AN ASSAY OF GRAFT-HOST INTERACTIONS ACROSS STRONG AND WEAK HISTOCOMPATIBILITY BARRIERS IN MICE*

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The basic concepts of homograft immunity were first established by the fundamental studies of Medawar (1, 2). These studies, together with succeeding work, have clearly defined the reactions of the host after exposure to homologous and heterologous antigens contained in the graft. Such reactions have been demonstrated to be of differing intensities, occurring in response to strong or weak histocompatibility stimuli, and have been elucidated by observations in inbred strains of animals (3).

The reactions produced by the graft against the host occur when cells capable of an immune response are a significant or major portion of the graft. This concept has been present for almost a decade, and has been evaluated by many workers in transplantation experiments, beginning with Simonsen (4, 5) and Billingham (6). The Simonsen discriminant spleen assay, which assesses the degree of splenomegaly of the unresponsive recipient after injection of donor lymphoid cells (7), has served as a valuable tool with which to determine the presence of donor and host components of these reactions (8). Other models employing elegant detection techniques, such as tritiated thymidine labeling (9), chromosome markers (10), and immunologic reactions to a third party antigen (11) have been utilized to identify one of the two components.

The simplicity of the Simonsen assay has been counterbalanced by its relative insensitivity. Howard, Michie, and Simonsen (12) had difficulty in finding the donor cell component in the F_1 hybrid suffering from "runt disease" as a result of the injection of parental strain cells. There has also been difficulty in demonstrating the donor component in tolerant chimeras that received viable lymphoreticular cells at birth (8), presumably because of the large quantity of spleen cells of the chimera in ques-

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tion that must be injected to produce splenomegaly in the test system. The assay is also limited to recipients which are incapable of a measurable rejection response to the injected lymphoid cells, that is, they are immunologically immature, genetically tolerant F_1 hybrid, or x-irradiated recipients.

The present studies were undertaken on the assumption that graft-host interactions when both donor and recipient are capable of immune responses may be quite unlike the unidirectional graft *versus* host reaction. A new criterion of graft-host interactions is presented, based on the *early* finding of mononuclear cell infiltrates of donor origin in the livers of recipient mice after administration of homologous lymphoreticular cells. In the evaluation of donor cell reactivity these infiltrates are consistent in appearance as compared with spleen enlargement, a more variable and complex response. This model is sensitive enough to detect such graft-host interactions between strains with a common H-2 histocompatibility locus, and can detect differences in the degree of reactivity of strains to each other when injected reciprocally.

Materials and Methods

Mice.—Young adult mice, aged 1 to 3 months, of the following strains, were employed: A, $C_{57}Bl/1$ (abbreviated B_1), C_3H/Bi, and F_1 hybrids obtained by a cross of A females and $C_{57}Bl/1$ males (AB_1), each of the foregoing strains differing from all the others by the strong H-2 histocompatibility barrier; and Balb/C and DBA/2, which differ at minor histocompatibility loci. In all donor-recipient strain combinations, animals of the same sex were used to avoid any influence of the Eichwald-Silmser phenomenon (13).

All the mice were obtained from the stock of Dr. J. J. Bittner in 1956, and have been maintained in our own colonies by rigorous inbreeding procedures since that time. The animals were housed in plastic cages, and offered Purina fox chow and water *ad libitum*.

Spleen Cells.—Suspensions of viable mature spleen cells were prepared by methods previously described (14). In some instances, cells were sonically disrupted at 18,000 cycles per second for 3 minutes in an MSE ultrasonic disintegrator.[1] These samples were examined microscopically and showed no intact cells.

Pretreatment of Donors and Hosts.—In some experiments, donors and hosts were pretreated as follows: (*a*) Thymectomy within 24 hours after birth, using a technique similar to that of Dischler and Rudali (15). All these animals were sacrificed and examined for thymus remnants, and no remaining thymus could be found. (*b*) Lethal whole body x-irradiation, consisting of 700 r, 47 r per minute using 220 kv and 150 ma at a distance of 60 cm, with an ionmeter cone and 0.25 mm copper grid. (*c*) Preimmunization, by intravenous injection of 100 million viable homologous spleen cells at 2 months of age, followed in 1 week by a skin graft (14) from donors of the same strain as that from which the spleen cells were derived. The skin grafts were always rejected within 11 days; such animals were considered immunized.

Injection of Cells.—Host animals received 0.2 to 0.3 cc. of a suspension containing 100 $\pm$ 10 million intact or disrupted cells, administered into the tail vein through a 27 gauge needle. Cell counts were performed on each injection lot.

Assessment of Graft-Host Interactions.—Recipients were weighed and sacrificed at 1, 2, 3, 4, or 7 days after injection of the cells. Relative spleen weight per 100 gm of body weight was recorded. The difference between the means of all experimental and control groups in each

[1] Measuring and Scientific Equipment, Ltd., London.

study was subjected to statistical analysis. Tissue specimens were taken from the left lobe of the liver (found to be representative of the entire organ). The tissues were fixed in neutral formalin for routine hematoxylin and eosin staining. Liver infiltrates (to be discussed in the following section) in some experiments were quantitated by calculating their number per total number of low power fields in the group (average number of low power fields per animal being 30).

