

High Stimulatory Activity of Dendritic Cells from Diabetes-prone BioBreeding/Worcester Rats Exposed to Macrophage-derived Factors

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Abstract

Dendritic cells (DC) present antigen and initiate T cell-mediated immune responses. To investigate the possible association of autoimmunity with DC function, we compared the accessory activity of splenic DC from Wistar/Furth (WF) and diabetes-prone (DP) BioBreeding (BB) rats. The latter develop autoimmune diabetes and thyroiditis. DC function was quantified in vitro by measuring T cell proliferation in mitogen-stimulated and mixed lymphocyte reactions. When purified without macrophage coculture, WF and DP DC displayed similar levels of accessory activity. In contrast, when purified by a method involving coculture with macrophages, DC from DP rats consistently displayed greater accessory activity. This finding could not be explained by morphological or phenotypic differences between DP and WF DC. In accessory activity assays performed after reciprocal DC cocultures with DP and WF macrophages, DP DC exhibited higher accessory activity irrespective of macrophage donor strain. We also compared the accessory activity of WF and DP DC cultured in the presence of conditioned medium and a mixture of IL-1 and GM-CSF. In all assays, DP DC exhibited higher accessory activity. In studies of (WF × DP) F₁ hybrids, the high accessory activity of DP DC was observed to be heritable, and studies of WF and DP radiation chimeras indicated that the effect was an intrinsic property of the DP hematopoietic system. We conclude: (a) splenic DC from DP and WF rats possess similar basal levels of accessory potency; (b) after interaction with macrophages, DC of DP origin are capable of greater stimulatory activity than are WF DC; and (c) the mechanism responsible for this phenomenon involves differential responsiveness of DP and WF DC to macrophage-derived factors such as IL-1 and GM-CSF. (*J. Clin. Invest.* 1993. 91:2040–2048.) Key words: antigen presentation • autoimmunity • BB rat • dendritic cell • macrophage

Introduction

Dendritic cells (DC)¹ are antigen-presenting cells (APC) specialized to initiate antigen-specific T cell responses (1). T cell

stimulation requires both antigen presentation to the T cell receptor (TCR) in the context of MHC molecules and costimulatory signal(s), delivered by APC. In the absence of costimulatory signal(s) T cells remain unresponsive (2, 3). In vitro, DC, but neither B cells (4) nor macrophages (5), can induce primary T cell responses in mitogenic systems (5) and mixed leukocyte reactions (MLR) (5, 6). DC activity is highly sensitive to macrophage-derived cytokines (1). In vivo, DC capture, process, and present exogenous antigens to T cells (7, 8). DC may also constitutively process and present self antigens (9). These characteristics suggest that DC could play a role in autoimmunity as presenters of self antigens.

We investigated DC from the diabetes-prone (DP) BioBreeding (BB) rat, an animal that develops both autoimmune diabetes and thyroiditis (10–12). Many studies have indicated that APC and T cells both play crucial roles in these autoimmune processes. The pathological substrate of BB rat diabetes is pancreatic islet inflammation (insulitis). Macrophages and DC are among the earliest infiltrating cellular constituents of insulitis (13–16). Neonatal thymectomy (17), selective T cell depletion (18), and silica treatment (19) in vivo all prevent diabetes. Concanavalin A (Con A)-activated T cells adoptively transfer both diabetes and thyroiditis to MHC-compatible hosts (20, 21). We undertook to determine if abnormalities of the APC system, specifically DC, exist in the BB rat.

Splenic DC were isolated from young nondiabetic DP-BB and normal histocompatible Wistar/Furth (WF) rats. The stimulatory capability of these DC was then measured in T cell proliferation assays. The results indicate that, subsequent to interaction with macrophages and/or macrophage-derived factors, DC from DP rats have intrinsically greater capability for accessory activity than do DC of WF origin.

Methods

Animals

Diabetes-prone (DP) and diabetes-resistant (DR) BB Worcester (Wor) rats (RT1^{u/u}) were obtained from a viral antibody-free colony at the University of Massachusetts, Worcester (22, 23). About 60–80% of both male and female DP rats become diabetic between 60 and 120 d of age; < 1% of DR rats become hyperglycemic. Inbred WF (RT1^{u/u}), Copenhagen (COP; RT1^{a/a}), M520 (RT1^{b/b}), Lewis (LEW, RT1^{l/l}), and Fisher 344 (RT1^{l/l}) rats were obtained from the National Cancer Institute. (WF × DP)F₁ hybrid rats were produced in our colony using WF males.

Cell culture conditions

All cell cultures were carried out in media consisting of RPMI-1640 supplemented with 2 mM L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, and 10% heat-inactivated horse serum (Gibco Labora-

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1. Abbreviations used in this paper: APC, antigen-presenting cell(s); BB, BioBreeding; BM, bone marrow; BPA, bovine plasma albumin;

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DC, dendritic cell(s); DP and DR, diabetes prone and diabetes resistant; HD and LD, high density and low density; LNC, lymph node cell(s); MLR, mixed leukocyte reaction(s); PEC, peritoneal exudate cell(s); SPC, spleen cell(s); TCR, T cell receptor; WF, Wistar/Furth; Wor, Worcester.

tories, Grand Island, NY). Incubation was at 37°C in a humidified atmosphere of 7% CO₂ in air.

DC preparation

Depending on the individual experiment, most DC were prepared using one or a combination of three procedures: FcR rosetting (5), silica pulsing (24), and adherence to plastic (5). These procedures differed principally with respect to the method and timing of macrophage depletion. The initial isolation of DC was the same in all cases (5). Spleens of 30–45-d-old rats were removed aseptically and the cells separated on a discontinuous density gradient ($\rho = 1.086$ g/ml) of bovine plasma albumin (BPA, Armour Pharmaceutical Co., Biochemical Division, Kankakee, IL) into two fractions. The high density spleen cell (HD-SPC) fraction contained mainly small T and B cells, erythrocytes, and debris; the low density (LD-SPC) fraction contained mainly macrophages, DC, and large T and B cells. The LD-SPC fraction then underwent further treatment to enrich for DC by one of the three methods (FcR rosetting, silica treatment, or adherence to plastic) described below. As part of all three methods, radiosensitive cells were eliminated by γ -irradiation (1,000 R, ¹³⁷Cs, Gammacell 40, Atomic Energy of Canada) followed by 48–50 h of culture and a second BPA fractionation.

