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T Cell Responsiveness to Heat Shock Proteins in Patients with Juvenile Chronic Arthritis as an Example of Immunomodulation *in Vivo*

Introduction

In applying basic immunological research to human diseases generally most interest is focussed on adult autoimmune diseases. Reasons for this are obvious, such as unfamiliarity with pediatric diseases, less patients, and shortage in funding. Besides this, research in pediatric patients is often hampered by legal and ethical restrictions. On the other side, using certain pediatric autoimmune diseases as a model in clinical immunology can have important advantages. Pediatric autoimmune diseases are well defined, and the course of the disease is in general less influenced by other factors such as long term immunosuppressive treatment. Besides, some of the pediatric diseases have features that are not known in adult autoimmune disease and that can be of special interest for clinical immunology. A good example is the pediatric variant of Rheumatoid Arthritis, Juvenile Chronic Arthritis.

Juvenile Chronic Arthritis (JCA) is one of the most common chronic diseases in childhood. It is characterised by the chronic inflammation of one or more joints (1) (2). Although JCA and adult Rheumatoid Arthritis (RA) share some histopathological features, especially the differences between the two forms of chronic arthritis are striking. First of all, while severe forms of RA are associated with the occurrence of certain HLA haplotypes, the association between JCA and genetic background is much less clear (3) (4). Second, the clinical course of JCA is in general more favourable than that of adult RA. In a majority of patients the course of JCA is self-limiting. Even after years of chronic inflammation, the disease can come to a complete remission. In particular this marked difference in clinical course has been the focus of interest of our research over the last five years.

Heat shock proteins

Heat shock proteins (hsp's) or stress proteins are highly conserved intracellular proteins that are present in cells of almost all living organisms and fulfil critical functions in the protein house-keeping machinery of cells (5). The production of hsp's increases when a cell is subjected to any form of stress (5,6).

Hsp's are highly conserved during evolution resulting in extensive amino acid sequence identities between mammalian and microbial hsp's (7). In spite of this homology microbial hsp's have been found to be strong immunogens (8). On the basis of these seemingly opposite qualities of hsp's the capacity of microbial hsp's to induce autoimmunity through antigenic mimicry has been suggested. In this view, exposition to a bacterial hsp antigen could lead to a break of immunological tolerance for self-hsp and thus induce autoimmune disease (9). However, most evidence-collected sofar does not support this idea. On the contrary, evidence has been collected from animal models that development of the immunity to hsp's by priming with bacterial hsp leads to a raised resistance against the induction of autoimmunity. Moreover, immunisation with mycobacterial hsp's has been shown to lead to protection in virtually all forms of experimental arthritis, such as adjuvant arthritis, avridine arthritis and streptococcal cell wall induces arthritis (10,11).

The regulatory role of heat shock proteins in adjuvant arthritis

Adjuvant Arthritis (AA) is an extensively studied form of experimental arthritis with a pathological resemblance of rheumatoid arthritis. It can be induced in susceptible (Lewis) rats by immunisation with mycobacterial antigens. T-cell responses to hsp's play an important role in both the induction of AA and the protection from AA (12). AA can be passively transferred from diseased rats to syngeneic disease-free animals by a T-cell clone, recognising the non-conserved 180-188 amino acid sequence in mycobacterial hsp60. (13). This T-cell clone also responded to cartilage proteoglycan but not to rat hsp60. It therefore showed that although self cross-reactive or 'mimicry' T-cells are able to induce overt autoimmune disease, this is not due to the conserved nature of hsp's. Preimmunisation with mycobacterial hsp60 did on the other hand protect against subsequent induction of AA. Even more, preimmunisation with mycobacterial hsp60 has also been found to protect in other experimental arthritis models as collagen type II induced arthritis, pristane arthritis and streptococcal cell wall induced arthritis (14), (11).

The epitopes of mycobacterial hsp60 recognised after induction of AA with heat-killed Mycobacterium tuberculosis (Mt) and after (protective) immunisation with mycobacterial hsp60 have been mapped (15). It was shown that, after protective immunisation responses to several epitopes on the mycobacterial hsp60 molecule. Various peptides that included these epitopes were tested for their ability to protect against arthritis. Only peptides that contained conserved epitopes with a high degree of homology with the homologous rat peptide and capable of inducing T-cell responses to 'self'-rat hsp60 were found to protect not only in AA but also in other experimental non-microbial induced arthritis models (16)}.

The protective effect in experimental arthritis is a unique feature of hsp's

The protective effect of hsp's is not confined to hsp60, but has also been shown for hsp10 and hsp70(17,18).

