GRAFT-VS.-HOST-ASSOCIATED IMMUNE SUPPRESSION
IS ACTIVATED BY RECOGNITION OF
ALLOGENEIC MURINE I-A ANTIGENS

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The injection of immunologically competent adult F_1 hybrid mice with parental T lymphocytes can result in severe reduction of abrogation of immune potential of the F_1, with respect to both T cell (1–3) and B cell (4) responses. This loss of immune function is known as graft-vs.-host (GVH)[1]-associated immune suppression (5). It occurs rapidly (within 5 d) after intravenous injection of a single lymphoid cell inoculum, is profound in its effects, can be long-lasting (2 mo or more after a single parental cell injection), is not antigen specific in its effects, is mediated by suppressor T lymphocytes (1, 5, 6), and can be prevented by counter-suppressor T lymphocytes that are activated by treatment of the F_1 with specific anti-H-2 antibodies before injection of parental T cells (7). GVH-associated immune suppression is probably under the control of a number of genetic factors, including some linked to the H-2 complex (5) and some that may not be H-2 linked. The potent suppressive signal involved in this phenomenon appears to be initiated by parental T cell recognition of H-2 antigens expressed by the other parental haplotype of the F_1. However, the minimal major histocompatibility complex genetic differences between the F_1 recipient and the parental lymphocyte donor that are required to activate this suppression are not known.

The present study has used congeneic inbred, intra-H-2 recombinant, and mutant strains of mice on the C57BL/10 and C57BL/6 genetic background to determine which H-2 antigens expressed by the F_1 need to be recognized by parental spleen cells in order to induce GVH-associated suppression. The findings are discussed with respect to the H-2 genetics of this type of suppression, as well as the possible analogy of this phenomenon to clinical examples of severe immune suppression.

Materials and Methods

Mice. The following mouse strains (with the strain abbreviations used in this report) were obtained from The Jackson Laboratory, Bar Harbor, ME: C57BL/6J (B6); C57BL/10Sn (B); B10.A/SgSn (A); B10.A(2R) (2R); B10.A(5R) (5R). The B10.A(4R) (4R) strain was obtained from the Animal Genetics and Production Branch, National Cancer Institute (NCI). The B6.C-$H-2^{bm12}$ (bm12) I-A mutant strain (8–10) was a gift from Dr. T. H. Hansen, Merck Sharpe & Dohme, Rahway, NJ. The following strains were a gift from the colony of Dr. David H. Sachs, Immunology Branch, NCI: B10.AKM (AKM); B10. MBR (MBR); A.TL; A.TH (see Table I

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[1] *Abbreviations used in this paper:* AIDS, acquired immune deficiency syndrome; CTL, cytotoxic T lymphocytes; GVH, graft vs. host; SCID, severe combined immunodeficiency disorders; TNP, trinitrophenyl.

for the H-2 alleles of these strains). The F_1 hybrids used in this study were bred in our own animal colony. All experimental mice were males, 8–13 wk of age.

Injection of F_1 Mice with Parental Spleen Cells. Cell suspensions were prepared as described elsewhere (5) and injected via the tail vein into F_1 mice. The F_1 mice were inoculated with 4–6 $\times$ 10^7 cells per recipient in a volume of 0.5 or 1.0 ml.

In Vitro Generation and Assay for Cell-mediated Lympholysis. The cell-mediated lympholysis potential of spleen cells from normal control and injected F_1 mice was tested 7–14 d after the single inoculum of parental spleen cells by in vitro sensitization against trinitrophenyl-modified syngeneic cells (TNP-self) or allogeneic cells, as previously described (11). The effectors generated 5 d later were tested for lytic activity in a 4-h ^{51}Cr-release assay on 48-h concanavalin A-stimulated spleen cell blasts, each of which was tested in triplicate samples at three or four effector:target cell ratios (11). The percent lysis was calculated as previously described (11) and standard errors of the mean have been omitted from the results presented for simplicity since they were consistently <10% of the mean values.

