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Adoptive transfer of autoimmune diabetes and thyroiditis to athymic rats

(BB rat/RT6 alloantigen/major histocompatibility complex/nude rat)

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ABSTRACT **We describe the induction of autoimmune diabetes, insulitis, and thyroiditis in athymic rats following injections of major histocompatibility complex compatible spleen cells. Lymphocytes with these capabilities were found in normal rats of the YOS, WAG, PVG, and diabetes-resistant BB strains, and in diabetes-prone BB rats. Adoptive transfer was facilitated by prior *in vivo* depletion of RT6.1$^+$ regulatory T cells and *in vitro* mitogen activation of donor spleen cells. By RT6 depleting diabetes-resistant donors and using nude recipients, transfer of diabetes and thyroiditis was accomplished by using fresh, unstimulated spleen cells. The data suggest that organ-specific autoreactive cells may be present to various degrees but suppressed to a variable extent in many rat strains. The equilibrium between autoreactive and regulatory cells appears to determine the expression of autoimmunity.**

Clonal deletion of autoreactive lymphocytes during repertoire development and active suppression of autoreactive cells in peripheral tissues contribute to the prevention of autoimmune diseases (1). In mice, T-lymphocyte populations are deleted in the thymus following interaction with a major histocompatibility complex (MHC) class II (I-E) molecule (2). Nonobese diabetic mice do not express I-E and develop autoimmune diabetes (3). These mice harbor T-cell populations normally deleted in I-E-expressing strains (4). In B6AF1 mice, neonatal thymectomy induces organ-specific autoimmunity (5). Thymectomy earlier or later is ineffective, suggesting that a sequence of intrathymic events culminates in an immunological definition of self.

Evidence for suppression of autoreactive T cells in peripheral tissues is also accumulating. Transplantation of Lyt-1-depleted spleen cells from *nu*/+ mice (6) or thymuses from cyclosporin-treated *nu*/+ mice (7) into *nu*/*nu* recipients results in autoimmune endocrinopathy. This can be prevented by cotransplantation of a *nu*/+ thymus or unfractionated spleen cells together with the cyclosporin-treated thymus.

An imbalance between autoreactive and regulatory (RT6$^+$) cells may in part determine the expression of BB rat autoimmunity (8). Diabetes-prone (DP) BB rats are lymphopenic and deficient in T cells that express the RT6 alloantigen (9). They spontaneously develop insulitis, hyperglycemia, and thyroiditis (8, 10). Lymphocyte transfusions prevent disease in DP rats if RT6$^+$ donor T cells become engrafted (11). Adoptive transfer of BB rat diabetes has been demonstrated by using either young DP or immunosuppressed non-BB rats as recipients. This requires mitogen activation of donor cells (12, 13). Diabetes-resistant (DR) BB rats have normal numbers of RT6$^+$T cells. *In vivo* depletion of these cells induces diabetes in ≈50% of treated animals, and their spleen cells transfer diabetes to naive recipients (14).

The present studies present evidence for the existence of autoreactive cells not only in BB but also in non-BB rats. We demonstrate the adoptive transfer of diabetes, insulitis, and thyroiditis to nude rats by using spleens from three normal rat strains.

MATERIALS AND METHODS

Animals. DR and DP BB/Wor rats were obtained from the University of Massachusetts (15). DP animals were inbred and 60–80% become diabetic between 60 and 120 days of age. Before 60 days, <0.5% develop diabetes. Thyroiditis occurs at a rate of 30–60% by 90 days of age, but hypothyroidism is rare. DR rats were derived from DP forebears and are not lymphopenic. The frequency of diabetes, insulitis, and thyroiditis is <1% in DR rats. The MHC haplotype of BB rats is RT1^u (10); DR T cells express the RT6.1 alloantigen (14).

