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Tissue Graft Rejection in Mice

II. Graft Survival Across *H-2* Regional Barriers

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Abstract. The influence of *H-2* subregions on graft survival in a liver slice-to-kidney bed grafting system has been investigated. *H-2K*-region and *H-2IA*-region donor-recipient differences, either individually or in concert, cause acute graft rejection. *H-2D*-region donor-recipient differences cause chronic immunological reaction as evaluated by histological criteria. Grafts across this barrier may ultimately be rejected or may survive indefinitely. Several possible explanations for the variation in survival are proposed. The remaining known *H-2* regions (*IB, IC, S,* and *G*) all appear to cause immunological reactivity in a recipient animal which differs from the liver tissue donor at any of these regions. However, only an *IC*-region difference may ultimately cause complete graft destruction following an extended chronic immunological course. Grafts across background histocompatibility barriers of several genetic types show rejection patterns equivalent to those seen across *K* and *IA* barriers. These patterns are unchanged, whether or not the donor and recipient are congenic for *H-2* alleles. Different *H-2* allelic donor-recipient differences do, however, show different times of survival, indicating variation in strength or number of donor antigens or differences in recipient immune response.

Introduction

A recent report from this laboratory describes the use of a liver slice-to-kidney bed grafting system in the determination of the relative contribution of *H-2* and non-*H-2* barriers in tissue graft rejection in inbred mouse strains. Syngeneic grafts can be shown to survive for at least 70 days after grafting. The state of rejection of allogeneic grafts can readily be recognized by the various histological criteria described below. Rejection time is constant when donor and recipient tissues span a major histocompatibility barrier. However, minor histocompatibility barriers cause variable histological patterns of rejection and variable

times of rejection, probably dependent on environmental factors and the relative strengths of the barriers involved. Multiple non-*H-2* barriers (background histocompatibility barriers) cause graft rejection at least as rapidly as do *H-2* barriers. Skin and lymphocytes are the only normal tissues that have been studied extensively for their rejection when grafted between histoincompatible mouse strains. Skin graft rejection has been found to be under the control of the *K*, *IA*, and to a lesser extent, the *D* regions (Klein, 1972). CML (cell-mediated lymphocytotoxic) target antigens are controlled by the *K* and *D* regions. The MLC (mixed lymphocyte culture) reaction and graft-versus-host reactivities are controlled by genes located throughout the *H-2* complex (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). In the studies described below, we have used the same tissue grafting method previously described (Schultz *et al.*, 1976) to identify the regions and/or subregions of the *H-2* complex which influence rejection of liver tissue grafts. We have also compared liver graft survival times over different *H-2* barriers (between donor and recipient congenic animals with a variety of *H-2* chromosomes on the same background) and over different multiple non-*H-2* (background) barriers.

There appears to be a small but significant variation in the effect of various donor-recipient barriers when these barriers span the entire *H-2* complex. However, grafts across different background barriers are all rapidly rejected, no matter what group of minor histocompatibility alleles is found in the donor and recipient. Within the *H-2* complex, the influence of different subregion and region barriers vary considerably in their effects on graft survival. The major regions controlling rejection in this system are localized at the *K* end of the major histocompatibility complex.

Materials and Methods

Mice. Strains B10, A/J, A.BY, B10.A, B10.D2, and B10.BR were obtained from The Jackson Laboratory, Bar Harbor, Maine. Strains A.BY, A/J, B10.A(4R), B10.A(5R), B10.AQR, C3H.B10, A.TH, A.TL, B10.A(3R), C3H.OH, C3H.OL, B10.S(7R), B10.S(9R), B10.S, and B10.HTT were kindly donated by Dr. Donald C. Shreffler and maintained in the animal colony at the Ann Arbor V.A. Hospital. Strain B10.A(2R) mice were obtained from Dr. J. Stimpfling, and Dr. Jan Klein donated B10.AQR animals. All recipients and most donor animals were males of approximately 8 weeks of age.

