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Increased expression of HDJ-2 (hsp40) in carotid artery atherosclerosis: A novel heat shock protein associated with luminal stenosis and plaque ulceration

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Purpose: **Evidence suggests that both humoral and cellular autoimmune processes directed toward heat shock proteins (hsp) contribute to the pathogenesis of atherosclerosis. We characterized a human hsp distinct from those previously characterized in atherosclerotic lesions, termed *HDJ-2.***

Methods: **To determine the role of HDJ-2 in atherosclerosis, we compared the level of HDJ-2 mRNA expression with the level of hsp60 and hsp70 mRNA expression in 26 carotid endarterectomy specimens and 17 normal arteries. The level of expression of HDJ-2 mRNA was also correlated to the presence of plaque ulceration and the degree of luminal stenosis associated with the lesion.**

Results: **The expression of HDJ-2 and hsp70 was significantly higher in carotid artery plaques as compared with normal arteries: HDJ-2, 6.7 ± 1.6 vs 0.1 ± 0.04, (P = .001); hsp70, 9.5 ± 0.9 vs 3.7 ± 0.8, (P = .002). There was no significant difference in hsp60 expression between carotid artery plaques and normal arteries (21.0 ± 0.9 vs 20.6 ± 0.8, P = .65). Increased HDJ-2 expression in carotid artery plaques was independent of hsp70 (Pearson correlation, r = 0.11; Bartlett χ^2 analysis, P = .71). Within the ulcerated plaque group, there was a correlation between degree of stenosis and high HDJ-2 mRNA expression (r = 0.896, P = .016). However, there was no correlation between degree of stenosis and high HDJ-2 mRNA expression within the nonulcerated plaque group (r = 0.530, P = .076) or within the entire group of patients (r = 0.0085, P = .97).**

Conclusion: **These results demonstrate that expression of HDJ-2 is significantly increased in atherosclerotic carotid artery plaques as compared with hsp60 and hsp70 and correlates with luminal stenosis in ulcerated atherosclerotic carotid artery plaques. (J Vasc Surg 2001;33:1065-71.)**

The pathologic condition of atherosclerotic lesions is characterized by cellular infiltration, proliferation, and lipid accumulation within the vessel wall, as well as deposition of extracellular matrix, which results in luminal stenosis or occlusion. In recent years, increasing evidence has suggested that atherosclerosis is also associated with features of autoimmunity.[1] This immune response appears to be associated with elevated tissue expression of heat shock proteins (hsp), such as hsp60/65 and hsp70.[1] Additional studies have demonstrated diverse roles for hsps in atherogenesis, ranging from protection of endothelial cells from stress[2] to dysregulation of macrophage function and recruitment of T lymphocytes to sites of plaque formation.[3] Furthermore, there is evidence that increased levels of antibodies to hsp60/65 occur in patients with carotid artery atherosclerosis, suggesting that a humoral immune response to hsps may play a significant role in atherogenesis.[4]

We recently cloned and characterized a novel 46-kDa hsp from human tissue, termed *HDJ-2,* which represents a human homologue of the DNA-J hsp found in *Escherichia coli.*[5] Several studies have suggested that HDJ-2 may have an immunogenic role in transplantation, because its expression is upregulated during allograft rejection.[6,7] Given the evidence that hsps such as hsp60 and hsp70 are involved in the autoimmune response associated with atherosclerosis, it is important to define the role of other hsps during the development of the atherosclerotic plaque. No studies have been carried out to determine the role of HDJ-2 in the pathogenesis of atherosclerosis. Therefore, this study was designed to determine whether there is a correlation between the expression of HDJ-2 and the development of atherosclerotic plaques and to identify the specific cell types expressing HDJ-2 in the atherosclerotic lesion.