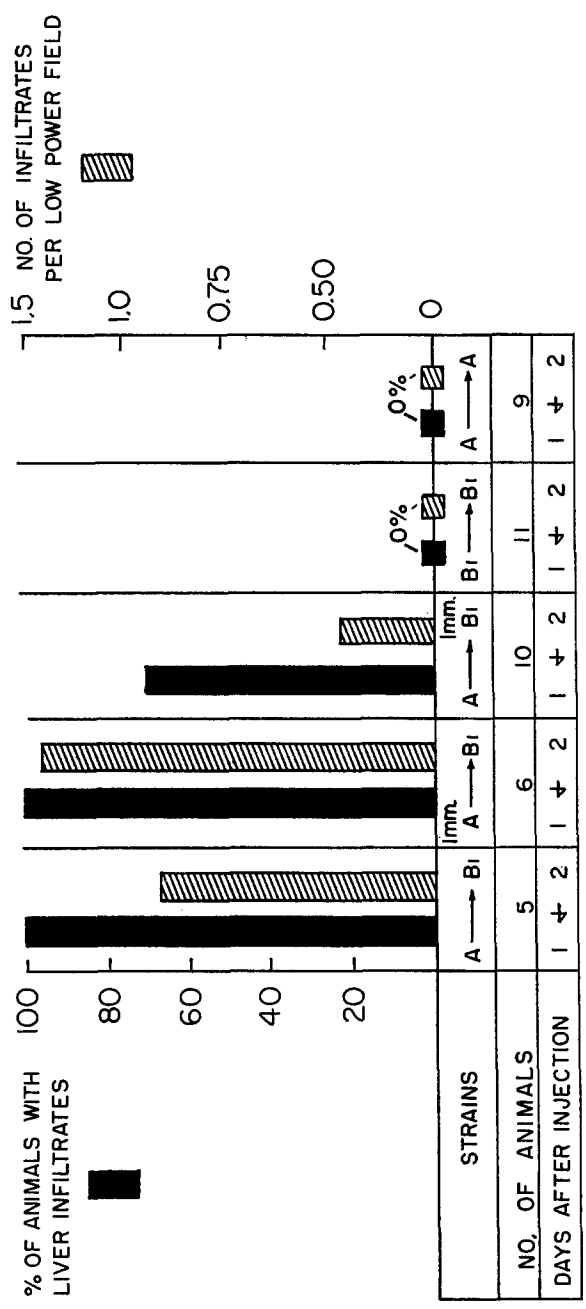
RESULTS

Liver Lesions.—When homologous spleen cells were injected intravenously into members of the strains listed below, a characteristic infiltrative lesion was observed in the periportal areas of the livers of the recipient animals (Fig. 1). These infiltrates consisted of collections of predominantly mononuclear cells of a moderately heterogeneous morphology: small cells with deeply stained nuclei and cytoplasm, and larger cells with reticular nuclei and lightly stained cytoplasm. The cells were apparently of the lymphocyte, reticulum cell, and macrophage varieties. Very few plasma cells were seen in these cell collections, even when preimmunized spleen donors were used. Polymorphs, erythroid, myelopoietic cells, and multinuclear cells were also very sparse.

The livers of animals in all control and experimental groups occasionally contained small foci of parenchymal damage, usually centrilobular, about which small to moderate aggregates of polymorphonuclear cells had gathered. The average incidence of these lesions was about 1 in 40 low power fields (× 100) and they were interpreted as being non-specific in nature. By contrast, the mononuclear cell infiltrates described above were consistently located just outside the wall of the venous radicle of the portal triad, in some instances completely encircling the vein, and in others ballooning to one side of a larger vein.

The experiments to be described are concerned with the incidence of the specific mononuclear cell infiltrates and spleen weight changes in various strain combinations of donor and recipient, and with the effects of various forms of pretreatment of donor and recipient on the frequency of these infiltrates.

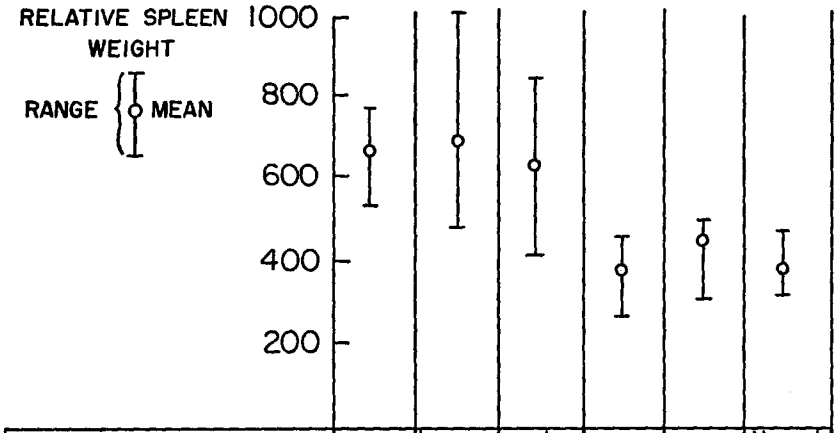
Liver Infiltrates in B_1 Strain Mice Injected with A Strain Spleen Cells.—As shown in Text-fig. 1, the characteristic liver infiltrate appeared in all the B_1 animals 1 and 2 days after they were injected with A strain spleen cells. The average number of such infiltrates per low power field was 0.89 (total low power fields counted—150). Both A and B_1 controls received isologous cells, and showed no evidence of the lesion. When the spleen cells were taken from A animals that had previously been immunized against the B_1 strain, all of the B_1 recipients developed the lesion (Fig. 2) and the average number of lesions was increased to 1.43 per low power field (total low power fields counted—180). By contrast, when the B_1 recipient animals had been previously immunized against A donors, the incidence of infiltrates was reduced to 70 per cent, and the average number of liver infiltrates per low power field was 0.49 (total low power fields counted—300).



