Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis

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Abstract
Atopic dermatitis (AD) is one of the most common chronic inflammatory dermatoses characterized by persistent itching and recurrent eczematous lesions. While the primary events and key drivers of AD are topics of ongoing debate, cutaneous inflammation due to inappropriate IgE (auto)antibody–related immune reactions is frequently considered. Highly conserved and immunogenic heat shock protein 90 (Hsp90), a key intra- and extracellular chaperone, can activate the immune response driving the generation of circulating anti-Hsp90 autoantibodies that are found to be elevated in several autoimmune disorders. Here, for the first time, we observed that serum levels of Hsp90 and anti-Hsp90 IgE autoantibodies are significantly elevated \((p < 0.0001)\) in AD patients \((n = 29)\) when compared to age- and gender-matched healthy controls \((n = 70)\). We revealed a positive correlation \((0.378, p = 0.042)\) between serum levels of Hsp90 and the severity of AD assessed by Scoring Atopic Dermatitis (SCORAD). In addition, seropositivity for anti-Hsp90 IgE has been found in 48.27% of AD patients and in 2.85% of healthy controls. Although further studies on a larger group of patients are needed to confirm presented data, our results suggest that extracellular Hsp90 and autoantibodies to Hsp90 deserve attention in the study of the mechanisms that promote the development and/or maintenance of atopic dermatitis.

Keywords Atopic dermatitis, AD · Allergy · IgE · Autoimmunity · Autoantibodies · Heat shock proteins, Hsps · Hsp90

Introduction
Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases, with an incidence of 15–30% in children and 2–10% in adults that is characterized by intense itching and recurrent eczematous lesions. Current therapy of AD consists of topical emollients for cutaneous barrier dysfunction and topical corticosteroids or calcineurin inhibitors for skin inflammation. In severely affected cases, phototherapy, systemic immunosuppressants, and biologic agents are indicated. In addition, immunoglobulin E (IgE)–selective immunoabsorption is proposed (Kasperkiewicz et al. 2018; Langan et al. 2020; Weidinger and Novak 2016). While the underlying events and key drivers of AD are subject to ongoing debate, there are at least two major and converging pathophysiological abnormalities of epidermal structure due to decreased filaggrin (FLG) expression and allergen-specific IgE- and/or autoreactive IgE-mediated dermatitis (Langan et al. 2020). Defects in the epidermal barrier lead to the penetration of the skin by allergens, activation of T helper 2 (Th2)–like cell polarization via Langerhans and dendritic cells, and IgE class switching (Guttman-Yassky et al. 2013; Varricchi et al. 2018). In addition, the activity of various other immune cells belonging to both innate and adaptive arm of immune responses including eosinophils and Th1, Th17, or Th22 cell populations appears to be critically involved in the initiation and the progression of AD, especially in European, American, and Asian populations (Czarnowicki et al. 2019; Weidinger and Novak 2016).

Highly conserved and intracellularly expressed Hsp90 (constituting 2–3% of the total cellular proteins) is a key molecular chaperone that plays an essential role in protein folding and the activation of its protein substrates (‘clients’) both inside and outside the cell. Hsp90 is responsible for biological activity of key signalling molecules, such as...
kinases, steroid receptors, cell cycle regulators, and transcription factors regulating various cellular processes. Extracellular Hsp90 with its chaperone activity is able to induce extracellular matrix remodelling via assisting in matrix metalloproteinase (MMP) activation. Hsp90 can also activate humoral immune responses driving to the generation of self-reactive antibodies to Hsp90 (Secli et al. 2021; Tukaj and Węgrzyn 2016). In fact, these autoantibodies are found to be elevated in several autoimmune diseases (Tukaj 2020; Tukaj and Kaminski 2019).

Hsp90 became the focus of interest of scientists in the context of the development of autoimmune/inflammatory conditions, since it is upregulated in inflamed tissues and its presence in the extracellular space had been associated with the autoimmune process (Tukaj 2020; Tukaj and Kaminski 2019; Tukaj and Węgrzyn 2016). Moreover, since IgE-dependent immune reactions to self-proteins have been associated with AD (Roesner and Werfel 2019; Tang et al. 2012), searching for the autoantigens that may play an important role in the pathogenesis of AD is desired. This study aimed to determine serum levels of Hsp90 and anti-Hsp90 autoantibodies of the IgE, IgG, IgM, and IgA isotype in a cohort of patients with AD and in age- and gender-matched healthy controls.

