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*

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Systemic graft-versus-host (GVH) disease arising in F1 animals injected with thymus-derived (T) lymphocytes of parental origin is a complex phenomenon (1). F1 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 F1 animals are comparatively resistant to GVH disease, the infusion of large numbers of parental cells being required to elicit GVH symptoms (2). In contrast, F1 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 F1 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 F1 rats by prior inoculation with subclinical doses of parental strain T cells. After immunization with lymphocytes from one parental strain (A), A/B F1 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 depleted of
specific alloreactivity to host alloantigen by negative selection procedures fail to induce specific local GVH resistance.

The present studies show that inoculation of F1 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 GVH mortality, is radioinsensitive, can be transferred adoptively to naive syngeneic F1 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 F1 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 F1 hybrids L/DA and DA/ Aug were derived from our own colonies.

F1 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 F1 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 re-described 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 x 10⁶ NH,Cl-treated splenic lymphocytes, in a total vol of 3 ml medium (RPMI-1640, supplemented with FCS (10% vol/vol) and Hapes buffer (1 mM) Microbiological Associates followed by 3 ml of medium were added drop by drop to the column. The ω 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. F1 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 x 10⁶ 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 x 10⁶ BN lymphocytes in ICFA.

For bulk treatment of cells with sera, 300 x 10⁶ 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 F₁ recipients | Mortality (dead/total) after inoculation with TDL |
|---------------------------|-----------------------------------------------|
|                          | L     | BN |
| X-Irradiation \( \times 10^6 \) |       |    |
| 200                      | 2/5   |    |
| 100                      | 0/5   |    |
| 450 rads                 | 24/24 | 4/4 |
| 30-100                   | 8/8   |    |
| 20                       | 4/4   | 5/5 |
| 10                       | 10/10 | 3/5 |

Results

Susceptibility of Irradiated Adult F₁ Rats to Systemic GVH Disease. The mortality in irradiated adult F₁ 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 \( 10 \times 10^6 \) L strain or \( 20 \times 10^6 \) BN strain TDL proved to be lethal to irradiated F₁ 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 F₁ rats were injected i.v. with TDL from parental strain donors (\( 30 \times 10^6 \) from L, or \( 50 \times 10^6 \) 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 F₁ rats before irradiation affords a marked degree of protection against lethal effects of systemic GVH disease. Furthermore, they display none of the clinical symptoms of GVH disease indicated above. The resistance appears to be quite specific; L/BN F₁ 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 F₁, Rats. Whether resistance to GVH disease in A/B F₁ 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
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TABLE II
Specifically Induced Resistance to Lethal GVH Disease

| Pretreatment of | Mortality† (dead/total) after inoculation with lymphocytes (≥ 30 × 10⁶) from | L    | BN  | L/BN (No cells) |
|-----------------|-------------------------------------------------|------|-----|-----------------|
| L/BN F₁ hosts* |                                  | 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 F₁ hosts received 450 rads X-irradiation; where indicated these were injected i.v. with lymphocytes from parental animals (30 × 10⁶ Lewis or 50 × 10⁶ BN) 7 days before irradiation.
† These results are derived from several experiments in which systemic GVH disease was induced with 30–500 × 10⁶ 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 F₁ animals could be protected against the lethal GVH effects of parental A strain lymphocytes by the concomitant administration of lymphoid cells from resistant A/B F₁ rats.

Table IV shows that 10 × 10⁶ L TDL or 20 × 10⁶ BN TDL injected together with 50–400 × 10⁶ splenic cells from normal L/BN donors results in lethal GVH disease in irradiated L/BN rats. However, if splenic cells from F₁ donors pretreated with L lymphocytes are cotransferred to irradiated F₁ 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 F₁ recipients.

The resistance conferring cells in the F₁ 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 F₁ 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 F₁ origin, and provide strong support for the possibility that GVH resistance reflects an immune response induced in the primary F₁ 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 F₁ 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 F1 Hybrid Rats

| Recipients (F1/F2) | Parental cells injected before X-irradiation (P1) | Mortality* after innoculation with parental cell (P1) (P2) |
|-------------------|-----------------------------------------------|-----------------------------------------------|
| L/DA              | -                                             | (L) (DA)                                      |
|                   | 30 x 10^6 L                                  | 2/12                                          |
| Aug/DA            | -                                             | (Aug) (DA)                                   |
|                   | 50 x 10^6 DA                                 | 4/4                                           |

