TOLERANCE TO PARENCHYMAL SELF

Regulatory Role of Major Histocompatibility Complex–restricted, OX8$^+$ Suppressor T Cells Specific for Autologous Renal Tubular Antigen in Experimental Interstitial Nephritis

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New information continues to emerge regarding basic immunologic mechanisms of self-tolerance. While tolerance is often defined at the T or B cell level (1), the overall process can also be categorized by mechanisms mediated either by the deletion of self-reactive clones, the effects of suppressor T cell surveillance, or the sequential action of both processes (2). Such mechanisms have been intensively analyzed in the context of strategies designed to induce immunologic unresponsiveness to histocompatibility antigens on lymphocytes, skin, and most transplantable organs (3–6). Current evidence, however, has not directly established whether such findings can be extended to the prevention of antigen-specific autoimmunity to non–major histocompatibility complex (MHC)[1] parenchymal self.

Therefore, taking advantage of the genetic polymorphisms that are involved with the susceptibility of rats to autoimmune interstitial nephritis (7), we have investigated the mechanism of tolerance to autologous renal tubular antigen. In this model, prototypically susceptible Brown Norway (BN) rats immunized with rabbit, but not with BN, renal tubular antigens develop anti–tubular basement membrane antibodies (α-TBM-Ab) and an intense mononuclear cell infiltrate producing severe interstitial nephritis. Previously, we showed that susceptibility to this lesion in rats principally requires both the genetically determined expression of the relevant cortical tubular antigen (TBM$^+$) (7, 8) and the capacity to mount an MHC-linked, cell-mediated immune response to the antigen (7). We now demonstrate that the induction of tolerance to TBM first requires antigen

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[1] *Abbreviations used in this paper:* α-TBM-Ab, anti–tubular basement membrane antibodies; BN-TBM, soluble BN renal tubular antigens; BN-SLA, soluble BN liver antigens; CFA, complete Freund's adjuvant; CTX, cyclophosphamide; DTH, delayed-type hypersensitivity; FITC, fluorescein isothiocyanate; MHC, major histocompatibility complex; NWA, nylon wool–adherent cells; NWT, nylon wool T cells; PBS, phosphate-buffered saline; PBS-5, 5% fetal calf serum in PBS; PPD, purified protein derivative; RITC, rhodamine isothiocyanate; TBM$^+$, genetically determined presence of target cortical tubular antigen; TBM$^-$, absence of cortical tubular antigen; (T), neonatally tolerized to BN alloantigens.

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expression, and is then largely attributable to the presence of an antigen-specific, cyclophosphamide-sensitive, $OX8^+$ suppressor T cell that is restricted by gene products in the MHC.

Materials and Methods

Animals. Brown Norway (BN) and Lewis (LEW) rats were obtained from the Charles River Breeding Laboratories, Wilmington, MA. Lewis.1N (LEW.1N), Fischer.BN (F.BN), (LEW.1N × LEW)F_1, and (BN × LEW)F_1 hybrids were bred at the University of Pennsylvania. MHC (RT1 in the rat) genotypes as well as the presence or absence of TBM^+ in the animals are as follows: BN ($RT1^n/TBM^+$), LEW ($RT1^l/TBM^-$), LEW.1N ($RT1^n/TBM^-$), F.BN ($RT1^n/TBM^+$), (LEW.1N × LEW)F_1 ($RT1^{n/l}/TBM^-$), and (BN × LEW)F_1 ($RT1^{n/l}/TBM^+$).

Induction of Neonatal Tolerance. Tolerance of BN antigens was induced by injecting neonatal LEW.1N and F.BN animals, <12 h old, with 50×10^6 BN spleen and lymph node cells according to procedures described elsewhere (9). Neonatal (LEW.1N × LEW)F_1 rats were rendered tolerant of BN antigens with 50×10^6 (LEW × BN)F_1 spleen and lymph node cells. In all cases tolerance was confirmed by the permanent acceptance of a BN skin graft.

Preparation of Renal Tubular Antigens. BN rat renal tubular basement membrane antigens and BN liver membrane antigens were isolated by a differential sieving technique (10). Highly enriched basement membrane fragments were sonicated, lyophilized, and stored at −70°C. Soluble renal tubular antigens (BN-TBM) and soluble liver antigens (BN-SLA) were made from these lyophilized membranes using collagenase digestion (7, 11).

