

IMMUNE DYSFUNCTION IN DIABETES-PRONE BB RATS  
Interleukin 2 Production and Other Mitogen-induced Responses Are  
Suppressed by Activated Macrophages

BY GÉRALD J. PRUD'HOMME,\* ABRAHAM FUKS, ELEANOR COLLE,  
THOMAS A. SEEMAYER, AND RONALD D. GUTTMANN

*From the McGill Cancer Centre; Department of Pediatrics, Montreal Childrens' Hospital; and the Transplantation Service, Royal Victoria Hospital and McGill University, Montreal, Canada*

BB Wistar rats are a partially inbred line of rats prone to develop insulin-dependent diabetes mellitus (IDDM),<sup>1</sup> usually between 60 and 140 d of age (1–5). The disease is characterized by the sudden onset of severe hyperglycemia, glycosuria, and weight loss followed by death within a few days unless insulin is administered. Thus, clinically, this disease closely resembles human insulin-dependent diabetes mellitus (type 1, or juvenile onset diabetes). Histologically, BB rats show pancreatic mononuclear cell infiltrates (PMI) (also called pancreatic lymphocytic infiltrates [PLI]), which are found initially around ducts and acini (before the onset of diabetes) and eventually involve islets of Langerhans (insulinitis). At this stage, there is severe beta cell destruction and hence, insulinopenia (5). By electron microscopy and/or immunohistochemical techniques these mononuclear cell infiltrates can be identified as macrophages, T cells, and B cells. Occasionally, eosinophils are also present.

In our hands, approximately one in three BB rats develops overt diabetes. However, some rats remain normoglycemic despite the histological presence of PMI. Since BB rats are not completely inbred this heterogeneity in expression of disease is not surprising. We are presently in the process of developing congenic lines by crossing BB rats with other rat strains. Our studies at this time suggest that development of IDDM correlates strongly with three independently inherited characteristics: (a) the major histocompatibility complex (MHC) haplotype RT1 (all diabetic rats are RT1<sup>u</sup> homozygous or heterozygous) (2); (b) lymphopenia, affecting primarily T cells (6, 7); and (c) PMI (6). Only rats with all three of these characteristics become overtly diabetic.

Several immune abnormalities have been described in BB rats. These rats have

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<sup>1</sup> *Abbreviations used in this paper:* Con A, concanavalin A; CAS, Con A supernatant; FCS, fetal calf serum; Ia, immune response region associated antigen; IDDM, insulin-dependent diabetes mellitus; IL-1, interleukin 1; IL-2, interleukin 2; LPS, lipopolysaccharide; 2-ME, 2-mercaptoethanol; MHC, major histocompatibility complex; MM,  $\alpha$ -methyl-D-mannoside; M $\phi$ , macrophage; PGE1 and PGE2, prostaglandins E1 and E2 respectively; PHA, phytohemagglutinin; PLI, pancreatic lymphocytic infiltrates; PMA, phorbol myristate acetate; PMI, pancreatic mononuclear cell infiltrate; PWM, pokeweed mitogen; RT1, MHC of rat; SLE, systemic lupus erythematosus; T<sub>h</sub>, T helper cell; T<sub>s</sub>, T suppressor cell; WF, Wistar Furth rats.

reduced T cell numbers and are susceptible to infections (3, 6–8). We and others (7) found a decrease in the T helper ( $T_h$ , W3/25<sup>+</sup>) to T suppressor ( $T_s$ , OX8<sup>+</sup>) ratios in BB rats. BB rats show reduced responses in mixed lymphocyte cultures (8), to concanavalin A (Con A), and to other T cell mitogens (7, 9). Autoantibodies to islet cells, lymphocytes, and other autoantigens have been described (10, 11). Furthermore, BB rats frequently develop thyroiditis (12), and B cell lymphomas (13, 14).

In this study we document deficient production of IL-2 by BB rat spleen cells in response to T cell mitogens. We present evidence that this defect is not due to an inability of macrophages to secrete IL-1, or to an inability of T cells to produce IL-2. Instead, the defect in IL-2 production is largely a consequence of macrophage suppression. Thus, the deficient production of IL-2 by BB spleen cells in response to Con A is greatly improved by partial depletion of macrophages. BB spleen cells and BB splenic macrophages strongly suppress the response of Wistar Furth (WF; MHC matched normal control rat) spleen cells to Con A. The prostaglandin synthetase inhibitor indomethacin partially reverses the suppressive effect of BB macrophages, while the enzyme catalase, and the tumor promoter phorbol myristate acetate have no effect. Furthermore, BB macrophages do not degrade IL-2. The possible significance of these findings to the disease complex of BB rats is discussed.

### Material and Methods

*Rats.* Male and female BB rats were supplied by Dr. P. Thibert, Department of Animal Resources, Department of Health and Welfare, Ottawa, Canada. These rats express the RT1<sup>u</sup> MHC haplotype. The genetic, endocrine, and histologic characteristics of these rats have been described elsewhere (1, 2, 4–6). The WF rats (RT1<sup>u</sup>) were bred and maintained in the McGill Cancer Centre animal colony. Rats used in this study ranged from 35 to 115 d of age.

