Molecular Chaperones and Cell Signalling

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Heat Shock Proteins Regulate Inflammation by Both Molecular and Network Cross-Reactivity

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16.1. Introduction

Heat shock proteins were initially identified as heterogeneous families of stress-induced proteins characterised by their chaperone activity [1]. Subsequently, they were identified as immunodominant antigens recognised by the host immune system following microbial infection [2] or during the course of autoimmune disease [3–6]. Recently, the role of heat shock proteins as endogenous activators of the innate and adaptive immune system has been unveiled [7]. In this chapter we discuss the relevance of heat shock proteins and their immune activities to the regulation of inflammation and autoimmune disease. We shall see that the regulatory activities of heat shock proteins on inflammation involve two types of cross-reactivity: *molecular* cross-reactivity exists between microbial and self-heat shock proteins and *network* cross-reactivity exists between different self-heat shock proteins.

16.2. Inflammation activates heat shock protein–specific T cells

Although the injection of incomplete Freund's adjuvant (IFA) to BALB/c mice induces local inflammation, Anderton and colleagues demonstrated that the injection of IFA also induces T cells reactive with the mammalian 60-kDa heat shock protein (Hsp60) [8]. These Hsp60-reactive T cells were TCR$\alpha\beta^+$, CD4$^+$ and major histocompatibility complex (MHC) class II-restricted [8]. Notably, Hsp60-specific cells could only be found in the local lymph nodes draining the site of IFA injection, and they were not present in distant lymph nodes. Hsp60-specific T cells are not only induced but also recruited to the site of inflammation [8].

The pro-inflammatory response which drives autoimmune disorders has also been shown to lead to an up-regulation of heat shock protein expression and the

recruitment of heat shock protein–specific T cells to the target organ. Mor and colleagues have described that, along with myelin-specific T cells, T cells specific for the mycobacterial 65-kDa (Hsp65) or 71-kDa (Hsp71) heat shock proteins are recruited to the central nervous system (CNS) in rats undergoing experimental autoimmune encephalomyelitis (EAE) [9]. This initial observation was subsequently extended to include self-heat shock proteins and T cells reactive to them, in both EAE and human multiple sclerosis [10–12]. Finally, transplanted organs undergoing rejection show increased levels of expression of endogenous heat shock proteins and are infiltrated by heat shock protein–specific T cells (reviewed in [13]).

In short, heat shock protein–specific T cells are induced by inflammation and are recruited to the sites of inflammation. In this chapter, we will discuss experimental data that support a regulatory role for heat shock proteins and heat shock protein–specific T cells in the control of inflammation.

16.3. Heat shock proteins control inflammation

Adjuvant arthritis (AA) in the Lewis rat [14] and spontaneous autoimmune diabetes in the non-obese diabetic (NOD) mouse [15] are experimental models for two of the most prevalent human autoimmune diseases: rheumatoid arthritis [16] and type 1 diabetes mellitus (T1DM) [17]. Although the clinical signs of the models are naturally different, both experimental diseases are linked by the observation that heat shock proteins can halt the autoimmune attack. We have used these experimental models to study the role of heat shock proteins in the control of autoimmune disease.

16.3.1. Adjuvant arthritis

AA is induced in Lewis rats by a subcutaneous injection of heat-killed *Mycobacterium tuberculosis* in IFA [14]. T cells specific for mycobacterial Hsp65 can both drive and inhibit AA. Although Hsp65-specific $CD4^+$ T cell clones cross-react with cartilage components and transfer AA [18], Hsp65 administered as a protein [19], encoded in a recombinant vaccinia virus [20] or administered as a DNA vaccine [21] can inhibit AA. The administration of Hsp65 can also regulate experimental arthritis triggered by the lipoidal amine CP20961 [22] or by pristane [23].

Inhibition of AA by Hsp65 is thought to involve cross-reactivity with self-Hsp60 [24]. We have studied the specificity of the regulatory immune response that controls AA using DNA vaccines coding for either human Hsp60 (pHsp60) or mycobacterial Hsp65 (pHsp65) [25]. Although both pHsp60 and

pHsp65 protect against AA, pHsp60 is significantly more effective [25]. Using DNA vaccines encoding fragments of Hsp60 to identify immunoregulatory regions within Hsp60, the anti-arthritogenic effects of the pHsp60 construct have been shown to reside in the amino acid (aa) 1–260 region of Hsp60 [26]. Using Hsp60-derived overlapping peptides, peptide Hu3 (aa 31–50 of Hsp60) is specifically recognised by T cells of rats protected from AA by DNA vaccination [26]. Vaccination with Hu3, or transfer of splenocytes from Hu3-vaccinated rats, prevents the development of AA, whereas vaccination with the mycobacterial homologue of Hu3 has no effect [26]. Prevention of AA by vaccination with pHsp60, DNA vaccines encoding the N-terminus of Hsp60, or Hu3 was associated with the induction of T cells that secrete IFN-γ, IL-10 and TGF-β1 upon stimulation with Hsp60 [25, 26]. Thus, Hsp60-specific T cells can control the progression of AA. However, what influence do T cells reactive with other heat shock proteins have on such processes?

T cell responses to the mycobacterial 10-kDa heat shock protein (Hsp10) [27] or mycobacterial Hsp71 have also been shown to control the progression of AA [28–30]. We studied whether self-heat shock proteins other than Hsp60 could inhibit AA using DNA vaccines encoding human 70-kDa heat shock protein (Hsp70) or the human 90-kDa heat shock protein (Hsp90). DNA vaccination with Hsp70 or Hsp90 shifted the specific arthritogenic T-cell response from a Th1 to a Th2/3 phenotype and inhibited AA [31]. Thus, Hsp70 and Hsp90 can also modulate arthritogenic T cell responses in AA.

Hsp60-specific responses in patients with rheumatoid arthritis [32, 33] or juvenile chronic arthritis [34] are associated with milder arthritis and a better prognosis. Although no information is yet available on T cell responses to Hsp70 or Hsp90 in human arthritis, these observations suggest that heat shock protein–specific T cells might also have a regulatory role in human autoimmune arthritis. The role of the 70-kDa heat shock protein BiP as a modulator of rheumatoid arthritis is described in detail in Chapter 14.

