Cell Stress and Chaperones (2008) 13:119–125
DOI 10.1007/s12192-008-0018-4

MEETING REVIEW

Heat-shock proteins: Inflammatory versus regulatory attributes

Verônica Coelho · Femke Broere · Robert J. Binder · Yehuda Shoenfeld · Kamal D. Moudgil

Received: 17 December 2007 / Revised: 9 January 2008 / Accepted: 11 January 2008 / Published online: 28 February 2008

The workshop on "Heat-shock proteins: inflammatory versus regulatory attributes" was held in August 2007 at the 13th International Congress of Immunology (ImmunoRio2007), Rio de Janeiro, Brazil. This report contains a summary of the presentations, the discussions during the workshop, and the ideas exchanged with experts as well as among presenters before the meeting.

Verônica Coelho and Kamal D. Moudgil contributed equally to this workshop and the preparation of the report.

V. Coelho
Heart Institute (InCor) University of São Paulo Medical School, and Institute for Investigation in Immunology-Millennium Institute, São Paulo, Brazil
e-mail: vecoelho@usp.br

F. Broere
Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

R. J. Binder
Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA

Y. Shoenfeld
Department of Medicine 'B' and Center for Autoimmune Diseases, Sheba Medical Center (affiliated to Tel-Aviv University), Israelheba Medical Center, Tel Hashomer, Israel

K. D. Moudgil (✉)
Department of Microbiology and Immunology, and Division of Rheumatology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA
e-mail: kmoud001@umaryland.edu

Organization and format of the workshop

The workshop was organized by Verônica Coelho (Brazil) and Kamal Moudgil (USA). The presenters included Yehuda Shoenfeld (Israel), Robert Binder (USA), and Femke Broere (The Netherlands), in addition to the two organizers. Coelho presented a brief introduction to the workshop, highlighting the inflammatory versus immunoregulatory properties of heat-shock proteins (Hsps) and emphasized the workshop as a discussion-oriented forum. To stimulate debate, she listed some unanswered hot questions on Hsps and the immune response that were gathered from different groups working in this field. The other presenters highlighted their experimental results obtained in different diseases or model systems that ranged from atherosclerosis (Shoenfeld), tumor immunology (Binder), arthritis and infectious diseases (Broere), and immune regulation (Moudgil). This first Hsp workshop held under the auspices of the triennial International Congress of Immunology (sponsored by the International Union of Immunological Societies; IUIS) took advantage of the considerable number of investigators working or interested in the field of Hsps. It was a successful initiative that promises to be continued in the following meetings. The novelty of this workshop was the effort to engage both the presenters and the audience participants in addressing open and controversial questions in the field of Hsps. This format created a very lively atmosphere for discussion and encouraged additional new questions. In addition, these interactions allowed one to see different points of view and to realize that there is no "one universal correct answer" to any particular question. The open questions debate revealed the need for further experimentation and also identified the areas of common interest among different research groups. Although the time was obviously too short to discuss all the

conflicting areas in Hsp research in inflammation and immunoregulation, the open debate gave a comprehensive view of the current perspectives on several critical issues pertaining to Hsps.

The scope of the workshop

The applications of immunology cover virtually all fields of medicine, but most notably the areas of autoimmune diseases, organ transplantation, tumors, and infectious diseases. Hsps are one of the common threads in these four areas of research. Immune responses to different members of the Hsp families have been the focus of intensive investigations in autoimmunity (van Eden et al. 1988; Durai et al. 2004a; Sherer and Shoenfeld 2006; Cohen 2007), transplantation (Pockley and Muthana 2005; Caldas et al. 2006), tumors (Srivastava 2002; Binder et al. 2004), and infection (Steinhoff et al. 1999; Prinz et al. 2002; Lo et al. 2004) over the last two decades. One of the perplexing aspects of Hsps that is posing a challenge to investigators is their dual role in different disease processes—an inflammatory (pathogenic) versus an anti-inflammatory (regulatory) activity. The objectives of this workshop were to discuss these contrasting roles of Hsps, to highlight the recent advances in the immunobiology of Hsps and the Hsp-based applications in medicine, and to raise and discuss open questions in the field.

