T-Lymphocyte Requirement for Diabetes in RT6-Depleted Diabetes-Resistant BB Rats

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Diabetes-prone (DP) BB rats develop spontaneous autoimmune insulin-dependent diabetes mellitus (IDDM). The cell populations involved in the expression of diabetes are not precisely known but probably include natural killer (NK) cells, macrophages, and T lymphocytes. Because the DP rat has few lymphocytes of the $CD5^+/CD^+$ phenotype, cytotoxic T lymphocytes (T_c) are not believed to be important in the process. Diabetes-resistant (DR) BB rats that are depleted of $RT6^+$ T lymphocytes also become diabetic and provide an additional model of IDDM. We report that diabetes in DR rats depleted of $RT6^+$ T lymphocytes is prevented by the concomitant depletion of either the $CD5^+$ or the $CD8^+$ population. In contrast, coadministration of anti-asialoganglioside$_{M1}$ (α-ASG_{M1}), an antiserum that principally recognizes NK cells, failed to prevent hyperglycemia in RT6-depleted rats. We propose that the initiation of diabetes in both DP and RT6-depleted DR rats is T-lymphocyte dependent. However, the final common pathway leading to autoimmune β-cell destruction in IDDM may be different in these models. The RT6-depleted DR rat requires a cell that is sensitive to anti-CD8 (possibly a T_c), whereas the DP rat requires an anti-ASG_{M1}–sensitive cell. *Diabetes* 40:423–28, 1991

The BB rat is widely used to model autoimmune insulin-dependent diabetes mellitus (IDDM). Rats belonging to the diabetes-prone (DP) subline develop spontaneous hyperglycemia with a cumulative frequency of 60–90%. As reviewed (1,2), the disease is associated with pancreatic insulitis, islet autoantibodies, and the $RT1^u$ major histocompatibility complex haplotype. DP diabetes is prevented by bone marrow allografts, thymectomy, and various immunosuppressive interventions (3).

DP rats are lymphopenic (4,5) and have reduced numbers of $CD5^+$ and $CD4^+$ cells (6,7). They are severely deficient in the phenotypic ($CD8^+/CD5^-$) (8) and functional (9,10) expression of cytotoxic/suppressor T lymphocytes (T_c). Cells expressing the RT6 T-lymphocyte alloantigen are also severely deficient (11,12). RT6 is expressed on ~70% of $CD8^+$ and 50% of $CD4^+$ T lymphocytes in the rat (13). DP rats have relatively higher natural killer (NK) cell number and functional activity than do diabetes-resistant (DR) rats (14). It has been shown in vitro that islet cells are sensitive to NK killing (15,16) and in vivo that NK cells are necessary for the development of diabetes in DP rats (17,18).

Rats belonging to the DR subline of BB/Wor rats were derived from DP forebears (19). Their cumulative frequency of spontaneous diabetes is <1%, and they lack the immunological deficits of the DP rat. They are not lymphopenic and express normal numbers of T lymphocytes expressing the RT6.1 phenotype (20). However, there have been spontaneous outbreaks of diabetes in DR colonies (21,22). Diabetes can also be induced in DR rats by low-dose irradiation (23), cyclophosphamide (24), media conditioned by spleen cells cultured in the presence of lectin (25), and in vivo depletion of $RT6^+$ T lymphocytes with a cytotoxic monoclonal antibody (MoAb) (20). About 50% of RT6-depleted DR rats develop diabetes within 4 wk. These data suggest that the expression of diabetes in the DR rat is under the control of a regulatory T-lymphocyte subset that expresses the RT6.1 antigen.

In this study, we examined the comparative cellular requirements for diabetes in DP and RT6-depleted DR rats. The data show that T lymphocytes, specifically cells sensitive to anti-CD8, are required for the development of diabetes in the latter system. We confirmed that anti-asialoganglioside$_{M1}$ (α-ASG_{M1})–sensitive cells, presumably NK cells, are necessary for diabetes in the DP rat but concluded from in vivo

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depletion studies that they are probably unnecessary in the RT6-depleted DR rat.

