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Tissue Graft Rejection in Mice:

I. Contributions of *H-2* and Non-*H-2* Genetic Barriers

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Abstract. A liver-slice to kidney-bed grafting system was used to study the course of rejection of a specific tissue across various genetic barriers in inbred strains of mice. Rejection or survival, scored histologically at various times after grafting, demonstrated that multiple non *H-2* differences cause rejection at least as rapidly as *H-2* differences. Differences at the *K* end of the mouse major histocompatibility complex cause tissue rejection more rapidly than do differences at the *D* end of the complex. The latter differences cause chronic rejection similar to that found across several minor *H* locus barriers. The *H-2* haplotype carried by the recipient or the strength of the *H-2* antigens of the donor affect the survival time in liver tissue grafts. Studies employing this model system will contribute to the definition of different immunogenetic parameters affecting survival of various tissues in a genetically well-defined animal model.

Introduction

The major genetic complex contributing to skin graft rejection in inbred strains of mice is the *H-2*. Skin grafts between inbred animals, which differ in this complex alone, have mean survival times of less than 2 weeks. Grafts between strains which differ in minor *H* loci have variable courses of rejection with mean survival times of 20 or more days (Graff and Bailey 1973). The *H-2* complex has recently been subdivided, by studies of numerous recombinants, into six subregions that control different aspects of immunological reactivity. Skin graft rejection is controlled by the *K*, *IA*, and, to a lesser extent, by the *D* regions (Klein 1972).

CML (cell-mediated lymphocytotoxic) target antigens are controlled by the *K* and *D* regions (Shreffler and David 1975). The MLC (mixed lymphocyte culture) reaction and graft-versus-host reactivities are controlled by genes located throughout the *H-2* complex (*Lad* loci, Klein 1975) and outside the *H-2* complex (*Mls* locus, Festenstein 1973). The immune response to various synthetic and natural protein antigens is localized in the *IA*, *IB*, and *IC* regions (Gasser and Silvers 1974).

Until recently it has been assumed that immunogenetic information obtained from skin graft survival studies and in vitro lymphocyte tests would predict the survival times of grafts of all or most tissues and organs. However, the

results of transplant studies in rat and man have made this a questionable assumption (Freeman and Steinmuller 1969, White and Hildemann 1968, Belzer *et al.* 1974, Salaman 1971). Technical difficulties have limited experimental kidney transplantation in mice to only one study (Skoskiewicz *et al.* 1973). Therefore it has not been possible to correlate findings concerning the control of other immunological parameters with kidney transplantation in this species, which has been so well defined genetically.

We are employing a liver-to-kidney grafting system modified from procedures described by Wheeler *et al.* (1966) and by Seller (1972) as a model system to elucidate the control of tissue graft rejection in inbred mous strains. This system has also been employed to study second set rejection reaction by Baldwin and Cohen (1974). In tis model, liver tissue slices are grafted to a prepared kidney bed and rejection scored histologically. We find that syngeneic liver tissue survives well in this system. Multiple non-*H-2* (background) differences cause rejection at least as rapidly as do *H-2* differences. In addition, animals differing at the *K* and *I* regions of the *H-2* complex reject each other's liver tissue much more rapidly than do animals with *D* region differences. Grafts across different *H-2* barriers show somewhat different survival times.

Materials and Methods

Mice. All experimental animals were inbred or congenic strains of mice obtained from the animal colony of the Jackson Laboratories, Bar Harbor, Maine. The following strains were used: C57Bl/10Sn (B10), B10.BR/SgSn, B10.A/SgSn, B10.D2/nSn, B10-$H\text{-}13^bA^w$/Sn, B10.129(10M)/Sn, B10.129(9M)/Sn, B10.C(45N)/Sn, B10.D2(57N)/Sn, B10.C(47N)/SN, B10.129(21M)/Sn, B10.129(5M)/oSn, B10.129(5M)/nSn, A.BY/Sn, and C3H/HeJ.

Grafting Procedure. The method employed is a modification of the procedure described by Wheeler *et al.* (1966). A clean but non-sterile technique is used throughout. One donor animal supplies the liver for 4 or 5 recipient animals and these grafts are performed simultaneously. Donors are anesthesized intraperitoneally with chloral hydrate (0.01 ml/g body weight of a solution containing 50 mg/ml). Recipients are anesthesized with methoxyfluorine (penthrane) administered through a nose cone prepared from a plastic 5 ml syringe. A midline incision is made from the sternum to the pelvic area of the donor animal and the liver exposed. An incision, about 1 cm long, is made into the left side of the recipient just below the rib cage. The left kidney is exposed and the fascia removed from around the kidney and renal vessels. The renal vessels are then constricted with an atraumatic clamp. A thin slice (approximately 1 to 2 mm thick) is removed from the dorsal side of the recipient kidney, using half of a stainless steel razor blade. A thin slice of approximately the same cross-sectioned area as that of the kidney bed is then cut from the donor liver using small curved surgical scissors. The graft slice is immediately placed on the prepared kidney bed and held in place gently for 30 seconds. The clamp is then removed and excess blood wiped from around the graft site with gauze. The kidney is replaced in the body cavity and the grafted area covered with the animal's pancreas to prevent fascia from adhering to the wound. The inner skin is sutured with 5× suture and the outer coat with 3× suture and the animal is allowed to recover from anesthesia under ordinary colony conditions.