Grafting Procedure. The grafting procedure, a modification of the method proposed by Wheeler and coworkers (1966) has been described previously. Briefly, a thin slice of liver tissue is transferred from the anesthetized donor to a recipient kidney site, prepared by removing a small slice from the kidney surface. The recipient skin is sutured and the animal maintained under ordinary laboratory conditions for a predetermined period of time. Four or 5 animals of each donor-recipient combination are grafted for each time period studied. After the graft has remained in place for the appropriate time period, the recipient animal is killed, the grafted kidney is removed, and the tissue is fixed, embedded, sectioned and stained as previously described (Schultz *et al.*, 1976). Three to 5 slides are prepared and examined histologically for each recipient animal.

Criteria for Evaluation of the State of Graft Survival. Graft survival is evaluated by histological inspection. A more detailed description of the histological characteristics of chronic and acute rejection phenomena over various genetic barriers and over the time course of rejection will be published elsewhere. The grafts were evaluated by the consensus of 3 observers with the heaviest weight on the opinion of one of us (T.F.B.), who is a pathologist and most experienced in histological observations. These slides were entirely randomized during the observation process so that all slides of one type of combination or one time period were never evaluated on the same day. For example, grafts which undergo chronic rejection and grafts which undergo acute rejection are likely to be evaluated on one day. Upon occasion, evaluation of a slice was repeated several weeks following the initial evaluation. These duplicate interpretations were always identical. The general criteria for the different types of graft evaluation are enumerated below.

1. The presence of healthy liver tissue in a well-ordered form with absence of lymphocytes or polymorphonuclear cells is indicative of graft survival. This pattern is typically found in syngeneic grafts or grafts that have overcome an episode of chronic rejection.

2. The presence of nodular aggregates of lymphocytes at the graft-kidney interface, complete absence of hepatocytes, or the presence of a few scattered, unhealthy liver cells is indicative of a graft which is being rejected acutely. During the early phases of graft rejection, the hepatocytes are replaced by plump active fibroblasts (Fig. 1 a). As the scar of the graft site ages, the immunologically competent cells are in as great or greater evidence, the fibroblasts become flattened, there is an increase in collagen, and the tissue contracts (Fig. 1 b). Several weeks after rejection, the immunologically competent cells have receded and only a few flattened fibrocytes are embedded in the dense contracted collagen of the scar. A graft is considered rejected when all hepatocytes have disappeared. In our initial studies, grafts were sampled for survival at 1, 2, 4, 6, and 8 weeks. When the earliest time of rejection had been determined, grafts involving the same combinations were then performed and sampled at a period one week prior to and one week following the time of rejection. For acute grafts, this narrowing down process continued until the day of disappearance of hepatocytes had been established within 3 days. Four grafts for each combination were performed at each time period. In every case, there was agreement among samples in rejection time for acutely rejected grafts. When rejection time had been determined for a particular type of genetic barrier, initial grafts for the same type of barrier (*e.g.*, an *H-2* barrier) were sampled at times 3 days before and 3 days after the rejection time for that barrier type. This simplification was necessary because of the time-consuming nature of the experimental procedure. In all cases, the limitation of observations to those times when changes were occurring in the grafts gave unequivocal measures of rejection time, even when these times turned out to be appreciably different from the original estimate.

3. In chronically rejected grafts, lymphocytes are scattered throughout the graft, often surrounding small foci of dying hepatocytes (Fig. 2). Sometimes these cell foci disappear, to be replaced completely by fibroblastic scar tissue. The immunologically competent cells subsequently recede. However, under certain environmental conditions and over certain immunological barriers, small nodules of hepatocytes will begin to divide, resulting in liver regeneration. The immunologically competent cells will then recede and the graft will take on the appearance of a syngeneic graft.

Chronic rejection was considered to commence at the time of appearance of lymphocyte infiltration and initial degeneration of hepatocytes. Grafts across barriers which were expected to give weak or no reactions were evaluated first at 10 weeks. If there were no signs of immunological reactivity and if the graft behaved as a syngeneic graft, no further studies of this combination were pursued. If the graft was rejected, grafts were performed at earlier periods. Rejection or recovery was considered to have occurred when all of the hepatocytes had been replaced by scar, or when the hepatocytes had begun to divide and assume an organized configuration. In each case, there was essential agreement among replicate grafts. However, a barrier was considered to have caused rejection when 3 of 4 grafts had disappeared and the one remaining contained only a few degenerating hepatocytes.

