Computers in Biology and Medicine 42 (2012) 1079–1085

Contents lists available at SciVerse ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/cbm

The possible role of HSPs on Behçet's disease: A bioinformatic approach

Younes Ghasemi [a,b,*], Fatemeh Dabbagh [a,b], Sara Rasoul-Amini [a,b], Afshin Borhani Haghighi [c], Mohammad Hossein Morowvat [a,b]

[a] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran
[b] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Neurology, Shiraz University of Medical Sciences, Shiraz, 71345, Iran

ARTICLE INFO

Article history:
Received 15 January 2010
Accepted 29 August 2012

Keywords:
Autoimmunity
Behçet's disease
Bioinformatics analysis
Heat shock protein (HSP)
Immunoinformatics

ABSTRACT

Current evidence lends increasing support to immunoinflammatory mechanisms as one of the prime pathogenic processes involved in the development and progression of Behçet's disease (BD). It has been observed that most human beings have cellular and humoral reactions against microbial heat shock proteins (HSPs). The observation that eukaryotic and prokaryotic HSPs have high sequence similarity promoted the hypothesis that HSPs might be potential candidates for molecular mimicry and could act as potentially dangerous autoantigens. In this study, using bioinformatics tools, we examined the hypothesis that HSPs (evolutionarily conserved proteins), which are present in pathogenic and commensal organisms and their hosts, provide the stimulus that initiates BD in susceptible individuals. In this regards, the nucleotide and amino acid sequences of the human HSP 60 kDa and bacterial HSP 60 kDa deposited in the NCBI and PDB databases were subjected to analysis using bioinformatics tools, including The CLC Sequence Viewer and MEGA softwares. These data showed that the sequence homology between bacterial and self HSPs (leading to cross-reactivity and molecular mimicry phenomenon) may be associated with the development of the disease; and suggesting that microbial HSPs, which cross-react with host tissues and elicit significant immune responses are possible pathogenetic agents involved in the development and progression of BD.

1. Introduction

Behçet's disease (BD) owes its name to the Turkish physician professor Huluci Behçet, who, in 1937, described the classic trisymptom complex of hypopyon, iritis, and orogenital aphthosis [1]. Today, BD is a more complicated entity and is defined as a chronic, relapsing, multisystemic idiopathic inflammatory problem with mucocutaneous (erythema nodosum, pustular vasculitis), ocular (anterior and posterior uveitis), arthritic, vascular (both arterial and venous vasculitis), and central nervous system (meningoencephalitis) involvements [2–4]. The etiology and pathogenesis of this disease have been explored extensively. Genetic susceptibility, environmental factors (viral and/or bacterial infections), inflammatory response abnormalities (heat shock proteins, dysregulated nitric oxide production) and abnormal immune response play also a major role in BD pathogeny [5]. Current evidence lends increasing support to immunoinflammatory mechanisms as one of the prime pathogenic processes involved in the development and progression of BD [6]. Common factors linking some of the possible pathogenetic agents are microbial stress or heat shock proteins (HSPs), which cross-react with host tissues and elicit significant immune responses [7].

Heat shock proteins or chaperonins, as they were previously called, are a group of evolutionarily conserved proteins that show high sequence homology between different species (mycobacterial and streptococcal HSP65s have over 90%, and human HSP60 over 50% homology) [6,8]. Despite their evolutionary sequence conservation, even between microbes and their host self-homologues, these microbial proteins are highly immunogenic and have been implicated in the control of autoimmune inflammation due to a cross-reactive immune response [9]. These constitutive proteins are found in all eukaryotes and prokaryotes [10]. In addition to being constitutively expressed (cHSP), under stress conditions (e.g. high temperature; radiation; viral and bacterial infections; hypoxia; ischemia; metabolic disruption; various inflammatory mediators; and deleterious substances including heavy metals, arsenic, ethanol, reactive oxygen and amino acid derivatives) synthesis of HSPs is markedly up regulated (induced HSP or iHSP) [11,12]. Intracellular HSPs (either cytosolic or resident within the endoplasmic reticulum) have many important functions: as protein folding machines, or chaperones; the protection of cells in response to stress; and the protection of cells against apoptosis [13,14]. Furthermore, depending

* Corresponding author at: Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Shiraz University of Medical Sciences, P.O. Box 71345-1583, Shiraz, Iran. Tel./fax: +98 711 242 6729.
E-mail address: ghasemiy@sums.ac.ir (Y. Ghasemi).

0010-4825/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.compbiomed.2012.08.009

on their localization, HSPs either exert immune activation or protection against environmental stress [15]. In addition to their physiological roles, they are implicated in the pathogenesis of various immune-mediated disorders such as infections (tuberculosis, chlamydia), auto-immune diseases (Behçet's disease, rheumatoid arthritis, multiple sclerosis), vascular thrombosis (atherosclerosis) and malignant disorders [8].

The observation that eukaryotic and prokaryotic HSPs have high sequence homology promoted the hypothesis that HSPs might be potential candidates for molecular mimicry and could act as potentially dangerous autoantigens [9]. An enhanced and dysregulated immune response has been suggested as the cause of BD manifestations, and this can be triggered by environmental agents, including certain microorganisms, in genetically susceptible individuals [16]. For example, the "Behçet's Disease Research Committee of Japan" reported systemic BD symptoms one to two weeks after contact with *Streptococcus sanguinis*, *S. pyogenes*, *S. faecalis* and *S. salivarius*, *Escherichia coli* and *Klebsiella pneumoniae* [17]. HSP60/65 is an immunodominant antigen that is derived from mammalian/bacterial 60/65-kDa HSP. The increased expression of both self and infective stress proteins and the sequence homology and cross-reactivity between microbial HSP (better known as groEL) and human HSP led to the concept that HSP might be involved in the aetiopathogenesis of Behçet's disease [18–20]. Studies showing elevated HSP levels and anti-HSP antibodies, increased expression of HSPs and their receptors (TLRs), and cellular immunity to HSPs, which are present in autoimmune inflammatory responses, such as BD, seem to support this hypothesis [9].

