The humoral immune response to heat shock proteins

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Abstract. Humoral immune reactions to heat shock proteins (hsp) from microorganisms are one aspect of microbial infections in humans. The production of antibodies which are specific to epitopes present on procaryotic hsp leads also to the appearance of cross-reactive serum antibodies in the host organism that react with human hsp. This article discusses the consequences of such autoreactive antibodies for the host in context with the development of immune tolerance and autoimmune diseases, especially rheumatoid arthritis (RA), and in experimental animal models for arthritis such as adjuvant arthritis in rats. On the basis of epitope cross-reactivity between hsp and other host proteins, a hypothesis is presented for the development of autoimmune disease following the production of hsp-specific antibodies.

Key words. hsp65; hsp60; autoimmunity; rheumatoid arthritis; adjuvant arthritis; mycobacteria.

Introduction

The humoral reaction against heat shock proteins (hsp) can be viewed from two aspects: 1) an antibody response against a foreign protein, for example from a microorganism, and 2) an antibody response against epitopes crossreactive between bacterium and host. The latter can be interpreted as the autoimmune response. Those two responses contain both trivial aspects of mere epitope reactivity, driven by the classical pathways of immune evocation, and at the same time, aspects that touch regulatory events within the immunological network and may be independent from epitope recognition. These statements are supported by the results from the T cell data obtained in this context, as described in other contributions to this issue.

The aim of this contribution is to summarize humoral immune responses against hsp described so far. However, this task is hampered by the direction that research has made in this field. Most studies have focused on the analysis of the T cell reaction against hsp. It is widely accepted that T cells hold the key for the destruction of microorganisms that have entered the body space and overwhelmed the host's passive barriers (see also Cohen and Young[11] for review). Until recently, antibody responses to hsp were used as a marker for an eventually successful vaccination of the host only, rather than as a tool for the analysis of the quality of the host's humoral immune response to hsp[8, 10, 15, 21, 22, 26, 27, 37, 42, 44, 48, 51, 52].

The hsp60 family

To allow a more specific discussion, the focus of this article lies on the members of the hsp60 family. They are best investigated within the wide range of heat shock proteins and seem to play the dominant role in immunological processes. Their properties are described elsewhere in this review. Herein, the designation 'hsp60' is used despite some diversity in molecular size of hsp60 in different organisms.

Basically, the immunological response of a host to bacterial hsp60 is trivial. The protein is widely abundant and present in large amounts in cells under metabolic stress. Therefore, a high probability exists for the generation of antigenic epitopes from such a protein by host cells with antigen processing and presentation capacity, i.e. macrophages. Usually, antibody titers against bacterial hsp60 in the infected or vaccinated organisms are quite high[5, 6, 8, 22, 26, 27, 32, 37, 43, 52, 55]. Under experimental conditions, antibodies or sera with high[15, 23] as well as with low[24, 37] species specificity, that means cross-reactivity, can be generated.

Induction of antibody response and persistence of disease

It seems that mycobacteria are the kind of microorganisms with the highest capacity to induce antibody response to hsp60. The reason for this property of mycobacterial hsp60 is unclear. It may even be the case that this impression derives from the dominant research interests in microbial infections (table 1) rather than from biological reasons. However, there is an interesting link between this immunological finding and the pathogenesis of such diseases: these microbial infections are strongly persistent, long-lasting processes. Another example is viral diseases with chronic perpetuations such as herpes simplex virus (HSV)-derived inflammations. They are also accompanied by immune responses to hsp60-related proteins, in the case of HSV to viral proteins with sequence homologies to hsp60 (see also below). This appears paradoxical. Why should a dominant immune response lead to a persistent infection in a host?

