GRAFT-VERSUS-HOST REACTION ACROSS DIFFERENT REGIONS OF THE *H-2* COMPLEX OF THE MOUSE*

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(Received for publication 2 January 1973)

The *H-2* complex of the mouse occupies a segment of chromosome no. 17, approximately 15 map units distant from the centromere (1). The segment is about 0.5 map units long (2). Operationally, the *H-2* complex can be divided into five or six regions (Fig. 1). These are (starting from the centromeric end): *K*, *Ir*, *Ss-Slp*, *X*, *D* (and *Tl*).

The *K* region consists of either a single gene (*H-2K*) or a gene cluster. If the latter is true, the cluster must be rather tight, since no crossover has yet been discovered inside this region. The products of the *H-2K* gene(s) are detectable by serological and transplantation methods as membrane alloantigens present on practically all types of cells of the body.

The *Ir* region is known to consist of more than one gene, most likely of a great number of genes. The products of these genes have not been identified yet, but the genes are known to control immune response to a wide variety of thymus-dependent antigens (3, 4). One very attractive possibility is that the *Ir* gene products are actually cell surface receptors for the different antigens (3). This hypothesis requires the *Ir* genes to be expressed only in one particular cell type (antigen-binding cells, most likely T lymphocytes) and, moreover, to be expressed clonally (each clone of T lymphocytes expressing only a few, perhaps only one, of the *Ir* genes, and different clones expressing different genes). If one assumes that all the intra-*H-2* recombinants detected so far are inter- rather than intracistronic (not an unreasonable assumption, considering the level of genetic analysis so far attained in the mouse), one can regard the following *H-2*-associated immune responses as controlled by separate *Ir* genes (Fig. 1): immune response to synthetic polypeptide poly-L(Tyr, Glu)-poly-D, L-Ala-poly-L-Lys (gene *Ir-1*, see reference 5), immune response to immunoglobulins of class A (gene *Ir-IgA*, see reference 6), and immune response to immunoglobulins of class G (gene *Ir-IgG*, reference 7).

The *Ss-Slp* region probably consists of at least two genes, *Ss* and *Slp*. The *Ss* gene controls a serum substance that is found in a high concentration (*Ss^h*) in some strains and in a low concentration (*Ss^l*) in other strains (8). The *Ss* antigen is detected by xenoantibody (rabbit antimouse). The *Slp* gene controls a sex-limited protein that is present in the serum of males of some inbred strains (9). The *Slp* antigen is detected by alloantibody. The *Ss* and *Slp* traits have so far not been separated by genetic recombination. The *Ss* region is seemingly functionally unrelated to the rest of the *H-2* complex. This, however, could be merely a reflection of the fact that we know nothing

* Supported by U.S. Public Health Service Research Grants GM 15419 and DE 02731.

about the function of the *Ss* and *Slp* proteins. As a matter of fact, very recently Hinzová et al. (10) reported that a gene residing probably in the *Ss* region controls a quantitative difference in the activity of hemolytic complement in the sera of normal mice. If this report is confirmed and the *Ss* region is indeed shown to be involved in the control of complement activity, then this region will no longer seem so unrelated to the rest of the *H-2* complex. After all, complement is very intimately involved with cell membranes, and one type or another of involvement with cell membranes is characteristic of the whole *H-2* complex.

The *X* region (*X* for unknown) occupies a chromosomal segment between the *Ss-Slp* and *D* regions. Of the 34 mapped intra-*H-2* recombinants, 21 are recombinants in the *X* region. It is thus very likely that the region consists of a considerable number of genes. However, none of these genes has so far been identified. The function of the *X* region and its relationship to the rest of the *H-2* complex is not known. The *D* region consists of a gene (*H-2D*) or gene cluster coding for serologically detectable and transplantation antigens similar to those controlled by the *H-2K* region.

FIG. 1. Genetic map of the *H-2* complex. Vertical bars indicate cases of observed recombinations inside the complex. Interrupted vertical bars indicate that the position of the crossover event is not certain.