Graft Evaluation. At selected times after grafting the recipient aminals are anesthesized with chloral hydrate, at the dose levels described above for donor animals. The grafted kidney is excised through a midline incision and the animal immediately killed with an overdose of sodium pentabarbital. The cuticle and renal pedicle are removed and the kidney fixed in Bouin's fluid. After 48 hours or more in Bouin's fluid, the fixed kidneys are bisected longitudinally, near the graft site, to obtain a flat area for embedding and to facilitate locating the grafted area for sectioning. The kidneys are washed overnight in 70% ethanol in embedding cassettes to remove excess fixative

before the routine Autotechnicon processing schedule for paraffin infiltration, using 50% ethanol and 1% butanol is applied. The kidneys are embedded in paraffin and 7 μ serial sections are cut from the graft area. Approximately 3 slides (1 × 3 inches), with 10 to 12 sections each, are prepared from each kidney to be sure that sections containing the graft site are obtained. The sections are stained by routine hematoxylin and eosin procedures.

Terminology. The term *K end* in the text, tables, and figure legends refers to the combined *H-2* subregions *K*, *IA*, *IB*, and *IC*; the term *D end* refers to *S*+*D*. The six *H-2* subregions are specified in the order *K*, *IA*, *IB*, *IC*, *S*, *D*, and are named according to their haplotype of origin. The designation *kkkkkk* indicates that the six *H-2* subregions had the $H\text{-}2^k$ chromosome as their haplotype of origin; *kkkddd* indicates that the *K* end of the haplotype originated from the $H\text{-}2^k$ and the *D* end from the $H\text{-}2^d$ chromosome.

Results

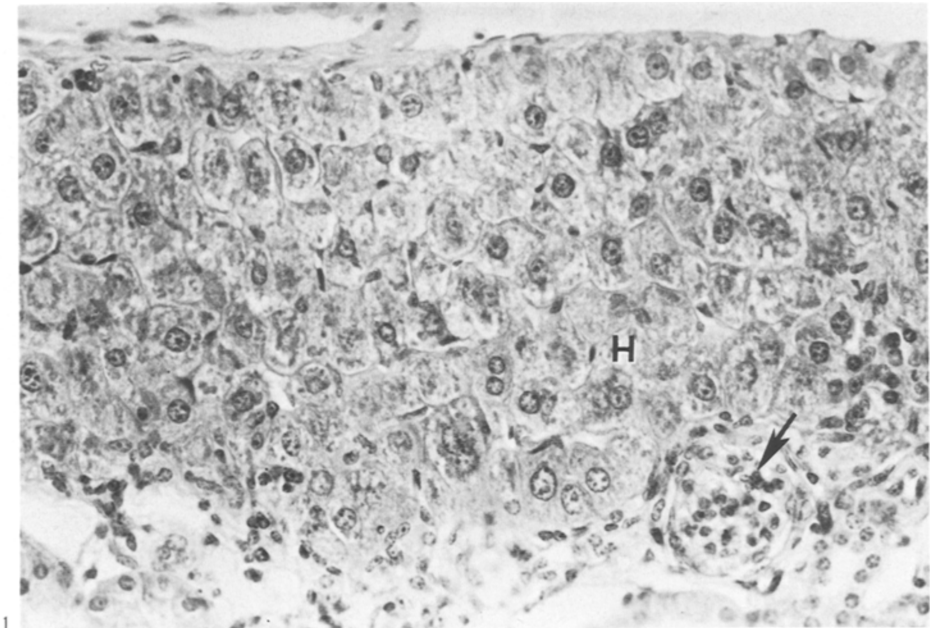
Histological Picture. Microscopic study of the graft site reveals a regular pattern of healing and/or rejection. At 1 week after grafting healthy residual liver parenchyma, as well as an intense inflamatory reaction, are present in all grafts. Foci of fibrin mixed with lymphocytes are observed. In syngeneic grafts, ballooning hepatocytes are seen which may indicate either metabolically active or degenerating cells.

After 2 weeks the inflammatory reaction has receded in the syngeneic grafts. In graft combinations in which rejection occurred in less than 4 weeks (A.BY to B10, C3H to B10, and so on) extensive lymphocytic infiltration is already evident and only a few viable hepatocytes survive 2 weeks after grafting. Fibrobasts are seen infiltrating these latter grafts. Whether these fibroblasts originate in the host or in the graft is unknown.

Syngeneic grafts (Fig. 1) show definite evidence of regeneration at 4 weeks. Cells with double nuclei, mitotic figures, metabolically active ballooning cells, and reactive Kupffer cells are evident. In some grafts across *H-2* barriers a similar pattern is seen at 2 weeks (Fig. 2). However, these grafts go on to rejection at 4 weeks.