*Preparation of Cell Populations and Culture Conditions.* Splenectomies, accompanied by pancreatic biopsies, were performed under ether anesthesia and aseptic conditions. After surgery the rats were kept alive and observed for development of diabetes mellitus by daily testing for glucosuria. This surgical procedure does not change the incidence of diabetes in BB rats (unpublished observation).

Cells were cultured in 96-well, flat-bottomed microtiter plates (Linbro 76-032-05; McLean, VA) in medium consisting of RPMI 1640 supplemented by L-glutamine, 25 mM HEPES (Gibco Laboratories, Grand Island, NY), 50  $\mu$ M 2-mercaptoethanol, penicillin (50 U/ml)-streptomycin (50  $\mu$ g/ml) mixtures (Flow Laboratories, McLean, VA), and 5% fetal calf serum (FCS) (lot 29101648, Flow Laboratories, Mississauga, Ontario). Cells were cultured in a humidified atmosphere at 37°C, in the presence of 5% CO<sub>2</sub>.

In some experiments B cells were depleted by panning twice on anti-rat immunoglobulin (Ig) coated plates as described by Wysocki and Sato (15). Spleen cells were incubated on these plates at 4°C at a concentration of 10<sup>7</sup> cells/plate for 90 min. Recovered unbound cells consisted of <5% B cells as determined by immunofluorescence.

T cells were depleted by treating spleen cells with biotin-conjugated W3/13 (Sera-lab, MAS 010C, a pan anti-T cell monoclonal antibody), followed by panning twice on avidin-coated plates as described by Basch et al. (16). <5% of nonadherent cells were W3/13<sup>+</sup> as determined by immunofluorescence. T-depleted populations failed to respond to concanavalin A (Con A).

Macrophages were depleted by passing spleen cells on Sephadex G-10 columns as described by Ly and Mishell (17). BB rat spleen cells consisted of 5–8% macrophages as determined by uptake of fluorescent microbeads (Polysciences Inc., Warrington, PA). Following passage on Sephadex G-10, <1% of the cells could be identified as macrophages

by this method.

In some experiments macrophages were enriched on the basis of cell density (18) and adherence in a two-step procedure. First, in order to deplete T cells, spleen cells were fractionated on a discontinuous Percoll (Pharmacia, Uppsala, Sweden) density gradient, consisting of three densities: 1.085, 1.052, and 1.030 g/ml. Spleen cells were layered on top of the gradient (at 4°C) and spun at 400 *g* for 30 min.

Fraction 1 at the 1.030/1.052 interphase consisted of 25–40% macrophages (by uptake of microbeads), 60–70% B lymphocytes, and <5% T cells. Fraction 1 cells failed completely to respond to Con A. Fraction 2 (1.052/1.085 interphase) comprised almost all splenic T cells (with 25–40% B cells, and 2–3% macrophages) and generated high responses to Con A.

T cell-depleted fraction 1 was further enriched for macrophages on the basis of cell adherence. Fraction 1 cells were added in various numbers to the wells of 96-well plates and incubated at 37°C for 4 h. Nonadherent cells were removed by vigorous flushing with medium using a pasteur pipette (three washes). Macrophage numbers were estimated by the number of adherent cells per high power field (hpf, 40× objective).

*Mitogen Assays.* Stimulation of spleen cells with T cell-dependent mitogens were performed at cell densities ranging from  $10^5$  to  $5 \times 10^5$  cells/250  $\mu$ l. Mitogen doses were 5  $\mu$ g/ml for Con A (Sigma C-2010; Sigma Chemical Co., St. Louis, MO), 1/30 dilution for phytohemagglutinin (PHA; Difco Laboratories, Detroit, MI), and 1  $\mu$ g/ml for poke-weed mitogen (PWM, Sigma L-9379). [ $^3$ H]Thymidine uptake was measured after 3 d of culture (18-h incubation with 1  $\mu$ Ci [ $^3$ H]thymidine per well), unless otherwise indicated.

In some experiments indomethacin (Sigma I-7378) 10  $\mu$ g/ml, prostaglandin E1 and E2 (PGE1 and PGE2 respectively, Sigma P-5515 and P-5640), catalase (Sigma C-100), or phorbol myristate acetate (PMA, Sigma P8139) were added to the cultures.

*Interleukin 2 (IL-2) Assay.* The IL-2 assay was performed as described by Gillis et al. (19) using the CTLL-2 cell line (a kind gift from Dr. H. Rode, McGill University, Montreal, Canada). Briefly,  $10^4$  CTLL-2 cells per well were cultured with various dilutions of Con A supernatants of spleen cells. [ $^3$ H]Thymidine uptake was measured after 24 h of culture, following a 4-h incubation with 1  $\mu$ Ci [ $^3$ H]thymidine per well. 1 U of IL-2 per ml was defined as that quantity of IL-2 producing one-half of maximal stimulation of CTLL-2. Test supernatants were compared to a standard IL-2 preparation.

*Incubation of IL-2 with Macrophages.* Con A supernatant (CAS) was used as a source of IL-2. CAS was added to graded numbers of macrophages (prepared as described above) in 96-well plates, and incubated at 37°C for 24 h in the presence or absence of 20 mg/ml  $\alpha$ -methyl-D-mannoside (MM, Sigma M-6882). Supernatants were then recovered, passed through 0.2- $\mu$ m filters, and stored at -20°C until used.

