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# Serum antibodies to heat shock proteins are of no diagnostic value for human kidney allograft rejection

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**Abstract** Because of the prevailing evidence that heat shock proteins (hsp) are involved in transplantation immunology, we investigate in this study the serum levels of anti-hsp60, and anti-hsp70 antibodies in human kidney allograft recipients. We analyzed 67 sera from 20 patients immediately before and 2 weeks after receiving a kidney allograft, and from 27 healthy age-matched controls with an ELISA. Eleven kidneys had normal allograft function, six had a mild rejection episode, all of which could be reversed successfully; three kidneys had to be removed later on because of resistant rejection. Hsp antibody frequency and titres were the same for transplant recipients and for healthy controls. In patients receiving a kidney allograft, no difference in the level of hsp-antibodies before and after transplantation was observed. Additionally, anti-hsp60 and anti-hsp70 antibody titres were found to be independent of the clinical course. These data suggest that the determination of anti-hsp60 and 70 antibody titers are of no diagnostic value for renal allograft rejection.

**Key words** Heat shock protein · Human kidney transplantation · Rejection

## Introduction

Heat shock proteins (hsp) can elicit responses of T-lymphocytes during kidney allograft rejection in man as well as in animal models [5, 6, 8]. Hsp are highly conserved proteins facilitating intracellular protein folding and transport. Their production in all cells is uniformly increased in response to exogenous or endogenous stress, essentially to protect themselves from damage. In addition, hsps have been shown to be involved in the immunology of e.g. autoimmune diseases and in cancer immunity [3, 10, 11]. In transplant immunology, they have been suggested to serve as new target molecules for the stimulation of allograft infiltrating T-cells, as T-cells reactive to mycobacterial hsp 65 and 70 were isolated from heterotopic rat cardiac allografts [5]. We have isolated hsp70-specific T-lymphocytes from human kidney transplants removed for irreversible rejection [8]. Furthermore, an increased expression of hsp60 mRNA has recently been described in biopsies of rejected human kidneys compared to organs without rejection [1]. An association of antibodies to hsp60 and high anti-heart antibodies in cardiac transplantation has been reported [4], but so far no reports are available on antibodies against hsps in renal allografts. It is therefore the aim of this study to investigate anti-hsp60 and -70 antibody titres in sera from human kidney allograft recipients, and to evaluate whether they are of diagnostic, predicative, or prognostic value.

## Materials and methods

### Patients

We analyzed 67 sera from 20 patients (11 women, 9 men, mean age 43,6 years) immediately before, and two weeks after, receiving a kidney allograft; and from 27 healthy age-matched controls (15 women, 12 men, mean age 46,2 years). The primary disease was

**Table 1** Heat shock protein 60 and 70 antibody levels before and after transplantation in all kidney allografts, allografts with slight rejection episodes and irreversible rejected kidneys. Values are mean ± SD Group differences were determined by Wilcoxons rank test

| | Before transplantation | After transplantation | *P* value |
|---|---|---|---|
| hsp 70: | | | |
| All allografts ($n = 20$) | 0.021 ± 0.037 | 0.015 ± 0.027 | $P = 0.33$ |
| Reversible rejection episode ($n = 6$) | 0.021 ± 0.034 | 0.007 ± 0.016 | |
| Irreversible rejection episode ($n = 3$) | 0.018 ± 0.026 | 0.014 ± 0.024 | |
| hsp 60: | | | |
| All allografts ($n = 20$) | 0.064 ± 0.122 | 0.063 ± 0.026 | $P = 0.16$ |
| Reversible rejection episode ($n = 6$) | 0.053 ± 0.071 | 0.035 ± 0.042 | |
| Irreversible rejection episode ($n = 3$) | 0.008 ± 0.012 | 0.022 ± 0.039 | |

chronic glomerulonephritis ($n = 10$), polycystic kidney disease ($n = 5$), congenital disorder ($n = 2$), chronic pyelonephritis, hemolytic-uremic syndrome and Alport syndrome ($n = 1$ each). Informed consent was obtained from the patients included in this study. Mean duration of dialysis before transplantation was 5,1 years. All patients received prophylactic immunosupression consisting of cyclosporine, azathioprine and prednisolone after transplantation. Postoperatively, six patients required dialysis for initial nonfunction: three of them once or twice, and the other three at least seven times. All of the latter three patients lost their kidney due to irreversible rejection. In eight patients, 19 biopsies were taken, 11 of which were carried out in patients with later graft loss. Biopsies were evaluated according to the Banff-classification [7]. Rejection episodes were treated with methylprednisolone (3 × 500 mg on 3 consecutive days). Three patients with later graft loss received six doses of plasmapheresis and anti-thymocyte globulin for the treatment of predominantly vascular rejection (Banff III).

