Significance of serum antibodies against HSP 60 and HSP 70 for the diagnostic of infectious diseases

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Heat shock proteins (HSP) represent important antigenic targets for the immune response, playing an important role in the pathology and infectious diseases control. The purpose of this work was to investigate the levels of HSP60 and HSP70 specific antibodies in the bloodstream of patients with different bacterial infections and cancer, in order to evaluate their potential role as diagnosis markers of different infectious diseases. Detection of specific anti-HSP 60 and HSP 70 serum levels was performed by ELISA. Statistical analysis of data by multivariate logistic regression was performed using GraphPad Prism software and statistical tests based on chi-square and Student t-test. High levels of anti-HSP60 were found in patients with localized infections, while the levels of anti-HSP70 were higher in the group with generalized infections. The serum levels of both anti-HSP 60 and anti-HSP70 were significantly increased in patients with Gram-negative bacterial infections, as compared with patients harbouring infections produced by Gram-positive and fungal strains, demonstrating their potential use as additional diagnosis and prognosis markers in infections with this etiology.

Introduction

Stress or heat shock proteins (HSPs), one of the most phylogenetically conserved superfamilies of chaperones could be expressed at very low levels under non-stressing conditions, including different stages of the cellular cycle, development, and differentiation, but are highly induced by environmental and patho-physiological stress factors.

During carcinogenesis, HSPs have been reported to show alteration of their expression levels, either increasing or decreasing. Although HSP expression was recognized as a factor of prognostic value in certain tumors, the data are limited and the results often are contradictory. HSP60 and its co-chaperone HSP10 are expressed early during the development of a malignant phenotype. For example, in colon and uterine cervix cancers, HSP10 and HSP60 expression levels are increased as cells progress from their normal state to dysplasia and cancer. HSP 60 over expression was associated with a poor prognosis in the ovarian cancer, but HSP10 over-expression was associated with a lower risk of progression in patients exposed to chemotherapy. HSP70 basal level is unusually high in cells or tissues from a wide range of tumors, contributing to tumor genesis through their pleiotropic activities on proteins that influence tumor cell growth or blocking the apoptosis at different levels, using caspase dependent or independent pathways.

Recognition of peptides derived from HSPs by the immune system can have an anti-inflammatory effect and down-regulate the chronic state of inflammation via modulation of cytokine secretion. So, despite their ubiquitous and high homology among different species, they also represent important antigenic targets of the cellular and humoral immune response, playing thus an important but yet, unclarified role in the pathology and infectious diseases control, as well as in the survival and virulence of pathogenic bacteria.

In this context, our aim was to investigate the serum levels of antibodies to HSP60 and HSP70 in patients with different localized and generalized infections, by comparison with cancer patients, used as positive control group, in order to evaluate their potential role as an aid in the diagnosis of different infectious diseases.

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Results

The highest levels of serum HSP60 and HSP70 antibodies were found in patients with laryngo-pharyngeal cancer (p = 0.002, Kruskal-Wallis test, Gaussian Approximation), probably due to increasing of plasma levels of intracellular proteins through cell cytolysis. Also, high levels of anti-HSP60 were found in patients with localized infections. The levels of anti-HSP70 were higher in the group with localized infections (Table 1).

Concerning the levels of the investigated antibodies inside the group of patients with generalized and localized infections with different aetiologies, it could be noticed that the levels of both anti-HSP60 and anti-HSP70 were significantly increased in patients with infections produced by Gram-negative microorganisms, as compared with those produced by Gram-positive and fungal strains (Fig. 1).

Discussion

In order to evaluate the potential role of antibodies to HSP60 and HSP70 in different infectious diseases, we have investigated the levels of these antibodies in the bloodstream of patients with different infections, comparatively with those occurred in cancer patients. Our results demonstrate that the patients with infectious diseases exhibited high levels of HSP 60, especially when the infections were caused by Gram-negative bacteria.