Nomenclature. The combined *K, IA,* and *IB* subregions of the major histocompatibility locus are referred to as the "*K* end" throughout the following discussions, while the combined *IC, S, G,* and *D* regions are referred to as the "*D* end". The designation $H\text{-}2K^b$ refers to the *K* region of the $H\text{-}2^b$ allele, the designation of $H\text{-}2D^d$ to the *D* region of the $H\text{-}2^d$ allele, etc. In all tables, the regions of the *H-2* haplotype are listed *K, IA, IB, IC, S, G,* and *D,* in that order.

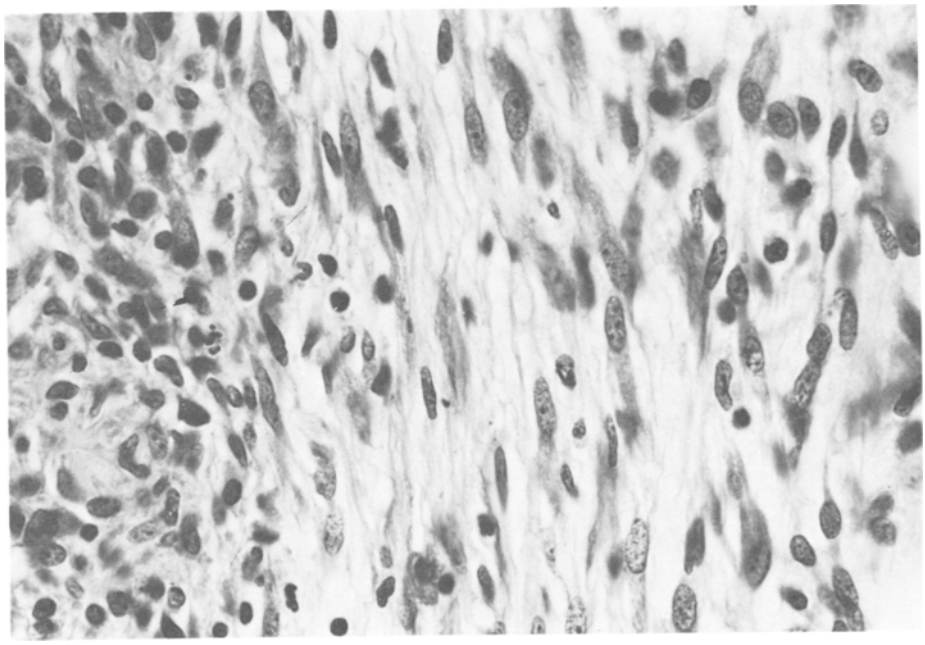

Fig. 1a. B10-to-B10.S graft at 10 days after grafting ($\times 800$). Hepatocytes have disappeared and the graft area is populated by active "plump" fibroblasts (early rejection)

