The cellular immune response to heat shock proteins

S. H. E. Kaufmann

Department of Immunology, University of Ulm, Albert-Einstein-Allee 11, D-7900 Ulm (Germany)

Abstract. T lymphocytes, which are central to almost every immune response, frequently recognize microbial hsp60. Such cells could provide an early defense mechanism against pathogenic microbes. However, T cells also recognize epitopes of hsp60 shared by microbe and host. Not only conventional α/β T cells respond to hsp60; γ/δ T cells do so, as well. In fact, certain γ/δ T cells seem to have a particular preference for this molecule. Recognition of stressed host cells expressing hsp60 could facilitate the scavenger function of the T cell system. On the other hand, such recognition could be involved in autoimmune disease.
Key words. T lymphocyte; heat shock protein; mycobacteria; scavenger function; autoimmune disease.

Introduction

T lymphocytes and antibodies represent the specific arm of the immune system. T cells, the central elements of almost every immune response, fulfil two major roles: first, they are responsible for effector functions such as the killing of altered host cells; second, they perform regulatory functions such as helping in antibody production by B cells, the activation of macrophage effector capacities, or the suppression of an ongoing immune response. To account for this multiplicity of biological functions, segregation into cellular subsets seems appropriate. The majority of human and murine T cells recognize antigen via a T cell receptor composed of a disulfide-linked heterodimer of an α and a β chain[1]. Hence, they are termed α/β T cells. A minority of peripheral T cells expresses another receptor composed of a γ and a δ chain, and hence they are called γ/δ T cells[4].

The biological properties of α/β T cells are the best understood. They recognize antigenic peptides in the context of products of the major histocompatibility complex (MHC). Antigen recognition is facilitated by molecules on the T cell surface which are expressed in a mutually exclusive way: CD4 T cells interact with MHC class II molecules, whereas the CD8 T cells utilize MHC class I molecules on target cells. CD4 T cells preferentially act as helper T cells and CD8 T cells are preferentially cytolytic[25].

The biological features of γ/δ T cells are less well understood[4]. Most peripheral γ/δ T cells are double-negative, i.e., they lack the CD4 and the CD8 molecules. Although evidence for recognition of antigenic peptides in the context of MHC molecules (similar to α/β T cells) has been established, several exceptions seem to exist and unconventional antigen recognition is not unlikely.

It is generally accepted that the antigen recognition repertoire of T lymphocytes is redundant. Limiting dilution experiments performed with α/β T cells indicate that the frequency of specific T lymphocytes for a given protein antigen ranges from approximately 1/100 to 1/1000[7]. Although fewer data are available for γ/δ T cells, increasing evidence suggests that these T cells are even more conserved in their antigen specificity. Several types of γ/δ T cells are sessile, e.g., the intraepithelial γ/δ T cells of the gut or the γ/δ T cells in the dermis. In this situation, it may be essential for the cells to focus on a few dominant antigens abundantly expressed in the microenvironment[4]. In contrast, for α/β T cells floating freely through blood, lymph, and lymphoid organs, such focussing is not essential.

Heat shock proteins (hsp) are among the most abundant and most conserved polypeptides of the biosphere[19]. Their unique features seem to pose interesting problems to the immune system. First, the omnipresence of hsp in the microbial world means that the chance that the immune system will frequently come into contact with them is extraordinarily high, and it is questionable whether the immune system can afford to ignore such prominent exogenous proteins. Second, the existence of hsp in mammalian cells may require that immune tolerance be established to avoid self-attack. Third, the hsp are highly conserved, and the high degree of homology between microbial and mammalian hsp cognates requires the immune system to decide whether it prefers to accept hsp as dominant microbial antigens, or rather sees them as harmful self-antigens. It appears that the immune system tries to do justice to all three possibilities. Often, this is achieved surprisingly well; sometimes, however, failures seem to be unavoidable.

In this review, corollaries of the potent antigenicity of hsp for T lymphocytes will be discussed. Emphasis will be laid on the members of the hsp60 family. The hsp60 of the pathogenic mycobacterial species *Mycobacterium tuberculosis* and *M. bovis* (the etiologic agents of tuberculosis), and *M. leprae* (the etiologic agent of leprosy) were cloned in 1985[35,37,38]. Sequence analysis revealed full identity between the hsp60 of *M. tuberculosis* and that of *M. bovis*, and 95% homology with the *M. leprae* cognate. Cognates have now been identified in a variety of microbial pathogens which all share a high degree of similarity[21]. Subsequently, the human homolog was also cloned and sequenced, and was found to share 50–60% sequence identity with the mycobacterial cognates[15].