**Materials and methods**

**Patients and controls**

Twenty-nine patients with atopic dermatitis (mean age: 25.86 ± 6.30; gender: 15 males and 14 females) (Table 1) and 70 age- and gender-matched healthy controls (mean age: 28.44 ± 8.81; gender: 31 males and 39 females) have been included in this study. Healthy volunteers who suffered from autoimmune, allergic, or any other skin disorder have been

| Table 1 Characteristics of atopic dermatitis (AD) patients |
|------------|-------|----------|-------|-------|-----------|-----------|-----------|
| No | Age | Gender | AD duration (years) | SCORAD | IgE (IU/ml) | Asthma | Allergic rhinitis | Allergic conjunctivitis |
| 1 | 21 | M | <2 | 51.2 | 1323.1 | + | + |
| 2 | 18 | M | <2 | 67 | 2794.3 | + | + |
| 3 | 10 | F | ≥2 | 84 | 1539.0 | + | + |
| 4 | 10 | F | ≥2 | 82 | 1264.6 | + | + |
| 5 | 53 | F | ≥2 | 80 | 2692.8 | + | + |
| 6 | 41 | F | ≥2 | 19 | 84.5 | + | + |
| 7 | 23 | F | <2 | 32.6 | 52.8 | + | + |
| 8 | 16 | F | <2 | 45 | 1120.3 | + | + |
| 9 | 20 | M | ≥2 | 19 | 3283.0 | + | + |
| 10 | 23 | F | <2 | 48.3 | 1674.3 | + | + |
| 11 | 20 | M | ≥2 | 38 | 158.4 | + | + |
| 12 | 17 | F | <2 | 32.4 | 1326.1 | + | + |
| 13 | 21 | M | <2 | 39.6 | 150.0 | + | + |
| 14 | 13 | F | <2 | 42.2 | 395.8 | + | + |
| 15 | 20 | F | ≥2 | 86 | 1105.0 | + | + |
| 16 | 15 | M | <2 | 34.8 | 1369.0 | + | + |
| 17 | 25 | F | ≥2 | 70.4 | 1349.0 | + | + |
| 18 | 59 | M | ≥2 | 70.4 | 1122.2 | + | + |
| 19 | 18 | M | <2 | 48.3 | 1032.7 | + | + |
| 20 | 25 | M | <2 | 71.5 | 1296.9 | + | + |
| 21 | 43 | M | ≥2 | 45.5 | 414.9 | + | + |
| 22 | 31 | F | ≥2 | 68.5 | 1022.1 | + | + |
| 23 | 27 | M | <2 | 43.3 | 1678.7 | + | + |
| 24 | 37 | M | <2 | 62 | 781.2 | + | + |
| 25 | 32 | M | <2 | 24 | 1221.7 | + | + |
| 26 | 22 | F | <2 | 58.5 | 1935.1 | + | + |
| 27 | 37 | M | <2 | 55.5 | 17.4 | + | + |
| 28 | 28 | M | <2 | 40 | 1039.5 | + | + |
| 29 | 25 | F | <2 | 43 | 834.5 | + | + |
excluded from the study. The use of human biological material was approved by a bioethics committee at the regional medical chamber in Gdańsk (Poland), and written informed consents were performed in accordance with the Declaration of Helsinki.

**Detection of circulating Hsp90**

Hsp90 was evaluated in serum by commercially available HSP90α (human) ELISA kit (Enzo Life Science), following the manufacturer’s instructions.

**Detection of anti-Hsp90 antibodies**

Levels of IgE, IgG, IgM, and IgA against human Hsp90 were evaluated in the serum samples by a home-made enzyme-linked immunosorbent assay (ELISA), as described previously (Mantej et al. 2019). Briefly, medium-binding 96-well plates were coated with commercially available full-length native human Hsp90 (Enzo Life Sciences) protein at a concentration of 0.5 μg/ml in 0.1 M bicarbonate buffer at 4 °C overnight. The wells were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at room temperature (RT) for 1.5 h. After a washing step (three times with PBS containing 0.05% Tween 20), the sera were diluted (1:50–1:200) in PBS containing 0.1% BSA, added to the wells and were incubated at RT for 1.5 h. Plates were then incubated with horseradish peroxidase (HRP)–conjugated anti-human IgE (Abcam), anti-human IgG (Sigma), anti-human IgM (Abcam), or anti-human IgA (BioLegend) secondary antibodies diluted in PBS containing 0.1% BSA at RT for 1 h. The TMB substrate solution (Sigma) was used to visualize HRP enzymatic reaction and the reaction was stopped by adding 0.5 M H₂SO₄. Optical density measurements were performed at 450 nm with an ELISA plate reader (VICTOR Multilabel Plate Reader, PerkinElmer).

