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Hjelholt, Astrid; Carlsen, Thomas Gelsing; Deleuran, Bent; Jurik, Anne Grethe; Schiøttz-Christensen, Berit; Christiansen, Gunna; Birkelund, Svend

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Increased Levels of IgG Antibodies against Human HSP60 in Patients with Spondyloarthritis



AALBORG UNIVERSITY

Billede

tc@hst.aau.dk

Astrid Hjelholt¹, Thomas Carlsen², Bent Deleuran^{1,3}, Anne Grethe Jurik⁴, Berit Schiøttz-Christensen⁵, Gunna Christiansen^{1,6}, Svend Birkelund^{2,6}

¹Department of Biomedicine – Medical Microbiology and Immunology, Aarhus University; ²Department of Health Science and Technology, Aalborg University; ³Department of Rheumatology, Aarhus University Hospital; ⁴Department of Radiology, Aarhus University Hospital; ⁵Aarhus Clinic for Rheumatic Diseases; ⁶Loke Diagnostics, Risskov, Denmark.

Introduction

Spondyloarthritis (SpA) comprises a heterogeneous group of inflammatory diseases, with strong association to human leukocyte antigen (HLA)-B27. SpA is suggested triggered by bacterial infection, and bacterial heat shock protein (HSP) seems to be a strong T cell antigen. Since bacterial and human HSP60, also named HSPD1, are highly homologous, cross-reactivity has been suggested in disease initiation. In this study, levels of antibodies against bacterial and human HSP60 were analysed in SpA patients and healthy controls, and the association between such antibodies and disease severity in relation to HLA-B27 was evaluated.

Methods

Serum samples from 82 patients and 50 controls were analysed by Enzyme-linked immunosorbent assay (ELISA) for immunoglobulin (Ig)G1, IgG2, IgG3 and IgG4 antibodies against human HSP60 and HSP60 from *Chlamydia trachomatis*, *Salmonella e.* Enteritidis and *Campylobacter jejuni*. Disease severity was assessed by the clinical scorings Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Metrology Index (BASMI).

Patients

Serum samples was collected from the outpatient clinic at Aarhus University Hospital after informed written consent was given, according to the Danish Data Protection Agency, the Local Ethics Committee (project number 20050046) and the Declaration of Helsinki.

References

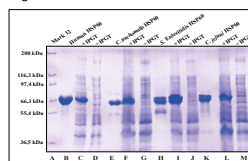
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Conclusions

- A statistical significant association between the SpA group and IgG1 & IgG3 antibodies against human HSP60 (Figure 1D, $p < 0.001$).
- Cross-reactivity to bacterial HSP60 could not be supported.
- IgG3 antibodies against human HSP60 correlated to disease activity for HLA-B27+ patients (Spearman, $r = 0.48$, $p = 0.001$).
- These results suggest that antibodies against human HSP60 in connection with HLA-B27 may be associated with SpA.

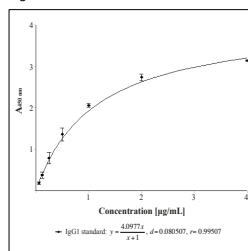
Results

Figure 1. SDS PAGE



Size and purity of each recombinant protein were evaluated by 10% SDS-PAGE gel. The different HSP60 proteins for ELISA were purified using a Ni²⁺ column and indicate as a competing reagent. Vector transformation was confirmed by PCR induction. Lane A: Molecular weight marker; Lane B, E, H, K: purified HSP60; Lane C, D, F, G, J, L & M: Total protein content from *E. coli* strain BL21 (DE3) with (+) and without (-) IPTG induction.

Figure 2. Standard curve



A standard curve made with Magellan data software used to quantify the A_{490} values from ELISA. An ELISA plate was coated with a known serial dilution (0.0625 - 4 µg/mL) of native IgG1 subsets from human myeloma plasma before incubating wells with HRP-conjugated secondary antibody. Correlation between A_{490} values and concentration was fitted by the regression output ($r = 0.99507$).

Figure 3. Antibody levels in the SpA & Control group

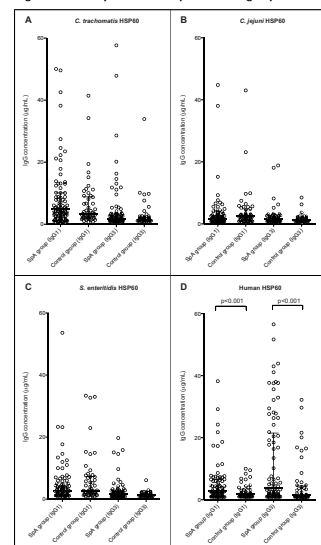


Table 1. Comparison of IgG1 & IgG3 antibodies against human & bacterial HSP60

	Median (IQR)		Statistical result of the Mann Whitney U test (p-value)
	IgG1	IgG3	
Human HSP60	2.91 (1.71;6.53)	3.38 (1.31;21.42)	$P = 0.53$
<i>C. trachomatis</i> HSP60	5.50 (3.07;10.81)	1.52 (1.07;3.08)	$p < 0.0001$
<i>C. jejuni</i> HSP60	2.14 (1.64;3.22)	1.64 (1.41;2.08)	$P = 0.0001$
<i>S. enteritidis</i> HSP60	3.22 (2.00;6.03)	1.55 (1.12;2.31)	$p < 0.0001$

Table 2. Spearman Rank Correlations coefficient (r) for the correlation between anti-human HSP60 IgG1 & IgG3 and BASDAI, BASFI & BASMI

	Total SpA group		HLA-B27 ⁺		HLA-B27 ⁻	
	IgG1	IgG3	IgG1	IgG3	IgG1	IgG3
BASDAI	0.13	0.05	0.18	0.05	0.05	0.02
BASFI	-0.03	0.01	0.08	0.00	-0.27	-0.13
BASMI	0.16	0.34*	0.29	0.48*	-0.13	0.05

** $p < 0.001$; * $p < 0.01$