Lymphocyte Transfusions Prevent Diabetes in the Bio-Breeding/Worcester Rat

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Abstract. The Bio-Breeding/Worcester (BB/W) rat develops spontaneous autoimmune diabetes similar to human insulin-dependent diabetes mellitus. Transfusions of whole blood from the nondiabetic W-line of BB/W rats prevent the syndrome in diabetes-prone recipients. We report three experiments designed to determine which blood component is protective. In all experiments, diabetes-prone BB/W rats 23 to 35 d of age were given four or six weekly intravenous injections. In the first experiment, animals received either saline or transfusions of erythrocytes, white blood cells, or plasma from W-line donors. Diabetes occurred in 7/22 (32%) erythrocyte, 2/27 (7%) white cell, 14/24 (58%) plasma, and 15/27 (56%) saline recipients ($P < 0.001$). At 120 d of age, peripheral blood was obtained from nondiabetic rats. Fluorescence-activated cell sorter analysis of OX 19 tagged leucocytes revealed 35% T lymphocytes in white cell recipients ($n = 13$), compared with 9% in saline recipients ($n = 7$; $P < 0.001$). Responsiveness to concanavalin A was also increased in the white cell group, whereas the frequency of both insulitis and thyroiditis was decreased. In the second experiment, 1/19 (5%) rats transfused with W-line spleen cells developed diabetes, as contrasted with 12/18 (67%) recipients of diabetes-prone spleen cells and 19/31 (61%) noninjected controls ($P < 0.001$). In the third experiment, diabetes-prone rats received either W-line blood treated with a cytotoxic anti-T lymphocyte antibody plus complement, untreated blood, or saline. Diabetes occurred in 8/20 (40%), 1/20 (5%), and 13/19 (68%) rats in each group, respectively ($P < 0.001$). We conclude that transfusions of W-line T lymphocytes prevent diabetes in the BB/W rat.

Introduction

The Bio-Breeding (BB)[1] rat spontaneously develops a heritable syndrome that closely resembles human insulin-dependent diabetes mellitus (IDDM) (1). The onset is abrupt. Untreated rats are hyperglycemic and ketonemic, and diabetic animals require insulin for survival. Genetic studies suggest that the disease is inherited as an autosomal recessive with incomplete penetrance (2). Three separate genes may be involved (3).

In the partially inbred Bio-Breeding/Worcester (BB/W) colony at the University of Massachusetts (Worcester), 40 to 60% of diabetes-prone animals become diabetic (4), usually between 60 and 120 d of age. The rats are lean, and both sexes are equally affected. The physiologic derangements seen in diabetic animals are similar to those observed in human IDDM and include hypoinsulinemia and hyperglucagonemia. Histologic study of acutely diabetic animals reveals lymphocytic infiltration of the islets, or insulitis. Chronically diabetic animals have end-stage islets, which are devoid of beta cells (4, 5).

The observation of pancreatic insulitis suggests that BB rat diabetes has a cell-mediated immune pathogenesis. Additional data that support this concept include adoptive transfer of diabetes by means of concanavalin A (con A) stimulated lymphocytes (6); prevention by immunosuppressive agents, including antilymphocyte serum (7), glucocorticoids (8), cyclosporin-A (8, 9), whole body irradiation (7), and total lymphoid irradiation (10); and prevention by immune modulations, in-

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1. *Abbreviations used in this paper:* BAT, brain-associated T cell antigens; BB, Bio-Breeding; BB/W, Bio-Breeding/Worcester; con A, concanavalin A; IDDM, insulin-dependent diabetes mellitus.

cluding neonatal thymectomy (11) and neonatal bone marrow allografts (12, 13).

Transfusions of whole blood from nondiabetic rats also prevent diabetes in the BB/W rat (14). The blood is obtained from the diabetes-resistant W-line of BB/W rats. Transfusion into young diabetes-prone BB/W rats prevents not only overt diabetes, but it also prevents insulitis and restores towards normal the depressed responsiveness of BB/W lymphocytes to the mitogen con A. The present studies were designed to determine which component of whole blood protects against diabetes in the BB/W rat.

Methods

Animals

All experiments used BB/W rats from the colony maintained at the University of Massachusetts. There are nine diabetes-prone and two diabetes-resistant lines of BB/W rats in this colony. Diabetes-prone transfusion recipients were drawn at random from the several lines being inbred. Male and female rats were used in approximately equal numbers.