Antibodies to hsp60 in rheumatic diseases

The expression 'rheumatic diseases and related diseases' in the meantime covers all kinds of connective tissue diseases, from osteoarthritis and rheumatic arthritis via ankylosing spondylitis and lupus erythematodes to Behcet's syndrome and Morbus Crohn. In most of these

Table 1. Disease-associated detection of anti-hsp antibodies

Type of disease (Diagnosis)	Specification of antibody reaction Antigen(s) for testing	Test system	Antibody reaction (spec., freq.)		Reference
Rheumatoid arthritis Tuberculosis Controls	rec hsp65 (*M. bovis* BCG) *M. tuberculosis* *M. avium, M. vaccae*	ELISA	RA: Tub.: Controls:	IgG, IgM IgG, IgM, IgA IgM	5
Rheumatoid arthritis Systemic lupus erythematodes Ankylosing spondylitis Crohn's disease	rec hsp65 (*M. bovis* BCG) *E. coli* hsp65 and hsp70 human hsp70 *M. tuberculosis* hsp70	ELISA	RA: SLE: IgA AS: CD: Cont.:	IgG, IgA IgA IgA (*E. coli*) occ. low titers?	31, 49, 50
Rheumatoid arthritis Ankylosing spondylitis Controls	Mycobact. rec. hsp65	ELISA	RA: AS: Cont.:	IgG, IgM, IgA (only sign.) all isotypes all isotypes	35
Crohn's Disease Controls	Mycobacteria Human cell line	Western blot	CD Cont.:	no titers found no titers found	33
Behcet's syndrome Controls	*S. sanguis*	Western blot	BS: Cont.:	IgG (70%), IgA (90%) IgG (70%), IgA (30%)	29
Systemic lupus erythematodes Disease controls	ubiquitin + peptides (bovine red blood cells)	ELISA, Western blot	SLE. Cont.:	general (80%) general (16%)	38
Acute rheumatic fever	*M. bovis*	ELISA	ARF	IgG	6
Candidiasis	*M. bovis* *C. albicans*	ELISA, Western blot	Cand.	not differentiated	23
Typhoid fever	Salmonellae	ELISA, Western blot	Typh.F.	not differentiated	8
Schistosomiasis	*S. mansoni* hsp70	ELISA		not differentiated	37
Lyme's disease	*M. tuberculosis*	Western blot	B.burg.	not differentiated	10
Leprosy	*M. leprae*	Western blot	M.lepr.	not differentiated	20
Bladder cancer	human BCG 65 kDa	ELISA	Bl.ca.	not differentiated	19

diseases a humoral reaction against heat shock proteins has been shown (see table 1). Some investigators have proved reactivity against microbial, others against human hsp, the latter including, by definition, an autoimmune reaction.

Fairly separated from these results are the observations in autoimmune diabetes (IDDM) research. These studies were all based on the examination of the antibody specificity found in autoimmune diabetes. Despite an initial idea of homology, most recent investigations have now demonstrated the immunological difference of the dominant diabetes autoantigen, a 60 kDa protein from islet cells from hsp60[2, 3, 45]. However, unless the 60 kDa protein has not been identified by alternative methods (protein chemical or molecular biological), it may well be that this protein is a member of the hsp family bearing some very individual immunogenic epitopes.

What are the target cells for such immunoglobulins? Despite the coexistence of IgG, IgM, and IgA subtypes (table 1), it is not clear whether or not hsp60 is exposed initially to such antibodies. The normal distribution of hsp60 is in intracellular compartments in the cytoplasm of the bacterial or host cells, and a possible cell surface exposure has not been proven finally. On the other hand, it is striking that just macrophages, and even more important, also chondrocytes in inflamed joints, are able to express quantities of hsp60 that are high enough to be detected in immunohistology[12, 24, 25]. As it could be shown in experimental arthritis in mice, antibodies to an epitope present in the cartilage may induce arthritis. For example, a passage of anti-collagen antibodies from a resistant mouse strain (SWR) to a susceptible strain (DBA/1) induced arthritis in the recipients[41]. It could well be that similar mechanisms work for hsp epitopes, considering hsp epitopes are present in inflamed cartilage[12–14, 24, 25].

