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Immunity to Heat Shock Proteins and Arthritic Disorders

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ABSTRACT

Adjuvant arthritis (AA) is a frequently used model of experimental arthritis. Because of its histopathology, which is reminiscent of rheumatoid arthritis in humans, AA is used as a model for the development of novel anti-inflammatory drugs. Recently, it has become evident that AA is a typical T-cell-mediated autoimmune condition. Therefore, novel immunotherapies targeted to T cells can be developed in this model. Analysis of responding T cells in AA have now led to the definition of various antigens with potential relevance to arthritis, including human arthritic conditions. One such antigen defined in AA is the 60kD heat shock protein. Both T-cell vaccination approaches and active antigen immunizations and antigen toleration approaches have turned out to be effective in suppressing AA. Infect. Dis. Obstet. Gynecol. 7:49–54, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS
heat shock proteins; arthritis

In adjuvant arthritis (AA), disease is induced by substances that have no obvious antigenic relationships with joint tissues. Originally this experimental model was discovered by Pearson when he observed the development of arthritis in rats after experimental immunizations using complete Freund's adjuvant containing heat-killed mycobacteria. Proof of the role of T cells was obtained by Holoshitz et al.[1] when they were able to select an arthritogenic T-cell line from cells obtained from mycobacteria-immunized Lewis rats.

Upon cellular cloning, several CD4+ T-helper subclones were obtained. One of these subclones was found to be virulently arthritogenic. This particular subclone was found to proliferate not only in the presence of mycobacterial antigens, but also in the presence of semi-purified preparations of cartilage proteoglycans.[2] These findings led to the concept of molecular mimicry between a mycobacterial antigen and a cartilage-associated self-antigen as the critical pathogenic mechanism explaining mycobacteria-induced arthritis.

Later the mycobacterial antigen was defined as the mycobacterial heat shock protein (hsp) 60, and the epitope of the arthritogenic T cell turned out to be nonconserved with a limited sequence homology with a rat proteoglycan link-protein sequence. Although supportive evidence for the relevance of this homology was obtained, for instance in human T-cell responses, it is uncertain whether this link-protein sequence is the target structure in AA.

Recently, the unique relationship of the 180–188 sequence with the arthritic process was further substantiated by tolerating rats for this particular sequence by administering this peptide in the nose (nasal tolerance) or by giving it subcutaneously in phosphate-buffered saline at high dosages (high dose tolerance). This procedure was seen to protect the animals from the subsequent induction of arthritis by either mycobacteria in oil or by

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the nonantigenic synthetic adjuvant avridine (CP20961).[3] Apparently, this single bacterial epitope, which resembles a (so far not identified) self epitope at the site of inflammation, is capable of inducing regulatory mechanisms of peripheral tolerance. The success of this regimen in suppressing disease, and especially in the case of nonmicrobially induced disease, seems to support the possibility that this microbial epitope has a unique relationship with a disease critical self-antigen in the joint. Apparently, the exposition of such antigen at the mucosal surface of the nose is already sufficient for setting reactive T cells in a regulatory mode with the capacity to enforce peripheral tolerance, leading to disease resistance.

CONSERVED HEAT SHOCK PROTEIN 60 EPITOPES INDUCE ARTHRITIS SUPPRESSIVE T CELLS

The identification of mycobacterial hsp60 as a critical antigen in arthritis has led to many studies on the potential of hsp60 in modifying arthritis development. Immunization experiments in mice and rats using hsp60 proteins or their derivative peptides have never led to induction of arthritis. On the contrary, resistance to arthritis was seen to develop. Prior immunization of experimental animals with mycobacterial hsp60 has been found to protect against subsequent induction of AA,[4] streptococcal cell wall arthritis,[5] avridine (a non-antigenic lipoidal amine) arthritis in rats,[6] and also pristane[7] and collagen arthritis[8] in mice. Careful analyses of differential T-cell responsiveness of whole mycobacteria- (AA induction protocol) versus mycobacterial hsp60- (protection protocol) immunized rats have now pointed out that the arthritis inductive capacity of whole mycobacteria does coincide with dominant responses directed to the mimicry epitope 180–188.[9] This is not the case when the hsp60 protein is used for immunization. Cellular cloning of responsive T cells in the latter protective protocol has revealed clones that recognize sequences conserved between mycobacterial and mammalian hsp60. Adoptive transfer experiments have shown that such T cells, recognizing conserved sequences and in particular the mycobacterial 256–265 sequence, were capable of transferring protection against the disease.[10] Furthermore, it was demonstrated that such cells had the capacity of responding to heat-shocked spleen cells. Immunizations with conserved peptide 256–265 were also inducing arthritis protection, and none of several other peptides containing nonconserved dominant T-cell epitopes tested was capable of inducing any protection. Testing of the same 256–265 peptide in a very different arthritis model in Lewis rats, in this case avridine arthritis, revealed that in a nonbacterially induced model the protective potential of the peptide was also present. Protection was also found using the homologous rat (self) peptide. Recent experiments have shown similar protective effects to result from immunization with mycobacterial hsp70 both in AA and avridine arthritis. Also, for the smaller subunit of the hsp60 complex (GroES of *Escherichia coli* of 10kD molecular weight), arthritis protective potential has been documented.

