

Graft-Versus-Host Resistance Induced By Class II Major Histocompatibility Complex-specific T Cell Clones

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Summary

Possible mechanisms of graft-vs.-host (GVH) resistance have been studied using a panel of seven class II major histocompatibility complex-specific T cell clones for elicitation and challenge. One clone recognized I-A^{k,d,f}, and expressed V β 8.3 together with J β 1.5. The remaining six clones were I-E^k specific and expressed V β 15 rearranged to J β 1.1 or J β 1.3. The I-E^k-specific clones were also homologous to each other and different from the I-A-reactive one in the D and N regions. Four of the seven clones exhibited I-E^k-specific cytolytic activity. Each clone, when injected in sublethal numbers into appropriate recipients, could induce resistance to a subsequent lethal dose of any other clone in the panel. The resistance did not require sharing of either T cell receptor β chains or antigen specificity, or MHC molecules by the eliciting and challenging clone. Cytolytic and noncytolytic clones were equally efficient in inducing GVH resistance. A prerequisite of resistance induction was the activation of eliciting clone subsequent to recognition of class II molecules in the host. Clones preactivated with high concentrations of recombinant interleukin 2, in vitro, could induce GVH resistance also in syngeneic hosts, suggesting that resistance induction was associated with the activated state of clone, rather than antigen recognition per se. In all instances of resistance, the challenging clones failed to induce vascular leakage, which was the cause of death in susceptible recipients (Lehmann, P. V., G. Schumm, D. Moon, U. Hurtenbach, F. Falcioni, S. Muller, and Z. A. Nagy. 1990. *J. Exp. Med.* 171:1485). Lipopolysaccharide (LPS) induced resistance to vascular leakage did not provide crossresistance to GVH and vice versa, suggesting that interleukin 1 α and tumor necrosis factor α implicated in LPS resistance are not involved in GVH resistance. Although the mechanism remains unclear, the most likely explanation for GVH resistance in this system is either the downregulation of permeability increasing effect in the challenging clone, or an induced refractoriness of blood vessels to this effect.

Injection of limiting numbers of lymphoid cells from MHC-homozygous donors into semisyngeneic or immunocompromised allogeneic recipients leads to resistance to a subsequent challenge with large numbers of lymphoid cells that would otherwise cause a graft-versus-host (GVH) reaction, manifested in lymph node swelling or lethality (1, 2, and reviewed in reference 3). This GVH resistance is of rapid onset (detectable from day 3 to 10) and short duration (rarely detectable after day 14). Experimental systems using polyclonal lymphoid cell populations for the induction of GVH resistance have yielded results suggesting that the major mechanism underlying GVH resistance is a T cell response of the recipient directed against antigen receptors of the injected T cells recognizing host alloantigens (2). Such receptors are forbidden in a healthy animal for reasons of self-tolerance (4), and thus, they may serve as immunogens in a GVH situation. Evidence favoring an antiidiotypic immune mechanism for GVH resistance includes, first, that the resistance extends only to T cells specific for the same MHC molecules, second,

that it is not restricted by the MHC of the resistance-inducing cells, and third, that it can be adoptively transferred to naive recipients (2). The nature of the effector cell mediating GVH resistance has remained unclear; CTL or other types of suppressive cells have been implicated (5-8).

In the experiments reported here, we have tested whether alloreactive T cell clones can induce GVH resistance, and, if so, by what mechanism. The data demonstrate that a short-term GVH resistance, similar to the one induced by polyclonal lymphoid cells, can also be induced by class II-reactive T cell clones. This resistance is dependent on the activation of cloned T cells in the recipients, and it seems to result from several different (including nonimmunological) mechanisms.

Materials and Methods

Mice. 8-wk-old specific pathogen-free mice of strains B10.A(2R), B10.A(4R), B10.AQR, B10.T(6R) (Olac Ltd., Bicester, UK) and BALB/c nu/nu (Iffa Credo, L'Arbresle, France) were used.

T Cell Clones. The T cell clones were isolated from MLR combinations B10.T(6R) (K^a,I-A^a,I-E^a,D^d) anti-B10.AQR (K^a,I-A^k,I-E^k,D^d), and B10.A(4R) (K^k,I-A^k,I-E^b,D^b) anti-B10.A(2R) (K^k,I-A^k,I-E^k,D^b). Thus, the former combination can generate I-A^k as well as I-E^k-specific clones, whereas the latter can only generate I-E^k-specific ones. Designation and properties of the clones are summarized in Table 1 (see also reference 9). The method of expanding cloned cells to numbers required for in vivo experimentation have been described previously (9).