Antibodies to hsp60 in rat adjuvant arthritis

Probably rat adjuvant arthritis is the most commonly used animal model for arthritic diseases. Injection of appropriate amounts of a suspension of inactivated mycobacteria (*M. bovis, M. butyricum, M. tuberculosis*) in mineral oil or paraffin oil at the basis of the tail or into the hind paw of a rat causes immediate severe inflammation followed by generalized arthritis within 3 to 6 weeks. Obviously all joints are involved, including other cartilage-bearing tissues like auricles. Ankylosing spondylitis and turbidity of the corneas are long-term consequences for the animals. A wide range of experiments has been reported within this model, including especially important studies with antirheumatic drugs.

Most recently, it has been reported that adjuvant arthritis can be modulated by immunization with mycobacterial hsp60 or peptide epitopes derived therefrom. The most recent data have been derived in context with the immune modulation of that disease via novel

drugs and, important for this contribution, via the involvement of intact (recombinant) hsp protein and partial sequences from mycobacterial hsp. The T-cell aspects are presented in other contributions to this multi-author review.

There are two possible interpretations of the antibody response to hsp60 in rats: 1) a direct B-cell response to the inoculation of the mycobacterial antigens, amongst them hsp60; and 2) a series of regulative events in the course of the disease in which the anti-hsp60 antibodies participate. An antibody response can be easily detected in rats treated with mycobacteria in oil or pristane [18, 47]. Nonetheless, only susceptible strains, such as Lewis or Wistar rats, develop arthritis, whereas BN rats are resistant. On the other hand, susceptible rats seem to carry natural antibodies against hsp60 without developing spontaneous arthritis [18]. Therefore, a circulating antibody is no immediate cause for the onset of the disease. On the other hand, treatment of adjuvant rats with immune modulatory, probably B cell suppressive, drugs like leflunomide reduce or even abolish arthritis in rats [40].

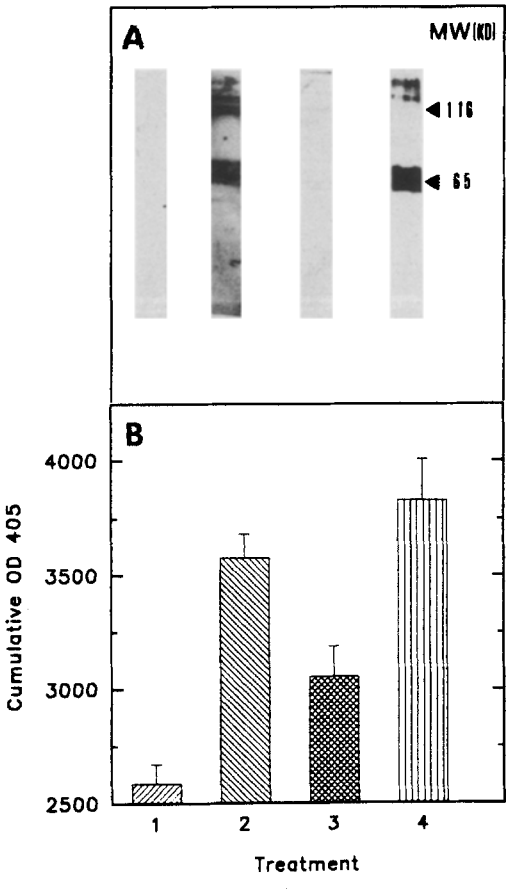

Figure 1. Antibody reactivities in *A*: Western blot and *B*: ELISA against chicken chondrocyte membrane proteins in sera from (1) normal rats, (2) rats with adjuvant arthritis, (3) rats after 3 i.p. treatments at day −35, −20, −5 with 0.1 mg peptide 180–188 emulsified in incomplete Freund's adjuvant, *without* induction of adjuvant arthritis, and (4) *with* induction of arthritis (for detailed methods, see refs 36, 53, 54). In *A*, each lane represents one typical rat serum; in *B*, the bars represent the mean titers plus standard error of 6 animals. Note the dominant reaction in *A*(2) at about 65 kDa, which is even enhanced in the peptide vaccinated rats with arthritis in *A*(4). Peptide 180–188 treatment alone leads to augmentation of the ELISA reaction against native antigens in *B*(3), but not to a signal with the denatured antigens in the Western blot *A*(3).

