IMMUNE DEFICIENCY SYNDROME

Correction of the Peripheral T-Cell Functional and Microenvironmental Defects in BB Rats by Bone Marrow Transplantation

A. Naji, W. K. Silvers, H. Kimura, D. Bellgrau, A. O. Anderson, and C. F. Barker

THE spontaneous diabetes of BB rats, which is characterized by severe insulinopenia, ketosis, and lymphocytic infiltration of pancreatic islets (insulitis), is the animal model that most closely resembles human insulin-dependent diabetes.[1] A cell-mediated immune etiology for the condition has been suggested by the fact that neonatal thymectomy or immunosuppression with antilymphocyte serum effectively prevents and/or ameliorates the disease.[2,3] Moreover, the finding that the incidence of diabetes in diabetes-prone BB rats (BBDP) is significantly reduced following neonatal inoculation with bone marrow from normal [but major histocompatibility complex (MHC) compatible] donors provides additional support for an immunologic etiology of this disease.[4] The present study further defines abnormalities in the lymphatic tissue microenvironments, complement of T and B cells, and mixed lymphocyte reactivities of untreated and bone-marrow-treated BBDP animals.

From the Departments of Surgery, Human Genetics, and Pathology, University of Pennsylvania, Philadelphia, Pa.

Supported in part by USPHS Grants AM26007 and CA18640, and by the Reynolds Foundation and the Thomas McCabe Fund.

Reprint requests should be addressed to Dr. A. Naji, Department of Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pa. 19104.

0041-1345/83/1501-0344$01.00/0

MATERIALS AND METHODS

Animals. The BB rats, as well as the other strains employed, were derived from stocks maintained at the University of Pennsylvania. Although not isogenic, BB rats are homozygous for RT-1^u at their MHC.[4,5] BBDP rats were rendered tolerant by injecting them intravenously with 50 × 10^6 Wistar-Furth (WF, RT-1^u) bone marrow cells.[6] Recipients that retained healthy donor (WF) skin grafts for >200 days were considered tolerant. ACI (RT-1^a), and Lewis (LEW, RT-1^l) rats were also used.

Antibodies and fluorescent reagents. Mouse anti-rat monoclonal antibodies W3/13 and OX6 were purchased from Pelfreez Biologicals (Rogers, Ark.). W3/13 labels all rat peripheral T lymphocytes. OX6 defines rat Ia (common part determinant) antigens and binds to B lymphocytes but not to T lymphocytes.[7] FITC goat F(ab')$_2$ anti-mouse IgG (GAMG) was purchased from Tago, Inc. (Burlingame, Calif.). For immunofluorescence staining, 5 × 10^6 lymph node cells (LNC) were incubated at 4°C for 30 min with a saturating concentration of the appropriate monoclonal antibodies, washed 3 times by centrifugation, incubated at 4°C for 30 min with saturating amounts of FITC-GAMG, washed twice, and analyzed by fluorescence-activated cell sorter (FACS IV).

Mixed lymphocyte reaction (MLR). The MLR was performed as previously described.[5] Briefly, 6 × 10^5 responding [thoracic duct lymphocytes (TDL)] and 3 × 10^5 irradiated stimulator LNC were cultured in 200 μl of

Table 1. Frequency of T and B Lymphocytes by FACS Analysis

Rat Strain	Percentage of Marker-Positive Cells*	
	W3/13	OX6
WF	73 ± 3	24 ± 4
BBDP	45 ± 1	53 ± 2
Tolerant BBDP	67 ± 4	20 ± 4

*Values are mean ± SEM of five separate analyses.

medium for a total of 4 days in microtiter plates, pulsed with 0.25 μCi ^{3}H-TdR for the final 16 hr, harvested, and counted in a liquid scintillation counter.

Histologic preparation. Specimens were fixed in Bouin's solution for routine paraffin sectioning and stained with hematoxylin and eosin. The cell density and volume of specific lymphatic compartments were assessed by morphometric techniques.

RESULTS

Lymphocytopenia

A consistent lymphocytopenia was observed in various lymphoid compartments of 16 BBDP rats (TDL, 167 ± 59 × 10^6/24 hr; LN, 79 ± 12 × 10^6) as compared with 5 normal WF animals (TDL, 630 ± 134 × 10^6/24 hr; LN, 176 ± 24 × 10^6). In 9 tolerant BBDP rats, the lymphocyte count was higher (TDL, 226 ± 83 × 10^6/24 hr; LN, 100 ± 35 × 10^6) than in untreated littermates, but nevertheless, lower than in normal animals.