RESEARCH DESIGN AND METHODS

DP and DR BB/Wor rats were obtained from the main breeding colony at the University of Massachusetts Medical Center (19). The cumulative incidence of diabetes in these animals averages ~60% in both sexes. Most cases (>85%) occur between 60 and 120 days of age. DR rats were derived from the DP line in the 5th generation of brother-sister mating and subsequently inbred for >20 generations before being bred in our own subcolony for 2–3 generations. During this study, no cases of spontaneous DR diabetes were observed. Animals were housed under standard laboratory conditions and given ad libitum access to food and water. Experimental animals were tested for glycosuria twice weekly, and diabetes was diagnosed on the basis of a plasma glucose concentration ≥11.1 mM. All animals were maintained in accordance with standard recommendations (26).

DS4.23 anti-RT6.1 MoAb was prepared with hybridomas grown in tissue culture for 72 h in serum-free RPMI-1640 (Gibco, Grand Island, NY) containing 1% Nutridoma-SP (Boehringer Mannheim, Indianapolis, IN). In some experiments, cell-free tissue culture supernatant was concentrated 100-fold by a filtration system with a molecular weight cutoff of 50,000 (Amicon, Danvers, MA). The protein content of concentrates was measured and the volume adjusted to permit intravenous injection of 2.5 mg protein/kg body wt in 0.5 ml RPMI (20). In other experiments, unconcentrated hybridoma supernatant was injected intraperitoneally at a dose of 2 ml/rat.

Hybridoma cell lines producing other mouse anti-rat lymphocyte MoAbs used in this study were obtained from A.F. Williams and D.S. Mason (Oxford Univ., Oxford, UK). W3/25 (anti-CD4) reacts with helper/inducer T lymphocytes (T_h)(27) and macrophages (28). OX19 (anti-CD5) reacts with all T lymphocytes (29). OX8 (anti-CD8) reacts with T_c (27) and NK cells (30). Antibodies were biotinylated as previously described (30). Phycoerythrin-avidin was obtained from Molecular Probes (Junction City, OR). Fluoresceinated goat anti-mouse IgG not cross-reactive with rat IgG was obtained from Cappel (Westchester, PA). α-ASG_{M1}, a rabbit antiserum that reacts with the neutral glycosphingolipid ASG_{M1} present on the surface of NK cells (31), was produced at the National Cancer Institute (Frederick, MD).

In vivo T-lymphocyte depletion. Diabetes was induced by depletion of $RT6^+$ T lymphocytes as follows. DS4.23 anti-RT6 antibody was injected into 30-day-old DR rats twice a week for 4–5 wk either intravenously (13) or intraperitoneally (32) as previously described. We previously determined that neither the route of injection nor the number of weekly injections greatly influences the overall frequency of diabetes among DS4.23-treated rats (33). Rats were tested for glycosuria throughout the period of injection. Diabetic rats were killed when diagnosed, and their pancreases were removed for histological examination. Nondiabetic rats were killed at the end of the experiment, and their pancreases and spleens were harvested.

Rats were depleted of $CD5^+$ or $CD8^+$ cells by intraperitoneal administration of 2 ml of OX19 or OX8 hybridoma culture supernatant. To deplete α-ASG_{M1}–sensitive cells, α-ASG_{M1} antiserum was given intravenously, diluted 1:35 in phosphate-buffered saline, in a volume of 0.5 ml every 3 days. This injection protocol has previously been found to reduce Wistar-Furth spleen cell cytotoxicity against NK-sensitive YAC-1 target cells by >95% (16). Spleen cells were harvested from α-ASG_{M1}–depleted DP and DR rats for immunophenotypic and functional studies after varying periods of injection. This dose of α-ASG_{M1} depleted NK cells and reduced NK activity but did not affect other T-lymphocyte subset percentages as assessed by immunophenotypic analysis.

Cell staining and analysis. All procedures were carried out at 4°C. For two-color fluorescence detection of OX8 and OX19 antigens, 100 μl of spleen cells at a concentration of 4×10^7/ml were incubated with OX8 supernatant for 30 min, washed twice, and incubated with fluoresceinated goat anti-mouse IgG for 30 min. The cells were next washed and incubated with medium containing 200 μg/ml mouse IgG to block remaining active sites on the fluoresceinated goat anti-mouse IgG, washed again, and incubated with 5 μg/ml biotinylated OX19 for 30 min. The cells were then washed, stained with phycoerythrin-avidin for 30 min, washed again, and fixed with 0.5% paraformaldehyde. Stained cells were analyzed by flow cytometry. For each determination, 10^4 viable lymphocytes selected according to their light-scattering characteristics were analyzed. The $CD8^-/CD5^+$ population was interpreted as T_h. $CD8^+/CD5^+$ cells were considered to be T_c. The $CD8^+/CD5^-$ population comprised NK cells (29).