*Interleukin 1 (IL-1) Assay.* IL-1 was measured by the mouse thymocyte proliferation assay, as described by Mizel (20). Briefly, supernatants derived from spleen cells cultured with or without 25  $\mu$ g/ml of LPS (*E. coli* 026:B6, Difco Laboratories), for 48 h were added at various dilutions to  $10^6$  BALB/c mouse thymocytes/250  $\mu$ l. [ $^3$ H]Thymidine uptake by the thymocytes was determined after an 18-h incubation with 1  $\mu$ Ci [ $^3$ H]-thymidine/well on day 3 of culture.

## Results

*Response of BB Spleen Cells to T-dependent Mitogens.* The proliferative responses of BB rat spleen cells to Con A, PHA, and PWM were very low when compared with MHC-matched normal control WF rats (Fig. 1). In fact, addition of these mitogens to BB spleen cells yielded counts lower than with medium alone in several BB rats (hence a negative  $\Delta$ cpm).

*IL-2 Production by BB Spleen Cells.* The mean IL-2 levels of 24-h Con A supernatants of BB spleen cells ( $5 \times 10^5$  cells/well) were approximately eightfold lower than those of WF controls (Fig. 2) ( $p < 0.001$  by the Student's *t*-test). Only 2 out of 10 BB rats tested had IL-2 levels above 10 U/ml, while WF IL-2 levels

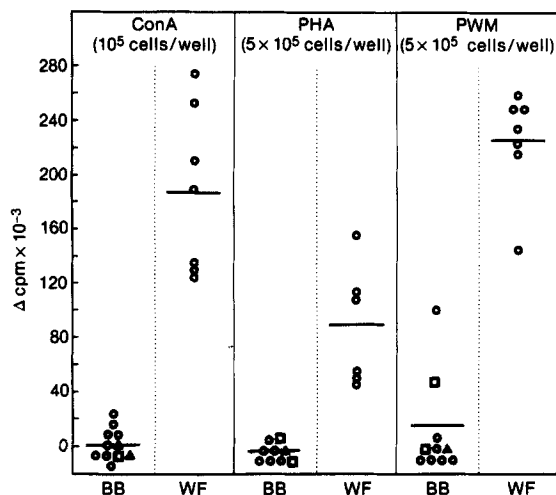


FIGURE 1. Proliferative responses of BB and WF spleen cells to T-dependent mitogens.  $\Delta\text{cpm} = [^3\text{H}]$ thymidine uptake of spleen cells with mitogens -  $[^3\text{H}]$ thymidine uptake of spleen cells with medium alone.  $\Delta\text{cpm}$  was measured on day 3 of culture. Con A = 5  $\mu\text{g}/\text{ml}$ , PHA = 1/30 dilution, PWM = 1  $\mu\text{g}/\text{ml}$ . O, rats that showed no clinical evidence of diabetes for at least 3 months following assay; □, rats tested before the onset of IDDM;  $\Delta$ , rats with IDDM at time of assay. Horizontal bars represent the mean values for BB and WF rats in each assay.

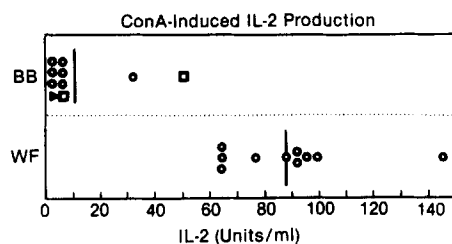


FIGURE 2. Con A-induced IL-2 production (U/ml) in BB and WF spleen cells. 24-h Con A (5  $\mu\text{g}/\text{ml}$ ) supernatants from  $5 \times 10^5$  spleen cells/250  $\mu\text{l}$  were tested for IL-2 in the CTLL-2 proliferation assay. O, □,  $\Delta$ , see legend to Fig. 1. Vertical bars represent the mean values for BB and WF rats.

ranged from 64 to 144 U/ml. However, there was no apparent correlation between the clinical status of BB rats, or the subsequent development of IDDM, and IL-2 levels. Low IL-2 production appears to be a feature of all BB rats.

*Suppressive Effects of BB Spleen Cells on WF Spleen Cells.* The addition of BB spleen cells to WF spleen cells resulted in a marked suppression of proliferation in response to T cell-dependent mitogens (Figs. 3 and 4), while addition of similar numbers of WF spleen cells instead of BB cells, resulted in an increase in  $\Delta\text{cpm}$ , rather than suppression (data not shown). The suppressive effect was equally apparent on IL-2 secretion in response to Con A (Table I). The level of suppression varied among BB rats but was always present. Less than 1 BB spleen cell per 10 WF spleen cells was sufficient to generate suppression of both proliferation and IL-2 production (Fig. 5 and Table I).