Frequency of B and T Lymphocytes in Untreated and Tolerant BBDP Rats

When the proportions of T and B lymphocytes were compared in BBDP, tolerant BBDP, and normal (WF) rats, significant differences were observed (Table 1). In normal rats, W3/13 antibody labeled 73% and OX6 antibody 24% of LNC. In BBDP rats, W3/13 antibody labeled 45% and OX6 antibody 53% of LNC, indicating a reduced proportion of T cells compared to B cells. The percentage of T (67%) and B (20%) cells in tolerant BBDP rats was similar to those of normal rats.

Alloreactivity of Untreated and Tolerant BBDP Rats

The alloantigen-induced proliferative response of lymphocytes from BBDP rats to an array of MHC-incompatible stimulator cells was markedly depressed as compared to the alloresponse of lymphocytes from tolerant BBDP animals (Table 2).

Morphology of Lymphoid Organs of Untreated and Tolerant BBDP Rats

Histologic examination of spleen and LN from BBDP rats (irrespective of whether they were diabetic or normoglycemic at the time examined) revealed a marked paucity in the number of small lymphocytes in T-cell-dependent anatomical zones. Thus, in the spleen, the periarteriolar lymphocyte sheaths (PALS), and in the LN, the paracortical (PC) regions were markedly underpopulated. In contrast, the PALS and PC regions of spleen and LN of tolerant BBDP animals displayed considerably more normal cellularity, although they were qualitatively different from the corresponding regions of normal rats. A majority of cells in the PALS regions of

Table 2. Primary MLR of BBDP and Tolerant BBDP Rats

Responder	Experiment	Stimulator Cells (cpm × 10^{-3} ± 1 SD)			
		WF	LEW	ACI	Unstimulated
BBDP*	1	0.4 ± 0.1	0.9 ± 0.8	0.8 ± 0.4	0.3 ± 0.2
	2	0.5 ± 0.1	1.5 ± 1.0	1.7 ± 1.1	0.4 ± 0.3
	3	0.3 ± 0.2	1.6 ± 0.4	0.8 ± 0.1	0.2 ± 0.1
	4	0.6 ± 0.4	0.8 ± 0.3	1.2 ± 0.2	0.5 ± 0.4
Tolerant BBDP	5	0.2 ± 0.1	18.5 ± 1.4	10.3 ± 1.3	0.3 ± 0.1
	6	1.1 ± 0.3	13.9 ± 1.5	13.9 ± 1.4	0.9 ± 0.1
	7	0.8 ± 0.2	10.3 ± 1.3	10.5 ± 0.9	1.5 ± 0.3
	8	0.6 ± 0.2	10.5 ± 1.0	15.9 ± 0.8	1.2 ± 0.4

*Two BBDP animals became diabetic within 4 weeks of the assay and two were diabetic at the time of the assay.

tolerant rats were lymphoblasts and plasma cells instead of the usual small lymphocytes. Despite the apparent functional and structural deficiencies of peripheral T cells and thymic-dependent microenvironments, the thymuses of both BBDP and tolerant BBDP rats were of normal size and contained normal densities of lymphocytes in the cortex and medulla.

DISCUSSION

There is ample circumstantial evidence in the BB rat model of human insulin-dependent diabetes mellitus to implicate a "cellular" autoimmune mechanism of pathogenesis[2,5] that proceeds from a lymphocytic and monocytic insulitis to selective destruction of beta cells and development of diabetes. The possibility that induction of diabetes might be tied to an immunologic deficiency of the peripheral T-cell compartment was suggested by the susceptibility of the BB rat to pulmonary infections, inability to quickly reject MHC-compatible allografts, and lymphopenia of peripheral T cells.[5] The ability of neonatal thymectomy[2] or ablation of peripheral T cells by antilymphocyte serum[2,3] to prevent spontaneous development of insulitis and diabetes in susceptible BB rats implicates T cells as the aggressors in the pathogenesis of diabetes. Since bone marrow transplantation replaces the T-cell compartment and restores T-cell reactivity in mixed lymphocyte reactions, and results in protection from the development of insulitis and diabetes, it seems likely that a T-cell subset or accessory cell present in normal bone marrow but absent in BBDP rats is involved in some immunoregulatory function that ordinarily prevents diabetes. The cellular or microenvironmental defect of the BBDP rats might be direct (i.e., a stem cell defect of immunoregulatory T-cell subsets) or indirect (inability to generate immunoregulatory cells from thymic precursors because of a deficiency of the T-dependent microenvironment). The presence of an apparently normal thymus that should be capable of providing mature T cells for peripheral lymphatic tissues in BBDP and diabetic BB rats suggests that the production of peripheral T cells may be normal but these cells cannot or are somehow prevented from gaining access to sites of recirculation and cell–cell interaction. Reversal of this tissue defect by bone marrow transplantation and restoration of MLR reactivity point to the possibility that the defect might be one of mononuclear cells responsible for antigen presentation or conditioning the T-cell microenvironment and not the T-cell perse.

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