DR-BB Rat Thymus Contains Thymocyte Populations Predisposed to Autoreactivity

Barbara J. Whalen, Aldo A. Rossini, John P. Mordes, and Dale L. Greiner

We have induced autoimmune insulin-dependent diabetes mellitus (IDDM) in athymic WAG rats by transfusing thymocytes from histocompatible phenotypically normal rats of the DR-BB strain. DR-BB rats rarely develop spontaneous IDDM, but readily become hyperglycemic if depleted in vivo of regulatory T-cells that express the RT6.1 maturational alloantigen. Successful adoptive transfer of IDDM by DR-BB thymocytes required that the athymic recipients be depleted of emerging populations of donor-origin RT6.1$^+$ T-cells. Thymocytes from both normal and RT6-depleted diabetic DR donors were equally capable of transferring autoimmunity. In contrast, thymocytes from normal histocompatible YOS rats failed to transfer IDDM. The autoreactive potential of DR-BB rat thymocytes was minimal from birth to 4 weeks of age and then increased substantially at 8–9 weeks of age. These results demonstrate that the DR-BB rat thymus harbors abnormal cell populations predisposed to autoreactivity. The data localize the developmental defect leading to diabetes in the BB rat to an abnormal intrathymic selection process. ***Diabetes*** **44:963–967, 1995**

DP-BB rats develop spontaneous hyperglycemia that is autoimmune in origin (1). They are used extensively as an animal model of human insulin-dependent diabetes mellitus (IDDM). Coisogenic DR-BB rats were developed from DP forbearers selected for normoglycemia. Fewer than 1% develop spontaneous autoimmunity, but IDDM can be induced by many interventions, including low-dose irradiation, cyclophosphamide administration, viral infection, and depletion of RT6$^+$ T-cells. RT6 is a rat T-cell maturational alloantigen acquired post-thymically that is expressed on regulatory T-cells which can prevent the development of autoimmunity in BB rats. DP-BB rats are severely lymphopenic and deficient in RT6$^+$ T-cells, whereas DR-BB rats are not lymphopenic and circulate normal numbers of RT6$^+$ T-cells (1). Both spleen cells and purified lymph node T-cells from RT6-depleted DR-BB donors adoptively transfer diabetes to naive athymic recipients (2), whereas cotransfer of cells from intact donors exerts a protective effect (3).

From the Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts.

Address correspondence and reprint requests to Dr. Dale L. Greiner, Diabetes Division, Biotech 2, 373 Plantation St., Worcester, MA 01605.

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FITC, fluorescein isothiocyanate; IDDM, insulin-dependent diabetes mellitus; KRV, Kilham's rat virus; mAb, monoclonal antibody; MHC, major histocompatibility complex; PE, phycoerythrin; poly(I·C), polyinosinic acid–polycytidylic acid; SA, streptavidin; TCR, T-cell receptor.

The pathological process that leads to the generation of autoreactive cells in BB rats is unknown, but previous studies have implicated intrathymic events. Neonatal thymectomy prevents DP-BB rat diabetes (4), and a population of DP-BB bone marrow–derived thymic dendritic cells is associated with the appearance of abnormal T-cells and the expression of IDDM (5,6). In addition, we have identified morphologically abnormal regions of thymic cortex and medulla devoid of thymic epithelium in DP and DR-BB rats (7). Either of the latter defects could contribute to abnormal thymic selection. Although these data and theoretical considerations imply the existence of thymocytes predisposed to autoreactivity, we are aware of no direct demonstration of their existence. The present studies demonstrate that the DR-BB rat thymus does harbor abnormal cell populations with autoreactive potential with the ability to induce both IDDM and autoimmune thyroiditis. They provide evidence for defective intrathymic selection in the BB rat model of IDDM.

RESEARCH DESIGN AND METHODS

Animals. Inbred, viral antibody–free DR-BB rats of both sexes were obtained from the colony at the University of Massachusetts Medical School (8). All BB rats express the RT1^u major histocompatibility complex (MHC) haplotype and the RT6.1 T-cell alloantigen (1). YOS (RT1^u, RT6.1) and athymic WAG *rnu/rnu* (RT1^u, RT6.2) rats were obtained from a colony maintained by us and previously described (2). Untreated YOS and WAG nude rats are free of diabetes, insulitis, and thyroiditis. Diabetes was induced in some DR-BB rats by treating them with the DS4.23 anti-RT6.1 monoclonal antibody (mAb) and polyinosinic acid–polycytidylic acid [poly(I·C)] as previously described (7). Diabetes was detected by testing for glycosuria (Tes-Tape, Lilly, Indianapolis, IN) and diagnosed on the basis of a plasma glucose concentration of 11.1 mmol/l (Beckman Glucose Analyzer II, Beckman, Fullerton, CA).

