Introduction

The spondyloarthropathies (SpA) are frequently occurring inflammatory rheumatic disease (1), in part leading to significant burden of disease with pain and disability probably not so much different from rheumatoid arthritis (RA, 2). Ankylosing spondylitis (AS) and undifferentiated SpA (uSpA) are the most frequent subtypes (3). The pathogenesis of the SpA has remained obscure although the extensive association with HLA B27 was reported already more than 25 years ago (4). Antigens derived from bacteria such as klebsiella (5) and autoantigens derived from the cartilage such as proteoglycan (6) have been tested but no convincing evidence for their involvement has been obtained to date.

1. Ankylosing spondylitis

Ankylosing spondylitis is a chronic systemic inflammatory disorder that primarily affects the axial skeleton (spine and sacroiliac), sacroiliac joint involvement is its hallmark (7). Hip and shoulder joints may be involved in some patients, but the involvement of the more peripheral limb joints occurs in only 20%. Ankylosing spondylitis may occur in association with reactive arthritis (Reiter's syndrome), psoriasis, ulcerative colitis or Crohn's disease, these forms of AS are called secondary AS. But most patients have no evidence of these associated diseases and are best classified as suffering from primary AS. The major clinical features of AS can be divided into skeletal and extraskeletal manifestations. Its typical presentation is with low back pain of insidious onset, arthritis of hips and enthesopathies are common. Extraskeletal complications include acute anterior uveitis, aortic valvular disease, and the cauda equina syndrome. Of which, acute anterior uveitis is the most common extraskeletal involvement in patients with AS. It occurs in 25-30% of patients at some time in the course of their disease (8), and is relatively more common in HLA-B27^{+} than HLA-B27^{-} patients with AS (9). Clinical manifestations of AS usually begin in late adolescence or early adulthood, onset after age 40 is uncommon. The disease is three times more common in men than women (9), and the clinical and radiographic features of the disease probably evolve more slowly in women. Recent studies have clearly shown that the demographic and clinical spectrum of AS is much wider. The disease affects women not infrequently and is of higher prevalence than previously appreciated (10). The course of AS is highly

variable and can be characterized by spontaneous remissions and exacerbations, particularly in early disease. The outcome is generally favorable because the disease is often relatively mild or self-limited, and the majority of the patients remain fully employed (11). Only rarely does AS show persistent disease activity that results in early and severe disability.

2. Genes and AS

2.1 Association of AS with HLA-B27

In AS, the aetiology is a mystery. The joint destruction is considered to be the result of the interaction between the host's immune response and enviromental factors, but we do not know what triggered this self-destructive process and why it persists. AS occurs mostly in immunogenetically susceptible hosts, its most important feature is an association with the histocompatibility antigen HLA-B27 (12) which is demonstrated in about 90-95% of cases. The prevalence of HLA-B27 in the general population, however, shows considerable geographic variation, occurring in 50% of Haida Indians of northern Canada (13), but being virtually absent among Black Africans (14) and Cuatemalan Indians (15). At present, there exists no satisfactory explanation of the great variation in the frequency of this genetic marker amongst various ethnic groups.

Class I antigens of the major histocompatibility complex (MHC) are 44kDa polymorphic molecules that are noncovalently associated with a monomorphic protein, β2 microglobulin, and expressed as a heterodimer on the surface of many cell types. Allelic variations in the amino acids of class I MHC molecules have classically been identified by antisera. HLA-B27 is a serologically defined allele of the HLA-B locus, one of the three classical loci encoding class I MHC molecules. Examination of HLA-B gene products that react with HLA-B27 typing alloantisera has revealed a family of allelic subtypes, twenty of these have been designated by the World Health Organization HLA Nomenclature Committee as HLA-B2701 through to HLA-B2720, of which, HLA-B2701 has been found in only a few individuals (16), and thus has not been amenable to population studies. However, association of ankylosing spondylitis with four of the other subtypes has been observed (17). By contrast, the study about African Blacks from Gambia indicated that there does not seem to be an association between HLA-B 2703 and ankylosing spondylitis (18). HLA-B2703 is a very unusual HLA-B molecule, containing a histidine at position 59

rather than the tyrosine that is present in all other HLA class I molecules, this feature might explain its lack of association with ankylosing spondylitis. There is also no evidence supporting an association of HLA-B2706 and B2709 with AS. However, it is likely that ankylosing spondylitis is associated with each of the other HLA-B27 alleles. Whether HLA-B27 alleles are also associated with the other spondyloarthropathies is currently unkown, but appears to be a good possibility.