In Vivo Assays with T Cell Clones. Activation of cloned T cells in the recipients was measured by an in vivo [³H]thymidine incorporation assay as described previously (9). Briefly, 60 h after injection of cloned cells, 0.5 mCi [³H]TdR was injected intravenously, and 18 h later, thymidine incorporation was determined in Soluene-dissolved organ samples. Under the conditions of this assay, the bulk of injected thymidine is taken up by the cloned cells themselves (9). The additional host component of uptake is dependent on the antigen-specific activation of the cloned cells in vivo. Homing of cloned cells was determined by injecting ⁵¹Cr-labeled cells intravenously, and the radioactivity of organs was measured by gamma counting 24 h later (10). Vascular leakage induced by T cell clones was determined by extravasation of ¹²⁵I human albumin (11).

Determination of TCR-β Gene Segment Usage by T Cell Clones. Total RNA was prepared from 10⁷ T cells as described (12). 5–10 μg of total RNA was used for the synthesis of first-strand cDNA using reverse transcriptase (M-MuLV) and oligo p(dT)15 (Boehringer, Mannheim, FRG). The reaction was stopped by diluting 30-fold with water and heating to 95°C for 5 min. The cDNA (1/100 to 1/300 of each sample) was amplified by PCR using one of the Vβ primers together with the Cβ primer (Table 2) in each reaction, at 1 μM final concentration. Amplification was performed in 50 μl with 1.25 U of Taq polymerase (Perkin Elmer, Küssnacht, Switzerland), and a thermocycler (Cetus/Perkin Elmer) under the following conditions: 94°C melting for 20 s, 55°C annealing for 20 s, and 72°C extension for 30 sec. The amplified products were separated on a 1.6% agarose gel, and the respective Vβ gene usage was determined by the appearance of the relevant PCR fragment. After identification of the Vβ genes expressed, several identical PCRs were set up and pooled to obtain sufficient product for sequencing. The PCR fragment was purified either by anion exchange chromatography (Quiagene tip 20; Diagene, Düsseldorf, FRG), or by preparative agarose gel electrophoresis and isolation of the DNA

Table 1. Characteristics of T Cell Clones Used in This Study

Clone designation	MLR of origin				MHC molecule recognized	Cytolytic activity*
	R	α	S	Exp.		
4Ra	4R	α	2R	1	I-E ^k	–
4Re	4R	α	2R	1	I-E ^k	–
4Rh	4R	α	2R	1	I-E ^k	+
4R5	4R	α	2R	2	I-E ^k	+
4R9	4R	α	2R	2	I-E ^k	+
6Re	6R	α	AQR	3	I-E ^k	+
6R2	6R	α	AQR	4	I-A ^{k,d,f}	–

Data are based on that in reference 9.

* Specific for the class II MHC molecule recognized.

Table 2. Primers Used for PCR and Sequencing

Specificity	Sequence	Reference
Vβ1	AGCGCTGAGAAGCCGCCAGA	42
Vβ2	TCAAGCTGTGAACCTACGCT	42
Vβ3	GATGAGGTGTATCCCTGAAA	43
Vβ4	TTCATGTTTTCTACAGCTA	44
Vβ5	ATTCTGGGGTTGTCCAGTCT	45
Vβ6	CATGGTGATGGTGGCATCAT	44
Vβ7	CCTGGTCTGGGGCTACAGCT	46
Vβ8	AACACATGGAGGCTGCAGTC	45
Vβ9	TGATAAGATTTTGAACAGGG	44
Vβ10	ACGAAACAGTTCCAAGGCCG	44
Vβ11	CAAGCTCCTATAGATGATTC	44
Vβ12	TTCCCCCTTATGGAAGATGG	44
Vβ13	CCTAAAGGAACTAACTCCAC	44
Vβ14	CCAGGTAGAGTCGGTGGTGC	47
Vβ15	ACTGTGAACTCAGCAATCAA	47
Vβ16	AATGGTAAAGTCATGGAGAA	47
Cβ	GATGGCTCAAACAAGGAGAC	48

with GeneClean (Bio 101; La Jolla, CA). Purified DNA was sequenced directly using the dideoxy-chain termination method (13), and the T7 polymerase as provided in the T7 sequencing TM kit (Pharmacia Fine Chemicals, Dübendorf, Switzerland). The primers used for sequencing were the ones used for PCR (Table 2).