**Total IgE levels**

 Serum IgE levels were detected by commercially available ELISA kit performed according to the manufacturer’s instructions (Invitrogen).

**Statistical analysis**

Statistical analyses were performed using the GraphPad Prism 5 software (San Diego, CA). Data was analysed by the Mann–Whitney U test or Spearman’s rank correlation test. p values less than 0.05 were considered significant.

**Results**

**Serum levels of Hsp90 are significantly elevated in patients with AD**

In the analysis, 29 AD patients and 70 healthy controls were included. Clinical characteristics of the patients are stated in Table 1. Here, we found for the first time that serum levels of autologous Hsp90 were significantly elevated (2.79-fold increase; \( p < 0.0001 \)) in AD patients when compared to age- and gender-matched healthy controls (mean ± SD; 215.41 ± 146.14 pg/ml vs. 84.64 ± 79.96 pg/ml), as measured quantitatively by a commercially available ELISA kit (Fig. 1).

**Serum levels of anti-Hsp90 IgE autoantibodies are increased in patients with AD**

Using home-made indirect ELISA assay, we found for the first time that serum levels of anti-Hsp90 IgE were significantly elevated (\( p < 0.0001 \)) in AD patients (\( n = 29 \)) as compared to age- and gender-matched healthy controls (\( n = 70 \)), whereas levels of anti-Hsp90 IgG, IgM, or IgA were similar between both groups (Fig. 2a). Values of sera reactivity with Hsp90 measured above the mean values for bovine serum albumin (BSA) reactivity (negative control) were regarded as positive. In addition, taking into account the cut-off value calculated as 3 × standard deviation above the mean of the control, 14 out of 29 (48.27%)
AD patients were anti-Hsp90 IgE positive, while only 2 out of 70 (2.85%) healthy controls matched such criteria (Fig. 2b).

**Serum levels of Hsp90 are associated with the clinical severity of AD**

Using Spearman's rank correlation coefficient test, relationships between higher levels of circulating Hsp90 or anti-Hsp90 IgE autoantibodies and selected parameters of AD patients (Table 1) including disease activity, serum levels of IgE, or comorbidities were analysed. While a significant positive correlation ($r = 0.378, p = 0.042$) between serum levels of Hsp90 and the clinical severity of AD (SCORAD) was observed (Fig. 3), there were no significant associations between Hsp90 and serum levels of total IgE (0.317, $p = 0.093$) or anti-Hsp90 IgE ($-0.055, p = 0.776$) and the presence of comorbidities such as asthma ($-0.086, p = 0.654$), allergic rhinitis ($-0.074, p = 0.700$), or allergic conjunctivitis ($0.057, p = 0.765$) could be recorded. Levels of either Hsp90 or anti-Hsp90 IgE were not dependent on the duration of AD (data not shown).

**Discussion**

Heat shock proteins (Hsps) are a diverse group of molecules, either expressed constitutively or stress-induced, that are classified into several families based on their molecular weight and the characteristic structural domains. They mediate a range of essential cellular functions, including folding of newly synthesized polypeptides and renaturation or stabilization of biologically active proteins (Tukaj 2020; Tukaj and Kaminski 2019). Highly expressed Hsp90 is a key molecular chaperone that can be
released to the extracellular milieu. Hsp90 facilitates the maturation of various ‘client’ proteins such as kinases, transcription factors, steroid hormone receptors, and E3 ubiquitin ligases that are involved in many different cellular pathways, including inflammation, growth, differentiation, and apoptosis (Schopf et al. 2017; Trepel et al. 2010). Since aberrant expression and secretion of the Hsp90 has been observed in cancer cells and chronically inflamed tissues, this chaperone has attracted scientists’ attention particularly in terms of development, progression, and treatment of cancer and autoimmune/inflammatory diseases (Tukaj 2020; Tukaj and Kaminski 2019; Tukaj et al. 2013, 2015b; Tukaj and Węgrzyn 2016). Here, for the first time, we found that serum levels of Hsp90 were significantly elevated in AD patients when compared to age- and gender-matched healthy controls and positively correlated with the clinical severity (SCORAD) of patients. While the pathological role of Hsp90 in AD needs to be further elucidated, we here propose non-exclusive explanations to the potential contribution of this chaperone in AD. It has been previously reported that MMPs are ‘client’ proteins of Hsp90 and dependent on its chaperone function (Secli et al. 2021). As the interactions between MMPs and extracellular Hsp90 have been previously described in the context of tumour cell invasion and metastasis (Eustace et al. 2004; Garcia-Carbonero et al. 2013), we may speculate that elevated serum levels of Hsp90 could be involved in AD progression via promotion of MMP activation, the latter being known as an important pathophysiological factor in AD (Devillers et al. 2007; Harper et al. 2010).