TEXT-FIG. 1. Occurrence of periportal mononuclear infiltrates in the liver of recipients at 1 and 2 days when 100 million spleen cells are injected intravenously into homologous, immunized homologous, and isologous recipients, and when 100 million immunized cells are injected into normal homologous recipients. (Imm. = immune.)

Spleen weights at the time of sacrifice were increased when A strain spleen cells were injected into B_1 recipients, but not when A and B_1 strain donor cells were injected into A and B_1 recipients respectively (Text-fig. 2).

Liver Infiltrates in Graft-Host Interactions Involving Immunologically Incompetent Donors or Recipients.—As demonstrated in Text-fig. 3, when cell products or cells unable to proliferate were injected, *i.e.* sonically disrupted or lethally x-irradiated cells, no liver infiltrates were seen. In addition, an infiltrate inci-

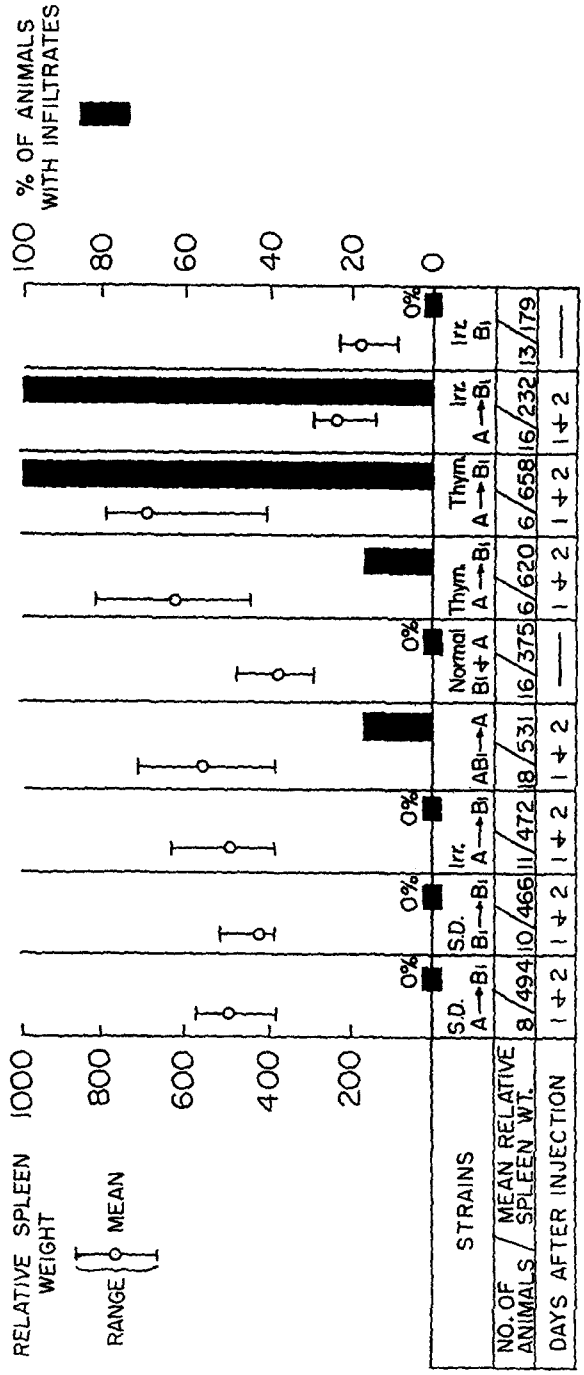


STRAINS	A→B1	Imm. A→B1	Imm. A→B1	B1→B1	A→A	Normal B1
NO. OF ANIMALS / MEAN RELATIVE SPLEEN WT.	5/670	6/690	10/627	11/358	9/440	8/375
DAYS AFTER INJECTION	1 + 2	1 + 2	1 + 2	1 + 2	1 + 2	1 + 2

TEXT-FIG. 2. Relative spleen weights (spleen weight per 100 gm body weight, hereafter referred to as relative spleen weight) 1 and 2 days after injection of isologous, homologous, and immune homologous donor spleen cells, together with the spleen weights of immunized recipients injected with homologous cells. (Imm. = immune.)

dence of 17 per cent was obtained both when AB_1 F_1 hybrid spleen cells were injected into A recipients, or when spleen cells from thymectomized A strain donors were injected into B_1 recipients. By contrast, A cells injected into thymectomized or x-irradiated B_1 recipients produced liver infiltrates in all the animals. Thus, it appears that these infiltrates, present after the injection of immunologically responsive donor cells into unresponsive hosts, were of donor origin.