SUPPRESSION IN ARTHRITIS MODELS IS SPECIFIC FOR HEAT SHOCK PROTEINS

Given the observation that conserved epitopes of hsps induced resistance to arthritis by setting a focus of T-cell reactivity directed to recognition of self homolog (human) hsp, one could easily ask whether other conserved bacterial proteins would have similar arthritis protective capacities. However, in striking contrast with the protection in autoimmune disease models obtained with hsps, such as hsp60, hsp70, and hsp10, we have seen recently that a set of other conserved, but not stress inducible, bacterial proteins, such as superoxide dismutase, glyceraldehyde-3-phosphate-dehydrogenase, and aldolase, did not protect in experimental arthritis, despite their good immunogenicity and sequence homologies with their mammalian homologs (Prakken et al., submitted). Therefore, it seems that the protective quality of hsps is a unique aspect of hsps, possibly caused by their exquisite behavior of upregulating local expression under conditions of stress such as that existing at sites of inflammation.

HEAT SHOCK PROTEINS IN AUTOIMMUNE AND OTHER INFLAMMATORY DISEASES

Despite the frequent occurrence of hsp antibodies in normal individuals, it seems that in the majority of inflammatory diseases raised levels of hsp antibodies can be found.[11] This has been reported for rheumatoid arthritis (RA), juvenile RA (JRA), reactive arthritis, Behcet disease, systemic lupus erythematosus (SLE), Crohn Disease, insulin-

dependent diabetes mellitus (IDDM), and multiple sclerosis (MS). Significant hsp-specific T-cell responses have been observed in RA, JRA (see below), Behcet disease, MS, and in graft infiltrating lymphocytes during transplant rejection episodes. Raised expression of hsps in diseased tissues has been documented in sarcoidosis, SLE, inflammatory liver diseases, chronic gastritis (gastric ulcer), celiac disease, MS, IDDM, and atherosclerosis. Despite the general perception that hsps seem to play a role in different autoimmune diseases, there is no consensus on cause-and-effect relationships. Evidence in favor of hsps being a trigger leading to autoimmunity, because of their conserved nature, is hardly available. A more plausible possibility is that inflammation in general causes raised tissue expression, leading to the generation of hsp-specific T- and B-cell responses.

Similar to what has been documented in many other autoimmune conditions, rheumatic diseases have also been seen to feature the sequelae of immune responses to hsps. This is most evident from serology studies that have shown the presence of raised levels of hsp60-specific antibodies in patients. Raised expression of hsp in inflamed tissues has been documented for arthritic synovium in both RA and JRA.[12] In children, this raised expression has been seen to be an event that occurred early in the development of disease. Despite earlier reports also claiming prominent T-cell responses to mycobacterial hsp60 in advanced RA, more recent studies have suggested that proliferative T-cell responses are, however, more confined to early RA and that in advanced RA, such responses are less prominent. Alternatively, responses have been detected in functional assays sensitive for cytokine production, such as in assays measuring the effect of mononuclear cells on cartilage proteoglycan turnover in vitro.[13] In reactive arthritis and in oligoarticular juvenile rheumatoid arthritis (OA-JRA), T-cell responses to hsp60 were again more prominent. In contrast to adult RA, these conditions are characterized by a remitting course of disease development.[14,15]

In adult RA patients, T-cell responses to human hsp60 were detectable by culturing the cells in the presence of added interleukin (IL) 4. Bacterial hsp60 was found to stimulate cells without added IL4. This suggests a Th2 nature of the T cells responding to the human molecule in particular.[16]

In children with OA-JRA, responses to human hsp60 coincided with raised expression of IL4 in the synovial cells (as determined by reverse transcription-polymerase chain reaction). Furthermore, stimulation of both synovial and peripheral blood T cells resulted in a raised CD30 expression (a possible marker for Th2 cells) in activated (CD45RO+) CD4+ and CD8+ T cells. In addition OA-JRA patient-derived hsp60-specific T-cell lines were shown to produce IL4 and transforming growth factor-β (TGF-β).[15]

Altogether the data obtained from human arthritis patients have shown the potential of hsps to trigger the release of Th2-associated and suppressive cytokines.