An important question is whether HLA-B27 itself is a direct disease susceptibility factor, or alternatively, merely a marker for a disease susceptibility gene in close linkage disequilibrium with HLA-B27. Indirect evidence from clinical epidemiology strongly suggests that HLA-B27 itself is the disease susceptibility gene (19). More recently, direct evidence that the HLA-B27 molecule itself can predispose to the spondyloarthropathies has come from studies of transgenic rats expressing HLA-B2705. These animals spontaneously develop a broad spectrum of disease manifestations closely resembling human HLA-B27 associated disease (20).

It was observed that infectious agents can trigger reactive arthritis in HLA-$B27^+$ individuals (21), One hypothesis has been constructed to account for that a small region of a human antigen is identical to amino acid sequences of proteins encoded by the triggering microorganism (22). This hypothesis has been termed molecular mimicry, which suggested that a structure in the triggering bacterium with a homology to HLA-B27 incited an antibody response which was then perpetuated by the presence of HLA-B27. Mimicry may also be of a structural gene product or the HLA-B27 molecule itself. Although it is not clear how mimicry of a class I MHC molecule would lead to an anatomically localized disease as is the case in the spondyloarthropathies, there is considerable evidence for the sharing of antigenic determinants between HLA-B27 and different bacterial products.

Monoclonal antibodies against HLA-B27 have been observed to react with bacterial cell envelope glycoproteins from shigella flexneri, Klebsiella pneumoniae, and Yersinia enterocolitica (23). Similarly, one of these HLA-B27 reactive antibodies (B27M2) has identified two cross-reative proteins of 80kDa and 60kDa from a K.pneumoniae isolate (24). Using computer search for bacterial proteins with amino acid sequence homology to HLA-B27 has yielded positive results. The nitrogenase enzyme in K.pneumoniae was found to contains a six amino-acid region that is homologous to HLA-B2705 residues 72-77 (25), and what is more, a elevated levels of antibodies against an HLA-B27-derived peptide containing the region of homology

with K.pneumoniae was shown in 54% of reactive arthritis patients and 30% of ankylosing spondylitis patients (26). These results clearly indicate that there is an amino acid sequence homology between HLA-B27 and the K.pneumoniae nitrogenase gene product. But, its relevance to disease pathogenesis is unclear.

2.2 Involvement of other genes

For most autoimmune disorders, the pattern of inheritance is very complex. The major histocompatibility complex (MHC) gene complex has been implicated as the major genetic component in the predisposition to these diseases but backcross studies in animal models of SpA suggest that multiple genes contribute to disease susceptibility (27). Furthermore, findings of human family studies of twins and sibpairs support the notion that genetic factors other than B27 determine which B27-positive individuals develop arthritis (27). Association of AS with HLA-B27 has been known for 25 years, however, there has been little progress in establishing whether other genes, particularly non-HLA genes, may play a role. The frequency of ankylosing spondylitis in randomly selected HLA-$B27^+$ individuals is only about 8%, but that in HLA-$B27^+$ family members of an HLA-$B27^+$ patient with AS approaches 20% (28). One explanation for these results is that other genes may play a role in determining disease susceptibility. The T cell receptor (TCR) was considered as one of the candidate gene product, since the specificity of the TCR involved in recognition of HLA-B27 and HLA-B27-bound peptides is likely to be an important determinant in the pathogenesis of the spondyloarthropathies (29). It is likely that specific residues of the HLA-B27 molecule recognized by T cells or binding peptides that are recognized by specific T cells play an essential role in determining the specific TCRs involved (29). Non-random usage of the various segments of the β chain of the TCR has been found in human T cell clones recognizing HLA-B27 (30), supporting the conclusion that limited numbers of TCR gene products may be involved in recognizing HLA-B27 and, perhaps, HLA-B27-bound peptides.

