JCI The Journal of Clinical Investigation

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*J Clin Invest.* 1986;77(4):1399-1401. https://doi.org/10.1172/JCI112448.

**Research Article**

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# Spleen Cell Transfusion in the Bio-Breeding/Worcester Rat

## Prevention of Diabetes, Major Histocompatibility Complex Restriction, and Long-term Persistence of Transfused Cells

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## Abstract

**We report that transfusions of RT1$^u$ Wistar-Furth (WF) spleen cells prevented spontaneous diabetes in the RT1$^u$ BB/W rat while RT1$^b$ Buffalo rat spleen cells did not. In addition, donor origin WF T lymphocytes were detected in nondiabetic-susceptible BB/W recipients 5 mo after transfusion. Survival of donor-origin lymphocytes may provide the cellular mechanism by which major histocompatibility complex–compatible WF spleen cell transfusions prevent BB rat diabetes.**

## Introduction

The BB rat develops spontaneous autoimmune diabetes that resembles human insulin-dependent diabetes mellitus (IDDM)[1] (1). Affected rats abruptly become hyperglycemic and ketonemic between 60 and 120 days of age; without insulin treatment they succumb from ketoacidosis. Previous studies strongly suggest that this is an autoimmune disease process (2). The pancreatic islets of acutely diabetic animals are infiltrated with mononuclear cells (insulitis) (3). Concanavalin A (Con A)–activated lymphocytes from acutely diabetic donors adoptively transfer diabetes to other rats (4). Islet and other autoantibodies are detectable both before and after the onset of diabetes (5, 6). The animal also exhibits depressed cell-mediated immune responses (2) due at least in part to a severe T cell lymphopenia (7, 8).

BB rat diabetes can be prevented by various immunosuppressive and immunomodulatory procedures (2). We reported prevention using transfusions of whole blood from a diabetes-resistant subline of these rats (the "W-line") (9). Transfusion into young diabetes-prone BB rats not only prevented diabetes and insulitis, but also restored towards normal the depressed responsiveness of BB lymphocytes to Con A. Subsequent studies identified the protective element in W-line blood and spleen cells as a T lymphocyte (10), but the mechanism of its action has remained unclear.

In the present study we obtained spleen cells from various strains of rats and compared their efficacy in preventing diabetes when given to susceptible BB rats. Using immunofluorescence methods, we then determined that protection from diabetes was associated with long-term engraftment of transfused lymphocytes.

## Methods

*Animals.* Transfusion recipients were diabetes-prone BB rats obtained from the colony of the University of Massachusetts, Worcester (BB/W rats). The frequency of spontaneous diabetes varies from 40 to 70% (11). Male and female rats were used in approximately equal numbers.

Transfusion donors included diabetes-resistant (W-line) BB/W rats and WF and Buffalo (BUF) rats from Charles River Laboratories, Wilmington, MA. W-line rats were originally derived from diabetes-prone BB/W forebears, but have been bred for resistance to the disease (11). Through 18 to 20 generations of inbreeding, <2.0% (54:>3,000) of W-line rats have become diabetic. Both W-line and Wistar-Furth (WF) rats share the same RT1$^u$ major histocompatibility complex (MHC) haplotype of the diabetes-prone BB/W rat (12); the haplotype of the allogeneic BUF rat is RT1$^b$.

*Preparation of spleen cells for transfusion.* Donor rats were killed in an atmosphere of 100% $CO_2$. Their spleens were removed aseptically, mechanically disrupted, and suspended in RPMI-1640 medium (Gibco, Grand Island, NY) as previously described (10).

*Experimental protocols.* Two experiments were performed in which randomly assigned littermate BB/W rats 25–30-d-old received five intravenous spleen cell transfusions in a volume of 0.5 ml within a 7-d period. In exp. 1, WF and W-line spleen cell transfusions were compared. Exp. 2 studied WF and BUF spleen cell transfusions. The mean number (±SD) of cells per transfusion was $12.6\pm5.0 \times 10^7$ W-line cells and $13.3\pm4.8 \times 10^7$ WF cells in exp. 1, and $6.4\pm6.9 \times 10^7$ BUF cells and $5.6\pm3.2 \times 10^7$ WF cells in exp. 2. Control animals received no treatment. The rats in exp. 1 were tested for diabetes through 165 d of age and those in exp. 2 through 175 d. Diabetes was diagnosed on the basis of 4+ glycosuria and a plasma glucose >200 mg/dl.

*Histologic studies.* Nondiabetic survivors in exp. 1 were killed with 100% $CO_2$. Their pancreata were excised, fixed in Bouin's solution, stained with hematoxylin and eosin, and evaluated for the presence of insulitis. The histologist (Dr. Appel) was unaware of the treatment status of the animals. The rats in exp. 2 were not studied histologically.