Results

Demonstration of GVH Resistance Induced by T Cell Clones. As described previously, class II-reactive T cell clones induced lethal GVH reaction between day 3 and 5 after injection of 8 × 10⁶ or more cells into recipients expressing the stimulating MHC antigens (9). Sublethal numbers (2–4 × 10⁶) of cloned cells caused no disease, or only transient symptoms of disease developed, and the animals became resistant to a subsequent injection of lethal numbers (up to 4 × 10⁷) of cloned cells. As shown in Table 3, each T cell clone was capable of inducing resistance to itself. The resistance was manifested not only in lack of mortality, but also in the absence of GVH symptoms. The resistant animals survived for at least 1 yr. The resistance developed 2–3 d after injection and lasted for 3–5 wk, depending on the clone administered (data not shown).

Effect of In Vivo Activation of T Cell Clones on GVH Resistance. A critical step in the induction of lethal GVH was activation of injected cells in the recipient (9). It was therefore conceivable that in the resistant animals activation might have failed to occur upon secondary injection of the clone. We tested this possibility by using an in vivo [³H]TdR incorporation assay, which reflects proliferation of the injected clone as well as its capacity to induce increased DNA synthesis in the target organs (lungs and liver). As shown in

Table 3. Demonstration of GVH Resistance: Each Clone Induces Resistance to Itself

T cell clone injected		Recipient strain	Mortality by day 13
Day 1	Day 7		
6R2 (3×10^6)	6R2 (10^7)	B10.AQR	0/4
-	6R2 (10^7)	B10.AQR	3/3
6Re (3×10^6)	6Re (10^7)	B10.AQR	0/5
-	6Re (10^7)	B10.AQR	4/4
4R9 (2×10^6)	4R9 (8×10^6)	B10.A(2R)	0/3
-	4R9 (8×10^6)	B10.A(2R)	3/3
4Rh (4×10^6)	4Rh (10^7)	B10.A(2R)	1/4
-	4Rh (10^7)	B10.A(2R)	4/4
4R5 (3×10^6)	4R5 (10^7)	B10.A(2R)	0/3
-	4R5 (10^7)	B10.A(2R)	3/3

Fig. 1. the noncytolytic clones 6R2 and 4Ra induced comparable thymidine incorporation in susceptible and resistant mice. In contrast, the cytolytic clones 6Re, 4R9, and 4R5 failed to incorporate, or incorporated significantly less thymi-

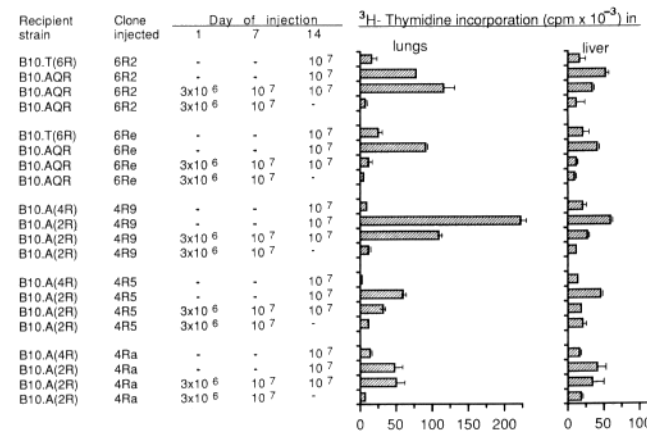


Figure 1. Proliferation of T cell clones in vivo in GVH-resistant mice. The development of GVH resistance elicited by 3×10^6 cells on day 1 was tested by a lethal dose (10^7) of T cells on day 7. In vivo proliferation was determined 3 d after a third injection of T cells on day 14. 18 h before the termination of experiment, 0.5 mCi [^3H]TdR was injected intravenously, and thymidine incorporation in lungs and liver was measured as described (9). Each column represents the mean (and SD) for three mice.

