Chapter 1

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1. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is one of the most common human autoimmune diseases characterized by chronic inflammation of the synovium of diarthrodial joints (1). It can lead to long-term joint damage, resulting in chronic pain, loss of function and disability. Primarily the small joints of the extremities are affected, but as the disease progresses more of the large joints become involved too. The chronic inflammatory process induces changes in the cellular composition (cellular infiltration) and the gene expressing profile of the synovial membrane, resulting in hyperplasia of the synovial membrane, which causes structural damage of cartilage, bone and ligaments (2). Extra-articular disease affecting a variety of organs occurs in the majority of patients and is a significant factor in morbidity and mortality of people with RA (3). The severity of RA encompasses a wide spectrum, ranging from self-limiting disease to chronic progressive disease, causing varying degrees of joint destruction and clinically evident extra-articular organ involvement.

RA occurs in 0.5-1.0% of the population worldwide (4,5). The prevalence is about two to three times more common in women than in men. Although the cause or causes of RA remain elusive, the general consensus is that factors contributing to its occurrence and course (clinical heterogeneity) are probably both genetic and environmental. The main risk factors for the disease include genetic susceptibility, sex and age, smoking and infectious agents. In addition, hormonal, dietary, socioeconomic, and ethnic factors seem to contribute (4-6).

The major goals of treatment of the arthritis are to reduce pain and discomfort, prevent deformities and loss of joint function, and to maintain a productive and active life. Inflammation must be suppressed and mechanical and structural abnormalities corrected or compensated by assistive devices. The introduction of new therapies such as tumor necrosis factor-alpha (TNFα)-blocking agents and new treatment strategies, especially early and aggressive therapy, including combinations of several disease-modifying anti-rheumatic drugs (DMARDs) have improved the outcome for RA patients (7). Unfortunately, these therapies form not a cure, as continuous systemic immunosuppression is required to maintain clinical benefits. Consequently, the long-term side effects are unsure while the costs are high (8,9).

2. Immune responses in RA

RA is a chronic inflammatory condition that involves many elements of the immune response (10). The synovium (or synovial membrane) is normally a relatively acellular delicate structure consisting of one or two layers of synoviocytes. In RA, the synovium becomes hypertrophic and edematous. Angioneogenesis, recruitment of inflammatory cells due to production of chemokines, local retention and cell proliferation do all contribute to the accumulation of cells in the inflamed synovium. Locally expressed degradative enzymes digest the extracellular matrix and destroy the articular structures (11). The synovial membrane that extends to the cartilage and bone is known as pannus. It actively invades and destroys the periarticular bone and cartilage at the margin between synovium and bone. Multiple cell types participate in the pathogenesis of RA and the following section will introduce the major contributing cell populations.
Chapter 1

T cells
Consideration of the possibility that T cells might be actively involved in the pathogenesis of RA first became prominent in 1980s (12). The innate immune system might prepare the synovium for infiltration by T cells and the subsequent immune events in the joint (13). Activated T cells that are abundantly present in the inflamed joints of RA patients can stimulate other cells (e.g. B cells, macrophages and fibroblast-like synoviocytes) (11,13-15). These T cells are found to participate in the complex network of cell- and mediator-driven events leading to inflammation and joint destruction (13,16,17). However, several parts of the T cell pathway are still hypothetical and further research is needed to provide conclusive evidence. The role of T cells in RA will be described in more detail in the next paragraph.

B cells
B cells play several critical roles in the pathogenesis of RA (18). They are the source of autoantibodies being produced in RA and contribute to immune complex formation and complement activation in the joints (8,19). Patients with RA frequently have rheumatoid factors (RF); antibodies of the IgM or IgG subclasses mostly reactive with the constant region of their autologous IgG molecules. Multiple other autoantibodies have been found in RA, with recent interest focused on those directed at cyclic citrullinated peptides (20). Antibodies that are directed to citrullinated protein (21) detect cyclic citrullinated peptides in many different proteins and are present in about 80% of the RA patients. Several lines of evidence suggest that citrullinated antigens have direct involvement in the rheumatoid disease process. Anti-cyclic citrullinated peptide antibodies precede the clinical development of synovitis by many years (22,23). B cells are also very efficient antigen-presenting cells, and can contribute to T cell activation (24). B cells both respond to and produce chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyperplasia. The important role of B cells in the disease etiology is supported by the recent success of B cell depletion therapy using rituximab (25).