This study demonstrates that in situ HDJ-2 expression is increased in the atherosclerotic carotid artery plaque to a greater extent than hsp60 and hsp70. In addition, this

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Table I. Primer pairs and oligonucleotide probes used for RT-PCR and Southern blot analysis

HDJ-2 (1438 bp mRNA) (Genbank reference NID g306713; accession no. L08069)
Forward primer: 5´-CCGCACCGGCAGTAGAAGATGGTGAAAGAA-3´ (bp 65-94)
Reverse primer: 5´-TCTCTCGAACTATCTTCCTTCCGTTGCAGC-3´ (bp 666-695)
cDNA probe: 5´-AAAGAAAAGGGAATTATATGAC-3´ (bp 256-277)
Expected amplification product: 630 bp
Hsp70 (1180 bp mRNA) (Genbank reference NID g188491; accession no. M59829 M34268)
Forward primer: 5´-ACCATCACCAATGACAAG-3´ (bp 2469-2486)
Reverse primer: 5´-TGTGATGATAGGGTTACA-3´ (bp 2772-2789)
cDNA probe: 5´-AATGCCTTAGAATCCTAT-3´ (bp 2583-2600)
Expected amplification product: 320 bp
Hsp60 (2202 bp mRNA) (Genbank reference NID g184411; accession no. M34664)
Forward primer: 5´-GGTTATGATGCTATGGCT-3´ (bp 1528-1545)
Reverse primer: 5´-GCAGTACACTGGTACATG-3´ (bp 2007-2024)
cDNA probe: 5´-AACTGTGACAGGAAGCCC-3´ (bp 1778-1795)
Expected amplification product: 496 bp
β-actin (1138 bp mRNA) (Genbank reference NID g2116654; accession no. AB004047)
Forward primer: 5´-TGACGGGGTCACCCACACTGTGCCCATCTA-3´ (bp 478-507)
Reverse primer: 5´-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3´ (bp 1109-1138)
cDNA probe: 5´-GGCTGGCCGGGACCTGACTGACTACCTCAT-3´ (bp 550-579)
Expected amplification product: 660 bp

study shows a significant correlation between increased in situ HDJ-2 expression and plaque ulceration plus degree of luminal stenosis associated with the lesion. Therefore, the data presented herein indicate that increased in situ HDJ-2 expression plays an important role in the pathogenesis of advance atherosclerotic plaques.

METHODS

Patients and tissues. Twenty-six atherosclerotic carotid artery plaques were obtained from patients undergoing carotid endarterectomy as approved by the Human Studies Committee at Washington University School of Medicine. As controls, 17 nonatherosclerotic arteries of similar size were obtained from cadaveric organ transplant donors (11 carotid arteries, 3 internal iliac arteries, and 3 femoral arteries). The size of the specimens varied between 1 and 2 g of tissue for both patients and control subjects. Necrotic lipid core and debris were removed from the atherosclerotic carotid artery plaques by means of microscopic dissection at 4°C. The adventitia and the media layer of the nonatherosclerotic intimas were also removed by means of microscopic dissection at 4°C, to match the processing of the atherosclerotic carotid artery plaques. Each specimen was then cut into two pieces. One piece was fixed in 10% buffered formalin for paraffin embedding, and the other was immediately frozen in liquid nitrogen for RNA isolation. The degree of luminal stenosis associated with the atherosclerotic carotid artery plaque was measured before operation in all patients by angiography by means of the following formula: $100 \times [1 - (MML/DL)]$ in which *MML* is the smallest luminal diameter at the stenotic site and *DL* is the luminal diameter distal to MML at the site in which the arterial walls become parallel. The presence of ulceration in the atherosclerotic carotid artery plaque was determined by histologic analysis, as well as angiography as described above.

Reverse transcription-polymerase chain reaction. Tissue samples were homogenized at –20°C with a Tissue Tearer (Biospec Products, Bartesville, Okla), and total RNA was isolated by use of Trizol (Gibco BRL, Gaithersburg, Md). All RNA samples were treated for 15 minutes at 37°C with RNase-free RQ1 DNase (Promega, Madison, Wis), then examined by 1% agarose electrophoresis to verify RNA integrity. Subsequently, reverse transcription (RT) was performed with total RNA 5 μg in RT buffer 20 μL (oligo dT 1 mg/mL; random pDN6 primer 1 mg/mL; Tris pH 7.6 1 mol/L; potassium chloride 1 mol/L; magnesium chloride 250 mmol/L; deoxynucleotides triphosphate, 5 mmol/L each; ditiothreitol 0.1 mol/L; RNasin, 100 units, and actinomycin D, 0.1 μg/mL) with Moloney murine leukemia virus reverse transcriptase at 37°C for 2 hours. After RT the reaction was heated at 95°C for 5 minutes and used as a template for polymerase chain reaction (PCR). Varying concentrations of cDNA were used as the template for PCR (1 μL of undiluted, 1:5, 1:10, and 1:20 dilutions) to determine the level of HDJ-2, hsp60, hsp70, and β-actin mRNA expression. The cDNA (1 μL) was amplified for 30 cycles with a Coy thermal cycler (Laboratory Products Inc, Ann Arbor, Mich) with these parameters: denaturation at 95°C for 60 seconds; annealing at 58°C (for β-actin, HDJ-2, and hsp60) or at 52°C (for hsp70) for 30 seconds; and extension at 72°C for 60 seconds. At the end of the amplification, 30 μL of each sample was analyzed on 1% agarose gel (FisherBiotech, Fair Lawn, NJ).

