IMMUNOLOGICAL STUDIES OF T-CELL RECEPTORS

I. Specifically Induced Resistance to Graft-Versus-Host Disease in Rats Mediated by Host T-Cell Immunity to Alloreactive Parental T Cells*

By DONALD BELLGRAU and DARCY B. WILSON

(From the Immunobiology Research Unit, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104)

Systemic graft-versus-host (GVH)[1] disease arising in F_1 animals injected with thymus-derived (T) lymphocytes of parental origin is a complex phenomenon (1). F_1 animals express the transplantation antigens of both parents codominantly and it is generally assumed that they are unable to reject grafts of immunocompetent parental lymphoid cells; as a consequence, they are susceptible to GVH disease. Normal adult F_1 animals are comparatively resistant to GVH disease, the infusion of large numbers of parental cells being required to elicit GVH symptoms (2). In contrast, F_1 hosts first given sublethal whole body irradiation are susceptible to much lower dosages of parental cells (2–5). There are several possible explanations that might be offered to account for the relative resistance of intact F_1 animals to GVH disease compared to irradiated recipients. It may reflect, for example, the ability of the host to develop an immune response against receptors present on donor parental strain T lymphocyte subpopulations specific for host antigens (6). For reasons of self-tolerance these receptors are considered to be lacking in the host and therefore they could be immunogenic.

Recent studies (7) supporting this argument have demonstrated that a state of specific resistance to local GVH reactions can be induced in F_1 rats by prior inoculation with subclinical doses of parental strain T cells. After immunization with lymphocytes from one parental strain (A), A/B F_1 animals no longer display the expected degree of lymph node enlargement in popliteal lymph node assays when they are subsequently injected with lymphocytes of the same parental strain. This resistance to local GVH reactions is specific for the immunizing parental strain (A), since cells from the other strain parent (B) continue to cause enlarged nodes. Furthermore, it depends on the presence in the immunizing strain A lymphocyte population of T cells with competence for strain B alloantigens; lymphocytes depleted of T cells, or T cells depeleted of

* Supported by grants from the U. S. Public Health Service AI-10961, CA-15822, and CA-09140.

[1] *Abbreviations used in this paper*: Aug, August; GVH, graft-versus-host; HBSS-FCS, Hanks' phosphate-buffered saline supplemented with fetal calf serum; ICFA, incomplete Freund's adjuvant; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; TDL, thoracic duct lymphocytes.

specific alloreactivity to host alloantigen by negative selection procedures fail to induce specific local GVH resistance.

The present studies show that inoculation of F_1 animals with subclinical dosages of parental lymphocytes also induces a state of specific resistance to systemic GVH disease. This resistance affords a marked degree of protection against GHV mortality, is radioinsensitive, can be transferred adoptively to naive syngeneic F_1 hosts, is mediated by host T cells, and is directed against specific anti-major histocompatibility complex (MHC) receptors on donor T cells.

Materials and Methods

Rats. Lewis (Ag-B1), BN (Ag-B3), and L/BN F_1 hybrids were purchased from Microbiological Associates, Walkersville, Md. L.B3 rats were obtained as a gift from Dr. Charles DeWitt, University of Utah. This congenic strain carries the B3 AgB-MHC haplotype of BN rats on the Lewis background. DA (Ag-B4), August 28807 (Aug; Ag-B5), and the F_1 hybrids L/DA and DA/Aug were derived from our own colonies.

F_1 hybrid B rats were prepared by thymectomy at 6 wk of age, lethal irradiation, and reconstitution with small numbers of T-cell-deficient marrow cells (8). Lymphocyte populations from these rats are unreactive in mixed lymphocyte cultures (MLC) (9) and local GVH (9) reactions and do not respond to phytohemagglutinin (10). A control group of F_1 rats was similarly treated except that they were sham thymectomized.

Lymphocyte Populations. Lymphocytes were obtained from the peripheral blood, lymph nodes and spleen, as previously described (11). The first 24 h drainage of thoracic duct lymphocytes (TDL) was collected at 4°C into Hanks' phosphate-buffered saline supplemented with fetal calf serum (2% vol/vol, HPBS-FCS) and heparin (10 U/ml). Thoracic duct cannulations were performed as recently redescribed by Ford and Hunt (12).

