Letters to the Editors

Increased immune reactivity towards human hsp60 in patients with primary Sjögren's syndrome is associated with increased cytokine levels and glandular inflammation

Sirs,

Although the etiology of primary Sjögren's syndrome (pSS) is largely unknown, T-cells as well as B cells are suggested to play an important role in the immunopathology of the disease. A crucial role of CD4 T-cells in pSS is indicated by the association of autoimmunity and polymorphisms in MHC class II molecules in pSS and their abundance in the lymphocytic infiltrates of labial salivary glands (LSG) (1), including high numbers of activated Th1 and Th17 cells (2). In addition, adoptive transfer of

CD4 T-cells induces experimental Sjögren's syndrome in a mouse model (3). The involvement of B cells in pSS is evident from elevated serum IgG levels, characteristic antinuclear auto-antibodies including SSA and SSB antibodies and the increased number of IgG and IgM producing plasma cells in glandular tissue.

Since self-heat shock proteins (hsps) are immunogenic proteins that have the capacity to induce immuno-regulatory effects in different auto-immune diseases (4) we studied expression of human hsp60 in the LSG and the type of immune response human hsp60 elicits in pSS patients compared to patients with non-Sjögren's sicca syndrome (nSS). Hsp60-specific humoral (IgG, IgG1 and IgG2) and cellulair (cytokine induction) immune responses were assessed and studied in relationship to disease parameters and circulating cytokines. In concordance with the Helsinki declarations LSG biopsies, serum and PBMC were taken from 10 pSS and 10 nSS sicca patients.

Increased hsp60 expression in LSG of pSS patients was demonstrated (Fig. 1A). Furthermore, anti-human hsp60 IgG and IgG1 antibody levels were significantly elevated in pSS patients compared to nSS controls (Fig. 1B) (IgG1 level in pSS group 1103 compared to 474 in the nSS group (p=0.0002). In contrast, no significant differences in antibody titers to control antigen (BSA) were detected (data not shown). The anti-hsp60 antibody levels signifcantly correlated with local (lymphocyte focus score and decrease in % IgA+ plasma cells) and systemic disease parameters (ESR, serum total IgG, Fig. 1C, all at least p<0.05). Human hsp60 induced the production of several inflammatory cytokines by PBMC of pSS patients (IL1B, IL6 and IL10) (Fig. 1D). Serum levels of these hsp60-induced cytokines positively correlated with hsp60



Fig. 1. A) The number of hsp60 positive cells in LSG of pSS patients was increased in pSS (bold squares) compared to nSS patients (open squares).

B) In serum samples of pSS patients anti human hsp60 IgG1 levels were significantly increased compared to nSS patients. Anti-human hsp60 IgG2 levels were reduced although not statistically significantly (*p*=0.14)

C) Total anti human hsp60 IgG1 correlated significantly with disease parameters such as ESR, LFS, serum IgG (r=0.808 p=0.000), and %IgA+ cells (r=0.604 p=0.005).

D) Human hsp60 significantly stimulated production of IL1 β , IL6 and IL10 compared to unstimulated cells (*p*<0.05).

E) Anti human IgG1 levels correlated significantly with serum levels of these hsp60-induced cytokines.

F) In addition, serum IL1 α , IL12, IL15, MIG, IP10, sRANKL and sVCAM1 were significantly increased in pSS patients (n=10) compared to nSS patients (n=10). Geometric mean was calculated for each group and represented in a heatmap

G) Increased anti-human IgG1 and decreased anti-human IgG2 levels (data not shown, both p < 0.05) correlated with increased serum cytokine levels of IL1 α and IL12.

Each dot represents one individual, the boxes show the inter quartile ranges (IQR) for each group of data, the horizontal lines show the median. Spearman's correlation coefficients (R) and two-tailed *p*-values (p) are given.

IgG1 levels (Fig. 1E) and negatively with IgG2 (all p < 0.05). In addition, proinflammatory cytokines and chemokines are increased in the serum of pSS patients (IL-1 α , IL12, IL15, MIG, IP10, sRANKL and sV-CAM1 – Fig. 1F). Of these cytokines and chemokines, IL-1 α and IL-12 significantly correlated with increased anti-hsp60 IgG1 (Fig. 1G) and decreased IgG2 (data not shown). Furthermore, concentration of IL-15, IP10 and MIG positively correlated with IgG1 levels.

The present study indicates that immune reactivity to human hsp60 contributes to immunopathology in pSS. Whether human hsp60 triggers the immune response or whether hsp60 titers are secondary to another inflammatory trigger remains unclear and has to be studied in more detail. Nevertheless, our data indicate that anti-human hsp60 antibodies could contribute to sialadenitis in pSS. Our report is an additional step towards better understanding of the role of human hsp60 in pSS and the potential use of this antigen in the treatment of this disease. Delaleu et al. recently demonstrated that immunisation with (eukaryotic) hsp60 as well as an epitope derived from human hsp60 (aa437-460) in an experimental pSS model (5) potently inhibited SS symptoms. Recently we have identified several human hsp60-derived peptides that induce regulatory T-cell responses in arthritis models (6, 7). These hsp60-derived epitopes could be used for epitope specific immunosuppression via bystander suppression or infectious tolerance. Modulation of

the immune response by activating hsp60specific T-cells via nasal, oral or subcutaneous tolerance, resulting in the subsequent homing of these hsp60 T-cells to the site of inflammation to fulfill their anti-inflammatory effects, has already been shown to be successful in human clinical trials for rheumatoid arthritis and type I diabetes (8-10). Potentially several of the above mentioned epitopes could be used in the treatment of pSS. Future studies need to document the type of immune responses that are induced by these peptides and how these modulate immune responses in pSS.

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