The aim of the present study is to provide bioinformatics proof, in addition to a brief review for the possible role of HSPs in pathogenesis of BD. Here we examine the hypothesis that HSPs (evolutionarily conserved proteins), present in pathogenic and commensal organisms and their hosts, provide the stimulus that initiates autoimmune disease in susceptible individuals. Evidences supporting the possible role of HSPs in BD are outlined briefly in following sections.

1.1. HSPs and their receptors (TLRs)

Besides modulating inflammatory responses via the induction of HSP-reactive regulatory T cells, HSPs can directly activate the immune system through surface receptors such as toll-like receptor [9]. Toll-like receptors (TLRs) have been identified as a group of receptors, which recognize specific patterns of microbial components, and regulate the activation of both innate and adaptive immune systems [21]. Toll-like receptors, which are expressed on phagocytes and other cells, recognize "pathogen-associated molecular patterns" in microbes and mediate inflammatory signal transduction [22]. Human HSP60 induces a potent inflammatory response in the innate immune system via its ligands, toll-like receptors, and operates in a similar manner to that of classical pathogen-derived ligands and can thus activate non-specifically the innate immune system and stimulates the maturation of dendritic cells [23]. These data are consistent with the interpretation that the immune response against TLR4 ligands, such as HSPs and LPS, plays an important part in development of BD [24].

1.2. HSP60 expression as an autoantigen

Due to high conservation and immunogenicity of HSPs, it is generally assumed that self HSPs might serve as the endogenous targets of the immune response initiated by the homologous foreign HSPs [25]. Besides their immunodominance as microbial antigens, under various circumstances HSPs do elicit immune responses also when (over-)expressed as self antigens by cells or tissues. And this seems to be a peculiar feature of HSP, especially because in many cases immune responses to this self antigen are not associated with pathogenic autoimmunity [26]. Recently it has been demonstrated that autologous HSP60 which serves as a danger signal to the innate immune system, leads to production of proinflammatory cytokines like TNF-alpha, IL-12 and IL-15 and mediates monocyte adhesion to endothelial cells [6].

1.3. Cellular immunity to HSP60/65

It has been observed that most human beings have cellular and humoral reactions against microbial HSPs. Autoantibody levels against HSPs are significantly increased in patients with BD and T lymphocytes specifically responding to HSPs have been demonstrated in these patients [6]. Increased T and B-cell activity against 60/65 kDa HSP is observed in different ethnic populations in BD with both $\alpha\beta$ and $\gamma\delta$ T-cell responses [8]. HSP60 is a lymphocyte activating agent that causes vigorous proliferation of T cells in an antigen-specific fashion [20,27]. After uptake of HSP-peptide complexes by antigen-presenting cells and "cross-presentation" of HSP-chaperoned peptides on major histocompatibility complex class I molecules, a CD8-specific T-cell response is induced [7]. Self-HSP and/or microbial HSP homologous to the self-HSP activates self reactive T cells specific to the HSP peptides. Bacterial HSPs may activate self-HSP reactive T cells which have been rendered unresponsive by self tolerance mechanisms. This process would lead to positive selection of autoreactive T cells in BD [28].

T-cell epitope mapping has identified four peptides derived from the sequence of the 65 kDa HSP that stimulate specifically TCR+ lymphocytes from patients with BD. These peptides (111–125, 154–172, 219–233 and 311–325) show significant homology with the corresponding peptides (136–150, 179–197, 244–258 and 336–351) derived from the human 60 kDa HSP. B-cell epitopes within mycobacterial HSP65 or human HSP60 overlap with the T-cell epitopes and both IgG and IgA antibodies have been identified. Among the four immunodominant T- and B-cell epitopes, peptide 336–351 of the 60 kDa HSP is significantly associated with BD [29–36].

1.4. Antibodies against HSP60

Patients with Behçet's disease have been shown to have antibodies against HSP60s, but the results of the ELISA antibody titer assay shows that, although the various HSP60s share a common basic antigenicity, they differ in reactivity to the anti-HSP60 antibodies in the sera of the BD patients [20,37]. Anti-HSPs are detected in inflammatory diseases, thus, antibodies to HSPs, regardless of Ig classes (IgG, IgM, IgA), may be useful to detect inflammatory response of host cells [12].

2. Materials and methods

In order to assess the hypothesis of molecular mimicry between microbial and self HSPs in BD pathogenesis, it would be helpful to compare nucleotide and amino acid sequences of human and microbial HSPs, using bioinformatics tools. Therefore, the nucleotide and amino acid sequences of the *Homo sapiens* mitochondrial heat shock 60 kDa protein 1 variant 1 (HSPD1) were retrieved from the NCBI database. The nucleotide and amino acid sequences of the 60 kDa HSPs present in pathogenic and commensal organisms, which were reported to have role in BD pathogenesis [2,4,12,38–55], were also retrieved from the NCBI database. The GI and GenBank accession numbers of studied organisms are summarized in Table 1. The obtained sequences