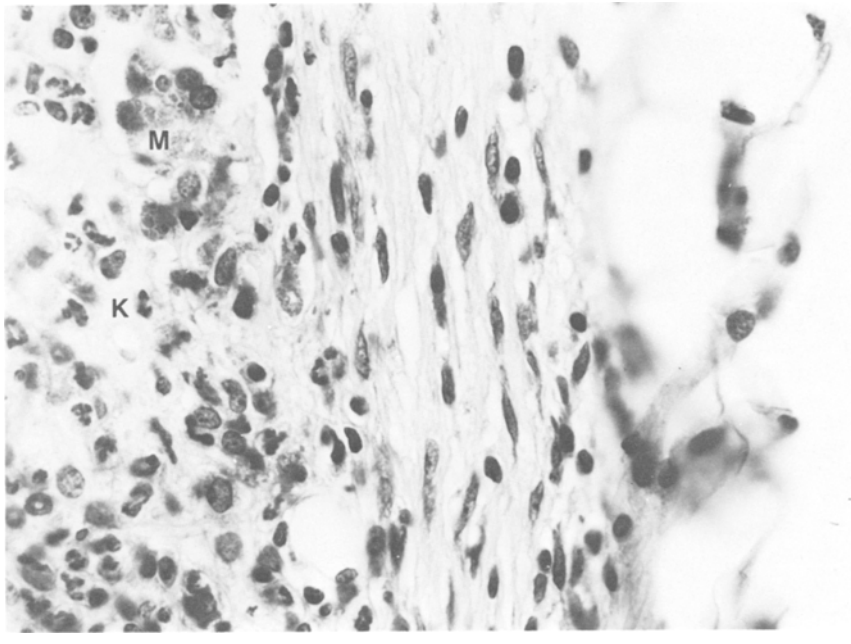

Fig. 1b. B10-to-B10.S graft at 17 days after grafting ($\times 800$). Scar has now contracted and fibroblasts are flattened and relatively inactive. The kidney bed is clearly visible (K) and hemosiderin-laden macrophages are in evidence (M)

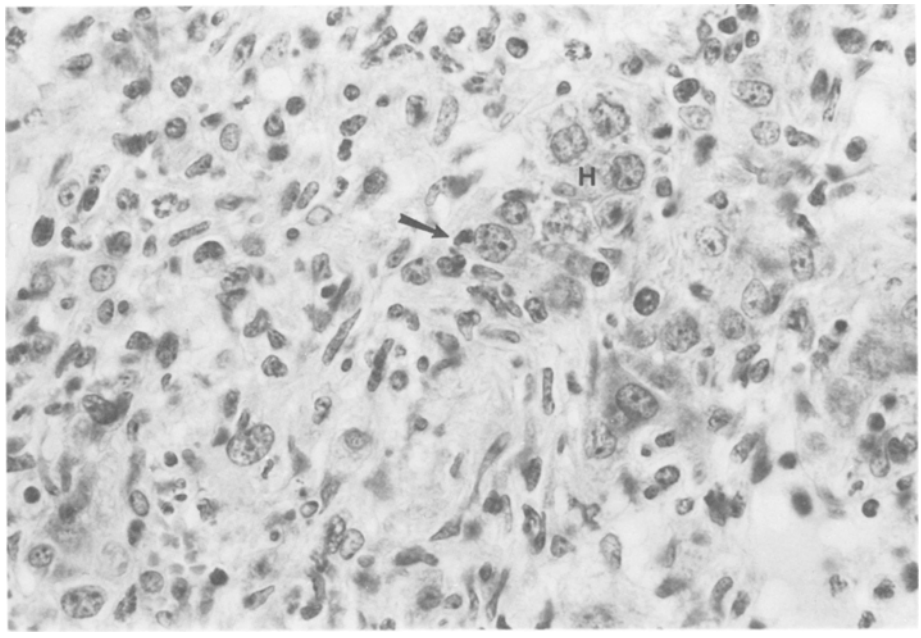

Fig. 2. B10-to-B10.D2 graft at seven days after grafting (× 800). Lymphocytes (arrow) are surrounding and presumably attacking residual hepatocytes (H), evidence of chronic rejection reaction

Results

The survival times for grafts between different *H-2*-disparate donor-recipient combinations are found in Table 1. In each case the donor and recipient animals have identical background genomes (A or B10) but differ for the entire major histocompatibility complex. All barriers were sampled at 10, 14, 21, and 24 days after grafting. In those combinations in which the graft had been rejected at day 10, a seven-day time period was studied as well. *H-2*^{b}-to-*H-2*^{d} and *H-2*^{b}-to-*H-2*^{k} grafts show chronic immunological reactivity and a variable rejection pattern

Table 1. Effects of Different *H-2* Barriers on Tissue Graft Rejection

Donor	Recipient	Donor *H-2*	Recipient *H-2*	Survival Times (days) and Immunological Activity
B10	B10.D2	*bbbbbbb*	*ddddddd*	Chronic rejection 7–14 Rejected at 18
B10.D2	B10	*ddddddd*	*bbbbbbb*	Survives < 14 days
B10	B10.A	*bbbbbbb*	*kkkdddd*	Survives 10 days
B10.A	B10	*kkkdddd*	*bbbbbbb*	Survives < 10 days
A.BY	A	*bbbbbbb*	*kkkdddd*	Survives < 14 days
B10.BR	B10	*kkkkkkk*	*bbbbbbb*	Chronic rejection 14–21 days
B10	B10.BR	*bbbbbbb*	*kkkkkkk*	Chronic rejection 10–14 days Rejected by day 18
B10	B10.S	*bbbbbbb*	*sssssss*	Survives 7 days