WF (RT1^u, RT6.2) rats were from the National Cancer Institute (Frederick, MD). YOS (RT1^u, RT6.1) rats were bred by us with stock provided by H. Kunz (University of Pittsburgh). WAG (RT1^u, RT6.2) rats were from our colony established with breeding stock from the Institute of Applied Radiobiology and Immunology (TNO) (Rijswijk, Netherlands). PVG (RT1^c, RT6.1) rats were from Harlan–Sprague–Dawley. WF, WAG, YOS, and PVG rats are inbred and normally free of autoimmune diseases.

Athymic *rnu*/*rnu* nude PVG rats were from Harlan–Sprague–Dawley. WAG nude rats were bred in our colony with stock from the TNO Institute. Nude rats were housed in laminar flow cabinets at 20°C–22°C and were given acidified water, autoclaved food (Purina 5010), and sterile bedding. At the time of these studies, the *rnu* gene had been backcrossed 11 generations in WAGs and 12 generations in PVGs. Nonnude rats were housed under standard conditions.

At the time of these studies, sera from sentinel animals in our facility indicated the presence of Sendai, PVM, H-1, and SDA viruses, and, occasionally Kilham's rat virus. Diabetes was diagnosed on the basis of glycosuria and a plasma glucose concentration of >200 mg/dl.

Adoptive Transfer Procedures. Spleen cells were prepared for adoptive transfer by mitogen activation with concanavalin A (14). Cell viability was determined, and recipient rats were injected via the tail vein with 40×10^6 viable cells in a volume of 0.4 ml. In some experiments, donor cells were not mitogen activated. After three washes in RPMI medium, these freshly

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Abbreviations: MHC, major histocompatibility complex; DP, diabetes prone; DR, diabetes resistant; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; GVHD, graft-vs.-host disease.

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isolated splenocytes were injected into recipients (10^8 cells in 0.5 ml per rat).

Flow Microfluorimetry. To determine the number of native T cells present in athymic rats and to assay for chimerism in adoptive recipients, lymph node cell suspensions were labeled with OX19 mouse anti-rat CD5 (pan-T cell) monoclonal antibody (mAb), with the rat anti-RT6.1 mAb DS4.23, or with the rat anti-RT6.2 mAb 6A5 (9). Cells were developed for immunofluorescence by using an $F(ab')_2$ fragment of a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse (H+L; Cooper Biomedical) or a FITC-conjugated goat anti-rat IgG (H+L; Caltag, San Francisco) (9). OX19, DS4.23, and 6A5 mAbs were obtained from cultures maintained in our laboratory (16). Controls included FITC conjugate alone. At least 5×10^4 formalin-fixed cells were analyzed on a FACS IV (Becton Dickinson).

Histology. Pancreas and thyroid specimens were fixed in Bouin's solution. Sections were stained with hematoxylin and eosin and were examined by a pathologist (M.C.A.) not aware of the treatment status of the specimens. Postmortem specimens of other tissues were also obtained from selected athymic recipients, processed identically, and examined for evidence of inflammation.

Experimental Protocols. Three experiments were performed in which spleen cells were transfused into nude rats. Recipients were weighed and tested for diabetes three times weekly from 10 days after transfusion until the end of the experiment. Diabetic rats were killed when diagnosed. Percentages of $CD5^+$ lymph node cells were determined in all nude recipients either at diabetes onset or at the end of the experiment.

Experiment 1 was designed to detect autoreactive cells against pancreas and thyroid in non-BB strain rats. In a preliminary experiment, $RT1^u$ YOS rats were depleted of $RT6.1^+$ cells, after which their spleen cells were mitogen activated and transfused into 30-day-old DP rats. Appearance of diabetes before 60 days of age in DP rats treated in this way is accepted as evidence of the adoptive transfer of the disease (12, 13). To deplete $RT6.1^+$ cells, DS4.23 mAb was given intraperitoneally as unconcentrated culture supernatant at a dose of 2 ml per rat, five times per week beginning at 30 days of age for 2–4 weeks (14).