Southern blot analysis. At the end of the PCR, 30 μL of each sample was analyzed by Southern blot analysis with Genescreen plus membrane (NEN/Dupont, Boston, Mass). The Southern blots were prehybridized according to the manufacturer's directions and hybridized with a phosphorus 32–labeled internal probe specific for each of the genes. The membranes were washed under stringent conditions according to the manufacturer's directions and exposed to XR5 film (Eastman Kodak Co, Rochester, NY)

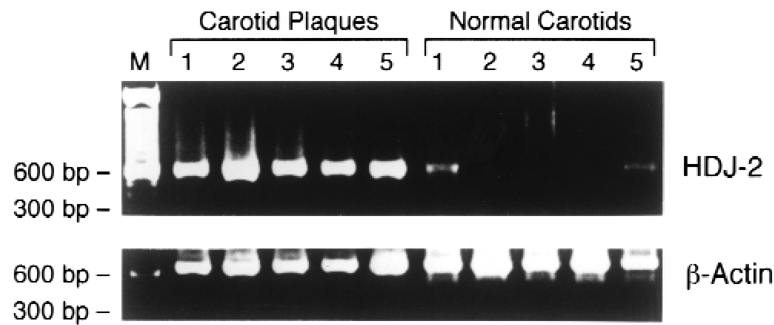

Fig 1. Southern blot analysis of HDJ-2 and β-actin RT-PCR products of representative atherosclerotic carotid artery plaques and nonatherosclerotic intimas. Sizes of HDJ-2 and β-actin bands are 630 base pairs and 660 base pairs, respectively. Briefly, tissue samples were homogenized and total RNA was isolated. Subsequently, RT was performed with M-MLV reverse transcriptase. After RT, reaction was used as template for PCR. cDNA was amplified with specific primer pairs for HDJ-2 and β-actin. At end of PCR, each sample was analyzed by Southern blot with ^{32}P-labeled internal probe specific for each of genes. Membranes were then exposed to XR5 film. PCR primers and oligonucleotide probes used in this study are shown in Table I.

overnight at –70°C. The PCR primers and oligonucleotide probes used in this study are shown in Table I. Autoradiograms of the Southern blots were analyzed by use of a laser densitometer (Pharmacia LKB, Piscataway, NJ). Data are expressed as arbitrary mRNA units representing the densitometry values obtained for HDJ-2, hsp60, and hsp70, corrected for the dilution factor, and then divided by the densitometry values obtained for the corresponding β-actin values.

Immunohistochemistry. Paraffin-embedded tissues were sectioned at 5 μm, attached to glass slides, and deparaffinized as described.[8] Endogenous peroxidase activity was blocked by incubating the slides in 0.3% H_2O_2 for 30 minutes at room temperature. An affinity-purified mouse immunoglobulin G (IgG) monoclonal antibody raised against human HDJ-2 was used as the primary antibody at 1:100 and 1:500 dilutions.[9] An isotype-matched IgG monoclonal antibody (MOPC; Cappel, Durham, NC) was used as negative control in all experiments at similar concentrations. A biotinylated goat-antimouse IgG (Cappel) (1:200 dilution) was then used as a secondary antibody. The immune complexes were detected by use of a Vectastain ABC kit (Vector Laboratory, Burlingame, Calif). Slides were counterstained with Harris hematoxylin (Sigma Chemical Co, St Louis, Mo).

Statistical analysis. Statistical analysis of the data was performed by use of the Mann-Whitney statistical analysis ($P < .05$), the Pearson correlation analysis, and the Bartlett χ^2 analysis ($P < .05$).