Immunizations. Groups of rats were immunized with 1 mg of BN-TBM in complete Freund's adjuvant (CFA) (0.5 ml; *Mycobacterium tuberculosis*, 2 mg/animal) by footpad and/or subcutaneous injection. Control animals received CFA alone. Selected groups of animals received intraperitoneal cyclophosphamide; chronic administration consisted of 15 mg/kg on the day of immunization, followed by 2 mg/kg daily until sacrifice (day 28). Other animals received a single injection of 50 mg/kg 72 h before sacrifice (12).

Elicitation of Delayed-Type Hypersensitivity (DTH) Responses. DTH responses to BN-TBM and purified protein derivatives (PPD) were induced by subcutaneously immunizing rats with BN-TBM in CFA (13). 12–14 d after such immunizations, groups of rats were challenged in one footpad with 50 μg of soluble antigen (BN-TBM, BN-SLA, or PPD) in 50 μl of phosphate-buffered saline (PBS). Footpad swelling, as an index of DTH, was measured 24 h later using a spring-loaded engineer's micrometer (13) (Schlesingers for Tools Ltd., Brooklyn, NY). The magnitude of swelling was expressed as the increment between the challenged footpad and the control, PBS-challenged footpad in inches $\times 10^{-3}$ ± SEM. All measurements were cage blind.

Adoptive Transfer Experiments. Spleens and/or draining lymph node cells were harvested from donors 12–14 d after immunization and made into a single-cell suspension. The cells were washed, counted, and injected intravenously ($20–25 \times 10^6$ cells per recipient) using 0.9% NaCl as the vehicle. 2–3 h after cell transfer, recipients were footpad challenged with antigens or PBS and footpad swelling was measured as described above.

Preparation of Immune Cell Subpopulations. Cell suspensions were T cell depleted with a monoclonal IgM anti-RT7.1 antibody (from BC84.5 hybridoma [14, 15] provided by Dr. John Ely and Dr. Frank Fitch, University of Chicago, Chicago, IL) and a mixture of rabbit and guinea pig complement. Nylon wool T (NWT) and nylon wool–adherent (NWA) cells were obtained from packed nylon wool columns ($3–4 \times 10^8$ cells added to a 35-ml column) according to standard methods (16). Monoclonal antibody OX8 (cytotoxic/suppressor subset) was purchased from Accurate Chemical & Scientific Corp., Westbury, NY (17). The OX8 monoclonal antibody was used to enrich T cells by an indirect panning technique as described previously (18, 19). Briefly, 2.5 ml of affinity-purified rabbit anti–mouse IgG (Zymed Laboratories, San Francisco, CA) in a concentration of 1 mg/ml in PBS was incubated on 60 × 15 mm polystyrene dishes overnight at 4°C. After removal

of the IgG solution, the plates were washed and blocked with 5% fetal calf serum in PBS (PBS-5) for 30 min. The fetal calf serum was then washed out before the addition of the T cells. 25×10^6 NWT were preincubated in 0.2 ml of a 1:40 dilution of OX8 antibody (ascites fluid) for 30 min at 4°C. After extensive washing in PBS, the cells were transferred onto the anti-IgG–coated dishes for 60 min at 22°C. Midway through the incubation the dishes were gently rocked. Nonadherent cells were removed from the plates with three cycles of swirling and washing with PBS-5. After the nonadherent cells were removed, 3 ml of chilled PBS-5 was added to the dishes, which were then placed at 4°C for 30 min. The adherent cells were then resuspended by vigorous pipetting and further washed with chilled PBS-5. The presence of Ig^+ cells in the separation mixture was determined by direct immunofluorescence using rhodamine isothiocyanate (RITC)–anti-rat Ig (gift of Dr. Susan Webb, Scripps Clinic and Research Foundation, La Jolla, CA) and the percentage of $OX8^+$ cells was determined by indirect fluorescence using FITC–anti-mouse IgG (Cappel Laboratories, Cochranville, PA).