The remaining trials in this experiment used untreated and RT6-depleted YOS donors with (*i*) WAG nude recipients, (*ii*) untreated and RT6-depleted PVG +/+ donors with nude PVG recipients, or (*iii*) untreated WF and WAG +/+ donors with WAG nude recipients (see Table 1). Depletion of $RT6.2^+$ cells in WAG and WF donors was not performed because of low cytotoxic activity of our anti-RT6.2 antibody.

Experiment 2 studied adoptive transfer of diabetes and thyroiditis using acutely diabetic DP rat donors and either mitogen-activated or freshly isolated spleen cells. Recipients were either MHC compatible WAG or incompatible PVG nude rats. Donors were used within 3 days of diabetes onset and were given insulin daily until then. Recipients were tested for diabetes until 38–88 days after transfusion except for two nondiabetic PVGs killed at 22 days.

Experiment 3 was identical except that spleen cell donors were either untreated or RT6-depleted DR rats. The RT6-depleted donors included both diabetic and nondiabetic animals. Previous studies have shown that activated cells from nondiabetic RT6-depleted DRs adoptively transfer diabetes (14).

Statistical Procedures. Parametric data are means ± SEM. Means were compared by *t* tests or analyses of variance with the LSD procedure for *a posteriori* contrasts. Nonparametric data were analyzed by Fisher exact or χ^2 statistics, and temporal changes were analyzed by linear regression (17). Because some histologic samples were unobtainable or technically unsatisfactory, the number of rats studied for diabetes occurrence is sometimes greater than the number reported as studied for diabetes plus insulitis.

RESULTS

There were no detectable $CD5^+$ lymph node cells in WAG nude rats at 36 days of age (Fig. 1*A*). A few were detected at 50 days of age and ≈23% were detected by 116 days of age ($P < 0.01$). No $RT6^+$ cells were detected through 116 days ($n = 10$), and between 116 and 208 days of age ($n = 5$) the mean percentage of $RT6.2^+$ cells was 8% ± 3%.

Experiment 1: Non-BB Rat Donors. Transfusion of mitogen-activated cells from RT6-depleted YOS rats into 30-day-old DP recipients induced diabetes in 6/36 (17%) recipients before 60 days of age. Thyroiditis was found in 15/23 (65%) specimens, a rate similar to that observed in unmanipulated DP rats of this age. No RT6-depleted YOS donors developed diabetes or insulitis ($n = 6$).

Although this result suggests that YOS rats harbor autoreactive cells, the outcome could represent nonspecific activation of DP effector cells. To avoid this possibility in subsequent studies, we used nude rats as adoptive recipients. Consistent with the preliminary result, RT6-depleted, mitogen-activated YOS cells produced diabetes or insulitis in 55% of nude recipients (Table 1). Thyroiditis was found in 1/6 specimens. An unexpected observation was exocrine pancreatitis. The commonest lesions were of moderate severity and were characterized by focal interstitial mononuclear cell infiltrates and some edema in the acinar stroma. Occasionally, severe infiltrative pancreatitis and acinar necrosis were observed. Pancreatitis did not correlate uniformly with the presence of diabetes or insulitis; it occurred in 8/9 nondiabetic recipients of mitogen-activated cells, but only half of the animals with pancreatitis also had insulitis.

Diabetes did not occur in recipients of activated cells from non-RT6-depleted donors, although insulitis was found in 2/4. No thyroiditis or pancreatitis was observed. Non-mitogen-activated YOS cells, whether untreated or RT6 depleted, did not induce insulitis or thyroiditis.

When mitogen-activated cells from untreated WAG +/+ rats were adoptively transferred to WAG nude hosts, insulitis and thyroiditis occurred, but diabetes and pancreatitis did not (Table 1). Neither freshly isolated WAG cells nor activated WF cells induced thyroid or pancreatic pathology in WAG nude rats. There was no evidence of graft-vs.-host disease (GVHD) in any WAG nude recipients. With few exceptions, none appeared ill; none had splenic enlargement when killed. Adrenal, gonad, stomach, tongue, skin, and liver histology from diabetic WAG recipients of fresh RT6-depleted DR cells revealed no inflammation.

