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Transfusions enriched for W 3/25^{+} helper/inducer T lymphocytes prevent spontaneous diabetes in the *BB/W* rat

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Summary. Transfusions of spleen cells are known to prevent spontaneous autoimmune diabetes in susceptible BB/W rats, while T cell-depleted transfusions are ineffective. To characterize further the protective cell(s), we transfused young diabetes prone rats with splenocytes from diabetes resistant BB/W rats that were treated in vitro to enrich them in either OX8^{+} (suppressor/cytotoxic) T cells or W 3/25^{+} (helper/inducer) T cells. Diabetes subsequently occurred in 19 of 29 (66%) recipients of OX8-enriched, W 3/25-depleted cells and 20 of 37 (54%) controls, but in only 7 of 30 (23%) recipients of W 3/25-enriched, OX8-depleted cells ($p < 0.005$). Transfusion of spleen cells from diabetes resistant donor rats pretreated in vivo to deplete OX8^{+} cells also prevented diabetes in susceptible BB/W recipients. We conclude that transfusions of W3/25^{+} helper/inducer splenic T lymphocytes obtained from diabetes resistant animals prevent spontaneous diabetes in the BB/W rat.

Key words: Diabetes, BB rat, immunology, transfusion, prevention, lymphocyte, helper/inducer subset.

Evidence from many laboratories suggests that spontaneous diabetes in the BB rat is an autoimmune disorder [1, 2]. Islets of acutely diabetic animals are infiltrated with lymphocytes. Adoptive transfer of BB diabetes has been demonstrated, and the disorder can be prevented by immunosuppressive drugs and various immune modulations including neonatal thymectomy, bone marrow allografts, total lymphoid irradiation and silica injections [1, 3].

Diabetes prone (DP) BB rats have a marked T-cell lymphopenia [4, 5] and a decreased lymphocyte response to mitogen [6]. T-cell subsets found in abnormally low numbers in DP-BB rats include the RT-6^{+} [7], W3/25^{+}, OX8^{+} and OX19^{+} phenotypes [4, 5, 8]. We have previously demonstrated that transfusions of whole blood from diabetes resistant (DR) BB rats prevent the disease in DP animals [9]. Transfusion into young DP rats also prevented insulitis and restored their lymphocyte response to mitogenic stimulation towards normal. Subsequent studies showed that buffy coat and unfractionated spleen cells, but not T cell-depleted lymphocytes, protected DP-BB rats from diabetes [10]. These studies suggested that a T-cell was the protective blood element. We now report that transfusions of W3/25^{+} (helper/inducer) T lymphocytes prevent BB rat diabetes.

Methods

Animals

Transfusion recipients were 25 to 30-day-old diabetes prone BB rats of both sexes obtained from the University of Massachusetts, Worcester, Mass, USA (DP-BB/W rats). The cumulative incidence of diabetes in these rats varies from 40 to 70%. Over 85% of all cases of diabetes appear between 60 and 120 days of age. The frequency of diabetes before 60 days of age is $< 0.5\%$. Transfusion donors were diabetes resistant BB/W (DR-BB/W) rats from our colony. DR-BB/W rats have been derived from diabetes prone forebears, but have been bred for resistance to the disease [11]. Splenic and peripheral lymphocyte numbers and subset percentages in DR-BB/W rats are similar to those of normal Wistar Furth rats [7]. At the time of these experiments, DR-BB/W rats had been inbred for 18-20 generations, and fewer than 2.0% (54 of > 3000) had become diabetic.

Experimental protocols

Three experiments were performed. In Experiment 1 aliquots of spleen cells from DR-BB/W rats were treated in vitro to enrich them for specific lymphocyte subsets. Litters of 30-day-old DP-BB/W rats were then randomized and given either five transfusions of spleen cells enriched for the helper/inducer (W3/25^{+}) subset [12], five transfusions enriched for the suppressor/cytotoxic (OX8^{+}) subset [12] or no treatment (Table 1). Transfusions were given over a 2-week period. Experiment 2 was identical to the first experiment except that the total number of cells transfused was greater (Table 1). In experiment 3, DR-BB/W transfusion donors were pretreated for 2 weeks in vivo with either W3/25 or OX8 monoclonal antibody or vehicle. A total of six transfusions from pretreated donors were then given to 30-day-old DP-BB/W rats.