The question was raised whether this protective effect could be extended to other highly conserved microbial antigens. We therefore immunised Lewis rats with conserved microbial antigens (superoxide dismutase, aldolase, myosine, glyceraldehyde-3-phosphate-dehydrogenase) and with heat shock proteins such as hsp70 (DnaK) of *E. coli* and mycobacterial hsp70. Rat (self) glutathione S-transferase that has a conserved microbial homologue was used as negative control. We found a protective effect of mycobacterial and, to a lesser extend, *E. coli* hsp70 in AA and avridine arthritis, whereas other conserved microbial antigens, though immunogenic as measured in T cell proliferation assays and delayed type hypersensitivity reactions, did not influence the course of experimental arthritis. In addition we found evidence that the protective effect of hsp70 was T cell mediated, since we succeeded to passively transfer protection using an hsp70 specific T cell line. Hsp70 immunisation was found to induce a switch in the subclasses of hsp70 specific antibodies upon immunisation from preferentially IgG2b and IgG2a to IgG1, which suggests the induction of a Th2-like response (Prakken & van Eden, article in preparation). The protective effect of conserved microbial proteins in experimental arthritis is therefore a unique feature of hsp's. Findings in patients with JCA have stressed the important role of T cell responses to hsp's in chronic arthritis.

Juvenile Chronic Arthritis

Juvenile Chronic Arthritis (JCA) is one of the most frequent occurring inflammatory disease of childhood (1). JCA is characterised by chronic inflammation of one or more joints. Three distinct subtypes are distinguished on the base of clinical parameters at the onset of disease: oligoarticular, polyarticular and systemic JCA. The histopathological abnormalities found in these three subtypes are similar and resemble the abnormalities found in adult rheumatoid arthritis (1). However, there is a striking difference in the clinical course. Oligoarticular JCA has a relative benign clinical course, whereas polyarticular and systemic JCA usually are non-remitting and crippling diseases often requiring aggressive immunosuppressive treatment. It is still largely unknown which pathogenic factors determine the different outcome of the three subtypes of JCA. However, evidence is accumulating that T cell responses to hsp's play a crucial regulatory role in the course of JCA.

Heat shock proteins and Juvenile Chronic Arthritis

Several observations have suggested that heat shock proteins (hsp's) are key regulatory antigens in patients with JCA.

First, an increased expression of endogenously produced hsp60 was found in the membranes of synovial lining cells of patients with JCA (19). In addition, IgG antibodies to human hsp60 could be detected in both serum and synovial fluid from patients with JCA (20). Subsequently, T cell reactivity to mycobacterial and human hsp60 was documented in patients with JCA (21). Other groups also found responses to (different) hsp's in patients with JCA (22,23).

Further studies revealed that most T cell responses to human hsp60 were found in HLA B27 negative oligoarticular JCA, the subtype of JCA with the best prognosis (24). Altogether, the data obtained from these studies showed that in patients with JCA responses to self-hsp's can be found and that these responses particularly are seen in patients with a relatively mild form of arthritis, namely oligoarticular JCA.

Reactivity to human hsp60 correlates with disease remission in patients with oligo articular JCA

However these first cross-sectional studies did not allow us to draw any conclusions on the role of this reactivity in the course of oligoarticular JCA. On the basis of the recent findings in the model of Adjuvant Arthritis (AA) we hypothesised that T lymphocyte reactivity to endogenous human hsp60 may play a more protective role in the course of oligoarticular JCA. For this reason we started a prospective longitudinal study in patients with a newly diagnosed HLA B27 negative oligoarticular JCA (25). We were able to show in this study that early in the course of HLA B27 negative oligoarticular JCA significant responses to human hsp60 can be found and that these responses are highly specific for this type of JCA. These anti-human hsp60 responses were correlated with responses to mycobacterial hsp60 and hsp70, suggesting that these responses are directed too more conserved epitopes of the hsp60 molecule.

Interestingly, during remission of their disease the oligoarticular JCA patients in majority lost their previous positive responses to human hsp60. In vitro priming of non-responder cells during remission restored the responsiveness in oligoarticular JCA patients, but not in patients with polyarticular and systemic JCA. When patients with oligoarticular JCA were seen to exacerbate their disease again positive T-cell responses to human hsp60 were found. However, all patients with positive responsiveness to hsp60 in the onset of the disease, eventually reached a disease remission.

One cannot formally exclude the possibility that the T cell responses to self-hsp60 found in this study are related to disease activity. The absence of responses to self-hsp60 during remission might in that case be due to the natural remitting course of the disease. In this concept, self-hsp60 plays a central role as a disease inducing autoantigen in oligoarticular JCA. However, we believe that these results strongly suggest that this T-cell reactivity is associated with the development of remission in JCA for the following reasons. First, these responses seem to be absent during the initial development of arthritis, since we could not find these responses within 4 weeks after the onset of disease. Second, similar T-cell responses could not be found in patients with polyarticular and systemic JCA and all oligoarticular JCA patients with positive responses developed a disease remission. Third, the reappearance of hsp60 responsiveness during disease exacerbation was followed by again a phase of disease remission. Finally, these findings complement the above mentioned findings in experimental arthritis in rats.