Results

General Pattern of GVH-associated Cytotoxic T Lymphocyte (CTL) Suppression. The results presented in this report illustrate data from individual experiments. However, it should be noted that each donor-recipient strain combination was run in at least three independent experiments, in which CTL potential was tested for responses to TNP-self and alloantigens at various times after parental cell inoculum. The results of such repetitive experiments were essentially identical. Similar results were obtained for the responses to TNP-self and alloantigens, although the level of suppression to alloantigens was often less dramatic than that to TNP-self. For each experiment, only the TNP-self (Figs. 1 and 2) or allogeneic (Fig. 3) CTL responses are shown to conserve space. In some experiments, spleen cells from nonparental, H-2-recombinant strains were injected (e.g., Fig. 1 A). Nevertheless, all donor-recipient combinations were selected such that (*a*) the F_1 recipients should not recognize the donor as allogeneic; and (*b*) the donor cells should recognize one or more H-2 gene products expressed by the recipient as allogeneic. It should also be noted that we have previously found that the F_1 mice that are heterozygous at H-2D are resistant to induction of CTL suppression with parental cells that express H-2D^b, but that such resistance can be overridden by injecting >2 $\times$ 10^7 parental spleen cells (5). To exclude any failure to detect suppression as being due to F_1 resistance to the parental inoculum, 4–6 $\times$ 10^7 spleen cells were inoculated in all experiments.

Mapping of GVH-associated Suppression within the H-2 Complex. The results of Fig. 1 summarize a series of six F_1 combinations in which suppression of CTL potential to TNP-self was studied after injection of parental spleen cells. (B $\times$ A)F_1 mice injected with A parental spleen cells were used as an example of parent into F_1 in which products of the entire H-2^b haplotype can be recognized as allogeneic by the A parent. As shown in Fig. 1 A, injection of 6 $\times$ 10^7 parental A, as well as 2R and 4R recombinant spleen cells, 7 d before CTL testing completely abrogated the ability of the F_1 (and that of any immunocompetent donor cells) to generate effector activity to TNP-self. These results are similar to previously published data (3, 5, 6) and are used here for comparative purposes and as an internal control for this study (see below). The results shown in Fig. 1 B, in which (2R $\times$ B)F_1 mice were injected with 6 $\times$ 10^7 2R or B spleen cells, indicate that recognition of F_1 K–S region H-2 products by parental lymphocytes was sufficient to induce suppression to TNP-self. The data of Figs. 1 C and D, using (5R $\times$ A)F_1 and (4R $\times$ B)F_1 injected with 4 $\times$ 10^7 parental
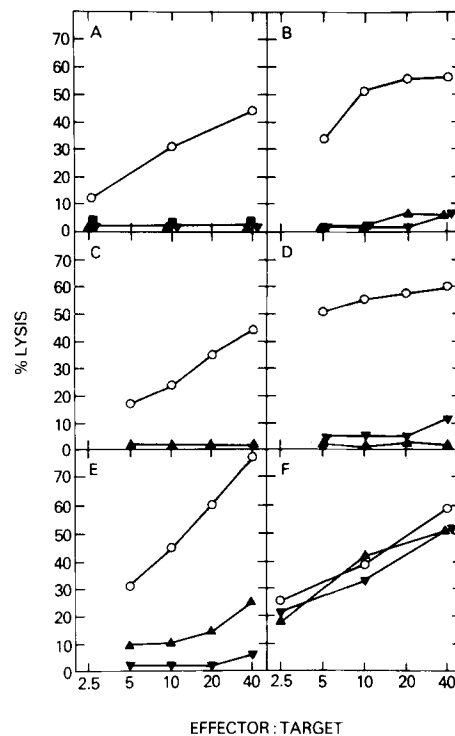
FIG. 1. In vitro CTL potential to TNP-self of spleen cells from F_1 mice. (A) (B × A)F_1 mice were untreated (○) or injected with 6 × 10^7 A (■), 2R (▲), or 4R (▼) spleen cells and tested 7 d later. (B) (2R × B)F_1 mice were untreated (○) or injected with 6 × 10^7 2R (▲) or B (▼) spleen cells and tested 7 d later. (C) (5R × A)F_1 mice were untreated (○) or injected with 4 × 10^7 5R (▲) spleen cells and tested 13 d later. (D) (4R × B)F_1 mice were untreated (○) or injected with 4 × 10^7 4R (▲) or B (▼) spleen cells and tested 7 d later. (E) (B × MBR)F_1 mice were untreated (○) or injected with 6 × 10^7 MBR (▲) or B (▼) spleen cells and tested 14 d later. (F) (4R × A)F_1 mice were untreated (○) or injected with 6 × 10^7 A (▲) or 4R (▼) spleen cells and tested 13 d later.