16.3.2. NOD diabetes

NOD mice spontaneously develop diabetes as a consequence of a T cell–mediated autoimmune process that destroys the insulin-producing β cells of the pancreas [17]. NOD mice have a high frequency of self-reactive T cells [35], which is reflected by a highly self-reactive B-cell repertoire [36]. Several antigens are targeted by diabetogenic T cells, including insulin [37] and glutamic acid decarboxylase (GAD) [38]. Similar to the situation found in AA, T cell reactivity to Hsp65 is a double-edged sword. A peak of Hsp65-specific

T cell reactivity precedes the onset of diabetes [39], and immunisation with Hsp65 can induce a transient hyperglycaemia [39]. However, vaccination with Hsp65 can also inhibit the development of diabetes [39]. These initial reports may be explained by cross-reactivity between mycobacterial Hsp65 and self-Hsp60.

We have shown that self-Hsp60 is targeted by the diabetogenic attack; T cells reactive with the Hsp60 peptide p277 (aa 437–460) can induce diabetes in irradiated NOD recipients [40]. On the other hand, vaccination of NOD mice with peptide p277 has been shown to arrest the development of diabetes [40] and can even induce remission of overt hyperglycaemia [41]. Successful p277 treatment leads to the down-regulation of spontaneous T cell proliferation to p277 and to the induction of a Th1-to-Th2 switch in the immune response to p277 [42]. Other peptides of Hsp60 can also inhibit the development of spontaneous diabetes in NOD mice [43].

NOD mice can also develop a more robust form of diabetes induced by the administration of cyclophosphamide, termed cyclophosphamide-accelerated diabetes (CAD) [44]. Cyclophosphamide is thought to specifically deplete regulatory T cells [44], thereby unleashing a Th1 response which is rich in IFN-γ secreting cells and leads to overt diabetes [45].

We have studied the effect of DNA vaccination with pHsp60 or pHsp65 on CAD. Vaccination with pHsp60, but not with pHsp65, protects NOD mice from CAD [46]. Thus, the efficacy of the pHsp60 DNA vaccine in this situation can be explained by regulatory Hsp60 epitopes that are not shared with Hsp65; indeed well-characterised regulatory epitopes from Hsp60 are not conserved in the sequence of Hsp65 [46]. Vaccination with pHsp60 modulates the T cell responses to Hsp60 and also to GAD and insulin. T cell proliferative responses are significantly reduced, and the cytokine profile induced by stimulation with Hsp60, GAD or insulin revealed an increased secretion of IL-10 and IL-5 and a decreased secretion of IFN-γ, a finding which is compatible with a Th1-to-Th2 shift in the autoimmune response [46].

In conclusion, the administration of Hsp60 peptides, or of whole Hsp60 as a recombinant protein or a DNA vaccine, can halt autoimmune NOD diabetes. Several antigens are targeted during the progression of diabetes [17] and it is therefore remarkable that the immunoregulatory networks triggered by Hsp60 can control diabetogenic T cells that are directed to a range of other antigens, such as insulin and GAD.

B and T cell responses to Hsp70 [47], Hsp60 and p277 [6, 48] have also been described in patients with T1DM. Indeed, a double-blind, phase II clinical trial was designed to study the effects of p277 therapy on newly diagnosed patients

[49]. The administration of p277 after the onset of clinical diabetes preserved the endogenous levels of C-peptide (which fell in the placebo group) and was associated with lower requirements for exogenous insulin, thereby revealing an arrest of β cell destruction [49]. Treatment with p277 led to enhanced Th2 responses to Hsp60 and p277 [49]. Thus, like NOD diabetes, human T1DM appears to be susceptible to immunomodulation by Hsp60 therapy.

Taken together it appears that heat shock proteins can control the progression of inflammation and, in particular, self-heat shock proteins seem to be quite efficient in doing so. However, do we need exogenous heat shock proteins to trigger heat shock protein–based regulatory mechanisms?

16.4. Triggering of heat shock protein–based immunoregulation by innate immune activation

Bacterial DNA stimulates the innate immune system via Toll-like receptor 9 (TLR9) [50] due to the presence of DNA motifs consisting of a central unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′ pyrimidines [51]. Such a sequence is referred to as a CpG motif. We have demonstrated that bacterial CpG motifs can inhibit spontaneous diabetes in NOD mice [52], but not CAD [46]. The prevention of diabetes was characterised by a decreased insulitis [52]. Moreover, we have detected a decrease in the spontaneous proliferative responses of T cells to Hsp60 and its p277 peptide, concomitant with the induction of Th2-like antibodies of the same specificity, thereby revealing a Th1-to-Th2 shift in the autoimmune response of the treated mice [52].

To investigate the mechanisms involved in the regulation of spontaneous NOD diabetes by CpG motifs, we studied the expression of Hsp60 in splenocytes from NOD mice stimulated with a synthetic oligonoucleotide containing CpG motifs (CpG). *In vitro* stimulation with CpG led to a dose-dependent upregulation of intracellular Hsp60 levels, as demonstrated by Western blot analysis, and also to the release of Hsp60 into the supernatant. A control oligonucleotide containing an inverted CpG motif (GpC) had no significant effect on the intracellular levels of Hsp60 or on Hsp60 secretion [Quintana and Cohen, manuscript submitted].

CpG also affected the responses of T cell clones specific for the Hsp60 peptides p12 (aa 166–185) or p277 (aa 437–460). In the presence of irradiated antigen-presenting cells (APCs), CpG triggered the dose-dependent proliferation of both Hsp60-specific T cell clones, but not of an anti-ovalbumin T cell line [Quintana and Cohen, manuscript submitted]. All the T cells were activated by their

target antigen, but not by lipopolysaccharide (LPS), thereby ruling out the possibility that some of the observed proliferation was due to the presence of contaminating B cells [Quintana and Cohen, manuscript submitted]. The analysis of cytokine secretion revealed that CpG stimulation triggered the secretion of higher amounts of IL-10 and lower amounts of IFN-γ than did activation with the target Hsp60 peptides (p12 or p277) [Quintana and Cohen, manuscript submitted]. The Hsp60-specific T cell lines were not activated by CpG in the absence of APCs, and CpG-induced proliferation was inhibited by anti-MHC class II antibodies [Quintana and Cohen, manuscript submitted]. Thus, CpG activates Hsp60-specific T cells by stimulating the presentation of peptides derived from endogenous Hsp60 in the MHC class II molecules of the APC. Because IL-10 is known to have suppressor effects on immune responses [53], the relative increase in IL-10 secretion by Hsp60-specific T cells might explain the protective effect of CpG on NOD diabetes. The reader should refer to Chapters 13 and 14 for a discussion of chaperones that selectively induce IL-10 over IL-1/tumour necrosis factor synthesis.

