

ANALYSIS OF T CELL POPULATIONS THAT INDUCE AND MEDIATE SPECIFIC RESISTANCE TO GRAFT-VERSUS-HOST DISEASE IN RATS

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Previous studies (1) have shown that F₁ rats (A/B) that recover from graft-versus-host (GVH)¹ disease that was deliberately induced with parental (A) T cells become profoundly and specifically resistant to GVH disease, revealed by subsequent reinoculation of T cells from the same parental strain. Specific GVH resistance appears to be the outcome of an immune response mediated by host T cells with specificity directed against immunogenic idiotypic determinants (specificity-associated markers) of anti-major histocompatibility complex (MHC) receptors (anti-MHC^b) present on the immunizing parental T cell population (2, 3). While it is known that GVH resistance is induced by immunization with parental T cells and that it is adoptively transferable to secondary F₁ recipients with host T cells, it is not yet clear which T cell subpopulations are involved in these two events. We show here that the parental T cell subpopulation which induces GVH resistance resides solely in the W3/25⁺ T helper (Th) subset that causes local GVH responses, i.e., enlarged popliteal lymph nodes, while host T cells of both the W3/25⁺ Th and the OX8⁺ T killer/suppressor (Tk/s) can adoptively transfer GVH resistance to secondary recipients, although Tk/s cells are more effective.

Materials and Methods

Rats. DA (RT1^a), Lewis (L) (RT1^l), and their F₁ hybrids were purchased from Trudeau Institute, Saranac Lake, NY. Only male animals were used, to avoid complications with the H-Y antigen. Where required, rats were irradiated at 83 rad/min from a ¹³⁷Cs source.

Cells. Suspensions of thoracic duct lymphocytes (TDL), spleen and lymph node lymphocytes were prepared according to standard procedures (4).

Antibodies. Monoclonal antibodies that identify various lymphocyte subsets in rats, kind gifts of Dr. D. W. Mason, MRC Cellular Immunology Unit, Oxford, were W3/25, Th cells (5); OX8 Tk/s cells (6); OX6, class II MHC gene products (7); and OX12, Igκ chain (8). Fluorescein-conjugated rabbit F(ab')₂ anti-mouse Ig and affinity-purified sheep F(ab')₂ anti-mouse Ig antibodies, purchased from Cappel Laboratories, Cochranville, PA,

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¹ *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; GVH, graft versus host; MHC, major histocompatibility complex; PLN, popliteal lymph node; SRBC, sheep erythrocyte; TDL, thoracic duct lymphocyte; Th, helper T cell; Tk, killer T cell; Ts, suppressor T cell.

were depleted of cross-reactivity to rat Ig by passage through a Sepharose 4B column coupled with rat Ig.

Local GVH Reactions. These were measured by the quantitative popliteal lymph node weight assay (9).

Cell Fractionation. TDL and spleen cells were depleted of selected subpopulations using modifications of the rosetting procedures described by Parish and Hayward (10) and by Mason (11). Briefly, 400×10^6 lymphocytes were resuspended in monoclonal antibodies (100 μ l) and incubated for 1 h on ice. These were washed twice in Hanks' balanced salt solution, and then mixed with 4×10^9 sheep erythrocytes (SRBC) that had been coated, using chromic chloride, with affinity-purified sheep F(ab')₂ anti-mouse Ig. This mixture was incubated (8 ml, 30 min at 4 °C) with gentle agitation, and then 0.5 ml of DA anti-SRBC or L/DA anti-SRBC hyperimmune serum added to facilitate further agglutination and this mixture incubated as above. Rosettes were separated by sedimentation at 1 g over 4 ml of fetal bovine serum. The nonrosetted population was recovered from the fluid layer above the serum interface; the purity of the recovered populations was monitored with appropriate antibodies and fluorescence-activated cell sorter (FACS) analysis. For preparations of enriched Th cells, OX8 and OX12 monoclonal antibodies were used in the negative selection rosetting procedure; for Tk/s, W3/25 and OX12; and for B cells, W3/25 and OX8.