spleen cells, indicate that recognition of K-IB and K-IA products, respectively, is all that is required to induce suppression of CTL potential to TNP-self. Since all examples shown above (Figs. 1 A–D) have involved recognition of F_1 K and I region products by parental lymphocytes, the (B × MBR)F_1 and (4R × A)F_1 combinations were used to determine whether K and/or IA need to be recognized as a minimal requirement for the induction of CTL suppression. The results indicate that injection of 6 × 10^7 MBR or B parental spleen cells into (B × MBR)F_1 greatly reduced CTL potential to TNP-self (Fig. 1 E), whereas the injection of 6 × 10^7 A or 4R parental spleen cells into (4R × A)F_1 mice had no detectable effect on CTL potential (Fig. 1 F). This same preparation of A and 4R cells, which did not suppress (4R × A)F_1 mice, was shown to be functionally viable by the cells' ability to suppress the appropriate F_1 mice in which K and IA would be allogeneic to the donor inoculum (Fig. 1 A). Thus, these results are consistent with the recognition of F_1 I-A antigens by the parental lymphocytes being sufficient for the induction of CTL suppression.

Mapping GVH-associated Suppression to the I Region. To investigate this phenomenon further, F_1-parent combinations were chosen such that only F_1 K, D, or I region products would be allogeneic to the parental cells. (AKM × MBR)F_1 mice were

injected with 6×10^7 AKM or MBR parental spleen cells (which differ only at H-2K), and CTL potential was tested 13 d later (see Fig. 2). No reduction in CTL potential to TNP-self was observed when AKM cells were injected. Only a marginal reduction was detected when MBR cells were injected (Fig. 2A). In contrast, the same inoculum of MBR cells drastically reduced the CTL potential of (B × MBR)F$_1$ (compare Fig. 2A with Fig. 1E). Thus, recognition of only H-2K region products did not lead to CTL suppression. (2R × A)F$_1$ mice were injected with 6×10^7 A or 2R spleen cells to determine whether recognition of H-2D only would lead to suppression. The data of Fig. 2B indicate that no suppression was detected. The same preparation of 2R and A spleen cells was capable of inducing suppression when injected into (B × A)F$_1$ mice (see Fig. 1A). Thus, recognition of only H-2D region products does not result in suppression. To determine whether recognition of the I region without K would result in suppression, (A.TH × A.TL)F$_1$ mice were injected with 5×10^7 A.TL spleen cells, and the CTL potential to TNP-self was tested 13 d later. The results (Fig. 2C) illustrate that I region recognition results in abrogation of CTL potential.

Mapping of GVH-associated Suppression to I-A. Since recognition of F$_1$ I region determinants by parental lymphocytes is all that is required to induce CTL suppression, we have investigated whether recognition of only I-A determinants would induce suppression. This was done by taking advantage of the H-2^{bm12} loss-gain mutation that maps within I-A (8-10). (B6 × bm12)F$_1$ mice were injected with 5×10^7 B6 or bm12 spleen cells. The same preparations of B6 and bm12 cells were also injected into (B6 × C3H)F$_1$ and (A × bm12)F$_1$, respectively, as positive controls, to demonstrate that these cells were capable of suppressing F$_1$ mice in which an entire H-2 haplotype could be recognized. Thus, the B6 cells suppressed (B6 × C3H)F$_1$ (Fig. 3A), and the bm12 suppressed (A × bm12)F$_1$ (Fig. 3B) CTL allogeneic responses. Most interesting was the observation that B6 and bm12 spleen cells completely abrogated CTL potential of (B6 × bm12)F$_1$ spleen cells to B10.D2 (*H-2^d*) alloantigens (Fig. 3C). These results indicate that I-A antigens are all that need to be recognized by the inoculated lymphocytes in order to induce profound CTL suppression.