FcR rosetting. This method of DC purification avoided culture of DC in the presence of macrophages and was used to obtain DC for studies of basal accessory activity. Immediately after isolation, LD-SPC were depleted of macrophages by exploiting the high levels of FcR receptor expression on macrophages; FcR are low or absent on DC (5). Sheep red blood cells (SRBC) were incubated for 30 min at 4°C in the presence of subagglutinating doses of rabbit α -SRBC antiserum. FcR-SRBC (5% vol/vol) were added to equal volumes of LD-SPC cells (20×10^6 cells/ml), incubated for 20 min on ice to form rosettes linking macrophages to the immunoglobulin-coated SRBC. The rosetted and nonrosetted cell populations were gently resuspended and fractionated on a BPA density gradient as described above. SRBC-macrophage rosettes sedimented in the pellet. The FcR⁺ LD fraction then underwent final irradiation, culture, and BPA gradient fractionation as described above.

Silica treatment. This method of DC purification included culture of DC in the presence of activated macrophages. After isolation, LD-SPC were cultured for 36–38 h and then pulsed with silica (1 mg/ml, Sigma Chemical Co., St. Louis, MO) for an additional 10–12 h. This procedure exploits the fact that macrophage phagocytic activity persists after prolonged culture, whereas DC lose phagocytic capability after 10–12 hours (25). During the final BPA fractionation, silica-laden macrophages segregated into the HD fraction and were removed. DC isolated by this procedure were cocultured with macrophages for a total of 48–50 h. “Conditioned medium” used in certain experiments was obtained by collecting supernatants of silica-treated LD-SPC cell cultures (26). Cells were spun down and culture supernatants were frozen immediately, stored at –70°C, and used within 2 wk. Fresh culture medium (10% vol/vol) was added to conditioned media just before use.

Purification of DC using adherence: syngeneic and reciprocal macrophage coculture experiments. The method was devised to compare WF and DP DC cultured in the presence of either syngeneic or heterologous macrophages. LD-SPC from DP and WF rats were cultured for 4–5 h and separated into two fractions by plastic adherence. The nonadherent fraction contained mainly DC and T and B blasts; the adherent fraction contained mainly macrophages. Nonadherent cells were recovered by swirling the dish and gently washing with warm medium (37°C) four times. Syngeneic (DP-DP; WF-WF) and reciprocal (DP-WF; WF-DP) DC-macrophage cocultures were then performed. After 30–36 h of incubation, both the syngeneic and reciprocal cocultures were pulsed with silica for 10–12 h and the DC purified as described above.

Viability and yield of DC. After purification by any of these methods, DC viability was always > 98% by trypan blue dye exclusion. Every preparation of DC was examined by light microscopy; the number of cells displaying typical DC morphology was determined and

used to calculate the concentration of DC to be used in each experiment. Consistent with previous reports (27), DC yield was 0.02 to 0.1% of the initial total spleen cell number.

Preparation of responder cells

Cervical and mesenteric lymph nodes were removed aseptically from male rats. DC and macrophages were removed by fractionation on a BPA density gradient. The efficacy of this procedure in producing “accessory cell-depleted” responder populations has been documented previously (5, 27) and was confirmed in our experiments. High density lymph node cells (HD-LNC) were collected from the pellet, with an average recovery of 65–80%. In some experiments, T cell enrichment was performed by eliminating B cells with a biotin-avidin rosetting procedure using anti-rat Ig (28). Viability of purified T cells was > 95% by trypan blue dye exclusion. T cell purity was determined by flow microfluorimetry; the percentage of CD5⁺ (OX19⁺) T cells was usually 92–96% and was never < 89%.

Preparation of peritoneal exudate cells (PEC)

Resident PEC were recovered from exsanguinated animals by flushing the peritoneum with 20 ml of cold Dulbecco's PBS. The cell suspension was enriched for macrophages by fractionation on a BPA density gradient. The LD-PEC preparations were examined by light microscopy and $\geq 95\%$ of cells were found to exhibit typical macrophage morphology. This result was confirmed by nonspecific esterase staining and electron microscopy. LD-PEC were > 95% viable by trypan blue dye exclusion.

Assays of DC accessory activity

DC accessory activity was quantified by measuring the proliferative response of accessory cell-depleted HD-LNC or purified T cells cocultured in the presence of increasing concentrations of DC. The following assay systems were used: (a) oxidative mitogenesis using sodium periodate (NaIO₄); (b) lectin-stimulated mitogenesis using Con A; (c) allogeneic and syngeneic MLR. These systems were selected on the basis of two previous observations (5, 27). First, accessory cell-depleted lymphocyte (HD-LNC or purified T cells) exhibit negligible proliferation in the absence of DC. Second, the magnitude of T cell proliferation varies as a function of the number of added DC.

Assay cultures were performed in triplicate in flat-bottomed 96-well plates (Falcon, Becton Dickinson Labware, Lincoln Park, NJ) in 0.2 ml of culture medium as previously described (5, 6, 27). All assays were performed using 5×10^5 responder cells per well and graded doses of splenic DC. In each experiment, DC from both WF and DP rats were isolated and assayed in parallel. To quantify T cell blastogenesis, cultures were pulsed during the final 4 h of incubation with medium (5 μ l per well) containing [³H]thymidine (TdR) and unlabeled TdR at saturating conditions (final TdR concentration in each well: 1×10^{-5} M; sp act 300 mCi/mM). Under these conditions, [³H]TdR incorporation correlates with the number of responding cells as determined by autoradiography (29). [³H]TdR incorporation in the cultured cells was measured using a liquid scintillation counter. The incorporated radioactivity was calculated as the average number of counts per minute (cpm) of triplicate cultures.

Periodate assays. In the NaIO₄ assays (27, 30), responder cells were incubated on ice at a concentration of 1×10^7 cells/ml in freshly prepared PBS containing 1.2 mM NaIO₄ (Fisher Scientific Co., Pittsburgh, PA). After incubation for 15 min, periodate-treated cells were washed twice in RPMI-1640 and resuspended in culture medium in the presence or absence of DC. Cell proliferation was measured by incorporation of [³H]TdR after 48–52 h of culture. In the absence of DC, the background [³H]TdR incorporation of periodate treated responder cells was usually < 500 cpm and < 10% of DC-stimulated responses.

Lectin-stimulated assays. In these assays, responder cells were incubated with Con A at a concentration of 5 μ g/ml for 30–60 min at 37°C. They were then added without washing to DC, yielding a final Con A concentration of 2.5 μ g/ml. [³H]TdR uptake was measured

after 62–66 h of culture. In the absence of DC, the response of Con A-treated responders was usually < 500 cpm.

