explanation for the blocking activity observed in some patients. The cytotoxicity induced by normal effectors was not affected in our assay by the addition of several sera containing more than 200 mg of urea per 100 ml. There is experimental evidence that inhibition of ADCC can be produced by immune complexes (*6, 12, 13*) and by antibodies against lymphocyte surface antigens (*9*) via a common mechanism, namely competition for Fc receptors (*19*). Immune complexes that can inhibit ADCC are generated in mixtures of antibodies with lymphocytes (*19*). In kidney patients anti-HLA and anti-B cell antibodies, which are potent inhibitors of ADCC (*10, 18, 20, 23*), are often present. The autologous blocking found in some of our transplant and dialysis patients may be because of the formation of immune complexes between antibodies and soluble antigens from transfusions or kidney grafts.

E. Dupont[2]
G. Opelz
L. A. Gustafsson
S. M. Mikulski
P. I. Terasaki
Department of Surgery
School of Medicine
University of California
Los Angeles, California 90024

LITERATURE CITED

1. Barnett IG, MacLennan ICM: 1972 Ann Rheum Dis 31: 425
2. Campbell AC, Skinner JE, MacLennan ICM, et al: 1976 Clin Exp Immunol 24: 249
3. Cordier G, Brochier J, Revillard JP: 1976 Clin Immunol Immunopathol 5: 351
4. d'Apice AJF, Morris PJ: 1974 Transplantation 18: 20
5. Descamps B, Gagnon R, Debray-Sachs M, et al: 1975 Transplant Proc 7: 635
6. Dickler HB: 1974 J Exp Med 140: 508
7. Ettenger RB, Terasaki PI, Ting A, et al: 1976 N Engl J Med 295: 305
8. Fauci AS, Dale DC: 1975 Blood 46: 235
9. Halloran P, Schirrmeimer V, Festenstein H: 1974 J Exp Med 140: 1348
10. Hersey P, Cullen P, MacLennan ICM: 1973 Transplantation 16: 9
11. Jewell DD, MacLennan ICM: 1973 Clin Exp Immunol 14: 219
12. MacLennan ICM: 1972 Transplant Rev 13: 67
13. MacLennan ICM: 1972 Clin Exp Immunol 10: 275
14. Perlmann P, MacLennan ICM: 1974, p 347 *In* Brent, L, Holborow J (eds). *Progress in Immunology II*. North Holland Publishing Co., Amsterdam
15. Perlmann P, Perlmann H, Wigzell N: 1972 Transplant Rev 13: 91
16. Rachelefsky GS, McConnachie PR, Ammann AJ, et al: 1975 Clin Exp Immunol 19: 1
17. Scheinberg MA, Cathcart ES: 1976 Clin Exp Immunol 24: 317
18. Schirrmacher V, Festenstein M: 1976 Transplant Rev 30: 140
19. Schirrmacher V, Halloran P, Ross E, et al: 1975 Cell Immunol 16: 362
20. Solheim BG, Thorsby E, Moller E: 1976 J Exp Med 143: 1568
21. Terasaki PI, McClelland JD: 1964 Nature 204: 998
22. Ting A, Terasaki PI: 1974 Cancer Res 34: 2694
23. Trinchieri G, deTiarchi M, Mayr W, et al: 1973 Transplant Proc 4: 1631

[2] Recipient of a Fellowship from the Belgian Fonds de la Recherche Scientifique et Medicale.

Received 15 September 1976.
Accepted 22 October 1976.

ELEVEN AND TWO-THIRDS YEARS' SURVIVAL AFTER CANINE ORTHOTOPIC LIVER TRANSPLANTATION[1]

The first animal to survive chronically after orthotopic liver transplantation was a mongrel dog that received a hepatic homograft on March 23, 1964. The recipient was estimated to be 2 years old. The unrelated mongrel donor was of a different color and appearance. Immunosuppression was with azathioprine which was stopped after 120 days. Treatment was never resumed. The transplanted liver was biopsied on several occasions during the next 5 years and was always thought to be normal (*2, 4*).

After a brief illness, the dog died on the night of December 8, 1975, at the estimated age of 14 years and after a total survival post-transplan-

[1] This work was supported by Research Projects MRIS 8118-01 and 7227-01 from the Veterans Administration and by Grants AM-17260 and AM-0772 from the National Institutes of Health.

tation of 11 years and 8½ months. He had appeared to be well clinically until 1 or 2 days before death.

For most of the post-transplant period, the dog had normal liver function tests (Fig. 1). However, during the last 12 months of life, there was intermittent transaminasemia and elevation of the serum alkaline phosphatase. A few weeks before death, the serum bilirubin was increased to 2.5 mg/100 ml.