Splenomegaly.—As is shown in Text-fig. 3, a small but significant degree of splenomegaly was obtained when sonically disrupted B_1 strain cells were injected into the isologous B_1 recipients. Similar results were obtained when A strain disrupted cells were injected into B_1 recipients. In another experiment,



TEXT-FIG. 3. Comparison of liver infiltrates and spleen weights in recipients of sonically disrupted homologous and isologous spleen cells; and of spleen cells from homologous lethally x-irradiated, F1 hybrid, and neonatally thymectomized donors—all contrasted to thymectomized and x-irradiated recipients of competent homologous spleen cells. Control groups were either untreated non-injected, or lethally x-irradiated non-injected animals. (S.D. = sonically disrupted; Irr. = lethally x-irradiated; Thym. = neonatally thymectomized.)

spleen cells from lethally x-irradiated A strain animals were injected into B_1 recipients, and AB_1 F_1 hybrid cells into A recipients. In both cases only a slightly greater spleen enlargement was obtained (not significant). When cells from thymectomized A strain donors were injected into B_1 recipients, a splenomegaly significantly greater than that of the former groups was observed. There was also a significant splenomegaly when A spleen cells were injected into neonatally thymectomized B_1 animals. In the x-irradiated B_1 recipients of A spleen cells, spleen weights decreased, but were significantly greater than those found in lethally x-irradiated but non-injected controls. Thus, the quality or quantity of injected antigen may have played a role in inducing spleen enlargement in the recipients. In addition, splenomegaly may have been due to specific immune responses on the part of the donor cells against the recipient. Also demonstrated, however, was an element of non-specificity in the recipient response (*viz.* disrupted isologous cells producing mild splenomegaly).

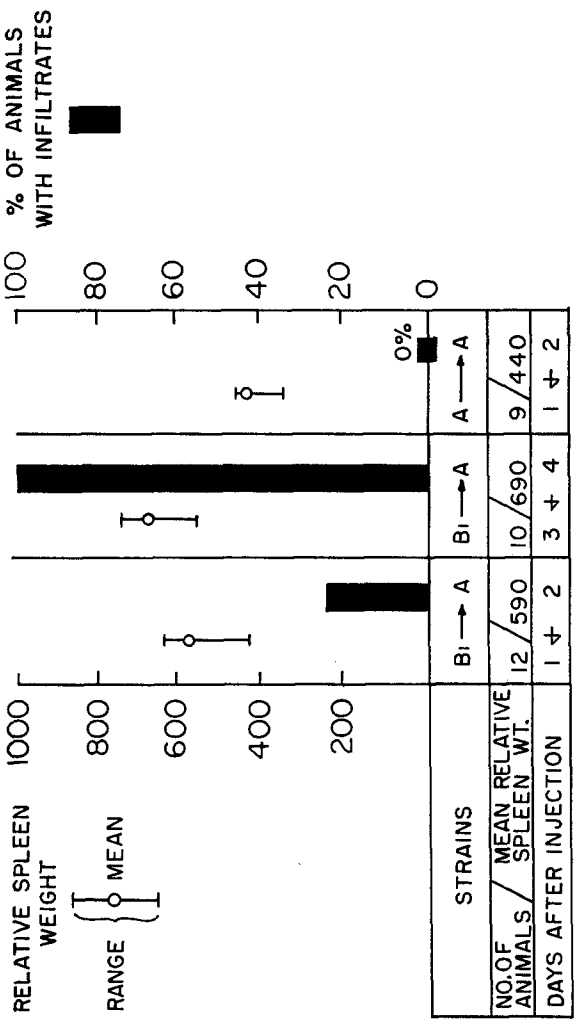
Spleen enlargement, then, stems from a more complex combination of causes while the infiltrative lesions of the liver appear to be only proliferating donor cells.

Liver Infiltrates—the Result of Other H-2 Histocompatibility Differences.—When A strain recipients were injected with B_1 strain cells (Text-fig. 4), 25 per cent of the animals revealed liver infiltrates at 1 and 2 days and a moderate splenomegaly was evident. However, at 3 days splenomegaly was markedly increased, and the incidence of infiltrates had risen to 100 per cent.

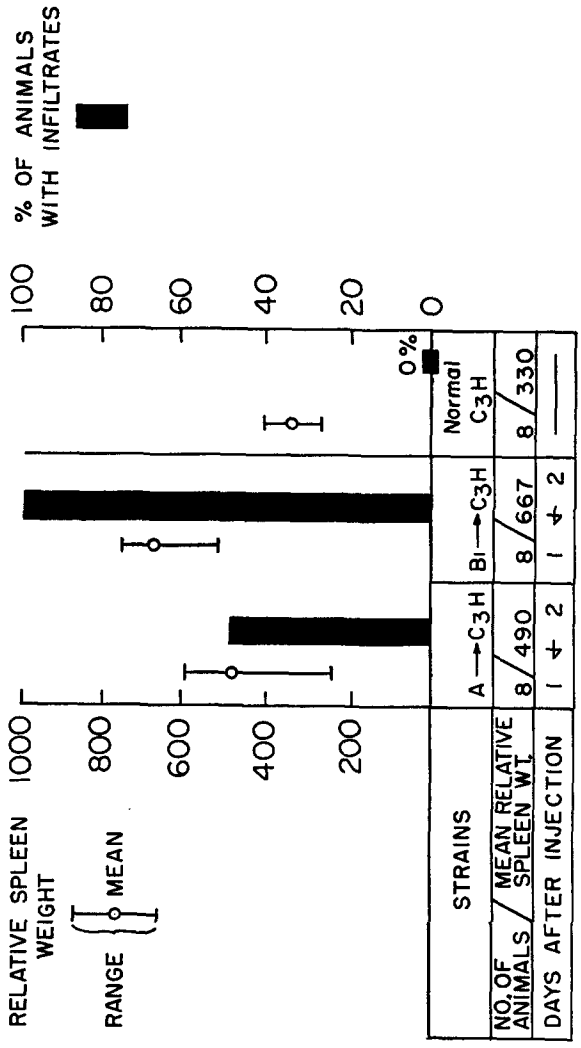
As can be noted in Text-fig. 5, whereas 45 per cent of C_3H hosts had liver infiltrates at 1 and 2 days after A strain cells were injected, all of the C_3H recipients had liver infiltrates when mice of the B_1 strain were used as spleen donors. A strain cells produced only a moderate splenomegaly in C_3H recipients, while B_1 strain cells produced a marked enlargement.

