0270-9139/89/1005-0861$02.00/0
HEPATOLOGY
Vol. 10, No. 5, pp. 861-866, 1989
Printed in U.S.A.

1034

Orthotopic Liver Transplantation and the Cytosolic Estrogen-Androgen Receptor Status of the Liver: The Influence of the Sex of the Donor

DELAWIR KAHN, QIHUA ZENG, LEONARD MAKOWKA, NORIKO MURASE, YASUAKI NAKAJIMA, PATRICIA K. EAGON, ANTONIO FRANCAVILLA, THOMAS E. STARZL AND DAVID H. VAN THIEL
Departments of Surgery and Medicine, The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Mammalian liver is known to contain cytosolic receptors for both estrogens and androgens. Furthermore, certain mammalian hepatic functions are known to display a sexual dimorphism. However, in clinical liver transplantation, the sex of the donor is not taken into consideration in selection of the donor. In this study, the effect of liver transplantation on the estrogen and androgen receptor content of the liver was determined.

Adult male and female rats were subjected to orthotopic liver transplantation, using donors form both the same and the opposite sex as the recipient. The animals were killed on the tenth postoperative day, and the livers were assayed to determine their cytosolic estrogen and androgen receptor content.

Transplantation of a liver from a male donor into a male recipient, from a male donor into female recipient and from a female donor into a male recipient produced similar changes in the number of cytosolic estrogen and androgen receptors in hepatic cytosol. In all three situations, the estrogen receptor content in the cytosol of the transplanted liver was the same as that found in an unoperated male liver, and the cytosolic content of the androgen receptor was the same as that of an unoperated female liver. After transplantation of the liver from a female donor into a female recipient, the estrogen and androgen receptor content in the cytosol of the transplanted liver was the same as that of an unoperated female. A consistent finding in this study was a reduction in the cytosolic androgen receptor activity of the transplanted liver when a male was either the recipient or donor. This reduction was maximal at 10 days post-transplantation. At 40 days following transplantation, a partial recovery in the androgen receptor acitivy of hepatic cytosol had occurred. These studies demonstrate that orthotopic liver transplantation changes the cytosolic content of both the estrogen and androgen receptors of the liver, and their ratios may have important effects during the immediate postoperative period and profound long-term consequences relative to gender differences in hepatic function.

Receptors for both estrogens and androgens have been identified in the liver of both rats and man, and their properties have been studied extensively (1-6). However, the precise function of these receptors remains unresolved. Nonetheless, in mammals certain functions of the liver are known to display a sexual dimorphism (7-9). Testosterone present *in utero* and after puberty appears to be the major determinant of the difference between the sexes in hepatic function (8).

In recent years, orthotopic transplantation of the liver has become an established alternative in the management of patients with endstage liver disease (10, 11). Donors for liver transplantation are currently selected with regard only to the ABO blood group and size compatibility with the recipient. The sex of the donor is not taken into consideration. In view of the sexual dimorphic character of hepatic function, the choice of a particular donor sex may be important for the initial success of the transplant procedure as well as subsequent long-term normal function of the graft (12). In this study, the effect of orthotopic transplantation on the estrogen and androgen receptor content of the liver in male and female rats, using donors from both the same and the opposite sex as the recipient, was determined.

MATERIALS AND METHODS

Animals and Chemicals. Adult Lewis rats weighing 200 to 350 gm were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Sodium molybdate, diethylstilbestrol, Tris base and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). New England Nuclear (Boston, MA) provided the [^{3}H]methyltrienolone (^{3}H-R1881; 85 Ci per mmole) and unlabeled R1881. Aqueous counting scintillant was obtained from Amersham Corp. (Arlington Heights, IL). All other chemicals were obtained from Fisher Scientific Co. (Pittsburgh, PA).