The *Tl* region has thus far not been considered to be a part of the *H-2* complex. However, recent developments have shown that it should be at least taken into account in most of the *H-2* studies. The region is marked by the presence of the *Tla* gene, which is about one map unit away from the *H-2D* gene (11). The *Tla* gene codes for membrane alloantigens that can be detected serologically on the thymocytes and certain leukemias, but not on other cell types. However, it has been recently shown (12) that a difference in the *Tl* region can lead to skin graft rejection. The rejection is rather peculiar, in the sense that some grafts are destroyed relatively rapidly whereas others are not rejected at all. The rejections indicate either that the *Tla* gene is expressed on skin cells in a form which is not detectable serologically, or that there are histocompatibility genes closely linked to *Tla* in the *Tl* region.

The interpretation of the *H-2* system as outlined above is relatively new (13, 14) and different from the traditional interpretation, which envisioned the *H-2* as a series of histocompatibility loci. The new interpretation raises a number of questions about the involvement of the different *H-2* regions in the different phenomena in which the *H-2* complex has been shown to play a role. In the present communication we have attempted to evaluate the role of the different *H-2* regions in graft-vs.-host reaction (GVHR).¹

¹ Abbreviations used in this paper: GVHR, graft-vs.-host reaction; MLR, mixed lymphocyte reaction; MSI, mean spleen index.

Materials and Methods

Theoretical Basis of the Experiment.—The testing of the different *H-2* regions is made possible by the availability of *H-2* recombinant strains. An *H-2* recombinant is defined as an animal that received the *H-2K* gene from one chromosome and the *H-2D* gene from another chromosome of the *H-2* heterozygous parent. The crossover event can be mapped inside the *H-2* complex by determining the *Ss* and/or *Slp* genotype of the recombinant (provided that the parental chromosomes differed in their *Ss* and/or *Slp* alleles). The *Ss-Slp* determination places the crossover event either to the left (between *H-2K* and *Ss*) or to the right of *Ss* (between *Ss* and *H-2D*). In those cases in which the crossing-over occurred to the left of *Ss*, the crossover position can be determined more precisely by testing for the alleles of the *Ir-1* gene and the crossover placed either to the left (between *Ir-1* and *H-2K*) or to the right of the *Ir-1* gene (between *Ir-1* and *Ss-Slp*). No markers are so far available for more precise localization of the crossovers that occurred in the *X* region (between *Ss* and *H-2D*).

The parental and the recombinant strains are different in some *H-2* regions and identical in others. By using recombinants in which the crossing-over took place at different positions inside the *H-2* complex, in combination with each other and with the parent strains, a situation can be created in which the reaction between graft and host is directed against only a portion of the *H-2* complex (one, two, or more regions of the complex). This is shown diagrammatically in Fig. 2. In this particular instance, B10.BR(*H-2^k*) spleen cells injected into

FIG. 2. Principle of the test for measuring the contribution of individual *H-2* regions to GVHR. Explanation in text.

newborn B10.D2(*H-2^d*) recipient mice (Fig. 2 *a*) encounter difference in four regions of the *H-2* complex. However, B10.A(*H-2^a*) cells injected into the same recipient encounter difference in only two *H-2* regions, *K* and *Ir* (Fig. 2 *b*); regions *Ss-Slp* and *D* are shared by *H-2^a* and *H-2^d* chromosomes. (It is assumed that the *H-2^a* chromosome is an *H-2* recombinant that received its *D* end from the *H-2^d* or *H-2^d*-like chromosome.) Therefore, in combination B10.A (graft) → B10.D2 (host), any observed GVHR can be attributed to the *K* and *Ir* regions.

The combinations used in the present paper are shown in Figs. 3–7. In each combination, the graft can react only against those regions of the host marked by solid or shadowed rectangles; regions marked by open rectangles are shared by the donor of the graft and the host. In these diagrams, as well as in all later considerations of the *H-2* complex structure, the *X* and the *Tl* regions are left out. This is done because there is no known marker available for the *X* region and the region thus remains purely hypothetical, and because the *Tl* region, although apparently involved in skin graft rejection, has no effect on GVHR (J. Klein, unpublished results).