After A strain cells were injected into AB_1 F_1 hybrid recipients, (Text-fig. 6) there was a 30 per cent incidence of animals with liver infiltrates. The complementary experiment of B_1 into AB_1 resulted in 20 per cent of the recipients possessing infiltrates. The spleen weights were also moderately increased in both parent F_1-hybrid combinations. By 7 days, however, all the mice in both groups possessed liver infiltrates, cell collections whose morphology was similar to the 1 and 2 day samples. This is in contrast with the more immediate response of both the liver and the spleen at 1 and 2 days when A strain cells were injected into B_1 animals.

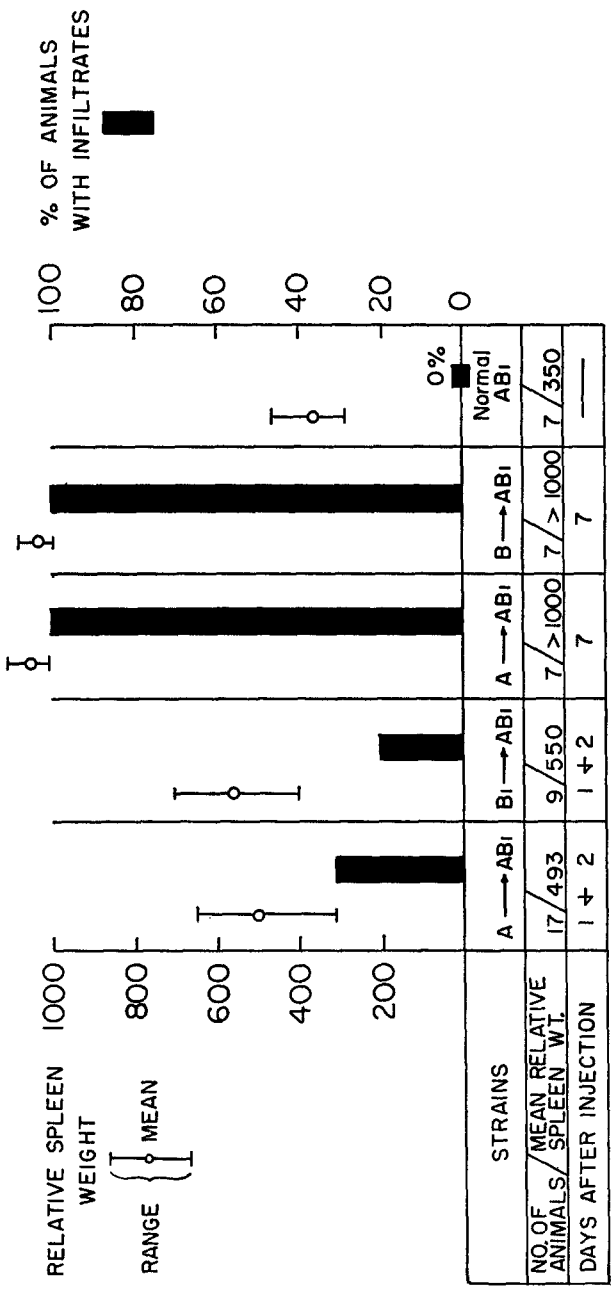
Liver Infiltrates and Splenomegaly—the Result of Minor Non-H-2 Histocompatibility Differences.—In an attempt to evaluate the response across a minor histocompatibility barrier, Balb/C strain cells were injected into DBA/2 recipients. In this instance 100 per cent of the recipients revealed liver infiltrates at 0 and 2 days following injection (Text-fig. 7), and a large degree of splenomegaly was observed. On the other hand, when DBA/2 strain cells were in-



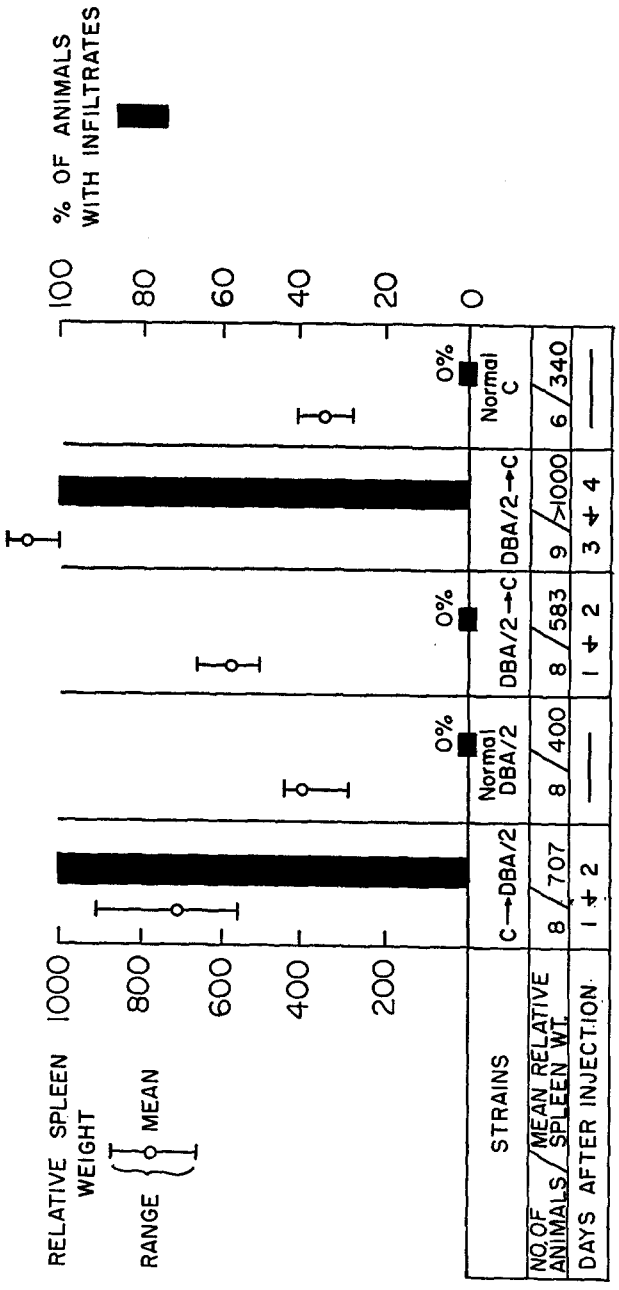
TEXT-FIG. 4. Liver infiltrate incidence and spleen size in A strain recipients 1, 2, 3, and 4 days after the intravenous injection of B_1 strain spleen cells.