Assessment of Renal Disease. Kidney tissue was prepared for immunofluorescent and light microscopy by standard methods for this laboratory (20). The degree of interstitial involvement was quantitatively determined from coded, paraffin-embedded kidneys stained with hematoxylin and eosin. Cellular infiltration and tubular damage leading to cortical interstitial destruction was graded on a scale of 0 to 4 as follows: 0, normal; 0.5, small focal areas of cellular infiltration and tubular damage involving <10% of the cortex; 2, involvement up to 25% of the cortex; 3, involvement up to 50–75% of the cortex; and 4, extensive damage involving >75% of the cortex. Scores for each experimental group were read blind, averaged, and expressed as a mean ± SEM. Direct immunofluorescence of tubular staining by α-TBM-Ab was graded $0–4^+$ and expressed as a mean ± SEM for each group.

Statistical Analysis. Differences between experimental groups were determined by Student's *t* test.

Results

Characterization of the Immune Response to BN-TBM. In an introductory series of experiments we observed that BN rats, when immunized with BN antigen in CFA (BN-TBM/CFA), made small amounts of α-TBM-Ab but did not develop histologic tubulointerstitial nephritis nor a significant DTH response to BN-TBM. To determine whether such unresponsiveness correlated with antigen presence in the kidney, we also immunized $RT1^n$, TBM^+ (F.BN), and TBM^- (LEW.1N) strains. As noted in Table I, LEW.1N and F.BN rats both developed a DTH response to BN-TBM after immunization whereas BN rats did not; F.BN rats also developed significant tubulointerstitial nephritis, indicating that BN-TBM is capable of producing disease. When alloreactivity to the BN-TBM preparation was attenuated, however, by neonatally tolerizing (T) LEW.1N and F.BN rats to BN alloantigen, we obtained very different results. Although LEW.1N(T) animals still displayed a DTH response to BN-TBM, F.BN(T) developed neither histologic disease nor a DTH response after immunization with BN-TBM. The DTH response to PPD was comparable among all groups. These results suggest that, with abrogation of alloreactivity, background disparate TBM^+ strains fail to respond to BN-TBM, whereas TBM^- strains continue to recognize BN-TBM.

Further studies demonstrated the feasibility of using the DTH response of an immunized $RT1^n/TBM^-$ strain as a measure of cell-mediated immunity to BN-TBM. As noted in Table II, immune lymphocytes from LEW.1N(T) donors can adoptively transfer this response into naive LEW.1N recipients. The lack of

TABLE I
Immune Response to BN-TBM

Strain*	Immunization	Direct fluorescence of bound α-TBM-Ab	Histologic‡ disease	DTH Response§	
				BN-TBM	PPD
BN	+	0.6 ± 0.2	0.0	3.8 ± 1.4	29.6 ± 5.5
BN	−	0.0	0.0	5.0 ± 1.0	27.1 ± 2.1
F.BN	+	2.8 ± 0.4	2.4 ± 0.3	27.6 ± 2.9‖	26.0 ± 2.5
F.BN	−	0.0	0.0	6.6 ± 0.3	28.0 ± 1.8
LEW.1N	+	0.0	0.0	31.6 ± 1.8‖	31.0 ± 4.5
LEW.1N	−	0.0	0.0	4.3 ± 0.9	28.3 ± 2.3
F.BN(T)	+	0.6 ± 0.1	0.1 ± 0.1	7.5 ± 1.8**	31.0 ± 3.1
F.BN(T)	−	0.0	0.0	3.5 ± 0.5	26.0 ± 2.0
LEW.1N(T)	+	0.0	0.0	33.5 ± 0.5‖	33.0 ± 7.0
LEW.1N(T)	−	0.0	0.0	6.5 ± 0.5	23.0 ± 4.2

* Animals were immunized with BN-TBM/CFA (+) or CFA alone (−).
‡ Kidneys were harvested 28 d after immunization.
§ Animals were challenged with antigens in the footpad of each hindleg; footpad measurements were made before and 24 h after antigen challenge. Data are calculated as the mean footpad increment in inches × 10^{-3} ± SEM.
‖ $P < 0.001$ compared with CFA-immunized controls.
** $P < 0.005$ compared with immunized F.BN.