Each experiment quantified the effect of spleen cell transfusion on the frequency of diabetes through 128 (experiments 1 and 2) or 175 (experiment 3) days of age. Diabetes was diagnosed on the basis of 4+ glycosuria and a plasma glucose > 13 mmol/l. In experiments 1 and 2 histologic studies of the frequency of insulitis and thyroiditis were also performed. All rats were housed in quarters with a 12-h light/dark cycle and fed Purina rodent chow ad libitum. All animals that became diabetic or reached the conclusion of an experiment were killed using 100% carbon dioxide anaesthesia.

In vitro fractionation of T lymphocyte subsets

Spleens of DR-BB/W rats were teased apart with a cell sieve and suspended in RPMI-1640 medium supplemented with 10% fetal calf se-

rum (Gibco Laboratories, Grand Island, NY, USA) and 4% HEPES buffer (Sigma Chemical Co., St. Louis, MO, USA) as previously described [10]. Erythrocytes were lysed hypotonically by suspending the spleen cells in 18 ml distilled water for 10 s, followed immediately by the addition of 2 ml 10 × concentrated Hank's balanced salt solution to restore normal osmolality.

Erythrocyte-depleted spleen cells (2×10^8) were applied at 37 °C to a column comprised of a 20-ml syringe barrel packed with 1 g nylon wool (ABS, Inc., Buffalo, NY, USA). The nonadherent cells were eluted from the column with two bed volumes of media, collected and labeled with either OX8 or W3/25 mouse monoclonal antibody (Accurate Chemical and Scientific, Westbury, NY, USA) [13]. The labeled cells were then applied to 10-ml columns containing Degalan methylacrylate beads (Accurate Chemical and Scientific) coated with rabbit anti-mouse IgG (Cappel, Malvern, PA, USA) as previously described [14]. The columns were eluted with 3 bed volumes of buffer. Recipient rats were each given an intravenous injection of 0.5 ml of non-adherent $OX8^-$ (W3/25-enriched) or $W3/25^-$ (OX8-enriched) spleen cells within 1 h of the completion of the separation.

The fractionation procedure yielded approximately twice as many $W3/25^+$ cells as $OX8^+$ cells. We attribute this ratio simply to the fact that there are more $W3/25^+$ cells (28.5%) than $OX8^+$ cells (15.6%) in the spleens of normal nondiabetic rats [15]. In experiment 1, cell numbers were adjusted after fractionation so as to transfuse similar quantities of W3/25-enriched and OX8-enriched T cells. In experiment 2, all recovered cells were transfused, and about 40% more W3/25-enriched than OX8-enriched cells were given (Table 1).

To assess the purity of fractionated T cell subsets, 20 aliquots of fractionated cells were developed for immunofluorescence analysis. The percentage of contaminating cells (i. e. antibody positive cells not removed by the column) was measured on a fluorescence activated cell sorter (FACS) as described below. In all instances, contamination of W3/25-enriched cells with $OX8^+$ cells (and vice versa) never exceeded 5%. Prior to transfusion, cell viability was measured by the method of trypan blue exclusion and was > 95% in all cases.

In vivo depletion of donor T lymphocyte subsets with monoclonal antibody

DR-BB/W rats 30–90 days old were randomized into three groups 2 weeks before their use as spleen cell transfusion donors. Two groups were then given 1.5 ml intraperitoneal injections of either OX8 or W3/25 monoclonal antibody three times weekly. The third group of rats was injected with media that contained no antibody.

The antibodies were produced in the laboratory of Dr. A. A. Like by hybridoma cell lines obtained from Drs. A. F. Williams and D. S. Mason, Oxford University [16, 17]. These cell lines were cultured under standard conditions, and the undiluted antibody containing tissue culture supernatants stored at −20 °C until used. Although the antibody concentrations in the supernatants were not quantified, each lot was tested by flow cytometry with normal rat spleen cells to ensure the presence of saturating concentrations of the specific antibody [18].

