Letter to the Editor

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Heat-Shock Protein 65 and Atherosclerosis in Patients on Regular Hemodialysis

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Dear Sir,

Atherosclerosis is one of the most significant yet unresolved problems in patients on regular hemodialysis (HD) with major impact on morbidity and mortality in patients with end-stage renal disease. A number of risk factors associated with the development of atherosclerosis in HD patients have been identified, such as hyperlipidemia, hypertension, diabetes, smoking and obesity [1]. There is growing evidence that atherosclerosis may – at least in part – result from autoimmune processes, in which the family of heat-shock proteins (HSP) may be involved [2]. HSPs are a family of about two dozen proteins which show highly homologous sequences between bacterial and mammalian species. They are induced during chronic inflammation and other forms of physiological stress. Experimental evidence has shown that expression of HSP 60 on endothelial cells triggers early inflammatory atherosclerotic changes [3]. Furthermore, immunisation of normocholesterolemic rabbits with a mycobacterial 65-kD HSP (mHSP 65) induced typical atherosclerotic lesions [2]. Crossreactivity of serum antibodies against mHSP 65 with the human 60-kD homologue present in high levels in atherosclerotic lesions was demonstrated by immunoblotting and immunofluorescence techniques [4]. In a series of 27 human subjects HSP 60 could be detected on endothelium, smooth muscle cells and/or mononuclear cells of carotid and aortic specimens with atherosclerotic lesions. No expression of stress protein was detectable on control specimens with normal intima [5].

In the present series we studied antibody titers against mHSP 65 in patients on regular hemodialysis (HD) (table 1). In total, 77 patients were included in the study (27 females, 50 males; mean age 59 ± 12 years). The major causes for end-stage renal disease were chronic glomerulonephritis, diabetic nephropathy and interstitial nephritis. Of the 77 patients 29 (6 females, 23 males; mean age 61 ± 12 years) showed typical manifestations of vascular disease as defined by cardiac ischemia and/or peripheral vascular disease. All 29 patients had typical clinical symptoms and angiography was performed to confirm the diagnosis. The remaining 48 patients (21 females, 27 males; mean age 57 ± 13 years) did not show symptoms of significant vascular disease. As a control, sera were obtained from healthy subjects (medical students and laboratory staff, n = 90, 48 females, 42 males, mean age 32 ± 12 years). Purified recombinant (r)mHSP 65 has been kindly provided by Dr. Jan van Embden from the WHO IMMTUB/IMMLEP Bank. The microtiter plates were coated with mHSP 65 overnight at 4°C. After a washing step, the wells were blocked with non-fatty dry milk powder (Merck) in PBS and after washing incubated with diluted patient sera. After extensive washing the plates were incubated with peroxidase-conjugated rabbit antihuman IgG antiserum. The plates were washed again and developed by addition of substrate. The absorbance was measured in an ELISA reader at 405 nm. In each assay one positive serum was included as a posi-

Table 1. Serum anti-HSP 65 antibody titers of indicated groups of patients on regular hemodialysis (OD ratio)

	Healthy controls	All HD patients	HD patients with AS	HD patients without AS
n	90	77	29	48
Mean age, years	32 ± 12	59 ± 12	61 ± 12	57 ± 13
Mean time on HD, months		58	42	68
Means	0.0475	0.156	0.227	0.116
+ SEM	0.004	0.137	0.144	0.116
Significance[1]		$p < 0.05$*	$p < 0.01$	$p > 0.05$

HD = Hemodialysis; AS = atherosclerosis.
* Kruskal-Wallis test.
[1] Compared to healthy controls.

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tive control and 3 negative sera as negative controls. Arbitrary units were calculated for accurate interassay comparison using the formula: OD ratio = OD sample/OD positive control.

In our study increased circulating antibodies to rmHSP 65 were detectable in only 2 of 90 healthy donors. The titers of rmHSP 65 antibodies of all HD patients were significantly elevated (OD ratio 0.156, $p < 0.05$) (table 1). The analysis of the two subgroups of HD patients with or without manifestations of vascular disease as defined by the criteria above, showed no significant difference of rmHSP 65 antibody titers between healthy controls and HD patients without vascular disease (OD ratio 0.116, $p > 0.05$). On the other hand, HD patients with vascular disease had clearly elevated antibody titers to rmHSP 65 as compared to healthy controls (OD ratio 0.227, $p < 0.01$). The comparison of the two subgroups of HD patients revealed the most striking difference in antibody titers to rmHSP 65 (OD ratio 0.227 vs. 0.116, $p < 0.001$).

The present study shows a correlation between serum anti-HSP 65 antibody titers and vascular events in end-stage renal disease. As HD patients with no major underlying vascular disease do not differ from the normal population in that respect, the intensity and duration of hemodialysis treatment by itself does not seem to be responsible for increased anti-HSP 65 antibodies. High serum antibodies to HSP 65 have been found in patients with symptomatic carotid atherosclerosis [1] or in patients with ischemic vasculitis [6]. None of these patients suffered end-stage renal disease. Whether or not serum antibodies increase with age or are the manifestations of atherosclerosis in elderly subjects remains controversial. Canadian investigators have attributed elevated serum anti-HSP 65 antibodies to age in patients with thrombotic stroke or transient ischemic attacks, but not to the number of cerebrovascular events [7]. Looking at our data, we cannot support this observation in our patient group. Although the age-related increase of serum anti-HSP 65 antibodies as well as atherosclerosis is well known [1], HD patients with vascular disease were only slightly older than those without vascular damage. In conclusion, our data provide further evidence for the hypothesis of an autoimmune induction of early inflammatory arteriosclerotic changes since HD patients with well-defined vascular lesions have high titers of anti-HSP 65 antibodies independent of age and methodology. Further studies are necessary to determine whether antibodies to HSP 65 are of diagnostic and prognostic value in patients on chronic hemodialysis.

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