MLR. In MLR, responder cell proliferation was measured in both allogeneic and syngeneic systems. Incubation times were 72–76 and 96–98 h for allogeneic and syngeneic MLR, respectively. Background response was usually < 200 cpm.

Choice of standard conditions. For each assay system, the time of incubation was chosen on the basis of preliminary experiments using WF DC and responder cells. Incubation times and mitogen concentrations were selected to generate optimal dose response curves with graded concentrations of DC. Standard deviations associated with the average of the triplicates in each assay usually ranged between 10% and 15% of the mean.

Flow microfluorimetry

Cell suspensions (10^5 to 10^6 cells per sample) were incubated for 30–50 min at 4°C with the appropriate monoclonal antibody (mAb), washed twice with RPMI-1640 containing 1% fetal calf serum and 0.05% NaN_3 , and developed for fluorescence as previously described (31). Mouse anti-rat antibodies used included MRC OX1 (anti-leukocyte common antigen, LCA), MRC OX6 (anti-Ia), MRC OX19 (anti-CD5), and R73.1 (anti- α/β T CR); all were purchased from Bioproducts for Science, Inc., Indianapolis, IN. The A3C (mouse IgG_{2a}) and C11B (mouse IgG₁) mAb have been developed in Dr. Bowers' laboratory; both antibodies are highly specific for rat DC. Neither binds to macrophages; A3C weakly stains B cells (unpublished observations). Mouse IgG₁ and rat IgG were obtained from Sigma Chemical Co. Hybridomas producing the anti-RT7.1 (BC84) and anti-RT7.2 (8G6.1) rat monoclonal antibodies are maintained in our laboratory (32). Analyses were performed on FACScan (Becton Dickinson, Mountain View, CA). A minimum of 5,000 cells per sample were analyzed.

Bone marrow chimeras

In certain experiments, DC obtained from bone marrow (BM) chimeras were studied. BM donors were 30–45-d-old DP or WF rats that were depleted of T cells by intraperitoneal injections of OX19 pan-T mAb 72, 48, 24, and 12 h before use. BM was obtained by flushing femurs and tibias with RPMI-1640. T cell depletion was confirmed by microfluorimetry using the R73.1 mAb on aliquots of donor cells. BM recipients were 28–30-d-old DP and WF rats that were fasted overnight, γ -irradiated (750 R), and then injected intravenously with $1.9\text{--}2.1 \times 10^8$ BM cells within 2–6 h of irradiation. Both reciprocal (WF \rightarrow DP, DP \rightarrow WF) and control syngeneic (WF \rightarrow WF and DP \rightarrow DP) reconstitutions were performed. Experimental animals were analyzed

four weeks after irradiation and BM reconstitution. The extent of chimerism was assessed by measuring the percentage of cells expressing the RT7.1 and RT7.2 rat alloantigens that are expressed by DP and WF rats, respectively (32). Both unfractionated spleen cells and splenic DC obtained from BM recipients were assayed.

Statistics

Analysis of variance was performed on each data set (33). The outcome analyzed was responder cell proliferation ($[^3\text{H}]\text{TdR}$) obtained by subtracting background cpm from test cpm. The factors or main effects were: (a) DC donor strain, (b) DC/responder cell ratio, and (c) day-to-day variability in cpm associated with individual experiments. The analysis of variance accounted for all three factors and their interactions simultaneously, with the primary goal of finding statistically significant main effects. In simpler terms, a statistically significant difference between DP and WF DC responses means that, as a group, the response of one strain averaged over all DC/responder ratios (excluding the zero point where no responders were present) was different. Parametric data are presented as means \pm SEM.

Results

The basal levels of accessory activity of DP and WF dendritic cells are similar. To determine basal levels of WF and DP DC accessory activity, DC were isolated using the FcR rosetting procedure that did not involve coculture of DC with macrophages. This procedure was chosen because DC are known to be extremely sensitive to the stimulatory effects of macrophage-derived factors (1). DC accessory activity was measured as T cell proliferation in an allogeneic MLR. We found that the basal levels of accessory activity of DC from DP and WF rats were comparable (Fig. 1 A).

After coculture in the presence of macrophages, splenic DP DC exhibit greater stimulatory activity than do WF DC. We next tested the accessory activity of DC that had been cultured with macrophages pulsed with silica. Assayed in an MLR, macrophage-cocultured DC of DP origin exhibited greater capacity than WF DC to stimulate responder cells from COP donors (Fig. 1 B).

This result was subsequently confirmed and expanded in several assays. These included MLR using purified T cells from MHC-incompatible M520, LEW, and COP rats (Fig. 2, A, B,

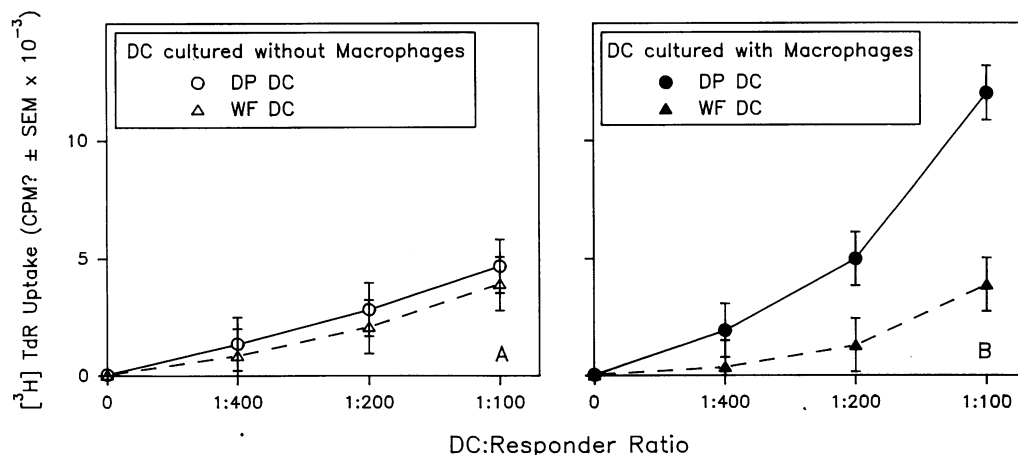


Figure 1. Basal DC stimulatory activity. DC were isolated in parallel by two different purification procedures as described in Methods. The first eliminated macrophages before LD-SPC culture (FcR rosetting, panel A). The second involved DC coculture with activated macrophages (silica treatment, panel B). The stimulatory activity of DC isolated by these two different methods was assessed in parallel by measuring responder cell proliferation ($[^3\text{H}]\text{TdR}$ uptake) in an allogeneic

MLR, using COP HD-LNC as responders (5×10^5 per well). DP DC (\circ) and WF DC (Δ) purified by the FcR rosetting method exhibited low levels of stimulatory activity that were statistically similar (A). When isolated by the silica method, DC of DP origin (\bullet) were more active than WF DC (\blacktriangle) isolated by the same method (B, $P < 0.001$). DP DC isolated by the silica pulse method were also more active than both DP and WF DC isolated by FcR rosetting ($P < 0.001$). Each data point represents the mean of four separate experiments performed as triplicate cultures.

and C). Additional data indicative of high DP DC activity were also obtained in a syngeneic MLR using accessory cell-depleted HD-LNC from (WF \times DP) F_1 hybrid rats (Fig. 2 D).

