Brief Definitive Report

IMMUNOGENICITY AND CROSSREACTIVITY OF SPECIFICITY-ASSOCIATED MARKERS ON ALLOREACTIVE T CELLS

Confirmation Based on the Model of Tolerance Abolition by Adoptive Transfer

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Previous studies have shown that clonotypic markers of alloreactive T cells, presumed to be antigen-specific receptors, can be potent immunogens or tolerogens. Immunization of adult F_1 hybrid rats of MHC-disparate matings (a × b) with T cells from parental (a) donors induces a state of resistance to GVHD that is rapid in onset, profound in effect, and specific for the immunizing parental strain cells (1–3). Likewise, inoculation of small numbers of parental T cells into newborn F_1 recipients results in a marked, specific, and longstanding sensitivity to GVHD caused by subsequently injected parental strain T cells (4).

These specificity-associated markers (SAM) of alloreactive T cells express some obvious diversity; immunization of a/b F_1 rats with a(anti-b) T cells does not induce resistance in these animals to b(anti-c) alloreactivity (1, 2). Quite unexpectedly, however, these SAM show no detectable polymorphism in different parental strains. Immunologically induced GVHD resistance in a/b hybrids to a anti-b alloreactivity also suppresses anti-b responses of T cells from other parental strains, c, d, e, . . . but not anti-a alloreactivity by T cells from these same strains (3). This unexpected crossreactivity of anti–MHC receptors in this in vivo GVHD model was recently confirmed with in vitro studies of the lytic specificity of T cells from GVHD-resistant F_1 rats. Cells from F_1 rats (a/b) primed with a T cells in vivo and reexposed in culture to a anti-b MLC blasts effectively lyse a anti-b and c anti-b mixed lymphocyte culture (MLC) blast targets, but not b anti-a, c anti-a, nor a anti-c MLC targets (5).

The nonpolymorphism of SAMs on alloreactive T cells from donors of different genetic backgrounds in the GVHD system was a surprising finding. Thus, for the sake of generalization, we considered it important to determine whether this crossreactivity could be demonstrated in a different model system. For this purpose, we exploited the findings that (*a*) long-tolerated skin grafts on adult

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animals rendered neonatally tolerant with bone marrow inocula are not easily rejected following adoptive transfer with normal syngeneic lymphoid cells unless large numbers of the latter are used (6, 7), and that (*b*) smaller numbers of adoptively transferred cells immunize their hosts so that larger numbers, even from sensitized donors, fail to abolish tolerance (8–10). In this study, we use this tolerance abolition model to confirm the immunogenicity of receptor-bearing alloreactive T cells, and we show, in this model also, the idiotypic crossreactivity of T cells from different strains reactive to the same MHC haplotype.

Materials and Methods

Rats. Lewis (L, RT1^l), DA (RT1^a), Brown Norway (BN, RT1^n), and L/DA F_1 hybrids were raised in our colonies or purchased from the Trudeau Institute (Saranac Lake, NY). L/DA rats were made tolerant of BN alloantigens by i.v. inoculation shortly after birth (<18 h) with 5×10^7 marrow cells from BN donors. These animals received full-thickness BN skin grafts that were allowed to remain in place ≧100 d before the animals were considered tolerant and could be used as recipients for adoptive transfer in these studies.

Adoptive Transfers. Thoracic duct lymphocytes (TDL) were obtained by standard procedures (11) from parental strain (L or DA) donors and negatively selected to host alloantigens by acute passage through irradiated L/DA rats (12). All cell transfers were made intravenously.

Graft Survival. Grafts were inspected daily, and the time of rejection was recorded as the day that the graft epithelium and its underlying dermis could be scraped, leaving a dry scab the next day.

Results

Experimental Plan. The object of this study was to determine whether the immunogenic determinants of alloreactive T cells, which induce in tolerant recipients a state of resistance to tolerance abolition, are shared by alloreactive T cells of a different strain reactive to the same MHC haplotype. As recipients to be immunized with alloreactive parental cells, we used L/DA F_1 rats tolerant of BN alloantigens, and which had been bearing BN skin allografts for periods in excess of 100 d.

To avoid complications due to GVHD, the adoptively transferred parental TDL populations (L, DA) used to immunize and to abolish tolerance of the BN skin allografts were first negatively selected with respect to the other parental MHC haplotype ($L_{\text{-DA}}$, $DA_{\text{-L}}$). The negative selection procedure involves acute filtration of parental cells through intermediate L/DA F_1 hosts, which have been heavily irradiated (1,000 rad). This procedure results in the recovery of highly purified fractions of recirculating donor T cells, having an insignificant B cell contamination due to the more rapid recirculation kinetics of T cells seen in normal and irradiated hosts (13–15).

The contribution of host lymphocytes to the recovered parental T cell fractions is also minimal due to the heavy dose of x-irradiation (16–18).

In a preliminary study, we established that 10^8 $L_{\text{-DA}}$, $DA_{\text{-L}}$ or $L/DA_{\text{-L/DA}}$ TDL populations consistently caused the rejection of long-term tolerated BN skin grafts on tolerant L/DA hosts within a subsequent 3 wk period. Doses of $3–5 \times 10^7$ $DA_{\text{-L}}$ or $L_{\text{-DA}}$ TDL, and doses of 1.5×10^7 $L/DA_{\text{-L/DA}}$ TDL rejected only 20% of the BN grafts over the subsequent 3–6 wk period; the remaining BN grafts remained intact for >60 d (data not shown). Consequently, we used these

numbers as the smaller priming dose of TDL; to determine whether this initial priming inoculum affected the tolerant hosts, they were given a secondary inoculation of 10^8 TDL 6 wk later. The crossreactivity of T cell markers associated with anti-BN alloreactivity was tested by comparing the effectiveness of high-dose inocula of T cells from the priming donor strain with that of the other parental strain in abolishing tolerance.