Peripheral blood for transfusion was obtained exclusively from the diabetes-resistant W-line of BB/W rats. These animals were originally derived from diabetes-prone BB/W progenitors but have been bred for resistance to the disease. Through 11 generations of brother-sister mating ($n > 1000$), only three diabetic W-line rats have appeared. Splenic lymphocytes for transfusion were obtained either from W-line rats or from normoglycemic diabetes-prone BB/W rats drawn at random from the various family lines.

Preparation of separated blood components

Heparinized blood for transfusion was obtained by cardiac puncture and kept at room temperature. Hematocrit and white cell count were measured, and the pooled blood was centrifuged for 20 minutes at 2,000 *g*. The supernatant plasma was decanted and saved. The buffy coat, which contained residual plasma, and some of the uppermost erythrocytes were washed twice with 15 to 25 vol of 0.9% NaCl containing 0.2% dextrose and 0.15% Na_2 EDTA and centrifuged at 500 *g* for 12 min. The supernatant containing most of the platelets was removed. The white cells were washed once more and then suspended in 0.9% NaCl and 0.2% dextrose.

To reduce white cell contamination, the packed erythrocyte concentrate was recombined with half the plasma and passed through a cellulose bed which contained equal amounts of microcrystalline cellulose (Sigmacell type 50) and α-cellulose (both from Sigma Chemical Co., St. Louis, MO) (15). The remaining plasma was centrifuged at 2,000 *g* for 20 min. The concentration of cells in all blood products was measured with a hemocytometer. No cells were observed in the plasma. No platelets were observed in any of the transfusions. White cell viability was ascertained by trypan blue exclusion for the first two white cell transfusions and was >99% in both instances.

Preparation of splenic lymphocytes for transfusion

Splenic lymphocytes were obtained from either diabetes-resistant W-line BB/W rats or normoglycemic diabetes-prone BB/W rats. Donors of both sexes and various ages were killed with intraperitoneal pentobarbital. Spleens were removed by sterile technique and mechanically disrupted with an autoclaved garlic press (Ekco Housewares Co., Franklin Park, IL). Extruded cells were collected in 10 ml of RPMI medium (Accurate Chemical and Scientific Corp., Westbury, NY). The volume of the suspension was increased to 45 ml with RPMI, and the cells were centrifuged at 1,200 *g* for 10 min. After one additional wash, the cells were resuspended in RPMI and counted. The volume of transfusion was 0.1 to 0.2 ml.

Preparation of T lymphocyte depleted whole blood

Blood donors were W-line BB/W rats of both sexes and various ages. Whole blood was withdrawn from the periorbital sinus of ether anesthetized rats into heparinized glass capillary tubes. The blood was separated into two aliquots, one of which was treated with a serum prepared against brain-associated T cell antigens (anti-BAT serum) and complement to deplete it of T cells.

Anti-BAT was a gift from Dr. M. Minami of Tokyo University. Anti-BAT serum is prepared in rabbits by immunization with rat whole brain tissue (16). It cross reacts with theta 1 positive rat T cells (17). The specific anti-BAT serum used in these studies was absorbed with mouse liver to remove class I and class II antibodies. After absorption, microcytotoxicity testing showed 50 to 60% splenocyte killing and 95% thymocyte killing. In further studies, rat whole blood was incubated with anti-BAT serum and R23 rabbit complement. After the erythrocytes were lysed with NH_4Cl, residual T lymphocyte activity was again measured by means of microcytotoxicity testing. The lymphocytes that had been treated with BAT serum demonstrated a kill rate of ~20%, a rate indistinguishable from background. In contrast, lymphocytes from untreated blood demonstrated almost 100% killing. It has further been demonstrated that the BAT antibody is a pan–T cell antibody and that it is not cytotoxic in the absence of complement (J. Davie, personal communication).

The whole blood to be treated with anti-BAT serum was centrifuged at 500 *g* for 10 min and then washed twice with 250 ml RPMI. Each time, the supernatant was carefully removed with pipette suction to assure that the buffy coat was not disturbed.

