Survival of Allografts after Hepatic Portal Venous Administration of Specific Transplantation Antigen

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PROLONGED survival of allogeneic tissue in "privileged" sites presumably occurs because of a barrier to the transport of transplantation antigen from the graft to the host lymphoid system.[10] This phenomenon can be exploited only with small quantities of foreign tissue since nourishment of the graft in privileged sites depends upon diffusion. Large, whole organs require direct vascular connections with the host for adequate sustenance, and these connections provide avenues by which transplantation antigens released from a graft may reach the lymphoid apparatus.[11]

Whole organ allografts may also release transplantation antigen directly by diffusion into contiguous host tissue, or by microvascular connections established between the graft and the host after implantation. The relative importance of these various routes of antigen access to the host lymphoid apparatus is not accurately known. If the significant avenue is through the graft's venous return, then successful removal of the antigen from its venous effluent may successfully retard initiation of the rejection response or diminish its intensity.

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The liver conceivably could function as such an "antigen filter." It is conspicuous as a reticuloendothelial organ with a large capacity for removal and degradation of foreign substances from the blood stream,[7] and its low pressure portal venous system can successfully be adapted to drain venous blood from a renal allograft. In addition, the absence of fixed lymphoid tissue in the normal liver precludes the local initiation of an intrahepatic immune response.[3, 8] Nevertheless, even if the reticuloendothelial cells of the liver possess the capacity to extract antigen from the portal venous blood, antigen introduced into the hepatic portal vein might still traverse the organ and be discharged into the systemic venous system if the quantity of antigen introduced is excessive or if the hepatic portal venous transit time is too brief.

Franzl [4, 5] has reported that labeled antigens injected into the portal vein and degraded in the liver are not immunogenic when subsequently recovered by disruption of the hepatic cells. In contrast, the same antigens sequestered in the spleen retained immunogenicity. Thus, it is possible that a process in the liver may degrade antigen so as to impair its capacity to induce an immune response. In keeping with this concept are some older but well confirmed observations on Rh hemolytic disease of the

newborn.[13] In man, small doses of strongly agglutinated red cells are rapidly removed from the circulation, chiefly by the liver. Group O mothers carrying Rh incompatible fetuses of blood group A are seldom stimulated to form significant anti-Rh antibody, whereas red blood cells from ABO compatible RH positive fetuses frequently sensitize their Rh negative mothers. In the former circumstance it has been supposed that fetal red cells entering the maternal circulation were promptly agglutinated by the mother's natural anti-A antibody, swept into the liver, and rendered non-immunogenic.

An effort therefore seemed warranted to determine the effect on development of transplantation immunity of diversion of transplantation antigen released in the venous effluent of an organ allograft through the portal system of the liver before the blood entered the systemic circulation. If transplantation antigen, released from renal allografts into the organ's venous blood, were trapped by the liver and degraded into a non-immunogenic form, the overall intensity of the allograft rejection mechanism might be modified. Even a partial blunting of the sensitization process might permit smaller doses of immunosuppressive drugs to control the rejection mechanism.

Experimental Design

Two experimental systems were developed. In Experiment I renal allografts were performed in mongrel dogs with the vein of the graft anastomosed to the hepatic portal vein. Since this experiment did not distinguish allograft immunity induced by the antigen leaving the graft (1) by diffusion and by microvascular connections with the host from immunity induced by (2) antigen leaving through the major venous pathway, Experiment II was performed. Experiment II utilized two inbred strains of mice. Known quantities of C3H antigen, in the form of a spleen-cell suspension or of spleen cell-free antigen, were injected into the hepatic portal vein of CBA mice at laparotomy. Five days after administration of the antigen. C3H skin was grafted to the CBA mice. The survival of the skin grafts in the animals pretreated with allogeneic antigen administered via the hepatic portal vein was compared with survival of control skin grafts on CBA mice that had received the same antigen delivered into the inferior vena cava.