Macrophages
The major effector cells in the pathogenesis of arthritis are synovial macrophages and fibroblasts (2). Activated macrophages are critical in RA, not only due to macrophage-derived cytokines (in particular TNFα and interleukin-1 (IL-1)) in the synovial compartments (26), but also because of their localization in strategic sites within the destructive pannus tissue (27,28). In addition beside tissue macrophages, also circulating monocytes and other cells of the myelomonocytic lineage contribute to disease (26,29-31). The variety and extent of macrophage-derived cytokines in RA and their widespread effect indicate that macrophages are local and systemic amplifiers of disease severity and perpetuation (32).

Fibroblast-like synovial cell
Among the many cell types present in the rheumatoid joint, the fibroblast-like synovial cell (FLS) is one of the most prominent. It is now accepted that the FLS is not only space-filling, but directly responsible for cartilage destruction,
and also drives both inflammation and autoimmunity (33). There is evidence for proliferation and expression of inflammatory cytokines and chemokines by FLS in inflamed synovia (34,35).

In summary, the cell-cell interactions that occur in RA synovium are multiple, complex and fundamentally important to the pathogenesis and outcome of this disease (36). In addition to the cell populations considered in detail above, osteoclasts, chondrocytes, mast cells, dendritic cells and other cell types are important in the events that occur within RA pannus and in adjacent cartilage and bone. Besides the cognate cell-cell interactions that require direct contact between two different types of cells, interactions between cell populations in RA synovium can also be mediated by secreted molecules, such as cytokines (36). Cytokines play a central role in the initiation and perpetuation of synovial inflammation (37). They are believed to activate resident synovial cells to produce protolytic enzymes that mediate destruction of the cartilage, ligaments and tendons of the joints. Many of the cytokines thought to play a role in initiating joint destruction are probably produced as a result of local T cell and macrophage activation. The cytokine system is a highly complex network with cytokines cross-regulating their expressions and function (2). Numerous cytokines, including IL-1, IL-8, TNFα and interferon-gamma (IFNγ), have been detected in synovial fluid (SF). TNFα and IL-1 have been identified as key players in synovial inflammation and are direct targets of successful anti-cytokine treatment of RA (2).

There is substantial evidence that RA has an autoimmune component, besides the fact that no infectious cause is proven. First, the fact that the disease affects multiple joints is consistent with the process being systemic (38). Second, the disease is associated with autoantibodies and the formation of immune complexes (39). Third, autoreactive T cells are present in the inflamed joints (40). Fourth, RA (like other systemic autoimmune diseases) is significantly more common in women than in men (39). Finally, there is a marked association between disease and the expression of specific HLA subtypes, which is consistent with the expansion of specific T cell subsets that are presumably activated by specific epitopes (41).

Over the last few years, susceptibility to autoimmunity has been regarded not so much as a predisposition to generating autoaggressive effector cells, but rather as a failure to regulate the immune response (42). Considering the current concepts of immunity, a defect in immunoregulation could be acquired at multiple points during both early and late phases of an autoimmune response. For CD4+ T cells, the initial ligand-receptor interaction between MHC-peptide and the T cell receptor (TCR) is crucial, since this determines qualitative and quantitative aspects of the signals transduced through the TCR. Useful understanding of the role of T cells in RA will need to place T cell function in context of the incompletely understood cell-cell interactions that are likely to be keys to the pathogenesis of this disease (43).

3. Role of T cells in arthritis
The extension of the contribution of T cells to the pathogenesis of RA remains a matter of intense debate. It is apparent that taking RA as simply a T cell-mediated or T cell-independent disease represents a too narrow view (13), as in complex diseases such as RA it is unlikely that there is a single ‘guilty’ effector cell. The association between
specific alleles encoded within the MHC class II region and the development of RA has provided the first strong evidence that CD4+ T cells play a role in the pathogenesis of this chronic inflammatory disease (42).