The results obtained in MLR were further confirmed in experiments using mitogenic systems. Oxidative treatment with NaIO_4 specifically induces T cells to undergo a single blastogenesis cycle in the presence of DC (30). NaIO_4 -treated lymph node T cells from WF rats exhibited significantly higher levels of proliferation in response to DP DC than they did to WF DC (Fig. 3 A). Finally, in the Con A-stimulated assay with WF T cells as responders, the highest level of accessory activity was again associated with DC of DP origin (Fig. 3 B).

DC preparations from WF and DP rats are similar in morphology, phenotype, and purity. To assess whether morphologic or phenotypic differences between DP and WF DC could account for their different levels of stimulatory activity, we analyzed silica-pulsed DC preparations by microscopy and flow microfluorimetry. DC preparations from DP and WF rats were morphologically similar by light microscopy. Electron microscopy of DC preparations from both strains revealed characteristic morphologic features including numerous projections, irregularly shaped nuclei, and scarce endoplasmic reticulum.

Surface phenotyping (Table I) revealed that $> 80\%$ of both WF and DP DC isolated by silica pulse expressed MHC class II and ICAM-1; they also stained with both the A3C and C11B anti-DC antibodies. T cell contamination, as assessed by anti-TCR staining with the R73.1 mAb, was $< 1\%$ in both cases. These results indicate $> 80\%$ DC purity and phenotypic similarity of DP and WF DC preparations. Expression of MHC class II and ICAM-1, taking into account both fluorescence intensity and cell size, was therefore comparable on activated DP and

WF DC. This observation is consistent with the results of Koide et al. (34), who found comparable levels of Ia expression on both resting and IL-1 stimulated mouse DC.

Since macrophages reportedly inhibit DC stimulated T cell proliferation (5, 35), we also assessed our DC preparations for macrophage contamination. Cytospins ($3\text{--}5 \times 10^4$ cells per slide) of DC and peritoneal macrophages (LD-PEC) from DP and WF rats were compared. DP and WF DC preparations isolated by all three methods contained $< 6\%$ macrophages by nonspecific esterase staining. Peritoneal macrophages, stained in parallel as a positive control, exhibited $> 95\%$ purity. The high purity and comparably low levels of contaminating macrophages in DP and WF DC preparations suggest that the number of contaminating macrophages is unlikely to account for the differential DC accessory activity we observed.

Macrophage-cocultured DC from DP-BB rats exhibit greater accessory activity than do WF DC irrespective of the strain of the macrophage donor. To determine whether our observations reflected a property of DC rather than a property of the macrophages with which they were cocultured, we developed a special cell culture system. Macrophages were removed from LD-SPC by plastic adherence; the nonadherent cell fraction containing DC was recovered. The percentages of adherent cells (macrophages) in LD-SPC preparations were comparable: $45 \pm 13\%$ ($n = 6$) in the case of the DP and $37 \pm 14\%$ ($n = 6$) in the case of the WF. The DP and WF DC isolated by this procedure exhibited basal levels of stimulatory activity that were comparable. The magnitude of this activity was similar to that exhibited by resting DC isolated by FcR rosetting and not exposed to macrophages in culture (see Fig. 3 B).

