sonication (*3*). These relatively mild methods seem preferable to papain digestion, which breaks peptide linkages and is difficult to control, and to extraction with complex salts and detergents, which are difficult to remove from the solubilized product. The KCl method is undoubtedly applicable to solubilization of other cell membrane activities. Indeed, analogous to the solubilization of tumor-specific antigens by sonication (*16*), 3*M* KCl has been shown to effectively solubilize tumor-specific antigenic determinants of diethylnitrosamine-induced guinea pig sarcomas (*17*).

The 3*M* KCl extraction procedure and electrophoretic purification technique, combined with the availability of cultured cell lines, should provide sufficient purified material to elucidate the chemical structure of the HL-A antigens, and thereby, the molecular basis of transplantation individuality.

R. A. REISFELD
M. A. PELLEGRINO
Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037
B. D. KAHAN
Department of Surgery, Massachusetts General Hospital, Boston 02114

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Compositional Relatedness between Histocompatibility Antigens and Human Serum Lipoproteins

Abstract. *Human histocompatibility antigens and the protein part of Lp(a) lipoprotein and low density lipoprotein of human serum appear to be related when the amino acid compositions are compared. The Lp(a) lipoprotein appears to be closely related also to murine H-2^d antigen. The results support the concept of a relation between serum lipoproteins and histocompatibility antigens on cell membranes.*

We have suggested that a relation may exist between lipoproteins in the serum and histocompatibility antigens in cell membranes (*1*). In an investigation of human donors and recipients of skin grafts, we found that the grafts survived significantly longer when donors and recipients had the same phenotype within the Lp system (*2*) of inherited antigens belonging to the serum lipoproteins. The material studied was, however, of limited size. The observation by Kasukawa *et al.* (*3*) that rabbits may produce antibodies to allotypic specificities of serum lipoproteins after transplantation of skin from other rabbits appears to be compatible with, and possibly in support of, the view that a relation exists between transplantation antigens and serum lipoprotein antigens.

Zmijewski *et al.* (*4*) have presented evidence that there is a substance in human serum which is variable in quantity and may either inhibit or augment the detection of HL-A antigens by the leukocyte agglutination technique. Van Rood *et al.* (*5*) have reported that antiserum of the specificity HL-A2 can be neutralized with whole serum from people possessing the corresponding antigen.

Charlton and Zmijewski (*6*) have shown that the HL-A7 antigen is present in the soluble state in the β-lipoprotein fraction of human serum. Thus, there is now direct evidence for the existence of a connection between serum lipoprotein components and transplantation antigens.

A comparison of the protein composition of transplantation antigens and serum lipoproteins is therefore of considerable interest, but information on amino acid sequence is, unfortunately, not available for histocompatibility antigens or serum lipoproteins.

However, Metzger *et al.* (*7*) have developed a method for assessment of compositional relatedness between proteins based on the amino acid composition. The method was developed as an aid to the study of possible relations between proteins, when sequence data are not available. By means of this method, two proteins are compared by determining the difference in their fractional contents of each amino acid. From the sum of these differences, a difference index (DI) is calculated for the two proteins. Two proteins with the same composition would have a DI of 0 and two proteins with no amino acid in common would have a DI of 100. Metzger *et al.* (*7*) conducted 630 comparisons of different proteins or subunits of proteins and listed the resulting difference indices. The lowest value observed was 9.1, four (0.6 percent) of the 630 values were lower than 10, and 32 values (5 percent) were 13.5 or lower. Metzger *et al.* (*7*) also compared some proteins or protein subunits which are known to be related. For instance, a DI of 7.5 was found in the comparison of triose phosphate dehydrogenase of man and of *Escherichia coli*.