*Cell Population Responsible for Suppression.* T cell depletion or B cell depletion had no effect on the level of suppression exerted by BB spleen cells. On the

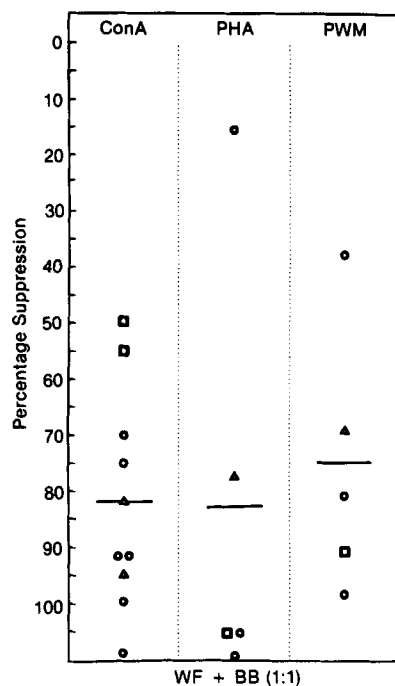


FIGURE 3. Suppression of WF proliferative responses to T-dependent mitogens by BB spleen cells.  $1.25 \times 10^5$  BB spleen cells were added to  $1.25 \times 10^5$  WF spleen cells and mitogens on day 0. [ $^3\text{H}$ ]Thymidine uptake was measured on day 3 of assay.

$$\% \text{ suppression} = \frac{\Delta\text{cpm WF} - (\Delta\text{cpm WF} + \text{BB})}{\Delta\text{cpm WF}} \times 100.$$

Addition of WF spleen cells instead of BB resulted in approximate doubling of  $\Delta\text{cpm}$  rather than suppression (not shown).  $\circ$ ,  $\square$ ,  $\triangle$ , and mitogen doses, see legend to Fig. 1.

other hand, macrophage depletion by passage of spleen cells on Sephadex G-10 completely abrogated the suppressive effects of BB cells (Fig. 4). In fact, following macrophage depletion, enhancement of responses rather than suppression was observed. This effect was seen in both diabetic and nondiabetic BB rats. Furthermore, macrophage depletion greatly increased proliferation and IL-2 production of BB cells in response to Con A (Table II). It should be added that macrophages were not completely depleted by passage on Sephadex G-10 (see Materials and Methods).

That macrophages were indeed the suppressor cell population (rather than some other adherent cell population) was further demonstrated by adding WF spleen cells to various numbers of BB splenic macrophages. Such macrophages were isolated on the basis of both cell density (on a Percoll gradient) and adherence and contained <5% contaminating T cells (see Materials and Methods). BB macrophages in small numbers (150–250 adherent cells per 10 high power fields) suppressed WF proliferative responses to Con A by >90%, and IL-2 production by >80% (Table III). Adherent cells derived from whole BB spleen cell populations (omitting the density gradient step) had a closely similar effect ruling out an effect of Percoll in this assay (data not shown). WF-derived splenic

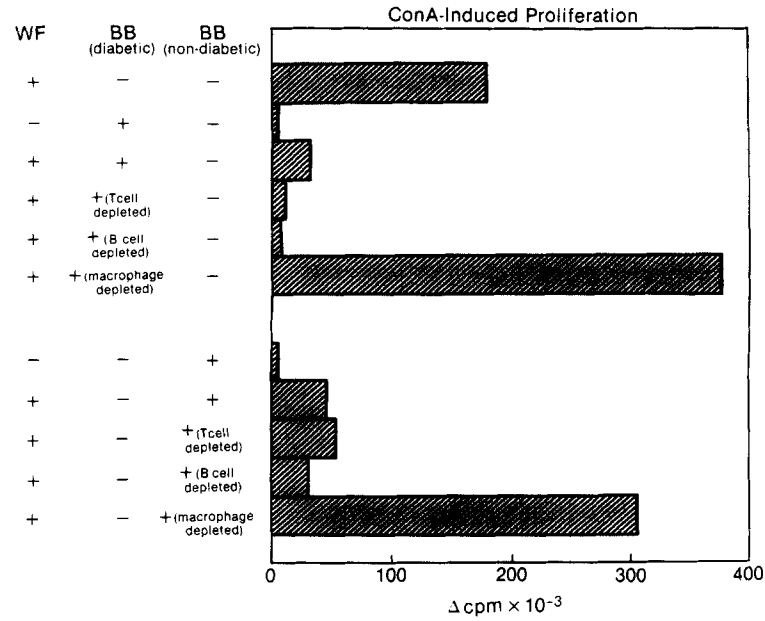


FIGURE 4. BB spleen cell subpopulation responsible for suppression of WF Con A-induced proliferation.  $1.25 \times 10^5$  BB spleen cells (with or without T cell, B cell, or macrophage depletion) were added to  $1.25 \times 10^5$  WF spleen cells and Con A ( $5 \mu\text{g/ml}$ ) on day 0. [ $^3\text{H}$ ]-Thymidine uptake was measured on day 3 of assay. Results of a representative experiment with a diabetic, and a nondiabetic BB rat are shown.