At autopsy, the liver was of normal size. It weighed 2.7% of the body weight, which was 12.5 kg. The liver had a nutmeg appearance and was hard. There were no abnormal venous collaterals suggestive of portal hypertension. The reconstructed vena cava above and below the liver and the portal vein were normal, with barely detectable sites of anastomosis. To arterialize the liver, a segment of donor aorta in continuity with the hepatic artery had been anastomosed to the infrarenal recipient aorta. Although the hepatic artery was grossly normal, the homograft aorta had extensive calcified atherosclerosis.

Biliary reconstruction was with cholecystoduodenostomy after ligation of the distal common duct. The gallbladder, the common duct, and all the intrahepatic ducts were filled with small or medium-sized stones or else with a soft, variably colored green to orange sludge. The stone analysis showed predominantly bilirubin with only trace amounts of cholesterol and bile salts.

Other significant findings at autopsy were pulmonary edema, coronary artery disease, and left ventricular hypertrophy. The immediate cause of death was congestive heart failure.

Histopathological evaluation of the homograft revealed portal fibrosis and linking of the enlarged portal tracts to one another by connective tissue septa (Fig. 2). There was concentric scarring around the interlobular and septal bile ducts and proliferation of ductules at the margins of the portal tracts. Many of the larger bile ducts were dilated and filled with inspissated bile. The epithelium of the affected ducts was ulcerated and there were aggregates of small lymphocytes, plasma cells, and macrophages

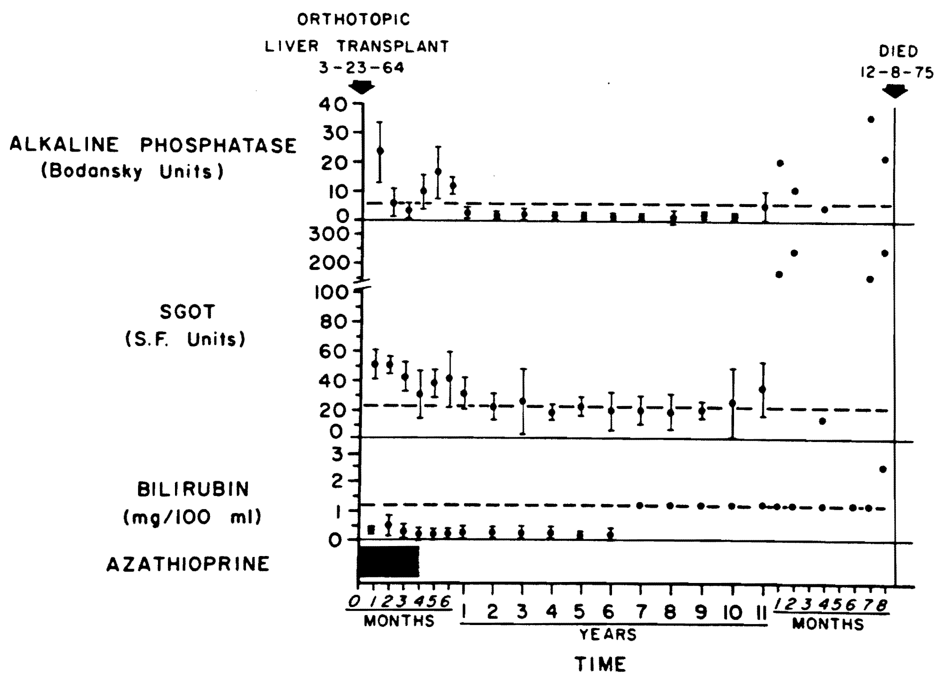

FIGURE 1. Serum biochemistries during the 11 years and 8½ months of survival following orthotopic liver transplantation on March 23, 1964. The values recorded during the last 8 months of life represent individual determinations. All other points on the graph are mean values for each time period; the brackets indicate 1 SD above and below the mean. Beginning with the 7th year of follow-up, bilirubin concentrations, if normal, were reported as ≤ 1.2 mg.%. The upper limit of normal in our laboratory for each test is marked by the horizontal broken line.