One day after the last injection, donor rats from all three pretreatment groups were killed and their spleen cells prepared for transfusion [10]. An aliquot of each spleen cell suspension was used for cell counts and lymphocyte subset analysis. Littermate DP-BB/W rats 30 days old were randomized into four groups to serve as recipients of transfusions or as uninjected controls. A total of six transfusions were given to each recipient over two weeks. The total number of cells transfused to each group is given in Table 3.

Lymphocyte subset analysis

Samples of transfused spleen cells as well as samples of peripheral blood from DP recipients obtained at various times before and after transfusion were used to measure splenic lymphocyte subset percentages and total white blood cell counts as previously described [13]. Cells were analyzed for light scatter and fluorescence intensity on a FACS IV (Becton-Dickinson, Sunnyvale, Calif, USA). For each sample the fluorescence signal from 10 to 20 thousand viable cells was measured. Because antibody was not available at the time of these studies, analysis of the $RT\text{-}6^+$ lymphocyte subset [7, 19] was not performed.

Concanavalin A stimulation

The mitogenic response of peripheral blood lymphocytes to concanavalin A (con A) was measured as previously described [9], using doses of 0, 0.125, 0.25, 0.5 and 1.0 μg/well. Measurements at each dose of con A were performed in triplicate and averaged. Background counts were obtained from wells to which no mitogen was added and were subtracted from the mitogen stimulated counts.

Histologic procedures

Pancreata and thyroids from DP rats obtained at the end of experiments were fixed in Bouin's solution and embedded in paraffin. Sections stained with haematoxylin and eosin were examined for the presence of insulitis or thyroiditis by a pathologist (Dr. A. A. Like) who was unaware of the treatment status of the rats.

Statistical analysis

Statistical analysis of the frequency of diabetes, insulitis and thyroiditis following transfusion of in vitro separated cells (experiments 1 and 2) used the method of logistic regression [20]. This analysis was chosen because of the design of the studies, which involved two dosage levels and two separate control groups. Logistic regression permits valid analysis of the entire data set as one unit and chooses predictive factors (OX8 transfusion, W3/25 transfusion or no transfusion) that are significantly associated with outcome (prevention of diabetes, insulitis or thyroiditis). We used a stepwise algorithm that cut off p values greater than 5%. In the study of transfusion of cells from in vivo treated donors, we analyzed the frequency of diabetes with the chi square statistic with correction for continuity [21]. For the analysis of the frequency of diabetes, only those animals surviving to either the end of the experiment or the onset of diabetes are included. Insulitis and thyroiditis were scored as either present or absent without regard to the severity of the lesion. Parametric data are presented as the mean ± SEM. Comparisons among three means used one-way analyses of variance and the least significant difference procedure for a posteriori contrasts [21].

Results

The effect of transfusing DR-BB/W spleen cells enriched in vitro for specific T lymphocyte subsets is shown in Table 1. The data for experiments 1 and 2 demonstrate that reduction in the frequency of diabetes and insulitis was significantly associated only with transfusion of W3/25 enriched (helper/inducer) T cell populations ($p < 0.001$). No dose effect was demonstrable, and the effectiveness of 30×10^6 and 193×10^6 W3/25-enriched DR-BB/W spleen cells were statistically comparable. When the data were analyzed with respect to the frequency of diabetes alone, significantly less diabetes was associated with transfusion of W3/25-enriched cells ($p < 0.005$) and also with membership in the control group of experiment 1 ($p = 0.03$). DR-BB/W spleen cell preparations enriched in vitro for $OX8^+$ (suppressor/cytotoxic) T cells had no effect on the frequency of diabetes or insulitis in susceptible recipients.

A total of 22 thyroids from the low dose experiment and 37 from the high dose experiment were examined

Table 1. Frequency of diabetes in DP-BB/W rats given 5 transfusions of splenic lymphocytes enriched in W3/25$^+$ or OX8$^+$ cells. Results of experiments 1 and 2

	Groups					
	W3/25-enriched		OX8-enriched		Control	
Experiment number	1	2	1	2	1	2
Total number of cells transfused ($\times 10^6$)	30.0	193.0	27.5	135.5	-	-
Number of rats studied	15	15	15	14	14	23
Number & (%) diabetic rats	4 (27%)	3 (20%)	8 (53%)	11 (79%)	5 (36%)	16 (70%)
Number of nondiabetic rats with insulitis	4	2	5	2	5	5
Number & (%) of rats with diabetes *or* insulitis	8 (53%)	5 (33%)	13 (87%)	13 (93%)	10 (71%)	21 (91%)