Surgical Procedures. The animals were allocated to five groups and treated as follows: 24 male rats (Group 1) were subjected to orthotopic liver transplantation using livers from male donors. At various times up to 8 weeks postoperatively, groups of animals were anesthetized with ether, and their livers

Received June 23, 1988; accepted April 28, 1989.
Supported by research grants from the Veterans Administration (Project Grant AM 29961), from the National Institute of Diabetes and Digestive and Kidney Diseases (Grant AM 32556) and from the National Institute on Alcohol Abuse and Alcoholism (Grant AA 06601). Dr. D. Kahn was supported by a grant from the Medical Research Council of South Africa.
Address reprint requests to: David H. Van Thiel, M.D., 1000J Scaife Hall, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261.

were removed, weighed and used to determine the level of the estrogen and androgen receptor content in the cytosol of the transplanted liver.

An additional five male rats (Group 2) were subjected to orthotopic liver transplantation using livers from male donors and, at 10 days postoperatively, the animals were anesthetized with ether, and the livers were removed, weighed and used to determine the content of the estrogen and androgen receptors in the cytosol of the transplanted liver.

Five male rats (Group 3) were subjected to orthotopic liver transplantation using a liver obtained from a female donor. On the tenth postoperative day, the recipient animals were anesthetized with ether, and the livers were removed, weighed and used to determine the content of estrogen and androgen receptors in the cytosol of the transplanted liver.

Five female rats (Group 4) were subjected to orthotopic liver transplantation using livers from male donors. On the tenth postoperative day, the animals were anesthetized with ether, and the livers were removed, weighed and used to determine the estrogen and receptor content in the cytosol of the transplanted liver.

Five female rats (Group 5) were subjected to orthotopic liver transplantation using organs obtained from female donors. On the tenth postoperative day, the animals were anesthetized with ether, and the transplanted livers were removed, weighed and used to determine the estrogen and androgen receptor content of the hepatic cytosol.

Orthotopic Liver Transplantation. Orthotopic transplantation of the liver in the rat was performed using the cuff technique as described by Kamada and Calne (13) for the portal vein, suprahepatic vena cava and infrahepatic vena cava.

Preparation of the Cytosol. The livers were homogenized in 4 volumes of ice-cold buffer consisting of 0.25 *M* sucrose, 1.5 m*M* EDTA, 10 m*M* mercaptoethanol and 10 m*M* Tris-HCl (pH 7.4) using a Brinkman Polytron homogenizer. The homogenate was centrifuged at 103,000 × *g* for 1 hr at 4°C, and the supernatant obtained was used for all of the assays performed.

Estrogen Receptor Activity. The specific binding of a saturating concentration of [^{3}H]estradiol was used to determine the cytosolic estrogen receptor content (1). The hepatic cytosol was diluted 1:1 with a buffer consisting of 40 m*M* sodium molybdate, 1.5 m*M* EDTA and 10 m*M* Tris-HCl (pH 7.4) in order to stabilize the estrogen receptor. A mixture consisting of 200 μl diluted cytosol, 25 μl 30 m*M* [^{3}H]estradiol and 25 μl ethanol was used to determine the total binding [^{3}H]estradiol. Nonspecific binding was measured in parallel assays in which the ethanol was replaced with 25 μl 3 μ*M* unlabeled diethylstilbestrol dissolved in ethanol. In each case, the mixture was allowed to incubate for 2 hr at 4°C, and the reaction was terminated by the addition of 0.4 ml 1% dextran-coated charcoal to remove unbound steroid. After centrifuging at 1,500 × *g* for 5 min at 4°C, the supernatant was transferred carefully to a scintillation vial containing 8 ml ACS scintillation fluid. The radioactivity in the supernatant was measured in a Packard Tri-Carb 460 CD liquid scintillation system (Downers Grove, IL).

Androgen Receptor Activity. The assay utilized to quantitate the cytosolic androgen receptor content was similar to the one described above for the estrogen receptor with minor exceptions (2). Total binding was measured using a mixture of 200 μl of the cytosol with 25 μl tritiated R1881, a synthetic androgen. Parallel assays in which 25 μl unlabeled R1881 (3 μ*M*) were added to the mixture were used to determine the nonspecific binding. The binding of R1881 to glucocorticoid receptors was blocked by adding 5 μ*M* triamcinolone acetonide to the assay mixture. After an overnight incubation at 4°C, the reaction was terminated by adding 0.41 ml 1% dextran-coated charcoal to remove unbound R1881. The mixture was centrifuged at 1,500 × *g* for 5 min at 4°C, and the supernatant was placed in scintillation vials with 8 ml ACS scintillation fluid. The radioactivity present in the supernatant was counted in a Packard Tri-Carb 460 CD liquid scintillation system.