Table 2. Effects of Different Background and Background Plus *H-2* Barriers on Tissue Graft Rejection

Donor	Recipient	Donor *H-2*	Recipient *H-2*	Survival Times (days) and Immunological Activity
A.BY	B10	*bbbbbbb*	*bbbbbbb*	14–18
A	B10.A	*kkkdddd*	*kkkdddd*	14
C3H.B10	B10	*bbbbbbb*	*bbbbbbb*	7–10
C3H.B10	B10.A	*bbbbbbb*	*kkkdddd*	10
C3H	B10	*kkkkkkk*	*bbbbbbb*	10
C3H.B10	B10.A[a]	*bbbbbbb*	*kkkdddd*	10
B10.AQR	A	*qkkdddd*	*kkkdddd*	14

[a] One-Year-old mice

(some grafts rejected and some in the process of rejection) between seven and 18 days and ten and 14 days after grafting, respectively. $H\text{-}2^b$-to-$H\text{-}2^a$ grafts are rejected at ten days, and $H\text{-}2^b$-to-$H\text{-}2^s$ grafts at seven days. Therefore, different recipient alleles show different immunological reactivity at the same time after grafting whether the donor and recipient backgrounds are B10 or A. When donor and recipient *H-2* alleles are reversed in otherwise identical strains, the rejection time is altered somewhat for some $H\text{-}2^b$ combinations. $H\text{-}2^k$-to-$H\text{-}2^b$ grafts survive longer than those from *H-2*-to-$H\text{-}2^k$ animals. The rejection of $H\text{-}2^a$ grafts by $H\text{-}2^b$ animals proceeds faster than the rejection of reciprocal grafts. However, $H\text{-}2^d$-to-$H\text{-}2^b$ grafts do not appear to differ from $H\text{-}2^b$-to-$H\text{-}2^d$ grafts in rejection time.

Survival times for grafts between animals which differ in background but match for *H-2* (congenic lines) are found in Table 2. Also in this table are graft survival results of background plus *H-2* combinations.

A-to-B10 grafts are rejected at 14 days or shortly thereafter, whether the *H-2* types of donor and recipient are $H\text{-}2^b$ or $H\text{-}2^a$. The C3H-to-B10 barrier causes rejection in from seven to ten days. This survival time is identical to the survival time whether the C3H-to-B10 background barrier is combined with an $H\text{-}2^k$-to-$H\text{-}2^b$ or an $H\text{-}2^b$-to-$H\text{-}2^a$ major histocompatibility barrier. Age of animals does not appear to affect rejection time over a background plus *H-2* barrier.

Survival times of grafts in which donor and recipient differ in *H-2* subregions are found in Table 3. Here too, reciprocal grafts frequently differ in survival times. Grafts across the *K* end of the complex (*K*+*IA*+*IB*) are rejected in 14 to 18 days for $H\text{-}2^d$-to-$H\text{-}2^a$ grafts, but in less than 14 days for $H\text{-}2^a$-to-$H\text{-}2^d$ grafts. *H-2D*-end-disparate grafts (*IC*-, *S*-, *G*-, and *D*-region differences) show a chronic rejection pattern between 28 and 56 days after grafting. By 70 days, grafts over a *D* end of $H\text{-}2^k$-to-*D* end of $H\text{-}2^a$ barrier have been rejected. However, when only the *D* region is involved in the donor-recipient difference ($H\text{-}2D^b$-to-$H\text{-}2D^d$), chronic rejection begins at 42 days and continues to 70 days, when the graft appears to be recovering. When the donor and recipient are reversed, however, chronic rejection begins at 21 days and continues to immunological destruction by 70 days after grafting. When the graft spans an $H\text{-}2D^k$-to-$H\text{-}2D^q$ barrier,