Mice.—The employed *H-2* recombinant strains and their genotypes are listed in Table I. Two of the eight strains (B10.A and B10.AKM) are “natural” *H-2* recombinants, i.e. the recombinational event occurred a long time ago, in the “prehistory” of these strains, and is therefore undocumented. The remaining six *H-2* recombinants were obtained intentionally in appropriate crosses. In addition to the recombinant strains, the following congenic resistant lines were also used: C57BL/10Sn(= B10; *H-2^b*), B10.D2(*H-2^d*), B10.BR(*H-2^k*),

and B10.G(*H-2^g*). All strains except one had the same genetic background, that of strain B10, from which they all differ (at least theoretically) by the *H-2* complex only (= congenic resistant strains). The one exception was strain B10.AQR, which was only partially congenic with B10. However, the B10.AQR animals used in this experiment were from a line that had undergone a total of four backcrossings to strain B10 and thus carried a considerable portion of the B10 genome. All mice were bred in our colony at The University of Michigan.

Assay for GVHR.—The GVHR was tested by the Simonsen spleen weight assay (15). Newborn mice, less than 24 h old, were injected intraperitoneally with 5×10^7 spleen cells suspended in Hanks' solution. The spleen cells were obtained from adult donors by pressing spleen fragments through a 50-mesh stainless steel screen. The cells were washed twice in cold Hanks' solution, and their viability was assessed by trypan blue staining.

Each litter of newborn mice was divided into two groups, experimental and control. The experimental mice were injected with the spleen cells; the control mice were injected with Hanks' solution. Both groups were sacrificed 10 days after injection, and body weight and spleen weight of the individual mice were recorded. Spleen indexes were calculated according to Simonsen and Jensen (15). Mean spleen indexes (MSI) were obtained in each combination by averaging the spleen indexes of several litters. In almost all combinations the mean spleen index was based on no fewer than 20 experimental animals.

RESULTS

The results are divided into five sections (Figs. 3–7). In each section (except section no. 5), the GVHR is directed against different regions of the same *H-2* chromosome. Thus in section no. 1, the GVHR is directed against regions of chromosome *H-2^b*; in section no. 2, against regions of chromosome *H-2^d*, etc.

FIG. 3. GVHR across *H-2^b* differences. Number of animals: control/experimental.

FIG. 4. GVHR across *H-2^d* differences. Number of animals: control/experimental.

FIG. 5. GVHR across H-2^k differences. Number of animals: control/experimental. FIG. 6. GVHR across H-2^q differences. Number of animals: control/experimental.

FIG. 7. GVHR across combined H-2 differences. Number of animals: control/experimental.

This, plus the fact that all the strains except one have the same genetic background, provides the highly uniform experimental conditions necessary in this type of experiment. Only in section no. 5 (Fig. 7) is the GVHR directed against regions of H-2 chromosomes of different origin. Pooled data from the different sections, arranged according to the tested H-2 region differences, are shown in Table I.

TABLE I
H-2 Recombinant Strains Used*

Strain		H-2 chromosome	Origin of H-2 regions				Origin of I ⁱ region	Reference
Full symbol	Abbreviation		K	I _r	S _s	D		
B10·A	—	a	K	K	D	D	A	16
B10·HTG	—	g-Go	D	D	D	B	B	17
B10·A(2R)	2R	h-2Sg	K	K	D	B	B	18
B10·A(4R)	4R	h-3Sg	K	K D	D	B	B	18
B10·A(5R)	5R	i-2Sg	B	B	D	D	A	18
B10·AKM	—	m	K	K	K	Q	Q	19
B10·AQR	AQR	y-Klj	Q	K	D	D	A	19
B10·T(6R)	6R	y-Sg	Q	Q	Q	D	A	20

* Vertical bar indicates position of genetic exchange.