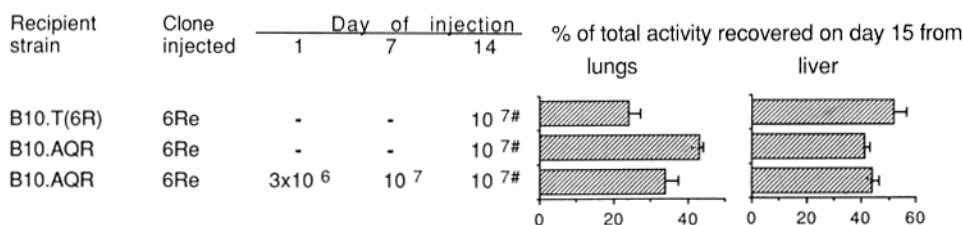


Figure 2. Homing of T cell clone 6Re in GVH-resistant mice. Percent activity relates to the total radioactivity recovered from all organs (corresponding to 70–80% of injected radioactivity). Each column represents the mean (and SD) for three mice. (#) Cr-labelled cells injected.

dine in GVH-resistant animals, although they exhibited a substantial primary thymidine incorporation. The failure of cytolytic clones to proliferate in resistant animals was not due to altered homing, since these clones, as exemplified by clone 6Re in Fig. 2, homed to lungs and liver in both susceptible and resistant mice. One possible interpretation for the lack of activation is that these clones have lysed the class II-expressing stimulator cells in the target organs after the first injection. Thus, the failure of secondary activation due to antigen depletion in the host may be a possible mechanism involved in GVH resistance, but it is certainly not an exclusive one, since noncytolytic clones have been found to become activated in resistant recipients.

GVH Resistance Is Neither MHC Restricted nor TCR- β Specific. We investigated whether or not GVH resistance resulted from an immune response of the host against the priming clone. As shown in Table 4, clones of B10.T(6R) and B10.A(4R) origin induced resistance against each other. Since these two strains differ at all class I and II MHC loci, the resistance is obviously not restricted by the MHC of the

Table 4. Induction of Crossresistance and Absence of MHC Restriction

T cell clone injected		Recipient strain	Mortality by day 13
Day 1	Day 7		
6R2 (3×10^6)	4R9 (10^7)	B10.AQR	1/7
-	4R9 (10^7)	B10.AQR	3/3
6R2 (3×10^6)	4R5 (10^7)	B10.AQR	0/3
-	4R5 (10^7)	B10.AQR	3/3
4Re (10^7)	6R2 (10^7)	B10.A(2R)	0/3
-	6R2 (10^7)	B10.A(2R)	3/3
4Re (10^7)	4R9 (10^7)	B10.A(2R)	1/3
-	4R9 (10^7)	B10.A(2R)	3/3
4R9 (2×10^6)	6Re (10^7)	B10.A(2R)	0/3
-	6Re (10^7)	B10.A(2R)	3/3
4Rh (3×10^6)	6R2 (10^7)	B10.A(2R)	0/3
-	6R2 (10^7)	B10.A(2R)	3/3
6Re (3×10^6)	6R2 (10^7)	B10.AQR	0/8
-	6R2 (10^7)	B10.AQR	3/3

Table 5. Junctional DNA and Protein Sequences of TCR β Chains Expressed by the Clones

