REPORT

Balancing the immune system: Th1 and Th2

W van Eden, R van der Zee, P van Kooten, S E Berlo, P M Cobelens, A Kavelaars, C J Heijnen, B Prakken, S Roord, S Albani

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CD4+ T cells are subdivided into Th1 and Th2 cells. Their relative presence or activation is thought to have a regulatory effect on immune behaviour. Until recently, the relative suppression of Th1 cells by the relative increase of Th2 activities, was thought to be a main mechanism of keeping or restoring the balance in a diseased immune system. Nowadays, however, a specialised subset of regulatory T cells is held to be responsible for the main effects of securing a balanced immune system. It is possible that heat shock proteins (hsps) are relevant antigens driving such regulation.

Heat shock proteins are known to be immunodominant antigens of bacteria. They are evolutionarily strongly conserved proteins present in all eukaryotic and prokaryotic cellular organisms and are upregulated by several forms of stress. Despite (the paradigm of) self tolerance, hsp-epitopes homologous to endogenous host hsp sequences have been implicated as T cell epitopes to endow cross reactive, hsp specific T cells with the capacity to regulate inflammation, such as in experimentally induced autoimmune diseases. Such T cells were found to produce regulatory cytokines like IL10, in contrast with T cells induced with other conserved microbial proteins that are not upregulated by stress. Hsps have been implicated in immune regulation not only as upregulated targets of adaptive immunity during inflammatory stress, but recently also as triggering factors for innate immunity through activation via Toll-like receptors (TLRs).

ARTHRITIS: A PROBLEM OF FAILING REGULATION?

Th1 cells or proinflammatory T cells are known to produce cytokines with proinflammatory activities. Therefore they are supposed to be critically involved in inflammatory conditions such as autoimmune arthritis. Th2 or helper T cells are known to produce cytokines that help B cells to become activated and to switch their class of antibody. Some of the cytokines produced by Th2 cells (IL4, IL5, IL10, and IL13) also have immune regulatory qualities. This has led to a perception of the balance between Th1 and Th2 T cells as the prime denominator of tolerance and the break of tolerance leading to disease. Experimental evidence now has contradicted this. In some cases Th2 cells have been seen to aggravate disease, depending on the timing of their administration and Th1 cells have been observed to inhibit disease. Furthermore, knock out mice lacking INFγ or TNFα were seen to develop Th1 dependent autoimmune conditions such as EAE and diabetes.

We (and others) have formulated several hypotheses on the relevance of immunological reactivity to hsps, since our initial observations on T cell reactivity to mycobacterial hsp60 in a rat arthritis model.[1 2] In some instances, such reactivity is seen as an example of "mimicry" in which an initial response against a pathogen component cross reacts with a self protein, triggering autoimmune pathology. Alternatively, the detection of autoreactive immune responses in people without disease suggests that they may be integral to normal immune function—possibly with a role in "immune surveillance". Finally, immune responses to hsps can be viewed as a secondary event, reflecting tissue breakdown and release of intracellular proteins after any pathological disturbance (necrosis). Chronic autoimmune diseases are the result of both organ specific and organ non-specific inflammatory reactions. Such inflammatory reactions now have been documented to be powerful inducers of stress responses at the cellular level, as demonstrated by raised synthesis of hsps at sites of autoimmune inflammation. Various studies carried out in a variety of distinct autoimmune and autoimmune inflammation related diseases have shown that the occurrence of disease coincided with the generation of immunity to hsps.[3] Now it has appeared that such immunity can represent the (failing?) regulatory T cell responses during disease.

HSP INDUCE REGULATORY T CELLS THAT HAVE THE CAPACITY TO SUPPRESS AUTOIMMUNITY

Initial evidence for the induction of regulatory T cells by hsp was collected in the rat model of adjuvant arthritis. Having defined the mycobacterial hsp60 as a relevant antigen for T cells in adjuvant arthritis,[1] various laboratories started experiments to induce disease with this hsp60 antigen. However, no induction of disease was seen to occur. Instead, immunisation with hsp60 was found to lead to the induction of protection against subsequent induction of disease. The same was found for other hsps, such as hsp70 and hsp10. One peculiar aspect of hsp is their sequence conservation, leading to homologies between bacterial and mammalian members of the same hsp families. When carefully analysed it turned out that immunisation with bacterial hsp led, among others, to the induction of T cell responses that included T cells with specificity for not only the bacterial sequence but also the self (rat) hsp. In other words these T cells cross recognised self hsp. Transfer of such T cells conferred protection and immunisation with peptides encompassing such conserved epitopes led to induction of protection.[4 5] Various mechanisms, including the induction of tolerance for bacterial hsp in the gut associated lymphoid tissues, are possibly endowing hsp reactive T cells with a regulatory potential. As a possible reflection of such mechanisms, in a number of studies, self hsp cross reactive T cells have been observed to be skewed toward the production of IL10, which can be a mediator of the regulatory effects of such T cells. The selective upregulation of hsp at sites of inflammation, attibutable to cellular stress caused by the locally produced toxic proinflammatory mediators, is possibly essential for the function of host hsp to attract such regulatory T cells and to let them exert their regulation.