Vaccination by hsp60 protein and a nonapeptide sequence derived from hsp60 helps to protect the animals from arthritis [17, 53, 54]. We found that antibodies directed against cartilage components [36, 39] may even increase in titer in such vaccinated, and therefore, disease-resistant rats after treatment with complete adjuvant (fig. 1). Additionally, antibodies against mycobacterial hsp60 or synthetic nonapeptide could be detected by ELISA (fig. 2). That means hsp60 itself or antibodies against hsp60 epitopes are not a limiting parameter for the disease. But hsp60 seems to modulate the disease both up and down, according to the dynamic parameters of the experiments, like time of treatment and concentration of the applied antigens.

Whatever these findings mean for the role of hsp60 and antibodies to hsp in diseased individuals, they explain the present difficulty to find causative relationships between hsp60 induction on the one side, and T- or B-cell responses at various stages of the disease on the other.

Antigenic cross-reactivity amongst hsp60 from different species and other proteins: What are the consequences for the development of autoimmune diseases?

The difficulties in discriminating between various reported anti-60–70 kDa reactions found in human sera may rise from another interesting feature of the humoral immunity to mycobacterial hsp65: the antigenic cross-reactivity of an increasing number of proteins with hsp65 epitopes and eventually even similar molecular weight (table 2). Concerning the putative specificity resp. cross-reactivity of 'naturally' occurring antibodies against hsp60 in rats [18] or in healthy control persons (table 1) only occasional information is available. Very often, the 'cross-reactivity' of animal or human sera to different members of the hsp60 family is compared without taking into consideration that cross-reactivities observed might be due to antibodies coexisting in a serum; in other words, the cross-reactivities observed are not real cross-reactivities based on epitope-sharing of a defined B-cell clone. On the other hand, examples of table 2 include proteins with cross-reactive epitopes to hsp which have apparently nothing else in common, such as lactoferrin and collagen type II. Moreover, there may exist circulating antibodies with no defined specificity that can be activated under certain conditions and to react with given epitopes, so-called 'silent antibodies', as part of the immunological network [7].

At present, it is rather difficult to find a hypothesis to explain findings as described above. One explanation could come from the chaperonin-function of hsp65. If this protein is involved in general protein folding and