WAG *rnu/rnu* and BB rats were housed either in sterilized cages in laminar flow cabinets or in sterile microisolators at 20–22°C and were provided with acidified water, autoclaved food (Purina 5010), and sterile bedding. YOS rats were housed under standard conditions in a specific pathogen-free facility. At the time of these studies, sera from sentinel animals in our facility tested negative for Sendai, pneumonia virus of mice, sialodacryoadenitis, H-1, GD7, Reo-3, and lymphocytic choriomeningitis viruses and for *Mycoplasma pulmonis* (Charles River, Wilmington, MA). Some sentinel animals were seropositive for Kilham's rat virus (KRV). All tested DR thymocyte donors were seronegative for KRV. A random sample of athymic adoptive recipients from each experimental and control group was also tested serologically for KRV at the conclusion of the experiment. These included at least one athymic recipient from every cage. Serologically positive animals were found in both diabetic and nondiabetic animals in both the control and experimental cohorts. All rats were maintained in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (Department of Health, Education and Welfare Publication; NIH publ. no. 78–23, 1985).

Adoptive transfer procedures. Thymocytes were harvested from donor rats aged 1 day to 8–9 weeks. Animals were killed in an atmosphere of 100% CO_2, and their thymuses were removed. Parathymic lymph nodes were identified and dissected away, and thymic cell

TABLE 1
Induction of diabetes, insulitis, and thyroiditis in athymic WAG rats after transfusion of thymocytes from DR-BB rats

Thymocyte donor strain	Donor treatment	Recipient treatment	Diabetic	Latency to diabetes (days)	Nondiabetic with insulitis	Diabetes or insulitis	Thyroiditis
DR	Anti-RT6 mAb + poly(I · C)	Anti-RT6 mAb	7/11 (64)*	47 ± 17 (33–82)	4/4 (100)	11/11 (100)†	4/10 (40)‡
DR	None	Anti-RT6 mAb	7/9 (78)*	48 ± 11 (36–61)	2/2 (100)	9/9 (100)†	6/8 (75)‡
YOS	Anti-RT6 mAb + poly(I · C)	Anti-RT6 mAb	0/5 (0)	—	0/5 (0)	0/5 (0)	0/5 (0)
YOS	None	Anti-RT6 mAb	0/4 (0)	—	0/4 (0)	0/4 (0)	0/4 (0)
DR	Anti-RT6 mAb + poly(I · C)	None	0/5 (0)	—	0/5 (0)	0/5 (0)	0/5 (0)
DR	None	None	0/10 (0)	—	0/10 (0)	0/10 (0)	0/10 (0)
YOS	Anti-RT6 mAb + poly(I · C)	None	0/5 (0)	—	0/5 (0)	0/5 (0)	1/5 (20)
YOS	None	None	0/4 (0)	—	0/4 (0)	0/4 (0)	0/4 (0)
No thymocytes	—	Anti-RT6 mAb	0/6 (0)	—	0/6 (0)	0/6 (0)	0/6 (0)

Data are n (%) or means ± SD (range). The dose of thymocytes transfused was $1.75–1.80 \times 10^8$ per recipient. The results shown are compiled from four separate experiments, each of which included recipients that received either normal DR or acutely diabetic DR thymocytes. Univariate statistical analysis revealed significant main effects associated with donor strain and recipient treatment status, i.e., treatment with anti-RT6 mAb: *$P < 0.001$, †$P < 0.0001$, ‡$P < 0.05$. There were no statistically significant effects associated with donor treatment status.

suspensions were prepared by gently extruding minced glands through mesh screens into RPMI-1640 medium at 4°C. Cells were washed twice and filtered to remove connective tissue. Viability of recovered thymocytes was confirmed by the method of trypan blue exclusion and was >95% in all cases. Cells were injected into recipients intravenously via the tail vein in a volume of 1 ml.

Reagents. The DS4.23 hybridoma, a rat IgG_{2b} mAb that depletes $RT6.1^+$ T-cells in vivo (9), was cultured in RPMI supplemented with 2% fetal calf serum. Culture supernatants were centrifuged to remove cells and filter-sterilized. Isotype-matched B21–2 anti-mouse $IA^{b,d}$ mAb culture supernatants (American Type Culture Collection, Rockville, MD) were prepared in the same manner. DS4.23 and OX8 (anti-rat CD8) mAbs were purified by affinity chromatography and biotinylated (10). All other mAbs were purchased from Pharmingen (San Diego, CA). Poly(I·C) was purchased from Sigma (St. Louis, MO).

