INDUCED TOLERANCE IN F₁ RATS TO ANTI-MAJOR HISTOCOMpatibility COMPLEX RECEPTORS ON PARENTAL T CELLS
Implications for Self Tolerance*

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Previous attempts to produce anti-idiotypic antibodies in F₁ rats against anti-major histocompatibility complex (MHC) receptors by immunization with populations of immunologically competent, alloreactive thymus-derived (T) lymphocytes of parental strain origin have shown, instead, that these F₁ animals produce strong T cell-mediated immune responses against anti-MHC receptors of parental T cells (1-4). Thus, inoculation of (A × B)F₁ rats with strain A lymphocytes renders these animals profoundly and specifically resistant to local graft-vs.-host (GVH) reactions (1) as well as the usually fatal systemic GVH disease caused by subsequent inoculation with strain A T cells (2, 3).

One of the particularly surprising features of specifically induced GVH resistance in F₁ rats is the ease and rapidity of its onset. A single intravenous inoculation of 30 × 10⁶ parental strain A lymphocytes (1 × 10⁶-5 × 10⁶ for T cell-enriched inocula), followed 7 d later by sublethal total body irradiation (450 rad), protects these F₁ animals from lethal GVH disease caused by doses of strain A lymphocytes (as high as 500 × 10⁶) administered the day after irradiation. Yet, such F₁ animals remain fully susceptible to GVH disease caused by low doses (10 × 10⁶-30 × 10⁶) of lymphocytes from the opposite parental strain B (2, 3). Additional adoptive-transfer studies demonstrated that the mechanism of specific GVH resistance is mediated by host T cells, and studies with congenic strains and with negatively selected T lymphocyte populations indicated that anti-MHC receptors of parental strain lymphocytes comprise the immunogen in this system (3).

The finding of the rapid onset of specific resistance to GVH disease after immunization suggested to us the possibility that this might represent a normal ongoing physiologic process, perhaps associated with the induction and/or maintenance of self tolerance to self MHC gene products. Thus, A × B animals may develop, at some stage in ontogeny, an effector T lymphocyte population with anti-MHC receptor specificity anti-(anti-a), and anti-(anti-b), which, in some way, controls the expression of T cell populations with anti-self a and anti-self b specificity. Such a possibility is also suggested by the well established, but poorly understood, finding that GVH...
disease is difficult to generate in normal intact F1 animals (3, 5); very high numbers of parental cells (>100 × 10^6) must be administered, whereas in lightly irradiated animals, much lower numbers (5 × 10^6-10 × 10^6) of parental cells will suffice (3, 6).

The present studies were conducted to explore this general model more fully, and to determine whether anti-MHC receptor-bearing T cell populations can be used to induce a state of tolerance in neonatal animals that could be reflected subsequently by an inability to induce specific GVH resistance. The results show that administering low numbers (1 × 10^6) of parental strain A T cells, or larger numbers (50 × 10^6) of parental marrow cells, to newborn (A × B)F1 rats has two important consequences for inducing GVH disease in these animals when tested later as adults: (a) the newborn rats become profoundly and specifically sensitive to fatal GVH disease, without any irradiation, caused by T cells from strain A, but not from strain B; and (b) these animals display a specific inability to develop resistance to GVH disease caused by strain A lymphocytes, although at the same time it is possible to induce GVH resistance by immunization with strain B lymphocytes.

Materials and Methods

Animals. Rats from the Lewis (L; Rt-1\(^{a}\)), DA (Rt-1\(^{b}\)), and Brown Norway (BN; Rt-1\(^{n}\)) strains and their F1 hybrids, maintained at the University of Pennsylvania, Philadelphia, Pa. were used in these studies.

Cells. Standard techniques were used for preparing lymph node (LN), thoracic duct (TDL), and bone marrow (BM) suspensions (7). Negatively selected L TDL populations, devoid of alloreactivity to BN MHC alloantigens (L-BN), were prepared by acute filtration through irradiated L/BN rats (8, 9).

GVH Resistance and Disease. Adult F1 rats (8-12 wk of age) were injected i.v. with 30 × 10^6 parental TDL or LN cells, with F1 cells, or left untreated. 7 d later, they were given 450 rad total body irradiation from a 137Cs source and injected i.v. with 30 × 10^6 parental strain lymphocytes to cause GVH disease. Animals with GVH disease routinely died within 4 wk; those showing no symptoms during this period were monitored for 1 mo longer, but none died during this time. Neonatal inoculations (0.05 ml) were given via the anterior orbital branch of the facial vein (10), within a few hours of birth.