The amino acid composition of the main lipoprotein fractions—the low density lipoprotein (LDL) and high density lipoprotein (HDL) of human serum—has been studied in several laboratories. Simons *et al.* (*8*) reported the amino acid composition of the Lp(a) lipoprotein, and Mann *et al.* (*9*) reported amino acid analysis of two samples of human histocompatibility antigen and two samples of murine histocompatibility antigen. Thus, the question of a possible compositional relatedness between cellular histocompatibility antigens and serum lipoproteins may now be approached by the method of Metzger *et al.* (*7*).

I now report the result of an analysis where the data of Simons *et al.* (*8*) were used for the different lipoproteins and data of Mann *et al.* (*9*) were used for human and murine histocompatibility antigens. More amino acids had

been analyzed in the lipoprotein fractions than in the preparations of histocompatibility antigens. In each comparison, data on amino acids for which the histocompatibility antigen had not been analyzed were omitted from the lipoproteins. The fractional values of the remaining amino acids in the lipoproteins were adjusted to unity. Since two more amino acids had been analyzed in the murine than in the human transplantation antigens, the fractional values of the amino acids in the lipoproteins were not the same in the two comparisons. Table 1 shows the comparison of the Lp(a) lipoprotein and one sample (RAJI) of human histocompatibility antigen. The resulting DI is 6.81. The comparison between LDL and human histocompatibility antigen yielded a DI of 8.90, and that between HDL and histocompatibility antigen gave a DI of 12.62. When human histocompatibility antigen was compared with Lp(a) lipoprotein or LDL, the values of DI were thus lower than any value obtained in the 630 comparisons made by Metzger *et al.* (*7*). Slightly higher values were found for the three lipoproteins when they were compared with the second sample (R-4265) of human histocompatibility antigen studied by Mann *et al.* (*9*), but the DI from the comparison with the Lp(a) lipoprotein was still lower (8.96) than any of the 630 values of Metzger *et al.* In this analysis the comparison with LDL gave a DI of 9.28 and with HDL a DI of 14.37.

The Lp(a) antigen resides on a distinct group of lipoprotein molecules (*10*). Although the Lp(a) lipoprotein carries less lipid and has different floating characteristics in the ultracentrifuge, it shares several properties with LDL. The low difference indices obtained in the comparison between histocompatibility antigen and Lp(a) lipoprotein or LDL are of interest in view of previous data (from this laboratory) which suggested an influence of compatibility with respect to the Lp(a) antigen on graft survival time.

Mann *et al.* (*9*) also reported the amino acid composition of two samples of murine histocompatibility antigen. The comparisons between the human serum lipoproteins and murine H-2^d antigen are presented in Table 2. The DI for the Lp(a) lipoprotein and H-2^d was as low as 6.71. In this comparison both the LDL and HDL of human extraction yielded considerably higher values of the DI (Table 2). Thus, the similarity with transplantation antigens seemed to be more pronounced for the Lp(a) lipoprotein than for LDL in all comparisons.

Table 1. Evaluation of difference index* for Lp(a) serum lipoprotein and a preparation (RAJI) of human histocompatibility antigen (*9*).

Amino acid	Mole fraction		|Δ|
	Lp(a) lipoprotein	Histocompatibility antigen (RAJI)	
Lysine	0.0656	0.0747	0.0091
Histidine	.0222	.0311	.0089
Arginine	.0421	.0344	.0077
Aspartic acid	.1033	.0983	.0050
Threonine	.0710	.0639	.0071
Serine	.0817	.0866	.0049
Glutamic acid	.1230	.1394	.0164
Proline	.0565	.0451	.0114
Glycine	.0784	.0640	.0144
Alanine	.0863	.0914	.0051
Half-cystine			
Valine	.0628	.0780	.0152
Methionine			
Isoleucine	.0454	.0388	.0066
Leucine	.0887	.0963	.0076
Tyrosine	.0363	.0204	.0159
Phenylalanine	.0368	.0377	.0009
Total			.1362

* Difference index: $50 \times 0.1362 = 6.81$.