TABLE 1  
Suppressive Effect of BB Spleen Cells on IL-2 Production by WF Spleen Cells

No. of BB spleen cells added to WF cells*	IL-2 (% suppression) <sup>‡</sup>	
	Con A <sup>§</sup>	Con A + indomethacin <sup>§</sup>
—	0	0
$10^4$	50%	29%
$2.5 \times 10^4$	44%	29%
$7.5 \times 10^4$	79%	25%
$1.25 \times 10^5$	84%	41%
$1.25 \times 10^5$ (macrophage depleted)	0% <sup>‡</sup>	0% <sup>‡</sup>

\*  $1.25 \times 10^5$  WF spleen cells per  $250 \mu\text{l}$ .

<sup>‡</sup> % suppression of IL-2 production =  

$$\frac{\text{WF IL-2 production (U/ml)} - [\text{WF + BB IL-2 Production (U/ml)}]}{\text{WF IL-2 production (U/ml)}} \times 100.$$

IL-2 was determined in 24-h culture supernatants. Results of a representative experiment are shown.

<sup>§</sup>  $5 \mu\text{g/ml}$  Con A,  $10 \mu\text{g/ml}$  indomethacin.

<sup>‡</sup> Negative values reflect enhancement rather than suppression.

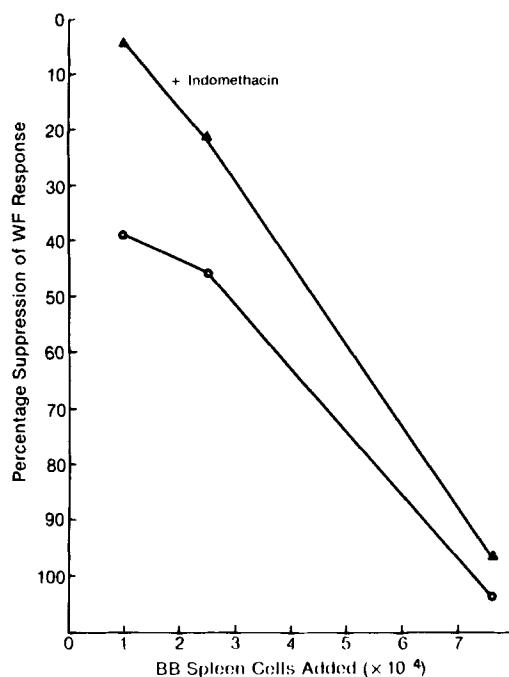


FIGURE 5. Effect of indomethacin on the suppressive effect of BB spleen cells on WF spleen cells. Graded doses of BB spleen cells were added to  $1.25 \times 10^5$  WF spleen cells and Con A ( $5 \mu\text{g/ml}$ ) on day 0, with ( $\Delta$ ), or without ( $\circ$ ) indomethacin ( $10 \mu\text{g/ml}$ ). % suppression, see legend to Fig. 3. Results of a representative experiments are shown.

TABLE II  
Effect of Macrophage Depletion and Indomethacin on Con A-induced Proliferation and IL-2 Production

Strain	Spleen cell no. ( $\times 10^{-5}$ )	Macrophage depletion	Proliferation ( $\Delta\text{cpm} \times 10^{-3}$ )		IL-2 (U/ml)	
			Con A <sup>‡</sup>	Con A + indomethacin <sup>‡</sup>	Con A	Con A + indomethacin
BB	1	—	-14.0	-11.3	3	6
	2.5	—	-16.1	-11.9	4	10
	2.5	+	148.5	146.8	46	32
	5	—	-1.2	0.4	4	38
WF	1	—	234.5	207.8	32	36
	2.5	—	357.9	352.5	33	30
	5	—	384.8	348.2	76	76

\* Proliferation determined on day 3 of culture. Negative values reflect lower counts with Con A than with medium alone. IL-2 levels determined in 24-h culture supernatants. Results of a representative experiment are shown.

<sup>‡</sup>  $5 \mu\text{g/ml}$  Con A,  $10 \mu\text{g/ml}$  indomethacin.

TABLE III  
*BB Macrophages Suppress Con A-induced Responses*

Macrophage* no. (per 10 hpf)	WF Spleen cells <sup>‡</sup> + Con A	
	Proliferation ( $\Delta$ cpm $\times$ $10^3$ )	IL-2 (U/ml)
0	238 (0%) <sup>§</sup>	28
30-50	106 (55%)	11
150-250	17 (93%)	5

\* Macrophages were isolated on the basis of both cell density (Percoll gradient) and adherence.

<sup>‡</sup>  $2.5 \times 10^5$  WF spleen cells per well. Results represent the mean of the effect of macrophages from three BB rats on WF spleen cells.

<sup>§</sup> Numbers in parentheses represent % suppression (see legend to Fig. 3).

macrophages did not suppress in this assay in the cells doses shown in Table III. However, much larger numbers of WF splenic macrophages (800-1,200 macrophages per 10 high power fields) could suppress WF responses to Con A by ~50% (not shown). Thus even normal spleens contain a population of suppressor macrophages which, however, are clearly more numerous in BB spleens than in normal spleens.