Materials and Methods

Experiment 1. Hepatic portal drainage of venous effluent from canine renal allografts.

Mongrel dogs from 15 to 25 kilograms in weight were typed for the A1, A2, C, and D erythrocyte antigens.[12] Recipient and donor pairs were chosen on the basis of erythrocyte compatibility. Leukocyte matching was not done.

The renal allograft was placed in the renal fossa of unilaterally nephrectomized recipient dogs. The vein of the allograft was anastomosed to the recipient's hepatic portal vein. The recipient's renal artery was used to provide the arterial blood supply for the graft. Urinary drainage was achieved by ureteroureterostomy. All anastomoses utilized continuous 6-0 arterial silk sutures. One of the recipient's own kidneys was left *in situ* in order to avoid uremia and obtundation of the rejection response. Five groups of animals were studied. Survival of the transplanted kidney was tested by performance of frequent intravenous pyelography, usually on alternate days, until loss of the ability to concentrate and secrete contrast material was detected. Then open renal biopsy was performed. Survival of grafted kidneys was taken on the last day on which good function, by intravenous pyelograms (IVP), was observed.

Group 1. Control Autografts

In three dogs, one kidney was excised from each, perfused free of blood with cold saline containing 1,000 units of aqueous heparin per ml. and reimplanted as an

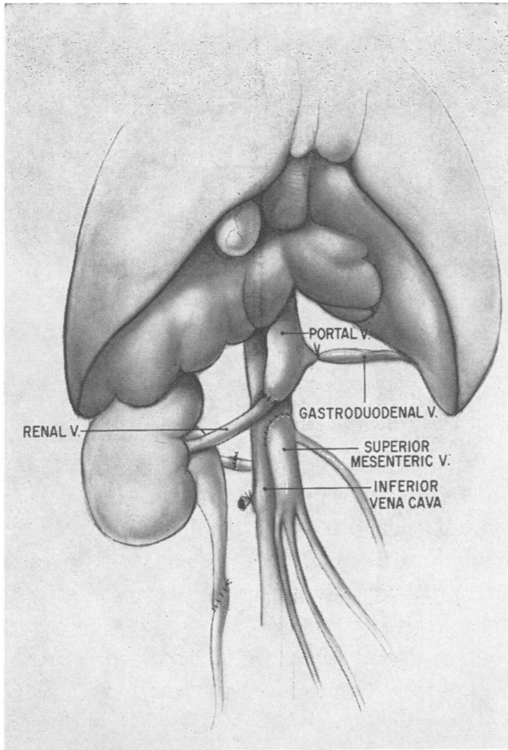


FIG. 1. The renal allograft is transplanted with its venous effluent passing into the hepatic portal system by means of an end-to-end renoportal venous anastomosis. Only renal venous effluent is introduced into the hepatic portal system because of prior construction of a portocaval shunt.

autograft with an end-to-side renoportal venous anastomosis.

Group II. Control Allografts

In five dogs kidneys were transplanted into the pelvis of the recipient animal and revascularized by means of the iliac artery and vein. Normal urinary drainage was provided by an ureteroureterostomy.

Group III. Experimental Allografts

A. In six dogs the allograft was placed in the renal fossa and its vein was implanted end-to-side into the hepatic portal vein so that all the venous blood from the renal allograft drained into the hepatic portal system.

B. In five dogs an end-to-side portocaval shunt was constructed immediately preceding renal transplantation. The vein of the renal allograft was then anastomosed end-to-end to the hepatic side of the transected hepatic portal vein (Fig. 1). All tributaries of the hepatic portal vein proximal to this anastomosis were ligated. Thus, only the venous blood from the renal allograft passed into the hepatic portal system.