The results of these analyses must be interpreted with caution. Metzger *et al.* (*7*) pointed out that the DI between hen egg lysozyme and bovine lactalbumin is 22.6 although these two proteins have about 35 percent sequence homology. Thus, there may have been sufficient compositional divergence between the two proteins so that their relation to each other, which can be determined by sequence analysis, has been obscured by this analysis. On the other hand, the DI values obtained when Lp(a) lipoprotein was compared with human or murine histocompatibility antigens are both lower than any value obtained in the 630 comparisons conducted by Metzger and his co-workers. These values are also lower than those reported from comparisons between different samples of immunoglobulin light chains from the same species, and lower than the difference indices found in comparisons between human and murine transplan-

Table 2. Evaluation of difference index* for human serum lipoproteins and a preparation (H-2^d) of murine H-2 antigen (*9*).

Amino acid	Mole fraction				|Δ|		
	Lp(a) lipoprotein	LDL	HDL	H-2^d	Lp(a) lipoprotein–H-2^d	LDL–H-2^d	HDL–H-2^d
Lysine	0.0634	0.0764	0.1021	0.0452	0.0182	0.0312	0.0569
Histidine	.0215	.0231	.0173	.0299	.0084	.0068	.0126
Arginine	.0407	.0335	.0452	.0481	.0074	.0146	.0029
Aspartic acid	.0998	.1074	.0822	.0949	.0049	.0125	.0127
Threonine	.0686	.0656	.0502	.0738	.0052	.0082	.0236
Serine	.0789	.0807	.0667	.0647	.0142	.0160	.0020
Glutamic acid	.1188	.1228	.1753	.1265	.0077	.0037	.0488
Proline	.0546	.0400	.0444	.0693	.0147	.0293	.0249
Glycine	.0758	.0524	.0444	.0716	.0042	.0192	.0272
Alanine	.0833	.0618	.0873	.0701	.0132	.0083	.0172
Half-cystine	.0180	.0060	.0074	.0225	.0045	.0165	.0151
Valine	.0606	.0554	.0609	.0581	.0025	.0027	.0028
Methionine	.0159	.0172	.0091	.0210	.0051	.0038	.0119
Isoleucine	.0439	.0571	.0099	.0375	.0064	.0196	.0276
Leucine	.0857	.1194	.1268	.0830	.0027	.0364	.0438
Tyrosine	.0351	.0305	.0329	.0499	.0148	.0194	.0170
Phenylalanine	.0355	.0507	.0379	.0354	.0001	.0153	.0025
Total					0.1342	0.2635	0.3495

* Difference index: Lp(a) lipoprotein–H-2^d: $50 \times 0.1342 = 6.71$; LDL–H-2^d: $50 \times 0.2635 = 13.18$; HDL–H-2^d: $50 \times 0.3495 = 17.48$.

tation antigens (*9*). At face value, the resemblance between Lp(a) lipoprotein of human serum and murine cellular histocompatibility antigen appears to be even greater than the similarity between transplantation antigens of man and mouse.

Within the limitations of the methods, I conclude that the results of the analyses support the concept (*1*) that a relation exists between lipoproteins in the serum and histocompatibility antigens on cell membranes.

KÅRE BERG
Institute of Medical Genetics, University of Oslo, Oslo, Norway

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Angiotensin II: Rapid Localization in Nuclei of Smooth and Cardiac Muscle

Abstract. *Five to ten nanograms of labeled angiotensin II rapidly injected in the left ventricle of adult rats was found to induce significant ultrastructural endothelial changes, resulting in net increases in number and size of pinocytotic vesicles as well as widening of intercellular spaces. This effect was followed by preferential localization of the compound in the nuclear zone of vascular and cardiac muscle cells. The selective cellular localization of angiotensin II suggests that this vasoactive agent or some of its metabolic fragments may have specific effects on nuclear function.*

The exact mechanism of action of the octapeptide angiotensin II at vascular cell level is not known. One of the current hypotheses is that this potent vasoactive agent binds to specific receptor sites on the muscle cell membranes (*1*), altering membrane permeability to several ions, thus leading to a physiological response (*2*). As part of studies on the effects of this and other vasoactive agents on vascular permeability, as well as on the initiation of atherosclerosis, we have investigated the short-term distribution of angiotensin II on endothelial and muscle components of the arterial wall and cardiac muscle.