Flow microfluorometry. Thymocytes were dual-labeled for immunofluorescence using phycoerythrin (PE)–anti-rat CD4 (OX-38) and fluorescein isothiocyanate (FITC)–anti-rat CD8 (OX-8) or were single-labeled using FITC–anti-rat αβ-T-cell receptor (αβ-TCR) (R73). Isotype controls were PE-mouse IgG2a and FITC-mouse IgG1 mAbs.

T-cell engraftment in athymic recipients was monitored by phenotyping cervical and mesenteric lymph node cell pools or by phenotyping individual recipient spleens. T-cell subsets were quantified by dual-label immunofluorescence using FITC–anti-rat TCR (R73) and biotinylated anti-CD8 (OX-8) and anti-CD4 (OX-38). These were followed by PE-streptavidin (SA) labeling. $RT6.1^+$ T-cells were detected with biotinylated DS4.23 mAb followed by PE-SA. Controls for background staining were FITC-mouse IgG1 and PE-SA alone. Labeled cells were fixed in 1% paraformaldehyde in phosphate-buffered saline and analyzed on a FACS IV (Becton Dickinson, Sunnyvale, CA).

Histology. Pancreas and thyroid specimens were fixed in Bouin's solution, washed, and postfixed in 10% buffered formalin. Sections were stained with hematoxylin and eosin and examined by a pathologist not aware of the treatment status of the specimens.

Experimental protocols. All experiments tested the ability of thymocytes to adoptively transfer IDDM and lymphocytic thyroiditis to athymic WAG nude recipient rats. Thymocyte donors included acutely diabetic DR rats that had been treated with DS4.23 anti-RT6.1 mAb and poly(I·C), nondiabetic YOS rats that had been treated in the same way, and untreated nondiabetic DR-BB and YOS rats. The age of thymocyte donors varied from 1 day to 8–9 weeks according to the specific experimental design. Recipients received either no further intervention, treatment with DS4.23 mAb (2 ml culture supernatant intraperitoneally 5 times/week), or treatment with an isotype-matched irrelevant mAb (B21–2) on the same schedule. Each protocol was tested on a minimum of four rats.

Recipients were weighed and tested for diabetes 3 times/week through the onset of diabetes or until 90–115 days after thymocyte transfer. Diabetic rats were killed within 1 week of diagnosis. T-cell subset percentages were determined using lymph nodes or spleens obtained at autopsy.

Statistical analysis. Parametric data are presented as means ± 1 SD. Means were compared using Student's t tests or analyses of variance with the least-squares difference procedure for a posteriori contrasts (11). Nonparametric data were analyzed by univariate analysis using Freeman-Halton (12), χ^2 (13), and Fisher's exact (13) statistics as appropriate for table dimensions. Because some histological samples were technically unsatisfactory, the number of rats studied for diabetes occurrence is sometimes greater than the number reported as studied for diabetes plus insulitis.

RESULTS

DR-BB rat thymocytes transfer IDDM. Thymocytes from DR-BB rats induced insulitis and diabetes after transfusion into histocompatible athymic WAG recipients (Table 1). The ability of DR thymocytes to transfer autoimmunity did not require any form of donor pretreatment but was critically dependent on treatment of recipients with anti-RT6.1 mAb. In the absence of recipient treatment with anti-RT6 mAb, none of the 15 athymic recipients of DR thymocytes shown in Table 1 developed insulitis or thyroiditis. YOS rat thymocytes were found to have no ability to transfer insulitis or diabetes irrespective of donor or recipient treatment status Table 1.

Results with respect to the induction of autoimmune thyroiditis were identical with the exception of a single recipient. That animal received thymocytes from an RT6-depleted YOS rat and no anti-RT6 mAb after transfer. The percentage of thyroiditis observed (40–75%) in athymic recipients of DR thymocytes was comparable to the frequency that is observed in diabetic DR donors after treatment with anti-RT6 mAb and poly(I · C) (7). Athymic WAG recipient control animals that did not receive thymocytes but did receive anti-RT6 mAb remained free of disease.

Phenotypic analyses of thymocyte recipients demonstrated *1*) the presence of donor-origin ($RT6.1^+$) T-cells in all untreated athymic recipients and *2*) the absence of such cells in recipients treated with anti-RT6.1 mAb (Table 2).