Reactivity to human hsp60 in oligoarticular JCA and the generation of suppressive cytokines in the joint

In this study we now were able to further substantiate this and show indications of the generation of regulatory cytokines by T cells recognising self hsp60 in oligoarticular JCA.

Firstly, we found an increased expression of CD30 on PBL and SF-derived lymphocytes from patients with oligoarticular JCA after *in vitro* activation with hsp60. CD30 is a cytokine receptor belonging to the TNF receptor superfamily. It was originally described as a marker of Reed Sternberg cells in Hodgkin's disease. Normally, in PBL the number of CD30+ lymphocytes is extremely low to zero. Although CD30 expression can also be found in Th1 or Th0 clones, there is accumulating evidence for a role of CD30 in both function and development of Th2-like human CD4+ cells (26). Until now, no data were available on the presence of CD30+ T cells in PBL or SF mononuclear cells from patients with JCA.

In unstimulated PBL from patients with JCA we found virtually no expression of CD30. In addition, we did find expression of CD30, though low, on unstimulated SF-derived mononuclear cells of patients with oligoarticular JCA. Subsequently, we found that *in vitro* activation with hsp60 induced a high expression of CD30 on CD4 and CD8 positive cells of patients with oligoarticular JCA, both in SF and PBL. CD30 expression was found mainly on activated (HLA DR-positive) memory (CD45RO-positive) cells, which suggests that these cells actually play a role in the ongoing inflammation process. In contrast, *in vitro* activation with hsp60 failed to induce a similar increased expression of CD30 on PBL of patients with polyarticular JCA.

Secondly, we found that after *in vitro* activation with either hsp60 or ConA the activated T cells from patients with oligoarticular JCA were capable of producing regulatory cytokines, such as IL-4 and TGF-ß. In contrast the activated T cells from patients with polyarticular JCA did not show IL-4 and TGF-ß production, although the proliferative responses to ConA were similar in both patient groups. The production of IL-4 correlated with an increased frequency of CD30-positive, CD4-positive cells and therefore supported the putative role of CD30 positive cells in promoting Th2-like cytokine production.

Finally, we found raised levels of IL-4 mRNA in SF-derived mononuclear cells obtained from patients with oligoarticular JCA, whereas no IL-4 mRNA was found in SF mononuclear cells of non-oligoarticular JCA patients. Expression and production of cytokines such as IL-1, IL-2, IL-6, TNF-α, TNF-í and interferon-γ has been described in patients with JCA and early stage RA (27),(28),(29). IL-4 mRNA expression, however, has only been scarcely found, mainly in patients with reactive arthritis. Now, we have documented for the first time the presence of IL-4 mRNA in SF of patients with oligoarticular JCA. The expression of IL-4 mRNA in oligoarticular JCA patients and not in non-remitting forms, is well in line with the supposed T cell control leading to remission in oligoarticular JCA.

Conclusion: the concept of the role of hsp's in Juvenile Chronic Arthritis

Altogether, the data as collected from the present study are suggestive of the following mechanistic principle in oligoarticular JCA.

During periods of active arthritis, inflammation in the joint leads to local cellular stress and therefore the upregulation of self hsp's in the synovial tissue. T cells recognising these self-hsp's are activated in the inflamed joint and are triggered to produce regulatory cytokines, such as TGF-ß and IL-4. Phenotypically such T cells are characterised by expression of HLA-DR, CD45RO and CD30 and mRNA expression of IL-4. Apparently, patients with polyarticular JCA are lacking these regulatory cells and develop a chronic, destructive inflammation. It is possible that the supposed activities of regulatory T cells are part of a common feed-back strategy of the anti-inflammatory immune response. The available T cell repertoire was initially positively selected on basis of low affinity cognate interactions with self-antigens in the thymus. Given the overrepresentation of self hsp's in the selecting thymus, the repertoire will include a high proportion of self hsp reactive T cells. On the re-encounter with self-hsp, as these proteins become upregulated at the site of inflammation, it is probable that the responding T cells, engaged in such low affinity interactions, will be driven in the direction of active tolerance. In this view, the Th2 phenotype of the responding cells of the oligoarticular JCA patients may well be the reflection of a common inflammation suppressive mechanism. If so, the present observations seem to in concordance with the known arthritis protective effects of hsp immunisations in experimental arthritis studies. Moreover, from this point of view one can even regard the non-remitting forms of JCA, especial the polyarticular and systemic subtypes, as diseases originating from a lack of regulatory feedback mechanisms and not so much as an autoimmune disease. As a possible consequence of the present findings, new ways for immunotherapies in chronic arthritis may be found in attempting to restore the natural regulatory responses in patients with polyarticular JCA, and possibly also in RA, e.g. through mucosal immunisation with hsp's.

In general clinical research focuses more on disease then on the recovery of a disease. With this approach useful information may be missed. As we have shown, it is especially the recovery of arthritis in JCA patients with a favourable course of disease that provides us with very valuable information and may help us to unravel the pathogenesis of the disease.

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