Figure 16.1 depicts our model for the action of CpG on spontaneous NOD diabetes. The activation of APCs or of other cell types via TLR9 leads to the up-regulation of intracellular levels of Hsp60 and eventually to its secretion. Hsp60 is then presented on the surface of the APC via MHC class II molecules. Hsp60-specific regulatory T cells are therefore activated, halting the progression of NOD diabetes.

A paper by Kumaraguru and colleagues reports that CpG triggers the up-regulation and release of Hsp70 from macrophages; however, the effects of CpG on Hsp70-specific T cell lines were not studied [54]. Based on the APC function of macrophages, it is likely that Hsp70 peptides presented in the MHC molecules of CpG-treated macrophages can modulate Hsp70-specific immunity.

TLR9-mediated activation has been shown to control several experimental models of autoimmune disease including EAE [55], colitis [56] and arthritis [57, 58]. Ligands for other TLRs, such as poly I:C [59] or LPS [60–62], have also been reported to inhibit experimental autoimmunity. Whether the activation of heat shock protein–based immunoregulatory mechanisms is a feature shared by several TLR-dependent signalling cascades remains to be seen. Nevertheless, our results suggest that regulatory Hsp60-specific T cell responses can be triggered by the activation of innate networks that lead to the release of endogenous heat shock proteins leading, in turn, to the activation of the specific T cell populations. Could we use these innate networks to diversify the heat shock protein–specific immune response? In other words, could we administer a particular heat shock protein and induce T cell responses directed to a different heat shock protein?

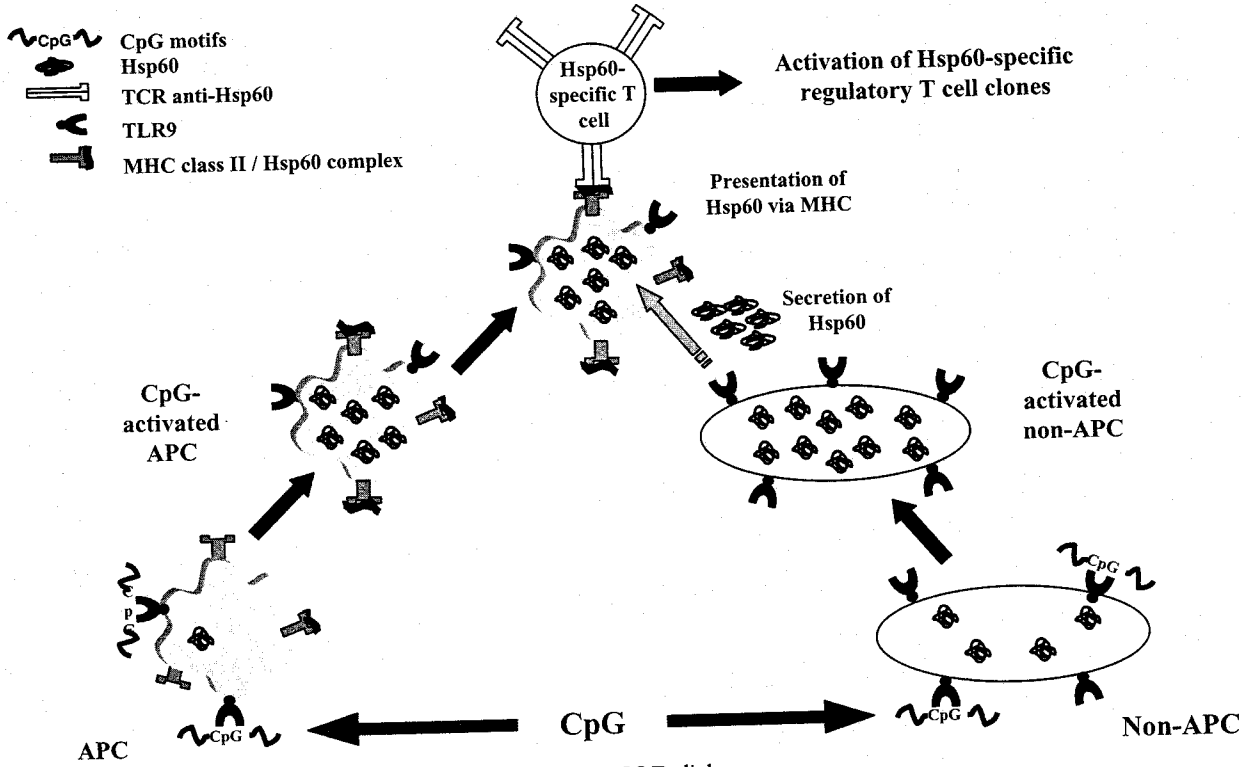

Figure 16.1. Model for the action of CpG on spontaneous NOD diabetes.