Results

Group 1. Control Autografts

Each of the three dogs with renal autografts, which drained into the hepatic portal vein, remained in good health as long as they were observed (85 days). IVPs were normal whenever carried out. Exploration of the graft on that day showed very little reaction, and the microscopic appearances of the autograft and its undisturbed autologous kidney were identical. Serial determinations of serum alkaline phosphatase and serum glutamic oxaloacetic transaminase (SGOT) were consistently less than 5 Bodansky Units and 25 Karmen units, respectively, in all dogs. The histology of the liver was normal in these animals at sacrifice 85 days after autotransplantation.

Group II. Control Allografts

In the five animals receiving renal allografts with systemic renal venous drainage cessation of renal function, as shown by IVP, occurred as early as the 4th day to as late as the 10th day after transplantation (Fig. 2). Immediate exploration and biopsy of the graft confirmed rejection. Invariably, the vessels of the renal allograft remained open with active blood flow, but the organ suffered varying degrees of ischemia, as shown by incisions of increasing depth, first into the cortex and then into the medulla. Frequently the cortex bled

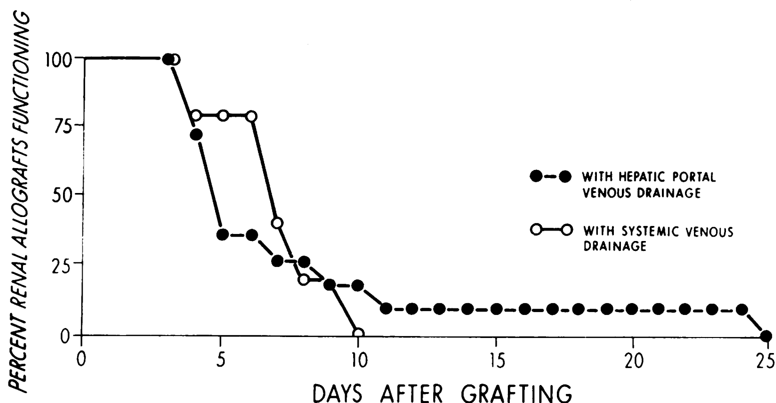


FIG. 2. Serial IVPs of the renal allografts with systemic venous drainage ceased to reveal secretion of hypaque on days 5–10. Hypaque ceased to be secreted by the allografts with hepatic portal venous drainage on day 4 to day 11 with the exception of one kidney which functioned at the time of excision on day 24.

poorly or not at all, while the medulla bled profusely when incised. The grafts appeared enlarged, firm, and hemorrhagic. Usually the hemorrhage was diffuse but occasionally it was patchy. Microscopically there were patchy infiltrations with mononuclear cells and broad areas of intestinal hemorrhage.

Liver function, as reflected in repeated determinations of alkaline phosphatase and SGOT, was not altered. At sacrifice, the livers were grossly normal, except for occasional adhesions between the hepatic surface and loops of bowel or omentum. Microscopically the liver showed only capsular inflammatory reaction, probably related to laparotomy.

Group III. Experimental Allografts

A. In this Group the IVP revealed the first evidence of rejection on the 4th day after transplantation. The longest surviving allograft ceased to secrete hypaque on the 11th day (Fig. 2). The IVP's of the remaining animals of the Group failed to be visualized within this interval. In each case patency of the allograft vasculature was confirmed and rejection was proven by biopsy as soon as the IVP failed to reveal function. All the allografts showed varying degrees of swelling, induration, and hemorrhage. The histologic picture of rejection was most typical in one dog in which there was a peri-glomerular mononuclear reaction and hemorrhagic necrosis of the renal parenchyma. Four allografts exhibited extensive hemorrhagic necrosis with a more marked polymorphonuclear interstitial reaction. The renal vessels showed mononuclear sheathing and many arteries of intralobar, arcuate, and intralobular size exhibited acute necrotizing arteritis.

Sections of the liver showed an acute inflammatory reaction, some with organizing granulation tissue, most severe on the capsular surfaces with increased numbers of polymorphonuclear cells in the sinusoids. One animal had moderately severe central fatty change in the liver. There was no prominence of the reticuloendothelial cells.