D and K Region Differences.—In 6 of the 35 strain combinations tested, the GVHR was directed either against the *D* region alone (four combinations) or against the *K* region alone (two combinations). In all six combinations, the spleen enlargement was either insignificant (range of MSI between 1.19 and 1.27) or only marginal (MSI between 1.47 and 1.66). Since the six strain combinations involved four different *H-2* chromosomes (*b*, *d*, *k*, and *q*), it was concluded that the lack of strong GVHR across the *D* or *K* regions was not limited to one particular *H-2D* or *H-2K* allele, but was characteristic for these types of differences. There was no difference between the GVHR reactivity of the *D* and *K* regions; the *K* region reactivity was as low as the *D* region reactivity. In addition to the six combinations testing single *H-2D* or *H-2K* differences, two combinations tested double *H-2D* + *H-2K* difference (with identity at the middle portion of the *H-2* complex). In one of these two combinations (AQR → 2R), no GVHR was observed (MSI = 1.13). This combination involved alleles at the *H-2D* and *H-2K* loci that singly also failed to stimulate GVHR (alleles *H-2D^a* and *H-2K^k*). In the second combination (2R → AQR), strong GVHR was observed (MSI = 2.55). The alleles against which the reaction was directed were the same as those that singly also stimulated significant GVHR (alleles *H-2D^d* and *H-2K^q*). However, the effect of the combined difference was stronger than the effect of each difference alone or than a simple arithmetic summation of the two differences taken separately (MSI of 2.55 as compared with MSIs of 1.47 + 1.63).

Ss-Slp Differences.—With the set of strains employed in the present experiment it was not possible to obtain a combination that differed in the *Ss-Slp* region only. However, some information about the effect of this region on GVHR was obtained from combinations in which the *Ss-Slp* difference was added to other differences, namely, *D* and *D* + *K*. Addition of the *Ss-Slp* difference to the *D* difference led to a slight increase of MSI in one combination (B10.A → B10.BR; MSI = 1.65 for the *D^k* + *Ss-Slp^k* difference, as compared

with $MSI = 1.19$ of the D^k difference alone) and no increase in another combination ($B10.AKM \rightarrow B10.A$; $MSI = 1.34$ for the $D^d + Ss-Slp^d$ difference, as compared with $MSI = 1.47$ of the D^d difference alone). Addition of $Ss-Slp$ difference to $D + K$ differences did not result in any increase of MSI in combination $B10.AKM \rightarrow AQR$ ($MSI = 2.41$ for the $K^a + D Ss-Slp^d + D^d$ difference, as compared with $MSI = 2.55$ for the $K^a + D^d$ difference). In another combination, namely, $AQR \rightarrow B10.BR$, the GVHR was also directed against the $D + Ss-Slp + K$ difference; and the MSI was 2.21. However, in this case it was not clear whether the addition of the $Ss-Slp$ difference caused any increase of the MSI , since the $K^k + D^k$ difference without the $Ss-Slp$ difference was not tested. (K^k alone and D^k alone did not cause any appreciable splenomegaly in the appropriate combinations.) Combinations identical in the $Ss-Slp$ region but differing in the rest of the $H-2$ complex ($2R \rightarrow B10.D2$ or $B10.HTG \rightarrow B10.A$) gave, paradoxically, a stronger GVHR than similar combinations differing in the whole $H-2$ complex ($B10.BR \rightarrow B10.D2$ or $B10 \rightarrow B10.A$, see Figs. 3 and 6). All of this suggests that the effect of the $Ss-Slp$ region on GVHR is minimal, and in many combinations, probably nonexistent.

Ir Differences.—The presently available $H-2$ recombinants cannot be arranged in a combination that would dissect out the Ir difference from the rest of the $H-2$ complex. The closest one can get to such a goal are combinations that differ in $Ir + Ss-Slp$ or in $Ir + K$. The former combinations are particularly informative. These combinations are not associated with any serological difference detectable by conventional methods (21 and J. Klein, manuscript in preparation). They are probably also not capable of causing skin graft rejection. Yet they are associated with significant GVHR as measured by the splenomegaly assay. This is demonstrated by combinations $4R \rightarrow 2R$ ($MSI = 1.77$) and $6R \rightarrow AQR$ ($MSI = 2.57$). The reciprocal combinations behave as follows. Combination $2R \rightarrow 4R$ shows no GVHR ($MSI = 1.15$) under the experimental conditions described above, whereas the $AQR \rightarrow 6R$ combination shows a strong GVHR (21). In the $4R$ strain the crossover occurred inside the Ir region (7). Consequently, the $2R \rightarrow 4R$ combinations test only a portion of the Ir region (which is indicated in Table II by the $\frac{1}{2}$ symbol and in Figs. 2, 4, and 6 by splitting the Ir region into two halves; both these symbols are not meant to indicate that the crossover in the Ir region occurred precisely in the middle of that region). Since the $Ss-Slp^d$ and $Ss-Slp^k$ regions involved in these combinations contribute very little to the GVHR (see above), the observed splenomegaly is most likely caused primarily by the Ir differences.