Immunogenicity and Crossreactivity of DA and L T Cells Reactive to BN Alloantigens. The results of this study are summarized in Table I. Four groups of L/DA rats tolerant of BN were used as recipients. The first were given large inocula of 10^8 T cells from parental ($DA_{\cdot L}$ or $L_{\cdot DA}$) or from F_1 donors; with one exception, they rejected their BN skin grafts promptly (≦3 wk). The second group were primed with a smaller dose of $DA_{\cdot L}$ T cells and then given a larger dose of either $DA_{\cdot L}$ or $L_{\cdot DA}$ T cells. Some of these BN grafts were rejected over a significantly more prolonged period of time (4–6 wk); others were not rejected at all (>50 d). The third group is the mirror image experiment; priming with $L_{\cdot DA}$ T cells gives the same result. The fourth group represents a control panel. It could be argued that the delayed rejection times of BN grafts on F_1 rats previously primed with smaller numbers of parental TDL reflected an immunosuppressed status of these animals associated with subclinical GVHD caused by the priming parental cell inoculum; this seems unlikely, since F_1 animals primed with syngeneic L/DA T cells show the same prolongation of graft survival times.

Discussion

The simplest and most direct conclusion that can be drawn from this study is that T cells from two different rat strains (DA and L) and from their F_1 hybrids (L/DA F_1) reactive to BN alloantigens are both immunogenic in tolerant L/DA recipients and share common idiotypic receptor-associated determinants. $DA_{\cdot L}$ T cells, transferred to tolerant F_1 recipients in numbers insufficient to abolish tolerance, induce in these recipients an actively acquired resistance, directed presumably against anti–BN receptor-bearing T cells, so that larger, normally effective dosages of these cells derived from either parental strain now fail to

TABLE I

Immunogenecity and Crossreactivity of Specificity-associated Markers on Alloreactive T Cells: L/DA ($RT1^{1/a}$) Hosts Tolerant of BN ($RT1^n$)

Primary innoculation (day −40)	Secondary innoculation (day 0)	Graft survival time (d)	Grafts surviving ≥25 d/*n*
	10^9 $DA_{\cdot L}$	14, 14, 15, 15, 16, 16, 17, 17, 19, 21, >50	1/11
	$L_{\cdot DA}$	11, 12, 13, 13, 14, 16, 18, 21	0/8
	$L/DA_{\cdot L/DA}$	12, 13, 13, 13	0/4
3×10^7 $DA_{\cdot L}$	10^9 $DA_{\cdot L}$	20, 29, 35, 40, 42, >50, >50	6/7
3×10^7 $DA_{\cdot L}$	$L_{\cdot DA}$	15, 33, 38, >50, >50	4/5
3×10^7 $L_{\cdot DA}$	10^9 $DA_{\cdot L}$	25, 25, >50	3/3
3×10^7 $L_{\cdot DA}$	$L_{\cdot DA}$	49, >50	2/2
1.5×10^7 $L/DA_{\cdot L/DA}$	10^9 $DA_{\cdot L}$	27, 29, 49, >50, >50	5/5
1.5×10^7 $L/DA_{\cdot L/DA}$	$L_{\cdot DA}$	18, 26, 32, 37, >50	4/5

abolish tolerance. These findings confirm earlier studies (1–3) that anti–MHC receptors are potent immunogens in the appropriate setting, that they induce in tolerant animals a suppressive immune response that inhibits the abolition of tolerance (8–10), and that anti–MHC receptors on T cells from different genetic backgrounds reactive to the same MHC haplotype share conserved idiotypic markers.

Earlier studies of Dorsch and Roser and their colleagues (19) have concluded that neonatally induced transplantation tolerance involves an active suppressive mechanism mediated by T cells derived from the tolerance-conferring bone marrow graft. It was cogently argued that the specificity of these suppressor cells was directed towards idiotypic determinants on receptors of the relevant alloreactive T cells. Our own previous studies (1, 2) with a different model system have concluded that specifically induced GVHD resistance in F_1 hybrid rats is mediated by a host-derived T cell suppressor mechanism with specificity directed towards idiotypic markers on alloreactive T cells. The present study shows that the immunogenic nonpolymorphic markers of alloreactive T cells first described for the GVHD resistance model (3) can also be shown with the transplantation tolerance model, a finding which implies that GVHD resistance and the maintenance of allograft tolerance involve very similar, if not identical, idiotype-antiidiotype regulatory T cell interactions.

Summary

Syngeneic or parental strain T cells adoptively transferred into hybrid rats tolerant of third party alloantigens (L/DA tolerant of BN), in numbers insufficient to abolish tolerance, induce instead an active resistance to tolerance abolition with larger, usually effective dosages of donor cells. Of particular interest is the finding that immunization with T cells from one parental strain donor (e.g., DA) inhibited the tolerance-abolishing alloreactivity (anti-BN) of subsequently transferred T cells from the same (DA) and the other (L) parental strain donor. We conclude that anti–MHC receptors on T cells from different genetic backgrounds reactive to the same third party alloantigens share the same conserved immunogenic specificity-associated markers (SAM). The nonpolymorphism of anti–MHC receptors shown here in the transplantation tolerance model is a confirmation of the same conclusion drawn from earlier studies with the GVHD-resistance model, and it therefore suggests that these two models of T cell MHC interactions involve very similar mechanisms of T cell idiotypic regulation.

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