Autoantibodies to heat shock proteins 60, 70, and 90 in patients with rheumatoid arthritis

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Received: 22 October 2018 / Revised: 11 November 2018 / Accepted: 14 November 2018 / Published online: 21 November 2018
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Abstract

Heat shock proteins (HSP) have been reported to impact immune responses and to be associated with rheumatoid arthritis (RA). Recently, we provided evidence for a role of autoantibodies to Hsp40 in patients with RA. In this study, we aimed at investigating the humoral autoimmune response to Hsp60, Hsp70, and Hsp90 in RA patients ($n = 39$). In comparison with healthy controls ($n = 40$), circulating IgG, IgM, and IgA autoantibodies against Hsp60, Hsp70, and Hsp90 were significantly increased in RA patients. Non-parametric statistical analysis, however, revealed no significant association between anti-HSP and disease activity or disease progression. On the other hand, positive correlations between serum levels of anti-Hsp60 IgG and IL-4 (Th2-like cytokine) or between serum levels of anti-Hsp90 IgG and IFN- γ (Th1-like cytokine) were found to be statistically significant in RA. In addition, a significant inverse correlation was found for serum levels of anti-Hsp70 IgM and TNF- α (Th1-like cytokine) in RA. Our results suggest a pronounced anti-Hsp60, anti-Hsp70, and anti-Hsp90 humoral autoimmune response in RA patients that seems not to be directly linked to RA pathophysiology, however, may have a potential modulatory impact on inflammatory status in this disease. Further investigations are needed to clarify the role of anti-HSP autoantibodies in RA.

Keywords Heat shock proteins, HSP · Rheumatoid arthritis, RA · Autoantibodies

Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of synovial joints that can lead to cartilage and bone destruction. Although much progress has been made in designing effective therapy, a lot of RA patients do not effectively respond to the current therapies and/or the therapy cause serious side effects (Guo et al. 2018). There is an increasing interest in the role of immunomodulatory heat shock proteins (HSPs) as potential treatment targets for a number of autoimmune diseases, particularly RA (Spierings and van Eden 2017). Highly conserved HSPs are constitutively expressed and/or stress-induced intracellular molecules that participate in proper protein folding and cell signaling (Tukaj 2014). Based on approximate molecular weights, HSPs are classified

into several major families including HSP40, HSP60, HSP70, and HSP90 (Kampinga et al. 2009). Beyond the classical role of intracellular HSPs, recent data showed that bacterial and self-HSP epitopes interact with both innate and adaptive arms of the immune cell components (van Eden et al. 2017). Interestingly, these interactions may lead to either stimulation of humoral (auto)immune response including production of HSP (auto)antibodies and/or activation of immune regulatory mechanisms. Although some independent observations have found that autoantibodies against HSP are present in the sera of healthy individuals (Pockley et al. 1998, 1999) and at significantly elevated levels in subjects with various autoimmune diseases including organ-specific coeliac disease (CD) and dermatitis herpetiformis, the last representing a cutaneous manifestation of CD (Kasperkiewicz et al. 2014; Tukaj et al. 2017), their role in autoimmune process is not completely understood.

Recently, we provided evidence for a role of autoantibodies to Hsp40 in patients with RA, a systemic autoimmune disease (Tukaj et al. 2010a, b; Kotlarz et al. 2013). In this study, we aimed at investigating the humoral autoimmune response to Hsp60, Hsp70, and Hsp90 in RA patients.

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Materials and methods

Patients and controls

Thirty-nine patients with RA (mean age 46.02 ± 16.40) with the mean disease duration = 8.47 ± 8.39 years and the mean disease activity score (DAS 28) = 4.13 ± 1.04 fulfilling ACR criteria for the classification of RA and 40 healthy controls (mean age 51.90 ± 6.70) were included in this study. The disease activity was assessed according to the Disease Activity Score including 28 joint counts (DAS 28) and joint damage was evaluated based on the Steinbrocker radiographic criteria. The use of human biological material was approved by the Ethics Committee of the Medical University of Gdańsk, Poland, and written informed consent was obtained according to the Declaration of Helsinki.