Having established the equivalence of the initial isolation

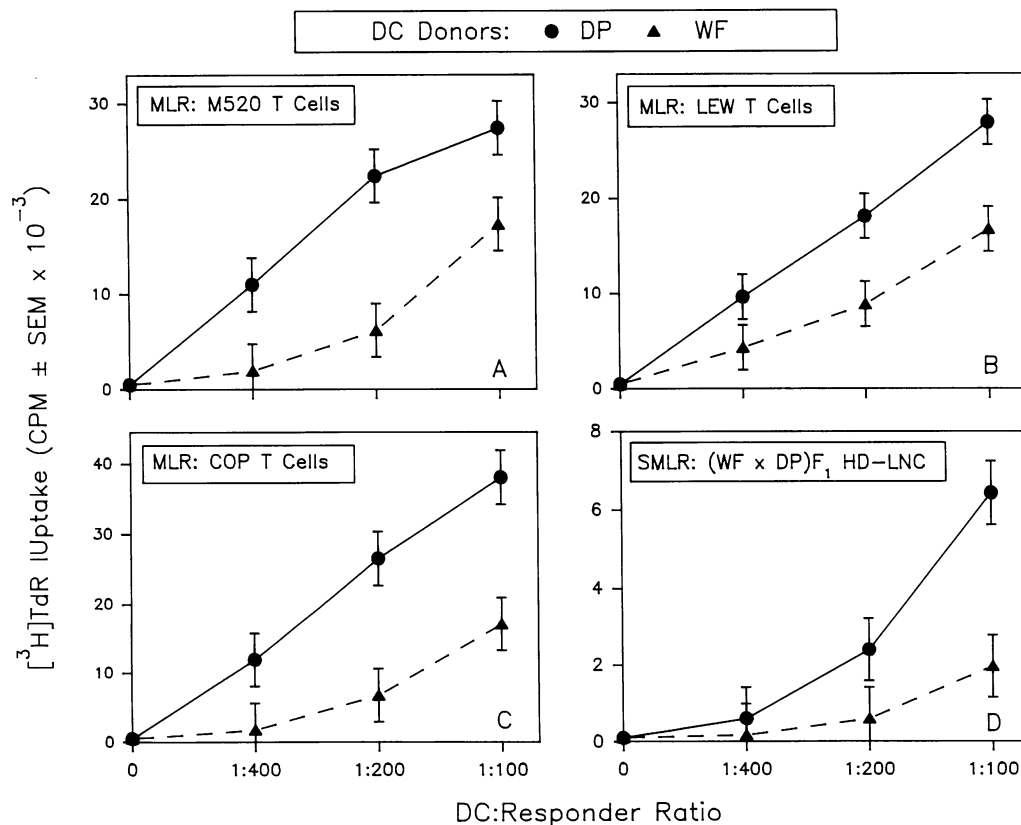


Figure 2. Differential stimulatory activity of DC from DP and WF rats in MLR. Splenic DC were isolated from DP and WF rats by macrophage-DC coculture and silica treatment as described in Methods. DC stimulatory activity was quantified as T cell proliferation ($[^3\text{H}]\text{TdR}$ uptake) in allogeneic (A, B, C) or syngeneic (D) systems. In the absence of DC, responder cells (5×10^5 per well) exhibited negligible proliferation. T cell proliferation varied as a function of DC number. DC of DP origin (●) consistently exhibited statistically significantly greater stimulatory activity than DC of WF origin (▲). (A) Response of M520 (RT1^{b/b}) T cells (DP DC vs. WF DC, $P < 0.001$, $n = 4$). (B) Response of LEW (RT1^{l/l}) T cells (DP vs. WF, $P < 0.001$, $n = 5$). (C) Response of COP T cells (DP vs. WF, $P < 0.001$, $n = 4$). (D) Response of syngeneic (WF \times DP) F_1 HD-LNC (DP vs. WF, $P < 0.02$, $n = 4$). Each data point represents the mean of separate experiments performed as triplicate cultures.

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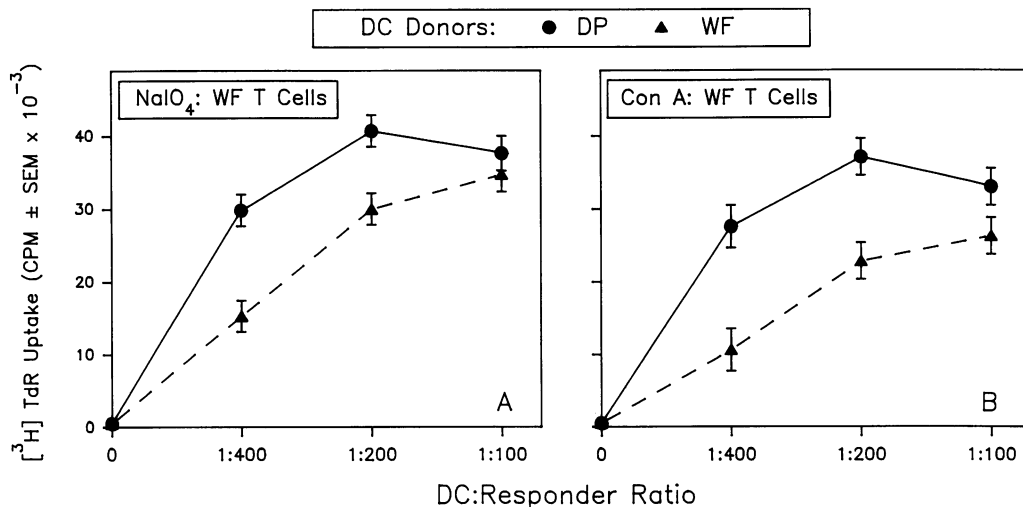


Figure 3. Differential stimulatory activity of DC from DP and WF rats in mitogenic assays. Splenic DC were isolated from DP and WF rats by macrophage-DC coculture and silica treatment. DC stimulatory activity was quantified as T cell proliferation ($[^3\text{H}]$ TdR uptake) in response to NaIO₄ (A) or Con A (B). The number of responder cells was constant (5×10^5 per well). In the absence of DC, there was negligible T cell proliferation. DC of DP origin (●) consistently exhibited significantly greater stimulatory activity than DC of WF origin (▲).

(A) WF T cell response (DP vs. WF, $P < 0.001$, $n = 6$). (B) Response of WF T cells (DP vs. WF, $P < 0.001$, $n = 4$). In each panel, data points represent the mean of separate experiments performed as triplicate cultures.

conditions, DP and WF DC depleted of adherent macrophages were then cocultured with syngeneic or heterologous macrophages using our standard conditions, pulsed with silica, and assayed for accessory activity. Again, DP DC exhibited greater T cell stimulatory activity than WF DC irrespective of whether cocultures were performed with macrophages of DP or WF origin (Fig. 4).

DC from BB rats exhibit greater accessory activity than WF DC in the presence of either DP or WF LD-SPC culture supernatant. We next assessed the possibility that WF and DP macrophages differed with respect to the elaboration of inhibitory or stimulatory factors relevant to DC accessory activity. To do so, we recovered supernatants from WF or DP silica-treated LD-SPC cultures (see Methods) and cultured macrophage-depleted LD-SPC from DP and WF rats in these "conditioned" media. Again, DC of DP origin exhibited greater stimulatory activity, regardless of the source of the conditioned medium in which they were cultured (Fig. 5, C and D).

DC from BB rats exhibit greater accessory activity than WF DC in the presence of exogenous IL-1 plus GM-CSF. Since the data obtained to this point suggested that DP DC might be more sensitive to macrophage-derived factors, we tested the

possibility directly by adding a mixture of human recombinant IL-1 β (10 U/ml) and mouse recombinant GM-CSF (25 U/ml) (Biosource Int., Camarillo, CA) to macrophage-depleted WF and DP LD-SPC. Each of these cytokines was used at concentrations previously shown to induce maximal DC stimulation (26). WF DC activity was stimulated two- to threefold, but again DC of DP origin exhibited much greater stimulatory activity despite the addition of equal amounts of IL-1 and GM-CSF (Fig. 5 B).

The high accessory activity of macrophage-cocultured DP DC is a heritable characteristic. To investigate the genetic basis of DC stimulatory activity, we isolated splenic DC from (WF \times DP) F₁ hybrids using the silica pulse method. (WF \times DP) F₁ hybrids do not become spontaneously diabetic (36). We tested DC from these hybrid rats in both the periodate assay using syngeneic responder cells (Fig. 6 A) and in an MLR using COP-HD-LNC as responders (Fig. 6 B). In both assays, the accessory activity observed using (WF \times DP) F₁ hybrid DC was intermediate between the higher DP and lower WF levels. Interestingly, we also observed in the course of these same experiments that the accessory activity of DC from the DR BB rat was also intermediate between the DP and WF (Fig. 6).