*Lymphocyte subset analysis.* Samples of peripheral blood from transfused and control rats in exp. 1 were obtained at 130 d of age and analyzed for the presence of T cells bearing the helper/inducer (W3/25) or suppressor/cytotoxic (OX8) antigenic phenotype as previously described (10).

We used monoclonal antibodies to the RT-6 and RT-7 (formerly termed ART-2 and ART-1, respectively) rat T cell differentiation al-

This work has been presented in part in abstract form. 1984. *Clin. Res.* 33:440A.

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*Received for publication 7 October 1985.*

1. *Abbreviations used in this paper:* BUF, Buffalo; Con A, concanavalin A; MHC, major histocompatibility complex; WF, Wistar-Furth.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/86/04/1399/03 $1.00
Volume 77, April 1986, 1399–1401

loantigens (13–16) to determine if donor-origin T cells were present in BB/W (RT-7$^a$) recipients of WF (RT-6$^b$ and RT-7$^b$) or BUF (RT-6$^a$ and RT-7$^b$) spleen cells (17). Spleen, lymph node, and thymus cells from nondiabetic BB/W recipients obtained 5 mo after WF or BUF spleen cell transfusions were analyzed for the presence of donor-origin lymphocytes. Monoclonal antibodies to the rat T cell alloantigens RT-6.1 (DS4.23) and RT-6.2 (6A5), and to the pan-lymphocyte alloantigen RT-7.2 (8G6.1) were directly conjugated with fluorescein isothiocyanate. The 6A5 monoclonal antibody was the gift of Dr. D. Lubaroff, University of Iowa, Iowa City, IA; it was originally developed and characterized by Dr. C. B. Carpenter (Harvard Medical School, Boston, MA) (18). Cells developed for immunofluorescence were analyzed for their relative light scatter and fluorescence intensity using a fluorescent antibody cell sorter. Each fluorescent antibody cell sorter analysis was performed on at least 50,000 cells.

*Statistical procedures.* Analysis of nonparametric data used the chi square statistic with Yates' correction where necessary. Comparisons of means used one-way analyses of variance.

## Results

The frequencies of diabetes and insulitis observed in exp. 1 are given in Table I. The effectiveness of W-line spleen cell transfusion in preventing diabetes was confirmed. In addition, MHC-compatible WF cells were found to provide a significant degree of protection from both diabetes and insulitis. In exp. 2, also shown in Table I, the effectiveness of WF spleen cells in preventing diabetes was independently confirmed. In contrast, MHC-incompatible RT1$^b$ BUF rat spleen cells did not prevent diabetes. These animals were not studied histologically.

In exp. 1, the absolute numbers ($\times 10^3$/mm$^3$±SEM) of T cells in the peripheral blood of BB/W recipients of WF spleen cells (5.2±1.4) or W-line spleen cells (4.5±0.7) were increased in relation to the absolute numbers of T cells in the peripheral blood of nontransfused BB/W controls (2.9±0.7). Furthermore, the W3/25/OX8 T cell ratios in BB/W recipients of WF (0.8:1, $P < 0.05$) or W-line (1.3:1, $P < 0.001$) spleen cells were significantly increased in relation to the ratio observed in nontransfused BB/W controls (0.4:1) (by analysis of variance, F ratio = 26.63, $P < 0.0001$).

Spleen and lymph node cells obtained from nondiabetic BB/W (RT-7$^a$) recipients of WF (RT-6$^b$ and RT-7$^b$) or BUF (RT-6$^a$ and RT-7$^b$) spleen cells were analyzed 5 mo after completion of transfusion. Donor-origin RT-6$^+$ T cells were present in the spleen and lymph nodes of the BB/W recipients of WF spleen cells (Table II). No donor-origin RT-6$^+$ T cells were detected in BUF spleen cell–transfused recipients. In addition, donor-origin RT-7$^{b+}$ lymphocytes were present in the spleen (41.0±5.6%) and lymph nodes (46.3±7.3%) of BB/W (RT-7$^a$) recipients of WF (RT-7$^b$) spleen cells, while <1% donor-origin RT-7$^{b+}$ lymphocytes were observed in BB/W recipients of BUF (RT-7$^b$) spleen cells.

In additional studies, we have found that four BB/W recipients of five WF spleen cell transfusions that subsequently became diabetic had no detectable RT-6$^{b+}$ cells, suggesting that without persistence of donor-origin lymphocytes diabetes is not prevented.