Addition of the Ir difference to the single K differences enhances GVHR considerably. For instance, the K^k difference alone causes no GVHR ($MSI = 1.19$), but the same difference combined with Ir^k difference in combinations $B10.D2 \rightarrow B10.A$ or $B10.HTG \rightarrow 2R$ causes very strong GVHR ($MSIs$ of 3.08 and 2.86, respectively). For the other $Ir + K$ differences, the MSI ranges between 2.63 and 2.94, which is the level of GVHR observed against the whole $H-2$ complex. K differences combined with only a portion of the Ir region

TABLE II
Splenomegaly Across Different Regions of the H-2 Complex

Strain combination	H-2 region difference	Mean spleen index $\pm$ SD
B10·A $\rightarrow$ AQR	K^a	1.63 ± 0.15
AQR $\rightarrow$ B10·A	K^k	1.19 ± 0.09
6R $\rightarrow$ B10·G	D^a	1.66 ± 0.21
B10·AKM $\rightarrow$ B10·BR	D^k	1.19 ± 0.14
B10·A $\rightarrow$ 2R	D^b	1.27 ± 0.16
2R $\rightarrow$ B10·A	D^d	1.47 ± 0.15
AQR $\rightarrow$ 2R	$K^k + D^b$	1.13 ± 0.10
2R $\rightarrow$ AQR	$K^a + D^d$	2.55 ± 0.32
B10 $\rightarrow$ 4R	$K^k + \frac{1}{2} Ir^k$	2.29 ± 0.24
4R $\rightarrow$ B10	$K^b + \frac{1}{2} Ir^b$	2.84 ± 0.23
B10·D2 $\rightarrow$ B10·A	$K^k + Ir^k$	3.08 ± 0.27
B10·HTG $\rightarrow$ 2R	$K^k + Ir^k$	2.86 ± 0.26
AQR $\rightarrow$ 5R	$K^b + Ir^b$	2.63 ± 0.22
B10·A $\rightarrow$ B10·D2	$K^d + Ir^d$	2.94 ± 0.16
2R $\rightarrow$ B10·HTG	$K^d + Ir^d$	2.76 ± 0.20
B10·A $\rightarrow$ B10·BR	$D^k + Ss-Slp^k$	1.65 ± 0.14
B10·AKM $\rightarrow$ B10·A	$D^d + Ss-Slp^d$	1.34 ± 0.19
2R $\rightarrow$ 4R	$\frac{1}{2} Ir^b + Ss-Slp^b$	1.15 ± 0.09
4R $\rightarrow$ 2R	$\frac{1}{2} Ir^k + Ss-Slp^d$	1.77 ± 0.24
6R $\rightarrow$ AQR	$Ir^k + Ss-Slp^d$	2.57 ± 0.26
B10·A $\rightarrow$ 4R	$\frac{1}{2} Ir^b + Ss-Slp^b + D^b$	1.55 ± 0.20
B10·A $\rightarrow$ 6R	$\left. \begin{array}{l} K^a + Ir^a + Ss-Slp^a \\ Ir^a + Ss-Slp^a + D^a \\ Ir^k + Ss-Slp^d + D^d \\ K^d + Ir^d + D^d \end{array} \right\} \text{with } Ir$	2.27 ± 0.19
AQR $\rightarrow$ B10·G		2.40 ± 0.14
B10·G $\rightarrow$ AQR		2.53 ± 0.23
2R $\rightarrow$ B10·D2		3.11 ± 0.16
B10·HTG $\rightarrow$ B10·A	$\left. \begin{array}{l} K^k + Ir^k + D^d \\ K^a + \frac{1}{2} Ir^k + Ss-Slp^d + D^d \\ K^k + \frac{1}{2} Ir^b + Ss-Slp^b + D^b \\ K^k + Ss-Slp^k + D^k \end{array} \right\} \text{with } \frac{1}{2} Ir$	3.37 ± 0.31
4R $\rightarrow$ AQR		2.63 ± 0.33
AQR $\rightarrow$ 4R		2.54 ± 0.28
AQR $\rightarrow$ B10·BR		2.21 ± 0.21
B10·AKM $\rightarrow$ AQR	$K^a + Ss-Slp^d + D^d$ without Ir	2.41 ± 0.30
B10 $\rightarrow$ B10·G	$K^a + Ir^a + Ss-Slp^a + D^a$	2.87 ± 0.24
B10·A $\rightarrow$ B10	$K^b + Ir^b + Ss-Slp^b + D^b$	2.84 ± 0.21
B10·D2 $\rightarrow$ B10·BR	$K^k + Ir^k + Ss-Slp^k + D^k$	3.03 ± 0.22
B10·BR $\rightarrow$ B10·D2	$K^d + Ir^d + Ss-Slp^d + D^d$	2.41 ± 0.23
B10 $\rightarrow$ B10·A	$K^k + Ir^k + Ss-Slp^d + D^d$	3.06 ± 0.29