The class II region contains 17 HLA class II genes and pseudogenes (31). It has been speculated that other MHC genes in the class II region might also be involved in AS susceptibility. The preliminary data derived from a genome-wide screening for susceptibility loci in AS confirm the strong linkage disequilibrium of the MHC region with AS but extends the MHC allelic association to about 8 cM across the MHC region, between markers D6S276 and DRB1 (32). This may be exclusively

due to linkage disequilibrium with HLA-B27, or alternatively, it may be due to extended haplotypes that may contain 2 or more genes relevant to AS susceptibility. Different studies suggest a weak effect of DRB1 on the susceptibility to AS in different populations (33). Further studies are needed to validate these observations and to elucidate whether this association is due to linkage disequilibrium with the relevant susceptibility genes.

TNF genes play an important role in inflammation, infection, and immune response (34). TNFα is a proinflammatory cytokine that plays a pivotal role in the inflammatory pathway and may have beneficial or deleterious effects depending on the extent of its release. Evidence exists indicating that individual TNFα responsiveness is genetically determined. Putative disease-causing TNFα alleles may either contribute to disease susceptibility or may only be in linkage disequilibrium with the causative gene. A suggestive idea is that differential TNFα production may contribute to the development of AS in B27 positive individuals, and an explanation for these differences could be due to an involvement of a TNFα promoter polymorphism (35,36).

Recently, a new molecule, tapasin, has been identified in the class II region (37), which plays a critical role in HLA class I expression by acting as a physical intermediary between TAP (transporters associated with antigen processing) and the class I heavy chain. Especially, the B2705 molecule achieves high levels of surface expression in the absence of tapasin (38). There are no data concerning the role of tapasin in AS susceptibility, but its properties, which are related to antigen processing, might contribute to the role of B27 in conferring susceptibility to SpA.

3. The role of T cells in rheumatic diseases

A central event in the etiology of rheumatic diseases appears to be the induction of immune reactivity to an antigen (foreign or self) by T cells. After activation by antigen, T cells can proliferate to serve as helper cells for B-cell antibody production or the generation of cytotoxic T cells. In addition, activated T cells can produce cytokines leading to functional changes, such as synovial cell proliferation in rheumatoid arthritis (RA) or collagen synthesis by fibroblasts in scleroderma (39).

T cells have been directly associated with rheumatic diseases because they represent the largest cell population infiltrating the affected tissue and interacting with

other blood-derived and resident cells. An important role for T cells in the pathogenesis of reactive arthritis is underlined by the finding that the synovial lining of affected joints is infiltrated with activated $CD4^+$ T cells in reactive arthritis (40).

Some T cells may contribute to disease through the secretion of cytokines acting as a bone and cartilage destructive factor, for instance, interleukin 17 (41). In addition, it increases production of proinflammatory cytokines by monocytes and further enhance their effects on extracellular matrix destruction.

The association of HLA-B27 with ankylosing spondylitis implies a role for CD8 T cells in its pathogenesis since the only known functions of the polymorphic portions of class I MHC molecules are selection of the TCR repertoire of CD8 T cells in the thymus and presentation of antigen to CD8 T cells in the periphery. Thus the principal function of MHC molecules is the presentation of antigenic peptides to T cells. While MHC class II molecules present peptides to $CD4^+$ T cells, CD8 T cells respond to the MHC class I/peptide complex. One attractive explanation for the HLA-B27 association is that presentation of a peptide by HLA-B27 to $CD8^+$ T cells might be an important step in the pathogenesis of the disease (4239). Direct evidence that CD8 T cells are likely to be involved in the pathogenesis of the spondyloarthropathies has come from observations made in individuals with acquired immune deficiency syndrome (AIDS). Despite suppression of CD4 T cells, these individuals are capable of developing reactive arthritis (43). AS has not been reported, although this may relate to the extended length of time required to develop symptomatic AS. Reactive arthritis developing in patients with AIDS frequently following a gastrointestinal or genitourinary infection, is usually associated with HLA-B27 and can be quite aggressive. CD8 T cell function is usually normal, although the number and function of CD4 T cells can be severely depleted. Moreover, the finding that reactive arthritis is extremely aggressive in AIDS patients, although perhaps not occurring at increased frequency, suggests that CD8 T cells may be involved in the development of reactive arthritis.