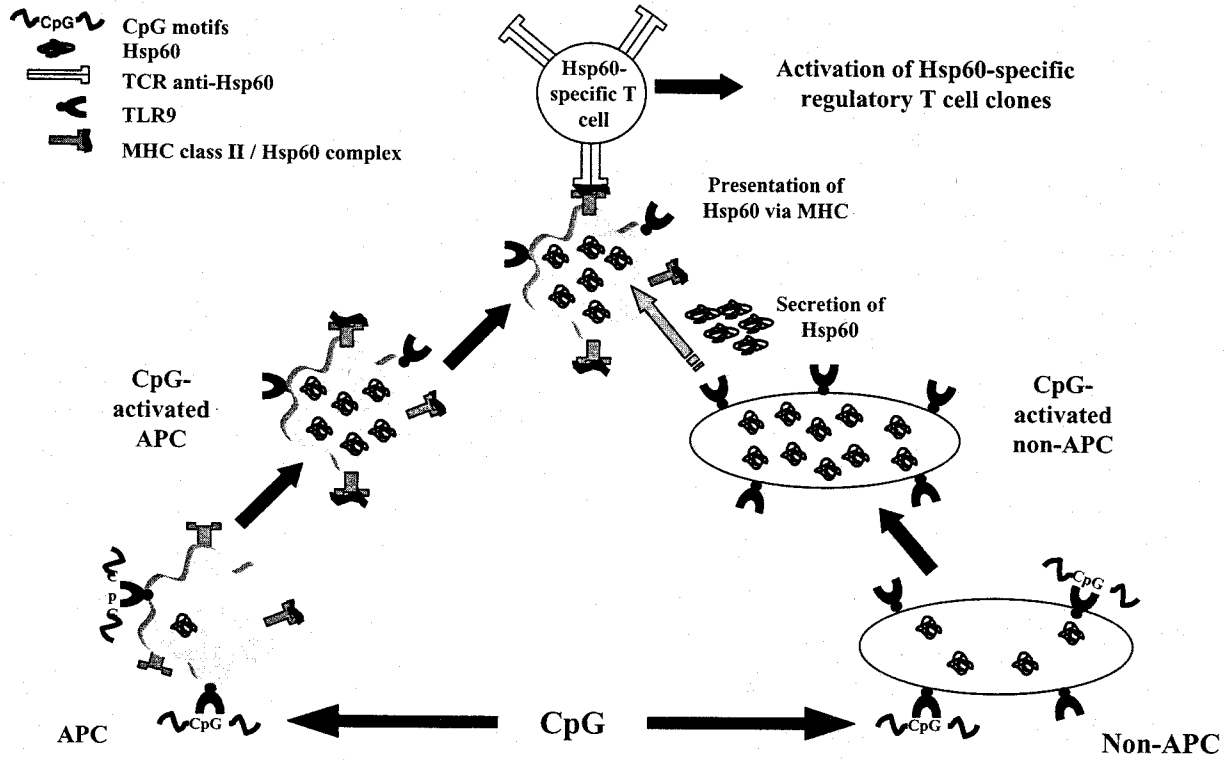

Figure 16.1. Model for the action of CpG on spontaneous NOD diabetes.

16.5. Connectivity between different heat shock protein–specific immune responses

We have demonstrated that DNA vaccines coding for Hsp60 (pHsp60), Hsp70 (pHsp70) or Hsp90 (pHsp90) can inhibit AA [25, 26, 30]. Moreover, DNA vaccines coding for Hsp70 or Hsp90 can modulate the Hsp65-specific T cell response which drives AA, in a similar manner to that previously demonstrated for Hsp60 [25, 26, 30]. Hsp60, Hsp70 and Hsp90 bear no significant sequence homology or immune cross-reactivity. However, might immunisation with an exogenous heat shock protein trigger the presentation of a different endogenous heat shock protein, leading to the diversification of the immune response induced by vaccination with a particular heat shock protein?

DNA vaccination with pHsp70 or pHsp90 induces antigen-specific proliferative responses: pHsp70-vaccinated rats manifest T cell responses to Hsp70, and pHsp90-vaccinated rats manifest T cell responses to Hsp90 [31]. However, DNA vaccination with pHsp70 or pHsp90 could also induce T cells that proliferated and secreted IFN-γ, TGF-β1 and IL-10 upon stimulation with Hsp60 [31]. Thus different heat shock protein molecules are linked immunologically.

To characterise this connection, we compared the epitope specificity of the Hsp60-specific T cell response induced by pHsp60 with that induced by pHsp70 using a panel of overlapping peptides derived from the human Hsp60 sequence [31]. We had previously found that pHsp60 DNA-vaccination-induced regulatory T cells were reactive with a single Hsp60 peptide epitope, Hu3 (aa 31–50) [26]. However, lymph node cells (LNCs) from pHsp70-vaccinated rats responded to several other Hsp60 peptides: Hu19 (aa 271–290), Hu24 (aa 346–365), Hu25 (aa 361–380), Hu27 (aa 391–410), Hu28 (aa 406–425), Hu30 (aa 436–455), Hu32 (aa 466–485), Hu33 (aa 481–500) and Hu34 (aa 271–290) [31]. Thus, although both pHsp60 and pHsp70 can induce Hsp60-specific T cells, the fine specificities of the T cell responses induced are different. The cross-talk between the Hsp60- and the Hsp70-specific T cell responses is reciprocal, in that pHsp60-vaccinated rats showed significant T cell responses upon stimulation with Hsp70 [31]. These findings are schematically represented in Figure 16.2.

Hsp60, Hsp70 and Hsp90 share no sequence homology and are not immunologically cross-reactive. One possible explanation for the induction of Hsp60-specific T cell responses by pHsp70 or pHsp90 is self-vaccination with endogenous self-Hsp60 which is induced and/or released as a result of the DNA vaccinations. Indeed, we could detect increased levels of circulating Hsp60 in pHsp70-vaccinated rats [Quintana et al., manuscript submitted]. The up-regulation of Hsp60 levels in the circulation was dependent on the presence

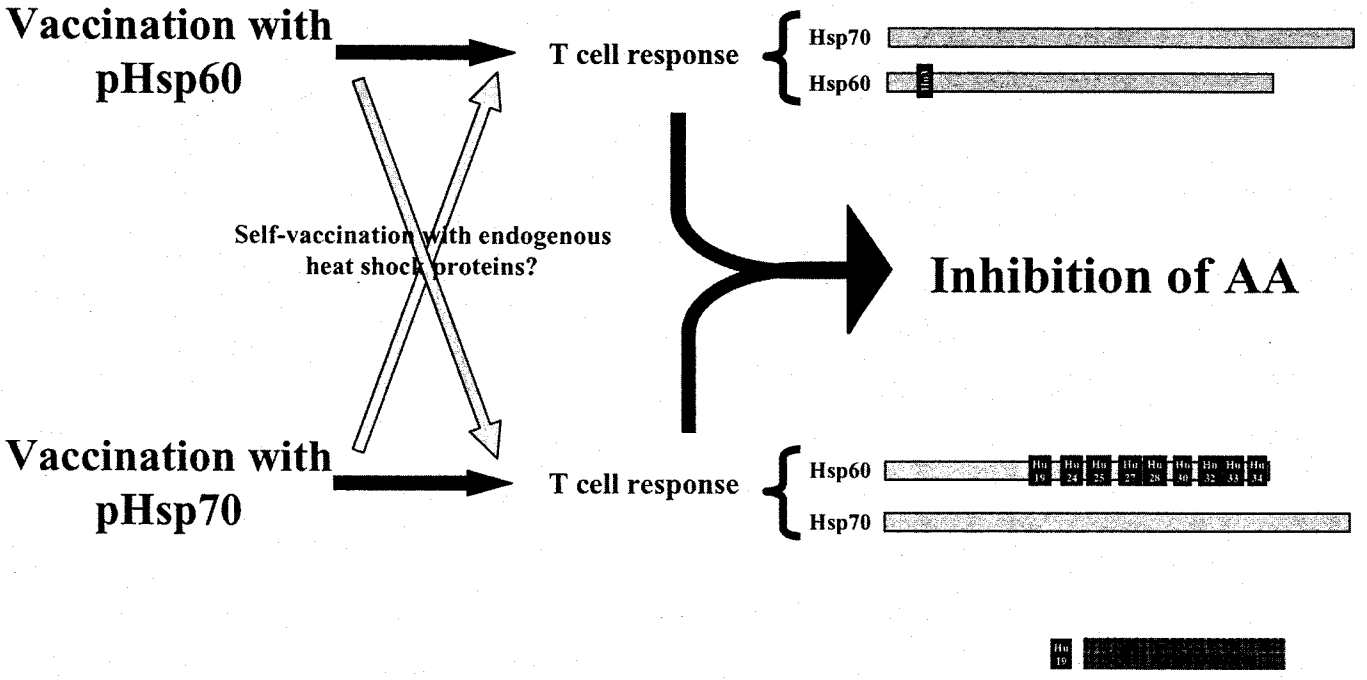

Figure 16.2. Connectivity between Hsp60- and Hsp70-specific T cell responses.