Autoimmunity was not restricted to rats of the $RT1^u$ MHC haplotype. Transfusion of mitogen-activated RT6-depleted cells from PVG rats produced diabetes in 1, and diabetes or insulitis in 3/7 PVG nude rats (Table 1). Transfusion of activated cells from untreated PVG +/+ donors produced no pancreatic pathology, and thyroiditis in only 1/6 specimens. The RT6-depleted PVG donors used in this experiment were treated with mAb for 2–8 weeks. None developed diabetes or insulitis; thyroids were not examined.

Experiment 2: Acutely Diabetic BB Donors. Activated cells from acutely diabetic DP donors adoptively transfer both diabetes and thyroiditis to WAG nude rats (Table 2). Considering both nondiabetics with insulitis together with diabetic rats, 100% of 20 nude recipients of mitogen-activated cells were affected, compared with no recipients of freshly isolated cells and no untreated controls ($P < 0.001$). Diabetes occurred 14–56 (mean = 27) days after transfusion. Thyroiditis occurred in 59% of recipients. Pancreatitis was observed in 55% of recipients of mitogen-activated and in 22% (2/9) of recipients of fresh DP cells ($P < 0.001$). Table 2 shows that pancreatitis did not correlate with diabetes, insulitis, or thyroiditis.

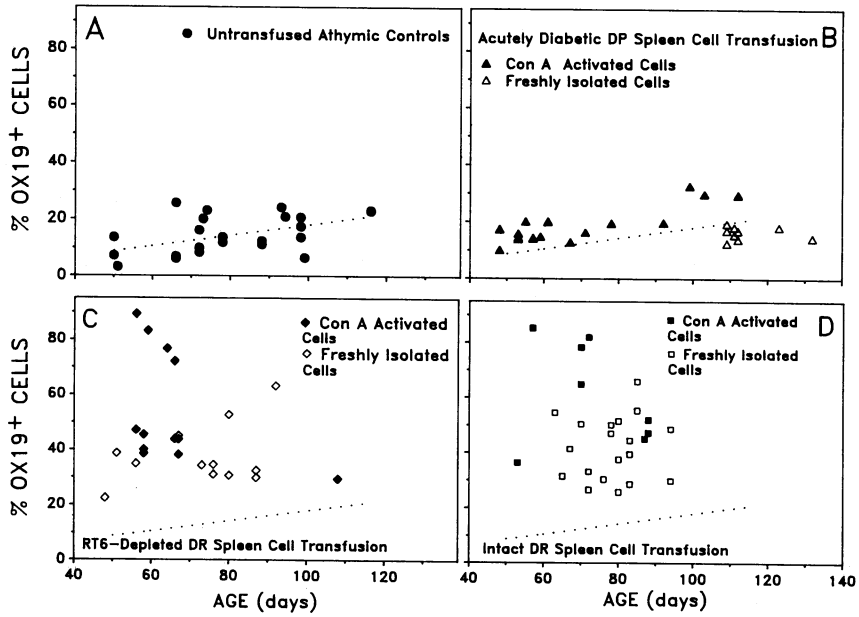

FIG. 1. Percentage of OX19$^+$ lymph node cells in control and experimental WAG nude rats of different ages. (*A*) Controls (n = 24) that received no cells (three points are superimposed). The increase in the percentage of CD5$^+$ (OX19$^+$) T cells over time is statistically significant; the regression equation is plotted as a dotted line [CD5 = (0.19 × age) − 0.68; $P < 0.01$]. (*B–D*) Control regression line from *A* together with the percentage of OX19$^+$ lymph node cells in nude rats after transfusion of either freshly isolated (open symbols) or Con A-activated (solid symbols) cells. Analysis of variance revealed a main effect for transfusion ($F = 35.04$; $P < 0.001$) but no statistically significant effect of age or interaction between age and transfusion status. The mean percentages of CD5$^+$ T cells in each of the three groups in *A* and *B* (untransfused and DP cell transfused) were statistically similar among themselves, but statistically significantly lower than the percentages observed in all four groups of DR cell recipients depicted in *C* and *D* ($P < 0.01$ for each paired comparison). In addition, in *C* and *D*, the transfusion of Con A-activated DR spleen cells was associated with a greater mean OX19$^+$ percentage than was the corresponding transfusion of freshly isolated DR cells ($P < 0.05$).