Moreover, in children with chronic arthritis (JIA) T cell responses to human hsp60 have been seen to coincide with disease remission.[6 7] These observations and others have now led to the initiation of clinical trials using selected hsp peptides as an immuno-intervention (therapeutic vaccine) in diabetes and rheumatoid arthritis.[8 9]

Abbreviations: hsp, heat shock protein; TLR, Toll-like receptor

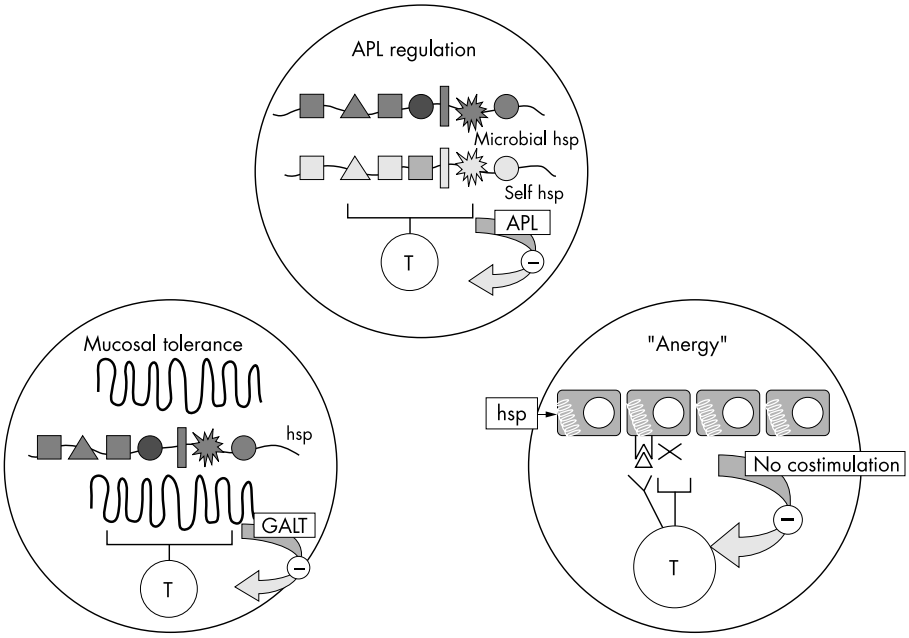

Figure 1 Three mechanisms of anti-inflammatory T cell induction by hsp. APL regulation: microbial hsp reactive T cells perceive self hsp homologues as partial agonists or APLs and develop a regulatory phenotype. Mucosal tolerance: hsp reactive T cells recognise microbial hsp in the tolerising GALT and display a tolerising activity when confronted with self hsp expressed elsewhere in the body. Anergy: non-professional or non-activated antigen presenting cells present constitutively self hsp in the absence of costimulation. The resulting self hsp specific "anergic" T cell can exert regulatory activity after the encounter with professional or activated APC presenting upregulated self hsp. Self hsp in lighter grey tone. Microbial hsp in darker grey tone.

In rat adjuvant arthritis and avridine induced arthritis, we have recently identified the induction of IL10 producing T cells upon hsp immunisation as one of the inflammation suppressive mechanisms.[5] Also in non-obese diabetes we have obtained evidence for IL10 production in hsp60 reactive T cells as one of the mechanisms contributing to inhibition of progression from insulitis to overt diabetes.[10]

HSP DO INDUCE REGULATION BY SEVERAL MECHANISMS

The arthritis protective regulatory nature of a self reactive T cell repertoire seems at odds with the perception that self reactivity will lead to the adverse effects of autoimmunity. None the less, there are explanations that may help to understand that in the case of hsps there is an exceptional situation and that indeed the self reactivity directed against self hsp controls inflammatory responses (see fig 1).

In the first place, the prominent presence of bacterial hsp in the close vicinity of the tolerising gut mucosa—the site known to induce so called "oral tolerance"—may play a part. T cells with specificity for conserved bacterial hsp may continuously perceive the presence of their epitopes in or close to the gut associated lymphoid tissue (GALT). From here they may well develop their regulatory phenotype. At a later stage, whenever confronted with the homologues present in mammalian hsp overexpressed in the inflamed joint (or other organ), they will express their adopted regulatory phenotype and control inflammation through bystander suppressive effects, such as through the production of IL10 or other suppressive cytokines.