*Effect of Indomethacin and Catalase on the Suppressive Effect of BB Spleen Cells.* Indomethacin was effective in reducing partial suppression of proliferation (50% or less) by BB cells of WF cells, but had less effect in situations of complete suppression (Fig. 5). Comparatively, indomethacin was more effective in reducing severe suppression of IL-2 production than reducing severe suppression of Con A-induced proliferation (Table I and Fig. 5). While indomethacin did little to increase proliferation of unfractionated BB spleen cells regardless of cell density in culture ( $10^5$  to  $5 \times 10^5$  cells/well), it nevertheless caused a marked increase in IL-2 production (Table II) in high cell density cultures ( $5 \times 10^5$  cells/well), while having little or no effect on WF responses.

On the other hand, addition of the enzyme catalase to cultures in amounts as high as 15,000 U/ml had no effect on BB spleen cells responses to Con A. In addition, catalase did not alter the effect of indomethacin (data not shown). It thus seems unlikely that the suppressive effect of BB macrophages is mediated by hydrogen peroxide generation.

*Suppression of IL-2 Production by PGE1 and PGE2.* The suppressive effects of PGE1 and PGE2 on mitogen-induced responses in humans and mice have been documented by other authors (21-24). As can be seen from Fig. 6, both PGE1 and PGE2 can suppress Con A-induced proliferation and IL-2 production in rat cells. Physiological concentrations of PGE1 and PGE2 (1-10 ng/ml) caused 40-50% suppression of Con A-driven responses. PGE1 and PGE2 in doses ranging from 1 to 1,000 ng/ml did not affect the response of CTLL-2 cells to IL-2, in the IL-2 assay, and thus did not interfere with IL-2 determinations in prostaglandin-containing supernatants (data not shown).

*BB Macrophages Do Not Inactivate IL-2.* No loss of IL-2 activity was found when IL-2 (from Con A supernatants) was incubated for 24 h with macrophages in numbers up to 800-1,200 macrophages per 10 hpf (Table IV). Similar results were obtained in the presence or absence of the Con A inhibitor  $\alpha$ -methyl-D-



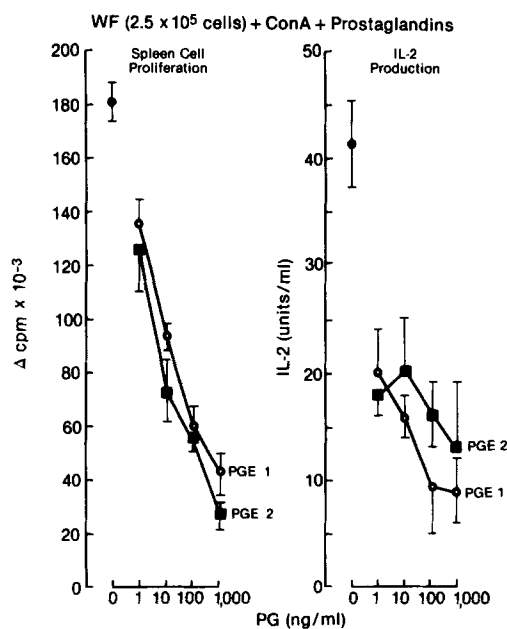


FIGURE 6. The effect of PGE1 and PGE2 on Con A-induced proliferation and IL-2 production in WF rats.  $2.5 \times 10^5$  WF spleen cells were cultured with Con A ( $5 \mu\text{g/ml}$ ), with or without PGE1 and PGE2. IL-2 levels were determined in 24-hour Con A supernatants, while proliferation ( $^3\text{H}$ )thymidine uptake) was measured on day 2 of assay. Results represent the mean  $\pm$  1 SD of three WF spleens. O, PGE1; ■, PGE2.

TABLE IV  
*BB Macrophages Do Not Inactivate IL-2*

Macrophage no. (per 10 hpf)	MM* (20 mg/ml)	IL-2 <sup>‡</sup> (U/ml)
0	+	34
	-	33
30-50	+	33
	-	35
150-250	+	36
	-	34
800-1,200	+	33
	-	35

\* MM,  $\alpha$ -methyl-D-mannoside.

<sup>‡</sup> IL-2 from rat Con A supernatant was incubated for 24 h with macrophages with or without MM. Results represent the mean effect of macrophages from three experiments on an IL-2 preparation.

mannoside, ruling out IL-2 production by possible residual T cells. Since such macrophage numbers had profound suppressive effects on IL-2 production these results rule out macrophage-mediated IL-2 inactivation as a suppressive mechanism.

*IL-1 Production by BB Spleen Cells.* The secretion of IL-1, as determined by the thymocyte proliferation assay, was as high in BB rats as in WF rats (Table V). This occurred despite the fact that the WF control rats tested produced levels of IL-2 severalfold higher than the BB rats. The addition of indomethacin

TABLE V  
*IL-1 Production in BB and WF Rats*

Treatment of spleen cells	IL-1 (cpm at 1/8 dilution)*	
	BB <sup>‡</sup>	WF <sup>‡</sup>
Medium	2,401 ± 887	1,813 ± 303
LPS <sup>§</sup>	6,342 ± 853	6,310 ± 1,781
LPS + indomethacin <sup>§</sup>	8,106 ± 315	6,667 ± 573

\* 10<sup>6</sup> mouse thymocytes cultured for 3 d with supernatants. Results represent mean [<sup>3</sup>H]thymidine uptake of three spleen ± 1 SD. Background proliferation of thymocytes = 1,358 ± 925; and thymocytes + LPS = 2,846 ± 735. No difference was seen between BB and WF at lower or higher dilutions of IL-1.