Miscellaneous Methods. The method of Lowry et al. (14) was used to determine the cytosolic protein concentration using bovine serum albumin as the standard.

All results are reported as mean values ± S.E. Statistical analysis of the data was performed using analysis of variance and appropriate paired comparison tests. A p value of 0.05 or less was considered to represent a significant difference.

RESULTS

The effect of orthotopic liver transplantation, from a male donor to a male recipient, on the cytosolic estrogen receptor activity of the liver is shown in Figure 1a. The cytosolic estrogen receptor content in the liver remained stable up to 20 days after the transplant. A small but significant reduction in the level of the estrogen receptor content was observed at 30 days postoperatively ($p < 0.05$). Thereafter, the level of estrogen receptor content increased steadily and returned to preoperative basal male levels by 50 days after transplantation.

The change in the cytosolic androgen receptor content in the hepatic cytosol after transplantation of the liver from a male donor to a male recipient is shown in Figure 1b. The baseline preoperative level of androgen receptor content in the hepatic cytosol was 3.45 ± 0.47 fmoles per mg protein. A significant ($p < 0.01$) reduction in the level of the cytosolic androgen receptor content in the liver was observed at 10 days after transplantation and persisted for at least 40 days. At 40 days, the androgen receptor content in the male allograft was still significantly ($p < 0.05$) lower than it was preoperatively, but a recovery toward levels seen preoperatively was evident.

The changes in the cytosolic estrogen and androgen receptor content of the liver at 10 days after transplantation in the case of a liver obtained from a male donor and transplanted into a male recipient are shown in Figure 2a. The level of the estrogen receptor content of the hepatic cytosol was unchanged compared to preoperative levels. In contrast, the level of the cytosolic androgen receptor content in the allograft liver was reduced significantly ($p < 0.01$) compared to the basal pretransplant value. When the ratio of estrogen receptor content to androgen receptor content at 10 days following orthotopic liver transplantation is compared to that of unoperated male animals, a significant increase is seen in the transplanted animals ($p < 0.01$) (Fig. 2b).

The effects of transplantation of a liver obtained from a female and transplanted into a male recipient in terms of the estrogen and androgen receptor content in hepatic cytosol at 10 days after the transplant are shown in Figure 3a. The level of cytosolic estrogen receptor content in the liver after transplantation was significantly ($p < 0.01$) less than that of a normal female liver but was similar to the level of estrogen receptor found in a normal male liver. In contrast, the level of the androgen receptor content in hepatic cytosol obtained from a fe-

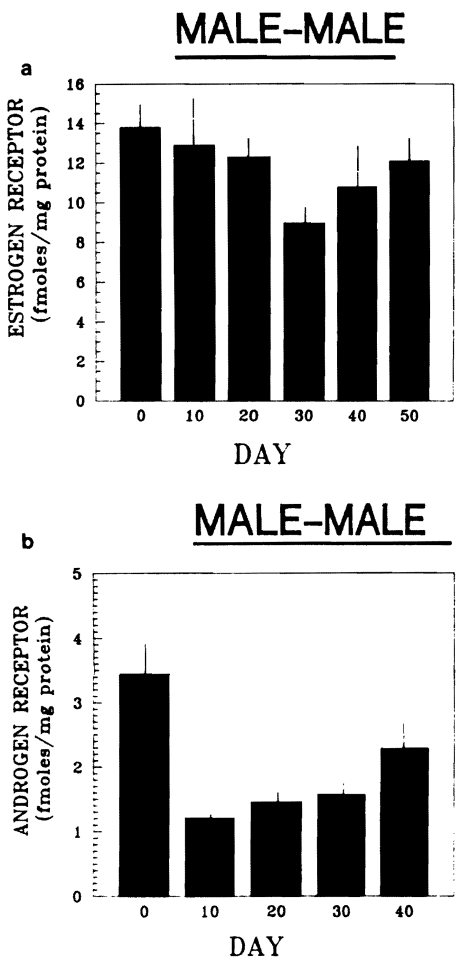

FIG. 1. (a) Estrogen receptor content (fmoles per mg protein) in the cytosol of livers transplanted from a male donor to a male recipient (n = 4 to 6 animals per group per time point). (b) Androgen receptor content (fmoles per mg protein) in the cytosol of livers transplanted from a male donor to a male recipient (n = 4 to 6 animals per group per time point).

