Review

The role of heat shock proteins in Behçet’s disease

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ABSTRACT

Heat shock proteins (HSP) are highly conserved molecules with scavenger activity that are involved in the correct folding of newly synthesized proteins. Increased T and B cell activity against 60/65 kD HSP is observed in different ethnic populations in Behçet’s disease (BD) with both αβ and γδ T cell responses. Although the specificity of these responses is not clear, animal models of uveitis treated with either subcutaneous and oral HSP-derived peptides suggest a significant role of HSPs in the immunopathogenesis of BD. Recent developments in the innate immune system with the description of toll-like receptors (TLR) and HSP60 as a ligand for TLR-2 and TLR-4 suggest also the role of HSP60 as an endogenous “danger” signal to the immune system with rapid inflammatory cytokine release and the enhancement of adaptive T1-type responses. Activation of both innate and adaptive responses with HSPs also fit well into the clinical spectrum of BD with both early, limited responses (recurrent ulcers, pathergy, etc.) and chronic lesions (posterior uveitis, thrombosis, neuro-BD, etc.).

Introduction

Behçet’s disease (BD) is a multi-systemic disorder with mucocutaneous, ocular, arthritic, vascular and central nervous system involvement (1). Neutrophil hyperreactivity with increased superoxide production, phagocytosis and the release of enzymes suggest an activated innate immunity (2). However, a Th1 type, oligoclonal peripheral blood CD4+ and CD8+ T cell repertoire is also present and implicates the role of an antigenic drive of the adaptive immune system (2-5). Main current candidates for a “Behçetogenetic antigen” are viral agents such as Herpes Simplex virus (HSV) and atypical Streptococcal (1-3). Clinical observations such as increased oral manifestations after dental treatments, streptococcal hypersensitivity in skin tests, dominance of atypical streptococcal species in the oral flora of BD patients and recent reports of beneficial anti-bacterial therapy have raised the possibility of a role of streptococcus in BD (6-8). As a wide variety of Streptococci (sanguis, salivarum, etc.) are implicated, antigens common to various species are logical candidates for immune stimuli in BD.

60/65 kD Heat Shock Protein (HSP60)

Heat-shock proteins are a group of intra-cellular proteins which scavenge for other intracellular proteins under denaturing stress conditions such as infections, hypoxia, trauma and toxic drugs (9). Significant sequence homology exists between mammalian and microbial HSPs (mycobacterial and streptococcal HSP65s have over 90%, and human HSP60 over 50% homology) (6). In addition to their physiological roles, they are implicated in the pathogenesis of various immune-mediated disorders such as infections (tuberculosis, chlamydia), autoimmune diseases (rheumatoid arthritis, multiple sclerosis), vascular thrombosis (atherosclerosis) and malignant disorders (9).

As HSPs form part of the intra-cellular protein transport mechanisms under stress, they are released from dying tumor cells in large amounts. These extra-cellular HSPs are taken by antigen-presenting cells (APCs) and can stimulate tumor-specific T cells directly (10). HSP60 with a molecular mass of 60KD is mainly expressed in the mitochondria. However, during stress an intracellular redistribution of HSP60 and cell surface expression has been reported. HSP65 is also expressed on monocytes after IFN-γ stimulation and on T cells undergoing apoptosis (11). Local HSP60 over-expression is present in BD in the epidermal regions of...
active skin lesions such as erythema nodosum and papulopustules, compared to other inflammatory skin disorders (12).

A molecular mimicry-based pathogenic mechanism for HSPs was first suggested by Lehner et al.; in their scenario human HSP-responsive T cells stimulated by microbial counterparts (cross-reactivity) might trigger T cell activation and memory responses, thus influencing the chronicity and relapsing-remitting nature of BD lesions (6). The first evidence supporting this hypothesis was the identification of anti-HSP65 antibodies cross-reactive with oral mucosal homogenates and oral streptococcal (13). Four epitopes of mycobacterial HSP65 (amino acid sequences 111-25, 154-72, 219-33 and 311-26) and their human counterparts with 50-80% homology were recognized to be immunodominant antigens for T and B cell responses in BD in studies from UK, Japan and Turkey (14-18).