<sup>‡</sup> Con A-induced IL-2 secretion for the rats tested in this experiment was 5 ± 1 U/ml for BB, and 105 ± 28 U/ml for WF spleen cells.

<sup>§</sup> 25 µg/ml LPS, and 10 µg/ml indomethacin. Indomethacin alone did not stimulate proliferation of thymocytes (not shown).

to spleen cells often resulted in apparent increases in IL-1. However, it should be remembered that indomethacin will reduce the prostaglandin concentration of such supernatants. Prostaglandins interfere with thymocyte proliferation (20) since this process depends on a IL-1 → thymocyte → IL-2 sequence of events, and prostaglandins E1 and E2 inhibit IL-2 secretion.

The addition to Con A-stimulated BB spleen cells of PMA in amounts ranging from 1 to 100 ng/ml did not improve proliferation or IL-2 production (data not shown). Since PMA is thought to act directly on T cells and to reduce the need for IL-1 in T cell activation (25), it seems unlikely that low IL-2 production in BB rats is secondary to an inability of T cells to respond to IL-1.

### Discussion

The BB rat represents one of the few animal models available for study that spontaneously develops IDDM (26). The autoimmune nature of the disease is supported by the findings of insulinitis (1), the presence of anti-islet cell antibodies in the sera of the animals (11), the possibility of preventing the disease by immunosuppressive treatment (27), the strict association of the disease with the MHC RT1<sup>u</sup> haplotype (2), and T cell lymphopenia (6). Studies showing a lower incidence of disease in neonatally thymectomized BB rats (28), and in rats treated in vivo with anti-lymphocyte antibodies (27) suggest an important role for T cells.

Paradoxically, BB rats are immune deficient, have low T cell numbers in blood and lymphoid organs (8), and respond poorly to T cell mitogens (7). In this study we document low production of IL-2 in BB rats following stimulation of BB spleen cells with Con A. Such a deficiency could be explained on the basis of T cell depletion, or changes in the T<sub>h</sub>/T<sub>s</sub> ratio in BB rats. We find (unpublished observation), as have others (7, 8), that BB spleen cells consist of approximately half as many T cells (percentage of total cells) as non-diabetes-prone controls, with a decrease in T<sub>h</sub>/T<sub>s</sub> ratios. The findings in this study clearly indicate that alterations in T cell numbers can account for only part of the decrease in IL-2 production. Addition of spleen cells from BB rats (both diabetic and nondiabetic)

to WF spleen cells strongly suppresses the proliferative responses to Con A, PWM, and PHA, as well as IL-2 production. This indicates that an active suppressive process is at work. Depletion of T cell or B cells from BB spleen cells had little effect on this suppressive process. On the other hand, macrophage depletion by passage through a Sephadex-G10 column completely abrogated the suppressive effect of BB spleen cells on the response of WF spleen cells to Con A. Furthermore, macrophage depletion greatly increased both proliferation and IL-2 production by BB cells in response to Con A. BB splenic macrophages (isolated on the basis of both cell density on Percoll gradients, and adherence) strongly suppressed WF spleen cells responses to Con A. Small numbers of BB macrophages were suppressive in this assay. On the other hand, up to 10-fold greater numbers of WF macrophages were necessary to obtain an equivalent degree of suppression. These results indicate that suppressor macrophages can be found even in normal spleens, however, this cell population is greatly increased in BB rats.

Although macrophage-mediated suppression is a well-known phenomenon, the mechanisms of suppression remain poorly understood. Metzger et al. (21) suggest that this phenomenon is mediated by activated macrophages. These authors found that following activation with thioglycollate, or *C. parvum*, peritoneal macrophages acquired the ability to suppress the response of lymphocytes to mitogens. This response could be partially reversed by a prostaglandin synthetase inhibitor (indomethacin), or catalase. They postulated that macrophage activation results in the production of prostaglandins and H<sub>2</sub>O<sub>2</sub>, both of which suppress lymphocyte responses. On the other hand, some authors believe that soluble protein mediators play an important role in macrophage-mediated suppression (22). We find that indomethacin partially reverses the suppressive effects of BB spleen cells, while catalase has no effect. Indomethacin also improves IL-2 production by unfractionated Con A-stimulated BB spleen cells. Furthermore, we find that the addition of PGE1 or PGE2 at physiological doses (1–10 ng/ml) to WF spleen cells suppresses Con A-induced proliferation and IL-2 production by ~50%. Clearly, PGE1 and PGE2 can strongly suppress T cell responses; however, other mediators are probably also involved in producing the marked suppressive effect (>90%) of BB macrophages. Interestingly, we find that BB macrophages have a strong cytostatic effect on tumor cells,<sup>2</sup> and can inhibit insulin secretion by a rat insulinoma cell line. This provides further evidence that BB rat spleens contain an unusually high number of activated macrophages.