Hsp60 as dominant microbial antigen for T cells

T cells with specificity for mycobacterial hsp60 have been identified both in the human and in the murine system [21]. Emmrich et al. were the first to provide strong evidence that hsp60 is a dominant antigen, using T cells of a patient suffering from tuberculoid leprosy [10]. Kaufmann et al. showed that about 20% of mycobacteria-reactive T cells from mice immune to *M. tuberculosis* respond to hsp60 [23]. These findings strongly indicate the dominance of hsp60 in the cellular immune response to mycobacteria. However, not only in immune individuals, but also in healthy controls, T cells which recognize hsp60 are frequently demonstrable. Thus, in a study by Kabelitz et al. a high number of T cells among peripheral blood T lymphocytes from healthy individuals were specific for hsp60 [17].

At least two reasons can be brought forward to explain these observations. First, hsp are abundantly produced inside the host. Mycobacteria are intracellular pathogens which survive in host macrophages at least for a certain time period [20]. During this time macrophages undertake several attempts to eradicate their intracellular predators. Thus, intracellular mycobacteria are attacked by toxic molecules, including reactive oxygen metabolites and reactive nitrogen intermediates. In addition, other insults may occur such as starvation and anoxia. Increased hsp synthesis may facilitate microbial survival inside host cells [5]. At the same time, their abundance may designate them as preferred targets for processing and presentation to the T cell system. During everyday life the host will frequently encounter microbes, most of which are nonpathogenic, but which may survive for a limited time period. Although such microbes will be eliminated before they cause clinical disease, hsp synthesis may be induced. Therefore, the host's immune system may be frequently boosted by microbial hsp derived from various microorganisms. In this way, constant T cell stimulation may occur, and T cells specific for epitopes of hsp shared among various microbes could provide an early defence mechanism against pathogenic microbes [22].

Self-hsp60 as T cell antigen

Hsp60 comprises several regions, at least 10 amino acids in length, which are fully or partially shared by the mycobacterial and human cognates [21]. Using peptides representing such regions it was shown that T cells with specificity for cross-reactive self epitopes exist in the peripheral blood of healthy individuals [26, 28]. The responsible T cells are presumably of the CD4 phenotype. These data, however, leave the question open as to whether such shared epitopes are also presented by host cells in the context of MHC molecules. In the murine system, T cells have been activated which are specific for mycobacterial hsp60 and recognize stressed macrophages [24]. Stress could be imposed by Interferon-γ stimulation, virus infection, and other signals. The responsible T cells are of the CD8 phenotype, and recognize stressed macrophages in a class I restricted fashion. Since the relevant epitope has not yet been identified, its cellular source remains as yet unknown. However, it is tempting to speculate that the self epitope represents a shared region of the self hsp60. Hence, these findings suggest that hsp60 serves as a self antigen for T cells. However, since this self-reactivity is even demonstrable in healthy individuals, this finding does not necessarily imply a role in autoimmune disease, which will be discussed after the next paragraph.

The particular predilection of γ/δ T cells for hsp60

O'Brien et al. found that several γ/δ T cell hybridomas derived from thymic T cells of newborn mice respond to mycobacterial hsp60 [29]. These γ/δ T cells recognize an epitope in the amino acid sequence 188–191 which shows partial homology with the mammalian cognate [3]. Indeed, a peptide which corresponds to the appropriate region in the mammalian hsp60 is also stimulatory. Among peripheral blood cells of healthy individuals γ/δ T cells which respond to hsp60 can be identified [17]. A T cell clone has been isolated from the peripheral blood of a healthy individual which recognizes both the mycobacterial and the human hsp60, which indicates that it is specific for a shared epitope [12, 13]. Evidence that at least certain γ/δ T cells interact with hsp60 on target cells in an unusual way has been presented [11, 30, 33]. In these studies, target cell recognition by γ/δ T cells could be inhibited by addition of anti-hsp60 antibodies. Although this may be interpreted as unconventional antigen recognition, it is also possible that hsp60 acts as an auxiliary molecule which facilitates cell-cell interactions. Evidence consistent with this notion has been presented recently [32].

T cells with specificity for self-hsp60: beneficial or detrimental?