There is also some evidence that CD4 T cells play an important role in AS. Patients with ankylosing spondylitis exhibit $CD4^+$ T cells responses to the human cartilage proteoglycan (44), adoptive transfer could induce a $CD4^+$ T cell mediated spondylitis (45). Another evidence supporting a role of T cells in both induction and perpetuation of rheumatic diseases is that therapy against T cell and its receptors could lead to a significant immunomodulation with marked changes in clinical and

laboratory parameters. Many studies have shown that CD4-mAb can prevent and, in a treatment setting, suppress activity in these disease models, including collagen-induced arthritis (46). Administration of the anti-CD4 Mab was followed by an immediate transient clinical benefit accompanied by a significant decrease in C-reactive protein levels and clinical improvement in patients with RA (47). To evaluate the roles of $CD4^+$ and $CD8^+$ T cell subsets in vivo in the induction of AS by immunization, Banerjee and coworkers (48) treated PG-immunized mice with isotype-controlled rat IgG2β monoclonal anti-CD4 or anti-CD8 antibodies, some of the mice were left untreated. They found that $CD4^+$ T cell depletion resulted in total inhibition of the disease with markedly decreased anti-PG antibody responses. $CD8^+$ T cell depletion, however, significantly enhanced the severity of the disease without affecting peak anti-PG antibodies, as compared to the control mice. These results demonstrate a crucial role for $CD4^+$ T cells in the pathogenesis of this disease, at least in the animal model.

4. Role of cytokines in autoimmune arthritis

A wide variety of cytokines and growth factors are produced in tissue of autoimmune arthritis, including IL1α and β, IL6, M-CSF and TNFα (49). In the synovium, these cytokines can be detected at the mRNA level by blotting and by in situ hybrization (50,51). Immunohistological localization of these protein products has demonstrated predominant expression in macrophages. These proteins were also detected in the short term in ex vivo cultures of the entire mixture of cells derived by enzymatic disaggregation of the synovial membrance (50). Of importance was the observation that IL1 and TNFα could be detected by bioassay of synovial membrane cultures, and hence they were present in quantities able to signal effectively. T cell do not survive in the absence of stimulatory signals from the T cell receptor or cytokines. Cytokines present in the joint that may be important in sustaining T cell survival and function include IL2 (low amount), IL7, and IL15 (relatively abundant) (52). IL10 reportedly prevents apoptosis in B lymphocytes and T lymphocytes (53), and as it is abundant in RA joints it may have a role in sustaining the survival of T cells there, although its inhibitory effects are also expressed.

In autoimmune arthritis, chronic immune responses and inflammatory reactions often cause severe destruction of cartilage and bone. This destruction progressively invades the bone and spreads over the cartilage, occurring in two

forms, one highly cellular, expressing essentially the same mixture of cytokines as in the active synovium and associated with active erosion. Another subset is relatively acellular and expresses chiefly TGF, TNFα and IL1 which have been shown to play a pivotal role in the pathogenesis of the synovitis and are regulators of osteoclastic resorption. Direct evidence in support of a role for IL1 in the pathogenesis of bone erosions in inflammatory arthritis is provided by examination of the findings in animals in which there is overproduction of this cytokine. Analysis of the joint changes in rabbits that constitutively overexpress IL1α due to intraarticular gene transfer revealed severe inflammatory arthritis with evidence of bone and joint destruction (54).

Mice bearing a human TNFα transgene that leads to dysregulated expression of this cytokine also develop spontaneous and progressive polyarthritis at an early age, consistent with the view that TNFα is an important cytokine in the initiation and perpetuation of inflammatory arthritis (55). When these animals are backcrossed into the DBA/1 background, arthritis is accelerated and severe bone erosions develop in association with multinucleated cells in bone resorption lacunae (56). Treatment with a monoclonal antibody to murine TNFα/β after the onset of CIA in mice leads to amelioration of arthritis as well as a reduction in histologic features of joint damage (57). In humans with RA, there is also evidence that blockade of IL1 and TNFα can modulate inflammation and in some instances retard bone erosions. These data suggest that both IL1 and TNFα play a role in the formation and progression of bone erosions.