Nylon wool purification of splenic lymphocytes utilized slight modifications of the procedure described by Julius et al. (13). Nylon wool was boiled in distilled water for 1 h before packing 1.2-g portions of wet wool into 20 cc syringe barrels to the 12 cc level. 300×10^6 NH_4Cl-treated splenic lymphocytes, in a total vol of 3 ml medium (RPMI-1640, supplemented with FCS (10% vol/vol) and Hepes buffer (1 mM) Microbiological Associates followed by 3 ml of medium were added drop by drop to the column. The column was then incubated for 1 h at 37°C. Nonadherent cells were collected by the drop addition of 30 ml of RPMI-FCS.

Negatively selected L T-cell populations, specifically depleted of alloreactivity to BN alloantigens, were prepared as described elsewhere (14). These TDL populations consist predominantly (> 95%) of T cells and are unreactive to BN alloantigens in MLC (10), local GVH reactions (10, 14), and, as demonstrated in Table VII, do not induce systemic GVH disease in irradiated L/BN rats. Their response to third party MHC alloantigens remains normal on a cell-for-cell basis.

GVH Mortality Assays. F_1 rats to be tested for susceptibility to systemic GVH disease were given sublethal whole body irradiation (450 rads). Parental cells were then injected immediately or on the day after irradiation. To eliminate cage effects, control animals expected to die, and experimental animals expected to live were mixed in the same cage. Animals succumbing to GVH disease usually died between 14 and 21 days; none lived longer than 30 days. Therefore, animals living longer than 60 days were scored as survivors.

Antisera. Rabbit anti-rat T-cell and B-cell sera were prepared and tested by procedures described elsewhere (15). Alloantisera (Lewis anti-BN) were raised by subcutaneous injection of 100×10^6 BN lymphocytes in complete Freund's adjuvant followed 2 wk later by a similar dose in incomplete Freund's adjuvant (ICFA). 1 wk before bleeding by cardiac puncture the rats were boosted with 100×10^6 BN lymphocytes in ICFA.

For bulk treatment of cells with sera, 300×10^6 nylon wool nonadherent splenic lymphocytes were suspended in 30 ml of RPMI-1640 (supplemented with penicillin, streptomycin, and glutamine) to which was added 3 ml of normal guinea pig serum, as a source of complement, and antisera (0.6 ml of Lewis anti-BN alloantibody, or 0.12 ml anti-B-cell serum or 0.15 ml anti-T-cell serum). The titers of the antisera were obtained by microcytotoxicity (11). The volumes of sera

TABLE I
Mortality Due to Systemic GVH Disease in L/BN Rats Inoculated With TDL From L or BN Donors

Treatment of F_1 recipients		Mortality (dead/total) after inoculation with TDL	
X-Irradiation	TDL injected $\times 10^6$	L	BN
–	200	2/5	
	100	0/5	
450 rads	100	24/24	4/4
	30–100	4/4	8/8
	20	4/4	5/5
	10	10/10	3/5

added for bulk treatment indicated above thus represent a final concentration calculated to be twice that of the last dilution which kills 100% of the cells. The assays were incubated for 1 h at 37°C with gentle agitation every 15 min. Viability was then assessed by trypan blue exclusion. Parallel controls using guinea pig complement or antisera alone were included.

Results

Susceptibility of Irradiated Adult F_1 Rats to Systemic GVH Disease. The mortality in irradiated adult F_1 rats caused by systemic GVH disease is shown in Table I. Various numbers of parental strain L or BN TDL were injected intravenously into L/BN rats that had been given 450 rads whole body irradiation 1–3 days earlier. As few as 10×10^6 L strain or 20×10^6 BN strain TDL proved to be lethal to irradiated F_1 rats within a 2–3 wk period. Animals with systemic GVH disease regularly die 14–21 days after injection with parental cells. Before death, they exhibit some of the classic signs of systemic GVH disease (5): reddening of ears and skin around the eyes, desquamation and swelling of feet, and they display a hunched posture and a wobbling gait.

Resistance to Lethal GVH Disease Induced by Inoculation of Parental Lymphocytes. L/BN F_1 rats were injected i.v. with TDL from parental strain donors (30×10^6 from L, or 50×10^6 from BN) or left untreated as controls. After 7 days they were irradiated (450 rads) and then injected i.v. with TDL from either parental strain in a dose which ordinarily causes lethal GVH disease.