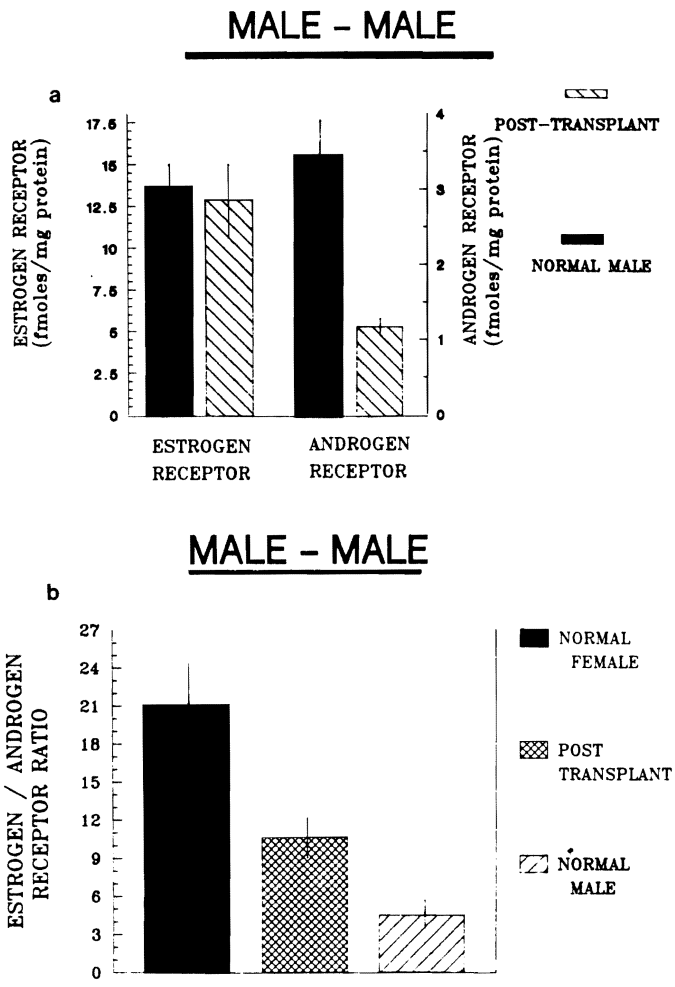

FIG. 2. (a) Estrogen and androgen receptor content (fmoles per mg protein) in the cytosol of the unoperated male liver and at 10 days after transplantation of livers from male donors into male recipients (n = 5 animals per group). (b) Ratio of the estrogen to androgen receptor content in the cytosol of unoperated male and female livers and livers transplanted from male donors to male recipients (n = 5 animals per group).

male liver transplanted into a male recipient was the same as that of a normal female liver, being significantly ($p < 0.01$) less than that of a normal male liver. The ratio of the estrogen to androgen receptor content in the transplanted liver was significantly greater than that found in the unoperated male liver but significantly lower than the value for an unoperated female liver (both $p < 0.01$) (Fig. 3b).

The levels of estrogen and androgen receptor content in the hepatic cytosol after transplantation of a liver obtained from a male donor into a female recipient are shown in Figure 4a. The estrogen receptor content of the liver at 10 days after the transplant was significantly ($p < 0.01$) less than that found in a normal intact female liver but was similar to the level present in a normal intact male liver. However, the androgen receptor content of the liver after transplantation was reduced to a level found in normal intact female liver ($p < 0.01$). The ratio of the estrogen receptor content to the androgen receptor content in the hepatic cytosol in this group of animals was significantly lower than that present in the unoperated female liver but significantly greater than the value obtained for unoperated male liver (both $p < 0.01$) (Fig. 4b).