of the *hsp70* gene on the pHsp70 DNA construct; as a control, empty plasmid had no effect on circulating Hsp60 levels [Quintana et al., manuscript submitted]. Reciprocally, vaccination with pHsp60 induced a T cell response to Hsp70; however, we have not yet been able to measure the levels of Hsp70 in the blood of pHsp60-vaccinated rats. Although the molecular mechanisms require further study, the present findings demonstrate that heat shock protein–specific responses are inter-regulated and highlight the multiple immune signalling activities of these molecules.

16.6. Immunoregulatory mechanisms triggered by heat shock proteins

Earlier, we have seen that inflammation induces heat shock protein–specific T cells and that, despite a lack of immunological cross-reactivity between the molecules, T cell responses to different heat shock proteins are connected. We have shown that heat shock proteins can control autoimmune disease, and we have referred to experimental data suggesting that heat shock protein–based regulatory networks can be triggered in the absence of exogenous immunisation with heat shock proteins. We are therefore left with the question of how heat shock protein molecules might control autoimmunity.

The autoimmune attack which leads to overt autoimmune disease is a process that simultaneously engages several cell types and molecular mechanisms that are not restricted to the immune system. Faced by such a multi-front attack, it is not surprising that heat shock proteins bear several features that might prove helpful for the control of the autoimmune response. Heat shock proteins, as described in Chapter 1, are intracellular chaperones which facilitate the correct folding of newly synthesised proteins [1]. Moreover, they also improve antigen processing and presentation by APCs [63]. Heat shock proteins facilitate the induction of T cell responses to free peptide epitopes which are bound by circulating heat shock proteins and taken up by APCs through heat shock protein–specific receptors [63, 64]. Circulating heat shock proteins, not loaded with any peptide, can directly activate several cell types via innate receptors [7]. Heat shock proteins can activate immune system cells, such as dendritic cells [65–67], and also non-immune cells, such as endothelial cells [68]. Finally, heat shock proteins bear regulatory T cell epitopes [25, 69]. However, as we have seen, heat shock proteins can also be targeted by the pathogenic T cells that characterise autoimmune diseases such as T1DM [6].

The sites at which heat shock proteins are expressed can influence their immunoregulatory functions. The intracellular levels of heat shock proteins are increased upon cellular stress. Viral or bacterial infections up-regulate heat shock

protein expression [70–73], and necrotic cells release heat shock proteins [74]. Inflammation is a source of cellular stress, and heat shock proteins are over-expressed at the sites of inflammation, such as in the synovium in arthritis [75]. Strikingly, heat shock proteins are also up-regulated in activated macrophages [76] and T cells [77]. Thus, heat shock proteins simultaneously mark the cells targeted by the autoimmune attack and the pathogenic immune cells that carry out the attack.

Based on the intra- and extra-cellular functions of heat shock protein and their localisation, several mutually non-exclusive mechanisms, involving adaptive and non-adaptive immunity, can contribute to the immunoregulatory properties of heat shock proteins.

16.6.1. Adaptive immunity

16.6.1.1. Environmental regulation of heat shock protein–specific immunity

Heat shock proteins are immunodominant bacterial antigens [78]. Because mucosal immunisation is known to induce antigen-specific regulatory responses [79], exposure to bacterial heat shock proteins from the intestinal flora might be a source of heat shock protein–specific regulatory T cells. Indeed, Moudgil and colleagues have demonstrated that environmental microbes can induce Hsp65-specific T cells directed to regulatory epitopes that are cross-reactive with self-Hsp60 [69, 80]. Vaccination with heat shock proteins or their peptides might simply amplify this naturally acquired regulation. However, based on this mechanism, any cross-reactive protein conserved through evolution from bacteria to mammals should be immunoregulatory. This is not always the case, as recently reported by Prakken and colleagues [81].

16.6.1.2. Boost of regulatory T cell responses

Heat shock proteins can bind free peptides and induce peptide-specific immune responses, even in the presence of low amounts of the target peptide [82]. Thus, heat shock protein molecules could be loaded *in vivo* with regulatory self-peptides and subsequently boost or amplify specific regulatory T cell responses. Indeed, Chandawarkar and colleagues have reported that gp96 can both induce and down-regulate tumour-specific immune responses [83]. Furthermore, heat shock proteins purified from the inflamed CNS of EAE rats (and not from naïve rats) can vaccinate naïve rats against EAE [84]. Thus, Hsp70–peptide complexes synthesised at the sites of active inflammation can trigger tissue-specific anti-inflammatory T cell responses [84]. Nevertheless, this mechanism does not

explain the immunomodulatory effects of heat shock protein–derived fragments or peptides. See Chapters 17 and 18 for more information on chaperones and peptide-specific immune responses.

16.6.1.3. Cytokine-mediated bystander inhibition

Inflammation leads to the local up-regulation of heat shock proteins. Heat shock protein–specific T cells might therefore be recruited to sites of inflammation, where they could control pathogenic T cell clones by the secretion of regulatory cytokines. Heat shock protein–specific T cells induced by vaccination with immunoregulatory DNA vaccines or peptides secrete regulatory cytokines (IL-10 and TGF-β1) [25, 29, 30, 46].

16.6.1.4. Anti-ergotypic regulation

T cells reactive to activated T cells (but not to resting T cells) can control experimental autoimmune disease [85–87]. The T cell receptor expressed by these regulatory T cells recognises peptides derived from activation markers (ergotopes), such as the α-chain of the IL-2 receptor [86, 87] or the TNF-α receptor [87]. These cells are termed anti-ergotypic [85]. Now it has been reported that mRNAs encoding for heat shock proteins are up-regulated upon T cell activation [77]. Thus, Hsp60 too might serve as an ergotope. We studied whether vaccination with DNA vaccines encoding Hsp60, or with the regulatory peptide Hu3, might induce anti-ergotypic responses. To serve as an ergotope, Hsp60 would have to fulfil two requirements. Firstly, Hsp60 must be up-regulated in activated T cells. Secondly, activated T cells must present Hsp60-derived peptides to Hsp60-specific T cells.