The results of several experiments (Table II) indicate that inoculation of parental lymphocyte populations into F_1 rats before irradiation affords a marked degree of protection against lethal effects of systemic GHV disease. Furthermore, they display none of the clinical symptoms of GVH disease indicated above. The resistance appears to be quite specific; L/BN F_1 animals pretreated with L lymphocytes, for example, remain susceptible to GVH disease caused by lymphocytes from BN strain donors.

Table III shows the results of similar experiments conducted with other rat strain combinations.

Adoptive Transfer of GVH Resistance to Irradiated F_1 Rats. Whether resistance to GVH disease in A/B F_1 hosts is mediated by cells or cell products of parental (A) origin or of host origin (A/B) is an important point to establish in understanding the underlying mechanisms of GVH resistance. To approach

TABLE II
Specifically Induced Resistance to Lethal GVH Disease

Pretreatment of L/BN F_1 hosts*	Mortality‡ (dead/total) after inoculation with lymphocytes (≥ 30 × 10^6) from			
	L	BN	L/BN	(No cells)
–	29/29	7/7	0/3	0/4
L	7/56	15/15	0/6	0/6
BN	9/9	0/7	ND	ND

* All F_1 hosts received 450 rads X-irradiation; where indicated these were injected i.v. with lymphocytes from parental animals (30 × 10^6 Lewis or 50 × 10^6 BN) 7 days before irradiation.

‡ These results are derived from several experiments in which systemic GVH disease was induced with 30–500 × 10^6 parental TDL. Within this range of cells there was no difference in the incidence of mortality, although the onset of mortality occurred somewhat later with lower dosages.

this question directly, it was necessary to develop an adoptive transfer system whereby naive, irradiated A/B F_1 animals could be protected against the lethal GVH effects of parental A strain lymphocytes by the concommitant administration of lymphoid cells from resistant A/B F_1 rats.

Table IV shows that 10 × 10^6 L TDL or 20 × 10^6 BN TDL injected together with 50–400 × 10^6 splenic cells from normal L/BN donors results in lethal GVH disease in irradiated L/BN rats. However, if splenic cells from F_1 donors pretreated with L lymphocytes are cotransferred to irradiated F_1 recipients along with GVH-inducing TDL suspensions, these animals are specifically resistant to GVH disease caused by L TDL, but remain susceptible to BN TDL. In addition, spleen cells from L/BN donor animals that were injected with L TDL and then irradiated (450 rads) also conferred some degree of resistance to GVH disease in secondary F_1 recipients.

The resistance conferring cells in the F_1 spleen cell population were fractionated in various ways to determine whether the adoptive transfer of GVH resistance is mediated by cells of parental or of F_1 host origin, and whether they are T cells or B cells. The results (Table V) show the following: (*a*) when separated into nylon wool adherent and nonadherent fractions, the latter were most effective, and (*b*) when nylon wool nonadherent cells were further treated with alloantibody specific for host alloantigens with C′ or cytotoxic anti-T-cell serum with or without C′, but not with anti-B-cell serum, the ability to transfer GVH resistance was abolished. These findings clearly indicate that the relevant cell population mediating GVH resistance consists of T cells of F_1 origin, and provide strong support for the possibility that GVH resistance reflects an immune response induced in the primary F_1 host against parental strain lymphocytes.

Adoptive Transfer of GVH Resistance to L/BN B Cell Rats. The studies described above show that specific resistance to systemic GVH disease induced by immunization of F_1 rats with parental lymphocytes before irradiation is mediated by a radioresistant population of host T cells. Furthermore, they suggest that the cellular precursors involved in the generation of GVH resist-

TABLE III
Induced GVH Resistance in Various F_1 Hybrid Rats

Recipients (P1/P2)	Parental cells injected before X-irradiation (P1)	Mortality* after innoculation with parental cell	
		(P1)	(P2)
		(L)	(DA)
L/DA	–	10/10	5/5
	30×10^6 L	2/12	5/5
		(Aug)	(DA)
Aug/DA	–	4/4	6/6
	50×10^6 DA	4/4	3/8

* 50×10^6 parental TDL injected 2 days after 450 rads irradiation.