The estrogen and androgen receptor content present in the hepatic cytosol after transplantation of the liver from a female donor to a female recipient is shown in Figure 5a. The levels of both the estrogen and androgen receptor content and their ratio in hepatic cytosol after the transplant were similar to those of normal intact female liver at all times studied from time of transplantation through 40 days following transplantation. The estrogen to androgen receptor content ratio in the cytosol of the transplanted liver was similar to the ratio found in the unoperated female liver at all times studied but was significantly greater than that found in the unoperated male liver (both $p < 0.01$) (Fig. 5b).

DISCUSSION

In this study, the estrogen receptor content present in the hepatic cytosol, except for a slight decrease seen at

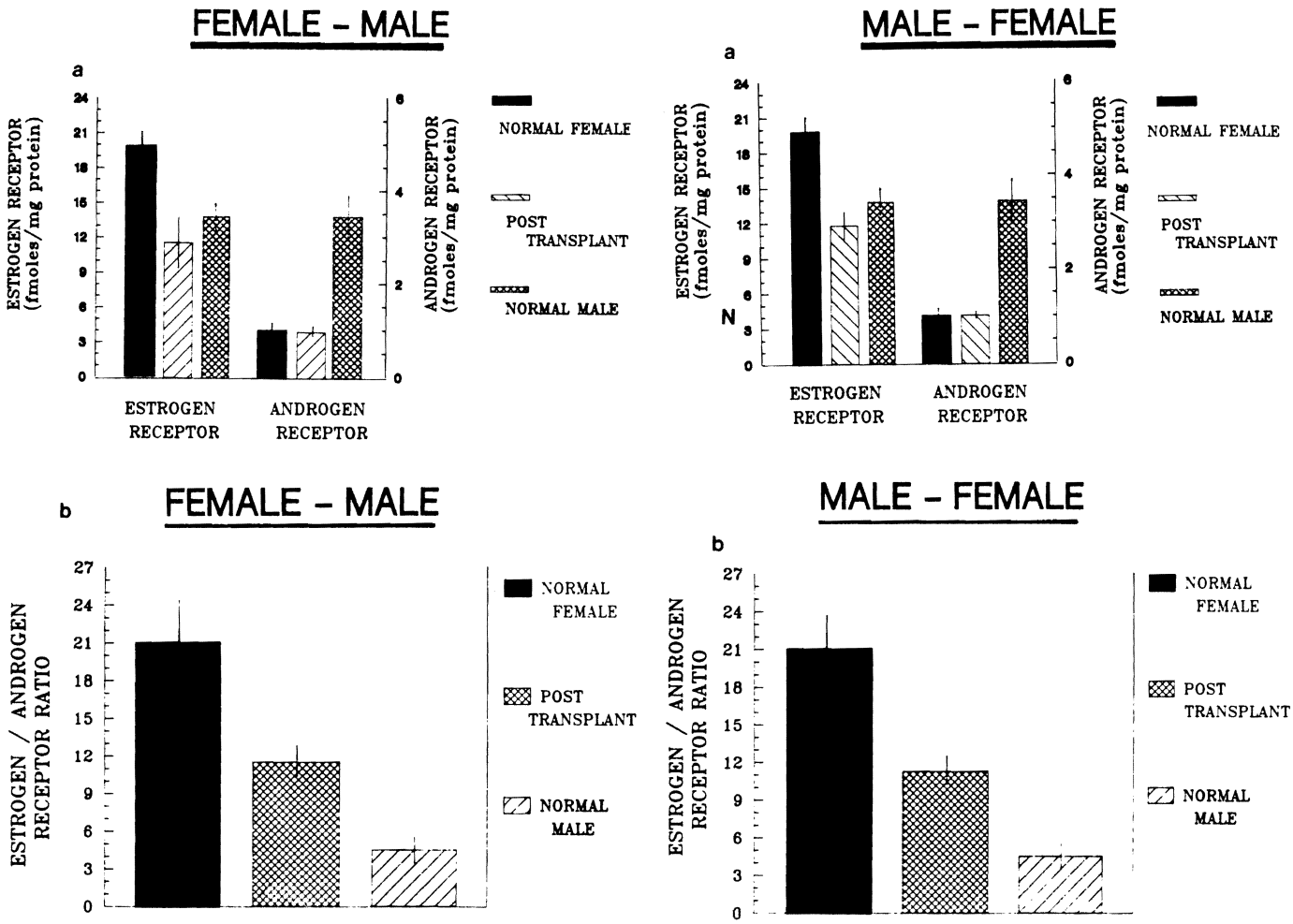

FIG. 3. (a) Estrogen and androgen receptor content (fmoles per mg protein) in the cytosol of the unoperated male and female liver and at 10 days after transplantation of livers from female donors into male recipients (n = 5 animals per group). (b) Ratio of the estrogen to androgen receptor content in the cytosol of unoperated male and female livers and livers transplanted from female donors to male recipients (n = 5 animals per group).

FIG. 4. (a) Estrogen and androgen receptor content (fmoles per mg protein) in the cytosol of the unoperated male and female liver and at 10 days after transplantation of livers from male donors into female recipients (n = 5 animals per group). (b) Ratio of the estrogen to androgen receptor content in the cytosol of unoperated male and female livers and livers transplanted from male donors to female recipients (n = 5 animals per group).