To quantify $RT6.1^+$ T lymphocytes, lymph node cells were stained with either the DS4.23 anti-RT6.1 MoAb (20) or the 6A5 anti-RT6.2 MoAb (34), followed by goat anti-mouse IgG. The number of RT6.1 cells was determined by subtracting the number of cells staining positively for RT6.2 from the number staining for RT6.1.

Mitogen stimulation, virus immunization, and microcytotoxicity assays. Spleen cells were stimulated with the T-lymphocyte mitogen concanavalin A (ConA) at a concentration of 2 μg/ml. Dose-response curves at concentrations of 0.1–10 μg/ml were generated for both DP and DR rats; 2 μg/ml was determined in preliminary testing to be the optimum dose. Cells were cultured in RPMI, 10% fetal bovine serum, 1×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 0.1 mM HEPES, and penicillin-streptomycin at a concentration of 5×10^5/ml. Triplicate cultures were performed at 37°C for 66 h in flat-bottomed microtiter plates (Falcon, Oxnard, CA) in an atmosphere of 5% CO_2/95% air. Cells were then pulsed with 0.5 μCi [^{3}H]thymidine (Du Pont-NEN, Boston, MA) and harvested 6 h later onto glass-fiber filters. The filters were air dried, placed into scintillation fluid, and counted.

For viral infection with lymphocytic choriomeningitis virus (LCMV, Armstrong strain, kindly supplied by R. Welsh), DR rats received 1.6×10^5 plaque-forming units intraperitoneally and 8×10^4 plaque-forming units in the hind foot pads. Seven days later, the spleens were removed, and spleen cells were used for microcytotoxicity assays.

Fetal fibroblasts from DP rats were established in culture for use as targets in viral cytotoxicity assays. Three days before assay, fibroblasts were infected with LCMV at a multiplicity of infection of 0.1. One day before assay, 1000 U/ml rat interferon (Lee Biomolecular, San Diego, CA) was

added. On the day of the assay, targets were trypsinized, and target cells (1×10^6) were labeled with ^{51}Cr (Du Pont-NEN) for 1 h at 37°C at a concentration of 100 μCi/10^6 cells. After labeling, cells were distributed into microtiter wells at a concentration of 10^4 cells/100 μl. Effector cells were added in 100 μl of medium at effector-target ratios ranging from 200:1 to 5:1. Assays were performed in triplicate. Medium was added to wells used for spontaneous lysis determinations, and 1% Nonidet P-40 was added shortly before harvest to a set of target cells to quantify counts associated with 100% lysis. Microtiter plates were incubated for 4 h at 37°C in a humidified atmosphere of 5% CO_2/95% air. Before harvest, plates were centrifuged at 200 × *g* for 5 min, and 100-μl samples were taken for counting in an LKB-Wallac γ-counter. Percent specific ^{51}Cr release was calculated as

$$\text{specific release} = \frac{\text{test sample cpm} - \text{medium control cpm}}{\text{Nonidet P-40 control cpm} - \text{medium control cpm}}$$

where cpm is counts per minute.

Fibroblast targets did not express detectable class I histocompatibility antigens and were treated with interferon to induce class I histocompatibility antigen expression as previously described (9). Target cells expressed LCMV antigens as assessed by immunofluorescence utilizing anti-LCMV antibodies. NK activity was assessed in a microcytotoxicity assay with the YAC-1 cell target as previously described (16).

For histological studies, portions of pancreas obtained at the end of the experiment were fixed in 10% buffered formalin or Bouin's solution, embedded in paraffin, and processed for routine light microscopic study. Sections were stained with hematoxylin and eosin and interpreted by B.A.W.

Parametric data are means ± SE. Means were compared with analyses of variance with the least significant difference procedure for a posteriori contrasts. Nonparametric data were analyzed with Fisher's exact or χ^2-statistics (35).