Like IL1 and TNFα, there is increasing evidence that IL6 may inhibit formation and induce bone resorption through its stimulatory effects on osteoclasts, for example IL6 gene knockout mice do not develop bone erosions (58). But it seems that interleukin-6 alone does not induce osteoclast formation, soluble interleukin-6 receptors (sIL-6R) triggered the formation in the presence of IL-6 in cocultures of murine osteoblastic cells and bone marrow cells (59).

The accumulating evidence suggests that TNFα is not only an inflammatory mediator in its own right but also is the key regulator of the production of other cytokines such as IL1, GM-CSF, IL6 and IL8. Other proinflammatory cytokines were also inhibited if TNFα was neutralised, leading to the new concept that the proinflammatory cytokines were linked in a network with TNFα at its apex. This led to

the hypothesis that TNFα is of major importance in autoimmune arthritis and as a therapeutic target. This hypothesis has been successfully tested in animal models of collagen induced arthritis, and these studies have provided the rationale for clinical trials of anti-TNFα therapy in patients with long-standing rheumatoid arthritis. Meanwhile, antagonists of IL1 and IL6 have also been tested in the clinic.

5. Cytokine pattern and rheumatic arthritis

Cytokines are involved in the regulation of growth, differentiation and function of hematopoietic and non-hematopoietic cells and play an important role in the regulation and outcome of an immune response. Subpopulation of human T cells (Th0, Th1 and Th2) can be distinguished by their cytokine-secretion pattern. Evidence is increasing from a lot of studies that the outcome of a human disease may depend on the subpopulation of T cells that predominates at the site of inflammation. It is now generally accepted that a balance between Th1 and Th2 cells determines the phenotype and progression of numerous diseases, such as inflammatory, allergic, or autoimmune diseases (60). The Th1 type cytokines interferon (IFN) γ and tumor necrosis factor (TNF) α are required for an effective cellular immune response and involved in the expression of chronic inflammatory diseases, while Th2 cells (secreting IL4 and IL5) are responsible for the induction of a humoral response. Cytokines of the Th1 spectrum are general elevated in successful responses to a variety of intracellular pathogens, and Th2 cytokines are elevated in allergic diseases and in helminth infections. The balance appears to be maintained not only by the cytokines considered originally to be of Th1/Th2 type but also by other inhibitory cytokines such as transforming growth factor β. Two other important cytokines which regulate the Th1 and Th2 responses are interleukin (IL)12 and IL10. IL12 selectively induces a Th1 cytokine pattern against which IL10 is an important negative regulator.

The immune responses driven by Th1 T cells and Th2 T cells are sometimes also influenced by a third T cell type whose main function is counterregulation or suppression of immune responses mediated by Th1 and Th2. It has been shown, for example, that the induction of oral tolerance by the feeding of relatively low amounts of myelin basic protein leads to the induction of immunoregulatory T cells, which prevent the development of experimental autoimmune encephalitis (61). Such T cells have a unique cytokine production pattern in that they produce high levels of TGFβ without necessarily producing either Th1 or Th2 cytokines, these subtype T cells have been

termed Th3 T cells. Th3 cells producing TGFβ have also been shown to occur in experimental models of colitis or diabetes or in $HgCl_2$ induced autoimmune disease (62). In these cases it is thought that such T cells play an important role in disease prevention or cure. Recently, another type of regulatory T cell has been identified that may be related to the aforementioned Th3 T cell. This cell, termed T regulatory cell 1(Tr1), is induced in vitro by stimulation of T cells in the presence of IL10 and is a T cell producing high levels of IL10 (63).

Cytokine profiles in RA patients have been characterized by a number of different approaches. The first studies investigated cytokine by enzyme-linked immunosorbent assay in synovial fluid or supernatant from T cells clones isolated from RA joint. Then, studies evaluated RA patients' joint cytokine mRNA by reverse transcription polymerase chain reaction or in situ hybridization. Rheumatoid arthritis is usually considered to be a Th1 disease, with the shift toward a Th1-mediated immune response (64) although there exist some contradictory results. It is now established that monocyte-derived pro-inflammatory cytokines such as TNFα and IL1 found in abundance in RA synovium play an important role in the pathogenesis of RA.