Table 3. Effects of *H-2* Subdivisions on Tissue Graft Rejection

Donor	Recipient	Donor *H-2*	Recipient *H-2*	Survival Time (days) and Immunological Reactivity	Donor-Recipient Incompatibility
B10.D2	B10.A	*ddddddd*	*kkkdddd*	14–18	*K* end
B10.A	B10.D2	*kkkdddd*	*ddddddd*	7–10	*K* end
B10.T(6R)	B10.A	*qqqqq?d*	*kkkdddd*	Less than 10 days	*K* end and *IC, S, G*?
B10.BR	B10.A	*kkkkkkk*	*kkkdddd*	Chronic rejection 28–56 days rejected at 70 days	*D* end
B10.A(4R)	B10.A	*kkbbbbb*	*kkkdddd*	Variable Course. Chronic rejection 18→49 days rejected at 56 days	*D* end + *IB* subregion
B10.A	B10.A(4R)	*kkkdddd*	*kkbbbbb*	18 days	*D* end + *IB* subregion
B10.A(2R)	B10.A	*kkkdd?b*	*kkkdddd*	Shows chronic rejection pattern after 42 days. Appears to be recovering at 70 days	*D* region
B10.A	B10.A(2R)	*kkkdddd*	*kkkdd?b*	14–42 days severe chronic rejection, rejected at 70 days	*D* region
B10.BR	B10.AKM	*kkkkkkk*	*kkkkkkq*	Chronic rejection 14–70 days	*D* region
B10.AKM	B10.BR	*kkkkkkq*	*kkkkkkk*	21–42 days immunological reactivity. Almost destroyed at 56 days	*D* region
B10.A(2R)	B10.A(4R)	*kkkdd?b*	*kkbbbbb*	Mild reaction 18 to 42 days	*IB, IC, S, G*?
B10.A(4R)	B10.A(2R)	*kkbbbbb*	*kkkdd?b*	At 70 days, like syngeneic with a few lymphocytes	*IB, IC, S, G*?
A.TH	A.TL	*sssssssd*	*skkkkkd*	Less than 14 days	*IA, IB, IC, S, G*
A.TL	A.TH	*skkkkkd*	*sssssssd*	Less than 14 days	*IA, IB, IC, S, G*
B10.S(7R)	B10.HTT	*sssssssd*	*ssskkkd*	Rejected at 56 days	*IC, S, G*
B10.S(7R)	B10.S(9R)	*sssssssd*	*sssdddd*	Chronic reaction, surviving at 56 days	*IC, S, G*
B10.S(9R)	B10.S(7R)	*sssdddd*	*sssssssd*	Chronic reaction, surviving at 56 days	*IC, S, G*
B10.A(5R)	B10.A(3R)	*bbbdddd*	*bbb?ddd*	Immunological reaction, survives more than 70 days	*IC*?
B10.AQR	B10.A	*qkkdddd*	*kkkdddd*	Less than 14 days	*K* region
C3H.OH	C3H.OL	*ddddddk*	*ddddkkk*	Immunological reaction, survives more than 56 days	*S, G*
C3H.OL	C3H.OH	*ddddkkk*	*ddddddk*	No reaction; like syngeneic at 56 days	*S, G*

chronic rejection begins at 14 days after grafting and continues through 70 days after grafting. These grafts are not completely rejected from this combination or its reciprocal at 70 days, but they are still in immunological difficulty. If the *IB* subregion barrier is added to the *H-2D*-end barrier, as in the case of a B10.A(4R)-to-B10.A graft, rejection follows a variable course, with some grafts healthy in appearance and some grafts showing only a few residual hepato-