TABLE IV
Adoptive Transfer of Resistance to Systemic GVH Disease

GVH induced with*	Status of F_1 spleen donors‡	Mortality (dead/total) number ($\times 10^6$) F_1 spleen cells in adoptive transfer					
		10	25	50	100	200	400
L TDL	Normal			10/10	12/12	6/6	4/4
BN TDL	Normal			11/11	4/4	3/3	
L TDL	L TDL			16/61	6/41	0/6	
BN TDL	L TDL			11/11	5/5	3/3	
L TDL	Normal-x	3/3	5/5				
L TDL	L TDL-x	3/5	2/6				

* $10–20 \times 10^6$ L or BN TDL injected into × 450 rads L/BN rats to cause GVH disease.
‡ Normal, not injected; L TDL, given 30×10^6 L TDL 10 days before cell transfer; normal-x, given 450 rads 3 days before transfer; L TDL-x, given 30×10^6 L TDL and irradiated (450 rads) 7 days later, 3 days before cell transfer.

ance are radiosensitive T lymphocytes, thus acounting for the comparative ease with which GVH disease can be induced in irradiated animals.

If GVH resistance is mediated by host T cells, it follows that adult thymectomized, lethally-irradiated, marrow reconstituted, stable F_1 B-cell rats, devoid of detectable T-cell functions (see Materials and Methods), should be particularly vulnerable to systemic GVH disease. This prediction was tested directly in the following experiment.

Table VI shows that (*a*) F_1 B-cell rats, but not sham-thymectomized control animals, are very susceptible to lethal GVH disease without any further irradiation, (*b*) this GVH susceptibility can be reversed by cotransfer of large numbers of syngeneic TDL, and (*c*) a spleen cell population capable of transferring GVH resistance adoptively cannot be generated in F_1 B rats.

Immunization with Alloreactive Parental T Cells. The induction of specific resistance to systemic GVH disease by administering parental strain lymphocytes before irradiation can be accounted for by one or more of a variety of mechanisms. One obvious possibility is the potential immunogenicity of parental T-cell receptors, lacking in the F_1 host, which are specific for host alloanti-

TABLE V

Adoptive Transfer of GVH Resistance to X-Irradiated L/BN F_1 Rats with Fractionated L/BN F_1 Spleen Cells

L/BN F_1 spleen populations		Mortality (dead/total) caused by L TDL‖ ($\times 10^6$) F_1 spleen cells transferred			
Status*	Treatment‡	25§	50	100	200
Normal	NH_4Cl		3/3	4/4	
L TDL		2/3	1/8	4/18	
Normal	Adherent				3/3
L TDL		3/3	4/7	0/9	
Normal	Nonadherent				4/4
L TDL		0/3	0/14	0/23	0/4
Normal	Anti-BN alloantibody			3/3	
	Anti-BN + C′			3/3	
L TDL	Anti-BN alloantibody			0/6	
	Anti-BN + C′			6/6	
	Anti-B-cell serum			2/8	
	Anti-B + C′		1/2	2/6	
	Anti-T-cell serum			6/6	
	Anti-T + C′			5/5	
	− C′			1/6	

* F_1 donor rats were either normal or injected with 30×10^6 L TDL 10 days before cell transfer.

‡ All populations passed through nylon wool were pretreated with NH_4Cl; Nonadherent cells were used for antiserum studies.

§ No. cells transferred refers to the number of live cells before a particular treatment. Cells treated with antisera alone, or with C′ alone were ≥ 95% viable; anti-BN alloantibody + C′ 0% viable; anti-B + C′ 90% viable; anti-T + C′ 10% viable. After treatment with anti-B + C′ aliquots of this suspension were retested with anti-B + C′ showing no further loss of viability, or with anti-T + C′, showing 0% viability.

‖ 10×10^6 L TDL injected into × 450 rads L/BN rats to cause GVH disease.

gens. Thus, immunization of F_1 animals with such parental T-cell populations could elicit a protective anti-receptor immunity directed to those parental T-cell clones responsible for GVH reactivity. As an alternative general explanation, GVH resistance induced with parental lymphoid cells could reflect a host immunity against recessive parental strain alloantigens not expressed in the host (16, 17).