Table I. Cell Surface Phenotype of Splenic DC and HD Spleen Cells

		OX-1	OX-6	α -ICAM-1	A3C	C11B	R73.1
		%					
DC	DP-BB	90 \pm 1 (5)	87 \pm 2 (6)	79 \pm 7 (7)	81 \pm 4 (5)	84 \pm 2 (5)	0 \pm 0 (3)
	WF	88 \pm 3 (5)	83 \pm 6 (6)	72 \pm 6 (7)	86 \pm 4 (4)	85 \pm 3 (4)	1 \pm 1 (3)
HD spleen cells	DP-BB	81 \pm 4 (5)	38 \pm 6 (4)	—	2 \pm 3 (5)	1 \pm 2 (5)	5 \pm 4 (4)
	WF	87 \pm 3 (5)	30 \pm 4 (5)	—	11 \pm 7 (4)	4 \pm 4 (5)	36 \pm 9 (3)

Phenotypic characterization of DC and HD-SPC from DP and WF rats. LD-SPC and HD-SPC were prepared by BPA density gradient centrifugation; DC were purified from the LD-SPC fraction by the silica procedure (See Methods). DC purity is indicated by staining with A3C and C11B, mAbs that are highly specific for DC (Dr. Bowers, unpublished observations). High levels of expression of both MHC class II (OX-6) and ICAM-1 are characteristic of DC. When DC were purified using FcR rosetting or a combination of adherence and silica pulsing, comparable levels of expression of both MHC class II and ICAM-1 were observed. The staining profile of HD-SPC from DP and WF are given for comparison. The low level of T lymphocytes (R73.1) in DP preparations reflects the T cell lymphopenia of these animals (10).

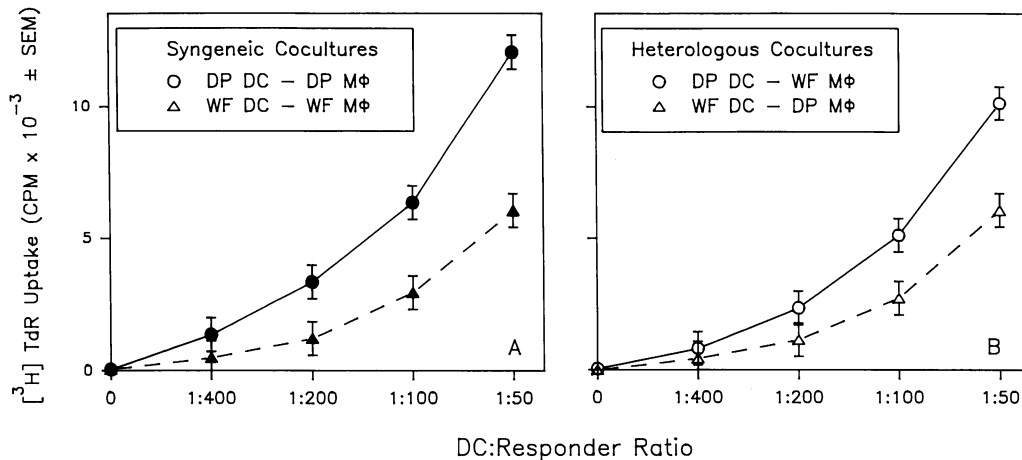


Figure 4. Stimulatory activity of DP and WF DC cocultured with either syngeneic or heterologous macrophages. LD-SPC were depleted of macrophages (MΦ) by plastic adherence as described in the methods. Non-adherent cells were removed and LD-SPC cocultures then reestablished either syngeneically (DP DC + DP MΦ and WF DC + WF MΦ) or heterologously (DP DC + WF MΦ, and WF DC + DP MΦ). After coculture, DC were purified by silica pulse and their stimulatory activity quantified.

as responder cell proliferation ($[^3\text{H}]$ TdR uptake) in an allogeneic MLR, using Fisher 344 HD-LNC as responders (5×10^5 per well). (A) Syngeneic cocultures. DC from DP (●) exhibited significantly greater stimulatory activity than DC of WF (▲) origin (DP DC vs. WF DC; $P < 0.001$). (B) Heterologous cocultures. DP DC (○) were again more active than DC of WF (△) origin (DP vs. WF, $P < 0.001$). Each data point represents the mean of four separate experiments performed as triplicate cultures.

The high accessory activity of macrophage-cocultured DP DC is intrinsic to the DP hemopoietic system. To investigate whether the high accessory activity we observed using DP DC is an inherent characteristic of the hematopoietic system, DP and WF BM chimeras were generated as described in the Methods. DC precursors reside in the BM (1, 37, 38), and the turnover interval for splenic DC is $\sim 2\text{--}4$ wk (39). Our experimental animals were studied 4 wk after irradiation and BM reconstitution. This helped assure both that the chimeras' DC would be

of donor origin and that they had matured in the radioresistant environment of the host. Chimerism of both total spleen cells and purified DC was confirmed by measuring the percentage of cells expressing the RT7.1 and RT7.2 rat alloantigens that are expressed by DP and WF rat DC, respectively (32). In three separate MLR assays and at two DC/responder cell ratios, we observed that DC of DP origin exhibited higher accessory activity than DC of WF origin, irrespective of the host in which they had matured (Fig. 7).