Treated animals were 30-day-old DP-BB/W rats. Cells were given in a series of 5 transfusions over a period of 2 weeks. Data on diabetes are through 128 days of age. Logistic regression [21] showed that transfusion of W3/25-enriched spleen cells was significantly associated with the prevention of diabetes ($p < 0.005$). Membership in the first control group was also significantly associated with prevention of diabetes ($p = 0.03$). When the data are analyzed with respect to the prevention of diabetes *or* insulitis, only transfusion of W3/25-enriched spleen cells produced a statistically significant effect ($p < 0.001$). There were no significant effects of dose with respect to W3/25 transfusion, and the effectiveness of 30×10^6 and 193×10^6 W3/25-enriched DR-BB/W spleen cells was statistically comparable

Table 2. Mitogenic response to concanavalin A of peripheral white blood cells from transfused and control nondiabetic BB/W rats

Groups of rats			Mean ± SEM ^{3}H-thymidine uptake ($\times 10^3$)			
Type of T cells transfused	Insulitis	Number studied	Con A dose (µg/well)			
			0.125	0.25	0.5	1.0
OX8-enriched	Yes	3	47.8 ± 28.1	70.4 ± 50.3	30.8 ± 14.0	1.1 ± 0.5
W3/25-enriched	No	8	53.4 ± 23.3[a]	196.1 ± 48.4[a]	149.3 ± 39.9[b]	13.9 ± 6.6
W3/25-enriched	Yes	4	13.1 ± 5.3	50.5 ± 43.8	10.0 ± 7.5	0.9 ± 0.9
None (control)	No	2	7.6 ± 7.6	9.1 ± 9.1	2.3 ± 2.3	0.0 ± 0.0
None (control)	Yes	5	18.2 ± 3.7	32.2 ± 13.5	28.4 ± 19.4	6.5 ± 5.4

Data were obtained from experiment 2. Transfused rats had received 193 million W3/25-enriched spleen cells. All rats were nondiabetic when studied. Some rats were > 128 days of age when tested because only a limited number of rats could be studied at one time. Background counts from wells to which no con A was added have been subtracted. There were no OX8-enriched spleen cell transfused rats without insulitis at the end of experiment 2. [a] $p < 0.05$ vs. both control groups and W3/25 with insulitis group. [b] $p < 0.05$ vs. all other groups. No other paired comparisons are statistically significant

for thyroiditis either at the onset of diabetes or at the conclusion of the experiment. There were no statistically significant differences in the frequency of thyroiditis associated with any of the experimental groups. We note, however, that the lowest incidence was observed in the recipients of W3/25-enriched transfusions. Overall, 5 of 24 (21%) of these animals had thyroiditis, compared with 7 of 15 (47%) recipients of OX8-enriched transfusions and 10 of 20 (50%) of controls.

Peripheral lymphocyte subset percentages were measured and compared in nondiabetic recipient rats both prior to the high dose cell transfusions and at the end of the experiment. The percentage of W3/25$^+$ cells in recipients of W3/25-enriched transfusions rose 16.5 ± 4.4% (from 5.4% ± 1.3% at age 30 days, to 21.9 ± 4.6% at age 120 days, $n = 9$), compared with in increase in controls of 1.0 ± 1.9% (from 8.8 to 9.8%, $n = 7$, $p = 0.01$). The percentages of OX8$^+$ cells (3.7 ± 0.9%) and B cells (11.9 ± 2.8%) in recipients of W3/25-enriched spleen cells at the end of the experiment were not statistically different from the percentages observed in controls (5.7 ± 3.1 and 8.5 ± 3.8% respectively). Only 2 of the 3 surviving nondiabetic recipients of OX8-enriched cells were tested for T-cell subsets at both 30 and 120 days of age. In those two rats OX8$^+$ cells increased an average of 6.7% (from 6.1% to 12.8%), as compared with an increase in controls of 1.9 ± 3.6% ($n = 7$). W3/25$^+$ cells also increased 6.7% (from 6.0% to 12.7%) in recipients of OX8-enriched transfusions.