Secondly, at the molecular level the amino acid differences between microbial and self hsp could be a critical factor. According to the current understanding of thymic selection, positive thymic selection (the thymic areas involved in positive selection do express hsp60) leads to a repertoire of T cells with receptors that have a low affinity for self, which is self hsp60 in our case. Those T cells that see conserved epitopes, will expand under the influence of selective pressure exerted by the microbial homologues in the (gut) environment. Naturally, such selective pressure will favour T cells with a relatively high affinity for the microbial homologues. And thus, the same resultant repertoire can see the microbial epitopes with good affinity, whereas the self hsp epitopes will be seen with relatively low affinity. From studies using so called altered peptide ligands (APL) it became apparent that peptides that trigger T cells with high affinity promote a more vigorous proinflammatory (Th1) T cell response—which is profitable in the case of infection—whereas modified (altered = subtle amino acid modifications) peptides with changes in the T cell receptor contacting positions leading to a lowered affinity, are able to promote a skewing of the resulting T cell response in the direction of a regulatory (Th2) or inhibited response. In this manner, the low affinity self hsp epitope would skew the responding T cell towards this regulatory and thus anti-inflammatory mode. For one of the arthritis suppressive T cells we showed that the self (stress induced) rat hsp60 molecule was perceived as an APL, which did not induce proliferative responses in the T cell, but which induced the selective upregulation of B7.2. The preferential interaction of this B7.2 with CTLA-4 on proinflammatory T cells we have interpreted as an additional T cell suppressive mechanism.[11]

Thirdly, the ubiquitous low level of constitutive hsp expression in every cell of the body will guarantee that T cells will notice the presence of self hsp epitopes also on non-professional antigen presenting cells that lack the costimulatory molecules needed to induce a T cell response. Recognition in the absence of proper costimulation is known to drive T cells into a state of anergy. Recent observations by others and us, have indicated that such "anergic" T cells are in fact regulators that inhibit proliferation of other T cells in coculture when confronted with their antigen on professional antigen presenting cells. Therefore, in the case of "anergic" self hsp reactive T cells, they may well exert a local suppressive effect when hsp60 is overexpressed by antigen presenting cells in the inflamed area.

Similarly, these mechanisms may work for other hsps.

HSP AND THE HYGIENE HYPOTHESIS

The hygiene hypothesis argues that in the modern western world we are relatively deprived of infection or otherwise

relevant exposure to microbial antigens. This may lead to an immune dysbalance, creating fertile grounds for chronic immune deviations such as allergies/asthma and certain autoimmune conditions such as type I diabetes. Heat shock proteins could well be part of the problem. Given the fact that hsp60 is known to be one of the most immunogenic bacterial antigens and hsp70 is an even more abundant protein present in bacteria, infections are likely to drive vigorous immunity for these proteins. And especially repetitive exposure to distinct bacterial organisms supposedly will lead to boosting of especially immune responses directed to conserved sequences of hsp. As argued above, especially responses to conserved sites of these molecules drive production of regulatory T cell responses. Therefore a relative lack of boosting such reactivities may contribute to the supposed disbalance leading to chronic disease conditions. New vaccines are again relatively deprived of these possibly useful proteins. While the older cellular pertussis vaccine still contained hsp and after vaccination antibodies to hsp were detected, the newer acellular pertussis vaccines do not contain hsp. Knowing the immune regulation inducing qualities of hsp one might well wish hsp to be a component of future vaccines.

HSP DO TRIGGER MECHANISMS OF INNATE IMMUNITY

Hsp of both bacterial and mammalian origin have immunomodulatory qualities, both at the level of innate defence mechanisms and at the level of antigen specific adaptive immunity. At the level of innate defence mechanisms hsp are now known to signal "danger" to antigen presenting cells (APC) such as macrophages and dendritic cells. This has been shown both for bacterial hsp60 and hsp70 and for, mainly, necrotic tumour cell derived mammalian gp96 and hsp70.[12–14] Recently, the first hsp receptors on APC, such as CD14, TLRs and CD91, were identified. CD14 has been identified as part of a receptor complex for mammalian hsp60 and hsp70. As a GPI linked protein without cytosolic tail, CD14 is incapable of signalling and must work in conjunction with signalling molecules.[15 16] Homologues of the IL1 receptor, the Toll receptors of Drosophila, have been proposed as possibilities. Previously TLRs were proposed to be associated with innate responses to bacterial infection and therefore with induction of proinflammatory responses. These receptors now appear to have multiple ligands, among which are members of the hsp families. TLR2 and 4 are primarily described as receptors for Gram positive bacterial products or LPS, respectively. TLR4 and TLR2 appear to be now also receptors for hsp60 and hsp70.[17–19] CD91 has now found to be a receptor for α_2-macroglobulin, but also the human hsp90 member, glucose regulated protein gp96.[20 21] TLR9 is now known to be a receptor for bacterial DNA CpG sequences.