Much attention has been focused on the role of T cells in autoimmunity for two main reasons. First, T helper cells are the key regulators of all immune responses to proteins. Second, several autoimmune diseases are genetically linked to the MHC, and the function of MHC is to present peptide antigens to T cells. T helper cell abnormalities may also lead to autoantibody production because T helper cells are necessary for the production of high affinity antibodies against protein antigens. An autoantigen has not been unequivocally identified in RA, but the synovium clearly is the site of antigen-specific T cell responses. Using in vitro proliferation assays with T cells from RA patients, many putative autoantigens have been identified as candidate antigens. Type II collagen (CII), proteoglycan (PG) aggrecan, cartilage link protein and many relatively joint-specific antigens (e.g. matrix metalloproteinases) have been implicated (44-46).

Tissue infiltrating T cells, in particular CD4+ T cells, are a consistent feature of rheumatoid synovitis (43,47). This antigen driven infiltration may predominate during the early phase of inflammatory response. T cells could be recruited through bystander activation, or by stimulation of self antigens released from inflamed tissue. Initial stimulation of T cells by complexes of antigenic peptide and disease-associated MHC class II molecules could profoundly influence the expression of pro-inflammatory cytokines. Data suggest that during the chronic phase of the disease it may be the cytokine milieu that sustains and maintains pathogenic T cells (Table 1) (42).

**Table 1.** Characteristics of chronically activated T cells in the synovium of patients with RA.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tbody>
<tr>
<td>T cells are found in follicular lymphoid aggregates</td>
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<tr>
<td>The cell-surface phenotype (e.g. CD45RO, CD69, CD44 expression) suggests chronic immune activation</td>
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<tr>
<td>T cells are terminally differentiated, with significant telomere loss</td>
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<tr>
<td>Synovial T cells are hyporesponsive to TCR ligation</td>
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<tr>
<td>Synovial T cells exist in an environment favoring cell survival</td>
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<tr>
<td>There is an imbalance of pro- and anti-inflammatory cytokines, with a predominance of macrophage products in inflamed joints</td>
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<td>There is a bias towards the development of T helper 1 cells</td>
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Adapted from Cope AP (42).

T cell hyporesponsiveness arises through subsequent sustained expression of inflammatory cytokines. The hyporesponsive state of synovial T cells in RA directly correlates with impaired TCR-mediated signaling transduction (48). Impaired T cell activation could promote and perpetuate the chronic inflammatory response through the loss of mechanisms of tolerance that are dependent upon intact TCR signal transduction pathways (49). Hyporesponsive T cells might function as effector cells and sustain the chronic inflammatory process through predominantly antigen independent mechanisms. It is proposed that by reversing T cell hyporesponsiveness, antigen-independent responses serve to regulate the inflammatory process.
In addition, to direct autoimmune attack by effector T cells, arthritis might result from defective homeostatic control of immunity by regulatory T cells (Tregs) (17). Synovial T cells can be divided into several subsets that either promote or inhibit inflammation. Both pathological responses to antigen and a failure of regulatory function need to be considered as ways in which T cells could perform a critical role in human arthritis.

The regulation of immune responses to self-antigens is a complex process that involves maintaining self-tolerance while retaining the capacity to mount robust immune responses against invading microorganisms (50). Peripheral tolerance mechanisms are needed to control autoreactive T cells. One control pathway is the absence of costimulatory signals that renders T cell activation incomplete and commits T cells to anergy or cell death (51). A second major control mechanism involves suppressive (immunomodulatory) signals that are delivered by Tregs (51). Two major subsets of Tregs can be distinguished: natural and adaptive (acquired) Tregs (50). Certain natural Tregs (CD4+CD25+ bright T cells) actively down regulate the activation and proliferation of autoreactive T cells (52). Such cells are capable of dampening an immune response by conventional CD4+ T cells specific for the autoantigen, as shown in transplantation. The production of Tregs is a key function of the thymus in self-tolerance. The development of Tregs in the thymus stresses the fact that there are many potentially autoreactive T cells that must be permitted to enter the periphery in order to assure protection from pathogens. Adaptive Tregs can develop either from CD4+CD25+ natural Tregs or by altering the activity of T helper cells and include type 1 Tr cells (Tr1) that secrete high levels of IL-10 (53), and Th3 cells which primarily secrete high levels of transforming growth factor-beta (54).

