

# LEVELS OF ANTI-STREPTOKINASE AND ANTI-MYCOBACTERIAL HEAT SHOCK PROTEIN 65 KILODALTON (ANTI-MHSP 65) ANTIBODIES IN PATIENTS WITH AUTOIMMUNE DISEASES

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## ABSTRACT

Bacterial and viral triggers are suspected agents in the initial etiology of autoimmune diseases. There are some studies on the etiology of autoimmune disorders which have focused on streptococcal infection and a possible relation with microbial heat shock proteins (hsp) which show significant homology with human heat shock proteins. In addition, some serotypes of streptococci cross-react with human hsp, namely 65kD hsp. Therefore, we have examined isotype specific antibody responses to streptokinase, the antigen released during infection with the common bacterium streptococcus, together with IgG responses to mycobacterium heat shock protein 65 (mhsp 65), a possible superantigen for autoimmune diseases. The levels of these antibodies were examined in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), thyroiditis and Henoch Schonlein purpura (HSP) and a group of normal controls. Patients with RA showed a statistically significant elevation in levels of IgM anti-streptokinase antibodies compared to the control group ( $p < 0.001$ ). In Henoch Schonlein purpura patients, the levels of IgG and neutralising anti-streptokinase antibodies correlated with the levels of IgG anti-mhsp 65 ( $r = 0.56, p < 0.09$  and  $r = 0.57, p < 0.08$ , respectively). According to these findings, we suggest that streptococcal infections may have an important role in the pathogenesis of rheumatoid arthritis and Henoch Schonlein purpura.

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## INTRODUCTION

Multiple mechanisms may lead to organ specific or sys-

temic auto-immune disorders.<sup>1</sup> Bacterial and viral infections have been suspected as antigens which may alter tolerance and produce autoimmune diseases. They may present antigen determinants similar to self components and produce cross-reactivity. Group A streptococci have similar antigens to human heart and glomeruli antigens and cause rheumatic fever, Henoch Schonlein purpura (HSP) and with

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possibly induces psoriasis.<sup>2</sup> Furthermore, immune responses to streptococcal infections have been associated with autoimmune diseases and sera with rheumatoid arthritis have been shown to contain high titers of anti-streptokinase antibodies.<sup>3</sup> The presence of the anti-streptokinase antibodies in patient sera is a result of previous streptococcal infection.<sup>4-6</sup> To investigate whether streptococcal infections have any role in initiating autoimmune disorders, the levels of anti-streptokinase antibodies were measured in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), thyroiditis and Henoch Schonlein purpura (HSP) patient groups.

Immune response to heat shock proteins (hsps) may lead to autoimmune disorders. These proteins were first shown to be released by cells cultured at temperatures above 37°C. Heat shock proteins (hsps) are a ubiquitous and highly conserved group of proteins and a major antigenic component of many bacteria.<sup>7</sup> The structural homology between foreign and self hsps may lead through cross-reactivity to an organ specific autoimmune disorder.<sup>8,9</sup> Autoantibodies against hsp 65, 70 and 90 have been detected in various autoimmune disorders, in particular arthritis patients show specific responses to the hsp 65 kD.<sup>10</sup> There are investigations on the etiology of autoimmune disorders which have focused on streptococcal infection which might have a common relation with microbial heat shock proteins (hsp) that show significant homology with the human heat shock proteins.<sup>11</sup>

Indeed, some serotypes of streptococci cross-react with 65 kD hsp.<sup>11</sup> Therefore, IgG anti-mycobacterial heat shock protein (mhsp 65) antibody levels were measured in the mentioned autoimmune patients groups and a group of normal individuals, to find if the presence of this antibody has any relationship with immune responses to streptococcal infections.

### MATERIAL AND METHODS

#### Patients and controls

Two-hundred and forty-six normal controls were studied for the presence of anti-streptokinase antibodies and twenty normal controls were studied for the presence of IgG anti-mhsp65. Fifteen patients with RA, twenty patients with SLE, twenty patients with thyroiditis and twelve patients with HSP were studied for the presence of anti-streptokinase antibodies and IgG anti-mhsp65. The age of normal controls ranged between 14 and 79 years at the time of venesection and they had no known current or recent illness. The sera samples were stored at -70°C. The patients' sera were obtained from the Immunopathology Laboratory, Western Infirmary, Glasgow University.

