Brief Definitive Report

INDUCED TOLERANCE IN F_1 RATS TO ANTI-MAJOR HISTOCOMPATIBILITY COMPLEX RECEPTORS ON PARENTAL T CELLS

Implications for Self Tolerance*

By D. BELLGRAU,‡ D. SMILEK, and D. B. WILSON

From the Division of Research Immunology, Department of Pathology, University of Pennsylvania School of Medicine; and The Wistar Institute, Philadelphia, Pennsylvania 19104

Previous attempts to produce anti-idiotypic antibodies in F_1 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 F_1 animals produce strong T cell-mediated immune responses against anti-MHC receptors of parental T cells (1-4). Thus, inoculation of (A × B)F_1[1] 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 F_1 rats is the ease and rapidity of its onset. A single intravenous inoculation of 30 × 10^6 parental strain A lymphocytes (1 × 10^6–5 × 10^6 for T cell-enriched inocula), followed 7 d later by sublethal total body irradiation (450 rad), protects these F_1 animals from lethal GVH disease caused by doses of strain A lymphocytes (as high as 500 × 10^6) administered the day after irradiation. Yet, such F_1 animals remain fully susceptible to GVH disease caused by low doses (10 × 10^6–30 × 10^6) 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

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‡ Present address: Basel Institute for Immunology, 487 Grenzacherstrasse, CH4005 Basel, Switzerland.

[1] For convenience, A, B, C... designate different homozygous strains of animals that differ with respect to a variety of loci, but in particular, they express different haplotypes—*a*, *b*, *c*...—of the MHC.

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disease is difficult to generate in normal intact F_1 animals (3, 5); very high numbers of parental cells ($>100 \times 10^6$) 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)F_1 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^l), DA (Rt-1^a), and Brown Norway (BN; Rt-1^n) strains and their F_1 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 F_1 rats (8–12 wk of age) were injected i.v. with 30×10^6 parental TDL or LN cells, with F_1 cells, or left untreated. 7 d later, they were given 450 rad total body irradiation from a ^{137}Cs 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 F_1 animals and the consequent inability to induce specific GVH resistance. The results of several experiments with (L × BN)F_1 and (L × DA)F_1 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 F_1 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 F_1 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 F_1 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 F_1 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§		
					Dead/total	MST	r
	× 10^6		× 10^6	× 10^6			
1		L × BN		30 L	11/11	18	15–21
		L × BN		30 BN	9/9	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	13/21	12	9–15
	0.25–1 L T cells	L × BN	30 BN	30 BN	0/5		
	0.25–1 L T cells	L × DA	30 L	30 L	7/7	11	9–24
4	50 L BM cells	L × BN	30 L	30 L	17/21	9	9–15
5	1 L_{-BN} T cells	L × BN	30 L	30 L	0.9		
	1 L_{-BN} T cells	L × DA	30 L	30 L	8/8	12	10–16

* L × BN or L × DA injected with L T cells or BM within 24 h of birth.

‡ Immunized at 8 wk with 30 × 10^6 L or BN lymphocytes.

§ 7 d later, rats were given 450 rad and 30 × 10^6 L or BN TDL to cause GVH disease; MST, median survival time, and r given in d.

‖ TDL populations were highly enriched for T cells (>95%) by passage through an irradiated syngeneic host (11). Because the modal blood to lymph transit time is shorter for T cells than for B cells, TDL populations collected 6–24 h posttransfer are virtually free of B cells (9).

TABLE II

Tolerance in F_1 Rats to Anti-MHC Receptors on Parental T Cells: Selective Sensitivity of Nonirradiated F_1 to Systemic GVH Disease

Group	Recipients	Cells injected at birth	Cells injected for GVH	GVH mortality		
				Dead/total	MST*	r
		× 10^6	× 10^6			
1	L × DA		30 L	0/6		
			30 DA	0/5		
2	L × DA	50 L BM	30 L	6/6	12	9–14
			30 DA	0/5		
3	L × DA	50 DA BM	30 L	0/6		
			30 DA	6/6	11	9–24

* Mean survival time.

median survival time is a full week shorter than for otherwise normal F_1 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 F_1 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 F_1 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 F_1 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 F_1 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 F_1 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 F_1 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 F_1 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 F_1 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 (unirradiated) F_1 animals are quite resistant to lethal GVH disease (3, 4), (*b*) allospecific parental T cells are able to induce an immunity in F_1 animals that protects irradiated F_1 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 specificity (12). 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 F_1 animals, and for the rapid onset of radioresistant 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 F_1 rats, they result in the specific inability to induce GVH resistance later on in adult life. Moreover, unlike normal animals, these F_1 rats are extremely sensitive to systemic GVH disease caused by T cells from the original donor parental strain.

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