TABLE II
Adoptive Transfer of DTH

Donor Cells*	Treatment	DTH Response‡		
		BN-SLA	BN-TBM	PPD
Immune LEW.1N(T)	—	5.0 ± 0.5	19.5 ± 1.5§	18.5 ± 0.5§
Immune LEW.1N(T)	C′	—	21.8 ± 1.1§	21.0 ± 1.0§
Immune LEW.1N(T)	α-RT7.1 + C′	—	3.8 ± 1.3	4.3 ± 0.3
Control LEW.1N(T)	—	4.7 ± 0.6	5.6 ± 0.9	26.0 ± 7.0‖
—	—	—	4.6 ± 0.3	5.0 ± 0.6

* Donor lymph node and spleen cells harvested from animals immunized 14 d previously with BN-TBM/CFA (immune) or CFA alone (control) were transferred intravenously into naive LEW.1N recipients (20 × 10^6 pooled lymph node cells and spleen cells per recipient.)
‡ Recipients were challenged at time of cell transfer and mean footpad increment in inches × 10^{-3} ± SEM ($n = 4$) was determined 24 h later.
§ $P < 0.001$ compared with recipients of no cells.
‖ $P < 0.05$ compared with recipients of no cells.

reactivity to BN-SLA suggests that the BN-TBM response is antigen specific. Treatment of the immune cells with an anti–T cell antibody (α-RT7.1) and complement completely abrogated the DTH response, suggesting that it was T cell mediated. There was no significant difference in DTH response to BN-TBM between recipients of cells from CFA-immunized donors and recipients of no cells. Therefore, in subsequent studies, either group was used as a negative control.

Genetic Restriction of the DTH Response to BN-TBM. We used this acute adoptive transfer assay to study the genetic requirements for expression of DTH reactivity. As seen in Table III, lymphocytes from immunized LEW.1N(T)

TABLE III
RT1 Restriction of DTH to BN-TBM

Donor Cells*	DTH response to BN-TBM‡		
	LEW.1N	LEW	(LEW.1N × LEW)F_1
Immune LEW.1N(T)	22.7 ± 0.9§	5.0 ± 0.4	—
Immune LEW(T)	4.8 ± 0.6	23.0 ± 1.2§	—
Immune (LEW.1N × LEW)F_1(T)	25.0 ± 2.2§	25.8 ± 1.6§	27.8 ± 1.1§
—	5.3 ± 0.3	4.0 ± 0.6	5.8 ± 0.8

* Pooled lymph node cells and spleen cells from animals immunized 14 d earlier with BN-TBM/CFA were transferred intravenously into naive recipients (20×10^6 cells per recipient). Animals were footpad challenged immediately after cell transfer.
‡ See Table II.
§ $P < 0.001$ compared with controls.

TABLE IV
Demonstration of Suppressor T Cells to BN-TBM in BN Rats

Donor cells*		Treatment of BN cells	DTH Response‡	
LEW.1N(T)	BN		BN-TBM	PPD
Immune	—	—	22.8 ± 3.4	21.3 ± 2.7
Immune	Immune	—	5.0 ± 0.5§	18.3 ± 1.7
Immune	Immune	C′	5.0 ± 0.4§	21.0 ± 1.7
Immune	Immune	α-RT7.1 + C′	21.8 ± 1.8	20.6 ± 1.0
Immune	Immune (CTX)	—	18.6 ± 2.4	—
Immune	Control	—	20.8 ± 2.4	20.2 ± 0.8
Immune	Normal thymus	—	7.0 ± 2.1‖	21.5 ± 1.6
—	Immune	—	4.6 ± 0.3§	19.6 ± 1.8
Control	—	—	5.6 ± 0.9§	22.3 ± 1.8

* Donor lymph node and spleen cells [LEW.1N(T)] or lymph node cells (BN) were harvested from rats immunized 10–14 d earlier with BN-TBM/CFA (immune) or CFA (control). CTX (50 mg/kg) was administered, where indicated, intraperitoneally to BN rats 72 h before sacrifice. Equal numbers (20×10^6) of LEW.1N(T) and BN cells were admixed and transferred intravenously into naive LEW.1N recipients.
‡ See Table II.
§ $P < 0.005$ compared with response with LEW.1N(T) donor cells.
‖ $P < 0.01$ compared with response with LEW.1N(T) donor cells.

donors could not transfer a DTH response into LEW recipients; LEW(T) donor cells could transfer this response into LEW, but not to LEW.1N recipients. The adoptive transfer of immunized (LEW.1N × LEW)F_1(T) donor cells into LEW.1N, LEW, and (LEW.1N × LEW)F_1 recipients confirmed previous observations that acute rejection is not an adequate explanation for the failure to observe a DTH response in RT1 disparate recipients (21).