For each 5.5 ml of packed blood cells, 220 lambda of anti-BAT serum was then added. The anti-BAT and blood cells were incubated for 30 min at room temperature. Every 15 s the cells were gently swirled. The blood was then diluted with RPMI and centrifuged at 500 *g* for 10 min. As the supernatant was removed with a pipette, care was taken not to disturb the pellet. Next, 100 lambda of R23 rabbit complement was added to the pellet, which was then placed in a shaker bath at 37°C for 30 min. During the incubation the tubes were swirled three additional times to ensure mixing. Finally, the cells were washed with RPMI and centrifuged at 500 *g* for 10 min. Control blood cells were processed identically, except that neither anti-BAT serum nor complement was added.

Lymphocyte subsets

Blood samples and spleens obtained from nondiabetic rats at the time of sacrifice were used to measure total peripheral white blood cell counts and peripheral and splenic lymphocyte subset percentages, as previously described (10, 18). Monoclonal antibodies directed against T helper cells (W 3/25), T suppressor cells (OX 8), and T cells (OX 19) were used. Cells were analyzed for light scatter and fluorescence intensity with a fluorescence-activated cell sorter (FACS III; Becton-Dickinson FACS, Becton-Dickinson and Co., Sunnyvale, CA). For each sample the fluorescence signal from 10,000 to 20,000 viable cells was measured.

Con A stimulation

The response of lymphocytes to con A was measured as previously described (14), using doses of 0.03, 0.125, 0.5 or 1.0 μg/well. Measurements at each dosage were performed in triplicate and averaged.

Measurement of autoantibodies

Blood samples were obtained from animals at death. Details of the measurement procedure have previously been described (19). The staining intensity of each sample was arbitrarily graded from 0 to 4+. Antibodies were considered to be present for any score of 1 or greater.

Histologic procedures

Pancreata and thyroids were fixed in Bouin's solution and embedded in paraffin. Sections were stained with hematoxylin and eosin and examined for the presence of insulitis (5) and thyroiditis (20). The examinations were performed by a pathologist (Dr. Like) who was unaware of the treatment status of the rats.

Statistical procedures

Statistical analysis of 2×2 tables used the Fisher's exact test for $n \leq 24$; all other tables were analyzed by the chi square statistic with Yates' correction where appropriate (21). Comparisons among three means used one-way analyses of variance; comparisons of two means used the unpaired t test with separate variance estimate (22). For the analysis of the frequency of diabetes, only those animals who survived either to the end of the experiment (120 or 170 d of age) or to the onset of diabetes were included. Animals that died before 120 d of age or the diagnosis of diabetes were excluded. All parametric data are presented as the mean±SEM.

Experimental protocols

Three experiments were performed; each studied the effect of transfusion on the frequency of diabetes in diabetes-prone BB/W rats. Diabetes was diagnosed on the basis of 4+ glycosuria (Tes-Tape; Eli Lilly and Co., Indianapolis, IN) and a plasma glucose > 250 mg/dl.

Experiment 1. Transfusion of separated blood components. Litters of diabetes-prone BB/W rats 23 to 30 d of age were randomly allocated to four groups which received either erythrocyte concentrate, white blood cell concentrate, cell free plasma, or 0.9% saline. Each transfusion or injection was administered intravenously into the tail vein in a volume of 1 ml. All rats received a total of six weekly injections.

The first half of the experiment studied 55 rats. Transfusions of erythrocyte concentrate contained an average of $9.36 \times 10^9 \pm 5.31 \times 10^8$ erythrocytes and $5.04 \times 10^4 \pm 2.29 \times 10^4$ white cells. Transfusions of white cell concentrate contained $1.21 \times 10^7 \pm 7.68 \times 10^5$ white cells and $1.10 \times 10^9 \pm 2.01 \times 10^8$ erythrocytes. Rats were tested for the presence of diabetes twice weekly between the ages of 60 and 120 d. Diabetic rats were sacrificed at the time of detection and not studied further.

Between 113 and 134 d of age, four nondiabetic rats were selected daily at random from among the rats that had received either white cell concentrate or saline. Approximately 1 ml of blood was obtained from the tail vein and used to measure white blood cell count and the proportion of OX 19, W 3/25, and OX 8 positive peripheral blood lymphocytes. 13 white cell and 8 saline recipients were studied.