(combinations $B10 \rightarrow 4R$ and $4R \rightarrow B10$) also cause strong GVHR (MSI of 2.29 and 2.84, respectively). In agreement with this, a combination that is identical in the *Ir* region but different in the rest of the *H-2* complex ($AQR \rightarrow B10.BR$) gives a significantly lower GVHR (MSI = 2.21) than a similar combination that involves a difference in the whole *H-2* complex ($B10.D2 \rightarrow B10.BR$; MSI = 3.03; see Fig. 4). In other words, omission of the *Ir* region from the *H-2* complex causes a significant decrease in the GVH reactivity of the corresponding combinations. We therefore conclude that the *Ir* region contributes considerably to the induction of GVHR.

DISCUSSION

The first indication that different regions of the *H-2* complex might not be equally important in GVHR was obtained unknowingly by Eichwald and co-workers (22) and by Lengerová and Viklický (23). These authors tested the severity of GVHR in combinations that were supposedly monoantigenic, i.e., the graft was supposed to react against a single H-2 antigen. They noticed that some H-2 antigens caused more severe GVHR than others. However, they failed to notice that the strong antigens were those controlled by the *K* region and the weak antigens were those controlled by the *D* region of the *H-2* complex.

In 1970, Rychlíková et al. (24) made the important observation that only the *K* end, and not the *D* end, H-2 antigens were able to stimulate in a mixed lymphocyte reaction (MLR). In the same year, Démant (25) reported that the same applies to GVHR. Here again, only the *K* end, not the *D* end, difference caused a measurable GVHR. It was inferred from these data that only the *K* end differences were important for both MLR and GVHR, and that the *D* end differences played an insignificant role, or no role, in these two types of reaction. However, in all the strain combinations used in these tests by the Prague group, the *H-2K* differences always involved differences in the *Ir* region as well. The question thus arose whether the unusual strengths of the H-2K antigens might not actually be due to their close association with the *Ir* region. This possibility was tested in the MLR assay by Bach and co-workers (26, 27), and the tests showed quite clearly that the *H-2K* locus alone was no stronger than the *H-2D* locus. It was the combination *H-2K* + *Ir* that was much stronger than the *H-2D* locus. We now show, in this paper, that the same is also true for the GVHR.

The experiments reported here allow us to reach the following conclusions. First, differences in the *D* region alone cause no splenomegaly in some strain combinations (e.g., $B10.AKM \rightarrow B10.BR$) and marginal but definite splenomegaly in other combinations (e.g., $6R \rightarrow B10.G$). Secondly, differences in the *K* region alone have the same effect as differences in the *D* region. They also cause either no ($AQR \rightarrow B10.A$) or only a marginal ($B10.A \rightarrow AQR$) splenomegaly. Thirdly, differences in the *Ss-Slp* region have only a minor or no effect on the degree of splenomegaly. Fourthly, differences in the *Ir* (+ *Ss-Slp*)

region can lead to moderate or strong GVHR. This latter reaction is usually as strong as the reaction caused by a difference in the whole *H-2* complex. Fifthly, differences in two or more *H-2* regions usually cause greater splenomegaly than differences in each of the regions alone.