**Autoantigens in RA**

In the classical model of autoimmunity, tissue specificity is determined by the tissue-specific recognition of autoantigens that elicit adaptive immune responses. Both T cell and antibody responses have been found to a variety of putative autoantigens in RA patients (55). RA associated antigens fall into two major groups: first, those that are locally expressed in the joint. Prominent candidate antigens are CII (56,57), human cartilage glycoprotein 39 (gp39) (44), and PG (58,59). These antigens can induce autoimmunity and arthritis in rodent models, but whether the reactivity participates in the primary pathogenesis of RA or reflects tissue degradation remains unknown. Second group of antigens are those proteins not associated with the joint, and consist of:

1) highly conserved foreign antigens with human homologues, in which the initiating antigenic stimulus may occur through infection. The Epstein-Barr virus (EBV) encoded glycoprotein, like other bacterial proteins, contains the QKRAA shared epitope motif (MHC class II determinant) and has been a strong candidate for over 25 years as environmental infectious agent involved in RA pathogenesis (60). Other conserved candidates include heat shock proteins (HSPs), which are major bacterial antigens. Antibodies and T cells reactive with HSP65 and DnaJ class of HSPs are abundant in the SF of RA patients (61).

2) post-translationally altered proteins, such as citrullinated filaggrin, to which autoantibodies show high specificity but low sensitivity for RA (21) and the Fc portion of IgG, which is recognized by rheumatoid factor.

3) ubiquitous proteins, such as glucose-6-phosphate isomerase (G6PI) (62), p205 (63), and HSPs secreted during stress, such as endoplasmic reticulum immunoglobulin binding protein BiP (64,65). Despite the presence of many
putative critical antigens there is for most of them no solid evidence yet that they are implicated in the pathogenesis of RA (40).

Taken together, numerous research studies have scrutinized the role for T cells and their mechanisms of action in RA. A crucial point remains unresolved: do T cells activated by one or more autoantigens initiate the disease process, or is the influence of T cells secondary to a synoviocyte-driven inflammatory response originating within the joints (17)? Probably the cause and the driving forces are polygenic and multifactorial, and understanding the disease will require a detailed basic analysis of disease mechanisms. Animal models are excellent tools for such analysis.

4. Experimental models for RA
To understand the complexity of the pathogenesis of RA and for preclinical testing of new therapeutic agents, animal models are a necessity. To be able to evaluate and select suitable animal models for RA it is crucial to reproduce some of the basic features of RA in such models (66). Hallmarks are first, tissue-specificity; RA is characterized by a tissue-specific, inflammatory attack affecting diarthrodial joints. Although systemic manifestation can be prominent, the predominant inflammatory attack is directed towards peripheral joints. Second, chronicity; in RA chronicity is an essential characteristic. The disease course may proceed with identifiable relapses, but there is usually steady progression of joint destruction. Third, MHC class II association; the genetic influence is significant though not prominent and points towards an important role of class II genes in the MHC. In particular, certain structures near the peptide-binding pocket of HLA-DR4 molecules are highly associated with RA (67,68).

Many models have been described and each represents different aspects of the disease. The models described for RA so far can be divided into three principal groups: 1) cartilage protein-induced, 2) adjuvant induced, and 3) spontaneous. As arthritis is mediated by a specific immune attack on cartilage in peripheral joints, it is not surprising that several cartilage proteins, such as PG (69), CII (70), gp39 (44) and cartilage oligomeric matrix protein (COMP) (71) have been shown to be arthritogenic in different animal strains.