The ability of DR thymocytes to transfer insulitis and IDDM was a function of the number of cells transfused (Table 3). A dose of 1.8×10^8 thymocytes consistently (Tables 1 and 3) induced either insulitis or IDDM in all recipients. Lower doses were less effective (Table 3). In contrast, adoptive transfer of lymphocytic thyroiditis did not display cell dose dependence. This experiment also confirmed the importance of recipient anti-RT6 mAb treatment; only one of nine athymic recipients of DR thymocytes developed insulitis in the absence of such treatment, and none developed IDDM.

TABLE 2
Lymph node T-cell analysis in athymic WAG rats after transfusion of thymocytes

Thymocyte donor strain	Recipient treatment	$CD4^+$ T-cells (%)	$CD8^+$ T-cells (%)	$RT6.1^+$ T-cells (%)
RT6-depleted DR	Anti-RT6 mAb	39 ± 2	7 ± 3	3 ± 2
Intact DR	Anti-RT6 mAb	34 ± 3	5 ± 3	4 ± 2
RT6-depleted YOS	Anti-RT6 mAb	32	9	3
Intact YOS	Anti-RT6 mAb	40	4	0
Nondiabetic DP-BB	Anti-RT6 mAb	20	8	0
RT6-depleted DR	None	46	13	21
Intact DR	None	41	7	28
RT6-depleted YOS	None	37	11	29
Intact YOS	None	36	9	29
No thymocytes	Anti-RT6 mAb	23	11	2

Lymph node cell phenotypes of animals presented in Table 1. Data were obtained on cells pooled from four or five nude recipients except in the case of the DR animals shown in the first two lines of the table. In these cases, three separate analyses were performed on individual rats or on cells pooled from two recipients (n = 5).

Ability of DR-BB rat thymocytes to transfer IDDM is low at birth and increases with age. Thymocytes from newborn, 1-, 2-, and 4-week-old DR-BB donors contain a low frequency of diabetogenic cells (Table 4). Only 3 of 24 recipients of thymocytes from animals in this age range developed diabetes. Considering both IDDM and insulitis together, only 5 of the 24 animals were affected. In contrast, when thymocytes from donors 8 weeks of age were tested, five of six recipients developed IDDM. Combining the results from Tables 1, 3, and 4 with respect to 8- to 9-week-old DR donors of thymocytes, 35 of 36 anti-RT6 mAb–treated recipients developed IDDM or insulitis.

As in the previous experiment showing that the adoptive transfer of thyroiditis by DR thymocytes was not dose-dependent, this experiment demonstrated that the transfer of thyroiditis was not dependent on the age of the DR donor. Overall, 12 of 28 recipients developed thyroiditis, but the rates varied from 17 to 75% in the various age-groups and displayed no temporal trends.

The composition of thymocyte transfusions was similar for all donor age-groups with respect to major phenotype subsets (data not shown). The mean overall composition of thymocyte transfusions was 8 ± 2% $CD4^+CD8^-$, 5 ± 1% $CD4^-CD8^+$, 84 ± 2% $CD4^+CD8^+$, 4 ± 1% $CD4^-CD8^-$, and 13 ± 4% TCR^{hi}. To exclude the possibility that the limited capacity of thymocytes from newborn to 4-week-old DR rats to transfer IDDM was due to failure of T-cell engraftment, four to six recipients in each donor age category were individually phenotyped for splenic T-cell subsets. No donor age–dependent variation with respect to subsequent engraftment of T-cells in athymic recipients was observed (data not shown). The overall mean percentage of R73 αβ-TCR^+ T-cells was 27 ± 9% (n = 19) in anti-RT6 mAb–treated athymic recipients of DR thymocytes, 34 ± 9% (n = 18) in isotype control treated athymic recipients of DR thymocytes, and 13 ± 3% (n = 6) in anti-RT6 mAb–treated athymic rats not given thymocytes.

DISCUSSION

These data demonstrate that DR-BB rat thymocytes are capable of adoptively transferring IDDM to naive athymic recipients. Such cells were absent in normal MHC-compatible YOS rats. In DR-BB rats, they were infrequent or nonfunctional in the neonatal thymus but readily demonstrable by 8 weeks of age.