The activation of T cells by the mitogen Concanavalin A, or by specific antigen, up-regulates intracellular levels of Hsp60 [Quintana et al., manuscript submitted]. Thus, the first condition is fulfilled: T cell activation triggers Hsp60 expression. Moreover, activated T cells can present Hsp60. Hsp60-specific T cells proliferate to activated T cells and secrete both IFN-γ and TGF-β1 [Quintana et al., manuscript submitted]. The activation of Hsp60-specific T cells was MHC class II (RT1.B) restricted, since it could be inhibited with the OX6 monoclonal antibody [Quintana et al., manuscript submitted]. Thus, Hsp60 can function as an ergotope *in vitro*; however, can functional Hsp60-specific anti-ergotypic responses be induced *in vivo*?

DNA vaccination with pHsp60 has been found to induce anti-ergotypic T cell responses that are MHC class II (RT1.B) and MHC class I restricted [Quintana et al., manuscript submitted]. In contrast, vaccination with Hu3 induced only an MHC class II restricted (RT1.B) anti-ergotypic T cell response [Quintana et al.,

manuscript submitted]. Thus, Hsp60-specific $CD4^+$ and $CD8^+$ anti-ergotypic T cells can be induced *in vivo*.

LNCs from rats with AA stimulated with the immunodominant 180–88 T cell epitope of Hsp65 (mt180) secrete high levels of IFN-γ [25]. Since T cells specific for this epitope have been shown to transfer AA [18, 88], the reactivity of LNCs of AA to mt180 is thought to reflect the behaviour of the arthritogenic T cells. LNCs of AA rats stimulated with mt180 in the presence of Hsp60-specific anti-ergotypic T cells (but not with a control anti-myelin bask protein (MBP) line) secrete significantly less IFN-γ [Quintana et al., manuscript submitted]. Thus, anti-ergotypic responses can control the arthritogenic response *in vitro*. Our model for the role of Hsp60-specific T cells in anti-ergotypic response is depicted in Figure 16.3. However, the contribution of the anti-ergotypic response to the regulatory functions of heat shock protein–specific T cells in AA and other autoimmune disorders *in vivo* is still unknown.

16.6.2. Innate immunity

16.6.2.1. Innate activation of regulatory T cells

Heat shock proteins are endogenous ligands for innate receptors. Hsp60 and Hsp70 activate TLR4 and TLR2 [89]; Hsp70 and Hsp90 have also been reported to signal via CD40 and CD91 [90, 91]. See Chapters 7, 8 and 10 for more details of the receptors for chaperones. Caramalho and colleagues have reported that regulatory $CD25^+$ T cells are activated via TLR4 [92]. Thus, it is possible that self-heat shock proteins directly activate regulatory cells via innate receptors. This hypothesis is partially supported by the findings made by Dr. Gabriel Nussbaum in our laboratory, who has generated NOD mice lacking a functional TLR4. NOD mice carrying a non-functional *tlr4* allele show an early onset and an increase in the incidence of spontaneous diabetes. Interestingly, the sensitivity of those NOD mice to CAD remains unchanged (Dr. Gabriel Nussbaum, personal communication). Cyclophosphamide is thought to deplete regulatory cells [44]; thus, these findings suggest that TLR4-mediated signals triggered by self-ligands do activate regulatory cells involved in the control of autoimmune diabetes.

16.6.3. Hsp60 triggers anti-inflammatory activities in T cells via TLR2

Hsp60 and p277 can directly inhibit chemotaxis and activate anti-inflammatory activities in human T cells, via TLR2 [93]. Human T cells activated by mitogen in the presence of Hsp60 or p277 also show a decreased secretion of IFN-γ and an increased secretion of IL-10 (unpublished observations). Thus, soluble Hsp60

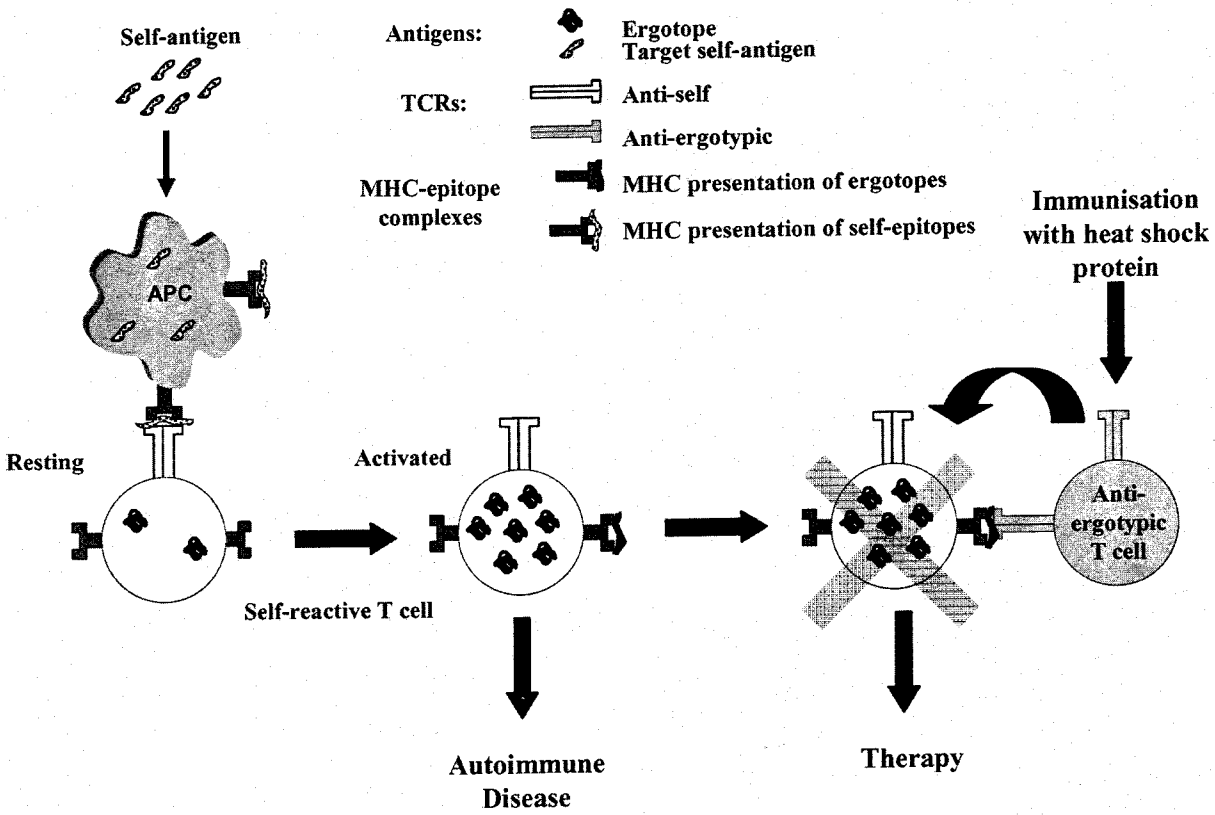

Figure 16.3. Anti-ergotypic response mediated by Hsp60-specific T cells.

or p277, acting via TLR2, can modulate T cells involved in the progression of inflammation.