Discussion

The findings of the present study indicate that parental lymphocytes injected into F$_1$ mice need to recognize only I-A determinants as allogeneic to induce profound
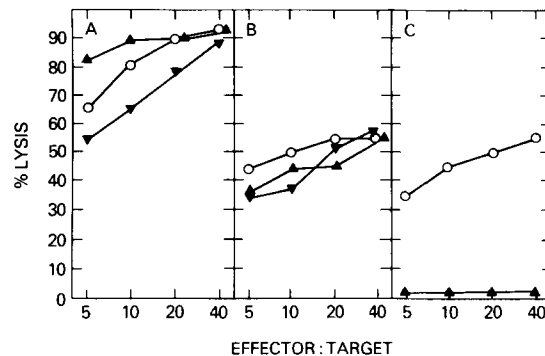

Fig. 2. In vitro CTL potential to TNP-self of spleen cells from F$_1$ mice. (A) (AKM × MBR)F$_1$ mice were untreated (○) or injected with 6×10^7 AKM (▲) or MBR (▼) spleen cells and tested 13 d later. (B) (2R × A)F$_1$ mice were untreated (○) or injected with 6×10^7 A (▲) or 2R (▼) spleen cells and tested 7 d later. (C) (A.TH × A.TL)F$_1$ mice were untreated (○) or injected with 5×10^7 A.TL (▲) spleen cells and tested 8 d later.
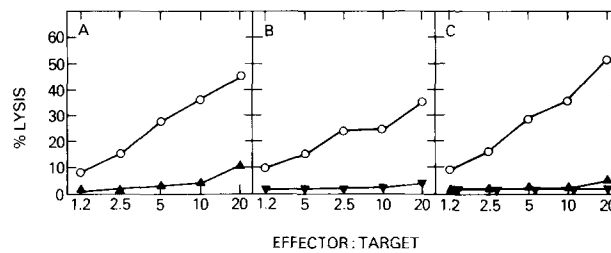
EFFECTOR : TARGET

FIG. 3.   In vitro CTL potential to B10.D2 alloantigens of spleen cells from F_1 mice. (A) (B6 × C3H)F_1 mice were untreated (○) or injected with 5 × 10^7 B6 (▲) spleen cells and tested 14 d later. (B) (A × bm12)F_1 mice were untreated (○) or injected with 5 × 10^7 bm12 (▼) spleen cells and tested 14 d later. (C) (B6 × bm12)F_1 mice were untreated (○) or injected with 5 × 10^7 B6 (▲) or bm12 (▼) spleen cells and tested 14 d later.

suppression of CTL responses to hapten-self (Figs. 1 and 2) and to alloantigens (Fig. 3). The results obtained using a series of B10 congenic and recombinant strains as well as B6 and the bm12 mutant are summarized in Table I. The data indicate that in any combination of parental spleen cells into F_1 hosts in which the parental lymphocytes have the potential to recognize I-A determinants of the F_1 as allogeneic, severe suppression of CTL potential was observed. Furthermore, the data indicate that potential recognition of a number of different region and subregion products that do not include I-A did not lead to suppression by this protocol. It should be noted that we have limited this study to the suppressive effects of a single injection of parental spleen cells and have not yet addressed the issue of whether multiple injections of parental cells into F_1 mice involving recognition of determinants encoded by genes mapping to the right of I-A would be suppressive [e.g., 4R into (4R × A)F_1; Fig. 1F]. Nevertheless, our results demonstrate a striking difference in suppressive potential initiated by I-A recognition vs. I-B-D recognition.