Fig. 1 shows that, despite the induction of diabetes, the proportion of CD5$^+$ lymph node cells in nude recipients of diabetic DP cells was not increased. Mean percentages were 14% ± 1% in unmanipulated nude rats, 18% ± 1% in activated DP cell recipients, and 16% ± 1% in recipients of fresh DP cells. Because nude recipients of DP cells became diabetic, at least a functional engraftment of DP cells can be inferred. We investigated the dearth of engrafted cells in a separate experiment, which revealed that the level of CD5$^+$ lymph node cells in 43-day-old nonthymectomized DP rats was 29% ± 4% (n = 4). This fell to 7% ± 2% (n = 4) and 2% ± 3% (n = 4) 11 and 25 days, respectively, after thymectomy at 4–6 weeks of age ($F_{2,9} = 79.3$; $P < 0.001$). The data suggest that CD5$^+$ T cells in DP rats could have a short life-span.

When acutely diabetic, activated DP cells were injected into MHC incompatible PVG nude rats, no endocrine autoimmunity was observed. The percentage of CD5$^+$ lymph node cells in transfused PVGs was indistinguishable from background (2% ± 1%; n = 8). Three untreated PVG nude rats had normal pancreases and thyroids.

Experiment 3: DR Donors. Transfusion of activated splenocytes from RT6-depleted DR rats produced diabetes in 85% of WAG nude recipients (Table 2). Two nondiabetics had insulitis and 5/5 had thyroiditis. Pancreatitis was observed in 7/12 specimens, but in only 5/10 obtained from diabetics.

In contrast to experiment 2, transfusion of fresh, nonmitogen-activated RT6-depleted DR cells produced diabetes and thyroiditis in WAG nude rats. Half the nondiabetics had insulitis. Considering diabetes and insulitis together, 87% of recipients were affected. Thyroiditis occurred in 5/21 cases. Pancreatitis occurred in only 2/17 diabetics and in 0/3 nondiabetics with insulitis. Table 2 shows that pancreatitis did not correlate with diabetes, insulitis, or thyroiditis.

Table 1. Induction of diabetes, insulitis, and thyroiditis in athymic rats after transfusion of splenocytes from non-BB rats

Donor strain	Spleen cell pretreatment	Nude recipient	Number diabetic (%)	Days to diabetes	Nondiabetics with insulitis (%)	Number with diabetes or insulitis (%)	Number with thyroiditis (%)	Number with pancreatitis (%)
RT6-depleted YOS	Con A	WAG	2/11 (18)	20, 24	4/9 (44)	6/11 (55)	1/6 (17)	8/9 (89)
	Fresh	WAG	0/2 (0)	—	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Untreated YOS	Con A	WAG	0/5 (0)	—	2/4 (50)	2/4 (50)	0/4 (0)	1/4 (25)
	Fresh	WAG	0/2 (0)	—	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Untreated WAG +/+	Con A	WAG	0/8 (0)	—	3/8 (38)	3/8 (38)	2/8 (25)	0/8 (0)
	Fresh	WAG	0/3 (0)	—	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Untreated WF	Con A	WAG	0/9 (0)	—	0/9 (0)	0/9 (0)	0/9 (0)	0/9 (0)
RT6-depleted PVG +/+	Con A	PVG	1/7 (14)	31	2/6 (33)	3/7 (43)	2/6 (33)	4/7 (57)
Untreated PVG +/+	Con A	PVG	0/6 (0)	—	0/6 (0)	0/6 (0)	1/6 (17)	0/6 (0)

Spleen cell transfusion into MHC-compatible WAG or PVG nude recipients (experiment 1). Average ages of the WAG and PVG nude recipients, respectively, were 39 (range, 25–67) and 39 (range 25–52) days. Spleen cell donors were of both sexes and 45–90 days old except for the YOS donors, which were 8–10 months old. The MHC haplotype of all listed rats is RT1^u except for the RT1^c PVG rats. Autoimmune islet and thyroid diseases do not normally occur in any of the donor strains.