Cross-reactivity between microbial and HSP supports the involvement of HSP in the autoimmune diseases pathogenesis. Zlacka et al. (2006) investigated the anti-HSP60 and anti-HSP70 serum levels in juvenile patients with idiopathic arthritis. While the anti-HSP60 levels were similar to those obtained for the control group, the anti-HSP70 level was much higher in patients versus controls, suggesting their implication in this disease. An altered immune response to HSP60 in rheumatoid arthritis has been also signaled by vanHalm et al. (2006).

The viral, bacterial, parasitic and fungal infections were incriminated to increase the levels of HSP antibodies; therefore we investigated their potential to be used as biomarkers for the severity of bacterial diseases. In our study, we have found a positive correlation between bacterial infections with Gram negative etiology and increased levels of anti-HSP antibodies, especially anti-HSP60. During infection, the host cells and microbial agent are engaged in a stressful relationship, requiring the rapid and increased synthesis of HSP. Some studies report the increased concentrations of serum anti-Helicobacter pylori HSP60 in patients diagnosed with H. pylori infection and peptic ulcers, the measurement of these antibodies proving to be useful for the monitoring of eradication therapy efficiency.

It has been shown that the HSP60 of Gram-negative bacteria are involved in the synthesis of cellular bacterial wall, suggesting their location at the surface of bacterial cells, accessible to antibodies. This could explain also the high levels of anti-HSP60 obtained in patients with Gram-negative infections. Another possible explanation for the increased anti-HSP60 serum levels in Gram-negative infections could be related to the fact that HSP60 stimulates the macrophage activity by specific LPS binding, through a HSP60 epitope region. Therefore, mammalian HSP60, specialized for the recognition and binding of microbial structures, may be involved in Gram-negative motifs recognition by phagocytic cells. Thus, the anti-HSP60 immune response activation is potentiated by the presence of bacterial LPS, as it happens in Gram-negative infections.

To our knowledge, this is the first report showing the specific association between the anti-HSP increased serum levels, particularly of the anti-HSP60 and the presence of Gram negative bacterial infections.

Table 1. The average of the HSP60 and HSP70 antibodies titers, according to the pathological stratification of the patients

|                  | Generalized infections | Localized infections | Laryngo-pharyngeal cancers |
|------------------|------------------------|----------------------|----------------------------|
| Anti-HSP60       | 103.8±129.6            | 153.2±145.9          | 210.8±142.4                |
| Anti-HSP70       | 209.5±153.0            | 156.4±78.32          | 232.8±163.2                |

**Figure 1.** Graphic representation of HSP60 (left) and HSP70 (right) antibodies values according to the microbial etiology of the infectious process.
Conclusion

The increased levels of both HSP60 and HSP70 antibodies could be used as diagnosis markers in patients with cancers, but also in those with different microbial infections. The level of anti-HSP60 was increased in patients with localized infections, while anti-HSP70 in those bearing generalized infections. Also, both tested antibodies were produced with high levels in patients with Gram-negative infections, demonstrating their potential use as additional diagnosis and prognosis markers in infectious diseases with this etiology.

Material and Methods

Patients
A total number of 42 patients were analyzed, being distributed in different groups: i) patients with generalized infections (15 patients with positive blood cultures with Staphylococcus sp., Pseudomonas sp., Candida sp.); ii) patients with localized infections (17 patients); iii) 10 patients with laryngeo-pharyngeal cancers.

Quantification of HSPs' antibodies serum levels
ELISA was performed to measure the amount of the human anti-HSP60-IgG, A, M and anti-HSP70-IgG, A, M antibody sera using anti-HSP60-IgG, A, M and anti-HSP70-IgG, A, M ELISA kit (Enzo Life Sciences BVBA, Belgium) according with producer instructions.

Statistical analysis
Data analysis by multivariate logistic regression was performed using GraphPad Prism software. Other statistical tests based on chi-square and Student t-test were also applied to the obtained results. Level of significance was considered when \( P<0.05 \) or \( P<0.01 \).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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