RESULTS

HDJ-2 expression is upregulated in atherosclerotic carotid artery plaques. The initial goal of the study described herein was to determine whether HDJ-2 expression is upregulated in atherosclerotic carotid artery plaques in situ. RT-PCR was performed with hsp-specific primers and total RNA isolated from individual carotid endarterectomy specimens or normal arterial tissues to determine the amount of hsp mRNA transcripts. Fig 1 shows the RT-PCR products derived from HDJ-2 and β-actin (internal control) mRNAs isolated from five atherosclerotic carotid artery plaques and five nonatherosclerotic intimas. These results demonstrate that HDJ-2 expression is significantly increased in atherosclerotic carotid artery plaques when

Table II. HDJ-2 expression in atherosclerotic carotid artery plaque

	Atherosclerotic carotid artery plaque	*Nonatherosclerotic intima*
	1.60	0.41
	1.27	0.35
	1.53	0.14
	0.90	0.15
	0.88	0.21
	14.35	0.41
	9.86	0.37
	25.83	0.10
	8.07	0.08
	10.37	0.07
	12.61	0.04
	15.66	0.04
	28.63	0.03
	18.10	0.03
	8.70	0.02
	3.80	0.01
	3.40	0.01
	1.90	
	1.40	
	1.20	
	1.10	
	0.97	
	0.87	
	0.67	
	0.65	
	0.62	
Mean ± SEM	6.73 ± 1.59	0.14 ± 0.04

Mann-Whitney analysis: $P = .001$.

Table III. hsp70 and hsp60 expression in atherosclerotic carotid artery plaque

	hsp70		*hsp60*	
	ACP	*NAI*	*ACP*	*NAI*
	6.77	5.90	18.79	19.67
	11.20	5.55	13.08	16.44
	9.95	1.92	13.09	29.74
	15.41	3.49	14.05	21.04
	15.51	5.88	25.72	21.86
	3.65	2.28	22.43	22.26
	8.94	1.02	17.44	13.29
	9.10		19.14	
	11.56		19.13	
	3.35		30.64	
	8.03		17.97	
	10.15		23.48	
	10.27		25.17	
	9.22		18.49	
			37.07	
Mean ± SEM	9.51 ± 0.94*	3.72 ± 0.78*	21.05 ± 0.91†	20.61 ± 0.78†

ACP, Atherosclerotic carotid artery plaque; *NAI,* nonatherosclerotic intima.
*Mann-Whitney analysis: $P = .002$.
†Mann-Whitney analysis: $P = .647$.

Table IV. HDJ-2 and hsp70 are independently expressed in atherosclerotic carotid artery plaque

Patient	*HDJ-2*	*hsp70*
1	0.88	3.65
2	0.90	15.51
3	1.27	9.95
4	1.53	15.41
5	1.60	6.77
6	8.07	3.35
7	9.86	9.10
8	10.37	8.03
9	12.61	10.15
10	14.35	8.94
11	15.66	18.49
12	25.83	11.56
13	28.63	9.22

Pearson correlation analysis: $r = 0.112$.
Bartlett χ^2 analysis: $P = .715$.

compared with nonatherosclerotic intimas. RT-PCR products were subjected to Southern blot analysis with specific internal oligonucleotide probes for HDJ-2 and β-actin to establish the specificity of the PCR reactions. The results shown in Table II indicate that, in spite of some degree of variability, the HDJ-2 mRNA expression in atherosclerotic carotid artery plaques is significantly upregulated when compared with nonatherosclerotic intimas (mean ± SEM 6.73 ± 1.59 vs 0.14 ± 0.04, respectively; $P = .001$). The expression of hsp70 and hsp60 mRNAs in atherosclerotic carotid artery plaques was also evaluated by means of RT-PCR and Southern blotting analysis. As shown in Table III, the expression of hsp70 mRNA is also significantly increased in atherosclerotic carotid artery plaques when compared with nonatherosclerotic intimas (mean ± SEM 9.51 ± 0.94 vs 3.72 ± 0.78, respectively; $P = .002$). In contrast, the expression of hsp60 mRNA is not significantly different in arterosclerotic carotid artery plaques when compared with nonatherosclerotic intimas (mean ± SEM: 21.05 ± 0.91 vs 20.61 ± 0.78, $P = .647$).

HDJ-2 and hsp70 are independently expressed in atherosclerotic carotid artery plaques. To determine whether the HDJ-2 mRNA expression correlated with that of hsp70 mRNA expression in the atherosclerotic carotid artery plaques examined, we reanalyzed the level of hsp70 mRNA expression in parallel with the level of HDJ-2 mRNA expression in situ. Table IV shows the values for all the samples ranging from 0.88 to 28.63 for HDJ-2 and 3.35 to 18.49 for hsp70. In addition, the results shown in Table IV indicate that the expression of HDJ-2 and hsp70 is not correlated with each other in atherosclerotic carotid artery plaques ($r = 0.112$, $P = .715$).