Summary of the workshop proceedings

The workshop was very well attended, engaging at least 150 congress members. This workshop employed a format consisting of short talks by five investigators with expertise in the area of Hsps, followed by an open discussion forum where the attending members were encouraged to participate actively by asking questions, making a comment, or sharing experimental data. Furthermore, all five presenters were available together for the discussion session after the presentations. This discussion was reinforced by the questions that had been submitted earlier in the workshop by other experts in the area of Hsps who could not attend the Congress.

(A) Presentations

1. *The involvement of Hsps in the pathogenesis of atherosclerosis* (YS). Autoantibodies towards Hsp60/65 (hereafter referred to as Hsp65 for simplicity) are associated with atherosclerosis in human and animal studies. Ultrasonographic assessment of carotid atherosclerotic lesions showed that subjects with such lesions had significantly raised levels of anti-Hsp65 antibodies compared with controls (Xu et al. 1993). In animal models, rabbits that were immunized with material containing Hsp65, either in the form of mycobacteria or recombinant Hsp65 alone, developed enhanced atherosclerotic lesions (Xu et al. 1992). In another study, C57BL/6 mice were injected with either Hsp65, Hsp65-rich *Mycobacterium tuberculosis*, or PBS, and early atherosclerosis was found to be significantly enhanced in mice fed a high cholesterol diet that were immunized with the two former immunizing agents (George et al. 1999). Interleukin (IL)-4 has a crucial role in the progression of early atherosclerosis mediated by inflammation, as it has been shown that IL-4- knockout mice immunized with Hsp65 had significantly less fatty streak formation than wild-type C57BL/6 mice (George et al. 2000). Another study determined the role of cellular and humoral immune responses to Hsp65 in murine atherosclerosis. Adoptive transfer of Hsp65-reactive lymph node cells increased fatty streak formation in comparison with mice treated with bovine serum albumin-primed cells. Similarly, repeated intraperitoneal administration of IgG from the serum of Hsp65-immunized mice enhanced fatty streak formation in experimental mice in comparison with controls (George et al. 2001). These studies provide direct evidence for the pro-atherogenic properties of cellular and humoral immunity to Hsp65, and raise the possibility that both arms of the immune system have a synergistic pro-atherogenic effect. Last but not the least, Hsp65 can be employed for therapeutic purposes as we showed when Hsp65 was used to induce oral tolerance for atherosclerosis (Harats et al. 2002).
2. *Priming antitumor immunity and regulating autoimmunity—a dual role for Hsps* (RB). Mammalian Hsps were first demonstrated to elicit antitumor immunity when they were isolated as tumor-associated antigens (Srivastava et al. 1986). Since then, Hsps have been shown to engage surface receptors on antigen-presenting cells (APCs; Binder et al. 2004). This interaction leads to internalization of the Hsp and cross-presentation of the chaperoned peptides on major histocompatibility complex (MHC) I and MHC II molecules. At the same time, Hsps also activate the APCs causing them to mature and co-stimulate the T cells. The result is the priming of a T-cell response, which is specific for the peptides chaperoned by the Hsp. These responses have been tested in numerous tumor, viral, bacterial, and model antigen systems and are

currently being used in clinical trials of cancer (Srivastava 2002). A contrasting aspect of Hsp immunity is its regulatory attribute. High doses of Hsps (at ten times the immunizing dose) elicit regulatory responses (Srivastava et al. 1986; Chandawarkar et al. 1999; Chandawarkar et al. 2004). As tested in several murine autoimmune diseases, tumors and tissue grafts, high doses of Hsps tend to shut down ongoing T-cell responses, thus drastically reducing the severity of the autoimmune disease, increasing tumor growth or prolonging survival of MHC-disparate grafts. CD4 T cells have been implicated in this phenomenon (Chandawarkar et al. 1999; Chandawarkar et al. 2004). We currently do not know what triggers the switch from immunity to a regulatory immune response and at what cellular and molecular level this occurs. The APCs and cell surface receptors are the likely candidates, and these are being investigated in different laboratories.