HEAT SHOCK PROTEINS ARE AN INTRINSIC COMPONENT OF THE SLOW-ACTING ANTIRHEUMATIC DRUG OM89

In various models of experimental autoimmunity, it is known that exposing animals to exogenous bacterial flora contributes to resistance against disease induction. In AA also, germ-free animals were more susceptible than their conventionally reared counterparts.[17] Furthermore, in Fisher rats, it was shown that the susceptible germ-free animals became resistant upon gut recolonization with *E. coli* bacteria.

In other words, exposure of the immune system to cross-reactive bacterial antigens, such as hsps, might well stimulate the immune system to resume control over unwanted self-reactive clones. In line with the known contribution of bacterial gut flora to tolerance, it seems best to effectuate such exposure through oral (or nasal) administration of bacterial antigens. Laboratoires-OM (Geneva, Switzerland) is producing a glycoprotein rich extract of *E. coli* (OM-89), which is marketed and used as a slow-acting drug for the treatment of RA. It is administered orally and has shown a therapeutic efficacy comparable with that of gold in trials in RA patients. Recent analyses have revealed that *E. coli* hsp60 (GroEL) and hsp70 (DnaK) are the dominant immunogens present in this material.[18,19] Furthermore, both in AA and in avridine arthritis in Lewis rats, OM-89 was found to protect against arthritis. Therefore, *E. coli* hsps, when administered orally, may trigger a T-cell regulatory event that contributes to the control of RA in a way simi-

lar to the effect of mycobacterial hsp60 in models of arthritis. It is therefore possible that the therapeutic potential of hsps in RA is already exemplified to some extent by the mode of action of OM-89.

MECHANISMS BY WHICH HEAT SHOCK PROTEINS PRODUCE PROTECTION IN AUTOIMMUNE ARTHRITIS

The early expression of hsp60 in inflamed synovial tissues (stress-response) and the findings of immunological recognition of the protein both at the level of antibodies and T cells, especially in remitting arthritic diseases, seem to tie in very well with the data obtained in the experimental animal models. The animal experiments have shown that, irrespective of the trigger that led to synovial inflammation, prior stimulation of immunity to self-hsp60 using bacterial hsp60 or its conserved peptide raised resistance to subsequent disease induction. Taken together, these findings have indicated that T-cell recognition of hsp60 (and also hsp10 and hsp70) at the site of inflammation does contribute to the control of the ongoing inflammatory response. The stimulation of such responses by prior immunization using bacterial hsp or its relevant peptides is therefore expected to facilitate such T-cell-mediated regulatory control.

Heat-shock proteins are, despite their conserved nature and therefore antigenic relationship with "self," immunogenic to an exceptionally high degree. Although the reasons for this are as yet unclear, this phenomenon can be understood in terms of repertoire selection.

Heat shock proteins are well expressed in the thymic medulla, at the sites where positive thymic selection occurs. This will lead to selection of a repertoire of T cells that have a receptor that allows low-affinity (antagonistic) interactions with self-hsp when it becomes overexpressed at sites of inflammation. High-affinity receptors were negatively selected and therefore deleted. During infection with an hsp expressing microorganism (any bacterium, parasite, etc.), the same repertoire will be expanded by recognition of the microbial hsp homolog. As the homolog will be antigenically close to the self-hsp, but not identical to it, this recognition will include high-affinity interactions. By their nature, these high-affinity interactions may lead to an aggressive (Th1) anti-infectious response. At the same time, this repertoire is numerically expanded. On re-encounter of these T cells with self-hsp as expressed in the inflammatory site, the interaction will again be of low affinity and lead to antagonistic T-cell regulation. In other words, exposure of the immune system to microbial hsps is expanding a repertoire of potentially anti-inflammatory T cells. The same repertoire expansion can be effectuated by directed stimulation the immune system with hsps or peptides thereof. Given the existing evidence that responses to hsps comply with peripheral tolerance mechanisms of immune deviation, it is likely that bystander suppressive mechanisms (secretion of IL4, TGF-β) are operational in hsp-induced anti-inflammatory mechanisms.