In our studies we used the proteoglycan (aggrecan)-induced arthritis (PGIA) model because this model reproduces several features of RA in which we are especially interested. PGIA is a chronic arthritis model which makes it especially useful to test immunomodulating agents over a longer period. Furthermore, PGIA is an antigen-induced arthritis model fundamentally controlled by T cells making it possible to generate a T cell receptor (TCR) transgenic (Tg) mouse and study the role of antigen-specific T cell responses in RA. In addition, PGIA is induced by a cartilage matrix component and there is growing evidence that, at least in a subset of patients with RA, antigen-specific T cell responses to cartilage matrix proteins do develop (45,59,72-76). Among the candidate autoantigens (58,77), cartilage PG aggrecan is one of the target autoantigens in RA joints (45,59,72-76).
5. Proteoglycan (aggrecan)-induced arthritis model

Aggrecan (Fig. 1) is a complex macromolecule consisting of a large core protein (~2,400 amino acids) to which glycosaminoglycan and oligosaccharide side chains are covalently attached (78,79). These side chains include approximately 100 chondroitin sulfate (CS) chains, 30 keratan sulfate (KS) chains and shorter N- and O-linked oligosaccharides. Link protein, a small glycoprotein, stabilizes the noncovalent linkage between aggrecan and hyaluronan (HA) to form the PG aggregate that may contain as many as 100 aggrecan monomers.

Figure 1. Schematic presentation of the structure of cartilage proteoglycan (PG) aggrecan. Cartilage PG consists of a central protein core to which glycosaminoglycan (GAG), chondroitin sulfate (CS), and keratan sulfate (KS) side chains are attached together with O-linked and N-linked oligosaccharides. (Hundreds of PG aggrecan molecules bind to a single hyaluronan chain, stabilized by link protein, forming large, multimillion Dalton size aggregates.) The various domains/subdomains of the central protein core are the G1 domain with A, B, and B’ loops; IGD (interglobular domain between G1 and G2 domains); G2 domain with B and B’ loops; KS (a KS-rich region between the G2 domain and the CS attachment region); CS attachment regions; and a G3 domain containing EGF (epidermal growth factorlike), LB (lectin-binding), and CRP (complement regulatory protein-like) subdomains. Approximately 100-120 CS side chains are attached to a long, but restricted, region of the core protein (CS attachment region). Although most of the KS chains are localized in a narrow region (KS), KS side chains are also present along the entire core protein and frequently mask T cell epitopes. CS and KS side chains mask the T cell epitopes of the core protein, and many of the T cell epitopes can be processed by the MHC only if the GAG side chains are removed. Adapted from Glant TT and Mikecz K (138) with the approval of Humana Press (Totowa, NJ).

The G1 and G2 N-terminal globular domains of aggrecan and its G3 C-terminal domain have distinct properties that function as integral parts of the aggrecan core protein. Approximately half of the aggrecan molecules in adult cartilage lack the G3 domain due to proteolytic cleavage during matrix turnover. The core protein of aggrecan is heavily degraded during inflammatory processes, which results in the loss of function of articular cartilage (58,77,80). Immunization of BALB/c mice with partially deglycosylated human PG (hPG) induces chronic progressive polyarthritis and spondylitis (Fig. 2) (81). This PGIA model has many similarities with human RA, as indicated by clinical assessments (Fig. 3), radiographic analyses, scintigraphic bone scans, laboratory tests, and histopathology of peripheral joints (69,81-83). The development of the disease is based upon T and B cell responses cross-reactive between the immunizing human and mouse (self) cartilage PG (69,83,84). Although the production of mouse PG-specific antibodies precedes inflammation and shows a high correlation with the incidence of arthritis, neither these antibodies nor PG-specific B cells alone can transfer the disease to naïve syngeneic mice (82,85,86).
Figure 2. Schematic presentation of the immunization scheme and arthritis course. To induce proteoglycan (PG)-induced arthritis young retired breeder female BALB/c mice are immunized with PG (100 µg human PG protein in 100 µl PBS) mixed with dimethyldioctadecylammonium bromide (DDA; 2 mg in 100 µl) on days 0 and 21. It is typical that, if an animal once develops any small symptom of inflammation e.g., in the interphalangeal joint of one paw, this mouse will develop severe arthritis sooner or later without additional injections. Usually, the small joints are first involved. Along the progression of the disease, more and more joints are involved until severe deformities and ankylosis develops. Disease may flare up in one or in a few joints, when the inflammation in other joints is regressed (95,138).