Demonstration of Suppressor T Cells in BN Rats. We wished to determine if active suppression might explain the lack of immunologic reactivity observed in BN rats immunized with BN-TBM. In Table IV it can be observed that admixture of immune lymph node cells from BN rats with immune LEW.1N(T) cells resulted in complete suppression of the DTH response to BN-TBM in naive LEW.1N recipients. This suppression was abrogated by pretreatment of the immune BN lymphocytes with the anti–T cell antibody and complement, indi-

TABLE V
Antigen-specific Suppression of DTH to BN-TBM by OX8$^+$ Lymphocytes

Donor cells*		Cell dose	DTH response‡	
LEW.1N(T)	BN		BN-TBM	PPD
Immune	—	—	23.0 ± 1.0	27.3 ± 2.2
Immune	Immune Unfractionated	25×10^6	5.0 ± 1.0§	23.3 ± 2.4
Immune	Immune NWT	15×10^6	6.3 ± 1.8§	23.0 ± 2.5
Immune	Immune NWA	15×10^6	24.3 ± 0.8	27.3 ± 2.7
Immune	Immune OX8$^+$	2.5×10^6	4.2 ± 0.9§	21.8 ± 0.8
Immune	Immune OX8$^-$	13.5×10^6	21.7 ± 1.1	23.2 ± 0.5
Control (CFA)	—	—	5.3 ± 0.8§	27.3 ± 0.8

* Pooled lymph node and spleen cells [LEW.1N(T)] and selected populations of lymph node cells (BN) were harvested from rats immunized 14 d earlier with BN-TBM/CFA or CFA, and injected intravenously into naive LEW.1N recipients. NWT and NWA represent nylon wool–nonadherent and –adherent populations; OX8$^+$, NWT that bind OX8 antibodies; OX8$^-$, NWT that do not bind OX8 antibodies.
‡ See Table II.
§ $P < 0.001$ compared with response with immune LEW.1N(T) donor cells.

cating that suppression was mediated by T lymphocytes. Suppression was also eliminated by cyclophosphamide pretreatment of an immunized BN rat cell donor.

As noted in Table V, nylon wool separation of the immune BN lymphocytes (a separation resulting in <3–5% Ig$^+$ cells in the nonadherent fraction) confirmed that the suppression resided in the enriched T cell preparation. These NWT cells were further separated into OX8$^-$ or OX8$^+$ subpopulations by indirect panning. The adherent population obtained by indirect panning was ~90% OX8$^+$. This represented 15–17% of the starting lymph node T cells. The nonadherent T cells were 90% OX8$^-$. Only the OX8$^+$ subpopulation displayed suppression. The data in Tables IV and V consistently demonstrate that the suppression was antigen specific, since the DTH response to PPD was unaffected. Although this suppressor cell could not be identified in the peripheral lymphoid organs of CFA-immunized BN rats (Table IV), it was present in harvested thymocyte preparations.

Genetic Restriction of the Antigen-specific Suppressor T Cell. We examined the ability of immune lymphocytes from BN rats to suppress the DTH reactivity of immune cells from disparate strains. We found that immune cells from BN donors suppressed the BN-TBM DTH reactivity of immune cells from LEW.1N(T) but not LEW(T) rats (Table VI). We did not have an RT1^l/TBM$^+$ strain to directly test its effect on DTH-reactive RT1^l/TBM$^-$ lymphocytes. Donor suppressor T cells from immune (BN × LEW)F$_1$ hybrids (TBM$^+$), however, were able to inhibit DTH-reactive cells from immune LEW.1N and LEW rats, indicating that the LEW response was also suppressible.