Fasting adult Wistar rats weighing between 200 and 220 g were anesthetized with ether, and a midline thoracotomy was made without delay. The heart was exposed and an 18-gauge needle was rapidly inserted into the left ventricle, avoiding the interventricular septum. A bolus of either 5 to 10 ng of cold angiotensin II [Hypertensin (Ciba)], or [isoleucyl-^{14}C]angiotensin II (*3*), or [tyrosyl-^{3}H]angiotensin II (*4*), diluted in 1 ml of Ringer's solution, was injected within 8 to 12 seconds through a three-way stopcock, followed by 1 to 2 ml of Ringer's solution and immediately thereafter by injection of 1 to 5 ml of a 1 percent solution of phosphate-buffered glutaraldehyde until induction of cardiac arrest. Average total perfusion times were 32.5 seconds (24 to 48 seconds). Slight tachycardia usually developed after injection of angiotensin II, and when approximately half the volume of fixative solution was injected, left ventricular contraction ceased, while the right ventricle continued contracting for another 30 to 40 seconds. Similar injection techniques were used for control studies with 50 μc of L-[^{3}H]tyrosine or 1-[^{3}H]isoleucine.

Immediately following this in vivo fixation, matching specimens (approximately 1 mm^3 in size) of the left and right ventricles, as well as full thickness specimens of the right and left coronary arteries and ascending portions of the arch of the aorta and thoracic aorta, were further fixed in 1 percent phosphate-buffered glutaraldehyde at 4°C for 30 minutes. One half of these samples was then processed for light microscopy–autoradiography following embedding in paraffin, and the other half was postfixed in buffered osmium tetroxide at 4°C for another 30 minutes, dehydrated in ascending concentrations of ethanol, and embedded in Epon resin for electron microscopy. For light microscopy–autoradiography, sections 6-μm thick were coated with diluted L-4 emulsion (Ilford) in a 0.01 percent solution of sodium lauryl sulfate and exposed in the dark at 4°C for 15 to 30 days. Sections 1 μm thick of Epon-embedded samples were stained with toluidine blue for orientation, and selected blocks were cut at a thickness of 300 to 600 Å in an LKB ultratome and stained with uranyl acetate–lead citrate. Sections mounted in Formvar-coated grids were also coated with L-4 emulsion and exposed in the dark at 4°C for periods of 60 to 140 days.

The injection of angiotensin II at the above concentrations failed to show any cytological changes by routine light microscopy. Electron microscopy, however, showed considerable increase in the number of pinocytotic vesicles in endothelial cells of both aorta and coronary arteries (Fig. 1a) with widening of intercellular gaps and occasional separation of desmosomes. Constantinides and Robinson (*5*) have previously reported that angiotensin II widens the interendothelial junctions by causing contraction of endothelial cells in superficial femoral arteries. However, in their study they used a much higher concentration of the peptide (66 μg/ml). Furthermore, in our studies, autoradiography consistently showed presence of radioactivity in the nuclear zone of endothelial cells, as well as in nuclei of smooth muscle cells of the aortic media and mitochondria of cardiac muscle. In contrast, little or no radioactivity could be shown in other cell organelles. In cardiac muscle, the label was not present in all nuclei and seemed to predominate in some muscle bundles more than in others, with higher radioactivity usually localized closer to blood vessels (Fig. 1b). Endothelial nuclei of intraparenchymal coronary branches, as well as surrounding pericapillary connective tissue, were often labeled. In order to demonstrate whether the intact peptide or individual amino