T cell clone	V β	Junctional region	J β	C β
4Ra	L Y L C G A TTATAATCTCTGTGGTGC V β 15	L R G G TCT CAGGG AGG D β 1	N T E V F F G K G T R L T V V AAACACAGAACTCTTTGGTAAAGGAACCCAGACTCACAGTTGTA J β 1.1	E D GAGGAT C β
4Re	L Y L C G A TTATAATCTCTGTGGTGC V β 15	R A G G TAGGG D β 1	S G N T L Y F G E G S R L I V V TCTGGAATACGCTCTATTTGGAGAAGGAAGCCGGCTCATTTGTTGTA J β 1.1	E D GAGGAT C β
4Rb	L Y L C G A TTATAATCTCTGTGGTGC V β 15	L R G G TCT CAGGG AGG D β 1	N T E V F F G K G T R L T V V AAACACAGAACTCTTTGGTAAAGGAACCCAGACTCACAGTTGTA J β 1.1	E D GAGGAT C β
4R5	L Y L C G A TTATAATCTCTGTGGTGC V β 15	L G G G CCTC D β 1	N T E V F F G K G T R L T V V AAACACAGAACTCTTTGGTAAAGGAACCCAGACTCACAGTTGTA J β 1.1	E D GAGGAT C β
4R9	L Y L C G A TTATAATCTCTGTGGTGC V β 15	L R G G TCT CAGGG AGG D β 1	N T E V F F G K G T R L T V V AAACACAGAACTCTTTGGTAAAGGAACCCAGACTCACAGTTGTA J β 1.1	E D GAGGAT C β
6Re	L Y L C G A TTATAATCTCTGTGGTGC V β 15	L R G G TCT CAGGG AGG D β 1	N T E V F F G K G T R L T V V AAACACAGAACTCTTTGGTAAAGGAACCCAGACTCACAGTTGTA J β 1.1	E D GAGGAT C β
6R2	L Y F C A S S TTGTACTCTGTGCCAGCAGT V β 8.3	E D R A GA GGACAG A D β 1	N N Q L R F L E R G L D F S V L AACAAACAGCTCCGCTTTTGGAGAGGGGACTCGACTTCTCTGTTCTA J β 1.5	E D GAGGAT C β

priming clone. Moreover, the I-A^k-specific clone 6R2 induced resistance against several I-E^k-specific clones and vice versa. The latter result indicates that sharing of antigen specificity by clones is not required for the induction of GVH resistance in this system. To further investigate whether an immune response against the antigen receptor of the clones may be involved in GVH resistance, we have characterized the β chain of TCR expressed by these clones. The results in Table 5 demonstrate that the I-A^k-specific clone 6R2 expresses V β 8.3 and J β 1.5. In contrast, all I-E^k-specific clones use V β 15. Five out of these six clones use J β 1.1, and exhibit identical amino acid sequence in the N and D regions. At least four of the latter five clones are distinct, as judged by the strain of origin (4R or 6R), the experiment of origin (see in Table 1), and a difference in the N region nucleotide sequence (clone 4R5). The remaining I-E^k-specific clone 4Re uses J β 1.3 and differs from the others in the N region. Thus, the I-E^k-specific clones exhibit a striking homology in terms of TCR- β usage, although they derive from three different groups of mice belonging to two different (fully MHC-disparate) strains. These data indicate that an immune response against TCR β chains could explain the cross-resistance between I-E^k-specific clones, but not the cross-resistance between the I-E^k- and I-A^k-reactive ones. The possible role of an anti-TCR α chain immunity remains to be investigated.

Induction of GVH Resistance in MHC-disparate Recipients and in Athymic Nude Mice. We have investigated whether immunization of recipients with MHC-disparate cells would induce a protective host response against a pathogenic clone sharing MHC with the priming cells. To this end, the I-E^k-specific clone 6Re was injected into MHC-disparate B10.A(4R) or B10.M (H-2^b) recipients, which do not express the stimulating class II molecule, and thus, the clone does not recognize any host antigens. 7 d later, the I-A^k- and I-A^f-specific clone 6R2 was injected in a lethal dose (Table 6).

Table 6. MHC Disparity Between Priming Clone and Recipient Does Not Induce Immunity to Protect from Lethal GVH

T cell clone injected		Recipient strain	Mortality by day 13
Day 1	Day 7		
-	6R2 (10 ⁷)	B10.M	3/3
6Re, 3 × 10 ⁶	6R2 (10 ⁷)	B10.M	4/4
6R2, 3 × 10 ⁶	6R2 (10 ⁷)	B10.M	0/3
-	6R2 (10 ⁷)	B10.AQR	3/3
6Re, 3 × 10 ⁶	6R2 (10 ⁷)	B10.AQR	0/3
-	6R2 (10 ⁷)	B10.A(4R)	3/3
-	6Re (10 ⁷)	B10.A(4R)	0/4
6Re, 3 × 10 ⁶	6R2 (10 ⁷)	B10.A(4R)	3/3
6Re, 10 ⁷	6R2 (10 ⁷)	B10.A(4R)	3/3
6R2, 3 × 10 ⁶	6R2 (10 ⁷)	B10.A(4R)	0/4