HDJ-2 expression is correlated with plaque ulceration plus degree of stenosis, but not with degree of stenosis alone. To determine whether HDJ-2 expression correlated with a particular clinical stage of the disease, we compared the level of HDJ-2 mRNA expression in individual carotid endarterectomy specimens with the severity of each atherosclerotic lesion and the clinical status of each patient. Specimens with different levels of HDJ-2 mRNA expression were analyzed in terms of the degree of carotid artery stenosis, the presence of plaque ulceration, as well as the patients' history of diabetes, hypertension, and tobacco use. As shown in Table V, the HDJ-2 mRNA expression was significantly higher in ulcerated plaques (patients 13-18) compared with nonulcerated plaques (patients 1-12) (mean ± SEM: 18.54 ± 2.97 vs 2.57 ± 0.90; $P = .0007$). Within the ulcerated plaque group (patients 13-18), there was a correlation between degree of stenosis and HDJ-2 mRNA expression ($r = 0.896$, $P = .016$). However, there was no correlation between degree of stenosis and HDJ-2 mRNA expression within the nonulcerated plaque group (patients 1-12) ($r = 0.530$, $P = .076$) or within the entire group of patients ($r = 0.0085$, $P = .97$). Similar analyses demonstrated a lack of correlation between the levels of hsp70 mRNA expression and plaque ulceration (mean ± SEM: 9.1 ± 1.89 for nonulcerated plaques vs 11.49 ± 1.84 for ulcerated plaques; $P = .38$) or the degree of stenosis ($r = -0.07$, $P = .82$).

Localization of the HDJ-2 protein expression in the atherosclerotic carotid artery plaque. The microscopic features of the atherosclerotic carotid artery plaques included an endothelial-lined fibrous cap containing smooth muscle cells, macrophage-derived foam cells, lymphocytes, collagen, elastin, and proteoglycans. In addition, the atherosclerotic carotid artery plaques included a necrotic lipid core containing cell debris, cholesterol crystals, and foam cells; and part of the media layer normally removed during endarterectomy procedures (data not shown). Immunohistochemical analysis with the anti–HDJ-2 monoclonal antibody was used to determine the tissue

Table V. HDJ-2/hsp70 expression and patient's clinical characteristics

Patient	*HDJ-2*	*hsp70*	*Hypertension*	*Diabetes mellitus*	*Tobacco usage*	*Degree of stenosis*	*Plaque ulceration*
1	0.62	NA	+	–	+	90%	–
2	0.65	NA	+	–	+	80%	–
3	0.67	NA	+	–	+	90%	–
4	0.88	3.65	+	–	–	70%	–
5	0.90	15.51	+	–	+	80%	–
6	0.97	NA	–	–	–	95%	–
7	1.27	9.95	–	+	+	80%	–
8	1.53	15.41	+	–	+	90%	–
9	1.60	6.77	–	–	+	85%	–
10	3.80	NA	+	–	+	90%	–
11	8.07	3.35	+	–	+	90%	–
12	9.86	9.10	+	+	+	99%	–
13	10.37	8.03	+	–	+	60%	+
14	12.61	10.15	+	–	+	75%	+
15	15.66	18.49	+	–	+	70%	+
16	18.10	NA	+	–	–	85%	+
17	25.83	11.56	+	–	+	85%	+
18	28.63	9.22	+	–	+	99%	+

Comparison of HDJ-2 expression between no plaque ulceration (patients 1-12, mean ± SEM: 2.57 ± 0.90) and plaque ulceration (patients 13-18, mean ± SEM: 18.54 ± 2.97): Mann-Whitney analysis, $P = .0007$.
Comparison of hsp70 expression between no plaque ulceration (patients 1-12, mean ± SEM 9.1 ± 1.89) and plaque ulceration (patients 13-18, mean ± SEM: 11.49 ± 1.84): Mann-Whitney analysis, $P = .38$.
Significant correlation between degree of stenosis and HDJ-2 expression in plaque ulceration (patients 13-18): Pearson correlation coefficient: $r = 0.896$, Bartlett χ^2 $P = .016$.
No correlation between degree of stenosis and HDJ-2 expression in no plaque ulceration (patients 1-12): Pearson correlation coefficient: $r = 0.530$, Bartlett χ^2 $P = .076$.
No correlation between degree of stenosis and HDJ-2 expression in all patients: Pearson correlation coefficient: $r = 0.0085$, Bartlett χ^2 $P = .97$.
No correlation between degree of stenosis and hsp70 expression in all patients: Pearson correlation coefficient: $r = -0.07$, Bartlett χ^2 $P = .87$.