16.7. Heat shock proteins: physiological modulators of inflammation

Inflammation is physiological [94]; it plays a role in processes ranging from wound healing [95] to neuroprotection [96]. However, uncontrolled inflammation can lead to disease and, as a consequence, precise mechanisms have been selected through evolution for the tight control of inflammation.

Inflammation induces heat shock proteins and heat shock protein–specific immune responses. However, heat shock proteins and the immune responses directed against them can both promote and inhibit inflammation. Heat shock proteins are central nodes in physiological networks that control inflammation; they integrate the intra-cellular response to stress with the inter-cellular signals that spread a cascade of pro- or anti-inflammatory responses (Figure 16.4A). The variety of the anti-inflammatory responses co-ordinated by heat shock proteins is as diverse as the biological activities of heat shock proteins. In the short term, heat shock proteins can activate regulatory mechanisms via innate receptors. In the long term, heat shock proteins can also trigger adaptive immunoregulatory T cell responses directed against heat shock proteins or other self-proteins. Heat shock proteins bridge the innate and adaptive immune responses involved in the physiological control of inflammation.

Regulatory networks centred on heat shock proteins can be boosted by several methods to treat autoimmune disease (Figure 16.4B). Indeed, these therapies could operate by simply mimicking the effects that the environment has on the immune system. The rise in the standard of living achieved during the past century in the developed world seems to have diminished the microbial stimulation of the immunoregulatory functions that keep immune balance. This reduction in immune stimulation might contribute to the increased incidence of autoimmune diseases observed in developed countries, as we have discussed elsewhere [97].

16.7.1. Network cross-reactivity

It is striking that immunisation with defined heat shock proteins leads to the induction of T cell responses directed to other structurally unrelated heat shock protein molecules [31]. The definition of immunological cross-reactivity, usually found in immunology textbooks, would not account for this unexpected finding. Herein, we would like to propose a new definition for cross-reactivity.

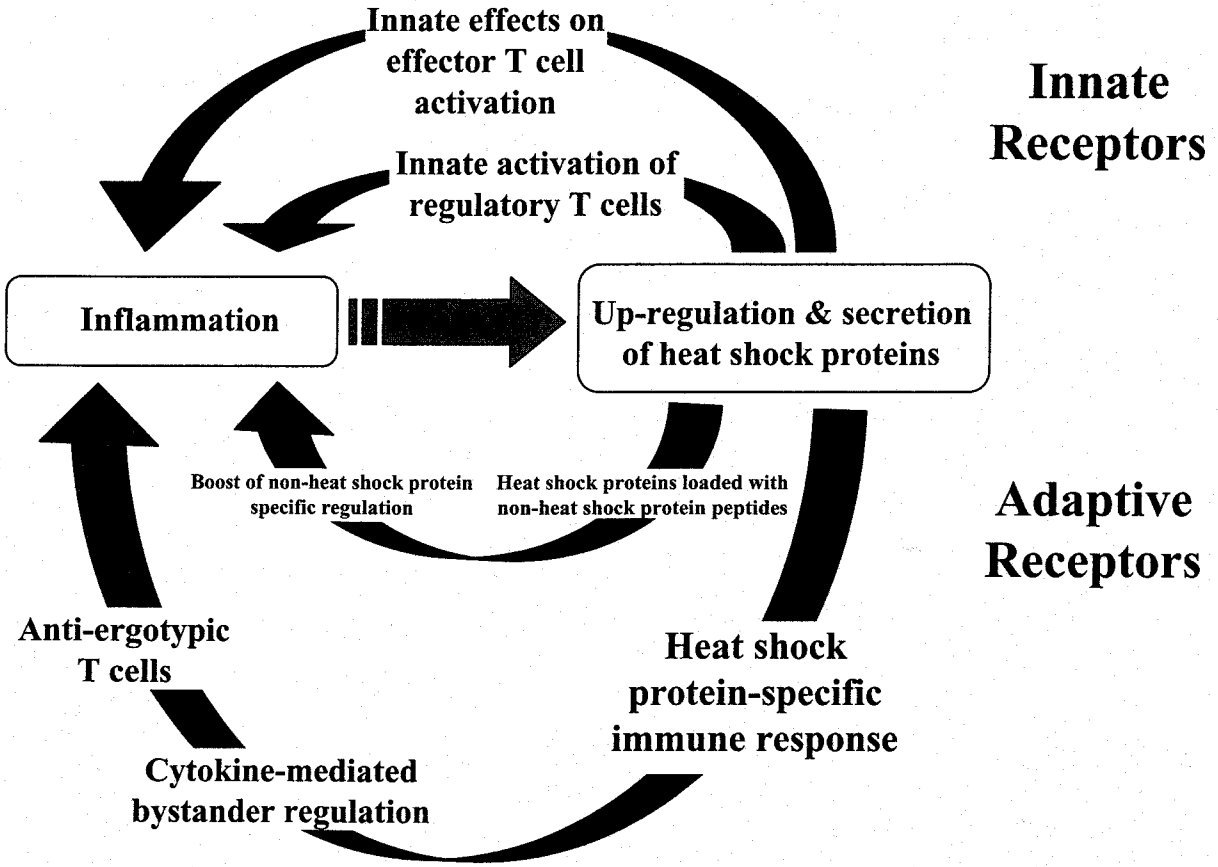

Figure 16.4. Heat shock proteins as regulators of inflammation: (A) physiological regulation of inflammation by heat shock proteins and (B) therapeutic/environmental regulation of inflammation by heat shock proteins.