Also between 113 and 134 d of age, 39 rats were selected at random from the four experimental groups and used to measure con A-induced lymphocyte stimulation. After the rats were anesthetized with ether, 4 ml of blood was obtained from the orbital sinus. At least 7 d separated the two blood drawings in rats subjected to both procedures. One rat in the plasma group died of anesthesia before 120 d of age and has been omitted from all analyses.

All rats were sacrificed between 135 and 143 d of age. Whole blood was collected in plastic syringes by cardiac puncture and centrifuged. The serum was then frozen and used for the subsequent measurement of anti-smooth muscle and anti-thyroglobulin antibodies. The thyroids and pancreata were removed for later histologic study.

The second half of the experiment used an identical transfusion protocol. 48 rats of both sexes were randomized into four groups. Transfusions of erythrocyte concentrate contained an average of $8.86 \times 10^9 \pm 1.59 \times 10^8$ erythrocytes and $8.64 \times 10^4 \pm 2.86 \times 10^4$ white cells. Transfusions of white blood cell concentrate contained $8.66 \times 10^6 \pm 1.41 \times 10^6$ white cells and $8.72 \times 10^8 \pm 5.92 \times 10^7$ erythrocytes. Two rats died before 120 d of age or the diagnosis of diabetes and have been excluded from all analyses. In this half of the experiment the rats were permitted to survive past 120 d of age to assess the duration of protection from diabetes. As of this writing, the surviving nondiabetic rats are 1 yr of age.

Experiment 2. Transfusion of splenic lymphocytes. 68 diabetes-prone BB/W rats 31 or 32 d of age were used. Experimental rats received four weekly transfusions of splenic lymphocytes ($25–50 \times 10^6$ cells/rat) from either diabetes-resistant W-line rats (n = 19 recipients) or from normoglycemic diabetes-prone BB/W rats (n = 18 recipients). Controls (n = 31) received no injections. All rats were tested for diabetes until 120 d of age. Diabetic rats were sacrificed and not studied further. Between 120 and 140 d of age, all nondiabetic rats were killed and their pancreata and thyroids were removed and prepared for light microscopy.

Experiment 3. Transfusion of T cell depleted whole blood. 59 diabetes-prone BB/W rats 30 to 32 d old were used. Litters were randomized into three groups which received either untreated whole blood (n = 20), whole blood treated with anti-BAT serum and complement (n = 20), or 0.9% saline (n = 19). All rats received a total of six weekly 1-ml injections. They were tested for diabetes twice weekly between 60 and 170 d of age. Diabetic rats were sacrificed and not studied further.

Beginning at 150 d of age, four rats were selected daily at random from the three groups. Approximately 1 ml of blood, obtained from the tail vein with heparinized capillary tubes, was used to measure the white blood cell count and the proportion of OX 19, W 3/25, and OX 8 positive peripheral blood lymphocytes.

Also beginning at 150 d of age, blood was obtained for the measurement of con A induced lymphocyte stimulation. At least 7 d separated the two blood drawings. All nondiabetic rats were sacrificed between 171 and 184 d of age by the use of 100% CO_2. The spleens were removed, placed in sterile Petri dishes that contained 15 ml RPMI at room temperature, and processed for the measurement of lymphocyte subsets. The thyroids and pancreata were also removed and processed for histologic study.

Results

Experiment 1. Transfusion of separated blood components

Frequency of diabetes. Through 120 d of age there were no differences in the outcome of the two halves of the experiment; the combined results are shown in Table I. Diabetes occurred least often in rats given white cell concentrate. Plasma and saline treated rats had similar high frequencies of diabetes. There was an intermediate but statistically nonsignificant effect among rats given erythrocyte concentrate.

The results of the second half of the experiment through 1 yr of age show a persistence of the trend observed for the whole group through 120 d of age. Diabetes has occurred in 2 of 12 (17%) white cell recipients, 5 of 9 (56%) erythrocyte recipients, 10 of 12 (83%) plasma recipients, and 10 of 13 (77%) saline recipients (chi square = 13.57, $P < 0.005$).

Table I. Frequency of Diabetes through 120 d of Age in BB/W Rats Transfused with Separated Blood Components or Saline

	Transfusion			
	White cells	Erythrocytes	Plasma	Saline
Diabetes	2	7	14	15
No diabetes	25	15	10	12
Percent diabetic	7%*	32%	58%	56%

Chi square = 18.82 ($P < 0.001$).
* $P < 0.001$ vs. plasma and saline groups. No other paired comparisons are significant.