TEXT-FIG. 5. Liver infiltrate incidence and spleen size when A and B_1 strain donor spleen cells are injected into C_3H recipients.



TEXT-FIG. 6. Liver infiltrate incidence and spleen size when A and B_1 strain donor spleen cells are injected into AB_1 F_1 hybrid recipients.



TEXT-FIG. 7. Liver infiltrate incidence and spleen size of recipients when Balb/C strain spleen cells are injected into DBA/2, and when DBA/2 spleen cells are injected into Balb/C.

jected into Balb/C recipients, there were no infiltrates at 1 and 2 days, and a moderate splenomegaly was present. At 3 and 4 days, however, liver infiltrates were demonstrated in 100 per cent of the animals together with a very large degree of splenomegaly.

DISCUSSION

The results of these experiments are of interest in the evaluation of graft-host interactions. These interactions usually are the result of both donor and host activity, and in many situations it would be useful to dissociate the two where both host and graft are capable of responding. Studies heretofore have, for the most part, dealt with hosts incapable of taking part in the interaction: the newborn, the x-irradiated host, or the F_1 hybrid recipient of parent cells. There is very little information about the quantitative aspects of the immune response of the donor cells against the host when the host is immunologically competent.

It seems clear from the experiments reported here that the liver infiltrates observed in recipients injected with immunologically competent cells are of donor origin. A similar conclusion was reached by Ozogoe from experiments in rabbits also injected with homologous lymphoid cells (16). The failure of infiltrates to appear when disrupted cells were injected, or when cells from x-irradiated or thymectomized donors were employed, is admittedly presumptive evidence. However, the abolition of the host response by lethal x-irradiation, and the appearance of liver infiltrates when immunologically competent cells were injected, is noteworthy. Similarly, the appearance of such infiltrates in thymectomized recipients, presumably also unresponsive (17, 18), adds to the proof.

By contrast, there was a degree of non-specificity to the response of splenic enlargement. This occurred in B_1 animals given sonically disrupted isologous cells, lacking in antigenic capacity. Splenomegaly due to an immune response in the recipient also occurred after the injection of homologous cellular debris or of intact homologous cells lacking graft *versus* host reactivity. Thus, the spleens of B_1 recipients were enlarged 1 and 2 days following the injection of sonically disrupted, lethally x-irradiated, and neonatally thymectomized A strain spleen cells, and A strain recipient spleens were also enlarged 1 and 2 days after injection of AB_1 lymphoreticular cells. Splenomegaly, therefore, occurred in strain combinations where few or no donor cell infiltrates were found in the liver, 1 and 2 days after the injection of homologous cells. In addition, it has been demonstrated in the past (9, 19) that the spleen is a site for proliferation of donor cells after their transfer to immunologically unresponsive recipients. Evidence for this is again found herein in the splenomegaly observed in the A into AB_1 F_1 combination, and in the small but significant spleen enlargement in x-irradiated recipients given homologous cells. We conclude that where both

donor and host cells are immunologically competent, the recipient spleen enlargement reflects proliferation of both.

To establish that this early graft-host interaction was not limited to the A and B_1 strains, C_3H animals were selected as recipients of both A and B_1 lymphoid tissue in additional experiments. Although B_1 animals had reacted more slowly to A strain antigens than had A animals to B_1 antigens, the opposite effect was observed in the C_3H mice. C_3H antigens apparently provoked a stronger and faster response among injected B_1 strain cells than among injected A cells. This is consistent with the observation that A cells induce tolerance in newborn C_3H mice with relative ease, while runt disease is the rule when B_1 lymphoid cells are administered (20). Thus, the observed liver infiltrates may be considered an index, albeit gross, of the degree of reactivity of donor cells to homologous recipient strain antigens, or as another method of identification of common transplantation genes between two strains, differentiating more closely related strains from more distantly related ones.

This model also permitted a comparison of the reaction that occurs in F_1 hybrid recipients receiving parent strain cells with the reaction that occurs in the parent strains receiving cells from each other. Both A and B_1 cells, in the time intervals studied, had relatively equal reactivity when injected into AB_1 F_1 hybrids (A strain reactivity was slightly greater at 1 and 2 days). The response in both instances was dampened when compared to the reactivity of the parent strains to each other. This relative lag in reactivity of the parent in the F_1 hybrid may well be another manifestation of the gene dose effect mentioned by Simonsen (7) and Prehn and Main (21). Although there is no evidence at this time for a dominant or recessive character to transplantation antigens, this is another possibility.