Table 7. GVH Resistance in Nude Mice

T cell clone injected		Recipient strain	Mortality by day 13
Day 1	Day 7		
6R2 (10 ⁷)	-	BALB/c nu/nu	0/4
6R2 (10 ⁷)	6R2 (2 × 10 ⁷)	BALB/c nu/nu	0/8
-	6R2 (2 × 10 ⁷)	BALB/c nu/nu	6/6

Although the priming and challenging clones were MHC identical, 6Re failed to induce resistance to 6R2. The failure of resistance induction was not due to altered homing (data not shown). In the same experiment, 6Re did induce resistance to 6R2 in B10.AQR mice expressing I-E^k to which 6Re was specific. Similarly, clone 6R2 induced resistance against itself in B10.M and B10.A(4R) recipients. Thus, antigen-specific activation of the priming clone in the recipient seems to be critical for the induction of GVH resistance, whereas a possible host response against strong alloantigens expressed by the clone (e.g., MHC) does not protect from lethal GVH reaction. We then investigated whether the host's T cell compartment was required for the development of GVH resistance. As demonstrated in Table 7, the I-A^d-reactive clone 6R2 could readily induce GVH resistance in young athymic BALB/c nu/nu mice, whose T cell system is severely deficient. Taken together, it appears that immune responses of the host do not play a major role in the development of T cell clone-induced GVH resistance.

Activation of the Priming T Cell Clone Is Required for the Induction of GVH Resistance. The data so far have indicated that the priming clone must recognize host antigens for the development of GVH resistance. To dissect whether antigen recognition itself or the subsequent T cell activation is rele-

Table 8. GVH Resistance Is Induced in Syngeneic Recipients by T Cell Clones Preactivated In Vitro

Preactivation*	Clone injected on:		Recipient strain	Mortality by day 8
	Day 1	Day 3		
rIL-2	4R9 (10 ⁷)	6R2 (10 ⁷)	4R	0/3
-	4R9 (10 ⁷)	6R2 (10 ⁷)	4R	3/3
rIL-2	4Rh (10 ⁷)	6R2 (10 ⁷)	4R	0/3
-	4Rh (10 ⁷)	6R2 (10 ⁷)	4R	3/3
rIL-2	4R5 (10 ⁷)	6R2 (10 ⁷)	4R	0/3
-	4R5 (10 ⁷)	6R2 (10 ⁷)	4R	3/3

* 1 wk after the last restimulation with 10 ng/ml rIL-2, the eliciting clone was cultured with 100 ng/ml rIL-2 for 12 h, washed, and injected into appropriate recipients. In the control group, the cells were left in the standard culture medium (with 10 ng/ml rIL-2, changed 7 d before) for the last 12 h of culture, washed, and injected as above.

Table 9. LPS Resistance Does Not Protect from Lethal GVH Induced by T Cell Clones

Recipient strain	Treatments			Mortality
	Day 1	Day 9	Day 15	
C3H/HeJ	LPS (400 µg)	-	-	0/3
C3H/HeN	LPS (400 µg)	-	-	4/4
C3H/HeN	LPS (100 µg)	-	-	0/4
C3H/HeN	LPS (25 µg)	LPS (400 µg)	-	0/4
C3H/HeN	LPS (25 µg)	6R2 (10 ⁷)	-	3/3
C3H/HeN	LPS (25 µg)	LPS (400 µg)	6R2 (10 ⁷)	4/4
C3H/HeN	-	4R9 (10 ⁶)	6R2 (10 ⁷)	0/3
C3H/HeN	-	-	6R2 (10 ⁷)	3/3
C3H/HeN	4R9 (10 ⁶)	6R2 (10 ⁷)	LPS (400 µg)	2/3

vant to GVH resistance, we have attempted to induce resistance in syngeneic recipients with clones in different states of activation. As shown in Table 8, cloned cells preactivated with high concentrations rIL-2 in vitro readily induced resistance in syngeneic mice, whereas resting cells failed to do so. Thus, resistance appears to be a consequence of T cell activation that may or may not occur via the antigen receptor.