Adoptive transfer of PGIA requires the presence of both T and B cells from arthritic animals (82,87,88), and a rapid accumulation of mouse PG-specific Th1 cells in the synovium appears to be the most critical component of the development of arthritis (69,89). It is very likely that an autoantigen-driven mechanism of joint inflammation becomes local and self-sustaining by PG (cartilage) degradation in the mouse joint. Autoreactive T cells subsequently migrate to and proliferate in the synovium and joint draining lymph nodes (85). Here self-peptides are present in relatively high concentrations as a result of the normal turnover of the cartilage matrix, which may be higher when an increased PG degradation occurs in the inflamed joint. Once an animal develops arthritis, more and more joints become involved and repeated “spontaneous” episodes of inflammation result in complete deterioration of articular cartilage and lead to deformities of the peripheral joints (69).

PGIA is postulated to be a fundamentally T cell-dependent, but PG-specific B cell and antibody mediated autoimmune model (69). Several lines of evidence indicate that CD4+ T cells play an important role in PGIA (69,84,90). First, susceptibility to PGIA is associated with MHC class-II (H-2d haplotype in BALB/c and H-2k in C3H mice) (69,84,91-93), and the disease is prevented when CD4+ T cells are depleted either in vivo (90) or in vitro prior to adoptive transfer (82). Moreover, immunization of BALB/c mice with hPG induces a dominant Th1 T cell response (89,94,95) and treatment of arthritic mice with IL-4 can prevent disease development by inducing a switch from a Th1-type to a Th2-type response. The importance of CD4+ Th1-cells was further underlined by the observation that IL-4-deficient BALB/c mice developed a significantly more severe form of the disease than wild-type BALB/c mice (96). Finally, the transfer of disease requires in vivo or in vitro-activated CD4+ T cells from arthritic animals (82,87), or an arthritogenic PG epitope-specific CD4+ T cell hybridoma (5/4E8) (97).

Although CD4+ T cells are necessary for the development of PGIA (89,90,97-99), both autoreactive T cells and B cells, and possibly autoantibodies are required for the development of a severe chronic relapsing arthritis in this model (69,83,84,99,100). PG-specific B cells regulate the initiation of autoimmune murine arthritis by functioning as antigen presenting cells and by producing autoantibodies (99). While arthritic serum alone did not induce disease (82), autoantibodies are important for initiating inflammation in the joint by binding to the cartilage surface (89), and initiating chemokine and cytokine responses in the joint (101).
6. Immune responses to proteoglycan in RA

Cartilage is one of the few immunologically privileged tissues in the body in that it is essentially avascular and therefore not subjected to close immunological surveillance. Only when degraded can its often uniquely antigenic cell fragments and matrix molecules become exposed, released and subsequently “recognized” by the immune system. Some structural changes, such as partial deglycosylation (102) or cleavage of the core protein of PG by various metalloproteinases (103,104), occur in vivo during the normal turnover of cartilage PG, but these processes are more extensive in inflammatory conditions. Fragments released during PG degradation may trigger and/or maintain local immune reactions in the synovial joints in arthritis-susceptible animals, and perhaps in humans as well. Indeed, immune responses to human cartilage PG have occasionally been detected in patients with RA, juvenile idiopathic arthritis (JIA), and ankylosing spondylitis (AS) (45,58,72,75) supporting the hypothesis that, among other candidate autoantigens, cartilage PG might be a target of the autoimmune inflammatory attack in arthritic joint lesions. Li et al. demonstrated the presence of G1 autoreactive T cells in peripheral blood (PB) and synovium of RA patients (45). Studies of Zou et al. showed that after antigen-specific stimulation with the G1 protein, CD4+ T cells of the majority of AS and half of the RA patients secreted significant amounts of IFNγ and TNFα, which clearly suggested recognition of the G1 domain of aggrecan (59). Recently they also showed that a G1 peptide-specific CD8+ T cell response is present in patients with AS and RA, but not in healthy controls (105).