3. *Hsps can induce protective immunity against arthritis as well as para-tuberculosis* (FB). Immunization studies with the recombinant Hsp65 protein or its defined epitopes in the adjuvant arthritis (AA) model showed that Hsp65− or Hsp65 peptide-immunized animals developed resistance against the induction of AA (van Eden et al. 1988; Anderton et al. 1995). Additional studies with Hsp70 also showed that only T cells recognizing highly conserved sequences had a regulatory phenotype and were cross-reactive with the mammalian homologous proteins (Wendling et al. 2000). These results demonstrate that immune exposure to microbial Hsp was capable of inducing a regulatory T-cell response and that such regulation depended on a T-cell response that included a repertoire of (endogenous) Hsp-specific T cells. In addition, Hsp-specific T cells responding to these self-specific Hsp peptides produce high amounts of IL-10, a known regulatory cytokine. This IL-10-inducing capacity is specific for Hsp, as other highly conserved proteins did not induce such an IL-10 response (van Eden et al. 2005). Hsp70 also can be used as a subunit vaccine in the chronic infection with *Mycobacterium avium paratuberculosis* (MAP; Koets et al. 2006). Clearing of the infection coincided with enhanced antibody titers against the MAP-Hsp70 and might therefore depend on B-cell-dependent mechanisms, whereas protection in autoimmune inflammation seems to depend more on T-cell regulation. Intriguingly, Hsp70 vaccination in arthritic mice also induced a strong Hsp70-specific antibody response, while a reduction in arthritis-associated antibodies was observed. Although both T cells and B cells apparently mediate the protective effect of Hsps, it is not clear what determines this specific capacity of Hsp-specific T cells in protection against autoimmunity and chronic infection.

4. *Self (rat) Hsp65 (Rhsp65) fails to induce arthritis, but affords protection against autoimmune arthritis* (KM). Self tolerance plays a critical role in preventing the induction of autoimmunity. Potentially self-reactive T cells are purged from the developing T-cell repertoire in the thymus via negative selection. However, subsets of autoreactive T cells escape this checkpoint for a variety of reasons, and make it to the periphery, where their activity is kept in control by CD4+ CD25+ T regulatory cells (Treg) and other mechanisms of peripheral tolerance (Bala and Moudgil 2006). In the AA model, the pathogenic T cells are directed against mycobacterial Hsp65. However, the identity of the precise self antigen targeted by these T cells is not fully defined. Rhsp65 is one of the putative target antigens implicated in the disease process (Moudgil 1998). In this context, we tested whether the activation of self Hsp65-reactive T cells in the Lewis rat might lead to arthritis induction. Interestingly, rats immunized with Rhsp65 with the objective of priming and expanding the self-reactive T cells did not develop arthritis. Instead, these rats were protected from subsequently induced AA (Durai et al. 2004a). The protective epitopes within Rhsp65 resided in the C-terminal region of the protein and were ‘dominant’ in terms of antigen processing and presentation. The pretreatment of rats with these C-terminal peptides of Rhsp65 also induced protective immunity against arthritis. Moreover, these epitopes were cross-reactive with the C-terminal counterparts in mycobacterial Hsp65, which otherwise are ‘cryptic’ but were subsequently revealed during the inflammatory milieu of AA (Durai et al. 2004b). Interestingly, both the self and the foreign C-terminal peptides were capable of downregulating the course of AA (Durai et al. 2004a, b). These observations on the C-terminal epitopes further validate and enlarge the scope of foreign-self cross-reactivity elaborated earlier by van Eden and colleagues with another epitope region of Hsp65, 256–270 (Anderton et al. 1995). In that epitope pair, the mycobacterial peptide, but not the self peptide, was protective against AA. With regard to the AA-protective effect of Rhsp65, the cytokine analysis of Rhsp65-reactive T cells revealed the predominance of the Th1 cytokine IFN-g over the Th2 cytokine IL-10.

A similar profile was evident when a single peptide of Rhsp65 (R465-479) was tested, and this peptide also induced protection against arthritis (Durai et al. 2004b; Durai et al. 2007). These results show that self-reactive T cells can be disease-regulating rather than being pathogenic. Paradoxically, the regulatory attribute of these T cells appears to be dependent on predominantly Th1 cytokines, highlighting another perplexing aspect of anti-Hsp immunity. Similar to Rhsp65, human Hsp65 has also been shown to induce protection against AA (Quintana et al. 2003). Furthermore, antibodies to Hsp65 in arthritic Lewis rats possess disease-regulating activity (Ulmansky et al. 2002; Kim et al. 2006). Thus, both T cell and antibody response to Hsp65 can contribute to regulation of autoimmune arthritis.

