1 Both TNF-alpha and IL-1 are considered to exert pivotal roles in the pathogenesis of RA. Both are present in synovial fluid and synovial tissue. TNF-alpha has been identified in approximately 40 per cent of lining cells and 5–10 per cent of sublining cells, while IL-1 is found in 20 per cent of lining cells and 25 per cent of sublining cells. Double-staining immunochemical experiments have demonstrated that cells expressing macrophage surface markers,^{27,28} in particular, produce these two cytokines.

TNF-alpha and IL-1 stimulate the development of a pro-inflammatory phenotype on responding cells. This gives rise to positive effects on chemotaxis, angiogenesis, vessel permeability, matrix metalloproteinase production (responsible for matrix degradation), and T- and B-cell recruitment and activation.²⁹ IL-1 and TNF-alpha have been shown to exert a synergistic effect, the

Panel: Effects mediated by TNF-alpha in RA

- Bone and cartilage resorption
- HLA class II expression
- Collagenase and PGE₂ induction
- Chemotaxis (IL-8)
- Increased production of other pro-inflammatory cytokines, for example, IL-1, IL-6, GM-CSF
- Endothelial cell activation via adhesion molecule
- T- and B-cell activation

addition of both factors resulting in even greater effector stimulus.

A homeostatic effect is considered to be exerted naturally by the presence of inhibitors or antagonists. There are two TNF receptors (p55 and p75) to which TNF binds and which after cleavage exist in soluble forms. These soluble receptors can act as antagonists, binding to soluble TNF, thereby preventing the latter from binding to cell-surface TNF receptors.³⁰ The two IL-1 receptors also occur in soluble forms, as does a naturally occurring IL-1 receptor antagonist.^{31,32}

From studies carried out in animal models, there is general agreement that the two cytokines IL-1 and TNF-alpha are crucial in RA pathogenesis. IL-1 is more responsible

for the destructive aspect of the disease, especially the damage to proteoglycans in cartilage. TNF-alpha is responsible for the proliferative and inflammatory aspects (see the Panel). TNF-alpha perhaps represents a more critical cytokine, as blocking its effect results in a dampening of the inflammatory cascade since IL-1 bioactivity is blocked as well.³³ Anti-TNF treatment was evaluated in animal models of RA, and this confirmed the reduction of joint inflammation and destruction.^{34,35} Compelling evidence stemmed from studies where marked clinical benefit was observed in RA patients treated with chimeric anti-TNF monoclonal antibodies.^{36,37} There are also other multiple confirmatory studies using other antibodies and soluble TNF-R Fc fusion proteins.³⁸ Double-blind, randomised clinical trials have confirmed the effectiveness of these therapies.^{39,40}

Antibodies to IL-1 have also been shown to ameliorate RA. The imminent availability of IL-1 blockade, via the use of an IL-1 receptor antagonist (Anakinra)^{41,42} and the evidence that combination blockade of both TNF-alpha and IL-1 is the most effective in animal models will stimulate further research in this area.

Persistent overproduction of IL-6 has been observed in patients with RA, suggesting a strong role for IL-6.^{43,44} Increase in platelet counts, C-reactive protein, serum amyloid A and gamma-globulin may be explained by the overproduction of IL-6.

Anti-IL-6 receptor antibody has been developed and tested in animal models with considerable success.⁴⁵ This has propelled the development of IL-6 blocking therapy in human IL-6 related diseases like RA. Preliminary data indicate that this treatment is beneficial and well tolerated.⁴⁶

PATHOGENESIS OF BONE DISEASE

The radiological hallmarks of RA include periarticular osteoporosis and focal bone erosions at the joint margins. Studies attempting to identify the mechanisms underlying the development of focal bone erosions have demonstrated that osteoclasts, predominantly, mediate the bone resorptive process. Other cell types, such as activated macrophages and fibroblasts also have the capacity to degrade mineralised bone, albeit to a more limited degree. The mechanisms involved in RA pathogenesis are shown in the Figure below.

Increasing evidence substantiating the principal role of osteoclasts is derived from studies of a potent inhibitor of osteoclast differentiation and activity, osteoprotegerin (OPG). OPG is a member of the TNF receptor family and acts as a decoy receptor to bind to a novel factor essential for osteoclast differentiation, known as ODF (osteoclast differentiation factor), RANKL (receptor activator of NF-kB ligand), or OPGL (osteoprotegerin ligand). This is a