B. The renal allografts of this Group showed evidence of rejection by intravenous pyelography beginning on the 5th day after transplantation. One allograft excreted contrast medium as late as day 24 (Fig. 2). On exploration after failure to secrete hypaque upon intravenous pyelography, with

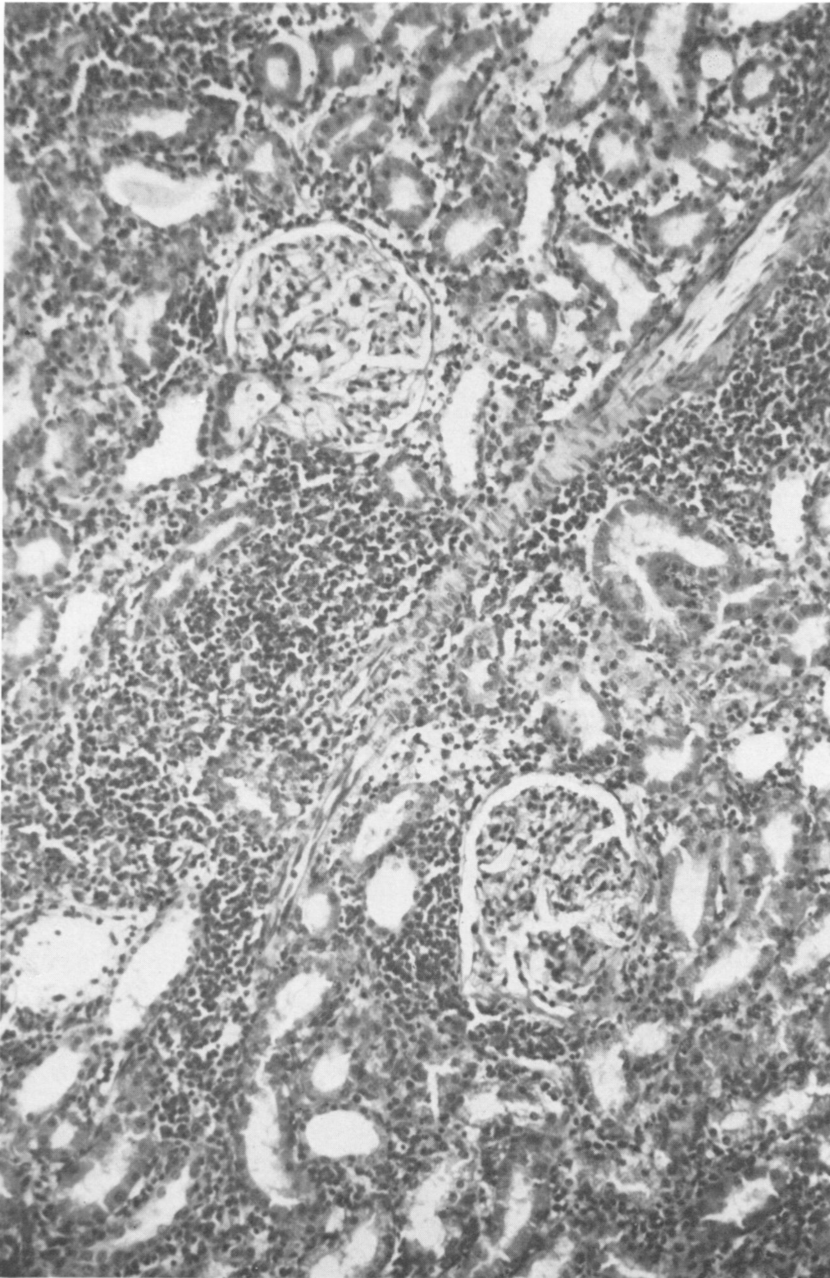


FIG. 3. A photomicrograph of the longest surviving renal allograft taken on day 25. There is a dense infiltration with mononuclear cells characteristic of immunologic rejection.