Results

Phenotypic Analysis of Parental Lymphocytes That Cause GVH Reactivity and Induce Specific Resistance to GVH Disease. Table I shows the effectiveness of subpopulations of Th, Tk/s, and B cells from DA donors in (a) causing local GVH reactions, and (b) inducing specific GVH resistance in L/DA F₁ recipients. 15×10^6 cells of the various subpopulations were injected into the right rear footpads of young adult F₁ animals (100 μ l vol) on day 0; 10 d later the draining popliteal lymph nodes (PLN) were removed and weighed to measure GVH reactivity. To assess resistance to GVH reactions, these F₁ animals were injected in the left rear footpads with DA or L lymph node cells (5×10^6) 2 d later and the weights of draining PLN were determined on day 19. It seems clear that only T cells of the W3/25⁺ Th subset are able to cause enlarged lymph nodes, evidence of their GVH reactivity, and, moreover, it is only cells of the Th subset that induce specific resistance to local GVH reactions.

Phenotype of F₁ T Cells That Adoptively Transfer Specific GVH Resistance. Lightly irradiated (100 rad) L/DA rats were injected intravenously with various numbers

TABLE I
GVH Reactivity and Induction of Specific GVH Resistance Caused by Various Subpopulations of Parental (DA) Lymphocytes in L/DA F₁ Hosts

Group	DA TDL (15×10^6) subpopulation injected on day 0 to cause GVH reactions and GVH resistance		GVH reactivity	GVH resistance	
	MAb used for rosette depletion*	Phenotype of remaining cells		PLN weights on day 19 [$\bar{x} \pm SD$ (n)] caused by LN cells on day 12 from:	
			DA	L	
1	OX8, OX12, OX6	W3/25 ⁺ (Th)	169.3 ± 8.5 (10)	6.0 ± 0.9 (5)	70.3 ± 4.4 (5)
2	W3/25, OX12, OX6	OX8* (Tk/s)	9.7 ± 0.8 (10)	67.1 ± 7.5 (5)	78.7 ± 8.8 (5)
3	OX8, W3/25	OX6*, OX12 (B)	4.5 ± 0.6 (6)	66.2 ± 7.6 (3)	85.9 ± 3.6 (3)

* The starting population consisted of 1.2×10^9 DA TDL; after rosette depletion with monoclonal antibodies (MAb) in groups 1, 2, and 3, the yield was 25%, 10% and 10%, respectively, with a purity of ≥ 95 –99% by FACS analysis.

of whole spleen cells or various lymphocyte subsets (or balanced salt solution, for controls) from syngeneic F_1 donors that had been immunized 12–14 d earlier by inoculation with 15×10^6 DA Th cells. 3 d after this cell transfer, these recipients were challenged with 5×10^6 lymph node cells of the immunizing (DA) parental donor in their right rear footpads and a similar number of lymph node cells from the other (L) parental strain in their left rear footpads. Draining PLN were removed and weighed 7 d later.

The results (Table II) show that specific GVH resistance can be transferred with either the $W3/25^+$ Th or the $OX8^+$ Tk/s subpopulation, although, on a cell-for-cell basis, the latter appears to be a somewhat more effective population. Fig. 1 shows the FACS profile of samples of the adoptively transferred cell populations, and Table III summarizes the results.

Discussion

These studies make two points. First, the parental T cell subset that bears the immunogenic markers responsible for the induction of GVH resistance in F_1 rats consists of $W3/25^+$ Th cells, and it is the same subpopulation which causes local GVH reactions (Table I). As such, this extends our previous finding (2) with negative selection protocols that these markers are associated with a particular subpopulation of T cells reactive to host alloantigens.

The second point is an extension of our previous findings with adoptive transfer experiments that GVH resistance is mediated by host T cells (2). Here, we show that T cells of both the Th ($W3/25^+$, $OX8^-$, $OX12^-$) and the Tk/s

TABLE II
Lymphocyte Subpopulations Involved in the Adoptive Transfer of Specific GVH Resistance