Successful transfer of autoimmunity was critically dependent on treatment of thymocyte recipients with a depleting anti-RT6 mAb. The RT6 alloantigen is not expressed on thymocytes but can be found on ~70% of T-cells within 4–8 days of their release from the thymus (9). Our data document that athymic rats circulate donor-origin $RT6^+$ T-cells after transfusion of thymocytes and that the development of such cells is prevented by continuous treatment of recipients with anti-RT6 mAb. These findings contribute additional support to our hypothesis that an imbalance between autoreactive $RT6^-$ and regulatory $RT6^+$ T-cells in part determines the expression of BB rat autoimmunity (1,14). Previous studies have demonstrated that DP-BB rats are deficient in $RT6^+$ T-cells (15) and that spontaneous diabetes in these animals is prevented by engraftment of transfused normal $RT6^+$ T-cells (16). Similarly, lymph node T-cells from diabetic RT6-depleted DR-BB donors adoptively transfer IDDM to athymic recipients (2), and this process of adoptive induction can in

TABLE 3
Diabetes and thyroiditis in athymic WAG rats after transfusion of varying numbers of thymocytes

Thymocyte donor strain	Nude recipient treatment	Cells transfused ($\times 10^6$)	Diabetic	Latency to diabetes (days)	Nondiabetic with insulitis	Diabetes or insulitis	Thyroiditis
DR	Anti-RT6 mAb	180	7/10 (70)*	50 ± 10 (41–68)	3/3 (100)	10/10 (100)*	4/10 (40)†
DR	Anti-RT6 mAb	36	1/9 (11)	45	1/4 (25)	2/5 (40)	2/9 (22)
DR	Anti-RT6 mAb	7.2	1/10 (10)	50	3/9 (33)	4/10 (40)	4/9 (44)
DR	None	180	0/9 (0)	—	1/9 (11)	1/9 (11)	2/9 (22)

Data are n (%) or means ± SD (range). The 1.8×10^8 cell dose is the same as that used in the experiments shown in Table 1. The other doses were successive 1/2-log dilutions. The DR thymocyte donors include both untreated and RT6-depleted animals. As documented in Table 1, there is no difference in the ability of thymocytes from treated DR and untreated DR donors to transfer diabetes, and data from these groups have been combined. *$P < 0.025$ vs. all other dosage groups; no other paired comparisons were statistically significant. †There were no statistically significant differences in the frequency of thyroiditis.

TABLE 4
Diabetes and thyroiditis in athymic WAG rats after transfusion of thymocytes from DR-BB rat donors of varying ages

Nude recipient treatment	Age of thymocyte donor (weeks)	Diabetic	Latency to diabetes (days)	Nondiabetic with insulitis	Diabetes or insulitis	Thyroiditis
Anti-RT6.1 mAb	1–3 days	1/9 (11)	68	0/8 (0)	1/9 (11)	3/9 (33)
	1	0/4 (0)	—	0/4 (0)	0/4 (0)	3/4 (75)
	2	1/5 (20)	41	1/4 (25)	2/5 (40)	2/5 (40)
	4	1/6 (17)	93	1/5 (20)	2/6 (33)	1/6 (17)
	8	5/6 (83)	50 (40–64)	0/1 (0)	5/6 (83)	3/4 (75)
Isotype control mAb	1	0/5 (0)	—	0/5 (0)	0/5 (0)	1/5 (20)
	2	0/5 (0)	—	0/5 (0)	0/5 (0)	1/5 (20)
	4	0/5)0)	—	0/5 (0)	0/5 (0)	0/5 (0)
	8	0/5 (0)	—	0/5 (0)	0/5 (0)	0/5 (0)

Data are *n* (%) or means (range). Thymocytes from 1- to 2-day-old donors were transfused at a dose of 1.3×10^8 cells; thymocytes from 3-day- to 8-week-old donors were transfused at a dose of 1.8×10^8 cells. Univariate statistical analysis revealed significant main effects associated with donor age and recipient treatment status, i.e., treatment with anti-RT6 mAb: For recipients treated with anti-RT6 mAb, donor age was associated with induction of diabetes at $P < 0.005$ and with the induction of either diabetes or insulitis at $P < 0.001$. The induction of thyroiditis was significantly associated with recipient treatment status, but among anti-RT6 mAb–treated recipients, there was no statistically significant effect of donor age.

turn be abrogated by cotransfer of $RT6^+$ T-cells from normal donors (3).