Beginning at 128 days of age, peripheral blood lymphocytes from nondiabetic recipients of high dose W3/25-enriched spleen cells and from surviving nondiabetic controls were tested for responsiveness to con A. As shown in Table 2, a significant increase in the mitogen response to con A was observed in lymphocytes obtained from transfused nondiabetic rats without insulitis ($p < 0.05$) as compared with those with insulitis or with controls. In contrast, the mitogen response of lymphocytes from recipients of W3/25-enriched cells that developed insulitis was statistically similar to that of controls.

The results of the third experiment are shown in Table 3. The lymphocyte subset composition of the transfused spleen cells obtained from rats pretreated with OX8 antibody indicates substantial depletion of the suppressor/cytotoxic subset. Pretreatment with

Table 3. Transfusion composition and frequency of diabetes in DP BB/W rats given six transfusions of spleen cells from donors pretreated in vivo with monoclonal antibody (MoAb). Results of experiment 3

	Experimental groups			
	1	2	3	4
Transfused	Yes	Yes	Yes	No
In vivo pretreatment of donor rats	W3/25 MoAb	OX8 MoAb	Medium alone	–
Total number of cells transfused ($\times 10^6$)	154.2	151.2	162.6	0
Number of T cells of different subsets transfused ($\times 10^6$)				
W3/25	46.2	54.0	49.8	0
OX8	25.2	2.4	30.0	0
Number of rats treated	15	15	12	15
Number and (%) diabetic	2 (13%)	1 (7%)	0 (0%)	8 (53%)

Cells for transfusion were obtained from rats pretreated for 2 weeks with injections of either W3/25 monoclonal antibody, OX8 monoclonal antibody or the vehicle used to dilute the antibody. Rats were studied for the development of diabetes through 175 days of age. Overall chi square = 15.91, $p < 0.005$. There were no statistically significant differences among groups 1, 2 and 3 in comparison with controls

W3/25 antibody, however, produced no significant depletion of helper/inducer T cells. Depletion of OX8$^+$ cells did not interfere with the ability of transfused spleen cells to protect DP-BB/W recipients from diabetes or insulitis. Because of the failure of the W3/25 antibody to deplete W3/25$^+$ cells in vivo, these spleen cell transfusions contained large numbers of W3/25$^+$ cells; they were effective in preventing diabetes.

Discussion

We previously showed that transfusions of whole blood [9], buffy coat cells and spleen cells, but not T cell-depleted lymphocytes [10], from DR-BB/W donors prevent spontaneous diabetes and insulitis in the DP-BB/W rat. Spleen cells from the histocompatible Wistar Furth rat were also shown to be effective [19]. The present study confirms and extends these observations by demonstrating that transfusions of DR-BB/W spleen cells enriched in vitro for the W3/25$^+$ helper/inducer T lymphocyte subset are protective, while those enriched for the OX8$^+$ suppressor/cytotoxic subset are not. We interpret the statistical significance associated with a reduced prevalence of diabetes in the control group of experiment 1 as an example of random fluctuation in the frequency of diabetes. This interpretation is supported by the observation that when insulitis, the pathological substrate of diabetes, is also taken into consideration, the "significance" associated with membership in this control group disappears.

We also observed that in vivo depletion of OX8$^+$ cells does not interfere with the protective effect of transfusion. Unfortunately, in vivo depletion of W3/25$^+$ T cells could not be achieved. Our failure to deplete these cells is consistent with previous reports that W3/25 antibody cannot be used for in vivo immune elimination [18]. Not surprisingly, spleen cells from rats pretreated with the W3/25 antibody contained normal numbers of W3/25$^+$ T cells and were protective.

In keeping with our previous reports [10], we also note that transfusion of protective spleen cells was associated with an increase in the percentage of W3/25$^+$ peripheral blood lymphocytes in long term nondiabetic survivors, and with enhancement of lymphocyte responsiveness to mitogenic stimulation in those nondiabetic rats that were also free of insulitis [9].

The reduction in the frequency of thyroiditis in transfusion recipients is less than that reported previously [10]. It should be noted, however, that in the present study the frequency of thyroiditis among controls was only 50%, compared with 100% in the previous report. The failure to demonstrate a significant effect on thyroiditis may reflect only its low overall incidence.