Role of the Suppression in Preventing the Development of Interstitial Nephritis to Autologous TBM. Since the presence of cyclophosphamide-sensitive suppressor T cells correlated with unresponsiveness to BN-TBM (Table IV) in BN rats, we examined the effect of cyclophosphamide (CTX) therapy on disease expression in immunized animals (22). In pilot studies we observed that BN rats receiving a single injection of CTX (50 mg/kg) immediately before immunization with BN-

TABLE VI
RT1 Restriction of BN Suppressor Cell to BN-TBM

Donor cells*		DTH response to BN-TBM‡
DTH reactive	Suppressor cell	
Immune LEW.1N(T)	—	28.3 ± 3.9
Immune LEW(T)	—	27.3 ± 1.5
Immune LEW.1N(T)	Immune BN	6.3 ± 0.3§
Immune LEW(T)	Immune BN	28.3 ± 2.2
Immune LEW.1N(T)	Immune (BN × LEW)F_1	5.7 ± 1.5§
Immune LEW(T)	Immune (BN × LEW)F_1	6.0 ± 1.0§
—	—	3.3 ± 0.3

* 20×10^6 immune cells from day-14 LEW.1N(T) or LEW(T) rats were injected alone or admixed with equal numbers of day-14 BN or (BN × LEW)F_1 immune lymph node cells, into naive (LEW.1N × LEW)F_1 recipients.
‡ See Table II.
§ $P < 0.005$ compared with response with LEW.1N(T) donor cells.

TABLE VII
Role of Suppressor Cells in Preventing Interstitial Nephritis in BN Rats

Immunization*	Treatment	Direct fluorescence of bound α-TBM-Ab	Histologic disease‡	DTH response§	
				BN-TBM	PPD
−	—	0.0	0.0	5.0 ± 1.6	22.0 ± 0.6
+	—	0.5 ± 0.3	0.0	5.5 ± 0.3	25.5 ± 0.6
+	CTX	3.0 ± 0.4	2.0 ± 0.8	19.5 ± 1.7‖	20.0 ± 2.0
−	CTX	0.0	0.0	4.7 ± 0.9	24.3 ± 5.8

* BN rats were immunized with BN-TBM/CFA (+) or CFA alone (−). Selected groups were concomitantly treated with cyclophosphamide (CTX) intraperitoneally, 15 mg/kg on the day of immunization and 2 mg/kg daily thereafter until sacrifice.
‡ Histologic disease was assessed 28 d after immunization.
§ DTH responses were measured 14 d after immunization as described in Table I.
‖ $P < 0.005$ compared with control CTX.

TBM/CFA did not develop histologic lesions (data not shown). Daily treatment of immunized BN rats with low dose CTX, however, resulted in a significant DTH response to BN-TBM as well as histologic interstitial nephritis (Table VII). This interstitial lesion was qualitatively indistinguishable from those seen after immunization with heterologous renal tubular antigen. CTX-treated rats displayed more marked deposition of α-TBM-Ab compared with non–CTX-treated BN rats; circulating α-TBM-Ab titers by radioimmunoassay also were significantly higher (data not shown).

Discussion

These studies illustrate several interesting findings regarding the control of the immune response to autologous parenchymal tubular antigen. BN rats immunized with BN-TBM failed to develop interstitial nephritis or a cell-mediated immune response. This is in sharp contrast to the severe, progressive lesion that develops in the same strain after immunization with rabbit antigen (7, 23). Previous work (13) in BN rats immunized with heterologous antigen has

shown that the DTH response to TBM correlates well with disease expression. By using $RT1^n$, TBM^+, or TBM^- strains neonatally tolerized to BN alloantigens, we observed that TBM^- strains mounted a cell-mediated immune response to BN-TBM, whereas TBM^+ strains did not. Acute adoptive transfer studies verified that the DTH response of immunized LEW.1N(T) rats was antigen specific and mediated by T lymphocytes. Expression of this effector function was restricted by gene products in RT1 (Table III). Admixture of immune BN cells with DTH-reactive lymphocytes from $RT1^n/TBM^-$ rats also resulted in antigen-specific suppression of the DTH response. This suppression was mediated by an RT1-restricted, $OX8^+$, cyclophosphamide-sensitive T cell present in immune peripheral lymph nodes. Suppression was also mediated by thymocytes from naive BN rats, and abrogation of this suppression by low-dose cyclophosphamide therapy resulted in significant α-TBM-Ab deposits, DTH responsiveness to BN-TBM, and histologic interstitial nephritis.