Exp.	Cell populations transferred ($\times 10^6$)	PLN weights [$\bar{x} \pm SD (n)$] caused by 5×10^6 lymph node cells from:			
		DA		L	
1	—*	47.6 \pm 1.7 (3)		55.5 \pm 3.9 (3)	
	Whole spleen (100)	19.1	4.5 (3)	39.1	2.9 (3)
	$OX8^+$ (30)	21.8	1.9 (3)	49.0	1.1 (3)
2	—	48.4 \pm 6.7 (3)		53.6 \pm 11.2 (3)	
	Whole spleen (400)	10.0	1.8 (3)	36.1	1.5 (3)
	$OX8^+$ (90)	19.0	1.0 (3)	53.8	2.9 (3)
3	—	39.2 \pm 5.5 (2)		36.2 \pm 5.6 (2)	
	Whole spleen (100)	27.8	1.8 (3)	38.1	1.9 (3)
	$W3/25^+$ (60)	26.8	3.8 (3)	33.0	1.7 (3)
4	—	48.4 \pm 5.8 (4)		59.5 \pm 8.9 (4)	
	Whole spleen (400)	11.1	1.7 (3)	54.3	4.1 (3)
	$W3/25^+$ (90)	24.9	1.0 (3)	51.2	7.2 (3)
5	—	75.1 \pm 5.7 (2)		70.8 \pm 1.1 (2)	
	Whole spleen (400)	14.9	0.8 (3)	72.0	5.3 (3)
	$OX12^+$ (90)	70.8	8.8 (3)	76.1	7.1 (3)

* Control recipients received Hanks' balanced salt solution; all recipients were given light irradiation (100 rad) 1 d before cell transfer.

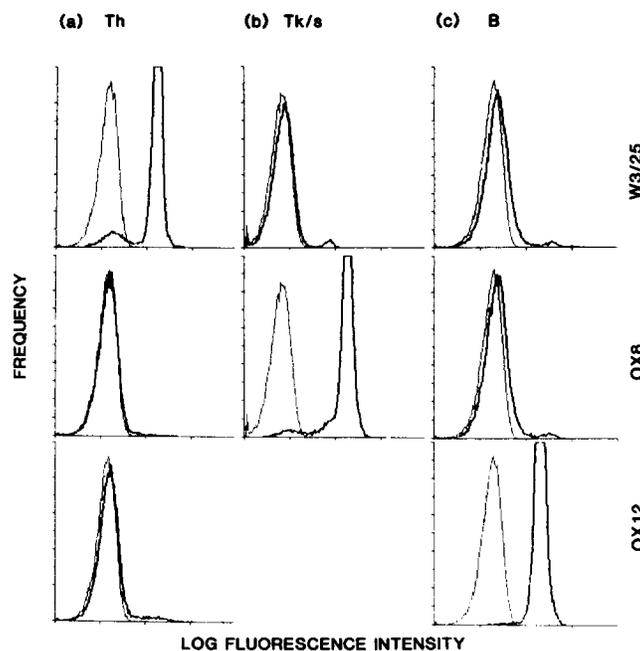


FIGURE 1. FACS profiles of (a) Th, (b) Tk/s, and (c) B cell populations prepared by negative selection rosetting techniques. In each case the staining with monoclonal antibodies (heavy lines) is compared with the autofluorescence control (light lines).

TABLE III

Summary of FACS Profiles of Enriched Th, Tk/s, and B Cell Populations Used for Adoptive Transfer Studies of Table II

Monoclonal antibody	Th (OX8 ⁻ , OX12 ⁻)	Tk/s (W3/25 ⁻ , OX12 ⁻)	B (W3/25 ⁻ , OX8 ⁻)
W3/25	90.0	3.0	6.1
OX8	2.2	94.6	5.2
OX12	5.5	ND	98.9
—*	2.2	2.9	4.5
Auto fluorescence [‡]	0.2	0.1	0.2

* No primary antibody with fluorescein isothiocyanate-labeled anti-mouse immunoglobulin.

[‡] No primary antibody and no secondary antibody.

(OX8⁺, W3/25⁻, OX12⁻) subsets can adoptively transfer resistance to local GVH reactions in secondary F₁ recipients (Table II). Coming to such a conclusion depends as much on the nature of the contaminating cell population as it does on the majority cell type present in the inoculum. FACS profiles of the transferred cells (Fig. 1) show that in this experiment the W3/25⁺-enriched Th population was contaminated with <2.5% OX8⁺ (Tk/s) cells and a somewhat larger population that expresses neither of the T cell markers nor Ig (W3/25⁻, OX8⁻, OX12⁻). Also, the enriched Tk/s population contained ~3% of contaminating Th (W3/25⁺) cells. It is most unlikely that these contaminating populations had any influence on the results, since the B cell population, which was

more heavily contaminated with Th (6%) and Tk/s (5%) cells, was ineffective in transferring GVH resistance.