cytes. By 56 days after grafting, rejection appears to be essentially complete. B10.A-to-B10.A(4R) grafts are, however, rejected in 18 days, a time period which does not differ from the period of rejection over a *K*-end barrier. Differences between donors and recipients involving only *IB*, *IC*, and *S* and *G* subregions do not cause rejection, but show signs of immunological reactivity in at least one combination, B10.A(2R)-to-B10.A(4R). Grafts from B10.S(7R) to B10.HTT animals, in which the *IC*, *S*, and *G* subregions alone are involved, are rejected prior to 56 days. In this case, the allelic combination is *s-k* rather than *b-k/d*, as in the 2R-to-4R combination. In the 7R-to-9R and 9R-to-7R combinations, which span the same regions (*IC*, *S*, and *G*), grafts survive longer than 56 days, although there is apparent lymphocytic activity within and around the graft. The *s* and *d* alleles are involved in the latter combinations. When the *IA* subregion barrier is added to the *IB*, *IC*, *S*, and *G* barriers (A.TH-to-A.TL and A.TL-to-A.TH), grafts are acutely rejected in less than 14 days after grafting.

B10.AQR and B10.A mice differ only for the *K* region (K^q to K^k) but grafts between animals of these strains are rejected in less than 14 days. However, grafts from C3H.OH-to-C3H.OL mice and from C3H.OL-to-C3H.OH mice involving and, *S* and *G* barrier survive for 70 days with only minimal immunological reaction. When *K*-end, *IC*, *S*, and possibly *G* barriers are combined [B10.T(6R)-to-B10.A], rejection occurs in less than ten days.

Discussion

Functional analysis of the *H-2* subregions has proceeded almost as rapidly as genetic analysis of these regions. The influence of *K*-, *D*-, and *I*-region differences on MLR reactions (Rychlíková *et al.*, 1971, Bach *et al.*, 1972, Meo *et al.*, 1975, 1976), the influence of *I*-region differences on T- and B-cell interactions in vitro (Katz *et al.*, 1975), a continuing controversy over the presence of *I*-region products on various T-cell factors (Taussig *et al.*, 1975, Tada and Taniguchi, 1976), and the relationship of these products to the immune response are all topics of vast current interest to basic immunologists. In order to apply in vitro test results to physiological situations, it will become increasingly important to understand what type of immunological activity is elicited, should tissue cells from a donor who differs from a recipient for any combination of *H-2* subregions be introduced into that recipient. The tissue-to-kidney bed grafting system is more tedious than a skin grafting system but presents opportunities for histological studies of the course of an immune reaction in vivo which are difficult to obtain from skin graft studies. Courses of rejection for various tissues may be investigated employing this system, which also presents a physiological grafting environment similar to that of a clinical organ graft. Although the evaluation of these grafts is, at present, somewhat subjective, the histological results do not leave any doubt of the rejection or survival of the graft in acute cases. The presence and extent of immunological reactivity is also apparent in cases of chronic rejection. A more detailed analysis of the histological picture of various rejection courses will be published separately.

The additional data presented in this paper confirm our original findings that multiple non-*H-2* (background) differences cause liver tissue graft rejection somewhat more rapidly than do *H-2* differences. The addition of an *H-2* difference to a background difference does not appear to accelerate the rejection, at least when recipients all carry the B10 background.

Donor-recipient differences for the entire *H-2* complex cause slightly different times and courses of rejection, with some grafts rejecting acutely and some showing chronic immunological reactivity for periods of up to 21 days before rejection is complete. Reciprocal grafts also show different time courses of rejection. These data indicate differences in antigenic strength for different donor *H-2* alleles and/or differing abilities among recipients carrying different immune response genes to respond to the challenge of the antigens of the liver graft.

The experiments discussed above present incontrovertible evidence that, as in the skin graft system, donor-recipient differences in the *K* end of the *H-2* complex cause uniformly acute rejection of liver grafts. *D*-end differences cause immune reactions which follow a chronic course and which may or may not result in rejection. In fact, histological studies of grafts over *D*-end barrier differences indicate that the immunological reactivity elicited may result in eventual stimulation of the grafted liver to regenerate.

