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Studies of Hepatic Synthesis *in Vivo* of Plasma Proteins, Including Orosomucoid, Transferrin, α_1-Antitrypsin, C8, and Factor B[1]

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Serum protein types were determined in eight recipients and donors in cases of hepatic homotransplantation. A change from recipient type to donor type was observed for factor B, C8, orosomucoid, haptoglobin, transferrin, α_1-antitrypsin, C3 and C6, but not for Gm and Inv immunoglobulin markers. The results indicate that all the proteins studied (except immunoglobulins) are produced primarily by the liver *in vivo*.

INTRODUCTION

Evidence for the sites of synthesis of plasma proteins has been obtained by organ perfusion, by examining tissues with fluorescent antibodies, by culturing of tissues *in vitro* and, in the case of the liver, genetic typing of the proteins in the recipient prior to hepatic homotransplantation and after transplantation, and in the liver donor prior to transplantation. By the last means, it has been possible to demonstrate that the liver is the primary site of synthesis of haptoglobin (1), Gc-globulin[3] (2), C3 (3) and, most recently, C6 (4). It is only by this last means that the relative contribution of the liver *in vivo* can be assessed.

In the present study, serum protein allotypes in eight persons undergoing hepatic transplantation and their donors were determined. Haptoglobin, Gc-globulin, orosomucoid, transferrin, α_1-antitrypsin, and the complement proteins, factor B, C3, C6, and C8 were examined. In addition, allotypes of IgG were analyzed.

METHODS

Serum samples. Serum was obtained from freshly clotted venous blood by centrifugation and was stored for up to 4 years at −70°C. Serum samples were

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[3] Abbreviations used in this paper to designate genetic types of plasma proteins: C3, C6, and C8, the third, sixth, and eighth components of complement; Bf, factor B of the properdin or alternative complement pathway; Gc, group specific component, Gc-globulin or vitamin D-binding globulin; Hp, haptoglobin; Or, orosomucoid or α_1-acid glycoprotein; Tf, transferrin; Pi, α_1-antitrypsin; Gm, γ1 (a, x, f) and γ3 (b^0, g) (heavy) chains of IgG; Inv, κ (light) chains of immunoglobulins.

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available for testing from eight recipients before and after hepatic transplantation and for eight of nine liver donors in these cases. In most instances, two or more samples from recipients after transplantation were available, and the last samples were obtained 1–27 months after operation.

Genetic typing of serum proteins. Haptoglobin types were determined by a modification of the method of Smithies (5) utilizing thin-layer polyacrylamide gel electrophoresis and peroxidase staining of hemoglobin–haptoglobin complexes with *o*-tolidine. Gc-globulin types (6) were ascertained by agarose gel electrophoresis and immunofixation (7) with monospecific antiserum to Gc-globulin obtained from Atlantic Antibodies, Westbrook, Maine. Prolonged agarose gel electrophoresis was used to type C3 (8) and factor B (9). Antiserum for factor B typing was purchased from Atlantic Antibodies. Orosomucoid (α_1-acid glycoprotein) types were analyzed by agarose gel electrophoresis of neuraminidase-treated samples (10) followed by immunofixation with monospecific antiserum to orosomucoid (Atlantic Antibodies). C6 (11) and C8 (12) types were determined by isoelectric focusing in thin-layer polyacrylamide gels and development of patterns of hemolysis in overlay agarose gels containing appropriately sensitized sheep erythrocytes and serum from C6-deficient rabbits (13) or a C8-deficient person (14). Transferrin subtyping (15) was performed by isoelectric focusing and protein staining. Gm and Inv types were performed by standard serological methods (16). Pi typing for α_1-antitrypsin was performed by isoelectric focusing (17) followed by immunofixation with specific antiserum (Atlantic Antibodies).

RESULTS

In general, the nonimmunoglobulin plasma proteins tested that showed a difference in allotype between liver donor and recipient changed type to that of the donor following homotransplantation of the liver. Table 1 presents these results in two cases. Recipient No. 1 was Bf F, whereas her first donor was Bf FS. Her Bf type changed to FS after transplantation. Although no serum was available from the donor of the second liver transplant to this patient, the patient's type again changed, this time to Bf S. This strongly suggested that the second donor was Bf S. Changes in type were also observed in C3, C6, and orosomucoid (second transplantation) as well as Gc-globulin, haptoglobin (first transplantation), and α_1-antitrypsin (both transplantations).

In recipient No. 2, a change in allotype to that of the donor was noted for Gc-globulin, C6, and C8, but α_1-antitrypsin did not change type to that of the donor following transplantation. In all the other cases studied, complete changes in allotypes to those of the donor were observed in a number of instances, as summarized in Table 2. In one instance, the recipient was Gc 1-1 and changed to 2-2 even though the donor's type was 2-1. In the latter, the Gc 1 bands were distinctly fainter than usual and less than half as intense as the Gc 2 band, suggesting that the donor had been transfused with blood containing Gc 1. Other serum protein types were the same in donor and recipient in that instance of liver transplantation.