LPS Resistance and GVH Resistance Are Mediated by Different Mechanisms. T cell clone-induced acute GVH, as well as LPS-induced lethal disease, are manifested in shock. The two conditions are also similar in that a first sublethal encounter induces resistance against a second lethal one. We therefore investigated whether LPS-induced resistance to shock would protect the animals also from lethal GVH. As shown in Table 9, pretreatment of LPS-sensitive C3H/HeN mice with 25 µg LPS induced resistance to a lethal dose (400 µg) of LPS. The same treatment, however, failed to provide protection against a lethal dose of clone 6R2. Conversely, GVH resistance induced by T cell clones failed to protect the animals against a lethal dose of LPS. It has been described that IL-1α

and TNF-α are involved in the induction of LPS resistance (14). Thus, the absence of LPS-GVH crossresistance indicates that GVH resistance is independent of IL-1α and TNF-α-related mechanisms.

Absence of Vascular Leakage in GVH-resistant Animals. We have shown previously that vascular leakage is a major symptom and probably also the cause of death in T cell clone-induced acute GVH reaction (9). We therefore asked whether the clones were also capable of causing vascular leakage in GVH-resistant animals. As shown in Fig. 3, the extravasation of ¹²⁵I albumine was not increased above the controls in animals resistant to clone 6R2. The same clone induced significant vascular leakage in nonresistant animals. GVH resistance paralleled the absence of vascular leakage in both euthymic and athymic mice (Fig. 3). The clone used in this experiment had been shown before to become activated after secondary injection in the host (see in Fig. 1), and yet, it failed to induce vascular leak syndrome. Thus, the absence of vascular leakage in GVH resistance is due to either an altered effector function of the inducing T cells upon secondary

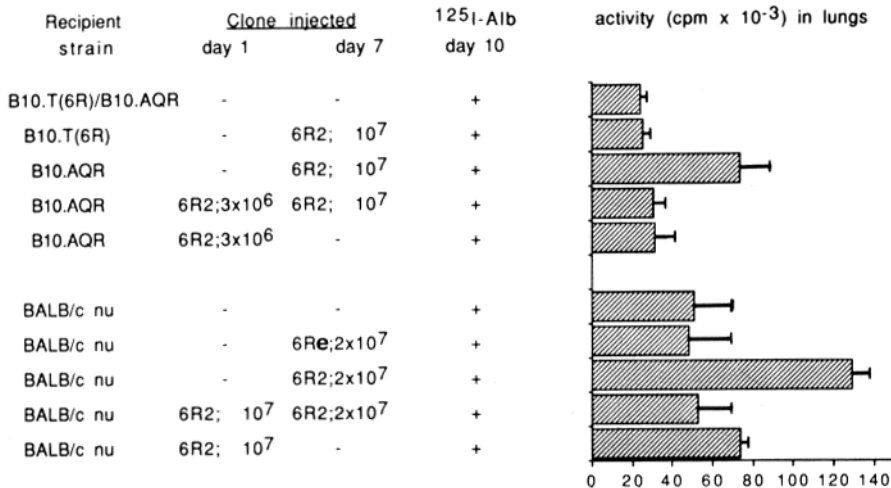


Figure 3. Extravasation of ¹²⁵I-albumine in GVH-resistant mice. Extravasation was measured 2 h after intravenous injection of 0.5 µCi ¹²⁵I human albumine as described (11). Each column represents the mean (and SD) for four mice.

injection, or a decreased sensitivity of capillary walls to the permeability-inducing effect.

Discussion

Resistance to local as well as systemic GVH reaction can be induced by injecting a small dose of parental lymphocytes into F₁ hybrid recipients (1, 2, 3, 7). Similarly, in animal models of autoimmunity, a resistance to disease develops after nonpathogenic administration of the autoantigen or the disease-inducing cells (15–21), or after recovery from a disease episode (21–24). Resistance to these disease conditions appears to be mediated by T cells (usually of the suppressor/cytotoxic type) that recognize the antigen receptors of the disease-inducing (usually helper type) T cells (6–8, 25, 26). However, this resistance mechanism is not found in certain GVH systems (8, 27). In addition to specific antireceptor immunity, several other host-derived mechanisms may contribute to the development of resistant state, including T cell-mediated undefined nonspecific mechanisms (27), suppression by antiertgotypic T cells directed against markers on activated T cells (28), suppression mediated by “inappropriate” APC (29), and decreased skin reactivity due to the action of Thy-1⁺ epidermal cells (30).