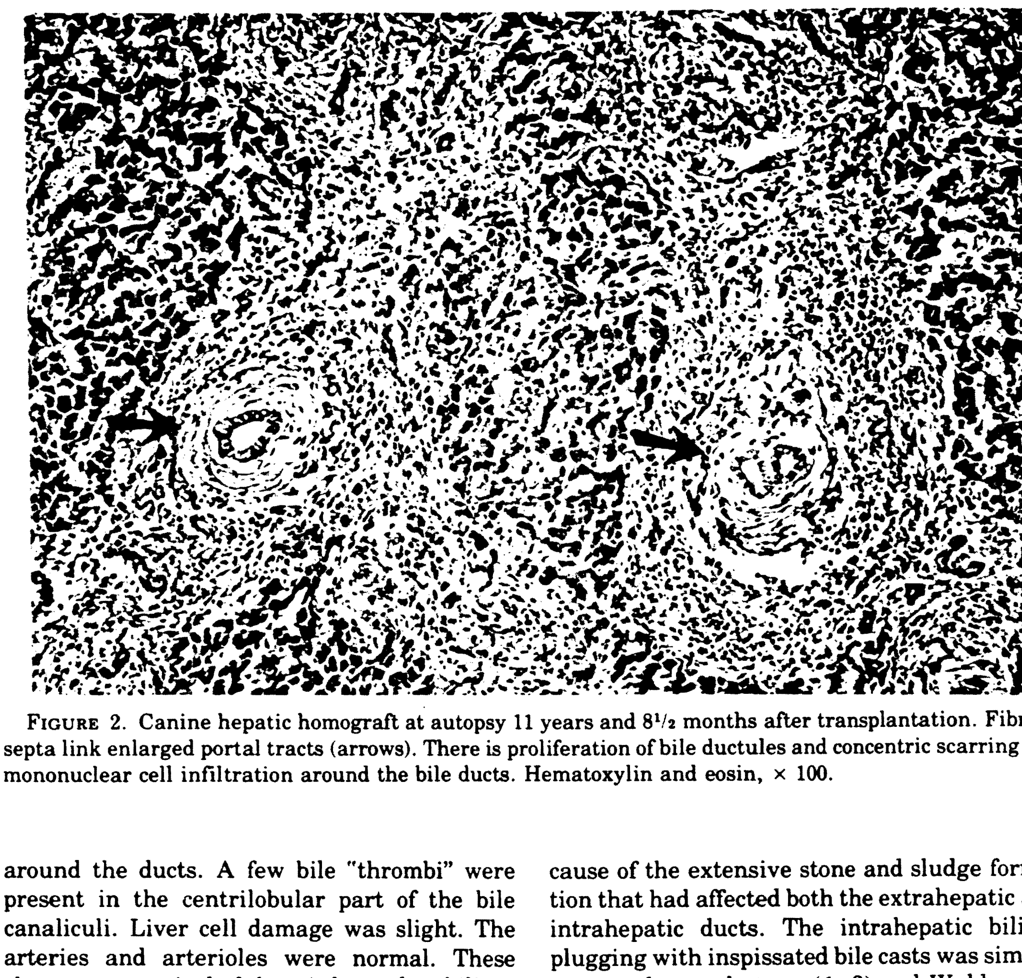

FIGURE 2. Canine hepatic homograft at autopsy 11 years and $8^{1}/_{2}$ months after transplantation. Fibrous septa link enlarged portal tracts (arrows). There is proliferation of bile ductules and concentric scarring and mononuclear cell infiltration around the bile ducts. Hematoxylin and eosin, × 100.

around the ducts. A few bile "thrombi" were present in the centrilobular part of the bile canaliculi. Liver cell damage was slight. The arteries and arterioles were normal. These changes are typical of chronic large duct biliary obstruction. There was no evidence of rejection.

The thymus, mesenteric and mediastinal lymph nodes, and Peyer's patches appeared to be normal.The spleen had been removed at the time of transplantation.

Examination of the rest of the tissues confirmed the narrowing of the coronary arteries by atherosclerosis, the hypertrophied left ventricle, and the congestion and edema of the lungs. The atherosclerosis in the homograft aorta was severe, with large patches of calcification and bone formation with marrow. By contrast the recipient aorta was normal. The kidneys were normal.

Thus, although the dog died primarily of cardiovascular manifestations of old age, a potentially lethal biliary duct complication was present which would have independently caused death within a short time. A partial obstruction of the cystic duct was apparently the main cause of the extensive stone and sludge formation that had affected both the extrahepatic and intrahepatic ducts. The intrahepatic biliary plugging with inspissated bile casts was similar to a syndrome that we (*1*, *3*) and Waldram et al. (*5*) have noted in humans. Such findings in animals and man point again to the reconstructed biliary duct system as the Achilles' heel of liver transplantation.

The completely normal findings in this dog's lymphoid system were of interest, since no immunosuppression whatever had been given for $11^{1}/_{2}$ years.