In the last study of this model, graft-host interactions were demonstrated in the DBA/2 and Balb/C strains, isogenic at the H-2 histocompatibility locus. Simonsen (7), reviewing the subject, expected that preimmunization would be required to reveal donor cell activity in such closely related strains. The lag in the appearance of liver infiltrates when DBA/2 spleen cells were injected into the Balb/C host, as compared with the reciprocal arrangement, parallels the degree of facility of reciprocal tolerance induction in adult animals of these strains. In our laboratories (22) tolerance to Balb/C skin grafts has been achieved in adult DBA/2 animals by a single intravenous injection of only 100 million Balb/C adult spleen cells. However, multiple intravenous injections of DBA/2 cells, or a single injection of DBA/2 cells following sublethal x-irradiation, were needed to achieve tolerance in Balb/C recipients.

CONCLUSIONS AND SUMMARY

1. A new assay of graft-host interactions has been presented. It consists of the early finding of liver infiltrates in responsive and unresponsive homologous

recipients 1 to 3 days after the intravenous injection of competent lymphoid tissue in the mouse.

2. Evidence is presented that infiltrates are of donor origin, and are influenced by a homologous antigenic environment. They can be quantitatively increased by preimmunization of donor to recipient and decreased by preimmunization of recipient to donor. Furthermore, they are present in lethally x-irradiated or neonatally thymectomized unresponsive recipients after intravenous injection of competent homologous spleen cells.

3. Splenomegaly, which also occurs in this early period, is a product of both donor and host-specific immune reactivity together with a small non-specific component of host reactivity.

4. A comparison of the responsiveness of lymphoid tissue from different strains to the same transplantation antigens is presented, together with the observation of differences in the reactivity of reciprocal donor-recipient injection combinations to each other.

5. Graft-host interactions with this model can be demonstrated in donor-host strain combinations isogenic at the H-2 locus without preimmunization of the donor. Differences in reactivity of reciprocal donor-recipient combinations of lymphoid tissue injections to each other can be detected in this group as well as in the groups differing at the strong H-2 locus.

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EXPLANATION OF PLATES

PLATE 62

FIG. 1. Periportal mononuclear cell infiltrate in the liver of an A strain recipient mouse 2 days after the intravenous injection of normal B_1 strain spleen cells.

(*a*). Note, in the lower left corner of the figure, the characteristic location of the lesion, ballooning to one side of a portal venous radicle. × 90.

(*b*). A higher magnification of (*a*). Note the completely extravascular location of the infiltrate which protrudes into the parenchyma. × 400.

(*c*). A higher magnification of (*b*). Note the absence of polymorphonuclear cells, plasma cells, and erythroid or myelopoietic cells, and the apparent heterogeneity of nuclear and cytoplasmic morphology. Several cells, however, are readily identifiable as lymphocytes. × 900.