The model of specifically induced GVH resistance is of particular interest since it represents a rather clear example of how MHC-reactive, receptor-bearing T cell populations can be down-regulated by antiidiotypic host T cells, possibly lytic T cells (12), to prevent the otherwise fatal consequences of GVH disease. It seems likely that this model may be akin to two others that involve regulation of T cells with specificity for MHC antigens. The first of these, recently described by Glazier et al. (13), involves the breaking of self-tolerance and induction of systemic autoimmune disease in irradiated rats that have been repopulated with their own or syngeneic marrow cells and then maintained for prolonged periods on a daily injection protocol with cyclosporin; lethal autoimmune disease ensues shortly after this drug is discontinued. This autoimmune state is adoptively transferable to irradiated animals with T cells, in the absence of cyclosporin, from diseased donors, but not to normal, nonirradiated recipients. It seems likely that this drug treatment has somehow crippled a suppressive mechanism that ordinarily maintains tolerance to self molecules, including self MHC, thus permitting the activation of self-reactive T cell clones when cyclosporin is discontinued. We suggest that the suppressive mechanism that ordinarily maintains self-tolerance is composed, at least in part, of host T cells, that this is the same population which resists nonspecific GVH disease in normal nonirradiated adult F₁ rats, and that it is the one which can be primed to cause specific GVH resistance in irradiated F₁ animals.

The second system involving suppression of MHC-reactive T cell clones, described by Roser and Dorsch and their colleagues (14), operates to maintain neonatally induced transplantation tolerance to allogeneic MHC antigens. Their studies used an experimental protocol where various cell populations were injected into irradiated syngeneic animals bearing a test skin allograft. As expected, normal T cell populations caused a prompt rejection and negatively selected T cells failed to affect delayed graft rejection typical of heavily irradiated animals. T cells from tolerant donors, however, caused an even longer prolongation of the rejection process and sometimes permanent graft survival. The suppressor cell in this system proved to be a thymus-derived, rapidly recirculating, radiosensitive T cell bearing the W3/13 marker (14, 15). In these respects, the phenotype of these cells is similar to that of T cells that cause resistance to GVH disease in nonirradiated F₁ rats (2), and is probably the same as the T cell population affected in the cyclosporin-induced autoimmunity model (13). What is surprising in the transplantation tolerance experiments, however, is that deletion of the Tk/s (OX8⁺) subset did not remove the suppressor effect (15). How should this result be interpreted?

The experiments described above show that GVH resistance can be adoptively transferred to secondary recipients with either the Th (W3/25⁺) or the Tk/s (OX8⁺) subset. This result agrees in part with the findings of the Roser group. Adoptively transferred Th subpopulations, lacking the Tk/s subset, can suppress both abolition of tolerance and GVH reactivity in secondary recipients. Therefore, the suppressive mechanism of these two systems appears to operate with two components to it. The first seems to involve killer cells of the OX8⁺ subset

that lyse other T cells bearing anti-MHC receptors; such antiidiotypic T cells having lytic specificity for specific mixed leukocyte culture blasts have been described among the lymphocyte populations of rats recovering from local GVH disease (12). The second component appears to be a Th cell that probably functions either by activating killer T cells or is required for triggering some other nonlytic suppressor cell. It is of some interest that cells with suppressor-like functions may be found in a population which has been depleted of the subset bearing the surface phenotype characteristic of Tk/s cells.

Summary

F₁ hybrid (A×B) rats immunized with parental strain (A) T cells, or which recover from graft-versus-host (GVH) reactions caused by parental T cells, develop strong T cell-mediated immune responses against anti-major histocompatibility complex (MHC) receptor structures on donor T cells specific for host (MHC^b) alloantigens. This immune response provides the basis for a profound and specific resistance to the subsequent induction in these animals of local or systemic GVH disease. Using subset-specific monoclonal antibodies and negative selection rosetting procedures, we attempted to determine which donor T cell subset possesses the immunogenic idiotypic markers, and which host T cell subpopulation mediates GVH resistance. We show here that the immunizing donor cell population belongs to the W3/25⁺ helper T cell (Th) subset, the same one that causes local GVH reactions, and that both the W3/25⁺ Th and the OX8⁺ killer/suppressor (Tk/s) subsets of host T cells are able to transfer GVH resistance to secondary F₁ recipients.

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