RESULTS

In vivo immune elimination. Among DR rats injected with DS4.23 anti-RT6 MoAb, the percent of lymph node T lymphocytes was 59 ± 8% (n = 29). T_c comprised 27 ± 4% (n = 10) and RT6$^+$ T lymphocytes 4 ± 2% (n = 12). In control DR rats (n = 9), the percent of lymph node T lymphocytes was 68 ± 2%, T_c cells 15 ± 1%, and RT6$^+$ T lymphocytes 29 ± 2%. In the rats that received both anti-RT6 and anti-CD8 MoAbs, the percent of CD8$^+$ lymph node cells was reduced to 3 ± 4% (n = 18). The degree of depletion of CD5$^+$ cells was more variable. In one experiment, CD5$^+$ cells were reduced to 8 ± 11% (n = 10). When all CD5-depletion experiments were analyzed together, however, CD5$^+$ cells were reduced only to 32 ± 21% (n = 28). Our inability to consistently reduce CD5$^+$ cells for long periods was presumably due to acquired antibody resistance.

Requirement of both T lymphocytes and anti-CD8–sensitive cell populations for diabetes. To determine the cell types necessary for the expression of diabetes in RT6-depleted DR rats, we also depleted CD5$^+$ cells or CD8$^+$ cells by in vivo injection of appropriate antibodies. When both CD5$^+$ and RT6$^+$ cells were depleted, only 7% of treated rats developed diabetes versus 63% of controls injected with anti-RT6 alone (Table 1). Histological examination of pancreases from nondiabetic RT6-depleted CD5-depleted rats revealed insulitis in only 1 of 17 (6%) specimens.

TABLE 1
Frequency of diabetes and insulitis in diabetes-resistant (DR) rats injected with monoclonal antibodies (MoAbs)

MoAb	*n*	%
Anti-RT6	41 of 65	63
Anti-CD5	0 of 14*	0
Anti-RT6 + anti-CD5	2 of 29*	7
Anti-RT6 + anti-CD8	1 of 18*	6

Experimental animals were 30-day-old DR BB/Wor rats of both sexes. RT6$^+$ T lymphocytes were depleted with DS4.23 MoAb given intravenously twice weekly. CD5$^+$ and CD8$^+$ cells were depleted by intravenous injection of the OX19 and OX8 MoAbs, respectively, every 3 days. All rats were treated with MoAbs from day 1 of the experiment until diabetes onset or 60–65 days of age. Overall χ^2 for diabetes frequency = 47.03, df = 3, $P < 0.001$.
*$P < 0.001$ vs. anti-RT6 only.

Depletion of CD8$^+$ cells almost completely prevented diabetes in RT6-depleted DR rats (Table 1). Histological examination of pancreases from nondiabetic RT6-depleted CD8-depleted rats revealed insulitis in 6 of 17 (35%) specimens.

We next determined whether we could prevent DR diabetes by depletion of CD5$^+$ cells after the autoimmune process had been initiated by the depletion of RT6$^+$ T lymphocytes. To do so, we began injections of anti-RT6 MoAb but did not start anti-CD5 injections until 8 or 15 days later. These experiments demonstrated that when the injection of anti-CD5 MoAb was delayed diabetes could not be prevented (Table 2).

Role of α-ASG$_{M1}$–sensitive cells in RT6-depleted diabetic rats. Injection of anti-CD8 MoAb prevented diabetes in RT6-depleted DR rats. It has previously been shown that the depletion of CD8$^+$ cells prevents diabetes in DP BB rats (17). Because the DP rat has a severe deficit in T_c, CD8 depletion presumably exerts its protective effect by depleting NK cells. Although the DR rat has relatively few NK cells and low NK activity (14), it is still possible that, in the DR rat, the effectiveness of anti-CD8 therapy in preventing diabetes was due to the depletion of NK cells. In the DR rat, we could not use anti-CD8 as an anti-NK reagent because

TABLE 2
Frequency of diabetes in RT6-depleted diabetes-resistant (DR) rats injected with anti-CD5 antibody at various intervals after 1st injection of anti-RT6 antibody

First injection of anti-CD5 MoAb	*n*	%
Day 1	2 of 29	7
Day 8	15 of 25*	60
Day 15	13 of 20*	65

DR rats were treated with anti-RT6 monoclonal antibody (MoAb) beginning at ~30 days of age (day 1). Injections of anti-CD5 MoAb were begun on the same day or 8 or 15 days after the 1st anti-RT6 MoAb injection. Once begun, all MoAb injections were continued either until onset of diabetes or until the rats were 60–65 days old. Overall χ^2 = 27.51, df = 2, $P < 0.001$.
*$P < 0.001$ vs. day 1; NS vs. each other.