(B) Comments by participants and other scientists in the field

1. *Hsps in allotransplantation* (VC). Although this topic was not specifically discussed in the workshop, it has been included here, as it is relevant to the ongoing debate on the inflammatory versus regulatory attributes of Hsps. The early observations regarding Hsp and transplantation included their cytoprotective effect in ischemia–reperfusion injury, increased expression in rejecting allografts, and the detection of graft-infiltrating T lymphocytes displaying reactivity to Hsp (Moliterno et al. 1995a; Moliterno et al. 1995b; Pockley 2001; Pockley and Muthana 2005). Autoreactivity to Hsp seems to play a relevant role in the immune response to the graft, and Hsps are likely to induce both proinflammatory and immunoregulatory responses both in humans and in mice (Pockley 2001; Granja et al. 2004; Pockley and Muthana 2005; Caldas et al. 2006). Hsp-derived molecules have been used for immunoregulation in experimental models of transplantation (Luna et al. 2007a; Slack et al. 2007). The precise conditions that determine these two opposing functional activities of Hsps are yet to be elucidated. These opposite outcomes are possibly influenced by a variety of factors such as the immunological microenvironment, the time point after transplantation, the inherent properties of different epitopes within the Hsps, and the balance between inflammatory and regulatory cytokines. The immunoregulatory epitopes of Hsps are promising candidates for immunoregulatory therapy in allotransplantation.
2. Healthy individuals harbor both antibodies and T cells reactive to self Hsp65, and soluble circulating Hsp (Abulafia-Lapid et al. 1999; Luna et al. 2007b; Merbl et al. 2007; Victora et al. 2007). Autoreactivity to Hsp may have a role in maintaining homeostasis and in immunoregulation, as proposed in the theory of the 'Immunological homunculus' (Cohen and Young 1991; Cohen 2007). The observations that subsets of CD4+ CD25+ regulatory T cells (Treg) are reactive to self antigens (Tarbell et al. 2004; Masteller et al. 2005; Tarbell et al. 2007) and that Hsp65 enhances Treg function (Zanin-Zhorov et al. 2006) support the concept that autoreactivity may be immunoregulatory and that Hsp may play an important role in this physiological activity.
3. Hsp65 induces different innate effects on different cell types that include both pro- and anti-inflammatory consequences. Also, Hsp65 is an innate activator of Treg and is an adaptive ergotope for anti-ergotypic T regulators. Thus, Hsp65 is a major biomarker for the immune system.
4. The seemingly contrasting effects of Hsp70 in AA in rats and paratuberculosis in cattle illustrate an interesting 'immune-organizing' function of Hsps. The underlying and integrating mode of action of Hsp needs further investigation.
5. The transfer of Hsp-specific CD8+ clones into recombination activating gene-knockout (RAG-KO) mice induces lesions in the small intestine. In mice bearing an Hsp-specific transgenic T cell receptor (TCR), about 50% of lymphocytes are Hsp-specific, but the mouse is quite healthy. The transgenic TCR was cross-reactive with Hsp of Bacillus Calmette-Guérin (BCG), but immunization of mice with BCG showed no effect. In a conventional (dirty) housing environment, these mice developed colitis. It may be inferred from these observations that in clean conditions, mice can have high anti-Hsp reactivity without developing immune pathology or disease.
6. A provocative proposition relates to the role of soluble Hsps in "catching" circulating lipopolysaccharide (LPS) and inducing effector immune responses, which in part might be attributable to the bound LPS–this induction of proinflammatory immune response could be viewed as a beneficial aspect of LPS contamination of Hsp preparation.
7. Besides the dose of the antigen, the route of delivery (e.g., nasal versus oral) might affect the outcome of antigenic exposure. Also, the Hsp-induced activation of immune cells might work via different routes in different species. For example, chemokine (C–C motif) receptor 5 (CCR5) can be activated via Hsp70 in human cells, but not in

murine cells (Floto et al. 2006). To circumvent such problems, peptide-specific effects can be studied in more detail in different species.