Grafts that show some signs of rejection at 2 weeks have disappeared by 6 weeks after grafting. At 6 weeks, the sites of the latter grafts are undergoining fibrosis with abundant collagen. Hemosiderin-laden macrophages (scavenger cells) and lymphocytes are present at the original interface of the graft tissue and the recipient tissue (Fig. 3).

In certain combinations a chronic rejection pattern has become apparent at 4 weeks, and is still evident 8 weeks after grafting. Replicate grafts vary within these groups and the degree of graft degeneration may be significantly influenced by minor technical variations. Some grafts have disappeared completely; others have variable quantities of hepatocytes remaining, but all grafts show a definite chronic inflammatory reaction and no signs of hepatocyte regeneration, although there are well-developed blood vessels in some. This variable pattern exists at 28, 32, 36, 42, and 56 days after grafting in donor-recipient combinations spanning the *H-2 D* genetic barrier (Fig. 4). In addition, two minor histocompatibility barrier combinations B10.D2(57N/Sn) to B10($H\text{-}8^b$ to $H\text{-}8^a$) and B10.129(21M)/Sn to B10($H\text{-}4^b$ to $H\text{-}4^a$) show similar variability 10 weeks after grafting.



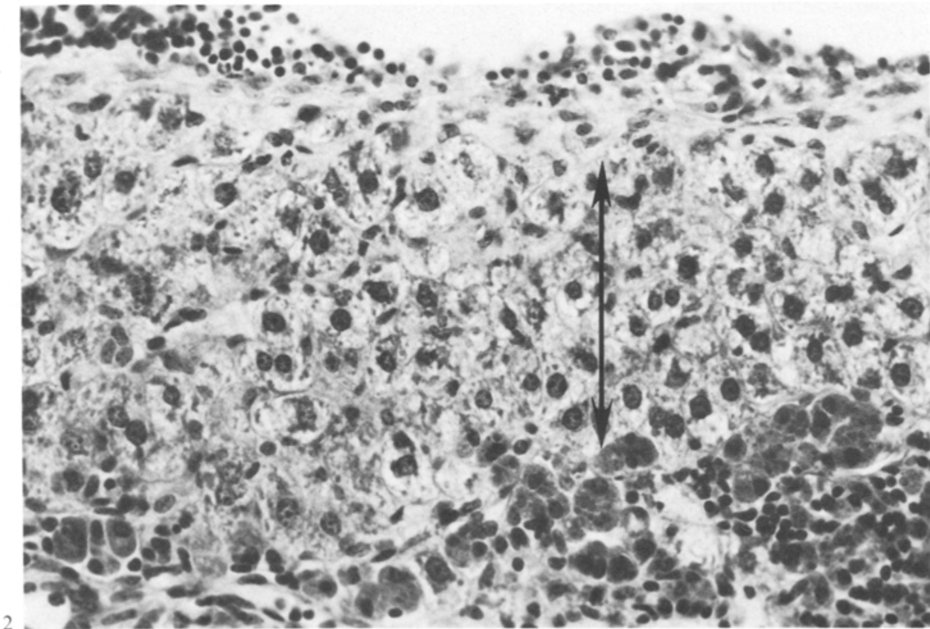
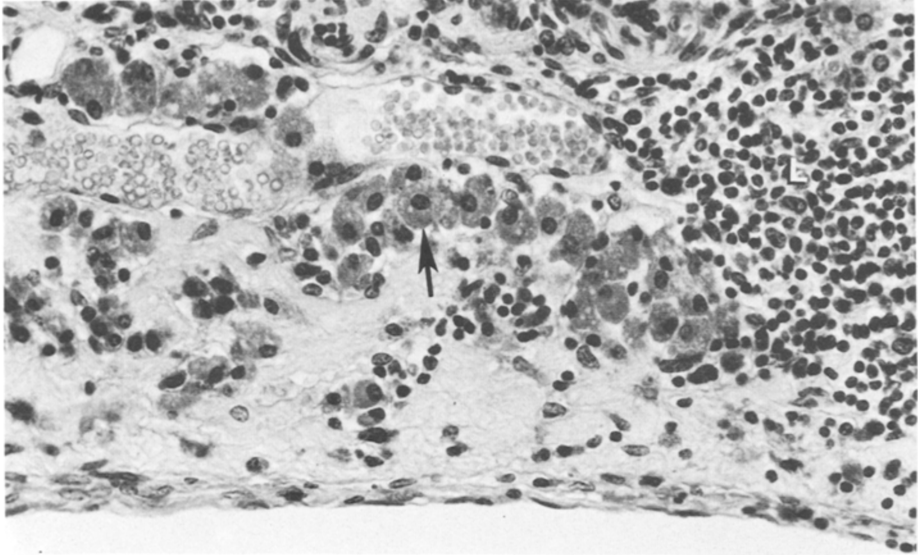