TABLE 3
Frequency of diabetes in diabetes-prone (DP) and RT6-depleted diabetes-resistant (DR) rats injected with antiserums

Treatment	*n*	%
DP		
α-ASG_{M1}	0 of 29	0
Normal rabbit serum	4 of 15*	27
Uninjected controls	13 of 25†	52
RT6-depleted DR		
Anti-RT6 alone	4 of 12	33
Anti-RT6 + α-ASG_{M1}	7 of 14‡	50

α-ASG_{M1}, anti-asialoganglioside$_{M1}$. Experimental animals were 30-day-old DP and DR BB/Wor rats of both sexes. DP rats were tested for diabetes through 120 days of age. DR rats were treated with the respective reagents until diabetes onset or 60–65 days of age. Overall χ^2 with respect to diabetes frequency = 13.25, df = 2, $P < 0.01$.
*$P < 0.02$ vs. α-ASG_{M1}.
†$P < 0.001$ vs. α-ASG_{M1}; NS vs. normal rabbit serum.
‡Fisher's exact statistic = 0.45, NS vs. anti-RT6 alone group.

anti-CD8 would react with both T_c and NK cells. To examine the proposed role of NK cells in this model, we depleted rats of α-ASG_{M1}–sensitive cells.

Administration of α-ASG_{M1} to DP rats between 30 and 120 days of age prevented diabetes, confirming the proposed role of NK cells in this model (Table 3). In contrast, administration of α-ASG_{M1} to RT6-depleted DR rats did not prevent diabetes (Table 3).

The immunologic effects of α-ASG_{M1} injection in DP rats were assessed on the 7th and 28th days of injection. In these rats, α-ASG_{M1} had no effect on the percentage of T_h or T_c, but it did reduce NK cell number and YAC-1 cell lytic activity (Table 4). To determine whether the administration of α-ASG_{M1} to DP rats inhibited functional T-lymphocyte responses, we measured the ability of DP spleen cells to respond to the T-lymphocyte mitogen ConA. α-ASG_{M1} treatment did not suppress the ConA response, and at the day-7 time point, it actually appeared to enhance mitogen reactivity.

The immunologic effects of α-ASG_{M1} injection in RT6-depleted DR rats were determined on the 7th and 21st days of injection. In these rats, we found no alteration of T_h or T_c percentages (Table 5). Because the activity of NK cells in DR rats is low (14), we did not attempt to quantify the functional depletion of these cells. α-ASG_{M1} injection had little effect on ConA responsiveness or the ability of DR rats to generate an antiviral cytotoxic response (Table 5).

DISCUSSION

These data show that the expression of diabetes in RT6-depleted DR rats requires both T lymphocytes and a population of anti-CD8–sensitive cells. Because the latter population also appears to be α-ASG_{M1} resistant, it is plausible to infer that they may be cytotoxic T lymphocytes. In this model of IDDM, NK cells appear not to play a crucial role, because treatment with the anti-NK reagent α-ASG_{M1} does not prevent diabetes. In contrast, both α-ASG_{M1} (18,36) and anti-CD8 (17) prevent DP rat diabetes.

We observed that T-lymphocyte depletion in the RT6-depleted DR rat prevents diabetes only if started at the same time as anti-RT6 treatment. When CD5 depletion is delayed for 1 wk, this form of serotherapy is ineffective. This result suggests that once the autoimmune process is triggered by RT6 depletion, it is difficult to arrest.