Detection of circulating autoantibodies to heat shock proteins

Serum IgG, IgM, and IgA autoantibodies against human Hsp60 (Abcam), human Hsp70 (Abcam), and human Hsp90 (Enzo Life Sciences) were evaluated by home-made ELISA as described previously (Tukaj et al. 2017). Briefly, medium-binding 96-well plates were coated with the respective HSP at a concentration of $0.5 \mu\text{g/ml}$ in 0.1 M bicarbonate buffer at 4°C for 18 h. The wells were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at room temperature (RT) for 2 h. After being washed with PBS + 0.05% Tween 20, sera diluted 1:100 in PBS + 0.1% BSA were incubated at RT for 1 h. Plates were then incubated with horseradish peroxidase (HRP)-conjugated anti-human IgG (Sigma)–, anti-human IgM (Abcam)–, or anti-human IgA (Biolegend)–specific secondary antibodies diluted 1:5000 in PBS containing 0.1% BSA at RT for 1 h. TMB substrate solution (Sigma) was used to visualize HRP enzymatic reaction, and the reaction was stopped by 1 M H_2SO_4 . Optical density measurements were performed at 450 nm with an ELISA plate reader.

Cytokine detection

Serum levels of IFN- γ , TNF- α , IL-10, IL-6, IL-4, and IL-2 were measured by a flow cytometric bead array (Becton Dickinson).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 (San Diego, CA). The Shapiro-Wilk test was used to verify whether the data had normal distribution. Non-normal distributed data was analyzed by the Mann–Whitney U test and Spearman's rank correlation test. P values less than 0.05 were considered as significant.

Results

Circulating anti-Hsp60, anti-Hsp70, and anti-Hsp90 autoantibodies are increased in RA patients

In comparison with sera from healthy individuals, we found that sera of patients with RA contained significantly higher levels of IgG, IgM, and IgA autoantibodies against Hsp60, Hsp70, and Hsp90, as measured by ELISA (Fig. 1).

Fig. 1 Levels of anti-Hsp60, anti-Hsp70, and anti-Hsp90 IgG, IgM, and IgA autoantibodies in sera of patients with rheumatoid arthritis (RA), as well as of age- and gender-matched healthy controls measured by enzyme-linked immunosorbent assay. The squares and horizontal bars indicate individual and mean values in each group, respectively. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Autoantibodies to HSP are associated with Th1-like and Th2-like cytokine levels in RA

Although the levels of circulating anti-Hsp60, anti-Hsp70, and anti-Hsp90 autoantibodies revealed no significant correlations with disease activity (DAS 28) and joint damage based on the Steinbrocker criteria (Table 1), some significant associations between anti-HSP and serum levels of Th1-like and Th2-like cytokine have been found in RA (Table 1). Positive correlations between serum levels of anti-Hsp60 IgG and IL-4 (Th2-like cytokine) and between serum levels of anti-Hsp90 IgG and IFN- γ (Th1-like cytokine), as well as anti-Hsp90 IgA and rheumatoid factor (RF), were found to be statistically significant in RA (Table 1). In addition, a significant inverse correlation was found for serum levels of anti-Hsp70 IgM and TNF- α (Th1-like cytokine) in RA (Table 1). No significant associations between serum levels of anti-HSP and the remaining cytokines IL-2, IL-6, and IL-10 were found (data not shown).