According to the first model for GVH resistance, negatively selected parental T-cell populations of one strain, A, specifically depleted of immune reactivity to alloantigens (A_{-B}) of a second strain, B, should fail to induce GVH resistance in A/C animals. (A, B, and C express different MHC haplotypes and also differ by multiple minor histocompatibility loci.) Thus, if GVH resistance reflects a host immunity against recessive parental strain alloantigens, negatively selected A_{-B} lymphocyte populations should continue to express such antigens and they should be as effective as unselected A lymphocytes in inducing GVH resistance in A/C F_1 rats.

To test this model directly, populations of L TDL were negatively selected to BN alloantigens by acute recirculation through irradiated L/BN hosts. The specificity of negative selection in these TDL populations (L_{-BN}) consisting

TABLE VI
Adoptive Transfer of GVH Resistance in L/BN B-Cell Rats or in Irradiated L/BN Recipients

F_1* cells used for adoptive transfer		F_1 Recipients‡	Mortality§ (dead/total) after GVH induced with parental TDL
–		L/BN Sham	0/6
–		L/BN B	12/12
L/BN Normal	1.5×10^9 TDL	L/BN B	0/4
	1.0×10^9 TDL	L/BN B	1/4
	0.5×10^9 TDL	L/BN B	4/4
	0.25×10^9 TDL	L/BN B	2/2
L/BN B α-L	0.1×10^9 Spleen	L/BN-x	7/7
L/BN sham α-L	0.1×10^9 Spleen	L/BN-x	1/6

* L/BN B α-L, L/BN F_1 B cell rat immunized with 30×10^6 L TDL 10 days before cell transfer; L/BN sham α-L, sham Tx control rats similarly immunized (see Materials and Methods).

‡ F_1 recipients were L/BN F_1 B cell rats or sham Tx control animals without any further irradiation, or normal F_1 rats irradiated with 450 rads.

§ GVH mortality induced with 30×10^6 L TDL in F_1 B-cell rats, and 10×10^6 L TDL in irradiated F_1 recipients.

TABLE VII
GVH Mortality Induced with Negatively Selected L_{-BN} TDL Populations in L/BN and L/DA Rats

Parental cells injected*	Mortality (dead/total) recipients	
	L/BN	L/DA
L TDL	4/4	10/10
L_{-BN} TDL	0/5	4/4

* 30×10^6 TDL injected 3 days after 450 rads X-irradiation.

mostly of T cells, was demonstrated in MLC (data not shown), and in systemic GVH reactions. These cells failed to cause GVH disease in irradiated L/BN rats when administered in numbers (30×10^6) which proved to be lethal in irradiated L/DA hosts (Table VII).

These same negatively selected T-cell populations (L_{-BN}) were tested for their ability to induce GVH resistance in L/BN and L/DA rats to L TDL. The results (Table VIII) clearly demonstrate the failure of L_{-BN} TDL to protect L/BN rats against L TDL despite their continued ability to induce GVH resistance to L TDL in L/DA rats. This finding demonstrates the requirement for alloreactive T lymphocytes in the immunizing parental lymphocyte populations and provides strong support for the premise that induced resistance to systemic GVH disease involves an immunity directed to parental T-cell receptors.

Evidence that Induced Resistance to GVH Disease is Directed to Anti-MHC Receptors on Parental T Cells. The results of the previous experiment indicate that the relevant antigen(s) on the immunizing parental lymphoid population used to induce GVH resistance is clonally distributed and that most likely it represents the parental T-cell receptors specific for host alloantigens. The receptors involved in the immunizing population are likely specific for host

TABLE VIII
Systemic GVH Resistance Induced with Negatively Selected L_{-BN} TDL Populations

Immunizing parental cell population*	Recipients	GVH Mortality caused by L TDL‡ (dead/total)
L_{-BN}	L/BN	6/6
L	L/BN	0/9
L_{-BN}	L/DA	0/4
L	L/DA	2/8

* 30×10^6 normal (L) or negatively selected (L_{-BN}) TDL injected 7 days before 450 rads whole body X-irradiation.
‡ 50×10^6 L TDL injected 3 days after irradiation to cause GVH disease.