Table 2. Adoptive transfer of diabetes and thyroiditis from acutely diabetic DP and RT6-depleted DR BB rats to athymic MHC-compatible WAG rats

Spleen donor strain	Spleen cell pretreatment	Number diabetic (%)	Mean days to diabetes (range)	Nondiabetics with insulitis (%)	Number with diabetes or insulitis (%)	Number with thyroiditis (%)	Number with pancreatitis (%)
			Experiment 2				
Acutely diabetic DP	Con A	16/21 (76)	27 (14–56)	4/4 (100)	20/20 (100)*	10/17 (59)†	11/20 (55)‡
	Fresh	0/9 (0)	—	0/9 (0)	0/9 (0)	ND	2/9 (22)
			Experiment 3				
RT6-depleted DR	Con A	11/13 (85)†	24 (19–34)§	2/2 (100)	13/13 (100)‡	5/5 (100)*	7/12 (58)¶
	Fresh	17/23 (74)	29 (18–53)	3/6 (50)	20/23 (87)†	5/21 (24)‖	2/22 (9)
Untreated DR	Con A	1/8 (13)	22	2/7 (29)	3/8 (37)‡	1/8 (13)	2/8 (25)**
	Fresh	1/19 (5)	28	0/16 (0)	1/17 (6)	0/19 (0)	0/17 (0)
			Unmanipulated WAG nude				
—	—	0/27 (0)	—	0/27 (0)	0/27 (0)	0/26 (0)	0/27 (0)

Spleen cell transfusion into WAG nude recipients 24–52 (mean = 34) days old. In experiment 2, spleen donors were spontaneously diabetic DP BB rats 60–120 days old. In experiment 3, donors were either RT6-depleted or untreated DR BB rats 30–109 days old. The bottom line presents control data from untreated WAG nude rats killed when 50–208 days old (mean = 87). In analyses involving the frequency of diabetes plus insulitis taken together, only animals for which both clinical and histological information were available have been analyzed. The MHC haplotype of all rats in this table is $RT1^u$. DP, DP BB/Wor rat; DR, DR BB/Wor rat; Fresh, freshly isolated, non-mitogen stimulated; ND, not done; *, $P < 0.005$ vs. fresh and control groups; †, $P < 0.001$ vs. control group; ‡, $P < 0.01$ vs. control group and not significant vs. fresh cell group; §, not significant vs. fresh cell group; ¶, $P < 0.001$ vs. control group and $P < 0.05$ vs. fresh cell group; ‖, $P < 0.05$ vs. control group; **, $P < 0.05$ vs. control group and not significant vs. fresh group.

Among athymic WAG recipients of fresh RT6-depleted DR cells, 74% became diabetic but only 9% had pancreatitis. When diabetes and pancreatitis did coincide, all gradations in the severity of pancreatitis were observed. Thyroiditis did not correlate with either diabetes or insulitis. Among 17 diabetic athymic WAG recipients of fresh RT6-depleted DR cells, gonads, adrenal, and stomach ($n = 16$) and skin, tongue, and liver ($n = 5$) were examined histologically for evidence of GVHD. All were free of inflammation.

When activated cells from non-RT6-depleted DR donors were transfused, diabetes, insulitis, and thyroiditis occurred in several WAG nude recipients (Table 2). Two animals with pancreatitis had neither diabetes nor insulitis. The nondiabetics were observed for 19–59 (mean = 42) days. When fresh, non-mitogen-activated cells from non-RT6-depleted DR rats were transfused into WAG nude rats, 1/19 (5%) developed diabetes without thyroiditis. No other animals developed insulitis or thyroiditis during 36–53 days of observation.