Thomas E. Starzl
Kendrick A. Porter
Charles W. Putnum
Veterans Administration Hospital
Department of Surgery
University of Colorado Medical Center
Denver, Colorado 80262
Department of Pathology
St. Mary's Hospital Medical School
London, England

LITE

1. Martineau B, I
Surgery 72: 6
2. Starzl TE, Mar
Surgery 58: 1
3. Starzl TE, Port
gery (in pres

THE CELLU

There have be
rejection and the
nomenom (9, 15
manifestation o
known to be the
their transformat
chemically activ
has also been dem
trate that occurs
to skin allografts
that the high bu
these cells could
(18).

It was original
antibody respon
much delayed, re
tion of the graft.
evidence that hu
significant role i
ever, antibodies a
soon after grafti
and have been sh
titer at about the
tion is complete
Algire and his co
moral antibodies
important role i
despite the fact t
high titers of spe
seem that, althou
allografts is prim
ically competent
play an ancillary
ing humoral facto
destruction of so
unsatisfactory, a
far as the majori
grafts are conce
by a cellular rath

Since the initi
mus-dependent

[1] Supported in p
GM-02236-02 from

LITERATURE CITED

1. Martineau B, Porter KA, Corman J, et al: 1972 Surgery 72: 604
2. Starzl TE, Marchioro TL, Porter KA, et al: 1965 Surgery 58: 131
3. Starzl TE, Porter KA, Putnam CW, et al: Surgery (in press)
4. Starzl TE, Putnam CW; 1969 *Experience in hepatic transplantation*. W B Saunders Co, Philadelphia
5. Waldram R, Williams R, Calne RY: 1975 Transplantation 19: 382

Received 7 October 1976.
Accepted 1 November 1976.

THE CELLULAR INFILTRATE IN CARDIAC ALLOGRAFT REJECTION IN MICE[1]

There have been many studies of allograft rejection and the cellular response to this phenomenom (*9, 15, 16*). The main cytological manifestation of the allograft response is known to be the activation of lymphocytes and their transformation into blasts (*4,20*). A histochemically active population of macrophages has also been demonstrated in the cellular infiltrate that occurs as an immunological response to skin allografts in mice and it was thought that the high build-up of lysosomal activity in these cells could be responsible for rejection (*18*).

It was originally thought that the humoral antibody response to an allograft was very much delayed, reaching its peak after destruction of the graft. This was cited as part of the evidence that humoral factors do not play a significant role in the rejection process. However, antibodies are demonstrable in the serum soon after grafting from the 4th day onward and have been shown to reach their maximum titer at about the same time the graft destruction is complete (*2*). The classic experiments of Algire and his colleagues (*1*) indicate that humoral antibodies alone probably do not play an important role in solid allograft destruction, despite the fact that such hosts produce fairly high titers of specific H-2 antibodies. It would seem that, although destruction of solid tissue allografts is primarily mediated by immunologically competent cells, humoral factors may play an ancillary role. The evidence incriminating humoral factors as major contributors to the destruction of solid tissue allografts remains unsatisfactory, and the current view is that, as far as the majority of types of solid tissue allografts are concerned, destruction is mediated by a cellular rather than a humoral immunity.

Since the initial observations that the thymus-dependent (T) line of lymphocytes was necessary for graft rejection (*17*), several T cell as well as non-T cell candidates for effector cells in cell-mediated immunity have been proposed. Antibody-dependent cell-mediated lysis of target cells in vitro may be carried out by a subset of B cells and/or surface adherent cells belonging to the monocyte-macrophage-reticulum cell line (*5, 6, 7, 19, 22*). In terms of in vivo graft rejection, however, it is likely that the effector cell population must come into contact with graft tissue and therefore be represented in the immune cellular infiltrate. Since it is now possible to identify lymphocytes in frozen tissue sections which bear surface antigens characteristic of the T cell and B cell (Ig bearing) lines (*8, 10–13*), we initiated a series of experiments to identify the cells involved in the immune cell infiltrates to cardiac allografts in mice.

The purpose of this study is 3-fold: to attempt to establish the type of cells present in the graft-host rejection site, the time relationships of the appearance of these cells, and finally to find out whether the type of cell changes as the graft becomes established.

Mice

Three groups of mice were used.

A. Allogeneic group. Donor hearts were from newly born (12 to 24 hr) BALB/cJ mice. The recipients were healthy adult C57BL/6J mice.

B. Preimmunized group. This group was similar to group A except that adult C57BL/6J recipients were preimmunized with 1×10^7 washed BALB/cJ spleen cells injected i. p. 10 days prior to transplant.

C. Syngeneic group. Both the newly born donor hearts and the recipient adult mice were of the BALB/cJ strain.

Antisera

A. Anti-T. Rabbit anti-BALB thymus serum was prepared as previously described (*12*). This

[1] Supported in part by Grants HL 13108-06 and GM-02236-02 from the National Institutes of Health.