Several of these findings require additional elaboration. It appears that alloreactivity to the immunizing antigen facilitates the development of autoimmune interstitial lesions, as evidenced by the results comparing F.BN and F.BN(T) rats immunized with BN-TBM. The alloreactivity exhibited by F.BN was conceivably directed against BN allo-determinants on the relevant nephritogenic moieties, or other allotypic markers in the immunizing preparation. Thus, although immune LEW.1N and LEW.1N(T) animals mount quantitatively similar DTH responses to BN-TBM, presumably these are qualitatively different responses; that is, an immunized LEW.1N rat probably exhibits (like F.BN) an antialloantigenic response in addition to an anti-TBM response.

The immunologic control of immune responses to BN-TBM by BN rats may occur at different levels by several mechanisms. Autoimmunity is probably contained to a certain degree by the fact that the T cell response to BN-TBM is MHC restricted. Although it is only incompletely mapped in rats, helper/inducer T cells in mice are restricted by MHC class II antigens while nephritogenic effector T cells are restricted by class I determinants (24, 25). Since class II antigen is variably expressed at the tubular level, autorecognition may be functionally minimized in the naive animal (work in progress). Since the BN rats that developed interstitial lesions with low-dose CTX (Table VII) were immunized with BN-TBM/CFA, an adjuvant effect on the expression of RT1 antigens at the tubular level cannot be fully excluded as a factor contributing to disease (26). Our data in this current report also suggest that immunization with parenchymal self-antigens elicits the expansion of antigen-specific suppressor T cells. These cells are readily detected in the thymus of naive rats, but require immunization before they are observed in peripheral lymphoid organs. The relevance of this suppressor mechanism to the maintenance of self-tolerance is supported by the effects of daily CTX administration. Daily CTX, in a dose range known to preferentially affect suppressor T cells (22, 27, 28), resulted in autoimmunity to TBM. Although a more direct demonstration of disease protection by these suppressor cells would use add-back strategies of appropriate T cell subpopulations, the requirement of daily CTX treatment to produce lesions in immunized BN rats eliminated this as an option. We also demonstrated that suppressor T cell function was RT1 restricted in its mediation of effect. This finding highlights

a probable role for thymic education in the development of tolerance to parenchymal self. Evidence that T tolerance to minor self-histocompatibility antigens is MHC restricted has also been recently reported by others (29, 30).

Thus, tubular antigen–reactive T cells are present in the BN rat, and their expression after antigenic challenge is inhibited by a potent suppressor mechanism. Our studies, however, do not rule out an immunoprotective role for clonal deletion. Indeed, the two mechanisms may coexist. The majority of self-reactive clones, for example, may be inactivated or deleted during ontogeny, and for those escaping deletion, suppressor T cells function as a backup mechanism preventing autoimmunity (2). In such a model, the relative importance of suppressor cells would be greater after an event eliciting expansion of quiescent self-reactive clones. The suppressor cells we describe here are also probably a distinct population from "veto" cells which serve to inactivate self-reactive precursor cells, as veto cells seem only to function during the generation of effector cell repertoires (31, 32).

Our results are also consistent with observations made in several other models of autoimmunity. Immunodeficient mice immunized with modified-self antigens can display antigen-specific DTH responses, but such responses are not seen in normal mice (33). Thymic or splenic T cells from normal syngeneic mice can suppress this reactivity (34). These suppressor cells are Lyt-2^+, I-J^+, and their function can be replaced by cell-derived soluble factors (34–36). Others, in studying the autoimmune lesions developing after reconstitution of nude mice with cells from euthymic (nu/+) heterozygotes, have found that defined T cell subpopulations can suppress the development of autoimmune disease (37). Similarly, the lack of an immune response to self-protein F may also be related, in part, to activation of suppressor cells (38). Thus, suppressor mechanisms similar to those described in the current studies may exist for other organ-specific autologous antigens.

Summary

BN rats develop interstitial nephritis after immunization with rabbit, but not rat renal tubular antigen. Using $RT1^n$ rat strains that differentially express tubular antigen, we investigated the unresponsiveness of BN rats to BN tubular antigen (BN-TBM) using delayed-type hypersensitivity (DTH) responses to BN-TBM as a measure of cell-mediated immunity. Our results indicate that rat strains expressing tubular antigen respond to immunization with BN-TBM with the clonal expansion of antigen-specific, cyclophosphamide-sensitive, $OX8^+$, MHC-restricted suppressor T cells. Such suppression appears to be relevant to the maintenance of tolerance to parenchymal self, since chronic cyclophosphamide therapy abrogates suppression and results in significant interstitial nephritis.

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