Frequency of insulitis and thyroiditis. The frequencies of insulitis and thyroiditis for the 41 nondiabetic rats sacrificed in the first half of the experiment are shown in Table II. Only those rats given white blood cell concentrate were uniformly free of insulitis at the time of sacrifice. Both erythrocyte and white cell recipients had significantly less thyroiditis than did saline recipients. The white cell recipients also had less thyroiditis than did plasma recipients, but this difference was not statistically significant ($P = 0.08$).

Frequency of diabetes or insulitis considered together. When the animals in the first half of the experiment who had either insulitis or frank diabetes were grouped together, a dramatic effect of white cell transfusion and an intermediate effect of erythrocyte transfusion were again seen. None of the 15 white cell recipients developed either lesion, as contrasted with 4 of 13 (31%) erythrocyte recipients, 10 of 12 (83%) plasma recipients, and 8 of 14 (57%) saline controls ($P < 0.001$).

Table II. Frequency of Insulitis and Thyroiditis in BB/W Rats Transfused with Separated Blood Components

	Transfusion			
	White cells	Erythrocytes	Plasma	Saline
Insulitis	0	2	4	3
No insulitis	15	9	2	6
Percent with insulitis	0%*	18%	67%	33%
Chi square = 11.99 ($P < 0.01$)				
Thyroiditis	0	1	2	5
No thyroiditis	14	10	4	4
Percent with thyroiditis	0%‡	9%§	33%	56%
Chi square = 12.10 ($P < 0.01$)				

Tissues were obtained from nondiabetic rats 135 to 143 d of age.
* $P < 0.005$ vs. plasma and $P < 0.05$ vs. saline.
‡ $P < 0.01$ vs. saline.
§ $P < 0.05$ vs. saline.

Frequency of autoantibodies. Autoantibodies were measured in the sera of the 41 nondiabetic rats sacrificed at 120 d of age. There was a significant reduction in the presence of anti-smooth muscle antibodies in the rats given white cell transfusions (1/15, 7%) compared with rats given plasma (4/6, 67%, $P < 0.02$) or saline (7/9, 78%, $P < 0.005$). In addition, there was a significant reduction in the occurrence of antibody in erythrocyte recipients (2/11, 18%, $P < 0.02$) compared with saline recipients. There were no statistically significant differences in the frequency of antithyroid colloid antibodies among the four groups. Antibodies were present in 3 of 15 (20%) white cell recipients, 3 of 11 (27%) erythrocyte recipients, 1 of 6 (17%) plasma recipients, and 5 of 9 (56%) saline recipients.

Con A induced lymphocyte stimulation. When cells were incubated in the absence of con A there were no significant differences in thymidine incorporation among the four groups of rats. At a dose of 0.03 μg per well, incorporation ($\log_{10}$cpm) was 5.42±0.08 in the erythrocyte group ($n = 11$) and 5.48±0.09 in the white cell group ($n = 14$). These results were similar and both were significantly higher ($P < 0.05$) than the incorporation in the plasma (4.93±0.25, $n = 6$) or saline (4.59±0.17, $n = 8$) groups. At a dose of 0.125 μg/well, the results were similar. The thymidine incorporation was 5.61±0.04 in the erythrocyte group and 5.56±0.05 in the white cell group. These results were both slightly but significantly ($P < 0.05$) greater than in the plasma (5.07±0.22) or saline (4.99±0.18) groups. At doses of 0.5 and 1.0 μg con A per well there were no significant differences in thymidine incorporation among the four groups.

Lymphocyte subsets. Peripheral blood lymphocyte subset percentages were measured in most of the nondiabetic white cell and saline treated rats between 113 and 134 d of age. The results are shown in Table III. They demonstrate a highly significant increase in total (OX 19 positive) T cells and in helper (W 3/25 positive) cells. The percentage of suppressor/killer (OX 8 positive) cells was also increased in the white cell recipients, but the increase was not statistically significant ($P = 0.09$). Transfusion did not affect total white blood cell count. The subset percentages observed in the white cell recipients approach

Table III. White Blood Cell Count and Peripheral Blood Lymphocyte Subset Percentages in Nondiabetic Diabetes-prone BB/W Rats Treated with White Cells or Saline Injections