Our data indicate that, in some OX19-treated RT6-depleted DR rats, $CD5^+$ cell numbers were depressed for up to 4 wk. In most rats, however, resistance to the antibody appeared to develop, and $CD5^+$ cells reappeared in peripheral lymphoid organs. Although the $CD5^+$ cells returned, these rats did not develop diabetes or insulitis. This observation suggests that there is a critical period during which the autoimmune process is initiated and after which it becomes difficult to reverse. If T lymphocytes are removed during the critical period, the autoimmune process is not initiated.

TABLE 4
Phenotypic and functional effects of anti-asialoganglioside$_{M1}$ (α-ASG_{M1}) on diabetes-prone (DP) rat lymphocytes

		Spleen cell populations (%)			Spleen cell functions	
Treatment/duration	*n*	T_h	T_c	NK*	Mitogen stimulation (cpm)†	YAC-1 cell cytotoxicity (% ^{51}Cr release)‡
7 Days						
α-ASG_{M1}	7	7 ± 1	0.7 ± 0.3	2.0 ± 0.2	20,844 ± 4723	6 ± 1
Normal rabbit serum	7	8 ± 1	0.4 ± 0.2	4.0 ± 0.4	3615 ± 1490	20 ± 5
28 Days						
α ASG_{M1}	3	7 ± 1	1.0 ± 0.1	2.0 ± 0.1	5462 ± 1594	6 ± 1
Normal rabbit serum	4	6 ± 1	1.0 ± 0.1	7 ± 1	1583 ± 378	31 ± 5
Uninjected controls	4	9 ± 1	1 ± 1	7 ± 1	1005 ± 111	21 ± 6

Values are means ± SE. T_h, $OX19^+/W3.25^+$ helper/inducer T lymphocyte; T_c, $OX19^+/OX8^+$ cytotoxic T lymphocyte; NK, $OX19^-/OX8^+$ natural killer cell; cpm, counts per minute; anti-ASG_{M1}, anti-asialoganglioside$_{M1}$. These phenotypic and functional data were obtained on a different set of animals than shown in Table 3. Antiserums were injected every 3 days. Data were obtained from rats 7 or 28 days after the 1st injection.
*Overall analysis of variance: $F_{4,20} = 28.6$, $P < 0.001$. Each α-ASG_{M1}–treated group is significantly lower than the normal rat serum groups and the uninjected control group at $P < 0.01$.
†Overall analysis of variance: $F = 7.70$, df = 4 and 20, $P < 0.001$. The day-7 α-ASG_{M1} group differs from all other groups at $P < 0.001$. No other paired comparisons are statistically significant.
‡Overall analysis of variance: $F = 5.88$, df = 4 and 20, $P < 0.005$. Each α-ASG_{M1} group differs from both normal rabbit serum groups and the control group at $P < 0.05$. Mitogen stimulation studies of spleen cells were performed with concanavalin A. There were no statistically significant differences among groups with respect to T_h or T_c.

TABLE 5
Phenotypic and functional effects of anti-asialoganglioside$_{M1}$ (α-ASG$_{M1}$) on diabetes-resistant rat lymphocytes

Treatment/duration	n	Spleen cell populations (%)			Spleen cell functions		
					Mitogen stimulation*		Virus-specific cytotoxicity (% ^{51}Cr release)†
		T_h	T_c	NK	cpm	n	
14 Days							
α-ASG$_{M1}$	4	29 ± 2	18 ± 4	2.0 ± 0.4	187,000 ± 36,230	7	28 ± 3
Normal rabbit serum	3	34 ± 3	11 ± 4	3 ± 1	215,750 ± 10,127	8	45 ± 4
21 Days							
α-ASG$_{M1}$	3	34 ± 2	18 ± 1	2.0 ± 0.2	208,250 ± 14,026	4	37 ± 9
Normal rabbit serum	3	26.0 ± 0.4	21 ± 1	4 ± 1	226,250 ± 10,127	4	38 ± 5

Values are means ± SE. Protocols and abbreviations are as described in Table 4, except that the treatment durations at the time of study are different. The viral cytotoxicity was assayed against cells infected with lymphocytic choriomeningitis virus at an effector-target ratio of 100:1 (see METHODS). There were no statistically significant differences among groups with respect to T_h or T_c percentages. With respect to both functional assays, overall analysis of variance of the 4 data sets indicates no statistically significant differences among any of the groups (*F = 0.41, df = 3 and 19, NS; †F = 1.95, df = 3 and 9, P = 0.2). The phenotypic and functional results from antibody-injected rats are similar to those obtained with noninjected controls (9,14,42).