Fig. 1. Syngeneic (B10 to B10) graft at 8 weeks after grafting ($\times 500$). Healthy liver tissue (H) and glomerulus (arrow) at the kidney bed-graft interface are in evidence

Fig. 2. B10.BR to B10 ($H\text{-}2^k$ to $H\text{-}2^b$) graft surviving at 2 weeks after grafting ($\times 500$). Double headed arrow marks extent of graft



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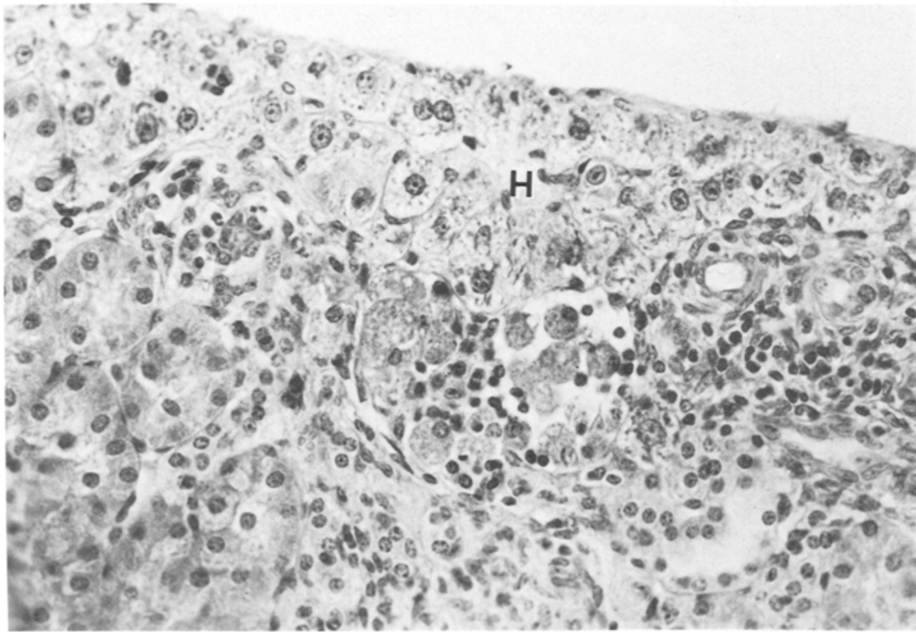

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Fig. 3. B10.BR to B10 ($H\text{-}2^k$ to $H\text{-}2^b$) graft rejected at 6 weeks after grafting ($\times$ 500). Graft interface is marked by hemosiderin-laden cells (arrow); lymphocyte nodule (L) can be seen in region of graft

Fig. 4. B10.BR to B10.A ($H\text{-}2^k$ to $H\text{-}2^a$) graft surviving at 8 weeks after grafting across a *D*-end barrier ($\times$ 500). Abundant liver tissue (H) remains but lymphocyte infiltration into the host kidney is evident