Thus, as in the case of MLR, the *K* end-*D* end asymmetry in the strength of GVHR observed by Eichwald et al. (22), Lengerová and Viklický (23), and Démant (24) does not mean that the antigens controlled by the *H-2K* locus are stronger than the antigens controlled by the *H-2D* locus. The asymmetry is explained by the fact that on the linkage map the *Ir* loci are located closer to the *H-2K* locus than to the *H-2D* locus. All the *K* end differences in the experiments of the above-mentioned authors were actually *K + Ir* differences, and the greater strength of the GVHR reaction was apparently caused by the presence of *Ir* differences. As soon as the *H-2K* locus is deprived of its *Ir* neighbors, it becomes relatively weak, no stronger than the *H-2D* locus.

The GVHR results reported here are amazingly similar to those obtained by Bach and co-workers (26-28) with the MLR test. As a matter of fact, in almost all combinations there is a direct parallel between the results of the two tests, indicating that the GVHR and MLR apparently measure the same differences.

If one accepts the hypothesis that the presently known serologically detectable antigens of the *H-2* complex are controlled by the *K* and *D* regions but not by the middle portion of the complex, then one has to postulate genes for GVHR distinct from the *H-2K* and *H-2D* genes. We shall call these the *G* genes. (This symbol is introduced for purely practical reasons, namely, to avoid lengthy expressions such as "genes whose products are responsible for GVHR reaction." We do not feel that it is necessary at this point to have an official symbol for these genes.) We explain the results reported in the present paper by the following hypothesis.

There is more than one *G* gene, most likely a large number of them. The genes are distributed all over the *H-2* complex, but are most dense in the *Ir* region. They are expressed in cells responsible for the GVHR; these are the thymus-derived (T) lymphocytes (29). Since in at least some instances skin grafts survive across a *G* gene difference (21), it is assumed that the *G* genes are not expressed in skin cells. Furthermore, since conventional serological methods fail to detect *G* gene differences even in spleen cells (21, and J. Klein, manuscript in preparation), it is also assumed that the *G* genes are expressed clonally, i.e., each clone of T cells expresses only a limited number of the *G* genes, perhaps only one. The population of T cells thus consists of a number of different clones, each clone expressing different *G* genes. For this reason, it is difficult to raise antisera against the *G* gene products and to detect serologically the small fraction of cells expressing a given gene. We postulate that the products of the *G* genes are present in the cell membrane and probably function as antigen recognition sites (T cell receptors). Further, these receptors are postulated to be identical with receptors involved in MLR and receptors responsible for the differences in the

immune response to different thymus-dependent antigens (3). On the basis of this hypothesis, the GVH reactivity of the *K* and *D* regions alone can be explained either by postulating some *G* genes closely linked with *H-2K* and *H-2D* loci, or by the assumption that antigens controlled by the *H-2K* and *H-2D* loci can also cause GVHR (and MLR).

SUMMARY

H-2 crossovers and their parental strains were arranged into 35 combinations in which the adult donor of spleen cells differed from the newborn recipient in the whole *H-2* complex, or in three, two, or one region of the complex. A Simonsen splenomegaly assay was then used to test the contribution of the individual *H-2* regions to the graft-versus-host reaction (GVHR).

It was shown that the strongest GVHR was associated with the *Ir* region. Differences in the *Ir* region caused significant splenomegaly in spite of the fact that no antigens detectable by conventional serological methods have thus far been associated with this region. Differences in the *K* and *D* regions showed only a borderline effect on GVHR in spite of the fact that these regions code for most, if not all, of the antigens detectable by conventional serological and transplantation methods. The *K* region alone caused no stronger GVHR than the *D* region alone; however, *K* + *Ir* region differences led to much stronger GVHR than *D* region differences. The *Ss-Slp* region also showed only a borderline effect on GVHR. Differences in two or more *H-2* regions usually caused greater splenomegaly than differences in each of the regions separately.

On the basis of these findings it is concluded that the strongest GVHR is caused by genes distinct from the known histocompatibility genes of the *H-2* complex. It is speculated that the GVHR genes are identical with the mixed lymphocyte reaction (MLR) and *Ir* genes and that the product of these genes are receptors on the surface of the thymus-derived lymphocytes (T cells).

We thank Miss Cristina Perou and Mr. Steven Gerson for technical assistance.

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