30 days posttransplant, remained stable up to 50 days after orthotopic transplantation of a male donor liver to a male recipient (Fig. 1a). In contrast, the cytosolic content of the androgen receptor was significantly lower at 10 days after transplantation of a male liver into a male recipient compared to preoperative levels (Fig. 1b). Thereafter, however, the androgen receptor content increased with time in the new host but still was reduced, compared to either preoperative or intact male levels at 40 days after the transplant procedure.

Transplantation of a liver obtained from a male or female donor into a male or female recipient produced quite different changes in the cytosolic estrogen and androgen receptor content and their relative density in the transplanted liver. Only the transplantation of a female donor liver into a female recipient produced results similar to those present in unoperated control animals of the same sex. Transplantation of a male liver into a male recipient resulted in the estrogen receptor content being the same as that present in a normal male before surgery, but the androgen receptor density declined to levels found in a normal female liver (Fig. 2a).

The changes in the estrogen and androgen receptor content of hepatic cytosol after transplantation of a liver obtained from a male donor transplanted into a female recipient and obtained from a female donor but transplanted into a male recipient were similar. In both situations, the estrogen receptor density of the cytosol was the same as in a normal intact male liver, and the hepatic androgen receptor density was the same as that found in a normal intact female. In other words, after transplantation of a female liver into a female recipient, the liver, with regard to the estrogen and androgen receptor content of hepatic cytosol, remains effectively identical to that of a female liver. In contrast, after transplantation of a male or a female liver into a recipient of the opposite