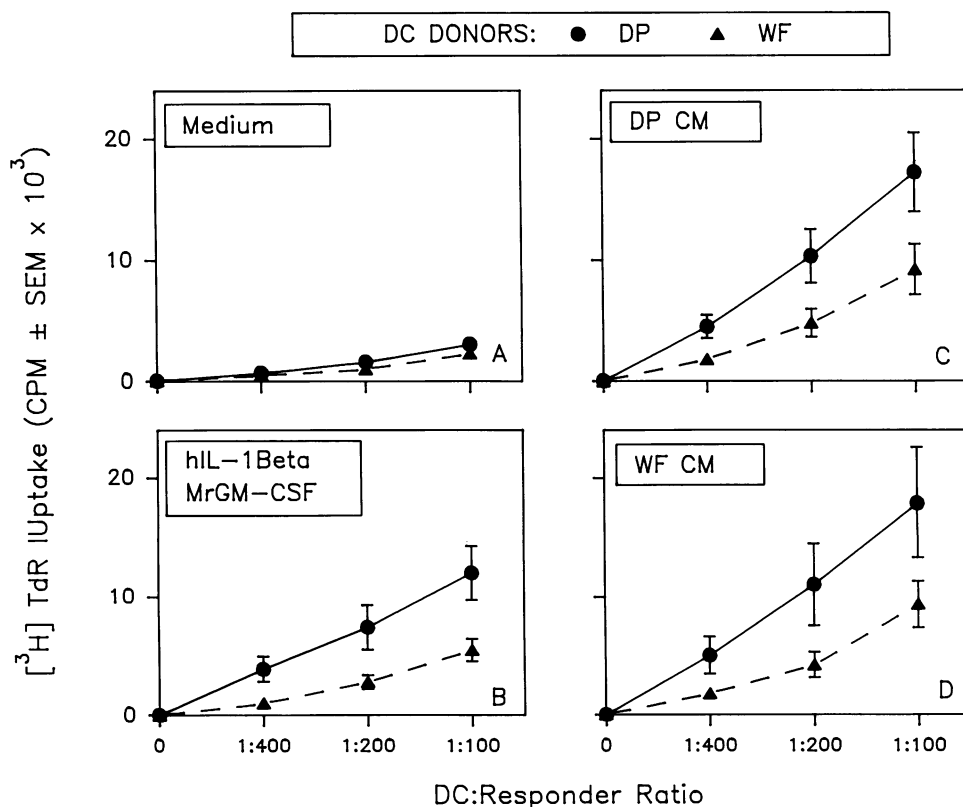


Figure 5. Differential effect of macrophage-derived cytokines on stimulatory activity of DP and WF DC. LD-SPC depleted of macrophages by FcR rosetting were incubated for 36 h in the presence of various added reagents: (A) Unmodified culture medium. (B) a mixture of human recombinant IL-1β (10 U/ml) and mouse recombinant GM-CSF (25 U/ml). (C) Conditioned culture medium (see Methods) from silica-treated LD-SPC from DP rats. (D) Conditioned culture medium from WF rats. DC stimulatory activity was quantified as responder cell proliferation ($[^3\text{H}]$ TdR uptake) in an allogeneic MLR, using Fisher 344 HD-LNC responders (5×10^5 per well). DP DC (●) consistently exhibited greater stimulatory activity than DC of WF (▲) origin (DP vs. WF, $P < 0.001$ for B-D, $P = \text{NS}$ for A). Each data point represents the mean of three separate experiments performed as triplicate cultures.

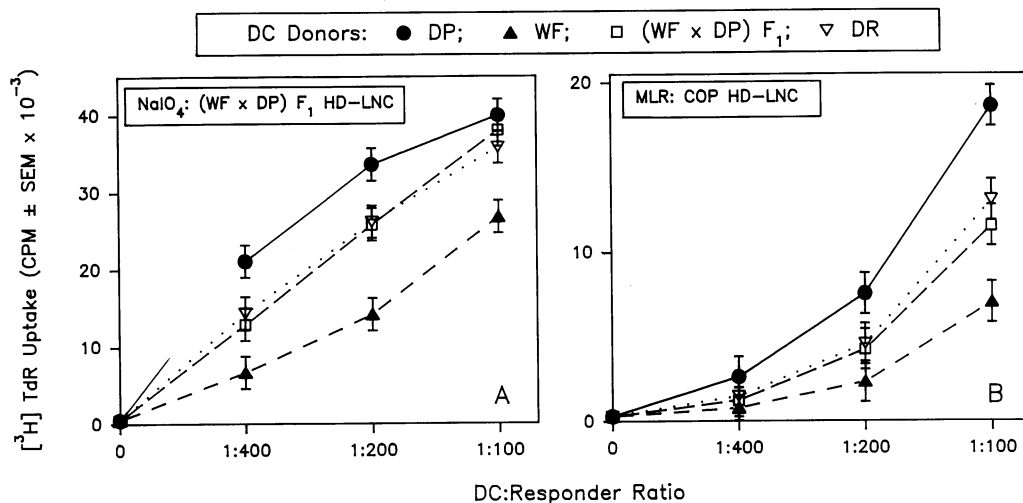


Figure 6. Stimulatory activity of DC from (DP \times WF) F_1 hybrids and from DR rats. DC were purified using macrophage coculture and silica pulse as described in the Methods. DC stimulatory quantified as HD-LNC proliferation ($[^3\text{H}]$ TdR uptake) in a NaIO₄ assay (A) or an allogeneic MLR (B). DC of DP origin (●) consistently exhibited significantly greater stimulatory activity than DC of WF origin (▲); DC from (WF \times DP) F_1 hybrids (□) possessed intermediate levels of activity, differing significantly from both DP and WF DC. The stimulatory activity

of DC from DR rats (▽) was similar to that of (WF \times DP) F_1 hybrids. (A) (WF \times DP) F_1 HD-LNC responders (DP vs. WF, $P < 0.001$; DP vs. F_1 and DR, $P < 0.01$; WF vs. F_1 and DR, $P < 0.001$; DP vs. F_1 , $P = \text{NS}$) (B) COP HD-LNC responders (DP vs. WF, $P < 0.001$; DP vs. F_1 and DR, $P < 0.001$; WF vs. F_1 and DR, $P < 0.01$; DR vs. F_1 , $P = \text{NS}$). Each point represents the mean of separate experiments performed as triplicate cultures.

Discussion

These studies investigated the hypothesis that susceptibility to autoimmunity in DP BB rats is associated with abnormalities of DC function. Our results demonstrate that, after culture in the presence of macrophages or their secretory products, DC from DP rats exhibit greater in vitro stimulatory activity than DC from normal, histocompatible WF rats.

Using DC isolated without macrophage coculture, we found that the basal accessory activity of DP and WF DC were comparable. We interpret this result to indicate that DP and WF DC behave similarly with respect to basal stimulatory activity. In contrast, after coculture with activated macrophages, splenic DC from DP rats consistently displayed greater in vitro stimulatory activity than DC from WF rats. This characteristic was evident in both mitogenic and MLR systems.