Results

The first series of experiments were undertaken to explore the possible antigenicity of receptor-bearing parental T cell populations in terms of the potential of these cell surface markers for causing tolerance in newborn F1 animals and the consequent inability to induce specific GVH resistance. The results of several experiments with (L × BN)F1 and (L × DA)F1 rats, arranged according to treatment groups, are presented in Table I; they show the following:

(a) Groups 1 and 2 show, as before (2-4), that immunization of F1 rats with parental lymphocytes before irradiation affords a significant protective effect against otherwise supralethal numbers of lymphocytes from the same parental donor strain.

(b) Groups 3 and 4 demonstrate that inoculation of newborn F1 rats with small numbers of parental peripheral T cells or with larger numbers of marrow cells results in an inability to induce GVH resistance by subsequent immunization with parental lymphocytes as adults. Apparently, neonatal inoculation of F1 animals with parental T cells results in a specific state of tolerance to the anti-MHC receptors on these cells which is reflected in the inability to induce specific GVH resistance. In addition, these tolerant animals appear to be more vulnerable to the effects of systemic GVH disease caused by parental lymphocytes from the strain employed to induce tolerance; the
Table I

Tolerance in F1 Rats to Anti-MHC Receptors on Parental T Cells: Failure to Induce GVH Resistance

| Group | Cells injected at birth* | Recipients | Immunizing inoculation§ | GVH inoculation | GVH mortality§ |
|-------|--------------------------|------------|-------------------------|-----------------|----------------|
|       | × 10⁶                    | × 10⁸      |                         |                 |                |
| 1     | L × BN                   | 30 L       | 30 BN                   | 0/5             | 19 16-21       |
|       | L × BN                   | 30 L × BN  | 0/5                     |                 |                |
|       | L × DA                   | 30 L       | 7/7                     | 18 16-22        |
| 2     | L × BN                   | 30 L       | 30 L                    | 1/7             | 15             |
|       | L × BN                   | 30 BN      | 30 BN                   | 0/4             |                |
|       | L × DA                   | 30 L       | 30 L                    | 0/7             |                |
| 3     | 0.25-1 L T↓ cells        | L × BN     | 30 L                    | 30 L            | 13/2          |
|       | 0.25-1 L T cells         | L × BN     | 30 L                    | 30 BN           | 0/5           |
|       | 0.25-1 L T cells         | L × DA     | 30 L                    | 30 L            | 7/7           |
| 4     | 50 L BM cells            | L × BN     | 30 L                    | 30 L            | 17/22         |
| 5     | 1 L as T cells           | L × BN     | 30 L                    | 30 L            | 0/9           |
|       | 1 L as T cells           | L × DA     | 30 L                    | 30 L            | 8/8           |

* L × BN or L × DA injected with L T cells or BM within 24 h of birth.
§ Immunized at 8 wk with 30 × 10⁶ L or BN lymphocytes.
§ 7 d later, rats were given 450 rad and 30 × 10⁶ L or BN TDL to cause GVH disease; MST, median survival time, and
r given in d.

Table II

Tolerance in F1 Rats to Anti-MHC Receptors on Parental T Cells: Selective Sensitivity of Nonirradiated F1 to Systemic GVH Disease

| Group | Recipients | Cells injected at birth | Cells injected for GVH | GVH mortality |
|-------|------------|-------------------------|------------------------|---------------|
|       |            | × 10⁶                   | × 10⁶                  |               |
| 1     | L × DA     | 30 L                    | 0/6                    | 12 9-14       |
|       |            | 30 DA                   | 0/5                    |               |
| 2     | L × DA     | 50 L BM                 | 30 L                   | 6/6           |
|       |            | 30 DA                   | 0/5                    |               |
| 3     | L × DA     | 50 DA BM                | 30 L                   | 6/6           |
|       |            | 30 DA                   | 0/6                    | 11 9-24       |

* Mean survival time.

median survival time is a full week shorter than for otherwise normal F1 animals undergoing systemic GVH disease.

c Group 5 shows the specificity of tolerance induction. Lymphocyte populations from L donors negatively selected for reactivity to BN alloantigens, and hence lacking anti-BN receptor-bearing T cells, fail to induce tolerance in L × BN hosts. Therefore, it is possible to induce specific GVH resistance to L lymphocytes in these animals. L × DA newborn animals given the same inocula, on the other hand, are rendered tolerant and cannot be immunized against L T cells.

Table II demonstrates the selective sensitivity of adult F1 animals to systemic GVH disease if they have been injected neonatally with parental cells. No irradiation is
used in this experiment, and group 1 shows the nonspecific resistance of normal rats
to GVH caused by either parental (strain A or B) T cell population. Groups 2 and 3
show that this nonspecific GVH resistance is selectively abolished for parental cells of
the strain used to inject F1 animals at birth.