The GVH reaction has been divided into two broad phases. The proliferative phase is considered (a) to be reflected in vivo by the splenomegaly or popliteal lymph node assay; (b) to have an in vitro counterpart in the mixed lymphocyte reaction; and (c) to be stimulated mainly by I region (class II) antigens, particularly those mapping to I-A (12–15). The effector phase is considered (a) to be reflected in vivo by the wasting syndrome and mortality; (b) to have an in vitro counterpart in the CTL assay; and (c) to involve the recognition of K and D region antigens (class I), as well as I region antigens (13, 15). In the effector phase, K, D, and I region-encoded products all can contribute to mortality (14). GVH-associated acquisition of host H-2 antigens by donor-derived T cells also has been mapped to K, D, and I-A (16). The results of the present study of GVH-associated immune suppression indicate a role for I-A, but not for K, D, or any other I subregion (Table I). The recognition of I-C determinants resulting in GVH mortality was reported only after multiple injections of cells (15). Studies are in progress to determine whether GVH-associated suppression can be induced via I-E/C recognition by multiple injection of F_1 mice with parental cells that can recognize I-B-D-encoded determinants expressed by the F_1. It should also be noted that the recognition of I-E determinants in GVH results in the production of autoantibodies with lupus-like properties (17). This observation, together with our demonstration that recognition of I-A antigens results in immunosuppression, raises the possibility that the complex nature of GVH disease can be further resolved into distinct entities by such genetic mapping studies.

TABLE I

Summary of Mapping of H-2 Recognition Required for Suppression

F$_1$ recipient and H-2 alleles	Parental spleen cell donor	F$_1$ subregions allogeneic to donor	Suppression detected	Data shown in
(C57BL/10 × B10.A)F$_1$				
bbbbbbbb	B10.A			
kkkkkddd	kkkkkddd	K–D	Yes	Fig. 1 A
[B10.A(2R) × C57BL/10]F$_1$				
kkkkkddb	B10.A(2R)			
bbbbbbbb	kkkkkddb	K–S	Yes	Fig. 1 B
*kkkkkdd*b	C57BL/10			
bbbbbbbb	bbbbbbbb	K–S	Yes	Fig. 1 B
[B10.A(5R) × B10.A]F$_1$				
bbbkkddd	B10.A(5R)			
*kkkk*kddd	bbbkkddd	K–IB	Yes	Fig. 1 C
[B10.A(4R) × C57BL/10]F$_1$				
kkbbbbbb	B10.A(4R)			
*bb*bbbbbb	kkbbbbbb	K, IA	Yes	Fig. 1 D
*kk*bbbbbb	C57BL/10			
bbbbbbbb	bbbbbbbb	K, IA	Yes	Fig. 1 D
(C57BL/10 × B10.MBR)F$_1$				
bbbbbbbb	C57BL/10			
b*kkkkkkq*	bbbbbbbb	IA–D	Yes	Fig. 1 E
b*bbbbbbb*	B10.MBR			
bkkkkkkq	bkkkkkkq	IA–D	Yes	Fig. 1 E
[B10.A(4R) × B10.A]F$_1$				
kkbbbbbb	B10.A(4R)			
kk*kkkddd*	kkbbbbbb	IB–D	No	Fig. 1 F
kk*bbbbbb*	B10.A		No	Fig. 1 F
kkkkkddd	kkkkkddd	IB–D		
(B10.AKM × B10. MBR)F$_1$				
kkkkkkkq	B10.AKM			
*b*kkkkkkq	kkkkkkkq	K	No	Fig. 2 A
*k*kkkkkkq	B10. MRB			Fig. 2 A
bkkkkkkq	bkkkkkkq	K	No	
(B10.A(2R) × B10.A)F$_1$				
kkkkkddb	B10.A(2R)			
kkkkkdd*d*	kkkkkddb	D	No	Fig. 2 B
kkkkkdd*b*	B10.A			
kkkkkddd	kkkkkddd	D	No	Fig. 2 B
(A.TH × A.TL)F$_1$				
*ssssss*d	A.TL			
skkkkkkd	skkkkkkd	IA–S	Yes	Fig. 2 C
(C57BL/6 × B6.C^{bm12})F$_1$				
bbbbbbbb	C57BL/6			
b*b**bbbbbb	bbbbbbbb	IA	Yes	Fig. 3 C
b*b*bbbbbb	B6.C^{bm12}			
bb*bbbbbb	bb*bbbbbb	IA	Yes	Fig. 3 C