localization of the HDJ-2 protein in carotid endarterectomy specimens.[9] As shown in Fig 2, *A* and *B,* high levels of the HDJ-2 protein were detected in the atherosclerotic carotid artery plaques. Immunoreactive HDJ-2 protein was localized to macrophage-derived foam cells (Fig 2, *A*), surface endothelial cells (Fig 2, *B*), and vascular smooth muscle-like myointimal cells (Fig 2, *B*). In contrast, no significant levels of the HJD-2 protein were detected in the nonatherosclerotic intimas (Fig 2, *C* and *D).* No significant reactivity was detected with the MOPC monoclonal antibody (negative control) in either the atherosclerotic carotid artery plaques or the nonatherosclerotic intimas (data not shown).

DISCUSSION

Hsps were first described as proteins produced by organisms in response to heat stress. In addition to their rapid induction by heat stress, it has recently been recognized that these proteins may also be upregulated by exposure to oxygen radicals,[10] cytokines,[11] hemodynamic stress,[12] ischemia,[13] surgical stress,[14] and viral infection.[15] In addition to their role in protecting the organism from stress, hsps have also been found to be involved in the pathogenesis of autoimmune diseases such as adjuvant-induced arthritis in rats,[16] diabetes mellitus in the nonobese diabetic mouse,[17] rheumatoid arthritis in human beings,[18] and systemic sclerosis in human beings.[19]

Traditionally, atherosclerosis has been thought of as a lipid disorder. However, there is increasing evidence suggesting that atherosclerosis may have an autoimmune component against hsps. For example, it has been shown that normocholesterolemic rabbits immunized with mycobacterial hsp65, which has a high homology with mammalian hsp60, developed atherosclerotic lesions.[20] In addition, hsp65-reactive T cells were also isolated from these lesions.[21] Hsp60 expression was also found in human atherosclerotic lesions by immunohistochemical analysis.[22] Moreover, hsp60 has been found to be coexpressed with ICAM-1 and to increase the adhesion of monocytes and T cells to aortic endothelium.[3] Several studies have confirmed the presence of a high number of activated CD4+ and CD8+ T cells in human atherosclerotic plaques.[23-28] Taken together, these results indicate that the hsp60 expression plays an important role in the immunopathogenesis of the atherosclerotic lesion.[1] However, hsp60 expression may not be the only factor in the immunopathogenesis of the disease since hsp60 is also expressed in normal arterial tissues.[6,7,20-22] Moreover, Xu et al[22] also found that a large population of the T cells infiltrating the atherosclerotic lesion did not respond to hsp60.

In contrast to hsp60, we report herein that HDJ-2 expression is significantly elevated in arteriosclerotic carotid artery plaques when compared with nonatherosclerotic intimas (Table II). Perhaps more important, we also found a significant correlation between HDJ-2–expression and plaque ulceration (Table V). These results suggest that HDJ-2 may play an important role in

