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