8. Hsp10 is also being considered for use in the treatment of arthritis. A therapeutic efficacy and safety clinical trial using Hsp10 in the treatment of rheumatoid arthritis (RA) has shown some promising results (Vanags et al. 2006). The issue of LPS contamination of Hsp preparations is critical in clinical trials. It is very difficult to eliminate LPS for injection of Hsp or other proteins into humans. However, blood contains minute amounts of LPS, causing the injected Hsp to bind to LPS. Also, the Hsp10 treatment might show a beneficial effect in patients, but not necessarily in mouse models, and a reverse outcome might be observed for some other proteins. Hsp10 has been shown to induce suppression of AA (Ragno et al. 1996; Agnello et al. 2002), which further supports the rationale for testing of Hsp10 in RA patients. Similarly, Hsp10 has been shown to reduce clinical signs of experimental autoimmune encephalomyelitis (Johnson et al. 2005), and therefore, it might also be of utility in the treatment of multiple sclerosis.
9. An Hsp peptide has been shown to have a therapeutic effect in RA patients (Prakken et al. 2004). The administration of this peptide is associated with increased Treg activity. Although the eventual clinical impact of this approach in RA needs to be tested in a larger clinical trial, it is an important development in Hsp research that follows the extensive and promising clinical testing of another Hsp peptide in patients with type 1 diabetes mellitus (T1D; Raz et al. 2007).

(C) Some open questions pertaining to the immunobiology and practical applications of Hsps

The comments and questions below that were discussed during the workshop cover many of the cutting-edge areas of ongoing research and point to various challenging areas for future research.

1. As Hsp can induce both proinflammatory and regulatory immune responses:
   (a) What are the necessary conditions to direct the immune response?
   (b) Is the origin of Hsp (self versus foreign) relevant?
   (c) Do different regions of Hsp direct distinct (inflammatory versus regulatory) immune responses?
   (d) Is it important to have an inflammatory microenvironment to trigger Treg specific for Hsp?
2. Why do animal models develop either arthritis or atherosclerosis? Why does anti-Hsp65 immune response not result in generalized autoimmunity? What makes Hsp65 a special target antigen in different diseases?
3. Which Hsp receptors, besides CD91, are involved in the interaction of Hsps with APCs?
4. There is a continuing controversy on Hsp70 and the triggering of responses in dendritic cells (DCs) and possibly in other cell types. Several investigators have claimed the induction of pro-inflammatory responses by Hsp70. However, van Eden and colleagues have observed only minor or no activation and no or minor production of pro-inflammatory cytokines. Instead, they observed the development of a regulatory phenotype. Bonorino and colleagues have also shown that mycobacterial Hsp70 inhibits DC maturation and suppresses the proliferation of T cells (Motta et al. 2007). What factors might cause these opposite outcomes?
5. What are the differences between mammalian and prokaryotic Hsps with regard to their immunogenicity, despite their high level of evolutionary conservation?
6. Hsps that are upregulated in inflamed tissues can be targets for Hsp-specific regulatory T cells. How do the levels of endogenous expression of Hsp at sites of inflammation or elsewhere in the body influence the capacity of endogenous Hsp reactive T cells to maintain or display regulatory activity?
7. The likelihood that the TLR2/4 receptor association with Hsps could actually be due to the contaminating LPS should be probed thoroughly. The potential endotoxin contamination of Hsp preparations is a major concern to some investigators, whereas others might perhaps find the low amount of the contaminant immaterial. What precise information exchange among researchers working on different models, particularly humans versus experimental animals, would be of help in sorting out this issue?
8. Hsps trigger an innate response through surface receptors, but Hsps also can activate the adaptive system. Are there models that reveal these two effects of Hsps simultaneously?