one exception, the grafts had become large, indurated, and discolored by diffuse or patchy hemorrhage. The graft which functioned on the 24th day after transplantation was removed on day 25. This kidney was large, indurated, and had a thickened capsule but was a normal, pink color. This dog had responded uniquely to the presence of the renal allograft with a brisk leukocytosis to 22,600. The leukocytosis subsided after the 7th day and remained at normal levels until removal of the graft. The

TABLE 1. *Survival of C3H Allografts on CBA Mice After Pretreatment with C3H Spleen Cells*

Group	No. of Mice	Antigen Dose (nucleated spleen cells per mouse)	Route of Administration	Median Survival Time of Skin Graft (days)
I (control)	11	None	—	14.3 ± 0.9
II	7	2.5×10^6	Hepatic portal vein	11.7 ± 3.1
III	8	2.5×10^6	Inferior vena cava	11.0 ± 3.6
IV	10	1.25×10^6	Hepatic portal vein	10.7 ± 2.0
V	10	1.25×10^6	Inferior vena cava	9.8 ± 0.9

other animals of Groups IIIA and IIIB developed increasing leukocytoses as rejection progressed.

Microscopically the kidneys revealed infiltration with mononuclear cells and extensive hemorrhagic necrosis. There was sheathing of renal arterioles and frequent necrotizing arteritis. The one allograft which functioned 24 days was unusual inasmuch as it showed only a dense infiltration with mononuclear cells, no interstitial hemorrhage, and little if any arteritis (Fig. 3).

The livers of this Group of animals resembled those of Group IIIA grossly and microscopically. There were no changes which could not be related to the trauma of surgery. In three animals serial determinations of serum alkaline phosphatase and SGOT were done and showed progressive increases as rejection of the kidney developed.

Experiment II. Effect of hepatic portal vein injection of allogeneic transplantation antigen on skin graft survival in mice.

Murine transplantation antigen was prepared in two forms: First, as a C3H spleen cell suspension,[2] and second, as a cell-free antigen made from C3H spleens after the technic of Monaco, Wood, and Russell.[9] The cellular antigen was measured in terms of numbers of nucleated spleen cells, and the cell-free antigen was measured in terms of spleen equivalent weights. Only female mice were used.

Cellular Antigen Mouse Groups

Eleven CBA mice received C3H skin grafts without any pretreatment except for an intraperitoneal injection of 50 international units of aqueous heparin followed by a laparotomy 5 days before grafting. These animals formed Control Group I. In Groups II to V (Table 1) the hepatic portal vein was exposed by means of a midline laparotomy incision, and the antigen preparation was injected into the inferior vena cava or the hepatic portal vein by means of a No. 30 stainless steel hypodermic needle shortly after the intraperitoneal injection of 50 international units of aqueous heparin. The point of the needle was introduced into the hepatic portal vein through the pancreatic capsule to avoid bleeding. After injection the incision was closed with a running suture of fine silk in the rectus muscles and with Michel clips in the skin. Each CBA mouse of experimental Groups II and III received 2.5×10^6 C3H nucleated spleen cells. The seven mice in Group II received hepatic portal vein injections; the eight mice in Group III received inferior vena cava injections. The CBA mice in Groups IV and V received 1.25×10^6 nucleated C3H spleen cells per mouse, via the portal vein in Group IV and via the inferior vena cava in Group V (Table 1). Five days after injection of each CBA mouse, C3H skin was grafted to its right

TABLE 2. *Survival of C3H Skin Allografts on CBA Mice after Pretreatment with C3H Cell-free Antigen*

Group	No. of Mice	Antigen Dose (equivalent to mg. of C3H spleen)	Route of Administration	Median Survival Time of Skin Graft (days)
A (control)	14	1.5	Subcutaneous	8.1 ± 0.9
B	10	1.5	Hepatic portal vein	13.6 ± 2.5
C	11	1.5	Inferior vena cava	12.5 ± 1.6
D	10	0.3	Hepatic portal vein	11.9 ± 2.6
E	11	0.3	Inferior vena cava	12.6 ± 1.5

hemithorax and the survival time of the skin graft was recorded.