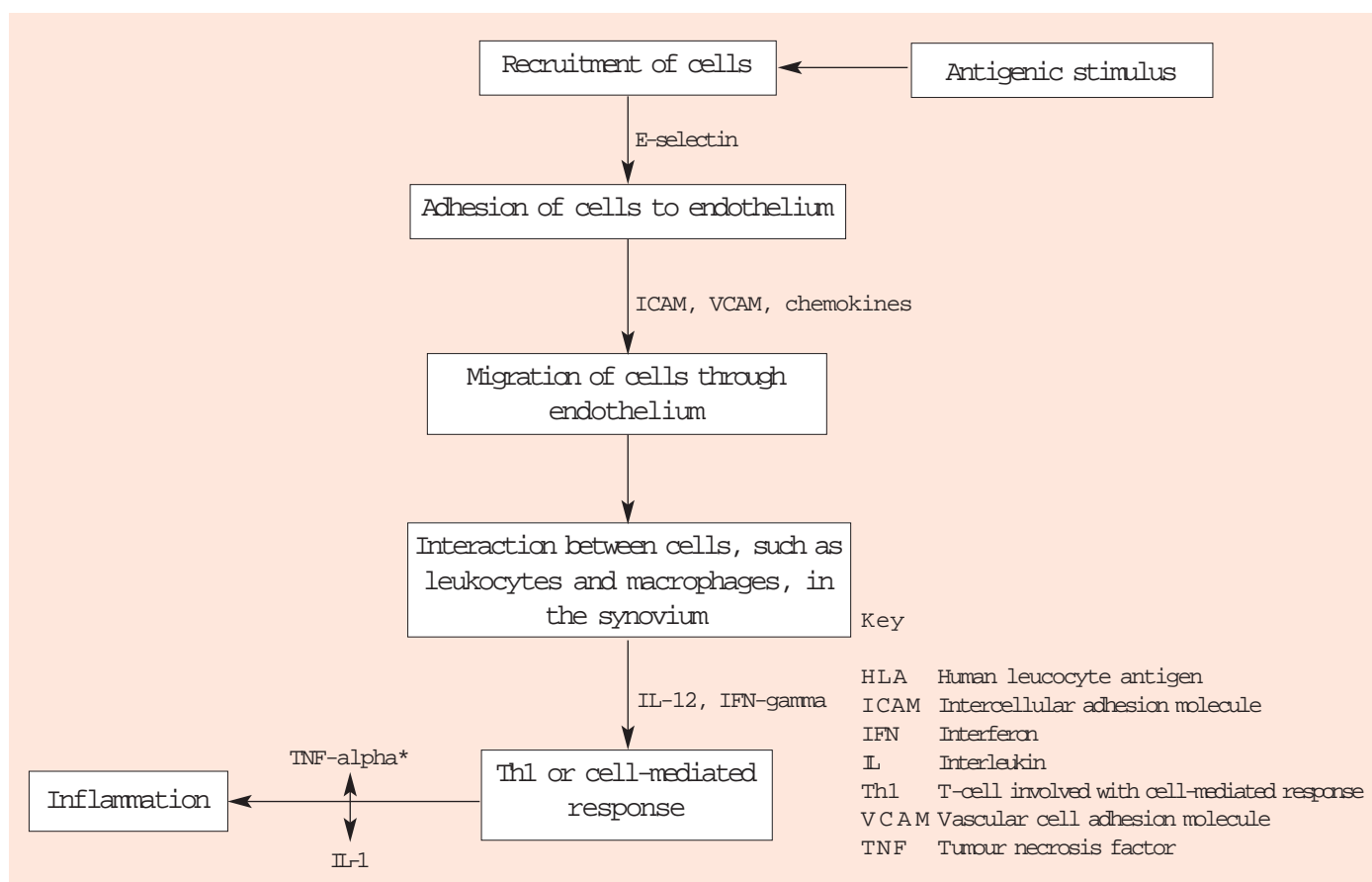


Figure: mechanisms involved in the pathogenesis of RA *The relative contribution of TNF-alpha and IL-1 to the inflammatory response is open to debate

member of the TNF ligand family of cytokines which regulates osteoclast differentiation and activity through the RANK receptor on osteoclast precursors. Studies in animal models confirm that treatment of arthritis with RANKL almost completely abolishes erosions. Further studies have shown that human synovial fibroblasts as well as CD4+ and CD8+ T-lymphocytes can also express RANKL.

A range of cytokines, IL-1 being the most potent, can stimulate the production of RANKL. Although TNF and IL-17 are less potent as single agents, they show considerable synergy when used together. It remains to be identified whether RANKL is a good target for prevention of bone erosion.

Cell recruitment The increased cellularity in the rheumatoid synovium implies that there are mechanisms for increasing cell input. The increase in cellularity is accompanied by angiogenesis in the synovial membrane, thus increasing delivery of cells and molecules to areas of inflammation.