Reactive arthritis, which is induced by several intracellular bacteria and remnants of bacteria or even live bacteria can be found in the joint, serves as a useful model of chronic inflammatory diseases, because the triggering antigen can be identified, whereas for others, the triggering events are unknown. Our previous studies indicate that the key cytokine for a Th2 response, IL4, was found in synovial membrance of ReA patients more frequently than in rheumatoid arthritis patients using the technique of PCR and in situ-hybridisation (64). In another study the Th1/Th2 cytokine secretion pattern upon stimulation of synovial fluid mononuclear cells with the triggering bacterium was investigated in ReA patients, in whom low amounts of IFNγ and TNFα but high amounts of IL10 were found also hinting towards a Th2 pattern (40).

The concept of Th1/Th2 balance has attracted much interest recently in attempts to understand the pathogenesis of AS. In contrast to ReA, a pathogenic role of bacteria in AS is less clear and very little is known about cytokines in AS. A lot of researchers have been trying to assess whether the T cell cytokine pattern in AS might also be polarized towards a Th2 like pattern since ReA and AS are related clinical conditions. A cytokine pattern different from RA was recently observed in SpA (65), where low Th1 cytokines were found in AS patients. A similar situation was

demonstrated in another study (36): the T cell production of IFNγ and TNFα of AS patients was on average significantly decreased whereas the T cell production of IL4 did not differ from controls, which indicates that AS can be regarded as a low TNFα/IFNγ disease. However whether a typical Th2 type pattern characterised by the predominance of IL4 positive T cells is present remains to be further identified.

6. Putative antigens

Inflammatory responses provoked by pathogens are antigen-specific in their induction but are nonspecific in their effects. Consequently, they are potentially damaging to the host that produces them. In addition, the immune system can respond specifically to self antigens, thereby giving rise to autoimmune diseases. Ankylosing Spondylitis is a T cell dependent inflammatory possibly autoimmune disease, its pathogenesis is regarded as a consequence of the activation of T cells by yet unknown antigens and the co-stimulatory molecules CD3 and CD28. A lot of potential antigens have been proposed for this process, including type II collagen, aggrecan G1 domain, glycoprotein gp39 and heat shock proteins, and others. Following activation, T cells initiate the inflammatory cascade through secretion of either interleukin 2 or interferon gamma, or through direct cellular interaction with macrophages and synoviocytes.

6.1 Antigens derived from cartilage

In joint diseases, there is a loss of the normal balance between synthesis and degradation of the macromolecules that provide articular cartilage with its biomechanical and functional properties. Articular cartilage is a multiphasic material with 2 major phases: a fluid phase composed of water and electrolytes, and a solid phase composed of collagen, proteoglycans, glycoproteins, other proteins, and the chondrocytes. Each phase contributes to its mechanical and physiologic properties.

Autoimmune reactions to auto-antigens may play a key role in the pathogenesis of inflammation in various rheumatic diseases (66). The major components of the matrix of articular cartilage and the intervertebral disc, namely type II collagen and the large aggregating proteoglycans, have both been incriminated as autoantigens in these diseases (66). Cartilage is normally secluded from immune surveillance due to its avascular structure (67); however, local

inflammatory processes (for example: from trauma or infections) may stimulate the production of various factors and enzymes which could degrade the cartilage matrix in sites which include diarthrodial joints and the intervertebral disc. This could then expose the cartilage components to the immune system leading to an autoimmune reaction to cartilage (66,67). The autoimmune attack on the joints could also be triggered by a cross reactive immune reaction in response to unrelated antigens by the mechanism of "molecular mimicry" (68). The net result of such autoimmune reactions could be further destruction of cartilage and release of more autoantigens. This could lead to a chronic, self-perpetuating inflammation in genetically predisposed individuals who are prone to develop these autoimmune reactions (67). Relevant to this is the finding that removal of all cartilage from an arthritic joint at arthroplasty leads to a loss of synovial inflammation in that joint (69). In addition, end-stage arthritis with a total destruction of cartilage leads to a gradual decrease in inflammation of the synovium in the affected joints (69).