As can be seen in Table 1, Gm and Inv types, where they differed between donors and recipients, did not change following liver transplantation. In addition

TABLE 1
SERUM PROTEIN TYPES OF LIVER DONORS AND RECIPIENTS

Subject		Bf	C3[a]	C6	C8	Gc	Hp	Or	Tf	Pi	Gm					Inv a
											a	x	f	g	b[a]	
Recipient No. 1 pretransplant	9/17/75	F	—	AB	AB	2-1	2-1	F	C1,2	M_1M_2	0	0	+	0	+	0
Donor No. 1	9/17/75	FS	FS	AB	AB	1-1	2-1	F	C1,2	M_1	0	0	+	0	+	+
Recipient No. 1 post-transplant No. 1	10/17/75	FS	FS	AB	AB	1-1	2-1	F	C1,2	M_1	0	0	+	0	+	0
Recipient No. 1 post-transplant No. 2[c]	8/26/77	S	S	A	AB	1-1	2-1	S	C1,2	M_1M_2	0	0	[b]	0	+	[b]
Recipient No. 1 post-transplant No. 2[c]	11/11/77	S	S	A	AB	1-1	2-1	S	C1,2	M_1M_2	0	0	+	0	+	0
Recipient No. 2[d] pretransplant	11/15/74	S	—	AB	A1A	1-1	2-1	F	C1	M_1	+	0	+	+	+	+
Donor No. 2	11/27/74	S	—	A	AB	2-2	2-1	F	C1	M_1M_2	0	0	+	0	+	0
Recipient No. 2[d] post-transplant	2/28/75	S	—	A	AB	2-2	2-1	F	C1	M_1	+	0	+	+	+	+
Recipient No. 2[d] posttransplant	8/ 5/75	S	—	A	AB	2-2	2-1	F	C1	M_1	+	0	+	+	+	+

[a] C3 types indicated by hyphens could not be determined owing to conversion.
[b] Gm type could not be determined owing to the presence of antibody.
[c] Serum from the donor for the second liver transplantation not available.
[d] Pi types (α_1-antitrypsin) did not change, as expected, to that of the liver donor.

TABLE 2
CHANGES IN SERUM PROTEIN TYPES IN INFORMATIVE SETS OF DONORS AND RECIPIENTS

Protein	No.	Recipient prior to transplantation	Donor	Recipient after transplantation
Bf	2	S	FS	FS
	2	FS	S	S
	1	F	FS	FS
	1	S1	S	S
C3	1	S	FS	FS
	1	FS	—[a]	F
	1	FS	—[a]	S
C6	2	AB	A	A
	1	AB	—[b]	A
	1	B	AB	AB
C8	1	A1A	AB	AB
	2	A	AB	AB
	1	AB	A1A	A1A
Gc	1	1-1	2-2	2-2
	2	2-1	1-1	1-1
	1	1-1	2-1[c]	2-2
Hp	1	2-1	2-2	2-2
	1	2-2	1-1	1-1
	1	1-1	2-1	2-1
Or	1	FS	F	F
	1	F	FS	FS
	1	F	—[b]	S
Tf	2	Cl	Cl,2	Cl,2
	1	Cl,2	Cl	Cl
Pi	1	M_2	M_1	M_1
	1	M_1	M_1M_2	M_1[d]
	1	M_1M_2	M_1	M_1
	1	M_1	—[b]	M_1M_2

[a] A dash indicates either that C3 was untypable or the donor was not available.
[b] Donor serum not available.
[c] The donor was probably Gc 2-2 but transfused with Gc 1-1 or 2-1 plasma.
[d] The recipient's α_1-antitrypsin genetic type did not change to that of the hepatic donor. See Table 1 and text.

to the two instances shown in Table 1, two additional informative pairs showed no change in recipient immunoglobulin types after operation.

DISCUSSION

These results provide strong evidence that the liver is the primary site of synthesis of factor B, C8, transferrin, α_1-antitrypsin, and orosomucoid in man. They also confirm previous findings that the same is true for haptoglobin (1, 18), Gc-globulin (2), C3 (3), and C6 (4). Complete change in type could be assessed for orosomucoid, Gc-globulin, haptoglobin, and factor B in instances where there was

no shared type between donor and recipient. For example, in the case where Bf S changed to Bf S, there was no detectable Bf Sl post-transplantation in the recipient. This suggests that the synthetic contribution by tissues other than the liver or cell types other than hepatocytes is minor. In the guinea pig, macrophages produce factor B (19) in culture. The latter cells, as well as human monocytes (20), synthesize C3 but, again, the liver appears to be the primary source of this protein *in vivo*. For C8 and C6, informative donors and recipients shared an allotype, but typing patterns after transplantation were identical to those produced by donor serum. In the previously reported changes in C3 and C6 types after liver transplantation (3, 4), donors and recipients had no shared allotypes.

As can be seen in the tables, in one out of four informative liver transplantations, α_1-antitrypsin in the recipient did not change to that of the donor. In the remaining three instances, the change in Pi type occurred as expected. This finding is puzzling. It is unlikely that samples were somehow mislabeled since all other donor nonimmunoglobulin serum protein types were identical to the recipient after transplantation (Table 1). It seems unlikely that the major organ of synthesis of α_1-antitrypsin is different in different individuals.

The failure of immunoglobulin types of liver recipients to change to those of the donors confirms what has long been known; B lymphocytes and their descendants, the plasma cells, and not hepatocytes produce these molecules. This lack of change also provides the proper control for the liver-synthesized proteins. We did not in the present study observe, as did Kashiwagi and co-workers in an earlier study (21), that some liver recipients had Gm types of their donors (as well as their own preoperative types) for long periods after transplantation. Very recently, Witherspoon and colleagues (22) have shown that immunoglobulins after marrow transplantation for acute leukemia and aplastic anemia are of donor origin. The current experiments confirm in detail that virtually all plasma proteins except for the immunoglobulins are synthesized by the liver *in vivo*, an observation first made 25 years ago in studies of the isolated perfused liver and hepatectomized rat (23, 24).

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