HSP MAY BE EFFECTIVE WHEN ADMINISTERED AT THE GUT MUCOSA

The gut mucosa is the major contact area, over 400 squared metres, between the host and the external world of microflora. The intestinal wall is densely populated with cells of the lymphoid system and there are secondary lymphoid organs, Peyer's patches, specialised in monitoring the gut for the presence of threatening invaders. The gut mucosa is oriented towards the maintenance of tolerance,[22] which is reflected by the production of regulatory cytokines such as IL10 and TGFβ, not only by cells of the adaptive immune system, but also, such as in the case of IL10, by intestinal epithelial cells. For LPS a tolerance promoting activity has been demonstrated at the level of the gut. Antigen specific oral tolerance was shown to be more difficult to induce in germ free animals as compared with conventional animals. Altogether, it seems that both at the side of innate and at the side of adaptive immunity, contact with microbial antigens in the gut contributes to the development of a tolerant state towards the microflora.

Given the potential of heat shock proteins to interact with receptors of the innate immune system and the immunodominance of these proteins with respect to the adaptive immune system, the gut may well constitute the prime site of interaction between hsp and host tolerance promoting immune effector mechanisms.

The impact of gut microflora composition on the susceptibility to arthritis has been reported by Nieuwenhuis *et al*.[23] Reduction of Gram positive flora, leading to an expansion of Gram negatives such as *E coli*, by oral administration of Vancomycin, was found to impair the severity of induced adjuvant arthritis. This effect was neutralised when tobramycin/colistine, which reduced also Gram negative flora was added to the antibiotic regimen.

More direct evidence for a role of hsp at the level of the gut mucosa was obtained by Cobelens *et al*[24 25] when they showed a dramatic and instantaneous arthritis suppressive effect of intragastric hsp, in combination with a soybean trypsin inhibitor, when given at the start of disease development in adjuvant arthritis.

CYTOKINE TARGETED INTERVENTIONS CAN CONTRIBUTE TO MUCOSAL TOLERANCE

The experiments of Cobelens *et al* on intragastric hsp were performed with a low dose of 30 μg of hsp given at four alternating days. Most probably, the effects seen were similar to those seen in the low dose oral tolerance regimens leading to stimulation of IL10 and TGFβ producing T cells. Cobelens *et al* have shown more recently that β_2 agonists, such as Salbutamol, have the capacity to promote production of IL10 and TGFβ in intestinal cells. When given orally in combination with hsp60, a suppressive effect on development of adjuvant arthritis was noted. A separate series of experiments using a classic protocol of oral OVA induced tolerance in combination with Salbutamol showed a remarkably long lasting (several months) tolerance promoting effect of Salbutamol.

An alternative approach was taken by Roord *et al*. In their experiments they administered intranasally an hsp60 epitope (180–188), previously reported to be a target of arthritis producing T cells in adjuvant arthritis.[1] It turned out that the arthritis suppressive effect of this nasally administered peptide in adjuvant arthritis was dramatically increased by the simultaneous parenteral administration of TNFα inhibitor Enbrel. At the level of peptide specific T cells this combined intervention was demonstrated to lead to a shift towards the production of IL4 and IL10, thus indicating that cytokine targeted interventions may give direction to tolerance enhancing mechanisms.

In conclusion, it turns out the hsps can be targets for strategies of therapeutic immune interventions and that in combination with cytokine targeted immunointervention strategies the therapeutic potential of these proteins may be increased. In this way hsp and cytokine interventions in combination may offer therapeutic possibilities beyond the mere balancing of Th1/Th2 distributions.

Authors' affiliations
W van Eden, R van der Zee, P van Kooten, S E Berlo , Division of Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands
P M Cobelens, A Kavelaars C J Heijnen, B Prakken, University Medical Centre Utrecht, "Wilhelmina Children's Hospital", Department of Paediatric Immunology, Utrecht, Netherlands
S Roord, S Albani, Center for Pediatric Rheumatology, University of California San Diego, La Jolla, CA, USA

Correspondence to: Dr W van Eden, Division of Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584CL Utrecht, Netherlands; w.eden@vet.uu.nl

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