Fig. 1 shows that DR cell transfusion was associated with increased percentages of $CD5^+$ cells in nude recipients. Among recipients of RT6-depleted DR cells, the percentages were 54% ± 6% for activated cells and 37% ± 3% for fresh cells ($P < 0.01$). Among untreated DR cell recipients, CD5 percentages were 61% ± 7% for activated cells and 42% ± 3% for fresh cells ($P < 0.01$). We also measured the percentage of $RT6.1^+$ cells and found them to be 10% ± 2% ($n = 12$), 19% ± 2% ($n = 12$), 21% ± 3% ($n = 8$), and 18% ± 2% ($n = 17$), respectively. Since untreated WAG +/+ rats are $RT6.2^+$, these data indicate that many $CD5^+$ cells in nude recipients were donor origin T cells.

DISCUSSION

These data demonstrate the presence of autoreactive cells capable of adoptively transferring insulitis, thyroiditis, and diabetes in three normal non-BB rat strains, one of which has a non-$RT1^u$ MHC haplotype. They also extend previous studies of autoreactivity in the BB rat. We suggest that organ-specific autoreactive cells may be present to various degrees, but suppressed to a variable extent, in many rat strains.

The existence of autoreactive cells in normal animals has been suggested by Penhale *et al.* (18, 19), who induce thyroiditis in rats by thymectomy and irradiation. In a preliminary communication, they report inducing diabetes in PVG rats.‖ Induction of autoimmunity in these studies was attributed to selective depletion of suppressor T cells with sparing of unspecified "autoreactive" cells.

The cells responsible for adoptive transfer of endocrine autoimmunity in the BB rat have not been identified (10). *In vivo* depletion of $CD5^+$ T cells in BB rats may prevent adoptive transfer of diabetes in RT6-depleted DR rats (21), and $CD4^+$ T cells may mediate the adoptive transfer of DP BB diabetes.** The identification of these effector cells is complicated by the possibility that various types may exist. For example, in the BB rat, *in vivo* depletion of natural killer (NK) cells by anti-asialo-GM1 prevents diabetes in the DP rat but not in the RT6-depleted DR (23), and *in vivo* depletion of $OX19^+$ cells prevents diabetes in the RT6-depleted DR (B. Woda, personal communication). In our study, the induction of autoimmunity in adoptive recipients of all but acutely diabetic DP cells was associated with detectable engraftment of T lymphocytes in the nude recipients. With respect to the DP, this observation could be interpreted to suggest that a different cell type is responsible for adoptive transfer. Alternatively, our thymectomy data suggest that their $OX19^+$ T cells may simply have a short life-span, thus accounting for our failure to detect them in the nude recipients.

One may speculate that the cell responsible for autoreactivity is a T lymphocyte that has escaped clonal deletion. In several animal models, failure to delete specific V_β^+ T-cell populations correlates with the development of autoimmunity (4, 24). In the BB rat, two thymic abnormalities have been reported. First, a population of DP BB bone marrow-derived thymic dendritic cells associated with the appearance of abnormal T cells and the expression of diabetes has been observed (25). Second, we have described defective MHC class II expression by thymic epithelium in DP and DR rats (26). Either of these defects could result in the release of autoreactive T cells from the thymus of the BB rat (27). Neither of these thymic defects accounts for the lymphopenia or RT6 deficiency in the DP rat (28). We suggest that DP BB rats have two defects, one causing autoreactive cells to

‖Penhale, W. J., Stumbles, P. A., Huxtable, C. R., Sutherland, R. J. & Pethick, D. W., 7th International Congress on Immunology, Aug. 1989, Berlin, p. 502 (abstr.).

**Dayer-Métroz, M.-D., Mouland, A., Brideau, C., Duhamel, D. & Poussier, P., Immunology of Diabetes 10th International Workshop, March 1990, Jerusalem, p. 18 (abstr.).

appear in peripheral tissues and another at the prothymocyte level causing lymphopenia.