Italics indicate H-2, K, IA, IB, IJ, IE, IC, S, D, F$_1$ alleles that are allogeneic to the parental donor.
* Indicates gain-loss IA mutation of B6.C-H-2^{bm12} strain.

The use of the B6 and bm12 differences in the parent into F_1-induced immuno-suppression has permitted the mapping of this phenomenon to the I-A subregion, which indicates that recognition of other regions and subregions are not required. In fact, no differences were detected in the severity of CTL suppression in this study when combinations in which the entire H-2 could be recognized as allogeneic (Figs. 1 A and 3 A and B) compared with combinations in which only I-A was recognized as allogeneic (Fig. 3 C). However, it is possible that the recognition of products of other H-2 regions may contribute to some aspect of suppression such as the number of parental cells required to induce suppression or the time required for recovery from suppression. Furthermore, this study has not addressed the issue of whether I-A recognition is all that is required for the induction of other GVH parameters such as splenomegaly, mortality, wasting syndrome, and the production of autoantibodies.

This study also provides new information concerning the mutual recognition of the I-A differences between the C57BL/6 wild type and B6-C-H-2^{bm12} mutant strains. Thus, B6 and bm12 can be distinguished from each other not only by graft rejection (9), mixed lymphocyte reactivity (9), and generation of CTL (18), but also by allogeneic I-A recognition leading to severe immunosuppression (Fig. 3 C). Thus, these findings indicate that the I-A of the B6 and bm12 differ from each other at determinants responsible for inducing immune suppression.

The question can be raised whether the parental lymphocytes recognize only I-A of the other parental haplotype (B6 × bm12)F_1 expressed by the F_1, or whether a unique I-A determinant could possibly be recognized similar to that reported for combinational I-A molecules of (C57BL/6 × B10.A)F_1 origin (19). It is unlikely that such a combinational I-A molecule can exist in the (B6 × bm12)F_1 since the bm12 mutation is limited to the A_b chain (20). This does not necessarily exclude the possibility that recognition of an F_1 combinational I-A molecule in the appropriate strains would initiate suppression.

The mechanism of immune suppression associated with GVH has been extensively investigated. Arguments that suppression is effected both by parental anti-F_1 CTL (21) and by other types of noncytotoxic suppressor cells (22) have been made. Although the present study was not designed to address this issue, it does provide some insight concerning this question. The fact that recognition of allogeneic I-A determinants is required for induction of expression does not exclude the possibility that suppression is due to CTL, since the antigeneic differences between B6 and bm12 are capable of eliciting CTL responses (18). However, the fact that recognition of K or D differences only does not induce suppression, whereas such differences are capable of eliciting potent CTL responses, indicates that there is not necessarily a direct correlation between those loci that elicit CTL responses and those that induce GVH-associated suppression. It is possible, however, that CTL generated by I-A differences uniquely function as suppressor cells in this system.