Type II collagen is found in cartilage, as well as in the vitreous humor of the eye, which forms the basic fibrillar structure of the extracellular matrix. Like collagen type II, type IX and XI are also cartilage specific and are present together with type II collagen. There is evidence that many collagens, including type II, IX and XI in cartilage, exist as hybrid molecules. The induction of polyarthritis in rats, mice and primates on immunization with type II collagens has strongly suggested that this antigen possibly play an important role in the pathogenesis of various human rheumatic disease (70,71). The investigation of an immune response to type II collagen in rheumatic diseases have been investigated both at the cellular and humoral levels (72,73). Several lines of evidence point to type II collagen as a significant autoantigen, at least in RA. Autoantibodies directed against type II collagen are found in the serum and joint fluid-derived T cells of a significant proportion of RA patients (74), cells obtained from the affected synovium of RA patients recognize type II collagen (75). Furthermore, oral administration of chicken collagen to patients and human collagen to mice significantly improves RA and collagen-induced arthritis in some studies (76). When injected into selected strains of mice and rats and into nonhuman primates, type II collagen causes an inflammatory arthritis resembling rheumatoid arthritis (77).

Another cartilage-specific molecule is the large proteoglycan called aggrecan, which is arranged in 3 globular domains (G1, G2 and G3). The G1 domain binds to

hyaluronan and link protein in cartilage, resulting in the formation of macromolecular PG aggregates. Cartilage proteoglycan has an Mr of 1-3 x 10^6 with a protein core of Mr 210, 000 to which are attached numerous glycosaminoglycan chains, namely chondroitin sulfate (CS) and keratan sulfate (KS) (78). Numerous N-linked and O-linked oligosaccharides are present interspersed on the protein core. The N-terminal G1 globular domain binds non-covalently to hyaluronate (HA) in the cartilage matrix and is known as the hyaluronate binding region (HABR). This linkage is stabilized by link protein to form macromolecular aggregates of proteoglycan and link protein about an HA chain. There are two N-terminal globular domains called G1 and G2. The around 30 KS chains of proteoglycan are attached to a region C-terminal to G2. Up to 100 CS chains are attached to a CS region C-terminal to the KS rich region. The C-terminal end is characterized by another globular domain called G3 which has lectin-like properties. This region has also been shown to be alternatively spliced and may or may not contain additional regions with homologies to epidermal growth factor and complement proteins (79). Immune response to aggrecan have been detected in rabbits with experimentally induced synovitis (80). Patients with inflammatory arthritis exhibit cellular immunity to this molecule (81).

In AS, antigen derived from the cartilage such as proteoglycan have been tested as a possible autoantigen, but no convincing evidence for its involvement in pathogenesis has been obtained to date (18,44). While the animal model of HLA B27 transgenic rats has not been able to fulfil the initially high expectations derived from the suggestive clinical disease of these rats, a mouse model for AS has recently gained some interest. Injection of the G1 domain of the main proteoglycan aggrecan into BALB/c mice induces not only peripheral arthritis but also spondylitis (82). It could be shown that T cells play an important role in this model and G1 derived immunodominant T cell epitopes have been identified (82).Of some interest in the context of SpA, aggrecan is present in fibrocartilaginous entheseal regions of the tendon but not in the human midtendon (83). Versican, another proteoglycan has a similar G1 domain. Furthermore, G1 is the major degradation product of intervertebral discs. There are limited data in humans about the cellular immune response to the G1 to date, mainly based on lymphocyte proliferation. With these technique, a T cell response to the G1-protein has been reported in AS and RA patients (84).

One of the major secreted proteins of human articular chondrocytes in monolayers or explant cultures and of synovial fibroblasts is a glycoprotein with an apparent molecular weight of approximately 39,000, referred to as human cartilage glycoprotein-39 (HC gp-39). HC gp39 has gained interest recently. Multiple levels of evidence suggest that HC gp39 is a target of the immune response in the joints of RA patients: both HC gp39 messenger RNA and protein have been detected in synovial specimens and cartilage from RA patients but not from normal subjects (85); HC gp39 is the predominant secretory protein of synovial fibroblasts and is produced by articular chondrocytes (86); and serum levels of HC gp39 correlate with joint disease(87). But so far there is no report about the relationship between gp-39 and the pathogenesis of AS.