	Transfusion		
	White cells	Saline	
White blood cell count (*cells/mm³*)	4,758±845	6,057±701 ($n = 7$)	NS
OX 19 cells (%)	30.6±3.6	8.4±1.5	$P < 0.001$
W 3/25 cells (%)	28.0±3.2	7.5±1.5	$P < 0.001$
OX 8 cells (%)	20.8±3.0 ($n = 11$)	13.5±4.1	NS

The results of unpaired t tests are indicated at the right. Results are from 12 white cell recipients and 6 saline recipients, except as indicated. NS, not significant.

those we have previously observed (10) in diabetes-resistant W-line rats: 55.6±5.8% OX 19 positive cells, 20.6±1.0% OX 8 positive cells, and 40.1±2.7% W 3/25 positive cells.

Experiment 2. Transfusion of splenic lymphocytes

The frequency of diabetes, insulitis, and thyroiditis through 120 d of age in the three experimental groups is given in Table IV. Diabetes occurred at a similar rate in both the untreated controls and in rats given transfusions of splenocytes from normoglycemic diabetes-prone rats. In contrast, rats given transfusions of W-line splenic lymphocytes were almost completely protected from the disease. Among the nondiabetic survivors, the frequency of insulitis was 7% in the W-line cell recipients, compared with 50% in rats given cells from diabetes-prone donors and 80% in controls ($P < 0.001$). There was a trend towards a reduction in the frequency of thyroiditis in the W-line cell recipients, but this did not achieve statistical significance ($P = 0.07$). A total of seven pancreas and six thyroid specimens were technically unsatisfactory and are not included in these analyses.

Experiment 3. Transfusion of T cell depleted whole blood

Frequency of diabetes, insulitis, and thyroiditis. These results are shown in Table V. Animals given whole blood were almost completely protected from diabetes and had a significantly lower frequency of diabetes than did rats who received either T cell depleted blood or saline. The frequencies of diabetes in these last two groups were statistically similar.

Table IV. Frequency of Diabetes, Insulitis, and Thyroiditis through 120 d of Age in BB/W Rats Transfused with Splenic Lymphocytes

	Transfusion		
	W-line spleen cells	Diabetes-prone spleen cells	None
Diabetes	1	12	19
No diabetes	18	6	12
Percent diabetic	5%*	67%	61%
Chi square = 18.62 ($P < 0.001$)			
Insulitis	1	2	8
No insulitis	14	2	2
Percent with insulitis	7%‡	50%	80%
Chi square = 13.99 ($P < 0.001$)			
Thyroiditis	2	2	6
No thyroiditis	13	2	5
Percent with thyroiditis	13%	50%	55%
Chi square = 5.43 ($P = 0.07$)			

* $P < 0.001$ compared with other groups.
‡ $P < 0.001$ compared with untreated group. No other paired comparisons are significant.

Table V. Frequency of Diabetes, Insulitis, and Thyroiditis through 170 d of Age in BB/W Rats Transfused with Whole Blood, T Cell Depleted Whole Blood, or Saline

	Transfusion		
	Untreated blood	T cell depleted blood	Saline
Diabetes	1	8	13
No diabetes	19	12	6
Percent diabetic	5%*	40%	68%
Chi square = 16.85 ($P < 0.001$)			
Insulitis	0	3	5
No insulitis	18	9	1
Percent with insulitis	0%‡	25%§	83%
Chi square = 18.16 ($P < 0.001$)			
Thyroiditis	2	4	6
No thyroiditis	17	8	0
Percent with thyroiditis	11%‖	33%‖	100%
Chi square = 16.67 ($P < 0.001$)			

* $P < 0.05$ compared with T cell depleted blood group and $P < 0.001$ compared with saline.
‡ $P < 0.001$ compared with saline group.
§ $P < 0.05$ compared with saline group.
‖ $P < 0.001$ compared with saline group.

In contrast to the findings with regard to overt diabetes, the frequency of insulitis and thyroiditis was reduced in both the untreated blood and T cell depleted blood recipient. It should be noted, however, that the lowest frequencies were found in the untreated blood recipients. Insulitis was entirely absent in this group.

When the rats are grouped according to the presence of either insulitis or diabetes, the outcome of the experiment is clearer. Only 1 of 20 (5%) rats given untreated blood had either lesion, as compared with 11 of 20 (55%) rats given T cell depleted blood and 18 of 19 (95%) saline controls ($P < 0.001$).