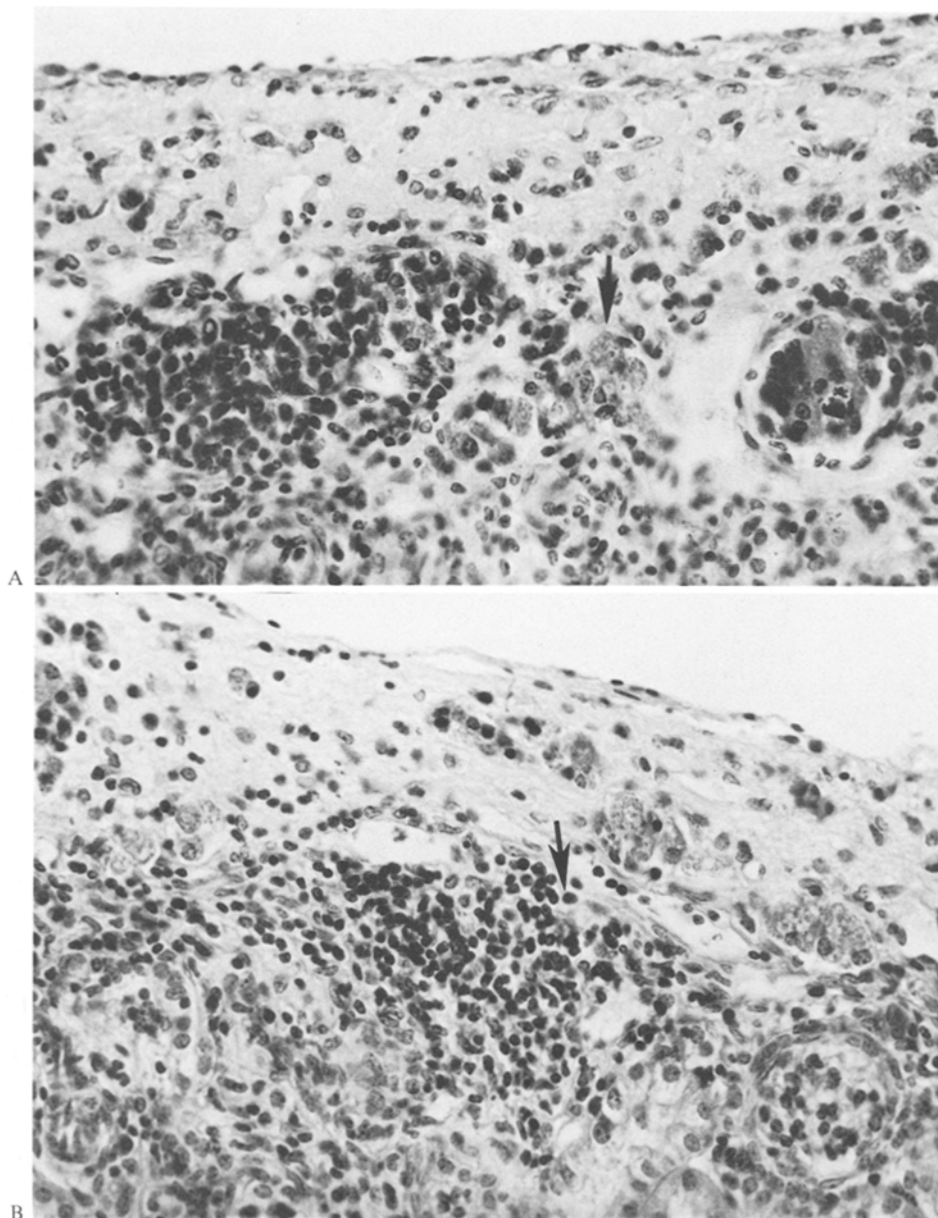

Fig. 5A and B

Revascularization of syngeneic grafts which has begun at 6 weeks after grafting is well developed at 10 weeks. By 6 weeks all graft combinations across background, *H-2* and *K* end barriers are totally resorbed, leaving only clustered hemosiderin-laden macrophages and some scar (Fig. 5).

The grafting procedure is relatively atraumatic to the underlying recipient kidney tissue. Healthy glomeruli and intact nephrons are presently directly

C

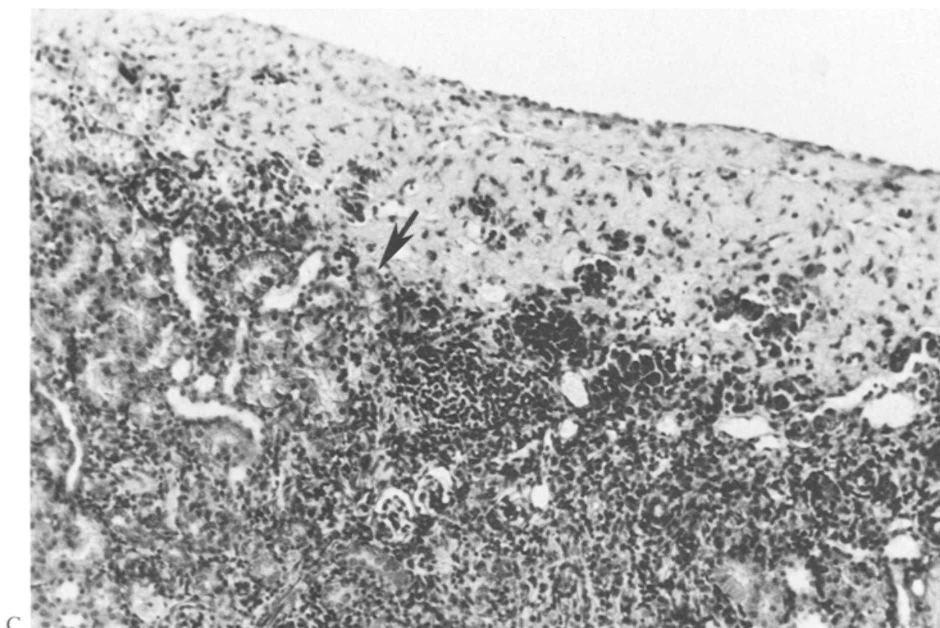

Fig. 5A–C. Rejected grafts. Host-scar interfaces are indicated by arrows. (A) B10 to B10.A (*H-2*^{b} to *H-2*^{a}) at 4 weeks after grafting (×500). (B) B10.D2 to B10.A (*H-2*^{d} to *H-2*^{a} K-end barrier) at 4 weeks after grafting (×500). (C) A.BY to B10 (multiple non *H-2* barriers) at 4 weeks after grafting (×200)

beneath the graft interface. There is some tubular atrophy in the host kidney in regions of massive lymphocytic infiltration in actively rejecting grafts. Therefore, renal atrophy appears to be associated with the rejection process and not with the graft technique.

Immunogenetic Picture. Rejection times for grafts across various *H-2* associated barriers and appropriate control data are presented in Table 1. Four animals were observed at each time interval for each donor-recipient combination. In each case, except that of the *H-2D* barrier, identical results are obtained in all animals at each time interval.