Cell-Free Antigen Mouse Groups

In order to define the immunogenicity of the C3H cell-free antigen, 1.5 mg. spleen equivalent weight of antigen was injected subcutaneously or retroperitoneally into CBA mice which had previously received 50 units of aqueous heparin intraperitoneally and had been submitted to a midline laparotomy. After administration of the antigen the incision was closed as described previously. These mice formed Control Group A (Table 2). Groups B and C consisted of 10 and 11 CBA mice, which received the equivalent of 1.5 mg. of C3H spleen in the form of cell-free antigen into the hepatic portal vein and inferior vena cava, respectively. Mouse Groups D and E received cell-free antigen equivalent to 0.3 mg. of C3H spleen into the hepatic portal vein (10 mice) or inferior vena cava (11 mice). Five days after the cell-free antigen injection each mouse was grafted with C3H skin which was then observed for survival.

Results

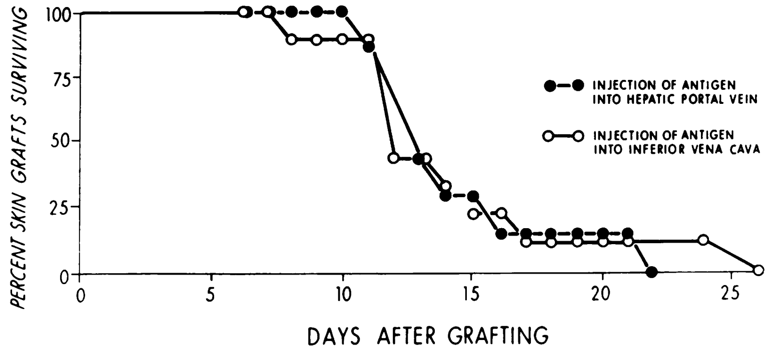
The median survival time of female C3H skin placed on untreated female CBA mice (Control Group I) was 14.3 ± 0.9 days. In all series of mice pretreated with C3H antigen, both cellular and cell-free, the median survival time was shortened, indicating the induction of transplantation immunity (Tables 1 and 2). Most significant shortening of the median survival time occurred in the mice receiving antigen subcutaneously or retroperitoneally. Administration of a given dose of antigen into the inferior vena cava or into the hepatic portal vein produced statistically the same degree of immunity. The skin graft survival curves (Figs. 4 and 5) suggested that the shortened median survival time was the result of a second set rejection of some of the grafts, whereas some grafts seemed to undergo a first set rejection response. This phenomenon occurred in the groups receiving antigen injected into the hepatic portal vein as well as in the groups receiving antigen injected into the inferior vena cava. The effect was particularly evident in the groups of mice in which higher doses of antigen had been given.

Discussion

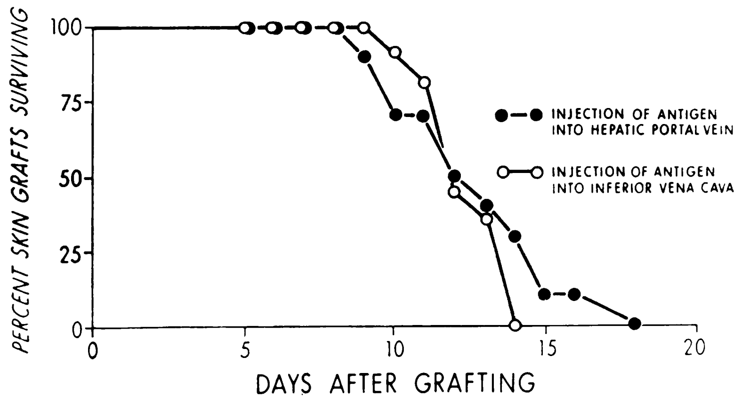
While these studies were in progress Barker *et al.*[1] and Fukuda *et al.*[6] reported their experience with canine renal allografts with venous drainage directly into the hepatic portal system. The former observed that survival of the graft was not prolonged. The latter investigators noted slight prolongation of allograft suvival, but interestingly, these animals failed to develop a state of sensitivity to specific donor antigen so that a second graft from the same donor was rejected in the first set fashion. They interpreted these observations as evidence for a difference in the processing of antigen when delivered to the host via the hepatic portal vein rather than a systemic vein.