The presence of PG-specific immunity/autoimmunity in RA has raised many questions as to whether the immunity is causal, a consequence of cartilage destruction or a result of a molecular mimicry. It may be a secondary event after cartilage destruction caused by other mechanisms. Nonetheless, the demonstration of such immune response in both PB and SF in RA patients is very interesting, particularly since immunity to G1 causes the induction of an inflammatory erosive polyarthritis in BALB/c mice (PGIA model) (69,84).
7. Heat shock proteins

Heat shock proteins (HSPs) constitute a group of proteins that are highly immunogenic, present in all organisms, with the potential to trigger immunoregulatory pathways (61). More specifically, immunity to HSPs can suppress immune responses that occur in various inflammatory conditions, such as in RA, JIA and related disorders (61,106-108). The synovial tissue of patients with arthritis is characterized by a chronic inflammatory process leading to an alteration of cellular homeostasis and finally to severe tissue damage. A variety of different stressors (e.g. proinflammatory cytokines) is present in the inflamed synovial tissue of RA patients, each of which has the potential to induce upregulation and unleash an HSP-specific immune response (109-111). It has been shown that HSPs are expressed in the synovial membrane in patients with RA and JIA but not in non-inflamed synovial tissue (111-113).

HSPs, also called stress-proteins, become markedly over-expressed by all cells under conditions of stress: such as increased temperature (fever); viral infection; exposure to pro-inflammatory mediators, such as TNF-\(\alpha\) and IFN-\(\gamma\); oxidative stress (114-117). HSPs carry out crucial housekeeping functions that are important for the survival of prokaryotic and eukaryotic cells. As molecular chaperones they interact with unfolded or partially folded protein subunits and facilitate correct folding, assembly and translocation of proteins and they protect cells from the effects of various stresses. HSPs are classified into families on the basis of their molecular weight (including the HSP10, HSP40, HSP60, HSP70, HSP90 and HSP100 families). HSPs are highly evolutionary conserved (115,118,119), with significant interspecies homologies e.g. some mammalian family members have highly conserved microbial homologues (120-122). HSPs are strongly immunogenic molecules; immune responses to bacteria are dominated by responses to HSP including the recognition of conserved bacterial epitopes giving rise to cross-recognition of self-HSP. The physiological significance of this aspect of immune behavior is possibly connected to the regulatory control of the inflammatory process, as shown in several systems described below.

Although HSPs have well described roles as chaperones for intracellular proteins, the significance of the immunoregulatory capacity of HSPs in arthritis is only now becoming clear (122-124). The first evidence for a role of HSPs as antigens in inflammatory responses was obtained in 1988 in the rat model of heat-killed-mycobacteria-induced adjuvant arthritis (125). From then on many studies have shown the potential role for immune responses to HSP in the immune regulation of arthritis, in experimental models with HSP60 (121,126-128) and first clinical trials in patients with HSP peptides (129,130). Less is known about HSP70 (antigenically unrelated to HSP60), which is one of the most conserved HSPs (118).