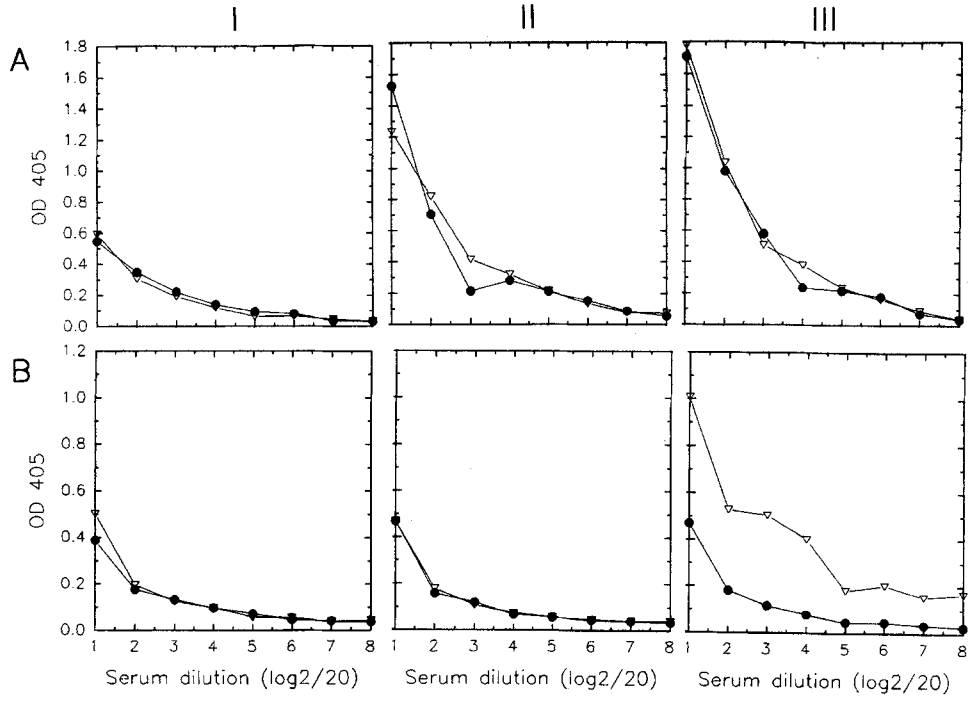

Figure 2. Inhibition – ELISA with peptide 180–188. ELISA plates were coated with chicken chondrocyte membranes (*A*) or peptide 180–188 (*B*) as test antigens. Sera were obtained from normal rats (I), or rats with adjuvant arthritis (II), or rats protected against adjuvant arthritis by i.p. pretreatment with peptide 180–188 emulsified in incomplete Freund's adjuvant (III) 35 days prior to adjuvant arthritis challenge with mycobacteria in oil (for detailed methods, see refs 36, 53, 54). Inhibition was performed in the presence of 0.01 mg peptide 180–188/ml to 1:20 prediluted sera.

Table 2. Antigenic cross-reactivities to mycobacterial hsp65

Protein	Source of antigen	Specification of cross-reactivity	Test system	Reference
Alpha-crystallin	Bovine lens	mab's to alpha-crystallin residue 98–108 react with *Drosophila* hsp	ELISA, immune affinity chr.	4
F protein	Measles virus	mab's to F protein recognize human host cell hsp70	Immunoprecipitation	44
Plasmodium chabaudi	*P. chabaudi*	IgG from infect. mice reactive to tubulin recognizes hsp70 & other cytoskeletal proteins	Immune affinity chr. & Western blot	46
Lactoferrin	Human	Anti-lactoferrin binds to mycobact. hsp65	Western blot, immunogold cytochemistry	16
Lactoferrin Transferrin HLA-DR beta subsets	Human	Antibodies to human proteins recognize mycobact. hsp65 ab's to hsp65 recognize lactoferrin	Western blot, immunogold AA sequence homology (tetrapeptide)	1
Collagen type II	Chicken	Murine autoreactive antibodies recognize CNBr fragment 11 with structural homology to hsp65, EBV protein BPLF-1, cytomegalovirus protein	Sequence comparison	9

mab's = monoclonal antibodies

transport processes (see also Burel et al., this issue), it may well be that an undefined number of proteins carry a domain responsible for the interaction with hsp65 during early steps of the protein synthesis, folding, and intracellular transport that remains stable in the further functional lifetime of the polypeptide[9, 28, 32]. This domain may include amino acid sequences or tertiary structures, as well, and should be highly conserved during evolution. A strong immune reaction against hsp65, provoked either by foreign antigens, or self antigens in the case of autoimmunity, would induce the production of anti-idiotypic network antibodies. Such antibodies may in part identify an epitope that is closely related to the domain structure of the patient's 'self' epitopes and therefore may be the source of the autoantibodies found in the serum of patients or animals with autoimmune diseases. If one assumes that such antibody networks exist, then an antibody reactive with 'self' could be a member in a network. An enhanced expression of such an antigen in a tissue could perpetuate and enhance the initial network reac-

tion to a pathophysiological level in immunologically 'labile' patients or animals. On the other hand, a suppression of such a network reaction in immunologically stabile patients may lead to a suppression of the initial reaction against hsp65 from infectious agents, thus allowing a persisting chronic bacterial disease, such as tuberculosis or leprosy. The immune system has to decide on the level needed for response, and therefore some microorganisms may provoke the failure of an appropriate reaction of the host's immune system as a survival strategy within the host.

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