Concluding remarks

This first Hsp workshop at ImmunoRio2007 was certainly a positive starting point to bring together a large number of investigators interested in the immunological properties of Hsps and their role in health and disease. One message

from this workshop is that Hsps may be involved in the initiation and propagation of certain diseases. The other message is that under another set of conditions, Hsps may function as immunoregulatory molecules. The challenging aspect of this dual function of Hsps is to determine how to direct the immune response to Hsps for therapeutic purposes. This workshop may be taken as a stimulus to continue identifying various hot and open questions in the field and to combine individual efforts to answer them as an international team. At this point, it is important to revisit with an open mind the concepts and the data from different groups, to verify which of these hot questions can be answered satisfactorily with supportive data, to identify open and controversial questions, and to openly discuss appropriate approaches to answer as many of these unanswered questions as possible. We hope that the immense success of this workshop will stimulate the Hsp researchers to keep an *Hsp workshop space* at each of the future ICI meetings and at other appropriate international meetings. In addition, creating an Hsp-open debate network, where the discussions may continue internationally throughout the year, can further reinforce the Hsp workshop's collaborative and productive activities.

Acknowledgment We would like to thank Irun Cohen and Willem van Eden for their contributions of provocative questions that they find relevant to the field of Hsps. We also would like to extend our thanks to all the workshop participants for their enthusiastic discussion, to Ernesto Luna for organizing open questions, and to Gabriel Victora for her help in taking notes on topics discussed in the workshop. Authors have no conflict of interest.

References

Abulafia-Lapid R, Elias D, Raz I, Keren-Zur Y, Atlan H, Cohen IR (1999) T cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. J Autoimmun 12:121–129

Agnello D, Scanziani E, Di GM, Leoni F, Modena D, Mascagni P, Introna M, Ghezzi P, Villa P (2002) Preventive administration of *Mycobacterium tuberculosis* 10-kDa heat shock protein (hsp10) suppresses adjuvant arthritis in Lewis rats. Int Immunopharmacol 2:463–474

Anderton SM, van der Zee R, Prakken B, Noordzij A, van Eden W (1995) Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. J Exp Med 181:943–952

Bala KK, Moudgil KD (2006) Induction and maintenance of self tolerance: the role of CD4+ CD25+ regulatory T cells. Arch Immunol Ther Exp (Warsz) 54:307–321

Binder RJ, Vatner R, Srivastava P (2004) The heat-shock protein receptors: some answers and more questions. Tissue Antigens 64:442–451

Caldas C, Luna E, Spadafora-Ferreira M, Porto G, Iwai LK, Oshiro SE, Monteiro SM, Fonseca JA, Lemos F, Hammer J, Ho PL, Kalil J, Coelho V (2006) Cellular autoreactivity against heat shock protein 60 in renal transplant patients: peripheral and graft-infiltrating responses. Clin Exp Immunol 146:66–75

Chandawarkar RY, Wagh MS, Kovalchin JT, Srivastava P (2004) Immune modulation with high-dose heat-shock protein gp96: therapy of murine autoimmune diabetes and encephalomyelitis. Int Immunol 16:615–624

Chandawarkar RY, Wagh MS, Srivastava PK (1999) The dual nature of specific immunological activity of tumor-derived gp96 preparations. J Exp Med 189:1437–1442

Cohen IR (2007) Biomarkers, self-antigens and the immunological homunculus. J Autoimmun 29:246–249

Cohen IR, Young DB (1991) Autoimmunity, microbial immunity and the immunological homunculus. Immunol Today 12:105–110

Durai M, Gupta RS, Moudgil KD (2004a) The T cells specific for the carboxyl-terminal determinants of self (rat) heat-shock protein 65 escape tolerance induction and are involved in regulation of autoimmune arthritis. J Immunol 172:2795–2802

Durai M, Kim HR, Moudgil KD (2004b) The regulatory C-terminal determinants within mycobacterial heat shock protein 65 are cryptic and cross-reactive with the dominant self homologs: implications for the pathogenesis of autoimmune arthritis. J Immunol 173:181–188

Durai M, Kim HR, Bala K, Moudgil KD (2007) T cells against the pathogenic and protective epitopes of heat-shock protein 65 are crossreactive and display functional similarity: novel aspect of regulation of autoimmune arthritis. J Rheumatol 34:2134–2143

Floto RA, MacAry PA, Boname JM, Mien TS, Kampmann B, Hair JR, Huey OS, Houben EN, Pieters J, Day C, Oehlmann W, Singh M, Smith KG, Lehner PJ (2006) Dendritic cell stimulation by mycobacterial Hsp70 is mediated through CCR5. Science 314:454–458

George J, Afek A, Gilburd B, Shoenfeld Y, Harats D (2001) Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice. J Am Coll Cardiol 38:900–905