It is not yet clear what the potential significance might be of immune suppression resulting from in vivo recognition of allogeneic I-A determinants. In allogeneic combinations involving immunocompetent recipients, the immune system of the host can reject the donor's lymphocytes, and the immunosuppression seen in the GVH model would not be observed. However, in hosts that are incapable of rejecting donor lymphocytes such as parent into F_1, or allogeneic combinations in which the immune system of the host is impaired (e.g., transplant patients) or not yet developed (e.g.,

neonates), donor-induced activation of the type of suppression described in this study could be critical. In this context it should be noted that Pollack et al. (23) have recently reported that some infants suffering from severe combined immunodeficiency disorders (SCID) are chimeras in that they possess maternal T lymphocytes. This observation raises the possibility that the immune deficiencies of these chimeric SCID are the result of an immune suppression mechanism similar to the GVH-associated immune suppression described here. If true, one might expect that all chimeric SCID patients would be recognized as allogeneic at the human equivalent of I-A by maternal T lymphocytes for activation of this suppression.

A second clinical situation in which "I-A-activated" immune suppression could be relevant is in hemopoietic transplantation. Because GVH reactions are observed in transplant patients, many of whom are already immune suppressed, there may not be a general awareness of the profound suppressive potential that can accompany GVH. It should be noted that patients undergoing chronic GVH reactions are unable to cope with infections and appear to be immune suppressed (24, 25). Because the murine model of GVH-associated suppression is nonspecific in that it suppresses responses to all antigens except those to which the host had been primed (L. Joseph and G. M. Shearer, unpublished observations), this type of suppression should render the host very susceptible to infections. If the donor bone marrow inoculum contains T lymphocytes and if the recipient and donor are mismatched at the human equivalent of I-A, e.g., DS (26), even though HLA matched at other loci, including possibly DR and SB (27), it is possible that an immunosuppressive signal similar to the one demonstrated in this report could impair the development of the immune system of the reconstituted recipient without there necessarily being other symptoms of GVH disease.

Finally, a third clinical situation in which GVH-associated immune suppression may be relevant is in the acquired immune deficiency syndrome (AIDS) recently reported mainly in male homosexuals (75%), but also detected in other groups such as intravenous drug users and Haitians (28–31). GHV-associated suppression is similar to AIDS, both in its lack of antigen specificity and in its severe and lasting effects. Thus, among the homosexuals affected, an etiology involving GVH-associated suppression could be a factor. For example, it has been recently observed that some homosexuals affected with AIDS have high antibody titers to sperm and appear to be undergoing anti-sperm autoimmune reactions (Rubenstein, personal communication). Such autoimmunity could affect the blood-testes barrier and result in abnormally high numbers of leukocytes in semen. These leukocytes could be transferred via semen to homosexual partners and induce GVH-associated immune suppression. In order that the allogeneic leukocytes not be rejected, it would be necessary for the recipient of the leukocytes to be at least transitionally immune suppressed, possibly by virus (32) or sperm (33). Alternatively, it would not be necessary for the recipient to be immune suppressed to accept the leukocytes if the recipient were HLA heterozygous and the leukocyte donors were HLA homozygous and matched with one of the recipient's haplotypes. Because the results of the present study demonstrate that I-A recognition is all that is required to initiate suppression, DS (26) recognition might be all that would be required to initiate AIDS.

The present study has demonstrated a new function for antigenic determinants of the murine I-A subregion, i.e., the activation of a potent suppressive signal that

rapidly abrogates immunity. Possible parallels between this phenomenon and three examples of clinical immune deficiency have been considered.

Summary

Several combinations of F_1 hybrid mice were injected intravenously with parental spleen cells to determine the minimal H-2 differences between F_1 and parent that are necessary to induce graft-vs.-host-associated immune suppression (GVH-associated suppression). 7–14 d after injection, the spleens of the F_1 mice were tested for cytotoxic T lymphocyte potential by in vitro sensitization against trinitrophenyl-self and H-2 alloantigens. The results indicate that parental T lymphocytes must recognize I-A allogeneic determinants of the F_1 recipient in order to induce suppression. Recognition of K or D alone or D with I region products other than I-A did not induce suppression. The recognition of I region without K and/or D and even the I-A difference between C57BL/6 and the B6.C^{bm12} mutation resulted in immune suppression that was as potent as that resulting from the recognition of K, D, and I together. The possible significance of this function for I-A antigens is discussed with respect to three clinical examples of immune suppression for which this phenomenon may be relevant.

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