The present data were generated using thymocytes from DR-BB rats. It is reasonable to presume that a similar pathological process occurs in the thymus of the coisogenic DP-BB rat. We have studied the DP thymus using similar protocols, but have been unable to document adoptive transfer of autoimmunity (data not shown). We believe that this failure reflects the short life span of DP-BB rat T-cells (17,18). We found that adoptive transfer of DP thymocytes did not lead to detectable levels of T-cell engraftment in athymic recipients (Table 2). These observations closely parallel previous studies. These have shown that DP splenic T-cells cannot adoptively transfer IDDM or thyroiditis to athymic recipients unless they are mitogen-activated, whereas unstimulated splenocytes from RT6-depleted DR rats readily transfer both disorders (2). In this context, it is appropriate to note that our data do not specify which thymocyte subpopulation (i.e., cortical versus medullary) is responsible for the adoptive transfer of IDDM. It is known, however, that double-positive ($CD4^+CD8^+$) thymocytes obtained from normal rats do not mature into functional T-cells after transfer to athymic recipients (19). It is, accordingly, reasonable to speculate that the induction of IDDM observed in these studies reflects the ability of medullary DR thymocytes to mature into effector T-cells in athymic recipients.

The present studies also document the ability of DR-BB rat thymocytes to induce lymphocytic thyroiditis, a disorder that also occurs spontaneously in DP-BB rats (1) and after RT6 depletion in DR-BB rats (20). Unlike insulitis, thyroiditis in the intact BB rat never progresses to frank hypothyroidism, suggesting that the pathological processes underlying the two diseases differ in certain respects. The present data document some additional differences. The adoptive transfer by thymocytes of thyroiditis, unlike that of IDDM, was not dependent on either cell dose or donor age. These data suggest that the pathogenic processes underlying the two disorders, although genetically linked, exhibit different developmental kinetics.

The thymus has been implicated in the generation of autoreactive T-cells in other experimental models of autoimmunity. In B6AF1 mice, for example, neonatal but not adult thymectomy induces organ specific autoimmunity (21,22). Fetal rat or neonatal mouse thymuses grafted into athymic recipient mice induce both organ-specific and systemic autoimmunity; adult thymus grafts are much less effective (23,24). The detection of autoimmunity after manipulation of the thymus in the neonatal period has led to the inference that neonatal thymocytes are enriched in populations with autoreactive potential or that the neonatal thymus fails to generate populations of mature regulatory cells. We are aware of only one previous demonstration of autoreactivity in adult thymocyte populations. Using an adoptive transfer design, Smith et al. (25) demonstrated that thymocytes with autoreactive potential directed against ovary and gastric mucosa are present in both neonatal and adult BALB/c mice.

Our ability to test for the presence of thymic autoreactivity in the absence of regulatory cell populations in the present study permits us to extend these previous observations and draw several conclusions. First, autoreactive thymocyte populations capable of causing IDDM are present in the thymus of the adolescent DR-BB rat but are not readily detectable in the newborn animal. Their number and functionality increases with age. It might be argued that this observation could simply reflect the presence of fewer medullary thymocytes at younger ages. Our phenotyping data suggest, however, that younger and older animals harbored comparable percentages of phenotypically mature medullary thymocytes. A second conclusion is that the DR-BB rat thymus also harbors $RT6^-$ cell populations that mature into $RT6^+$ regulatory T-cells after their release from the thymus. The regulatory process itself is documented to be a peripheral, not an intrathymic, event. Expression of autoimmunity is dependent on the absence of circulating $RT6^+$ T-cells.

It is known that viral infection, specifically KRV infection, can modulate the frequency of IDDM in the DR-BB rat (20,26). In the present study, tested thymocyte donors were serologically negative for KRV, but serologically positive recipients were observed. Because seropositivity for KRV was observed in both diabetic and nondiabetic animals and in both experimental and control groups, we can conclude that KRV did not exert an independent diabetogenic effect in these studies. We cannot, however, exclude the possibility of a contributory effect of recipient KRV seropositivity in concert with thymocyte transfer and regulatory cell depletion.

In conclusion, our studies have documented the presence of thymocytes with autoreactive potential in the DR-BB rat. In the context of previous studies, the present data suggest a

possible mechanism to explain the temporal characteristics of diabetes expression in the BB rat (1). The age at which thymocytes transfer autoimmunity is approximately the age at which it is possible to induce diabetes in DR rats by depletion of $RT6^+$ T-cells (27) and the age at which DP rats can be protected from autoimmunity by transfusions of regulatory T-cells (16). Finally, the time course associated with the appearance of thymocytes with autoreactive potential in the present study closely parallels the development of thymic epithelial abnormalities (7). We believe it reasonable to speculate that these parallel functional and morphological observations are causally related.

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