The role of highly immunogenic, extracellular Hsp90 in AD may also be associated with its ability to activate the humoral (auto)immune response. Under stress conditions, Hsp90 can be released to the extracellular milieu and activate both the innate and adaptive immune responses driving the generation of circulating anti-Hsp90 autoantibodies that are found to be elevated in several autoimmune diseases, e.g., diabetes type 1 (Qin et al. 2003), systemic lupus erythematosus (Ripley et al. 2001), rheumatoid arthritis (Mantej et al. 2019), dermatitis herpetiformis (Kasperkiewicz et al. 2014), and coeliac disease (Tukaj et al. 2017b). Here, for the first time, we found that serum levels of anti-Hsp90 IgE were significantly elevated in AD patients as compared to healthy controls, whereas levels of anti-Hsp90 IgG, IgM, or IgA were similar between both groups. It is well established that elevated serum levels of total IgE are found in about 80% of AD patients (Weidinger and Novak 2016). Here, seropositivity for anti-Hsp90 IgE has been found in about 50% of AD patients.

In the past, molecular mimicry hypothesis suggested that the immune response originally directed to the bacterial Hsps may be re-directed towards their human counterparts and in this way promote development of autoimmune reactions (Albani et al. 1995). While immune cross-reactions between foreign and self-antigens were experimentally confirmed in the case of Hsp40, Hsp60, or Hsp70 chaperones (Kotlarz et al. 2013; van Eden et al. 2017), clinical consequences of such immune cross-reactions have brought ambiguous outcomes (Tukaj and Kaminski 2019; Tukaj et al. 2021; van Eden et al. 2017). Information on a potential contribution of the bacterial Hsp90 (HtpG) in autoimmunity and the immune cross-reactions between HtpG and human Hsp90, however, is generally lacking. Since human Hsp90 shares about 40% sequence identity and 55% similarity with bacterial (E. coli) Hsp90 (Huai et al. 2005), immune cross-reactions between these molecules are theoretically possible. On the other hand, Kawano et al. (2004) found that set of antibodies raised against bacterial HtpG did not cross-react with all four human Hsp90 analogues, and vice versa. Therefore, we postulate that significantly higher levels of self-Hsp90 in the blood of AD patients led to the activation of the humoral autoimmune response and the production of anti-Hsp90 IgE autoantibodies. Since IgE-dependent immune reactions to self-proteins have been already associated with AD by many authorities on the matter (Roesner and Werfel 2019; Tang et al. 2012; Zeller et al. 2009), it is tempting to speculate that autoimmune reactions towards self-Hsp90 play an important role in the pathogenesis of AD. In fact, autoreactive IgE can also be found in other inflammatory skin diseases, such as autoimmune bullous diseases (AIBD). For example, patients suffering from the
bullous pemphigoid, the most common form of AIBD, are IgG-, IgA-, or IgE-sensitized to the hemidesmosomal BP180 NC16A protein of the dermal–epidermal junction (Liu et al. 2017). The relevance of anti-Hsp90 IgE in AD, however, needs to be further examined since no significant associations between the levels of anti-Hsp90 IgE and total IgE or the clinical severity of AD were found in this study. On the other hand, lack of significant associations between anti-Hsp90 IgE and comorbidities (i.e., asthma, allergic rhinitis, or conjunctivitis) in AD patients may suggest a disease-specific IgE-dependent immune response to Hsp90 in AD.

Finally, numerous studies have shown that pharmacological inhibition of Hsp90 is successfully used in murine models of (auto)inflammatory diseases including AIBD via modulation of humoral and cellular immune responses (Kasperkiewicz et al. 2011; Tukaj et al. 2017a, 2015a, 2014a; Tukaj and Węgrzyn 2016; Tukaj et al. 2014b; 2015b), further supporting a potential role of this chaperone in the inflammatory process.

Conclusions

Although further studies on a larger group of patients and additional experimental analysis are needed to confirm the present data, our results suggest that extracellular Hsp90 and autoantibodies to the Hsp90 deserve attention in the study of the mechanisms that promote the development and maintenance of atopic dermatitis.

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Declarations

Conflict of interest The authors declare competing interests.

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