6.2 heat shock protein (hsp)

Based on high interspecies sequence homologies, inducible tissue expression and a strong immunogenicity, hsp have been repeatedly incriminated to be involved in various autoimmune disease. By now, various lines of evidence suggest that hsp play an active role in the development of autoimmune diseases in animals (88), and in human autoimmune diseases (89). However, Van der Zee and coworkers (90) reported that only passive transfer of a T cell clone responding to mycobacterial hsp60 evoked disease in naive recipient animals. The disease could not be induced by immunization with hsp60, instead protection was established. A similar situation was found in experimental models of arthritis in which immunization of mice with an immunodominant epitope (amino acid 261-271) from hsp 65 can protect from the development of pristane-induced arthritis by T helper type 2 pattern (91). Immunization with Mycobacterium tuberculosis heat shock protein 60 has been shown to protect rats from experimental arthritis (92). It has been documented that preimmunization with microbial proteins belonging to different hsp families(mycobacterial hsp70, hsp60, hsp10) protect from subsequent induction of arthritis (93,94). These different results indicate that hsps might play a major role as a target molecule or as a protective factor.

6.3 Antigens derived from bacteria

The development of many autoimmune diseases has been etiologically linked to exposure to infectious agents (95,96). Models proposed to account for the

relationship between infection and autoimmunity include inflammation-induced presentation of cryptic self-epitopes, antigen persistence and molecular mimicry (97,98). There is a clear relationship between bacteria and reactive arthritis, which usually occurs after a genitourinary tract infection due to Chlamydia trachomatis or an intestinal infection due to Yersinia, Salmonella, Shigella or Campylobacter jejuni. Persistence of bacteria or bacteria antigens may play a pivotal role in the immune process that finally leads to the inflammatory characteristics of reactive arthritis. Yersinia enterocolitica is a common pathogen of reactive arthritis, the synovial T cell from patients with yersinia-induced ReA response is primarily directed against bacterial components, of which the 19-KD urease beta subunit is regarded as one of the immunodominant proteins (99). By now, no sufficient evidence supported a relationship between bacteria infection and AS or RA. Especially for AS, it could be of some significance to test T cell responses to bacterial epitopes because AS and reactive arthritis belong to the group of spondylarthropathies which comprise a spectrum of diseases characterized by shared clinical features and a variably strong association with HLA-B27, with 20-40% of HLA-B27 positive patients with ReA developing the full picture of AS (100).

7. Identification of antigenic T cell epitopes

The identification of T cell epitopes is of significance for the understanding of the host response during autoimmune diseases. The characterization of relevant T cell epitopes is generally based on the analysis of the specificity of T cell lines propagated in vitro (101). Alternative approaches are the biochemical purification and sequencing of MHC-bound peptides or the T cell-screening of expression libraries prepared from the organism of interest. A more recent method is the identification of unknown T cell epitopes by the screening of combinatorial peptide libraries resulting in millions of different peptides (102). Epitope screening with synthetic peptides is expedited by the use of peptide spot libraries, which allow the automated and economic synthesis of multiple peptides (103).

The identification of MHC class I-restricted T cell epitopes is further facilitated by the existence of relatively strict peptide binding motifs of individual MHC class I molecules (104). If the CD8 T cell target protein is already identified, the knowledge of MHC class I binding motifs allows the educated guessing of epitopes and thus greatly reduces the number of synthetic peptides required for epitope identification.

But epitopes that do not exhibit the typical binding motif may be overlooked. Regarding the identification of CD4 T cell epitopes, the less strict binding requirements and thus the limited predictive value of MHC class II motifs (105) makes this approach less suitable.

Recently, a new technique has been introduced which allows the determination of antigen specific T cell frequency, the analysis of cytokine release after antigen specific T cell stimulation (106), and the identification of the cytokine secreting cells according to its surface marker(107). The advantages of flow cytometry are largely based on its ability to analyze very rapidly, even in small samples, multiple cell properties simultaneously, including size, granularity, surface antigens and intracellular cytokines. These features make identification of T cell epitope of antigens faster and more accurate, and it is even possible to further analyze the phenotype of the TCR of antigen-specific T cells after separating the specific cells by MACS (magnetic activated cell sorting) or FACS (fluorescence activated cell sorting).