Depletion of CD8$^+$ cells prevented diabetes but not insulitis in RT6-depleted DR rats. This suggests that CD4$^+$ cells are sufficient for the induction of insulitis but are not by themselves adequate to produce β-cell destruction. It appears likely that CD8$^+$ cells are necessary for the destruction of DR β-cells, presumably by a classic cytolytic T-lymphocyte mechanism.

Although we have shown that DR rats have relatively few NK cells and low NK activity (14), it could still be argued that anti-CD8 serotherapy prevented diabetes in RT6-depleted rats because it eliminated those few NK cells rather than the T_c population. We addressed this possibility by testing the ability of another anti-NK reagent, α-ASG$_{M1}$, to prevent diabetes. Consistent with our interpretation of the anti-CD8 data, we observed that α-ASG$_{M1}$ prevented diabetes in the DP rat but not in the RT6-depleted DR rat.

We interpret these data to suggest that the initiator of the anti-islet response is similar in both models, but the effector limbs are different. Because the DP BB rat is immunodeficient and has a defect in T_c, diabetes develops relatively late in life. To compensate for the deficiency in T_c, the DP rat exploits its population of NK cells as effector elements. In the RT6-depleted DR rat, diabetes develops briskly via a classic T_c-effector pathway.

The ASG$_{M1}$ glycolipid is widely expressed on lymphoid cells but is expressed in highest density on NK cells (14). When utilized at an appropriate dilution, α-ASG$_{M1}$ is specifically cytotoxic for NK cells. In our experiments, α-ASG$_{M1}$ had little effect on the relative numbers of T_h or T_c. In DP rats studied after 7 or 28 days of injection, NK cell number and activity were decreased. We did not study NK cell activity in DR rats because we have previously shown that these rats have low NK activity (14), and it would have been difficult to detect any additional inhibition of NK cell function. α-ASG$_{M1}$ had little effect on the response to the T-lymphocyte mitogen ConA in the DR rat, but after 7 days of injection, it appeared to enhance ConA responsiveness in the DP rat. This is consistent with data showing that NK cells may downregulate T-lymphocyte responses (37). α-ASG$_{M1}$ also did not prevent the RT6-depleted DR rat from generating virus-specific cytolytic T lymphocytes. Of course, these data do not exclude an effect of α-ASG$_{M1}$ on some other cell type for which we did not specifically assay.

These data extend our characterization of the RT6-depleted DR model of autoimmune diabetes and highlight several areas of similarity and difference with respect to the DP rat. Both models require CD5$^+$ T_h for the initiation of the autoimmune response. Presumably, these cells recruit to the islets cytotoxic cell populations that effect actual β-cell destruction. Perhaps for this reason, it should not be surprising that cyclosporin is effective in the prevention of diabetes in both the DP (38) and the RT6-depleted DR rat (32).

However, DP rats are deficient in T_c (8–10) and require α-ASG$_{M1}$–sensitive cells for the expression of diabetes. In contrast, DR rats have normal numbers of T_c. We speculate that when these animals are depleted of RT6$^+$ regulatory T lymphocytes, these anti-CD8–sensitive T_c (and CD4$^+$ T_h) become released from suppression and capable of β-cell destruction. Hence, it is likely that DP rats utilize α-ASG$_{M1}$–sensitive effector elements as a compensatory mechanism for their deficiency of T_c. This in vivo distinction between the DP and RT6-depleted DR rats models with respect to effector populations is supported by preliminary data obtained with an in vitro islet cytotoxicity assay (39). Differences between the DP and the DR rat may also exist at other levels. For example, it has been reported that silica, an agent that acts primarily as a macrophage toxin, prevents diabetes in the DP (40) but not in the RT-6-depleted DR (41) BB rat.

In summary, these data suggest the presence of intriguing differences in the cellular mechanisms underlying the expression of autoimmune diabetes in DP versus RT6-depleted DR BB rats. Additional comparative studies of these two models may permit us to dissect apart the initiator limb of autoimmunity from the less stringently constrained final effector limb.

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