Discussion

Rheumatoid arthritis is a chronic systemic autoimmune disease characterized by dysregulation of the balance between

pro- and anti-inflammatory immune reactions. Therefore, therapeutic strategies which aim at the induction of immunoregulatory mechanisms and blockade of uncontrolled activation of effector cell populations are highly warranted. There is an increasing interest in the role of heat shock proteins (HSP) as potential treatment targets for a number of autoimmune diseases including RA. Both bacterial and autologous HSP interactions with host immune cell components lead to stimulation of humoral (auto)immune response including production of HSP (auto)antibodies and/or activation of immune regulatory mechanisms (van Eden et al. 2017). Recently, we found higher titers of IgG autoantibodies to Hsp40 in sera of RA patients which positively correlated with the stages of joint damage (Steinbrocker's criteria), disease activity according to Disease Activity Score (DAS 28), and serum levels of pro-inflammatory IL-6 (Tukaj et al. 2010a, b). In parallel, we found that Hsp40 modulates T cell response in RA, manifested by the reduction of T cell proliferation and stimulation of anti-inflammatory IL-10 in PBMC cultures isolated from RA patients (Tukaj et al. 2010a). It seems that despite the activation of the humoral immune response and concomitant positive correlations with disease activity and disease progression, as well as pivotal cytokine involved in the pathophysiology of RA, Hsp40 displays immunosuppressive properties, as was

Table 1 Correlation coefficients for the relationships between levels of circulating anti-Hsp60, anti-Hsp70, and anti-Hsp90 IgG, IgM, and IgA autoantibodies and selected parameters of RA patients including disease

activity (DAS 28), Steinbrocker radiographic criteria (RTG), rheumatoid factor (RF), and serum cytokine levels. *P* values ≤ 0.05 were considered significant

	DAS 28	RTG	RF	IFN- γ	TNF- α	IL-4
IgG						
Anti-Hsp60	-0.081 <i>P</i> = 0.622	0.339 <i>P</i> = 0.058	0.267 <i>P</i> = 0.188	0.253 <i>P</i> = 0.121	0.117 <i>P</i> = 0.479	0.328 <i>P</i> = 0.042
Anti-Hsp70	-0.004 <i>P</i> = 0.982	-0.220 <i>P</i> = 0.226	-0.111 <i>P</i> = 0.590	0.113 <i>P</i> = 0.493	-0.022 <i>P</i> = 0.896	-0.005 <i>P</i> = 0.977
Anti-Hsp90	-0.277 <i>P</i> = 0.088	-0.249 <i>P</i> = 0.169	-0.085 <i>P</i> = 0.681	0.372 <i>P</i> = 0.020	0.183 <i>P</i> = 0.264	0.147 <i>P</i> = 0.373
IgM						
Anti-Hsp60	0.179 <i>P</i> = 0.297	0.093 <i>P</i> = 0.633	0.199 <i>P</i> = 0.363	-0.073 <i>P</i> = 0.673	-0.161 <i>P</i> = 0.349	-0.290 <i>P</i> = 0.086
Anti-Hsp70	0.142 <i>P</i> = 0.409	0.047 <i>P</i> = 0.809	0.121 <i>P</i> = 0.582	-0.213 <i>P</i> = 0.212	-0.353 <i>P</i> = 0.035	-0.273 <i>P</i> = 0.107
Anti-Hsp90	-0.076 <i>P</i> = 0.660	-0.046 <i>P</i> = 0.811	0.087 <i>P</i> = 0.694	0.006 <i>P</i> = 0.971	-0.184 <i>P</i> = 0.282	-0.307 <i>P</i> = 0.068
IgA						
Anti-Hsp60	0.050 <i>P</i> = 0.785	0.245 <i>P</i> = 0.210	0.390 <i>P</i> = 0.089	0.014 <i>P</i> = 0.971	-0.049 <i>P</i> = 0.790	0.175 <i>P</i> = 0.339
Anti-Hsp70	0.102 <i>P</i> = 0.579	0.182 <i>P</i> = 0.353	0.390 <i>P</i> = 0.089	-0.156 <i>P</i> = 0.394	-0.075 <i>P</i> = 0.685	-0.018 <i>P</i> = 0.921
Anti-Hsp90	0.047 <i>P</i> = 0.796	0.248 <i>P</i> = 0.203	0.477 <i>P</i> = 0.033	-0.095 <i>P</i> = 0.603	-0.035 <i>P</i> = 0.850	-0.037 <i>P</i> = 0.840

earlier proved in clinical trials with DnaJp1 (bacterial Hsp40-derived epitope) in RA (Koffeman et al. 2009).