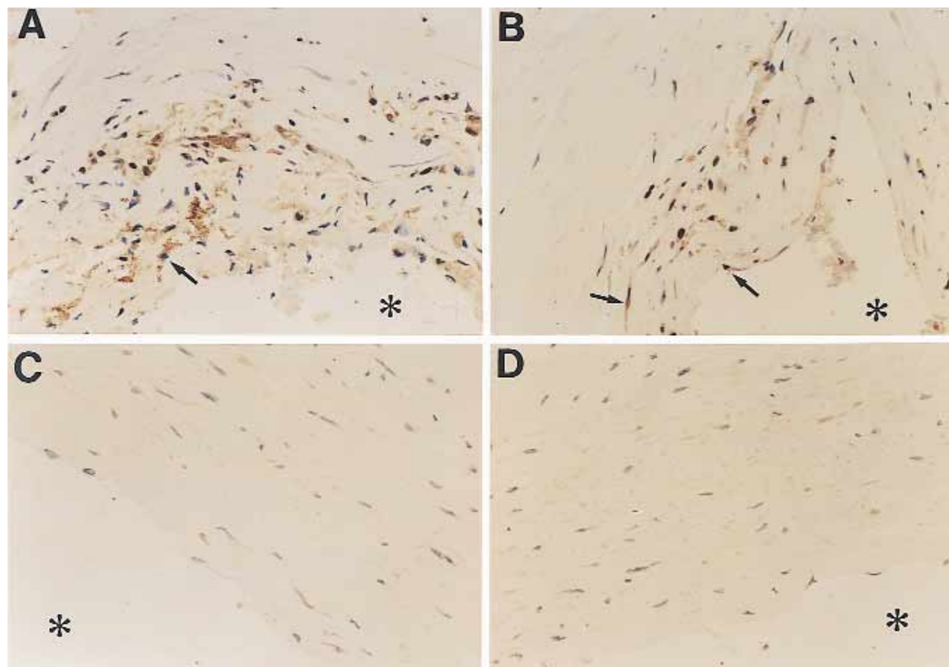

Fig 2. Localization of HDJ-2 protein expression in atherosclerotic carotid artery plaque. Immunohistochemical staining of atherosclerotic carotid artery plaques **(A** and **B)** and nonatherosclerotic intimas **(C** and **D)** was performed with anti–HDJ-2 mouse monoclonal antibody. Immunoreactive HDJ-2 protein was localized to macrophage-derived foam cells **(A,** *arrow),* surface endothelial cells **(B,** *right arrow),* and vascular smooth muscle-like myointimal cells **(B,** *left arrow). Asterisk* denotes lumen of vessels. Briefly, paraffin-embedded tissues were sectioned, attached to glass slides, and deparaffinized. Endogenous peroxidase activity was blocked by incubating the slides in H_2O_2. Monoclonal antibody raised against human HDJ-2 was used as primary antibody. Biotinylated goat-anti-mouse IgG was used as secondary antibody. Immune complexes were detected with Vectastain ABC kit (Vector Laboratory). Slides were counterstained with Harris hematoxylin (Sigma).

the immunopathogenesis of the atherosclerotic lesion. HDJ-2–reactive T cells must be demonstrated in the atherosclerotic lesion to prove this hypothesis. Current studies in our laboratory are aimed toward this goal. Up to this date, this is the first study demonstrating increased expression of HDJ-2 in atherosclerotic carotid artery plaques. The immunogenicity of HDJ-2 has been demonstrated by our previous studies in renal and lung transplantation.[6,7] The expression of this hsp has been shown to be increased in kidney[6] and lung[7] allografts undergoing rejection. Thus, HDJ-2 may also be responsible for T-cell activation during the immunopathogenesis of the atherosclerotic lesion.

Hsp70 expression was also found in this study to be significantly elevated in atherosclerotic carotid artery plaques when compared with nonatherosclerotic intimas (Table III), a result that is consistent with previous reports.[29,30] However, hsp70 is thought to play a protective rather than a causative role in atherosclerosis. Hsp70 was found to be differentially distributed in atherosclerosis and insufficient hsp70 accumulation in arterial smooth muscle cells resulted in cell necrosis.[29]

Hsps have also been shown to play a role in humoral response in atherogenesis. Anti-hsp65 antibodies have been found to be significantly elevated in subjects with carotid atherosclerosis, independent of other risk factors.[4] Complement-fixing autoantibodies to hsp60 were also shown to induce endothelial cytotoxicity.[31] Whether the antibody response to hsps represents a primary or secondary event in immunopathogenesis of the atherosclerotic lesion is still unknown. Because HDJ-2 expression is significantly upregulated in the atherosclerotic carotid artery plaques, current studies in our laboratory are aimed to screen for the development of anti–HDJ-2 antibodies in patients with atherosclerotic disease.

The results of this study suggest a possible role for HDJ-2 in the development or progression of atherosclerotic carotid artery plaques. Further studies are needed to elucidate the mechanisms by which HDJ-2 might participate in this process. HDJ-2 expression showed a significant correlation with the degree of luminal stenosis in ulcerated atherosclerotic carotid artery plaques. Therefore, understanding these mechanisms may provide new insights into the pathologic condition of atherosclerosis and potentially lead to new strategies for therapeutic intervention.

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