Syngeneic control grafts for B10 and B10.A mice were observed at intervals from 1 to 10 weeks after grafting. After the initial adjustment period described above, all grafts were accepted and contained well healed healthy tissue. Lymphocytic infiltration has totally disappeared by 8 to 10 weeks.

Although grafts across *H-2* plus non *H-2* barriers (C3H to B10) are nearly identical to syngeneic grafts at 1 week, by 2 weeks after grafting the liver tissues are uniformly necrotic and only a few viable hepatocytes remain. At 2 weeks the grafts have disappeared. A.BY to B10 combinations, in which donor and recipient share the *H-2* chromosomal region but differ in multiple background loci, follow a rejection pattern identical to that of the C3H to B10 allogeneic combination. Graft combinations of the same background, in which the donor carries the *H-2*^{k} haplotype (B10.BR) and the recipient carries the *H-2*^{b} haplotype

Table 1. Liver Graft Rejection Times – Major Barriers

Donor Strain	Donor *H-2*	Recipient Strain	Recipient *H-2*	Immunologic Barriers	Survival Time (Weeks)	No. of Animals/ No. used
B10	*bbbbbb*	B10	*bbbbbb*	None	Survives >10	4/4
B10.A	*kkkddd*	B10.A	*kkkddd*	None	Survives >10	4/4
B10.BR	*kkkkkk*	B10	*bbbbbb*	Entire *H-2*	2–4	4/4
A.BY	*bbbbbb*	B10	*bbbbbb*	Multiple non *H-2*	<2	4/4
B10	*bbbbbb*	B10.A	*kkkddd*	Entire *H-2*	<2	4/4
C3H	*kkkkkk*	B10	*bbbbbb*	Entire *H-2* + multiple non *H-2*	<2	4/4

Table 2. Rejection Course for *D*-end, *K*-end, and *D*-Plus *K*-end Genetic Barriers

Killing Time After Grafting (Days)	B10.BR to B10.A *D*-end Difference (*IC*+*S*+*D*)	B10.D2 to B10.A *K*-end Difference (*K*+*IA*+*IB*)	B10 to B10.A *K*-Plus *D*-end Differences
28	Some healthy hepatocytes in 3 of 4 animals	4 of 4 grafts rejected, lymphocytic nodules present	4 of 4 grafts rejected; massive inflamatory response
32	All appear healthy (4 of 4 animals)	As in 28 days	4 of 4 grafts rejected
36	Some healthy hepatocytes in all (4 of 4 animals)	As in 32 days	4 of 4 grafts gone
40	2 grafts rejected; 2 appear healthy	Old scars in 4 of 4 grafts	4 of 4 grafts gone
56	1 graft rejected; 3 have some remaining healthy hepatocytes	Well-healed mature scars in 4 of 4 grafts	Dense scar tissue in 4 of 4 grafts

(B10) appear to be surviving at 2 weeks but have totally degenerated by 4 weeks. However, when the *H-2* haplotypes of donor and recipient are $H\text{-}2^b$ (B10) and $H\text{-}2^a$ (B10.A), respectively, the graft is rejected before the second postgraft week.

Data from Klein (1972) show the strong influence that *H-2K* differences have on skin graft rejection and the lesser contribution of *H-2D* differences. We used recombinant congenic lines to test the relative contributions of these two regions to rejection of liver slices (Table 2). B10.BR and B10.A congenic lines differ at the *IC*, *S*, and *D* regions only, but are identical at the *K* end of the *H-2* complex (*K*, *IA*, and *IB*). Grafts from B10.BR to B10.A animals, evaluated at five postgraft periods from 28 to 56 days, show a chronic rejection pattern, with signs of rejection present in some, but not all, grafts from 28 days on. Three of four grafts still have some viable hepatocytes at 56 days, however. The B10.D2 to B10.A grafts, which differ genetically at the *K* end subregions (*K*, *IA*, and *IB*) but are identical at the *D* end (*IC*, *S*, and *D*) showed total rejection by 28 days after grafting. By 56 days, mature scar tissue is well formed

Table 3. Liver Graft Rejection Time – Minor Histocompatibility Barriers

Donor Strain	Recipient Strain	Locus at which Donor and Recipient Differ	Donor Allele	Recipient Allele	Skin Graft Survival (Days)[a]	Graft Results at 10 Wk After Graft
B10–*H-13*^{b}*A*^{w}/Sn	B10	*H-13.A*	*H-13*^{b},*A*^{w}	*H-13*^{a}, *A*	73	All surviving (5 of 5)
B10.129(10M)/Sn	B10	*H-11*	*H-11*^{b}	*H-11*^{a}	164	All surviving (5 of 5)
B10.129(9M)/Sn	B10	*H-10*	*H-10*^{b}	*H-10*^{a}	71	All surviving (5 of 5)
B10.C(45N)/Sn	B10	*H-9*	*H-9*^{b}	*H-9*^{a}	>300	All surviving (5 of 5)
B10.D2(57N)/Sn	B10	*H-8*	*H-8*^{b}	*H-8*^{a}	37	1 graft surviving, 2 grafts rejected, 2 grafts in process of rejection
B10.C(47N)/Sn	B10	*H-7*	*H-7*^{b}	*H-7*^{a}	33	4 grafts totally rejected; in 1 graft only a few cells remain
B10.129(21M)/Sn	B10	*H-4, p, d*	*H-4*^{b}*, p, d*	*H-4*^{a} +	20	4 grafts survive but not healthy; 1 graft rejected
B10.129(5M)/oSn	B10	*H-1, Ea-7*	*H-1*^{b}, *Ea-7*^{a}	*H-1*^{c}, *Ea-7*^{b}	>250	All surviving (5 of 5)
B10.129(5M)/nSn	B10	*H-1*	*H-1*^{b}	*H-1*^{c}	>250	4 of 5 surviving; a few cells remain in 1