TABLE IX
GVH Resistance Tested with Parental TDL from Congenic Donors

F_1 Recipients* ($\times$ 450 rads)	Mortality (dead/total) after GVH induced with:‡		
	BN	L	L.B3
L/BN Normal	7/7	7/7	4/4
L/BN Anti-BN	0/7	7/7	1/6

* Recipients ± immunized with 30×10^6 BN TDL and irradiated 7 days later.
‡ 50×10^6 TDL injected to cause GVH disease 1 day after irradiation.

MHC alloantigens but they also may be specific for one or more of the various minor alloantigens of the host.

The following experiment (Table IX) demonstrates that GVH resistance in F_1 animals is directed at least in part against parental T-cell clones specific for host MHC alloantigens. Three TDL populations were used to cause GVH disease: (*a*) BN, potentially reactive to B1 MHC and weak alloantigens of the Lewis genome; (*b*) L, potentially reactive to B3 MHC and weak alloantigens of BN; and (*c*) L.B3, potentially reactive to B1 MHC of Lewis, and BN minor alloantigens. Recipient L/BN rats were immunized with BN TDL, and tested for GVH resistance with these three TDL populations. As expected, they were resistant to GVH caused with BN TDL, but not with L TDL. In addition, they were also resistant to GVH disease caused by L.B3 TDL populations, showing that the suppression of GVH reactivity was directed at anti-B1 T-cell clones.

Discussion

In previous studies (7) we demonstrated that F_1 rats injected systemically with lymphocytes from parental strain donors no longer display the usual local popliteal lymph node GVH response to footpad injections of parental T cells from the same strain. This state of GVH resistance is highly specific for lymphocytes of the parental strain used to induce it, it is rapid in onset, and it depends on the presence of alloreactive T cells in the pretreatment inoculum. While it seemed likely that suppressed GVH reactivity represented a cell-

mediated immune phenomenon of some kind, it was not clear at the time whether it was mediated by cells of donor or of host origin; nor was it possible to determine what the target of this immunosuppressed GVH reactivity might be. These questions have now been resolved in the present studies which show that GVH resistance represents an immune response against specific alloreactive parental T lymphocytes mediated by host T cells.

Several findings directly demonstrate the importance of host T cells both in the induction of a state of GVH resistance in adult F_1 rats as well as in its expression. First, both F_1 B-cell rats and irradiated F_1 rats are quantitatively more vulnerable to systemic GVH disease than normal, intact F_1 recipients, and this extreme sensitivity to GVH disease is markedly reversed by the adoptive transfer of normal syngeneic TDL (Table VI). Second, a specific state of resistance to systemic GVH disease directed against lymphoid cells of one parental strain (A), but not to the other (B), can be adoptively transferred to naive, syngeneic F_1 recipients (A/B) from specifically immunized F_1 donors (A/B anti-A) (Table IV). Successful transfers of GVH resistance in this setting are abolished by treatment of the F_1 spleen cell population with cytotoxic antisera specific for host alloantigens and for T-cell surface markers in the presence of complement. Why anti-T sera in the absence of complement also effectively abolishes adoptive transfer of GVH resistance is not totally clear, however, it might indicate the opsonization in vivo of T-cell subpopulations coated with xenogeneic rabbit immunoglobulins (18). Third, spleen cells from B-cell rats immunized with parental T cells are not effective in adoptive transfer of GVH resistance (Table VI).

The results of this study also show that the immunogen responsible for inducing GVH resistance maps with functional alloreactivity of parental T cells, and thus it is presumed to be the receptors on parental T cells specific for host alloantigens. This conclusion derives from experiments designed to exclude the alternative possible explanation of a host-mediated immunity directed to ubiquitous, nonclonally distributed, surface antigens unique to parental cells, for example, recessively expressed alloantigens, or other surface structures associated with endogenous viruses peculiar to the immunizing parental strain. Negatively selected parental TDL populations (L_{-BN}), consisting predominantly (> 95%) of T cells specifically depleted of alloreactivity to BN alloantigens (Table VII), fail to induce GVH resistance to parental (L) lymphocytes in L/BN F_1 rats, but do so in L/DA F_1 recipients (Table VIII). This finding indicates that the relevant immunogen is present on alloreactive L T lymphocytes and that it is most probably the T-cell receptor(s) specific for BN alloantigens of the host.