George J, Shoenfeld Y, Afek A, Gilburd B, Keren P, Shaish A, Kopolovic J, Wick G, Harats D (1999) Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65. Arterioscler Thromb Vasc Biol 19:505–510

George J, Shoenfeld Y, Gilburd B, Afek A, Shaish A, Harats D (2000) Requisite role for interleukin-4 in the acceleration of fatty streaks induced by heat shock protein 65 or *Mycobacterium tuberculosis*. Circ Res 86:1203–1210

Granja C, Moliterno RA, Ferreira MS, Fonseca JA, Kalil J, Coelho V (2004) T-cell autoreactivity to Hsp in human transplantation may involve both proinflammatory and regulatory functions. Hum Immunol 65:124–134

Harats D, Yacov N, Gilburd B, Shoenfeld Y, George J (2002) Oral tolerance with heat shock protein 65 attenuates *Mycobacterium tuberculosis*-induced and high-fat-diet-driven atherosclerotic lesions. J Am Coll Cardiol 40:1333–1338

Johnson BJ, Le TT, Dobbin CA, Banovic T, Howard CB, Flores Fde M, Vanags D, Naylor DJ, Hill GR, Suhrbier A (2005) Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. J Biol Chem 280:4037–4047

Kim HR, Kim EY, Cerny J, Moudgil KD (2006) Antibody responses to mycobacterial and self heat shock protein 65 in autoimmune arthritis: epitope specificity and implication in pathogenesis. J Immunol 177:6634–6641

Koets A, Hoek A, Langelaar M, Overdijk M, Santema W, Franken P, Eden W, Rutten V (2006) Mycobacterial 70 kD heat-shock protein is an effective subunit vaccine against bovine paratuberculosis. Vaccine 24:2550–2559

Lo WF, Dunn CD, Ong H, Metcalf ES, Soloski MJ (2004) Bacterial and host factors involved in the major histocompatibility complex class Ib-restricted presentation of Salmonella Hsp 60: novel pathway. Infect Immun 72:2843–2849

Luna E, Postol E, Caldas C, Benvenuti LA, Rodrigues JM Jr., Lima K, Kalil J, Coelho V (2007a) Treatment with encapsulated Hsp60

peptide (p277) prolongs skin graft survival in a murine model of minor antigen disparity. Scand J Immunol 66:62–70

Luna E, Postol E, Caldas C, Mundel LR, Porto G, Iwai LK, Ho PL, Kalil J, Coelho V (2007b) Diversity of physiological cell reactivity to heat shock protein 60 in different mouse strains. Cell Stress Chaperones 12:112–122

Masteller EL, Warner MR, Tang Q, Tarbell KV, McDevitt H, Bluestone JA (2005) Expansion of functional endogenous antigen-specific CD4+ CD25+ regulatory T cells from nonobese diabetic mice. J Immunol 175:3053–3059

Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR (2007) Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. J Clin Invest 117:712–718

Moliterno R, Valdivia L, Pan F, Duquesnoy RJ (1995a) Heat shock protein reactivity of lymphocytes isolated from heterotopic rat cardiac allografts. Transplantation 59:598–604

Moliterno R, Woan M, Bentlejewski C, Qian J, Zeevi A, Pham S, Griffith BP, Duquesnoy RJ (1995b) Heat shock protein-induced T-lymphocyte propagation from endomyocardial biopsies in heart transplantation. J Heart Lung Transplant 14:329–337

Motta A, Schmitz C, Rodrigues L, Ribeiro F, Teixeira C, Detanico T, Bonan C, Zwickey H, Bonorino C (2007) Mycobacterium tuberculosis heat-shock protein 70 impairs maturation of dendritic cells from bone marrow precursors, induces interleukin-10 production and inhibits T-cell proliferation in vitro. Immunology 121:462–472

Moudgil KD (1998) Diversification of response to hsp65 during the course of autoimmune arthritis is regulatory rather than pathogenic. Immunol Rev 164:175–184

Pockley AG (2001) Heat shock proteins, anti-heat shock protein reactivity and allograft rejection. Transplantation 71:1503–1507

Pockley AG, Muthana M (2005) Heat shock proteins and allograft rejection. Contrib Nephrol 148:122–134