On the other hand, it may be that under normal conditions low-level expression of self-hsp epitopes by nonprofessional antigen presenting cells in the periphery or of conserved microbial hsp epitopes in the 'tolerating' gut environment is continuously noted by T cells. This recognition, however, in the absence of costimulation or in the otherwise tolerating mode, will drive such T cells into a regulatory phenotype or anergy. Upon subsequent involvement of such cells in autoimmune inflammation, they will exert regulatory activity, also when they recognize their overexpressed antigen presented by the professional antigen presenting cells. Recently, we demonstrated that anergic T cells suppressed, in coculture, proliferative responses of other T cells as soon as the antigen that was used to induce anergy was added to the coculture.[20] In the case of hsps, thus anergised cells could focus their regulatory activity to sites of inflammation where hsps become temporarily overexpressed (bystander suppression). In infection, the activity of such anergic regulators would be outweighed by a dominant frequency of T cells (vigorously) responding to nonconserved microbial hsp epitopes. Depending on the circumstances, the pro-inflammatory response will (temporarily) break peripheral tolerance. And indeed, upon immunization or infection, microbe-specific epitopes are more dominantly recognized. The hsp-induced protection against autoimmune inflammation is now explained by either the expansion of the self-hsp cross-reactive T cells that assume the anergic phenotype when encountering their antigen on nonprofessional antigen presenting cells, or by further

imprinting or spreading of the preexistent anergic state.

LESSONS FOR THE DEVELOPMENT OF SPECIFIC IMMUNOTHERAPY IN AUTOIMMUNITY

As argued above, immunity to bacterial antigens, such as hsps, may contribute to maintenance of self tolerance as a hedge against autoimmunity. To achieve a lasting restoration of such tolerance in the case of disease, it seems most adequate to target immunotherapy to the reinforcement of natural mechanisms that contribute to such maintenance of self-tolerance. In other words, exposure of the immune system to bacterial antigens, such as hsps, might well stimulate the immune to resume control over unwanted self-reactive clones. In line with the known contribution of bacterial gut flora to tolerance, it seems best to effectuate such exposure through oral administration of bacterial antigens. Although little support for the effectiveness of such an approach can be obtained from work in experimental disease models, so far from experience in human medicine such support can be obtained. As mentioned above, Laboratories-OM has been producing *E. coli* bacterial lysates containing bacterial hsps, which are used among others for the treatment of RA.[21] For obvious reasons, it would be of great interest to analyze such mechanisms at the level of T-cell responses in patients treated with this material.

Positive findings of such an analysis would then possibly lead to attempts to develop better defined pharmaceutical compounds, such as synthetic peptides. Such peptides could be composed of conserved sequences of bacterial hsps and be used to stimulate the frequency or activity of T cells with the potential to recognize self-hsp molecules, expressed at sites of inflammation.

On a limited scale, first experience with oral administration of a defined hsp peptide has been obtained by Albani.[22] Based on the sequence homology of an *E. coli* dnaJ sequence with the so-called "shared-epitope," a consensus sequence shared by RA-associated HLA-DR molecules, the peptide QKRAA was administered to RA patients in an open-label clinical trial. Upon analysis of the functional phenotype of responding T cells in these patients, it appears that the oral treatment did effectuate a relative shift from Th1 to Th2 in cells with specificity for the dnaJ peptide.

The manipulation of peripheral tolerance with hsp immunization may work through the reinforcing of natural protective mechanisms of T-cell regulatory events in inflammation. The approach is broad and can be useful for the treatment of inflammation as seen in various autoimmune diseases and other inflammatory diseases, such as allergy. Furthermore, spin-off treatments may be expected for infection (including vaccines) and cancer. The ubiquitous nature of hsp expression in diseased tissues may well ensure that antigenic spreading as a possible tolerance escape mechanism will not easily take place. This is in contrast to alternative, more conventional antigen-specific approaches.

Effective treatment leading to reduced hsp expression in the tissues will lead to a gradual loss of therapeutic impact as a useful built-in feedback mechanism, or, in other words no therapeutic overshoot.

In the exploration of mechanisms of peripheral tolerance, it has become evident that, besides control through specific elimination of cells, interactive regulatory effects of antigens do play a role. Work on antagonistic peptides, anergic T cells, and cells displaying a regulatory cytokine profile has provided an experimental basis for such effects. Heat shock proteins can be the example of how such interactive regulatory effects can be targeted to sites of cellular stress. Immunological recognition of hsps, as seen in association with virtually every inflammatory condition, including allograft rejection and autoimmune diseases, can be central to peripheral tolerance mechanisms meant to control inflammation beyond tissue-specific antigenic manipulation.[22]

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