B10.A-to-B10.D2 and the reciprocal grafts reveal the strong influence of *K*-end differences on rejection. Although grafts in both directions are rejected in seven to 18 days (as rapidly as grafts between mice with both *H-2* and non-*H-2* differences), the B10.D2-to-B10.A grafts survive longer than the B10.A-to-B10.D2, again indicating variation in strength or number of donor antigens and/or *H-2* controlled immune response of the recipient. Grafts between the only animals which differ for the *K* region alone (B10.AQR and B10.A; $H\text{-}2K^q$ and $H\text{-}2K^k$) are also rejected in less then 14 days with an acute rejection pattern. It is apparent, therefore, that the *K* region of the *H-2* complex codes for a major histocompatibility antigen in the liver-to-kidney grafting system. Since A.TH-A.TL combination grafts are also rejected in less than 14 days, and B10.A(2R)-B10.A(4R) combination grafts show only mild chronic immunological reaction, it appears that the *IA* subregion, the barrier included in the former combination but excluded in the latter, must also serve as a major histocompatibility barrier for this system.

D-region immunological barriers result in rejection courses with considerable variability. An $H\text{-}2D^b$-to-$H\text{-}2D^d$ barrier elicits chronic reaction beginning at 14 days after grafting and going on to eventual recovery of the graft at 70 days. The reciprocal combination is in immunological difficulty at 14 days and is rejected at 70 days. It is of interest to note that, of the *D*-series public antigens which have been defined, the $H\text{-}2D^d$ allele contains several antigens missing in the $H\text{-}2D^b$ allele. The $H\text{-}2D^b$ allele contains no antigens which are missing from the $H\text{-}2D^d$ allele. One could postulate, therefore, that, if the strong antigens of this region have already been defined, there are no strong specificities (except for one private specificity) present in $H\text{-}2D^b$ which might elicit a reaction in an animal carrying the $H\text{-}2D^d$ haplotype. This explanation is further supported by the results of B10.A(4R)-to-B10.A grafts and their reciprocals (see Table 3). The latter grafts include an entire *D*-end plus an *IB* barrier, but there is evidence

from other grafting combinations that the *IB*, *IC*, *S*, and *G* barriers cause only mild chronic rejection. Therefore, the rapid rejection of B10.A-to-B10.A(4R) is probably due almost entirely to the $H\text{-}2D^d$-to-$H\text{-}2D^b$ combination. The chronic reaction in B10.A(4R)-to-B10.A grafts reflects the absence of antigens in $H\text{-}2D^d$ not present in $H\text{-}2D^b$ which we have discussed above. The $H\text{-}2^q$-to-$H\text{-}2^k$ *D*-region difference (B10.AKM-to-B10.BR) also elicits a much stronger response than does the $H\text{-}2^k$-to-$H\text{-}2^q$ (B10.BR-to-B10.AKM) difference. Again, in this combination, the rejected donor carries at least twice as many defined antigens which may be recognized as foreign by the recipient than in the reciprocal situation.

The results of grafts among B10.S(7R), B10.S(9R), and B10.HTT animals, which differ at the *IC*, *S*, and *G* subregions (Table 3), are more difficult to explain.It appears from grafting studies between C3H.OL and C3H.OH mice that *S*- and *G*-region differences elicit only very mild lymphocytic infiltration in our grafting system. Therefore, *IC*-region barriers must be causing rejection prior to 56 days in the B10.S(7R) (IC^s)-to-B10.HTT (IC^k) combination. However, grafts of 7R to 9R and 9R to 7R (IC^s-IC^d combinations) are surviving and healthy at 56 days after grafting. This discrepancy cannot be explained either by the known Ia antigens present in the *IC*-subregion alleles involved, or by differences at recently defined *IJ* and *IE* subregions lying between *IB* and *IC* (Tada *et al.*, 1976, Murphy *et al.*, 1976). B10.S(7R) and B10.S(9R) differ in both *IJ* and *IE* subregions, while B10.S(7R) and B10.HTT differ only in the *IE* subregion. The final clarification of the influence of the *IC* and adjacent *I* subregions on graft rejection await further genetic definition of these regions.

It is clear from the data presented above that *H-2* and background differences contribute equally to tissue graft rejection. Moreover, the *H-2* effect is localized in the *K* end of the major histocompatibility complex, notably in the *K* and *IA* subregions. The contributions to immunological activity of the other subregions of the major histocompatibility complex vary with the region and the alleles involved.

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