Prakken BJ, Samodal R, Le TD, Giannoni F, Yung GP, Scavulli J, Amox D, Roord S, de Kleer I, Bonnin D, Lanza P, Berry C, Massa M, Billetta R, Albani S (2004) Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. Proc Natl Acad Sci U S A 101:4228–4233

Prinz I, Zerrahn J, Kaufmann SH, Steinhoff U (2002) Promiscuous peptide recognition of an autoreactive CD8(+) T-cell clone is responsible for autoimmune intestinal pathology. J Autoimmun 18:281–287

Quintana FJ, Carmi P, Mor F, Cohen IR (2003) DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. J Immunol 171:3533–3541

Ragno S, Winrow VR, Mascagni P, Lucietto P, Di Pierro F, Morris CJ, Blake DR (1996) A synthetic 10-kD heat shock protein (hsp10) from *Mycobacterium tuberculosis* modulates adjuvant arthritis. Clin Exp Immunol 103:384–390

Raz I, Avron A, Tamir M, Metzger M, Symer L, Eldor R, Cohen IR, Elias D (2007) Treatment of new-onset type 1 diabetes with peptide DiaPep277 is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. Diabetes Metab Res Rev 23:292–298

Sherer Y, Shoenfeld Y (2006) Mechanisms of disease: atherosclerosis in autoimmune diseases. Nat Clin Pract Rheumatol 2:99–106

Slack LK, Muthana M, Hopkinson K, Suvarna SK, Espigares E, Mirza S, Fairburn B, Pockley AG (2007) Administration of the stress protein gp96 prolongs rat cardiac allograft survival, modifies rejection-associated inflammatory events, and induces a state of peripheral T-cell hyporesponsiveness. Cell Stress Chaperones 12:71–82

Srivastava P (2002) Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 20:395–425

Srivastava PK, DeLeo AB, Old LJ (1986) Tumor rejection antigens of chemically induced sarcomas of inbred mice. Proc Natl Acad Sci U S A 83:3407–3411

Steinhoff U, Brinkmann V, Klemm U, Aichele P, Seiler P, Brandt U, Bland PW, Prinz I, Zugel U, Kaufmann SH (1999) Autoimmune intestinal pathology induced by hsp60-specific CD8 T cells. Immunity 11:349–358

Tarbell KV, Petit L, Zuo X, Toy P, Luo X, Mqadmi A, Yang H, Suthanthiran M, Mojsov S, Steinman RM (2007) Dendritic cell-expanded, islet-specific CD4+ CD25+ CD62L+ regulatory T cells restore normoglycemia in diabetic NOD mice. J Exp Med 204:191–201

Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM (2004) CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med 199:1467–1477

Ulmansky R, Cohen CJ, Szafer F, Moallem E, Fridlender ZG, Kashi Y, Naparstek Y (2002) Resistance to adjuvant arthritis is due to protective antibodies against heat shock protein surface epitopes and the induction of IL-10 secretion. J Immunol 168:6463–6469

van Eden W, Thole JE, van der Zee R, Noordzij A, van Embden JD, Hensen EJ, Cohen IR (1988) Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331:171–173

van Eden W, van der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5:318–330

Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D (2006) Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. Lancet 368:855–863

Victora GD, Bilate AM, Socorro-Silva A, Caldas C, Lima RC, Kalil J, Coelho V, Victora CG (2007) Mother-child immunological interactions in early life affect long-term humoral autoreactivity to heat shock protein 60 at age 18 years. J Autoimmun 29:38–43

Wendling U, Paul L, van der Zee R, Prakken B, Singh M, van Eden W (2000) A conserved mycobacterial heat shock protein (hsp) 70 sequence prevents adjuvant arthritis upon nasal administration and induces IL-10-producing T cells that cross-react with the mammalian self-hsp70 homologue. J Immunol 164:2711–2717

Xu Q, Dietrich H, Steiner HJ, Gown AM, Schoel B, Mikuz G, Kaufmann SH, Wick G (1992) Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. Arterioscler Thromb 12:789–799

Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, Kiechl S, Stulnig T, Luef G, Wick G (1993) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. Lancet 341:255–259

Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR (2006) Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. J Clin Invest 116:2022–2032