[a] From Graff and Bailey (1973).

and almost all lymphocytic nodules and hemosiderin-laden phagocytes have disappeared in this combination. Of the 20 grafts in this donor-recipient combination evaluated between 4 and 8 weeks, none survive.

Because of the rapid rejection exhibited by A.BY to B10 donor-recipient graft combinations, a series of grafts across minor *H* locus barriers were evaluated to determine the individual contributions of these loci to graft rejection in our model system. Five grafts of each combination were evaluated 10 weeks after grafting (Table 3). Two of these combinations show some indication of rejection 10 weeks after grafting. These include grafts over the *H-4* and the *H-8* barrier. Grafts across the *H-7* barrier are totally rejected at 10 weeks. The remaining graft combinations, which differ at minor histocompatibility barriers, are viable at 10 weeks and are identical to syngeneic control grafts.

Discussion

Histological analyses of liver-to-kidney grafts between genetically identical inbred mice show the presence of healthy surviving liver tissue. This result is uniform up to at least 70 days after grafting, when careful attention is paid to technical grafting procedures. Grafts at various stages of rejection can also be distinguished by changes in liver cell viability and the presence of lymphocytic nodules, hemosiderin-laden macrophages, and mature scar tissue in stained sec-

tions examined by light microscopy. Old scars are readily recognizable. Since we can easily distinguish histologically between the alternatives of survival and rejection, we are able to draw certain conclusions as to the effect of various immunogenetic barriers on the survival of grafts in this system.

Donor-recipient *H-2* complex differences cause graft rejection, but these grafts may, in some cases, survive longer than *H-2* matched grafts differing at multiple non *H-2* loci. Several background loci are doubtlessly contributing to the rapid rejection in the A-BY to B10 combination, the two strains are both $H\text{-}2^b$ but differ in minor *H* loci. Strains A and B10 differ at the *Mls* locus defined by Festenstein *et al.* (1971). Strain A carries the Mls^c allele, while the B10 strain carries the Mls^b allele. The *Mls* locus has a strong effect on MLC reactivity and has been shown to have a weak effect on graft-versus-host reactivity (Peña-Martinez *et al.* 1973) and bone marrow survival. It has not been detected serologically (Festenstein 1971) and does not influence other graft survival systems tested so far. The *Mls* locus is not linked to the *H-2* complex. We are planning to investigate the effect of this locus in other strain combinations.

It appears that minor *H* locus differences do not contribute uniformly to liver-graft rejection. Of the combinations studied, only the *H-7* locus difference causes definitive graft rejection before 10 weeks. These data differ from the results of skin grafting as *H-4* and *H-8* barriers have the same influence as *H-7* on skin graft rejection but cause only weak, chronic rejection of liver grafts at 10 weeks. Experiments now underway will yield the data necessary to determine the actual survival time of liver grafts across the *H-7* barrier, and will pinpoint the time at which rejection is first noted over the *H-4* and *H-8* barriers.

Grafts of B10.D2 liver and B10.BR liver to B10.A recipients were employed to score the relative contributions of the *K* and *D* regions to graft rejection. There animals are identical for the background (non *H-2*) portion of the genome. B10.D2 ($H\text{-}2^d$ or *dddddd*) and B10.A ($H\text{-}2^a$ or *kkkddd*) are identical at the *D* end of the *H-2* complex but differ at the *K* end. B10.D2 grafts are totally rejected by B10.A recipients earlier than 28 days after grafting. Grafts from B10.BR donors ($H\text{-}2^k$ or *kkkkkk*) to B10.A recipients, which were identical at the *K* end of the complex but different at the *D* end, showed signs of chronic rejection at 28, 36, 40, and 56 days, with massive lymphocyte infiltration; at least one graft in each time interval disappeared. There was, however, some liver parenchyma still present in three of the four 10-week grafts. It appears, therefore, that relative contributions of *K* and *D* regions to liver slice rejection are similar to their contributions to skin graft rejection (Klein 1972). *D*-end differences tend to behave the same way as minor *H* differences. Experiments to determine the relative contributions of the *K*, *IA*, and *IB* subregions are now in progress.