Lymphocyte subsets. These results are shown in Table VI. White blood cell count was greater in the control rats than in either of the two experimental groups, but there was considerable variability and this difference was not significant. In general, both blood recipient groups had higher subset percentages than did the controls. This was true of both peripheral and splenic lymphocytes.

Con A induced lymphocyte stimulation. At all dosages of con A, the response of the rats transfused with untreated whole blood was statistically similar to that of rats given T cell depleted

Table VI. White Cell Count and Lymphocyte Subsets in Nondiabetic BB/W Rats Transfused with Whole Blood, T Cell Depleted Blood, or Saline

	Transfusion		
	Untreated blood	T cell depleted blood	Saline
Peripheral white blood cell count (*cells/mm³*)	3,020±250 (19)	3,490±340 (12)	6,420±1,700 (5)
Peripheral lymphocyte subsets (%)			
OX 19	24.0±1.8 (19)	21.9±2.4 (12)	5.5±0.8* (5)
W 3/25	19.1±1.3 (19)	18.3±2.2 (12)	5.7±0.3* (4)
OX 8	8.3±0.8 (19)	8.0±1.4 (11)	3.1±0.7* (4)
Splenic lymphocyte subsets (%)			
OX 19	19.5±1.1 (18)	12.9±2.1 (11)	11.1±5.1‡ (5)
W 3/5	18.4±1.2 (18)	13.6±1.8 (12)	8.7±3.1* (5)
OX 8	9.7±0.8 (18)	7.0±1.3 (12)	5.5±1.0‡ (6)

Number of rats is given in parentheses.
* Saline significantly less than other groups ($P < 0.01$).
‡ Saline significantly less than other groups ($P < 0.05$).

blood, and the responses of both of these groups were greater than that of saline treated rats (Table VII).

Discussion

These data confirm and extend our previous observation that blood transfusion prevents diabetes in susceptible BB/W rats. The first experiment demonstrates that white blood cells are the protective component of transfused blood. Only five weekly transfusions were required for protection with white cells, as contrasted with the 11 transfusions of whole blood employed in our initial report (14). In this experiment the erythrocyte concentrate may also have provided some degree of protection. This is most readily explained by contamination of the erythrocyte concentrate with a small number of white blood cells. The white cell recipients in the second half of the experiment were not sacrificed and have been protected from diabetes through 1 yr of age.

The postmortem studies show that the white blood cell concentrate not only protected against diabetes but also ameliorated other immunologic abnormalities frequently observed in the diabetes-prone animal. Insulitis, the pathologic substrate of autoimmune diabetes, was totally absent in white cell treated animals. Thyroiditis, another autoimmune abnormality frequently observed in the BB rat (20), was also much reduced in animals receiving white cell concentrate.

Lymphocytopenia is also a characteristic immunologic abnormality of the BB rat (12, 23, 24, 25). Although the total white blood cell count was not increased by transfusion, animals receiving white cell concentrate had a greatly increased percentage of OX 19 labeled circulating T lymphocytes. In particular, the percentage of W 3/25 helper cells was substantially higher, and there was some suggestion that the percentage of OX 8 suppressor/killer cells was also increased by white cell transfusion.

The results of the con A studies are consistent with previous observations (14). Animals transfused with white cells displayed enhanced thymidine incorporation when compared with either plasma or saline treated animals. Again, a similar effect was also observed in those animals who received the erythrocyte concentrate.

The second set of experiments compared the degree of protection afforded by transfusion of spleen cells derived from W-line rats with that afforded by transfusion of spleen cells derived from nondiabetic but diabetes-prone BB/W rats. Again, nearly complete protection from diabetes was afforded by the transfusion of lymphocytes from W-line rats. Transfusion of lymphocytes from nondiabetic diabetes-prone BB/W rats was not at all protective.

The results of the first two experiments suggest that there is a defect in cellular immunity in the diabetes-prone BB/W rat which permits the development of diabetes mellitus and which is corrected by the transfusion of peripheral or splenic lymphocytes from diabetes-resistant rats.