The HSP70 family is very large with most organisms having multiple members. Most eukaryotes have at least a dozen different HSP70 found in a variety of cellular compartments. Some of the better known mammalian members are the constitutive cytosolic member (HSC70 or HSP73), the stress-induced cytosolic form (HSP70 or HSP72), the ER form immunoglobulin binding protein (BiP or glucose-regulated protein (Grp) 78), and the mitochondrial form mHSP70 (or mito-HSP70, or Grp75) (115). Some of the cytosol-resident HSPs of the HSP70 family belong to the group of HSPs most highly induced by stress. HSP70 is highly overexpressed in the synovium of patients with arthritis (65,111,120). Interestingly, non-steroid anti-inflammatory drugs (NSAIDs) (116) and gold (131) can also induce the expression of HSP70 which might promote HSP-directed immunoregulation. Moreover, animal studies
showed that treatment with the anti-rheumatic drug OM-89, containing HSP60 and HSP70, leads to HSP60- and HSP70-specific immunity, indicating that this immunity might be crucial for its clinical efficacy (132,133). Kingston et al. demonstrated that HSP70 from Mycobacterium tuberculosis has modulatory effects on experimental rat arthritis (134). Later Tanaka et al. showed that activation of T cells recognizing an epitope of Mt HSP70 can protect against adjuvant arthritis (135) and Wendling et al. showed that a conserved Mt HSP70 sequence prevented adjuvant arthritis upon nasal administration and induced IL-10-producing T cells that cross-reacted with the mammalian self-HSP70 homologue (136). Prakken et al. demonstrated that the induction of IL-10 and inhibition of experimental arthritis are specific features of HSPs that are absent for other evolutionarily conserved immunodominant proteins (137). Recently, a mammalian HSP70 protein, BiP (Grp78), reduced the severity of adjuvant arthritis and disease suppression was found to be related to the induction of regulatory T cells cross-reactive with self-HSP70 that triggered the production of IL-10 (136,137).

Recent studies showed that HSPs are critical antigens in the immune regulation of certain chronic inflammatory diseases, and are important in protection from disease (61,108). As HSPs are present at the site of inflammation and have been described as relevant targets of T cell responses, they provide promising immunomodulatory candidates for site-specific intervention of arthritis. Initial clinical trials with a DnaJP1 peptide (E. coli HSP40) in RA (129,130) have indicated the potential of HSPs as a source of immunomodulatory peptides. For further development of HSPs for therapy, better understanding of the immunomodulation of chronic inflammatory arthritis and the immunomodulation mechanism of HSPs in this disease is essential.
8. Outline of the thesis

The aim of this thesis is to extend our knowledge about the pathogenesis and immunomodulation of arthritis. In chapter 2 the application of dimethyldioctadecylammonium bromide (DDA), a powerful adjuvant that does not have the side effects of the conventionally used Freund's adjuvants, in the PGIA model is described. A significantly reduced onset period and a more severe arthritis was achieved in BALB/c mice immunized with cartilage PG in DDA, which makes the PGIA model much more convenient and interesting for studying the immune pathogenesis and immunoregulation. In the third chapter the anti-inflammatory role of HSP70 is further analyzed in the PGIA model. HSP70 treatment could dramatically suppress the development of inflammation and subsequent tissue damage in PGIA. Moreover, we demonstrated that HSP70 preimmunization resulted in an altered immune response as shown by proliferation and a regulatory cytokine profile to both HSP70 and PG resulting in a regulatory response. Together these data underline the therapeutic potential of HSP70 in arthritis via the induction of IL-10. In chapter 4 the generation is described of a new Tg mouse which has a PG-specific TCR. This Tg mouse will allow us to study the role of antigen-specific T cells in the development of autoimmune arthritis. The immunological features of these TCR-Tg mice are further characterized and explored in chapter 5. A single PG injection could provoke a severe form of PGIA, and splenocytes from naïve TCR-5/4E8-Tg mice, after a mild in vivo activation, or cells from hPG-immunized arthritic TCR-5/4E8-Tg mice could adoptively transfer arthritis into syngeneic BALB/c.SCID recipient mice. Given the relative paucity of useful models of chronic arthritis, these TCR-5/4E8-Tg mice may constitute a valuable and novel tool for studying mechanisms of autoimmune regulation of arthritis, and for developing T cell-directed immune modulating strategies. In chapter 6 the immune response to PG epitopes in RA patients is further investigated. The results indicate that some PG-specific peptides, mostly those located in the G1 domain of human PG, can induce T cell proliferation and cytokine production in vitro. The findings described in this thesis and their possible contribution to the understanding of the pathogenesis of RA and their implications for the design of HSP-derived therapeutic interventions are discussed in chapter 7.
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