We conclude that W3/25$^+$ helper/inducer T lymphocytes from DR-BB/W rats (or a subset of these lymphocytes) prevent diabetes and insulitis in susceptible BB/W rats. We cannot, however, conclude that they represent the only such protective element in transfused blood. Because of the small number of observations available to us, for example, we cannot state definitively that the OX8-enriched transfusions were associated with long term survival of transfused OX8$^+$ cells. The hypothesis that transfusions of OX8$^+$ spleen cells do not confer protection is, however, consistent with and supported by the observation that in vivo depletion of these cells prevents diabetes [18].

We note that in the present study the frequency of diabetes following transfusion of splenocytes enriched in vitro for W3/25$^+$ cells (23%) was higher than that observed following transfusion of splenocytes depleted in vivo of OX8$^+$ cells (7%). It was also higher than that observed in previous studies using buffy coat (0% diabetic) [9] or fresh spleen cell (5% diabetic) [10] transfusions. Several factors could plausibly account for these discrepancies.

First, the process of fractionation may affect the ability of transfused W3/25-enriched spleen cells to provide protection. It could be that in vitro processing in some way damages lymphocytes, rendering them less capable of affording protection in low numbers. Alternatively, the highly effective in vitro purification procedure may remove from transfusions a cell population with which the selected cells must interact to provide complete protection. Not only the absolute number and functional integrity of transfused W3/25$^+$ cells, but also the restoration of normal lymphocyte subset ratios (hence normal cell-cell interactions) may determine the effectiveness of transfused cells in preventing diabetes.

The incompleteness of protection observed in the present study could also be explained if the W3/25$^+$

phenotype were itself only a subset of the actual protective T-cell population. Recent studies in the BB rat indicate that the protective effect mediated by lymphocyte transfusions may involve the T-cell subset expressing the RT-6 alloantigen [19]. RT-6$^+$ T cells are absent in DP-BB/W rats [7], and donor origin RT-6$^+$ T cells from donor Wistar Furth rats can be detected in protected DP animals up to 4 months after lymphocyte transfusion [19]. In contrast, no RT-6$^+$ cells have been found in DP BB/W rats not protected by transfusion [19]. Since only 50% of W3/25$^+$ T cells and 80% of OX8$^+$ T cells in the rat express the RT-6 alloantigen [22], it is tempting to speculate that only RT-6$^+$, W3/25$^+$ T cells actually play a significant role in immune regulation and in the prevention of diabetes in the BB rat. Alternatively, OX8$^+$, RT-6$^+$ T cells may also be necessary for maximal protection. If either of these possibilities were correct, then transfusion of insufficient numbers of the relevant W3/25$^+$ T cell subset, or injection of OX8-depleted W3/25$^+$ T cells could account for the incomplete protection reported here. To achieve maximal protection, i.e. to obtain the appropriate number of relevant regulatory T cells, it may be necessary to transfuse a significant excess of W3/25-enriched cells or to transfuse appropriate mixtures of fractionated T cell subsets.

The cellular mechanism(s) by which helper/inducer T cell transfusions might mediate the prevention of autoimmune diabetes in the rat are unknown, and could involve either direct cell-cell contact or the secretion of a "protective lymphokine" by the appropriate transfused cells. In support of the latter hypothesis, we have recently demonstrated that lymphocytes from nondiabetic rats sequestered in diffusion chambers that are then implanted into susceptible BB/W rats protect the recipients against diabetes [23]. Based on these observations it is hypothesized that W3/25$^+$ T cells or a subset thereof may secrete a lymphokine required for diabetes prevention in the BB rat.