We have demonstrated previously that alloreactive, class II MHC-specific T cell clones can induce a lethal GVH reaction, when injected into recipients of the stimulator strain (9). In this communication, we have shown that the same T cell clones injected in sublethal doses render the host resistant to a subsequent lethal dose of the cloned cells. The resistance was demonstrable from day 3 after injection and lasted for ~1 mo. To investigate whether the clone-induced resistance exhibits immunological specificity, we have used different T cell clones for elicitation and challenge. Since the GVH resistance induced by bulk lymphoid cells has been considered to result from a specific immune response against TCRs expressed by the pathogenic T cells, the use of clones with different TCRs should allow us to address the question of specificity at the clonal level. The panel of T cell clones used in these experiments included six I-E^k-specific and one I-A^k-specific clone. Surprisingly, all I-E^k-specific clones expressed V β 15 with minimal variations in terms of J β usage and N region sequence, although they derived from different mice of two fully MHC-disparate strains. It remains to be investigated whether or not this observation represents another example of restricted TCR- β usage among I-E-reactive cells (31–33). In any case, this finding suggests that the cross-resistance between different I-E^k-specific clones could, in principle, be due to an immune response against TCR β chain-associated determinants. However, this explanation cannot apply to the observed crossresistance between I-E^k- and I-A^k-specific clones, since the latter expresses a completely different TCR- β (V β 8.3, J β 1.5, and a different N region sequence). The data leave open the possibility of an immune response to TCR α chain-associated determinants in the latter case of crossresistance. Alternatively, anti-TCR immunity may not be involved here.

Since the clone-induced GVH resistance failed to exhibit any known immunological specificity, we have sought for alternative criteria that may be relevant for induction of the resistant state. We have found that activation of the eliciting clone in the host is a prerequisite of resistance induction. This occurs normally upon specific recognition of host class II molecules by the clone, but the recognition phase can be artificially circumvented by preactivating the clone with high concentrations of IL-2 *in vitro*. Such preactivated cells can induce GVH resistance in syngeneic recipients. The latter finding indicates that it is not antigen recognition *per se*, but the subsequent activation of T cells, which is relevant to resistance induction. We then asked whether the activated state of the T cell clone induces a so far undetected (*i.e.*, not antireceptor) type of immune response. Several lines of evidence indicate that this may not be the case: first, GHV resistance lacks MHC restriction; second, it can be induced in athymic nude mice; and third, it cannot be transferred to naive recipients by either lymphoid cells or serum (P.V. Lehmann, unpublished observation). The latter finding is in contrast to the reported transfer of GVH resistance induced by bulk cell populations (2). This discrepancy may be explained by the different homing patterns of bulk lymphoid cells and clones: the former seed in lymphoid tissues (34), whereas the latter home almost exclusively to lungs and liver (35, 36).

Since immune responses of the host do not seem to be critical for GVH resistance in our system, it is reasonable to assume that resistance is induced directly by the injected clone. One possible mechanism revealed by this study is that cytolytic clones may induce GVH resistance by killing the class II-positive cells in the target organs, and thereby preventing the activation of the challenging clone. However, the finding that noncytolytic clones also generate resistance implies that depletion of stimulator cells may not be a major mechanism. One striking finding was the absence of vascular leakage in GVH resistance. Since the cause of lethality in clone-induced GVH was a circulation disturbance (9), the absence of vascular leakage alone can explain the resistance. Although activated T cells are known to increase vascular permeability (37, 38), the mechanism by which they perform this function is unclear. Perhaps an antigen-induced endoglycosidase (39, 40) or a lymphokine(s) is involved. In the latter case, the lymphokine is not likely to be either IL-1 α or TNF- α , since LPS resistance, where these two lymphokines are involved, does not provide crossresistance to lethal GVH reaction. It is possible that the permeability-increasing effect is associated with a so far unknown T cell lymphokine. GVH resistance could then be the consequence of altered lymphokine production upon secondary injection. Downregulation of some effector functions of the challenging clone by the eliciting clone (41) could bring about this alteration. Another possibility is that a refractoriness of capillary walls develops to the permeability-increasing effect. Further investigations aimed at clarifying the mechanisms whereby T cells influence blood vessels could be of clinical importance in overcoming the side effects of lymphokine therapy and in preventing shock.

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