#### Quantitation of neutralising antibodies to streptokinase

Serum samples were analysed for the presence of

neutralising antibodies to streptokinase (ASK) using a commercial kit (Bio-Merieux). This method analysed the biological activity of these antibodies to inhibit the thrombolytic efficacy of streptokinase. 25 µL of diluted sample (1/10 in PBS) was applied to a round-bottomed microplate and double diluted across the plate up to 1/640. Streptokinase (25 µL) was then added to each well and incubated at 37°C for half an hour. 50 µL of a mixture of fibrinogen and plasminogen was then added to each well. Rabbit erythrocytes were washed in PBS and resuspended in PBS to give a 5% suspension and then added to dried bovine thrombin. 25 µL of this suspension was then applied to all the wells and the plate was covered and incubated at 37°C for 2 hours. The last dilution that prevented the lytic effect of streptokinase on the formed clots and prevented the sedimentation of erythrocytes was considered as the end point titer of the sample and expressed in reciprocal titer of neutralising anti-streptokinase antibodies per mL.

#### Measurement of isotype specific anti-streptokinase antibodies

IgG, IgM, IgA and IgE isotype specific anti-streptokinase antibodies were measured by ELISA. Briefly, ELISA plates (Immulon type 4, Dynatech) were coated with a solution of streptokinase, 5 µg/mL in PBS, overnight at 4°C. After washing the plates with PBS-Tween (Tween in phosphate buffered saline to give 0.05% solution), the plates were blocked with gelatin 0.01%. Unknown and known positive samples for constructing standard curves were applied to the plates and incubated for 2 hours. The plates were washed and horseradish peroxidase conjugated to human IgG, IgA, IgM and IgE (Dakopatts) were applied to the plates and incubated for 1 hour. The plates were washed and the colour was developed using OPD (O-phenylenediamine, Sigma) and hydrogen peroxide. The reaction was stopped with 4N H<sub>2</sub>SO<sub>4</sub> and the colour reaction was read at 490 nm on a Dynatech ELISA reader.

#### Measurement of IgG anti-mhsp65 antibodies

IgG anti-mhsp65 antibodies were measured in the groups of patients by indirect ELISA. Briefly, ELISA plates were coated with recombinant mycobacterial mhsp65 (*Mycobacterium bovis*, molecular weight= 65kD) (A gift from Dr. J.D.A. Van Embden, National Institute of Public Health and Environment, Bilthoven, Netherlands), 1µg/mL in carbonate bicarbonate buffer coated overnight at 4°C. After washing, the plates were blocked with bovine serum albumin 1% (Type II, Sigma). Unknown samples and known samples for constructing standard curves were applied to the plates and incubated for 2 hours. The plates were washed and horseradish peroxidase conjugated rabbit anti-human IgG (Dakopatts) was applied to the plates and incubated for 1 hour. After washing, the colour was developed as described above.

### Measuring rheumatoid factor (RF) in the sera of rheumatoid arthritis patients

To evaluate the effect of rheumatoid factor (RF) on the levels of detectable anti-streptokinase antibodies, the RF in the sera of patients with rheumatoid arthritis was measured by nephelometry.

### Statistical analysis

Statistical analysis of data was performed using Spearman correlation test and Wilcoxon signed rank test.

## RESULTS

IgG, IgM, IgA and neutralising anti-streptokinase antibodies were detectable in the normal control group. IgE response to streptokinase was not detected in any of the normal controls. This data was used to construct normal ranges (Table I) and based on these ranges, levels in patient groups were assessed.

**Table I.** Mean, median and normal ranges of anti-streptokinase antibodies on assumption of a Log-normal distribution (values are expressed in unit/mL).

Isotype	Mean	Median	Normal range
IgG (u/mL)	5.7	3.8	0-45.8
IgA (u/mL)	31	15	0-324
IgM (u/mL)	1.9	0	0-11
ASK (u/mL)	29	20	0-166

The levels of IgG, IgA and neutralising anti-streptokinase antibodies were detectable in patients with RA, SLE, thyroiditis and HSP. In patients with SLE and thyroiditis detectable levels did not differ significantly from the levels of antibodies observed in the control group (Table II).