The recognition of self-hsp60 by T lymphocytes, be they of α/β or γ/δ type, as well as the finding that stressed macrophages are recognized by hsp60-specific T cells, can be interpreted in two ways which need not be mutually exclusive. Hsp60 recognition may be beneficial since it allows identification of stressed cells by T lymphocytes. Through such a scavenger mechanism T cells may be enabled to recognize cells suffering from a variety of insults such as inflammation, transformation, infection or trauma. In the case of γ/δ T cells which, at least in the mouse, represent a high percentage of the sessile intraepithelial lymphocytes, focus on a common stress indicator may be particularly valuable [21]. On the other hand, recognition of self-hsp60 may ultimately cause detrimental consequences. Because of the extremely high sequence homology between bacterial and host hsp60, such T cells could contribute to the pathogenesis of intracellular bacterial infections. The finding that stressed Schwann cells

are recognized by T cells reactive to hsp60 would underline such a possibility [34]. Moreover, striking evidence for an involvement of hsp60-reactive T cells in experimental autoimmune disease models has been presented.

The role of hsp60-specific T cells in experimental autoimmune disease will be dealt with by Yang and Feige and, therefore, need not be discussed in further detail here. Suffice it to say that T cells involved in adjuvant arthritis of rats recognize an epitope of mycobacterial hsp60 which is not shared with the mammalian counterpart, and that T cells involved in insulin-dependent diabetes mellitus (IDDM) of non-obese diabetic mice (NOD) are specific for an epitope shared by mycobacterial and mammalian hsp60 [8, 9, 36]. Clearly, these findings strongly imply hsp60 reactivity in experimental autoimmune diseases.

The situation in human autoimmune disease seems to be far more complicated. In human IDDM, an antigen with an apparent molecular weight of 60 kDa has been thought to be implicated for a long time. Although evidence for increased hsp60 expression in human IDDM has been presented, it appears more likely that the relevant auto-antigen is glutamic acid decarboxylase, which also has a molecular mass of 60 kDa [2, 16]. Arthritis particularly afflicts the joints. It appears in different clinical forms, and microbial etiology has been assumed for a long time. T cells with specificity for mycobacterial hsp60 have been isolated from the synovial fluid of patients with different forms of arthritis, and these findings have been taken as strong arguments for an involvement of hsp60 in arthritis [6, 14, 27, 31]. Reactive arthritis frequently develops subsequent to gastrointestinal infections, and is often self-healing. T lymphocytes specific for mycobacterial hsp60 have been isolated from rheumatoid arthritis patients. Several of these cells recognize a non-shared region. Recent studies showing that T cells from reactive arthritis patients respond to a variety of mycobacterial antigens other than hsp60 would argue against a unique role of hsp60 in this disease form [27]. Microbial deposition in reactive arthritis lesions is likely.

Taken together, these findings argue for the inititation of an inflammatory response by T cells with specificity for microbial antigens. More recently, however, a T cell clone has been isolated from the synovial fluid of a reactive arthritis patient which responds both to mycobacterial and human hsp60, raising the question of whether true autoimmunity participates as well [14]. Evidence for involvement of hsp60-specific T cells in chronic arthritis has been published [31]; however, the data did not demonstrate a link with autoimmune disease convincingly. Van Eden et al. have recently analyzed T cell responses from juvenile and adult arthritis patients [6]. They found that T cells from the synovial fluid of juvenile patients suffering from arthritis responded strongly to both mycobacterial and human hsp60. In contrast, such a response was not observed with T cells from adult patients [6]. Although the relevant epitope has not been identified as yet, this study provides strong evidence for the involvement of a true autoreactive T cell response against hsp60 in autoimmune disease. Even in this case, however, it remains unclear whether hsp60 is indeed the inducing autoantigen, or whether a primary tissue-specific antigen initiates autoimmune disease. The insults occurring at the site of the lesion could cause increased hsp60 expression and hsp60-specific T cells could then further contribute to disease progression. Although this has by no means been proved, such a facilitating role might also participate in other autoimmune diseases. Evidence for increased hsp60 expression in autoimmune lesions has been presented [16, 18, 30, 33]. Thus, heightened hsp60 levels have been found in the synovial lining and other cells present in rheumatoid arthritis lesions, oligodendrocytes of multiple sclerosis patients, B lymphocytes of patients with active lupus nephritis, and pancreatic cells of IDDM patients.

In summary, evidence for an involvement of hsp60 in autoimmune disease is still growing, although the initial enthusiasm about hsp60 as a universal link between infection and autoimmunity had to be cooled down on the basis of more in-depth studies.

Concluding remarks

This review has attempted to focus on the interesting consequences of the unique features of hsp60 for the immune system. The data available thus far show that the immune system does indeed attempt to produce a compromise in the face of the problem discussed at the beginning of the paper. That is, the immune system has decided to accept hsp60 as a dominant microbial antigen, and not to neglect T cells with specificity for shared epitopes. Rather, it seems to rely on regulatory mechanisms in the periphery, which can be more easily overcome in vitro than they can in vivo, where the danger of immunopathogenic corollaries of microbial infection, or even autoimmune disease, prevail.

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