We suggest that an imbalance between autoreactive cell populations and $RT6^+$ regulatory T cells may exist in the BB rat. Such an imbalance cannot, however, be the only factor that determines expression of autoimmunity in BB and non-BB rats. Only 60–80% of $RT6^{null}$ DP rats and ≈50% of RT6-depleted DR rats become diabetic (14). Furthermore, occurrences of spontaneous diabetes in nonlymphopenic DR rats have been reported (29). Additional factors could include environmental influences, non-T-cell regulatory populations, requirements for accessory cells, and the expression of specific MHC haplotypes (8). The present data do not allow us to determine which of these are operative in the adoptive transfer paradigm.

We speculate that mitogen activation may serve as an "environmental" stimulus in our adoptive transfer system. In other models, mitogen activation preferentially expands autoreactive T cells in class II-restricted secondary mixed lymphocyte reactions (30). In addition, mitogen activation reduces the relative proportion of T cells that express RT6 in the rat (20) and could preferentially expand effector as compared with regulatory T cells. We note that mitogen-activated cells from DR rats were not only more effective in inducing autoimmunity than were freshly isolated cells, but were also associated with higher percentages of $CD5^+$ T cells in nude recipients. However, insulitis, diabetes, and thyroiditis were induced in recipients of nonactivated RT6-depleted DR cells, and diabetes was observed once in the absence of either RT6 depletion or mitogen activation. This suggests that neither *in vitro* activation nor RT6 depletion is mandatory for autoimmunity in the BB rat. Rather, mitogen activation and RT6 depletion appear to enhance the expression of endocrine autoimmunity in the BB rat and our ability to detect it in non-BBs.

A major benefit of using nude hosts in our adoptive transfer system is that immunosuppressive pretreatment is not required. The absence of host $CD5^+$ T cells in young nude rats suggests that recruitment of host T cells is not required for induction of autoimmunity in adoptive recipients. Our data do not rule out recruitment of host non-T cells such as B lymphocytes or NK cells.

Finally, we observed exocrine pancreatitis in animals both with and without diabetes or thyroiditis. Because recipient animals were injected intravenously, direct toxicity cannot explain the observation. A second possibility is the induction of GVHD on the basis of minor histocompatibility differences between hosts and recipients. However, we believe that GVHD did not play a significant role in these results. The PVG into PVG *rnu/rnu* and WAG into WAG *rnu/rnu* transfer systems are both congenic. Each nonetheless yielded insulitis and thyroiditis, and in the case of the PVG system, pancreatitis. Pancreatitis did not occur in all nude recipients and did not correlate with islet or thyroid pathology. We also have shown in one fully allogeneic system (DP BB into MHC-incompatible PVG nude) that recipients did not develop endocrine autoimmunity. Similar results were obtained following adoptive transfer of fresh DR cells into PVG nude rats. Overt GVHD has been detected only when Con A-activated splenocytes are transferred from RT6-depleted DR rats into PVG nude rats (U.McK., unpublished observations). A third possibility is that an infectious agent caused the pancreatitis and endocrinopathy. It is impossible to exclude the presence of virus-infected cytotoxic lymphocytes when using an adoptive transfer protocol, but we think it unlikely that pancreatic infection played a role. First, diabetes, insulitis, and thyroiditis were often observed in the absence of pancreatitis, and vice versa. Second, other studies have shown that severe, viral pancreatitis does not cause diabetes in the mouse (22).

In summary, our data support the concept that various levels of organ-specific autoreactive cells may be present, but suppressed to varying degrees, in normal animals. We have observed a spectrum that ranges from the absence of detectable autoreactivity in WF rats to levels of autoreactive cells that produce spontaneous disease in the DP rat. A dynamic equilibrium between autoreactive and regulatory cell populations may determine expression of autoimmune disease. The nature of the autoreactive cells responsible for autoimmune diabetes and thyroiditis in the rat remains unknown.

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