In this study, we found that sera of RA patients contained significantly higher levels of anti-Hsp60, anti-Hsp70, and anti-Hsp90 autoantibodies as compared to healthy individuals. There was no significant association, however, between these autoantibodies and diseases activity or stages of joint damage (Steinbrocker's criteria) in RA. Autoantibodies to HSP, however, were associated with Th1-like and Th2-like cytokine levels in RA suggesting the immunomodulatory role of HSP/anti-HSP in RA patients.

A majority of studies regarding immunosuppressive HSP actions concern Hsp60 and Hsp70, despite higher titer of (auto)antibodies directed to these molecules under physiological and pathological conditions (van Eden et al. 2005, 2017; Tukaj 2014). For instance, naturally occurring as well as acquired *Mycobacterium tuberculosis* anti-Hsp60 antibodies protect against the induction of murine autoimmune inflammatory arthritis (Ulmansky et al. 2002) and humanized anti-Hsp60 mAb was found to be effective in protecting and suppressing autoimmune arthritis and colitis experimental models (Ulmansky et al. 2015). In human PBMC cultures, anti-Hsp60 mAb inhibited the secretion of pro-inflammatory IL-6 and IFN- γ (Th1-like cytokine) (Ulmansky et al. 2015), the last representing antagonist cytokine to the Th2 subpopulation. This is in line with our results since positive correlation between anti-Hsp60 IgG and IL-4 (Th2-like cytokine) was found to be statistically significant in RA patients.

Further, active immunization with Hsp70, which generates Hsp70-specific IgG, can prevent or arrest inflammatory damage in murine arthritis models via IL-10 and Treg induction (Wieten et al. 2009; van Herwijnen et al. 2012). This is also partly in line with the results obtained herein since a significant inverse correlation between serum levels of anti-Hsp70 IgM and TNF- α (Th1-like cytokine) was found in RA patients.

Hsp90 plays an important role in activation of innate and adaptive cells of the immune system (Srivastava 2002). It has been shown that Hsp90 participates in stabilizing and activating the key signaling molecules including transcription factors (e.g., NF- κ B) or kinases (e.g., p38/MAPK) which regulate multiple cellular processes like inflammation (Trepel et al. 2010). Moreover, its pharmacological inhibition has increasingly become the focus of research in RA and other autoimmune diseases (Tukaj and Węgrzyn 2016). On the other hand, several independent observations have revealed immunosuppressive activity of Hsp90 or its endoplasmic reticulum analog gp96 in preclinical studies (Quintana et al. 2004; Chandawarkar et al. 2004; Liu et al. 2009). In this study, however, the presence of positive correlations between serum levels of anti-Hsp90 IgG and IFN- γ (Th1-like cytokine), as well as between serum levels of anti-Hsp90 IgA and rheumatoid factor in RA patients, may suggest possible contribution of Hsp90/anti-Hsp90 in this disease.

In summary, although further research is needed to confirm the present data, based on our report and previous observations, humoral immune response against autologous Hsp60 and Hsp70 is not in contradiction with the known immunosuppressive activity of these chaperones in RA.

Acknowledgments We are grateful to Prof. Ewa Bryl and Prof. Jacek M. Witkowski from the Medical University of Gdańsk for providing us sera samples from RA patients.

Funding information This study was supported by the Polish National Science Centre (NCN), grant no. 2017/25/B/NZ6/00305.

Compliance with ethical standards

The use of human biological material was approved by the Ethics Committee of the Medical University of Gdańsk, Poland, and written informed consent was obtained according to the Declaration of Helsinki.

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