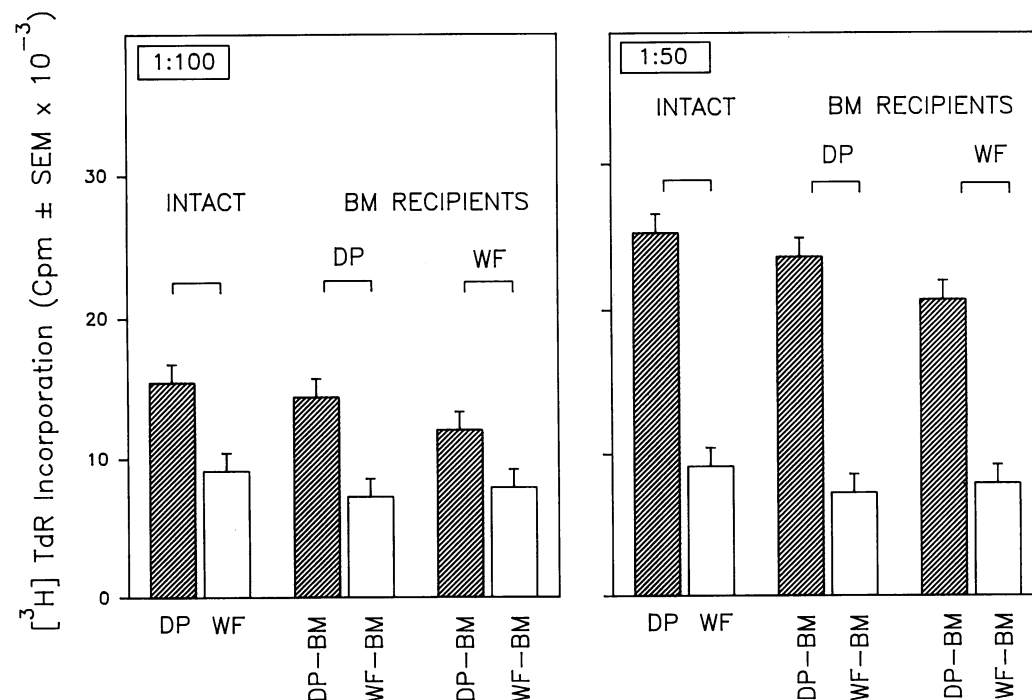


Figure 7. Stimulatory activity of DC from DP and WF reciprocal bone marrow chimeras. Splenic DC were obtained by the silica-pulse method from intact, unirradiated DP and WF rats and from irradiated, bone marrow reconstituted syngeneic (DP \rightarrow DP and WF \rightarrow WF) and reciprocal (DP \rightarrow WF and WF \rightarrow DP) chimeras. DC stimulatory activity was quantified as responder cell proliferation in an allogeneic MLR, using COP HD-LNC responders (5×10^5 per well). Left and right panels present data obtained at DC/responder ratios of 1:100 and 1:50, respectively. DC of DP origin are indicated by filled bars and DC of WF origin by open bars. DC donors are indicated above the bars. DC of DP origin consistently exhibited

statistically significantly greater stimulatory activity than DC of WF origin, irrespective of the environment in which they had matured. (Intact DC donors: DP vs. WF, $P < 0.0001$ at both DC/responder ratios; recipients of DP-BM: $P < 0.0001$; recipients of WF-BM: $P < 0.0002$). Each bar represents the mean of three separate experiments performed as triplicate cultures.

Additional experiments established that this differential activity persisted when DP and WF DC were (a) reciprocally cocultured with DP or WF macrophages and (b) incubated with conditioned medium derived from either DP or WF LD-SPC. These results indicate that properties inherent to DP vs. WF macrophages are unlikely to account for the difference between DP and WF DC accessory capability. This interpretation is strengthened further by the results of our assays performed using a mixture of IL-1 and GM-CSF, cytokines previously shown to account for most of the stimulatory activity of silica-treated LD-SPC culture supernatants (26). When DC from DP and WF rats were treated with a mixture of IL-1 and GM-CSF at doses corresponding to maximal stimulation, DP again exhibited greater stimulatory activity than WF DC. These findings suggest that DC from DP-BB rats are inherently more responsive to macrophage-derived factors than WF DC.

Our data do not exclude differential cytokine production by DP and WF macrophages. Stimulated DP-BB macrophages reportedly produce increased amounts of TNF (40). DP-BB macrophages also appear to inhibit mitogen-stimulated T cell responses by an undefined mechanism (41, 42). Given these observations, one might speculate that the DC-macrophage axis in the DP rat harbors abnormalities in both of its components. It is important to stress, however, that macrophage abnormalities alone could not account for the differential activity of DP and WF DC in vitro that we have observed.

Studies of (WF \times DP) F₁ hybrids, DR BB rats, and DP-WF reciprocal BM chimeras indicate that the differential activity of DP and WF DC is genetically determined. The cellular basis of these findings appears to be intrinsic to the hemopoietic system and is not influenced by the radioresistant maturational milieu.

Possible involvement of DC in the development of autoimmune processes has been suggested previously. Voorbij et al. (16) and Voorby et al. (43) have shown in DP-BB rats that DC are the first cells to infiltrate both islets and thyroid, suggesting their involvement in the initiation phase of both diseases. Georgiou and Bellgrau (44) have documented a defect in APC function in the BB rat thymus and suggest that such a defect might be more widespread. Finally, diabetes may be prevented in DP rats by in vivo treatment with silica, probably by interfering with antigen processing and presentation (19). In the non-obese diabetic mouse, another animal model of autoimmune diabetes, a preliminary report by Clare-Salzer et al. (45) suggests a possible role of DC in tolerization. Footpad injection of DC from peripancreatic lymph nodes of nonobese diabetic mice with active insulinitis reportedly conferred resistance to spontaneous and adoptively transferred diabetes. Interestingly, DC preparations derived from other lymphoid sites were not effective.

Our finding that DC from DP-BB rats become relatively hyperactive in response to macrophage-derived factors is compatible with two major hypotheses invoked to explain cell mediated autoimmune phenomena. Hyperactive DC could render immunogenic the constitutive tolerogenic presentation of self to the immune system. Alternatively, hyperactive DC could present tissue-specific neoantigens postnatally expressed or resulting from specific or nonspecific destruction, e.g., viral infection of β cells. One may further speculate that the comparatively modest response of WF DC in response to cytokines could in part explain WF refractoriness to insulin-dependent diabetes mellitus and other autoimmune disorders. Evaluation

of these possibilities will require extensive additional investigation and, in particular, expansion of the scope of study to include additional rat strains.

Interestingly, it has been recently shown that human thyroid epithelial cells produce IL-1 in the course of autoimmune thyroiditis (46). Furthermore, a human pancreatic beta cell line reportedly secretes IL-1 in response to viral infection (47). These observations suggest that both tissues could directly stimulate resident DC in the absence of other immune cell interactions. This could also account for the vulnerability of these tissues as targets of autoimmunity. The possibility that a defect in DC activity may underlie or contribute to susceptibility to autoimmune diabetes and thyroiditis warrants further study.

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