Other possible mechanisms to explain macrophage suppression in BB rats have been considered in this study. BB splenic macrophages secrete normal levels of IL-1, and do not inactivate IL-2. We tested the effect of PMA in Con A-stimulated cultures, since this substance has a direct effect on T cells and reduces or abolishes the need for IL-1 in T cell activation (25). However, PMA did not improve IL-2 production by BB Con A-stimulated spleen cells. It thus seems unlikely that low IL-2 secretion is due to an inability of BB T cells to respond to IL-1. Interestingly, PMA improves IL-2 secretion in some lupus-prone mouse strains (29).

<sup>2</sup> Prud'homme, G. J., A. Fuks, E. Colle, R. D. Guttman. 1983. Cytostatic effects of splenic macrophages derived from diabetes-prone BB rats on a rat insulinoma cell line. In preparation.

The finding of excessive macrophage-mediated suppression is not unique to BB rats. "Suppressor" macrophages have been described in rheumatoid arthritis (30) and Hodgkin's disease (31), as well as in human (32, 33) and murine (34) systemic lupus erythematosus (SLE, a systemic autoimmune disease). In fact, Gershwin et al. (34) demonstrated that the acquired  $T_h$  deficiency of older SLE-prone (NZB×NZW) $F_1$  mice is secondary to the presence of splenic macrophage suppressor cells, and is unrelated to the premature degeneration or involution of thymus or thymic epithelial elements in these mice. These findings are similar to our findings in autoimmune BB rats.

Interestingly, SLE, like IDDM in BB rats, is characterized by low IL-2 production (35–38), and other T cell defects (39, 40). Furthermore, a high incidence of lymphocytic infiltrates in the pancreas of NZB (80% incidence) and MRL (50% incidence) SLE-prone mice has been reported (41), as well as, a high incidence of diabetes in encephalomyocarditis virus-infected NZB, and (NZB×NZW) $F_1$  mice (42). BB rats and some autoimmune mice thus share the phenomenon of PMI, although only BB rats spontaneously develop IDDM.

The association of low IL-2 production, and other T cell deficiencies, with autoimmune diseases has been difficult to explain. In fact, it is not clear that low IL-2 production contributes to the polyclonal B cell activation found in SLE (43, 44), or to the occurrence of IDDM in BB rats. However, by crossing BB rats with other rat strains (e.g. Buffalo) we found that only animals with deficient T cell function developed IDDM (6). All diabetic offsprings of such crosses tested were found to be low producers of IL-2 (unpublished observation). It is clear that although deficient T cell function by itself does not cause IDDM in rats, this feature is nevertheless invariably associated with the disease. Expression of the disease only occurs if the RT1<sup>u</sup> haplotype, and other yet poorly characterized genetic factors are also present (2, 6).

The mechanism of macrophage activation in the BB rat has not been determined in this study. In experimental systems the signals that can activate macrophages are numerous and include microbial antigens, mitogens, and many chemicals, as well as lymphokines (22, 45–47). Studies are in progress to evaluate the possible presence of immune complexes or increased levels of interferon in the serum of BB rats. Conceivably, antigen-specific Ia-restricted T cell-macrophage interactions to a putative islet cell antigen could lead to both T cell and macrophage activation with secretion of monokines (e.g. IL-1) and lymphokines (e.g. IL-2, and  $\gamma$ -interferon); as well as, a whole battery of vasoactive, chemotactic, and cytotoxic products by macrophages. This model is supported by the prominent presence of both macrophages and T cells in islets of Langerhans during the acute phase of the disease.

Finally, the finding that indomethacin can increase *in vitro* IL-2 secretion in BB rats suggests that prostaglandin synthetase inhibitors may have useful immunopotentiating effects in diseases characterized by low IL-2 production.

### Summary

Spleen cells of diabetes-prone BB Wistar rats were found to generate excessively low proliferative responses, and interleukin 2 (IL-2) levels in response to T-dependent mitogens. This abnormality was not due solely to abnormal T cell

numbers since: (a) addition of BB spleen cells or BB splenic macrophages to normal major histocompatibility complex (MHC)-matched Wistar Furth (WF) spleen cells resulted in severe suppression of concanavalin A (Con A)-, phytohemagglutinin (PHA)-, and pokeweed mitogen (PWM)-mediated proliferation, and IL-2 production; (b) macrophage depletion from BB spleen cells, but not B cell or T cell depletion, removed completely the suppressive effects of BB cells on WF cells; (c) macrophage depletion greatly enhanced the response of BB lymphocytes to T-dependent mitogens. Although suppressor macrophages could also be found in the spleen of WF control rats they were present in much smaller numbers than in the spleen of BB rats. The suppressive effect of BB macrophages was partially reduced by addition of the prostaglandin synthetase inhibitor indomethacin to cultures. Furthermore, indomethacin (but not catalase or PMA) considerably augmented IL-2 secretion of Con A-stimulated BB spleen cells, but had little effect on WF spleen cells. In contrast, prostaglandins E1 and E2 (PGE1 and PGE2) suppressed IL-2 production. While IL-2 secretion was severely depressed in BB rats, unstimulated and lipopolysaccharide (LPS)-stimulated IL-1 secretion by splenic macrophages was normal. BB macrophages did not inactivate IL-2. Low IL-2 production and macrophage-mediated suppression were features of all BB rats tested.

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