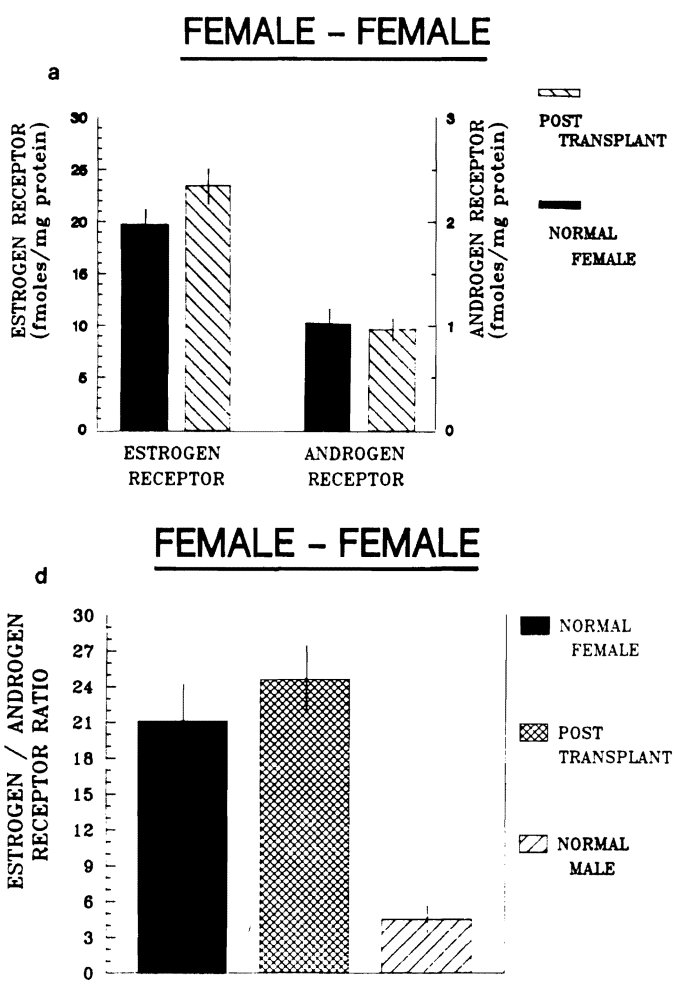

FIG. 5. (a) Estrogen and androgen receptor content (fmoles per mg protein) in the cytosol of the unoperated female liver and at 10 days after transplantation of livers from female donors into female recipients (n = 5 animals per group). (b) Ratio of the estrogen to androgen receptor content in the cytosol of unoperated male and female livers and livers transplanted from female donors to female recipients (n = 5 animals per group).

sex, the liver assumes a male identity with regard to its cytosolic estrogen receptor content and a female identity with regard to its cytosolic androgen receptor content.

The findings reported herein may have important implications. Because the liver is known to display a sexual dimorphism with regard to several important functions, these changes in the cytosolic estrogen and androgen receptor content of the liver after transplantation may be important both for the initial success of the transplant procedure and possibly also the long-term performance of the liver in the recipient.

A "feminization" of the liver of male rats has been reported to occur in association with regeneration and includes an increase in the serum level of estradiol and the hepatic content of the estrogen receptor. These changes occur while a reduction in the serum level of testosterone and the content of the androgen receptor within the liver is occurring (2, 6). Moreover, a translocation of the estrogen receptor from the cytosolic compartment of the cell to the nuclear compartment is seen prior to the initiation of DNA synthesis. These changes suggest an important role for nuclear estrogen and particularly that an estrogen-DNA interaction exists prior to the initiation of the regenerative process within liver (15). Thus, the changes in the estrogen and androgen receptor content seen in the transplanted livers reported herein may represent a regenerative response to some transplantation-related hepatic injury. Such a regenerative response in a transplanted organ may be crucial for the successful engraftment of the liver after transplantation.

During liver transplantation, the liver graft is injured in a number of different ways, which includes an ischemic injury related to the procurement process and the preservation time, an immunological injury occurring as a consequence of the attack mounted by the recipient's immune response, injury occurring as a result of an infection related to the transplant experience and, finally, injury occurring as a result of the toxicity of certain drugs, particularly cyclosporine, which are used to prevent graft rejection. Occasionally, because of a mismatch between the size of the donor and the recipient, a small liver is transplanted into a larger recipient, and hepatic regeneration of such grafts is required for the graft to meet the metabolic needs of the recipient (16, 17). Similarly, because of the lack of suitable-sized pediatric donors, some centers use partially hepatectomized (reduced) adult livers in pediatric recipients (18, 19). Both of these situations require a regenerative response on the part of the transplanted organ, and a normal regenerative response in these particular situations may be crucial for the ultimate success of the transplant procedure. Because estrogens and their receptors are thought to play an important role in the process of hepatic regeneration, the changes observed in the estrogen and androgen receptor levels in hepatic cytosol herein reported after transplantation may influence the regenerative response of such liver grafts. In the experiments performed, because an inbred strain of rats was utilized and cyclosporine was not, and the ischemia time of the transplanted liver was short, these variables were either not present or minimally important. In a clinical transplant situation, each of these variables is present and would only further enhance the need for a regenerative response.