PPD and HSP65 specific long-term T cell lines (mainly TCR ab+CD4+ or CD8+) are also highly reactive to human HSP60-derived peptides in both BD patients and healthy controls, showing that these self-reactive T cells are escaping central tolerance and are present in the peripheral repertoire (19). However, most PPD-stimulated lines responded to epitope 425-41 of HSP60 in BD patients (an epitope not described in primary cultures), whereas epitope 336-51 dominated in controls. The reaction pattern changes with HSP60 stimulation, which drives a dominant 336-51 response in both groups. This observation suggests that differential epitope recognition of the immune system associated with the balance of microbial versus human HSP expressions might determine the level of pathogenic self-reactivity in BD.

Although some in vitro data implicating Th2 activation has been reported, mainly in HLA type disorder with an interleukin-2 (IL-2), IL-12 and interferon-γ (IFN-γ) cytokine profile (1-3). In this context, stimulation of peripheral blood (PB) mononuclear cells with human HSP60 peptide 336-51 produced IFN-γ, tumor-necrosis factor-α (TNF-α) and IL-12, whereas Th2 cytokines IL-4 and IL-10 suppressed the proliferative responses in BD (20).

γδ-T cells and HSPs

γδ T-cells are a minor T cell population (1-10% of PB T-cells) that express T cell receptors (TCRs) comprised of γ and δ heterodimers (21). γδTδ+ T cells, a major subset of γδ T cells in the PB, recognize non-peptide antigens produced by bacteria. The second major subset, Vδ1+ T cells, are enriched in tissue mucosa and recognize self and foreign lipids presented by CD1 and the stress-inducible MHC class I related chains A and B (MICA and MICB). γδ T cells have important roles in immunity as a “first line of defence” against microorganisms, surveillance against tumors and possibly in modulating auto-immune responses. They are also thought to influence adaptive immune responses by secreting IFN-γ and IL-4, towards a Th1 or Th2 profile. Peripheral blood γδ T cells have been observed to be elevated in most (22-24), but not all studies in BD (4, 12). These γδ T cells are associated with active disease and show higher expression of CD29, CD69 and production of IFN-γ and TNF-α (22-24). Whereas PB γδ T cells are mainly Vδ2+, local fluids such as bronchoalveolar lavage and cerebrospinal fluid are dominated by Vδ1+ T cells (22), except in a study reporting elevated Vγ982 T cells in the intraocular fluid (25). Perhaps more significant is the local γδ T cell presence in active BD lesions where HSP65 expression is upregulated, with possible HSP-γδ T cell interactions (12).

γδ-T cell activation was also recently shown with oral flora extracts which might contain HSPs as antigens (24). KTH-1 (a crude extract of Streptococcus sanguis SSH-83) caused increased IL-6 and IFN-γ secretion by PB T cells of BD patients (26). KTH-1 also upregulates γδ T cells in short-term T cell cultures and KTH-1 specific γδ T-cell lines secrete pro-inflammatory mediators such as IL-6, IL-8 and TNF-α (27). HSP-derived peptide responsive T cells were mainly of the γδ T-cell subset in the UK (16), whereas CD4+ T cells were reported from Japan and Turkey (17, 28). However, in contrast to these data, in a recent study no response to HSP60 was observed in any T cell line derived from the intra-ocular fluid of uveitis patients with BD, whereas non-peptide prenyl pyrophosphate reactive γδ T cells were present (25). Although no functional data is available, the recognition of MICA alleles, which are upregulated in the epithelium by the Vδ1 subset merits further studies, as BD has been shown to have a genetic association with MICA alleles (29).

HSP60 and antibody responses

Similar to T cell studies, “cross-reactivity” has also been demonstrated for anti-HSP60 antibodies. Both anti-streptococcal and anti-retinal HSP60 antibodies are elevated in the sera of BD patients with uveitis (30). In competitive ELISAs both antigens inhibited the binding of anti-HSP60 antibodies to each other. Increased anti-HSP65 antibody responses are also present in the cerebrospinal fluid (CSF) of neuro-BD patients with parenchymal involvement (31).