Patients with RA showed a statistically significant elevation in levels of IgM anti-streptokinase antibodies compared to the control group ( $p < 0.001$ ) (Table II). In these patients the levels of rheumatoid factor (RF) were significantly elevated (mean = 602 IU/mL, range from 246 to 1370 IU/mL, normal range = 0-22 IU/mL). The elevated levels of IgM anti-streptokinase showed no relationship with the levels of RF as analysed statistically.

In the Henoch Schonlein purpura (HSP) patient group the levels of IgG and IgA anti-streptokinase antibodies were elevated when compared with the control group ( $p < 0.001$  and  $p < 0.02$ , respectively) (Table II). Three patients with HSP showed a positive IgE response to streptokinase, but no IgE positivity was observed in SLE, thyroiditis or RA patients. Levels of neutralising antibodies to streptokinase (ASK) did not differ significantly from the control group (Table II).

**Table II.** Mean values of anti-streptokinase antibodies in autoimmune patient groups and controls.

Isotype	Controls	SLE	RA	Thyroiditis	HSP
IgG (u/mL)	5.7	8.8	4.65	10.6	27
IgA (u/mL)	31	87	112	44	141
IgM (u/mL)	1.9	14.6	73	14.8	16.5
ASK (u/mL)	29	14	28.7	7	36

**Table III.** The mean, median and standard deviation of IgG anti-mhsp65 in rheumatoid arthritis (n = 15), systemic lupus erythematosus (n = 20), thyroiditis (n = 20) and Henoch Schonlein purpura patients (n = 12) and normal controls (n = 20) (The values are expressed in unit/mL).

Group	Mean	Median	Standard deviation
Normal controls	18.9	15	15.2
Rheumatoid arthritis	35.7	25.6	28
Lupus erythematosus	37.1	28	26.7
Henoch Schonlein p.	30.1	16.8	29.6
Thyroiditis	27.9	17.8	27

The mean values of IgG anti-mhsp65 antibodies in patients with RA, SLE, thyroiditis and HSP were higher than normal controls (Table III), although this elevation was not significant.

In Henoch Schonlein purpura patients, the levels of IgG and neutralising anti-streptokinase antibodies correlated with the levels of IgG anti-mhsp65 ( $r = 0.56$ ,  $p < 0.09$  and  $r = 0.57$ ,  $p < 0.08$ , respectively).

## DISCUSSION

Since the 1940's the influence of infectious diseases in the pathogenesis of RA has been the subject of much debate. The possibility of heat-shock proteins acting as superantigens and triggering autoimmune responses has restored these discussions.<sup>12</sup> In this study we have evaluated the extent of isotype specific anti-streptokinase antibodies in a normal population and compared them with the levels in patients with autoimmune diseases.

Antibodies to streptokinase were analysed as the general population are commonly exposed to this antigen during streptococcal infections (throat, skin, nose, ears, etc.). Surprisingly, patients with RA had significantly elevated levels of specific IgM anti-streptokinase antibody levels which did not show any correlation to RF. This finding has been shown in other studies and has also suggested that there is a strong correlation with the administration of streptokinase and the appearance of autoantibodies.<sup>3</sup> A brief remark to mention is that streptokinase contains similar epitopes

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with human antigens such as fibronectin and human mhsp65, therefore anti-streptokinase antibodies may combine with host constituents and provoke tissue damage.<sup>11,13</sup>

Heat shock proteins are a ubiquitous, highly conserved group of proteins used in a variety of cellular activities. They constitute a major antigenic component of mycobacteria and are considered as a major trigger for autoimmunity. Similar to the present study, elevated levels of anti-mhsp65 have been demonstrated before in RA patients.<sup>12</sup> Nevertheless, we could not strongly support this observation in our RA patients, or in patients with SLE, thyroiditis and HSP. This could be partly related to the British population studied which have a lower incidence of mycobacterial infections compared to eastern populations. However, in patients with HSP there was a correlation between IgG anti-mhsp65 levels and IgG anti-streptokinase antibody levels ( $p < 0.08$ ). This fact, although not highly significant, is based on a small number of patients; it does, however, raise the possibility that streptococcal infections and the release of heat shock proteins may play an important role in the etiology of Henoch Schonlein purpura.

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