That the immunogenic parental T-cell receptors include those specific for host MHC alloantigens of the Ag-B locus is demonstrated with the use of an MHC congenic strain. L/BN F_1 rats immunized with BN lymphocytes, a population containing anti-B1 receptors specific for L MHC antigens of the Ag-B1 haplotype and receptors specific for L minor alloantigens as well, suppress the potential GVH reactivity of L.B3 lymphocytes (Table IX). This second parental cell population is presumed to have receptors specific for Ag-B1 MHC alloantigens, but none specific for L minor alloantigens for reasons of self tolerance. It should be noted that the suppression of alloreactivity of both BN and L.B3 T cells in L/

BN rats immunized with BN lymphocytes implies a marked similarity, possibly shared idiotypy, of the BN anti-B1 and L anti-B1 T-cell receptors.

Anti-idiotypic immune responses against receptors of alloreactive T cells have been demonstrated directly in some studies (19–22) and inferred in others (7, 23–25). Some of these involve the production in F_1 animals of anti-idiotypic antibodies specific for anti-MHC receptors present on parental T cells and for the variable region idiotypes on alloantibody immunoglobulin molecules (22, 26, 27). Others involve immunization of parental strain animals with autologous anti-MHC blasts raised in MLC resulting in the production of killer/suppressor T-cell populations which inhibit specific MHC responses (28). To date, however, all of these anti-idiotypic responses require extensive and repeated immunizations, most in the presence of adjuvants, and many of these studies have been difficult to reproduce in other laboratories.

In this respect the present findings as well as those of our previous study are of particular interest. F_1 animals immunized with as few as 1×10^6 parental T cells and challenged 3 days later show reduced local popliteal lymph node reactions (11). Moreover, immunization of F_1 animals (which if unirradiated are quite resistant to systemic GVH disease) as few as 1–3 days prior to sublethal irradiation renders them resistant to systemic GVH disease caused by multilethal doses of parental lymphocytes (data not shown). The basis for this rapid onset of radioresistant specific immunity to alloreactive parental T cells is the subject of continuing study; it seems to reflect an ongoing process in the adult F_1 animal requiring the presence of an intact thymus.

Summary

The present studies extend our previous efforts to understand the immunological basis of specifically induced graft-versus-host (GVH) resistance in F_1 hybrid rats. Immunization of F_1 rats with alloreactive T-cell populations of parental strain origin induces a host-mediated T-cell response which is specific for anti-major hostocompatibility complex receptors on parental T cells. This protective immunity is rapid in onset and once induced, it provides a highly effective, specific resistance to lethal GVH disease which is radioresistant and can be adoptively transferred to syngeneic recipients.

We wish to thank Dianne Wilson, Mike Merva, and Karen Nowell for their technical assistance and Karen Nowell for preparation of the manuscript. We are especially grateful to Doctors Jonathan Sprent and Robert Woodland for suggestions concerning the conduct of these studies.

Received for publication 3 April 1978.

References

1. Elkins, W. L. 1971. Cellular immunology and the pathogenesis of GVH reactions. *Prog. Allergy*. **15**:78.
2. Gowans, J. L. 1962. The fate of parental strain small lymphocytes in F_1 hybrid rats. *Ann. N. Y. Acad. Sci.* **99**:432.
3. Schwartz, E. E., A. C. Upton, and C. C. Gongdon. 1957. A fatal reaction caused by implantation of adult parental spleen tissue in irradiated F_1 mice. *Proc. Soc. Exp. Biol. Med.* **96**:797.