In the present series of canine experiments certain modifications of the model

PRETREATMENT WITH CELL-FREE ANTIGEN EQUIVALENT TO 1.5mg OF C3H SPLEEN



PRETREATMENT WITH CELL-FREE ANTIGEN EQUIVALENT TO 0.3mg OF C3H SPLEEN



FIGS. 4 and 5. No difference in survival of skin allografts was noted in mice pretreated with cell-free antigen injected into the hepatic portal vein or the inferior vena cava. Each route of administration resulted in some shortening of skin graft survival.

were made in order to maximize any influence exerted by the liver on the processing of transplantation antigen and thus on the immune response. All transplants were made between dogs typed and cross-matched for erythrocyte antigens to avoid failure of the allograft due to erythrocyte incompatibility. In the dog Group IIIB, the intrahepatic residence time of the venous effluent from the renal allograft was increased by diversion of the host's entire mesenteric venous flow into the inferior vena cava through an end-to-side portal caval shunt. Furthermore, renal allografts from small dogs were transplanted into the portal circulation of large dogs in an attempt to minimize the antigen-loading of the portal circulation.

No statistically significant prolongation of survival was demonstrated by these maneuvers. A factor of possible importance in the failure to produce appreciable unresponsiveness to canine renal allografts by this technic may be that diffusion of transplantation antigens occurs from the allograft directly into contiguous host tissues, circumventing the liver and thus promoting direct sensitization of regional lymph nodes. The mouse model was designed to prevent direct sensitization in this manner. Allogeneic transplantation antigen was injected at laparotomy into the hepatic portal vein

so that regional lymphatic absorption was prevented. Pretreatment with two doses of antigen resulted in accelerated rejection of subsequent test skin allografts with no evidence of differential sensitization or of unresponsiveness, when the antigen was given by the hepatic portal or systemic venous routes.

These results are consistent with previous reports inasmuch as rejection of the primary renal allografts in dogs was not prevented. The prolonged survival of one portally drained allograft beyond 24 days is consistent with the random tissue matches occasionally encountered in this outbred animal. Even this kidney showed extensive histologic evidence of rejection at biopsy. The findings of sensitization after intrahepatic portal venous injection of transplantation antigen in the mouse model and of accelerated rejection of subsequent test skin grafts cannot be interpreted as inconsistent with Fukuda's results since a different species and an antigen of different character was employed.

Summary

Sixteen renal allografts were carried out in dogs which had been typed and matched for red cell antigens. In eleven dogs the renal venous effluent was directed into the hepatic portal venous system. The grafts with portal drainage underwent immune rejection of a quality and at a time statistically the same as control renal allografts with systemic venous drainage. In an effort to circumvent the effect of diffusion of transplantation antigen from the allograft directly into local lymphatic channels, allogeneic antigen in cellular and cell-free form was injected into the hepatic portal vein of mice. Survival of subsequent skin allografts (from the same strain as the donor of antigen) was not prolonged in comparison to controls. There was, indeed, slight shortening of graft survival consistent with sensitization induced by the intravenous route of antigen administration.

These experiments confirm previous reports that hepatic portal venous drainage of canine renal allografts does not prevent or delay rejection. In a murine model, particulate transplantation antigen delivered entirely into the hepatic portal vein resulted in sensitization of the host to subsequent test skin allografts.

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