Cyclosporine, the immunosuppressive agent used routinely in most organ transplant programs, has several untoward effects including hirsutism, increased lanugo hair growth, abnormal uterine bleeding, fibroadenomas of the breast, virilism in females and a tendency to develop diabetes, all of which appear to be mediated, at least in part, hormonally. Cyclosporine has been shown to potentiate hepatic regeneration (20, 21), but also to increase the estrogen receptor content present within hepatic cytosol (22). Thus, an interaction between the effects of cyclosporine on the estrogen receptor content in hepatic cytosol and the changes observed in hepatic cytosol after transplantation herein reported may be synergistic and have important implications.

REFERENCES

1. Eagon PK, Fisher SE, Imhoff AF, et al. Estrogen-binding proteins of male rat liver: influences of hormonal changes. Arch Biochem Biophys 1980; 201:486–499.
2. Francavilla A, Eagon PK, DiLeo A, et al. Sex hormone related functions in regenerating male rat liver. Gastroenterology 1986; 91:1263–1270.
3. Bannister P, Sheridan P, Losowsky MJ. Identification and characterization of the human androgen receptor. Clin Endocrinol 1985; 23:495–502.
4. Aten RF, Dickson EB, Eisenfeld AJ. Estrogen receptor in adult male rat liver. Endocrinology 1978; 103:1629–1635.
5. Eisenfeld AJ, Aten R, Weinberger MJ, et al. Estrogen receptor in mammalian liver. Science 1976; 191:862–865.
6. Eagon PK, Porter LE, Francavilla A, et al. Estrogen and androgen receptors in the liver: their role in liver disease and regeneration. Semin Liver Dis 1985; 5:59–69.
7. Roy AK, Chatterjee B. Sexual dimorphism in the liver. Annu Rev Physiol 1983; 45:37–50.
8. Bardin CW, Catteral JS. Testosterone: a major determinant of extragenital sexual dimorphism. Science 1981; 211:1285–1294.
9. Gustafsson JA, Mode A, Norstedt G, et al. Sex steroid induced changes in hepatic enzymes. Annu Rev Physiol 1983; 45:51–60.
10. Starzl TE, Iwatsuki S, Van Thiel DH, et al. Evolution of liver transplantation. Hepatology 1982; 2:614–636.
11. Iwatsuki S, Starzl TE, Todo S, et al. Experience in 1,000 liver transplants under cyclosporine-steroid therapy: a survival report. Transplant Proc 1988; 20:498–504.
12. Kahn D, Makowka L, Gavaler JS, et al. The outcome after clinical liver transplantation is influenced by the gender of the donor. Gastroenterology (in press).
13. Kamada N, Calne RY. Orthotopic liver transplantation in the rat: technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 1979; 28:47–50.
14. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.
15. Francavilla A, DiLeo A, Eagon PK, et al. Regenerating rat liver: correlation between estrogen receptor localization and deoxyribonucleic acid synthesis. Gastroenterology 1984; 86:562–567.
16. Van Thiel DH, Gavaler JS, Kam I, et al. Rapid growth of an intact human liver transplant into a recipient larger than the donor. Gastroenterology 1987; 93:1414–1419.
17. Kam I, Lynch S, Svanas G, et al. Evidence that host size determines liver size: studies in dogs receiving orthotopic liver transplants. Hepatology 1987; 7:362–366.
18. Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery 1984; 95:367–370.
19. de Hemptinne B, Salizonni M, Tan KC, et al. The technique of liver size reduction in orthotopic liver transplantation. Transplant Proc 1988; 20(Suppl. 1):508–511.
20. Kim YI, Calne RY, Nagasue N. Cyclosporin A stimulates proliferation of the liver cells after partial hepatectomy in rats. Surg Gynecol Obstet 1988; 166:317–322.
21. Kahn D, Makowka L, Lai H, et al. Cyclosporine augments the hepatic regenerative response in rats. Dig Dis Sci (in press).
22. Kahn D, Gavaler JS, Lai H, et al. Effect of cyclosporine on hepatic estrogen and androgen receptor levels before and after partial hepatectomy. Dig Dis Sci (in press).