*Table I. Frequency of Diabetes and Insulitis Among BB/W Rats Given Five Transfusions of Spleen Cells from WF, BUF, or W-line Rats*

| | Donor | | | |
|---|---|---|---|---|
| | WF | BUF | W-line | |
| Donor RT1 haplotype | u | b | u | No transfusion |
| Experiment 1 | | | | |
| *N* | 11 | — | 14 | 14 |
| Diabetic* | 1 | — | 0 | 5 |
| Insulitis‡ | 0 | — | 0 | 7 |
| With diabetes or insulitis (%)§ | 9 | — | 0 | 86 |
| Experiment 2 | | | | |
| *N* | 12 | 13 | — | 14 |
| Diabetic‖ | 1 | 6 | — | 8 |
| Diabetic (%) | 8 | 46 | — | 57 |

Only those animals surviving to either the end of the experiment or the onset of diabetes are included. Four rats in exp. 1 and three in exp. 2 died during transfusion, were found dead, or were killed while being bled and have been excluded from analysis.
* Chi$^2$ = 7.32, $P < 0.03$.
‡ Chi$^2$ = 23.69, $P < 0.0001$.
§ Chi$^2$ = 27.19, $P < 0.0001$.
‖ Chi$^2$ = 6.99, $P < 0.05$.

## Discussion

These data confirm and extend our previous observations that transfusions of whole blood, spleen cells, or T lymphocytes from appropriate donors can prevent spontaneous diabetes in the BB/W rat. They indicate that protection is not restricted to transfusions from the closely related diabetes-resistant W-line rat. Spleen cells from the MHC-compatible WF rat are equally effective. MHC-incompatible spleen cells are ineffective. Furthermore, this study extends our previous observations by using the RT-6 and RT-7 alloantigenic differences between the BB/W (RT-7$^a$) and WF (RT-6$^b$ and RT-7$^b$) strains of rats. We are now formally able to document the long-term persistence of donor origin (RT-6$^b$ and RT-7$^b$) lymphocytes in the BB/W (RT-7$^a$) recipients of transfused spleen cells.

At least two mechanisms could explain the presence of donor-origin T cells in the peripheral lymphoid tissues of BB/W recipients of WF spleen cells. The spleen contains a heterogeneous population that consists of stem cells, progenitor cells, and mature lymphocytes (19). Since the BB/W recipients were not analyzed until 5 mo after spleen cell transfusion, the WF lympho-

*Table II. Presence of Donor-origin Lymphocytes in Nondiabetic BB/W Rats Given Five Transfusions of Spleen Cells from WF or BUF Rats*

| Donor | Tissue | Percentage of donor-origin RT-6$^+$ T cells | Number ($\times 10^6$) of donor-origin RT-6$^+$ T cells |
|---|---|---|---|
| WF | Spleen | 8.5±2.5 | 7.5±3.0 |
| | Lymph node | 51.6±2.6 | ND |
| BUF | Spleen | <1.0 | <1.0 |
| | Lymph node | <1.0 | ND |

Results represent the mean±1 SD of four animals. ND, not determined. Values of <1.0 indicate that no donor-origin RT-6$^+$ cells were observed.

cytes could have been generated by the engraftment of an immature stem and/or progenitor cell population in the BB/W recipients.

A more likely explanation is that the mature T lymphocyte population in the spleen cell inoculum survived in the MHC-compatible BB/W hosts. Based on the assumption that the splenic T cell population represents ~40% of the total peripheral T cells in an adult rat (20), it can be calculated from the data in Table II that $19 \times 10^6$ donor-origin T cells were present in the WF spleen cell–transfused BB/W recipients. Since $\sim 84 \times 10^6$ T lymphocytes (30% of $280 \times 10^6$ WF spleen cells) were injected, and 25% of the T cells in the spleen are long-lived (>100 d) (20), survival of the mature T lymphocytes in the original inoculum ($21 \times 10^6$) could account for essentially all of the donor-origin T cells observed in the BB/W recipients.

Our results support the hypothesis that the protection afforded by transfusion results from persistence of donor-origin WF (RT-$6^b$ and RT-$7^b$) lymphocytes in the diabetes-prone BB/W (RT-$7^a$) rat. Moreover, we have shown that the absolute numbers of T cells, and the helper-inducer/suppressor-cytotoxic (W3/25/OX8) T cell ratios are increased towards normal in WF and W-line spleen cell–transfused BB/W recipients. These results are consistent with our previous observations (2, 4, 10) and suggest that the mechanism of protection may be the correction of regulatory T cell imbalances that are apparent in untreated BB/W rats. In other studies, we have shown that lymphocyte populations specifically enriched for W3/$25^+$ W-line rat spleen cells prevent BB rat diabetes, while transfusions enriched for $OX8^+$ cells do not (21). We have also demonstrated that BB/W rats lack the RT-6 T cell subset (17) that comprises ~70% of the peripheral T cells in normal rats (13–15). When taken together, these results suggest that the RT-$6^+$, W3/$25^+$ T cell subset may be of particular importance in the pathogenesis and/or prevention of BB rat diabetes. However, the exact cell type that confers protection as well as the mechanism by which it acts remain to be determined.

## Acknowledgments

We thank Anne Pelletier, Olita Treimanis, Glen Rouleau, Marianne Angelillo, and Barbara Papazian for their assistance.

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