Discussion

These and previous studies demonstrate the expression of a marker, clonally
distributed in a particular subset of parental strain T cells having a particular anti-
MHC specificity, that can be detected by the immune response by F1 T cells to it (2–
4); this marker can be used as an immunogen or a tolerogen to specifically increase or
decrease resistance to GVH disease caused by parental T cells. The simplest and most
direct interpretation that can be placed on these findings is that T cells of F1 animals
can be specifically stimulated or, alternatively, they can be tolerized by specificity-
associated determinants (idiotypes?) present on anti-MHC receptors of parental T
cells. The consequence of activating F1 T cells to anti-host MHC receptor determinants
is to render a radioresistant immunity which affords significant protection against
lethal GVH disease in host animals; the consequence of inducing tolerance in F1
animals to these receptors is to render these animals profoundly and specifically
sensitive to lethal GVH disease caused by subsequent inoculations with T cells from
this same parental strain.

It is clear from the experiments involving neonatal inoculation with small numbers
of purified T cells that the relevant tolerogen is a marker of parental T cells. Therefore,
it seems likely that it is the same one that induces GVH resistance in adult F1 rats (3,
4). This possibility is strongly supported by the finding with negatively selected
lymphocyte populations that the tolerogenic marker is a clonally distributed one
associated with alloreactivity to a particular MHC haplotype.

In this respect, the finding that GVH-resistance tolerance can also be induced with
large numbers (50 × 10^6) of marrow cells is of particular interest. It seems likely that
the relevant marker in this case is present on contaminating subpopulations of mature
T cells (11). However, preliminary attempts to deplete marrow of such T cells by
prolonged thoracic duct drainage (3–7 d) have not eliminated the tolerance-inducing
marker. This finding raises the possibility that this marker is also present on other
marrow cell subpopulations; for example, a nonrecirculating, immature pre-T cell.

The finding that a specificity-associated parental T cell marker can induce both
specific resistance in adult F1 rats and selective tolerance to GVH resistance in
newborn rats carries implications for the poorly understood basis of self tolerance,
particularly for self MHC gene products. Three facts seem clear: (a) normal (unirra-
diated) F1 animals are quite resistant to lethal GVH disease (3, 4), (b) allospecific
parental T cells are able to induce an immunity in F1 animals that protects irradiated
F1 rats against GVH disease caused by supralethal doses of parental T cells (3, 4) and
by comparison with anti-idiotypic antibody responses, this host T cell-mediated
immunity to parental T cells is very easily and rapidly induced, and (c) clonally
distributed markers (receptors?) on parental T cells, having a particular anti-MHC
specificity, induce a specific inability to develop resistance to GVH disease, and in
fact, render these animals as sensitive to GVH disease as irradiated or T cell-depleted
animals.

From these findings, it is tempting to consider the possibility that nonspecific GVH
resistance of normal animals and specific GVH resistance of immunized, irradiated animals may reflect the existence of an already ongoing immune mechanism responsible for the suppression of anti-self MHC T cell clones, thereby providing for tolerance to self MHC gene products. This model is based on the Jerne hypothesis of the generation of T cell specificities (11). T cell clones develop in the thymus reactive to one or another of the MHC haplotypes in the species (a, b, c...). Some of these clones bearing potential for reactivity to self MHC gene products emerge from the thymus. To deal with this threat, other T cells having specificity for anti-\( a \) and anti-\( b \) receptors, and comprising a self tolerance effector mechanism, become activated and suppress in some way the expression of clones with triggerable anti-self receptors. Such an anti-idiotypic regulatory mechanism might be under constant stimulation by anti-self clones chronically emerging from the thymus in post natal life, thereby accounting for the relative GVH resistance of normal intact F1 animals, and for the rapid onset of radioreistant immune reactivity towards anti-MHC receptors from homozygous donors.

**Summary**

The immunogenicity of cell surface markers associated with specific anti-major histocompatibility complex (MHC) alloreactivity of rat peripheral T lymphocyte subpopulations has been demonstrated in the past by the ability of such cell populations to induce a profound and specific resistance to systemic graft-vs-host (GVH) disease in adult rats. Our studies demonstrate that these specificity-associated anti-MHC parental strain T cell markers are also tolerogenic; if small numbers of parental strain T cells are administered to newborn F1 rats, they result in the specific inability to induce GVH resistance later on in adult life. Moreover, unlike normal animals, these F1 rats are extremely sensitive to systemic GVH disease caused by T cells from the original donor parental strain.

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