Neovascularisation involves angiogenic cytokines such as vascular endothelial growth factor, which promotes the growth of new blood vessels⁴⁷ and also renders them hyperpermeable.⁴⁸ Blood-borne cells would first have to adhere to the endothelium and then migrate through them to the synovial tissue. This is augmented by the expression of adhesion molecules capable of binding lymphocytes, polymorphs and monocytes.

Adhesion molecules Vascular adhesion molecules play a critical role in the recruitment of inflammatory cells into the synovium. These adhesion molecules are ligands for various integrins on leukocytes. Studies illustrate that the cellular components of the rheumatoid synovium can be regulated by the presence of adhesion molecules. Dysregulation of this process could be responsible for the pathogenesis of RA.

Leukocyte recruitment into inflammatory sites is generally thought to occur in the following manner. Selectins, for example, E-selectin initiates the first interactions between the circulating cell and the endothelium. The low-affinity binding causes the cell to slow down and roll along the blood vessel wall. Endothelial cells then release activating factors like IL-8 that cause the cells to stick firmly together. Cells then migrate through endothelial pores into the tissue.

The process in which the cells stick together is mediated through integrins and intercellular adhesion molecules (ICAMs). Immunohistochemical staining shows that ICAM-1 is found in sublining macrophages, macrophage-like synovial cells and intimal lining fibroblasts. Vascular cell adhesion molecules, or VCAM-1, can also mediate such immobilisation. VCAM-1 is expressed by RA synovial endothelium. The synovial

intimal lining has been identified as the region of the joint expressing the highest amount of VCAM-1. Various cytokines including TNF-alpha, IL-1 and interferon-gamma enhances the expression of these adhesion molecules.

Chemokines Chemokines, such as IL-8, are proteins that are involved in leukocyte chemotaxis and migration through the endothelial barrier into the inflamed synovium. Some chemokines are detected in significant quantities in the sera, synovial fluid and tissue of RA patients. These mediators have been implicated in angiogenesis and pro-inflammatory activities underlying RA.⁴⁹ Other chemokines observed include monocyte-chemoattractant protein-1 and macrophage inflammatory protein-1-a.⁵⁰ Pro-inflammatory cytokines like TNF-alpha and IL-1 stimulate the production of these chemokines.

Matrix metalloproteinases Matrix metalloproteinases are a family of enzymes involved in the remodelling and destruction of the extracellular matrix. They are produced by the fibroblast-like synoviocytes and high levels are found in RA. They are thought to be largely responsible for cartilage and bone degradation. Many factors have the capability to induce the biosynthesis of metalloproteinases. *In vitro* studies have demonstrated the role of cytokines, particularly IL-1 and TNF-alpha. The activity of matrix metalloproteinases is regulated by the presence of tissue inhibitors of metalloproteinases. Cytokines such as tumour growth factor-beta also enhance the production of metalloproteinases.

Arachidonic acid metabolites Prostaglandins and leukotrienes are synthesised by the synovium and synovial fluid cells. Phospholipase A₂ cleaves arachidonic acid from membrane phospholipids, and this is metabolised to prostaglandins by cyclooxygenase. Inducible COX-2 is largely responsible for prostaglandin production. COX-2 expression can be induced by IL-1 and suppressed by corticosteroids. Alternatively, the lipo-oxygenase enzymes metabolise arachidonic acid into leukotrienes. Prostaglandins potentiate vasodilation and vascular permeability and also inhibit interferon-gamma production by T-cells. Its role is supported by the benefits observed with the use of COX-inhibiting, non-steroidal anti-inflammatory drugs.

CONCLUSION

The aetiology of RA is unclear, but its pathogenesis is much better understood. It is probable that RA is initiated and driven through a T-cell mediated, antigen-specific process. The antigen remains unidentified

but could be an infectious agent. The presence of this antigen in a susceptible host in whom there is the appropriate HLA haplotype as well as other contributory genetic influences, initiates a T-cell response. This leads to the production of T-cell cytokines with consequent recruitment of inflammatory cells, including neutrophils, macrophages and B-cells.

Cytokines produced by macrophages and fibroblasts, in particular, TNF-alpha and IL-1, have been identified as being central to the pathogenesis of RA leading to a pro-inflammatory phenotype through a variety of mechanisms.

It is clear that T-cells, macrophages and fibroblasts perform key roles in the pathogenesis of RA. It is likely that RA disease is maintained by a contribution from all three sources. This is suggested by the less than complete success of therapies directed at one aspect, for example, T-cell therapies (anti-CD4) and the anti-TNF therapies, which do not produce full remission.

Advances in our understanding of the pathophysiology of RA has fuelled the search for further targets of therapeutic intervention. In addition, progress in drug development and design provide a great deal of optimism in the development of successful therapies for rheumatoid arthritis.

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