Two different donor-recipient *H-2* disparate strain combinations were used in these experiments. These strains show different rejection patterns across an *H-2* barrier. The $H\text{-}2^k$ to $H\text{-}2^b$ grafts (B10.BR to B10) are viable at 2 weeks but are rejected at 4 weeks. The $H\text{-}2^b$ to $H\text{-}2^a$ grafts (B10 to B10.A) are rejected prior to 2 weeks after grafting. It appears that the existence of different *H-2*

disparities in the donor and recipient can result in somewhat different graft survival times in the liver-to-kidney grafting system. These different survival times may be governed by differences in the immune response of the recipient and/or the strength of the donor *H-2* antigens. Grafts between B10-congenic resistant strains of animals are now being studied to determine the subregion(s) responsible for the genetic control of this difference in graft survival time.

Recent reports of clinical kidney transplant survival data show considerable differences among different centers, pertaining to correlation between HL-A matching and long-term graft survival. Some groups (Dausset *et al.* 1974) report that HL-A matching between donor and recipient is a good predictor of the course of transplant survival. Other centers find that multiple mismatched combinations do as well (or as badly) as full house (four antigen) matches. It has been shown, however, that HL-A matching does correlate with skin graft survival in humans.

Several reasons have been proposed for the reported discrepancies in typing and survival data. Among these are the lack of uniformity of typing in different centers, variation in genetic heterogeneity between different population groups, and contributions of tissue specific antigens to the rejection of transplants. Although our model system does not purport to be the animal equivalent of a human kidney transplantation system, it will contribute to the definition of different immunogenetic parameters that may affect survival of various tissues in a genetically well-defined animal model. It is important to elucidate the different systems that control the rejection of different tissues to better understand the immunological phenomena influencing human organ transplantation.

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References

Baldwin, W.M., III and Cohen, N.: "Weak" histocompability antigens generate functionally "strong" humoral immunity. *Immunogenetics 1*:33–44, 1974

Belzer, F.O., Fortmann, J.L., Salvatierra, O., Perkins, H.A., Kountz, S.L., Cochrum, K.C., and Payne, R.: Is HL-A typing of clinical significance in cadaver renal transplantation? *Lacet 1*:774–777, 1974

Dausset, J., Hors, J., Busson, M., Festenstein, H., Oliver, R.T.D., Paris, A.M.I., and Sachs, J.A.: Serologically defined HL-A antigens and long-term survival of cadaver kidney transplants. *N. Eng. J. Med. 290*:979–984, 1974

Festenstein, H., Sachs, J.A., and Oliver, R.T.D.: Genetic studies of the mixed lymphocyte reaction in H-2 identical mice. *In* A. Lengerová and M. Vojtíšková (ed.): *Immunogenetics of the H-2 System;* pp. 170–177, Karger, Basel, 1971

Festenstein, H.: Immunogenetic and biological aspects of in vitro lymphocyte allotransformation (MLR) in the mouse. *Transplant. Rev. 15*:62–88, 1973

Freeman, J.S. and Steinmuller, D.: Acute rejection of skin and heart allografts in rats matched at the major rat histocompatibility locus. *Transplantation 8*:530–533, 1969

Gasser, D.L. and Silvers, W.K.: Genetic determinants of immunological responsiveness. *Adv. Immunol. 18*:1–66, 1974

Graff, R.J. and Bailey, D.W.: The non-H-2 histocompatibility loci and their antigens. *Transplant. Rev. 15*:26–49, 1973

Klein, J.: Is the H-2K locus of the mouse stronger than the H-2D locus? *Tissue Antigens 2*:262–266, 1972

Klein, J.: *Biology of the Mouse Histocompatibility-2 Complex*. Springer-Verlag, New York, 1975

Peña-Martinez, J., Huber, B., and Festenstein, H.: The influence of H-2 and non H-2 M locus on spleen colony formation after allogeneic bone marrow transplantation in irradiated mice, assayed by ^{59}Fe uptake and colony counting. *Tranplant. Proc. 5*:1393–1397, 1973

Salaman, J.R.: Prolonged survival of renal allografts in rats mismatched at the major histocompatibility locus. *Transplantation 11*:63–70, 1971

Seller, M.J.: Prolonged survival of fragments of liver transplanted to an ectopic site in the mouse. *Anat. Rec. 172*:149–155, 1972

Shreffler, D.C. and David, C.S.: The *H-2* major histocompatibility complex and the I immune response region: genetic variation, function and organization. *Adv. Immunol. 20*:125–195, 1975

Skoskiewicz, M., Chase, C., Winn, H.J., and Russell, P.S.: Kidney transplants between mice of graded immunogenetic diversity. *Transplant. Proc. 5*:721–725, 1973

Wheller, H.B., Corson, J.M., and Dammin, G.J.: Transplantation of tissue slices in mice. *Ann. N.Y. Acad. Sci. 129*:118–125, 1966

White, E. and Hildemann, W.H.: Allografts in genetically defined rats: difference in survival between kidney and skin. *Science 162*:1293–1295, 1968