### Enzyme linked immunnosorbent assay

Antibody titres were tested by a solid-phase-bound antigen indirect enzyme linked immunnosorbent assay (ELISA) established in our laboratory and described in detail previously [9]. Shortly, flat bottom ELISA plates were coated with 100 µl/well human hsp70 (SPP 755, StressGen, Victoria, Canada) or human hsp60 (SPP 740, StressGen, 5 µg/ml each) in PBS (pH 7.2) and 0.1 M Ethanolamine in PBS 100 µl/well. It is important to note that in contrast to other studies, Ethanolamine instead of PBS was used as control. Antibody titres were stated as relative units/ml (U/ml) after subtraction of non-specific binding assessed with ethanolamine. Extensive specifity testing was performed by competitive one-site indirect ELISAs with either 100 µg/ml or 10 µg/ml (final concentrations) hsp60 or –70 [9]. Monoclonal antibodies against hsp 60 and –70 (SPA 806 and SPA 820, StressGene; both at 1:20,000 final concentration) served as positive controls. All densities were quantified without knowledge of serum identity, i.e. blindly.

### Statistics

Anti-hsp antibody levels were evaluated by Wilcoxon signed-rank test. For comparisons between groups, non-parametric tests (Mann-Whitney U-test) were used. The significance level was set at a value of $p < 0.05$. All values are given as mean ± S.D., and all tests were performed at least in duplicates.

## Results

### Antibobies against hsp60 and hsp70 in sera from patients with renal failure and controls

Antibodies to hsp70 were found in 6 patients (6/20, 30%), and to hsp60 in 10 patients (10/20, 50%), before transplantation. The mean ± S.D. optical density (OD) levels were 0.021 ± 0.037 for hsp70 and 0.064 ± 0.122 for hsp60, respectively. OD and frequency did not differ from levels found in healthy controls, in 44% (12/27) anti-hsp70 antibodies, and in 60% (16/27) anti-hsp60 antibodies were found. Levels in healthy controls were 0.034 ± 0.055 for hsp70 and 0.048 ± 0.081 for hsp60, respectively (mean ± S.D.).

### Antibobies against hsp60 and hsp70 in sera from patients after renal allograft transplantation

In the 20 transplant patients, hsp serum antibodies were present in sera as frequently prior to, as after transplantation. After transplantation, anti-hsp70 antibodies were found again in 30% (6/20), and anti-hsp60 antibodies in 45% (9/20). OD was 0.015 ± 0.027 for hsp70. OD for hsp60 was 0.063 ± 0.026 following transplantation (Table 1).

An ongoing reversible or irreversible rejection episode was not accompained by any change in anti-hsp60 or –70 antibody titres. In addition, no differences in antibody levels or frequency were found between patients undergoing rejection or having normal graft function (Table 1). The difference for hsp70 antibodies in the group with reversible rejection episodes before and after transplantation did not reach significance.

## Discussion

Recent studies report that T-lymphocytes infiltrating rejected heart and kidney allografts recognize hsps, thereby suggesting a participation of hsps in transplant rejection [5, 6, 8], accompanied by an increased expression

of hsps during the rejection of human kidney allografts [1]. It has been shown, that hsp expression is modulated in vitro by hypoxia, cytokines, glucocorticoids and cyclosporin [2]. As hsps are immunogenic; their specific participation in the rejection process is still not completely clear, although an active role in the pathogenesis of rejection seems be possible. Hsps are able to elicit humoral immunity, and antibodies against hsps have been described predominantly in autoimmune diseases. Although T-lymphocytes play a major role in the development of both cellular and inflammatory immune responses leading to rejection of an allograft, the humoral part of the immune system is also of critical importance. We therefore investigated for antibody levels against hsps in renal allograft recipients with different outcome.

In this study we had the possibility to relate antibody levels to different immunresponses and therapeutic strategies. No correlation was found either to severity of rejection or to an immunologically uncomplicated course. There was also no correlation with the severity of immunosuppression. A suppression of hsp expression by cyclosporin was described in vitro, but this seems not to be the case in vivo. However, as no changes of serum antibodies against hsps during rejection could be observed, a role in the pathogenesis of graft rejection seems to be unlikely. This is confirmed by the fact that in the healthy controls, anti-hsp antibodies were detected in the same range. These antibodies might represent a part of naturally occurring antibodies. They may play a role in autoimmunity via cross-sharing of epitopes [3, 11], due to high conservation on pathogens and on human hsps. But in transplant rejection, there is so far no evidence of a primary involvement of antibodies against hsps.

Tests on a representative sample of human kidney allografts with different graft outcome for hsp60 and -70 antibodies, show no changes in titres. We therefore conclude, that these antibodies are neither of prognostic nor of predictive value in the monitoring of human kidney allografts.

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