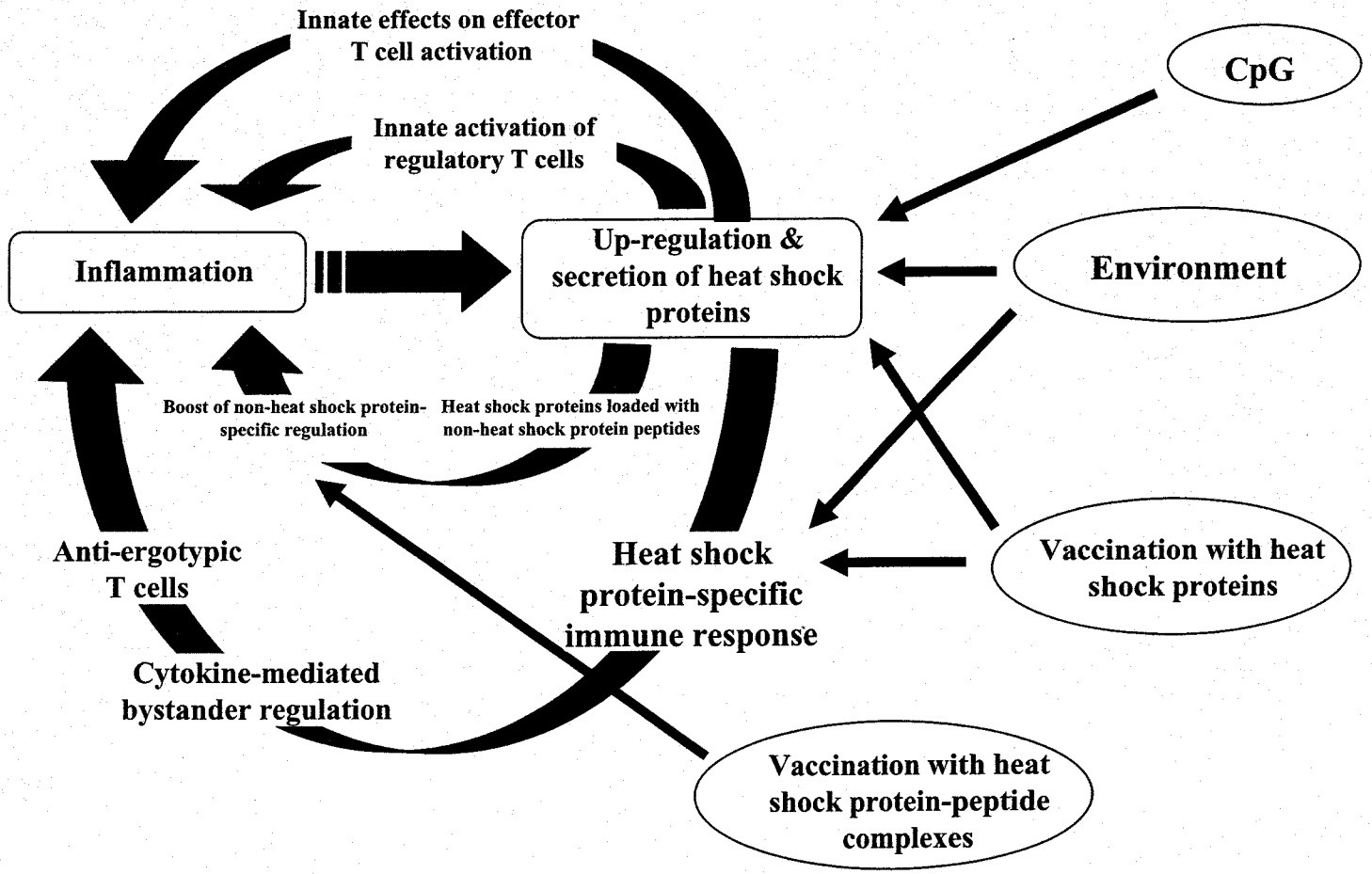

Figure 16.4. (*continued*)

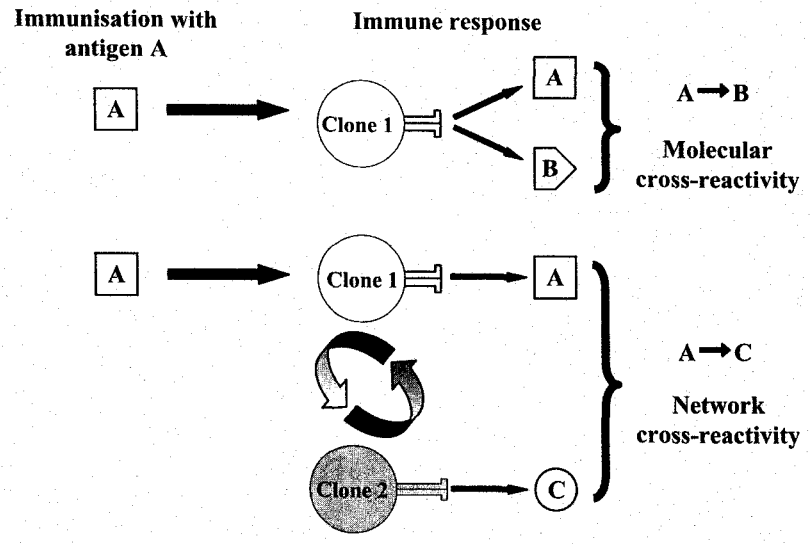

Figure 16.5. Immunological cross-reactivity.

We define *molecular* cross-reactivity as the classical cross-reactivity that exists between antigens that share sequence homology, leading to their recognition by the same T or B cell clones (Figure 16.5). We define *network* cross-reactivity as the immune connection existing between molecules that bear no sequence homology, like Hsp60 and Hsp70, but whose specific immune responses are somehow interconnected (by self-vaccination or another mechanism). Thus, the organisation of the immune network is such that immunisation with antigen 1 can induce an immune response that targets not only antigen 1, but also antigen 2, in the absence of any single T or B cell clone that recognises both antigens (Figure 16.5). The regulatory properties of heat shock proteins might result from the molecular cross-reactivity existing between self and microbial heat shock proteins and the network cross-reactivity that exists between different endogenous heat shock proteins.

The study of the pro- and anti-inflammatory mechanisms mediated by heat shock proteins could lead to the design of novel therapies for autoimmunity: therapies aimed at reinforcing the built-in mechanisms that are based on the physiological control of the immune function by heat shock proteins [94, 98]. The initial success of the Hsp60 peptide p277 in treating human T1DM shows the feasibility of this approach [49]. A deeper understanding of the multiple activities of heat shock proteins on the immune system and body homeostasis might allow us to extend these findings to other autoimmune disorders.

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