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References

1. Rossini AA, Mordes JP, Like AA (1985) Immunology of insulin-dependent diabetes mellitus. Ann Rev Immunol 3: 291-322
2. Marliss EB, Nakhooda AF, Poussier P, Sima AAF (1982) The diabetic syndrome of the 'BB' Wistar rat: Possible relevance to Type 1 (insulin-dependent) diabetes in man. Diabetologia 22: 225-232
3. Oschilewski U, Kiesel U, Kolb H (1985) Administration of silica prevents diabetes in BB-rats. Diabetes 34: 197-199
4. Jackson R, Rassi N, Crump T, Haynes B, Eisenbarth GS (1981) The BB diabetic rat: profound T-cell lymphocytopenia. Diabetes 30: 887-889
5. Poussier P, Nakhooda AF, Falk JF, Lee C, Marliss EB (1982) Lymphopenia and abnormal lymphocyte subsets in the "BB" rat: Relationship to the diabetic syndrome. Endocrinology 110: 1825-1827
6. Elder ME, Maclaren NK (1983) Identification of profound peripheral T lymphocyte immunodeficiencies in the spontaneously diabetic BB rat. J Immunol 130: 1723-1731
7. Greiner DL, Handler ES, Nakano K, Mordes JP, Rossini AA (1986) Absence of the RT-6 cell subset in diabetes-prone BB/W rats. J Immunol 136: 148-151
8. Yale J-F, Grose M, Marliss EB (1985) Time course of the lymphopenia in BB rats. Relation to the onset of diabetes. Diabetes 34: 955-959
9. Rossini AA, Mordes JP, Pelletier AM, Like AA (1983) Transfusions of whole blood prevent spontaneous diabetes in the BB/W rat. Science 219: 975-977
10. Rossini AA, Faustman D, Woda BA, Like AA, Szymanski I, Mordes JP (1984) Lymphocyte transfusions prevent diabetes in the Bio-Breeding/Worcester rat. J Clin Invest 74: 39-46
11. Like AA, Rossini AA (1984) Spontaneous autoimmune diabetes mellitus in the BioBreeding/Worcester rat. Surv Synth Pathol Res 3: 131-138
12. Mason DW, Arthur RP, Dallman MJ, Green JR, Spickett GP, Thomas ML (1983) Functions of rat T-lymphocyte subsets isolated by means of monoclonal antibodies. Immunol Rev 74: 57-82
13. Gilman SC, Woda BA, Feldman JD (1981) T lymphocytes of young and aged rats. I. Distribution, density and capping of T antigens. J Immunol 127: 149-153
14. Duarte AJS, Carpenter CB, Strom TB (1982) Expression of T cell differentiation antigens and Ia on rat cytotoxic T lymphocytes. J Immunol 128: 580-584
15. Dallman MJ, Thomas ML, Green JR (1984) MRC OX-19: A monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. Eur J Immunol 14: 260-267
16. Brideau RJ, Carter PB, McMaster WR, Mason DW, Williams AF (1980) Two sets of rat T lymphocytes defined with monoclonal antibodies. Eur J Immunol 10: 609-615
17. Jefferies WA, Green JR, Williams AF (1985) Authentic T helper CD4[W/325] antigen on rat peritoneal macrophages. J Exp Med 162: 117-127
18. Like AA, Biron CA, Weringer EJ, Byman K, Sroczynski E, Guberski DL (1986) Prevention of diabetes in BioBreeding/Worcester rats with monoclonal antibodies that recognize T lymphocytes or natural killer cells. J Exp Med 164: 1145-1159
19. Rossini AA, Mordes JP, Greiner DL, Nakano K, Appel MC, Handler ES (1986) Spleen cell transfusion in the BB/W rat: Prevention of diabetes, MHC restriction, and long term persistence of transfused cells. J Clin Invest 77: 1399-1401
20. Engelman L (1983) BMDPLR: Stepwise logistic regression. In: Dixon WJ, Brown MB, Engelman L, Frane JW, Hill MA, Jennrich RI, Toporek JD (eds) BMDP Statistical Software. University of California Press, Berkeley, pp 330-344
21. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH (1975) Statistical package for the social sciences, 2nd edn. McGraw-Hill, New York
22. Mojic CF, Greiner DL, Medlock ES, Goldschneider I (1985) Development of T cell subsets in the rat expressing the RT-6 alloantigen (Abstract). Fed Proc 44: 1301
23. Handler ES, Mordes JP, Nakamura N, Rossini AA (1986) Prevention of spontaneous diabetes in the BB/W rat by Wistar Furth (WF) spleen cells sequestered in diffusion chambers (Abstract). Diabetes 35 [Suppl 1]: 75 A

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