* 50 x 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 F1 spleen donor† | Mortality (dead/total) number (x 10^6) F1 spleen cells in adoptive transfer |
|-------------------|----------------------------|--------------------------------------------------------------------------------|
| 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 x 10^6 L or BN TDL injected into x 450 rads L/BN rats to cause GVH disease.
† Normal, not injected; L TDL, given 30 x 10^6 L TDL 10 days before cell transfer; normal-x, given 450 rads 3 days before transfer; L TDL-x, given 30 x 10^6 L TDL and irradiated (450 rads) 7 days later, 3 days before cell transfer.

ance are radiosensitive T lymphocytes, thus accounting 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 F1 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) F1 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 F1 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 F1 host, which are specific for host alloanti-
Table V
Adoptive Transfer of GVH Resistance to X-Irradiated L/BN F1, Rats with Fractionated L/BN F1, Spleen Cells

| L/BN F1, spleen populations | Mortality (dead/total) caused by L TDL\(\times 10^6\) F1 spleen cells transferred |
|-----------------------------|---------------------------------------------------------------------|
| Status* Treatment‡ 25§       | 50 100 200                                                           |
| Normal NH₄Cl 3/3           | 4/4                                                                  |
| L TDL Normal 2/3           | 1/8 4/16                                                             |
| Normal L TDL Adherent 3/3  | 4/7 0/9 3/3                                                           |
| Normal Nonadherent 4/4      | 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                   |                                                                      |

* F1 donor rats were either normal or injected with 30 × 10⁶ L TDL 10 days before cell transfer.
† All populations passed through nylon wool were pretreated with NH₄Cl; 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' 90% 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 × 10⁶ L TDL injected into × 450 reds L/BN rats to cause GVH disease.

Thus, immunization of F1 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 F1 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

| F1* cells used for adoptive transfer | F1 Recipients† | Mortality§ (dead/total) after GVH induced with parental TDL |
|--------------------------------------|----------------|----------------------------------------------------------------|
| L/BN Normal 1.5 x 10^9 TDL           | L/BN Sham      | 0/6                                                             |
| L/BN B 12/12                          | L/BN B         | 12/12                                                          |
| L/BN Normal 1.0 x 10^9 TDL            | L/BN B         | 1/4                                                            |
| L/BN Normal 0.5 x 10^9 TDL            | L/BN B         | 4/4                                                            |
| L/BN Normal 0.25 x 10^9 TDL           | L/BN B         | 2/2                                                            |
| L/BN Normal 0.1 x 10^6 Spleen         | L/BN-x         | 7/7                                                            |
| L/BN sham α-L 0.1 x 10^6 Spleen       | L/BN-x         | 1/6                                                            |

* L/BN B α-L, L/BN F1 B cell rat immunized with 30 x 10^6 L TDL 10 days before cell transfer; L/BN sham α-L, sham Tx control rats similarly immunized (see Materials and Methods).
† F1 recipients were L/BN F1 B cell rats or sham Tx control animals without any further irradiation, or normal F1 rats irradiated with 450 rads.
§ GVH mortality induced with 30 x 10^6 L TDL in F1 B-cell rats, and 10 x 10^6 L TDL in irradiated F1 recipients.

Table VII
GVH Mortality Induced with Negatively Selected L-α-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-α-BN TDL              | 0/5                              | 4/4                             |

* 30 x 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 x 10^6) which proved to be lethal in irradiated L/DA hosts (Table VII).

These same negatively selected T-cell populations (L-α-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-α-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
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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 × 10⁶ normal (L) or negatively selected (L–BN) TDL injected 7 days before 450 rads whole body X-irradiation.
† 50 × 10⁶ L TDL injected 3 days after irradiation to cause GVH disease.

TABLE IX
GVH Resistance Tested with Parental TDL from Congenic Donors

| F₁ Recipients* (× 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 × 10⁶ BN TDL and irradiated 7 days later.
‡ 50 × 10⁶ 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 F₁ 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 F₁ 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 F₁ rats as well as in its expression. First, both F₁ B-cell rats and irradiated F₁ rats are quantitatively more vulnerable to systemic GVH disease than normal, intact F₁ 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 F₁ recipients (A/B anti-A) (Table IV). Successful transfers of GVH resistance in this setting are abolished by treatment of the F₁ 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 F₁ rats, but do so in L/DA F₁ 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 F₁ 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 idiotype, 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 F₁ 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. F₁ animals immunized with as few as 1 × 10⁶ parental T cells and challenged 3 days later show reduced local popliteal lymph node reactions (11). Moreover, immunization of F₁ 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 F₁ 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 F₁ hybrid rats. Immunization of F₁ rats with alloreactive T-cell populations of parental strain origin induces a host-mediated T-cell response which is specific for anti-major histocompatibility 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.

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