4. Cole, L. J., and M. E. Ellis. 1958. Delayed deaths in sublethally x-rayed F_1 hybrid mice injected with parental strain spleen cells. *Science.* (*Wash. D. C.*). 128:32.
5. Field, E. O., and J. E. Gibbs. 1965. Affects of thymectomy and irradiation on graft versus host disease. *Transplantation* (*Baltimore*). 3:634.
6. Ramseier, H., and J. Lindenmann. 1969. F_1 hybrid animals: reactivity against recognition structures of parental strain lymphoid cells. *Pathol. Microbiol.* 34:379.
7. Woodland, W. T., and D. B. Wilson. 1977. The induction of specific resistance in F_1 hybrid rats to local graft versus host reactions: nature of the eliciting cell. *Eur. J. Immunol.* 7:136.
8. Howard, J. C. 1972. The lifespan and recirculation of marrow derived lymphocytes from the rat thoracic duct. *J. Exp. Med.* 135:185.
9. Howard, J. C., and D. B. Wilson. 1974. Specific positive selection of lymphocytes reactive to strong histocompatibility antigens. *J. Exp. Med.* 140:660.
10. Wilson, D. B., A. Marshak, and J. C. Howard. 1976. Specific positive and negative selection of rat lymphocytes reactive to strong histocompatibility antigens: activation with alloantigens in vitro and in vivo. *J. Immunol.* 34:1030.
11. Marshak, A., P. C. Doherty, and D. B. Wilson. 1977. The control of specificity of cytotoxic T lymphocytes by the major histocompatibility complex in rats and identification of a new alloantigen system showing no Ag-B restriction. *J. Exp. Med.* 146:1773.
12. Ford, W. L., and S. J. Hunt. 1973. *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications, Ltd. Oxford, England. 2nd edition. Chapter 23.
13. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
14. Ford, W. L., and R. C. Atkins. 1971. Specific unresponsiveness of recirculating lymphocytes after exposure to histocompatibility antigen in F_1 hybrid rats. *Nat. New Biol.* 234:178.
15. Howard, J. C., and D. W. Scott. 1974. The identification of sera distinguishing thymus derived and marrow derived thoracic duct lymphocytes in the rat. *Immunology.* 27:903.
16. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F_1 hybrid mice. *J. Exp. Med.* 134:1513.
17. Shearer, G. M., and G. Cudkowicz. 1975. Induction of F_1 hybrid antiparental cytotoxic effector cells. An in vitro model for hemopoietic histoincompatibility. *Science* (*Wash. D. C.*). 190:890.
18. Martin, W. J. 1969. Assay for the immunosuppressive capacity of antilymphocyte serum. I. Evidence for opsonization. *J. Immunol.* 5:979.
19. McKearn, T. J. 1973. Antireceptor antiserum causes specific inhibition of reactivity to rat histocompatibility antigens. *Science* (*Wash. D. C.*). 183:94.
20. Krammer, P. H., and K. Eichmann. 1977. T cell receptor idiotypes are controlled by genes in the heavy chain linkage group and the major histocompatibility complex. *Nature* (*Lond.*). 270:733.
21. Binz, H., and H. Wigzell. 1975. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. III. Physical fractionation of specific immunocompetent T lymphocytes by affinity chromatography using anti-idiotypic antibodies. *J. Exp. Med.* 145:1231.
22. Binz, H., and B. A. Askonas. 1975. Inhibition of mixed leucocyte cultures by anti-idiotypic antibodies. *Eur. J. Immunol.* 5:618.
23. McCullagh, P. 1973. Modification of the responsiveness of F_1 hybrid rats to lympho-

cytes from parental strain rats. *Aust. J. Exp. Biol. Med. Sci.* 51:753.

24. McCullagh, P. 1977. Anti-parental strain lymphocyte responsiveness of F_1 hybrid strain lymphocytes. *Scand. J. Immunol.* 6:967.
25. McKearn, T. J., Y. Yamada, F. P. Stuart, and F. W. Fitch. 1974. Antireceptor antibody and resistance to graft versus host disease. *Nature (Lond.).* 251:648.
26. Binz, H., J. Lindenmann, and H. Wigzell. 1973. Inhibition of local graft versus host reaction by anti-alloantibodies. *Nature (Lond).* 246:146.
27. Krammer, P. H. 1978. Alloantigen receptors on activated T cells, in mice. I. Binding of alloantigens and anti-idiotypic antibodies to the same receptors. *J. Exp. Med.* 147:25.
28. Binz, H., and H. Wigzell. 1977. Induction of specific immune unresponsiveness with purified mixed leucocyte culture activated T lymphoblasts as autoimmunogen. II. Proof for the existence of autoanti-idiotypic killer T cells and transfer of suppression to normal syngeneic recipients by T or B lymphocytes. *J. Exp. Med.* 147:163.