Animal models

In an animal model with subcutaneous HSP inoculation, human HSP-derived, immuno-dominant peptides caused an experimental uveitis without other symptoms of BD in rats (32). Oral administration of peptides also induced uveitis in contrast to most models of “oral tolerance” where mucosal immune encounter with pathogenic antigens suppress the immune activity (33). Heat-shock to oral mucosa also increases S. sanguis colonisation, oral inflammatory cytokine expression (IL-2, IL-6, IFN-γ and TNF-α) and mild iridocyclitis in mice, implying that stress might be crucial to the breakdown of mucosal defences and anti-HSP reactivity (34).

αβ-crystallin and HSP70

αβ-crystallin is a small stress protein constitutively abundant in the vertebrate eye lens and also to be found in several other organs including skeletal muscle, kidney epithelial cells and glia cells of the central nervous system (35). Serum and CSF IgG and serum IgM antibody responses to αβ-cryst-
Fig. 1. The dual role of HSP60/65 in the pathogenesis of Behçet’s disease. Cross-reactive epitopes of bacterial HSP65 might be presented by MHC class II molecules on APCs and stimulate self-HSP60 reactive T cell clones which escape high affinity clonal deletion in the thymus and are anergic in the peripheral blood. Self-HSP60 might also directly stimulate APCs by the HSP60 receptor and TLR/CD14 complex and upregulate MHC class II and CD80/86 molecules with increased IL-12 secretion. Self HSP60 reactive clones might then pass to the ocular compartment, be further activated with retinal-HSP60 and may cause a chronic ocular inflammation.

HSP: heat-shock protein; MHC: major histocompatibility complex; TCR: T-cell receptor; TLR-4: Toll-like receptor-4; APC: antigen presenting cell.

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of those that are simply “foreign” (47). An upregulated CD14 expression both on monocytes and neutrophils and elevated serum soluble CD14 levels are previously described in BD patients (48, 49). Recently, we have also observed a very early activation (CD69) and CD14 response to HSP60 on PB mononuclear cells of BD patients which might be associated with an HSP60-induced innate activation through APCs (50). Presence of highly expressed HSP60 in tissues such as oral mucosa induced by necrosis or inflammation (12) might upregulate CD14/HSPs and other immune mechanisms in BD. Elucidating the exact role of HSPs in BD pathogenesis might also pave the way to less toxic therapeutic approaches with HSP immunomodulation such as “oral tolerisation” recently reported with HSP peptide 336-51 linked to cholera toxin B subunit in an animal model (55) and in uveitis patients (56).

HSPs and other immune mechanisms

A final possible role of HPSs is their adjuvant function. In addition to the self presentation discussed previously, HSPs as molecular chaperones might transfer antigenic peptides to “professional” APCs which then activate specific T cells or enhance the presentation of MHC-peptide complexes by poorly immunogenic tumor cells (10). Deficiencies in HLA class I expression on tumor cells has been proposed as a mechanism to interfere with the anti-tumor cytotoxic T cell responses (CTL). HSP65 transfected clones of melanoma cell lines exhibit significantly increased levels of HLA class I expression and are effectively lysed by allogeneic CTL (51). Similarly, increased HSP60 expression of APCs may help to antigen presentation by BD-associated HLA-class I molecule HLA-B51 to the effector T cells and may enhance pathogenic immune responses. The reduced expression of HLA class I molecules on monocytes of HLA B51+ patients (which rose after IFN-α therapy) suggests an impaired expression of HLA-B51 in BD (52). Although an association of anti-HSP60 responses and HLA-B51 is not previously demonstrated (16), HSP-HLA interactions require further studies.

Recently, it was also demonstrated that both HSP65 and HSP70 upregulate CD8+ T cell derived β-chemokine expression (RANTES, MIP-1α and MIP-1β) both directly and also as an adjuvant linked to peptides indirectly, in non-human primates (53). This stimulation of innate immunity might drive adaptive responses and attract APCs (dendritic cells, macrophages) and effector T cells. Both α and β-chemokines are elevated in BD sera (54) and they might be linked to the chemotraction of inflammatory cells. In conclusion, HSPs might stimulate both innate and adaptive immune mechanisms in BD. Elucidating the exact role of HSPs in BD pathogenesis might also pave the way to less toxic therapeutic approaches with HSP immunomodulation such as “oral tolerisation” recently reported with HSP peptide 336-51 linked to cholera toxin B subunit in an animal model (55) and in uveitis patients (56).

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