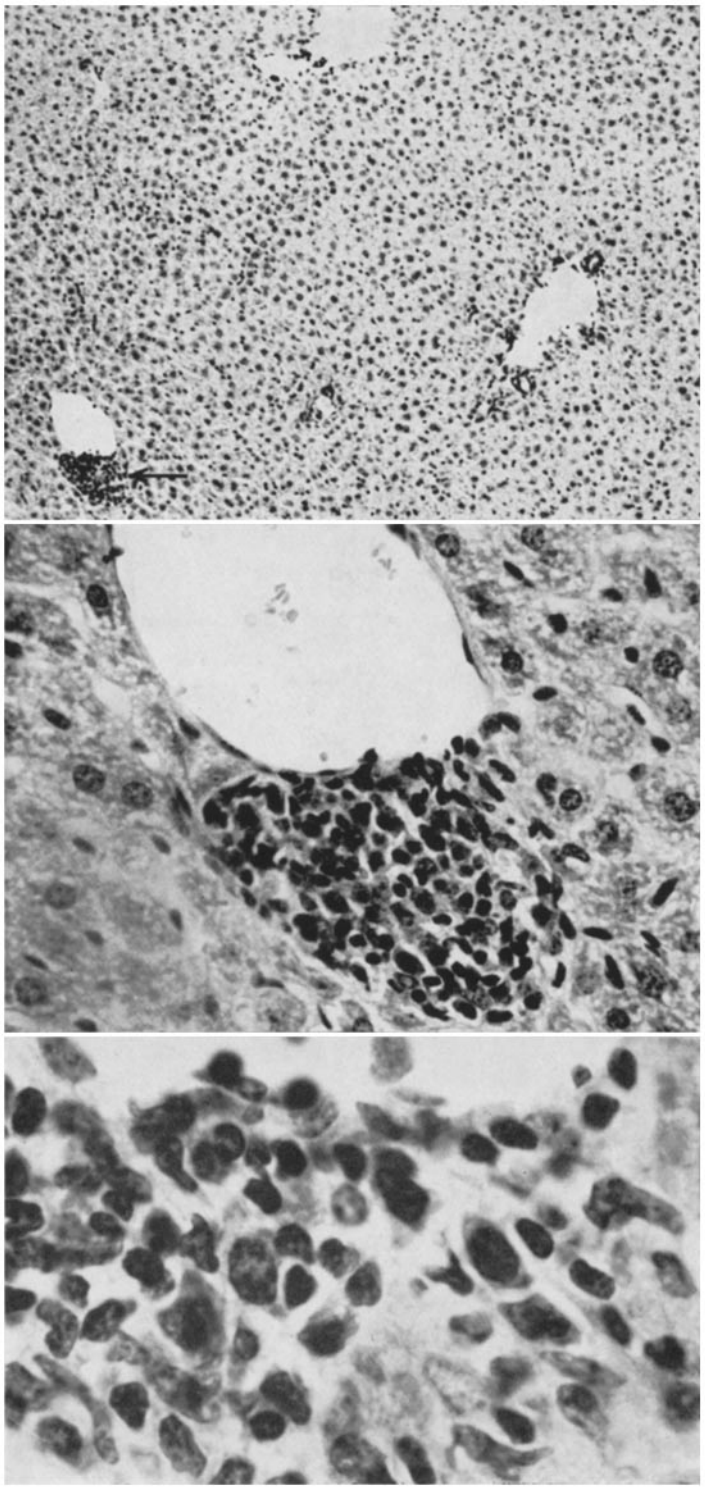

FIG. 1

(Miller *et al.*: An assay of graft-host interactions)

PLATE 63

FIG. 2. Periportal mononuclear cell infiltrates in the liver of an A strain recipient mouse 2 days after the intravenous injection of immunized (see text) B_1 strain spleen cells.

(*a*). Note the increase in frequency of the lesions over that produced by cells from unimmunized donors, and coalescence of some of the infiltrates. × 90.

(*b*). A higher magnification of the lesion in the lower center of (*a*). Note the complete encirclement of smaller vascular and biliary radicles, with greater infiltration into the parenchyma. × 400.

(*c*). A higher magnification of (*b*). Again, as in Fig. 1, note the lack of polymorphs, plasma cells, and erythroid or myelopoietic cells, and the heterogeneous nuclear and cytoplasmic morphology. Here also, several cells are readily identifiable as lymphocytes. × 900.

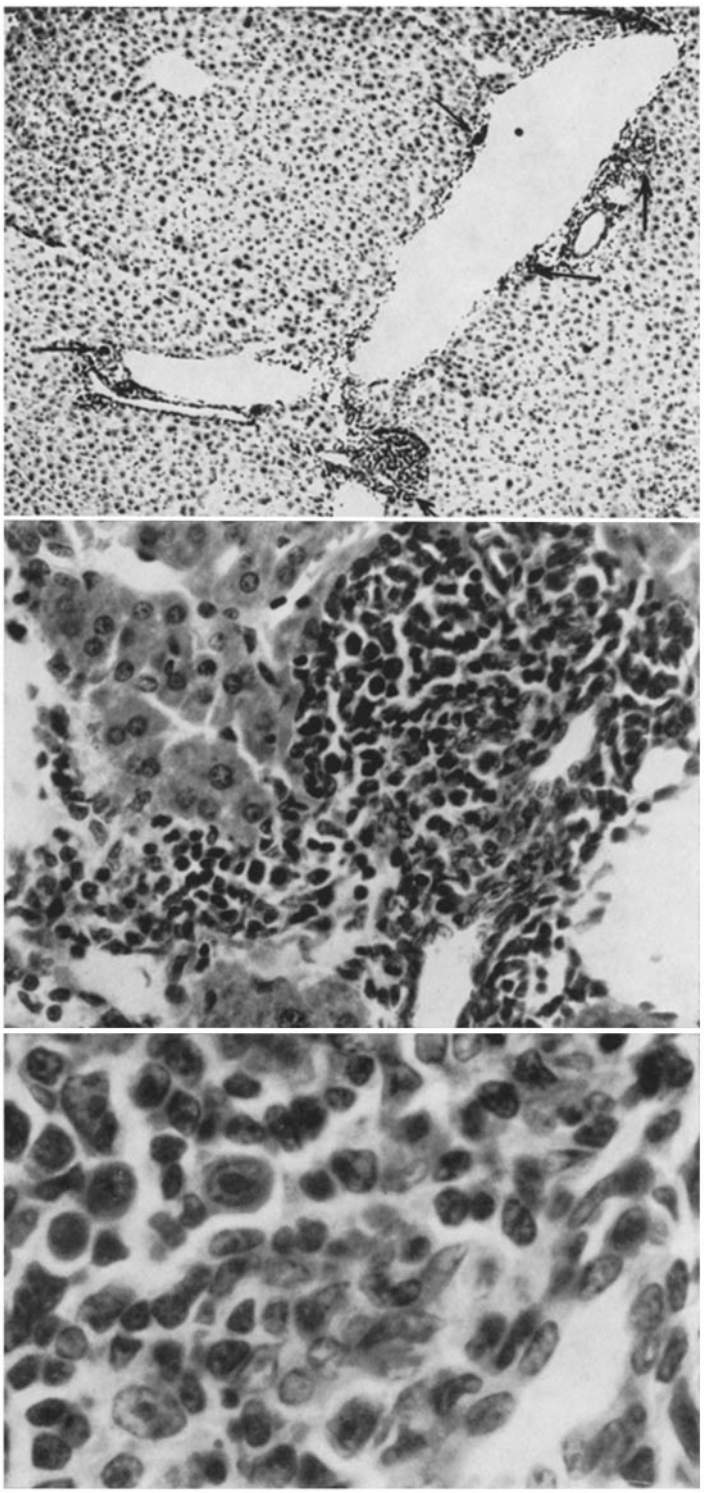

FIG. 2

(Miller *et al.*: An assay of graft-host interactions)