It might be argued that the effects that we have observed with transfusion are due to nonspecific allogeneic transfusion immunosuppression (26). However, it should be noted that the BB/W rat is not yet inbred. Skin grafts from one BB/W line are rejected by recipients from another line, albeit slowly when the recipient is a diabetes-prone rat (unpublished observations). In experiment 2, the splenic lymphocytes from diabetes-prone BB/W rats were obtained from various lines and pooled. Thus both the W-line spleen cells and the diabetes-prone spleen cells constituted allogeneic transfusions, but only the W-line cells

Table VII. Con A Response of Peripheral Blood Lymphocytes from Rats Transfused with Whole Blood, T Cell Depleted Whole Blood, or Saline ($Log_{10}cpm$)

	Transfusion		
Con A dose	Untreated blood	T cell depleted blood	Saline
μg			
0	3.79±.08	3.73±.08	3.30±.16*
0.03	4.86±.17	5.06±.17	3.57±.28‡
0.125	5.52±.05	5.41±.14	4.19±.43*
0.5	5.14±.12	5.01±.24	3.59±.20*
1.0	3.59±.20	3.72±.29	2.35±.16*

* $P < 0.05$ compared with other groups.
‡ $P < 0.01$ compared with other groups. There were no statistically significant differences between the untreated blood and T cell depleted blood groups.

were protective. The fact that one class of allogeneic transfusion failed to induce a protective effect suggests that allogeneic immunosuppression alone is an unlikely explanation for the W-line transfusion result.

The final experiment indirectly tested the possibility that a T lymphocyte is primarily responsible for the protective effect. The incubation of blood with anti-BAT serum plus complement depletes blood of peripheral T lymphocytes. Whole blood treated in this manner was only weakly protective against diabetes or insulitis, whereas untreated whole blood demonstrated a nearly complete protective effect. Although we cannot exclude the possibility that some other blood constituent was affected by the treatment with BAT serum plus complement, it appears most likely at this time that a peripheral T lymphocyte is a mediator of protection.

There were, however, no significant differences between the whole blood and the T cell depleted blood groups with respect to T cell subsets or con A responsiveness, and the responses of both of these groups were greater than those of the saline treated controls. These observations are consistent with those of the first experiment, in which rats transfused with erythrocyte concentrate were not protected from diabetes but exhibited some degree of immunologic restoration, as evidenced by enhanced con A responsiveness and a reduction in the frequency of both thyroiditis and anti-smooth muscle autoantibodies.

The most likely explanation for these observations is the presence of a small number of T cells that contaminate the erythrocyte transfusions and the anti-BAT serum treated blood. With regard to the BAT experiment, previous studies have demonstrated that as many as 20% of T cells may survive this procedure (J. Davie, personal communication). This small number of T cells may have been adequate to restore certain components of the BB rat immunodeficiency syndrome, but it was inadequate to prevent the occurrence of diabetes.

The fact that the protective effect of transfusion lasts for more than a year may imply either that the transfused cells permanently altered the effector cell population of the diabetes-prone rats at the time of transfusion or that progenitor T cells repopulated a deficient compartment in the recipients. The absence of markers able to detect chimers among these rats makes it impossible to select between these alternatives. The precise subset or number of T lymphocytes responsible for protection and the molecular mechanism by which such lymphocytes exert their effect remain to be determined.

We speculate that there exists a spectrum of immunodeficiency in BB rats, ranging from the acutely diabetic animal with a preponderance of effector cells and a deficiency of protective cells to the W-line animal in which the protective cells predominate. Whether these protective cells are suppressor or helper cells remains to be determined. However, it appears that the various immune modulations reported here can move the diabetes-prone BB/W rat along this spectrum by altering the balance of effector and protector cells. Animals who receive enough white blood cells, which presumably are rich in T lymphocytes, have this balance restored and are largely protected against diabetes. Animals who receive fewer appropriate cells exhibit less protection and move to an intermediate position along the immunodeficiency spectrum. This hypothesis is consonant with previous reports of profound T lymphocytopenia in the BB/W rat (12, 23, 24, 25). Lymphocytopenia is invariably present in spontaneously diabetic BB/W rats, and our studies have demonstrated a significant repletion of the T lymphocyte population in transfused animals.

Studies of humans with acute onset of IDDM have documented decreases in both the number and functional activity of suppressor T lymphocytes (27, 28). This observation suggests that altered lymphocyte function could play a role in the pathogenesis of IDDM. If so, restoration of normal lymphocyte function might provide the means to prevent or reverse the disease process.

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