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The Countervailing Actions of Myeloid and Plasmacytoid Dendritic Cells Control Autoimmune Diabetes in the Nonobese Diabetic Mouse

Vijay Saxena,* Jennifer K. Ondr,* Albert F. Magnusen,† David H. Munn,‡ and Jonathan D. Katz‡‡†

Islet Ag-specific CD4+ T cells receive antigenic stimulation from MHC class II-expressing APCs. Herein, we delineate the direct in vivo necessity for distinct subsets of macrophages and dendritic cells (DC) in type I diabetes mellitus of the NOD mouse by using diphtheria toxin-mediated cell ablation. The ablation of macrophages had no impact on islet Ag presentation or on the induction of insulitis or diabetes in either transfer or spontaneous models. However, the ablation of CD11b+CD11c+ DC led to the loss of T cell activation, insulitis, and diabetes mediated by CD4+ T cells. When the specific myeloid DC subset was “added-back” to mice lacking total DC, insulitis and diabetes were restored. Interestingly, when NOD mice were allowed to progress to the insulitis phase, the ablation of DC led to accelerated insulitis. This accelerated insulitis was mediated by the loss of plasmacytoid DC (pDC). When pDC were returned to depleted mice, the localized regulation of insulitis was restored. The loss of pDC in the pancreas itself was accompanied by the localized loss of IDO and the acceleration of insulitis. Thus, CD11c+CD11b+ DC and pDC have countervailing actions in NOD diabetes, with myeloid DC providing critical antigenic stimulation to naive CD4+ T cells and pDC providing regulatory control of CD4+ T cell function in the target tissue. The Journal of Immunology, 2007, 179: 5041–5053.

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toimmune or type I diabetes mellitus (T1DM) is a T cell-mediated disease in which insulin-producing pancreatic β cells are selectively destroyed (reviewed in Ref. 1). The immunopathology of T1DM is well modeled in the NOD mouse. Numerous studies in the NOD mouse show essential roles for CD4+ T cells in: 1) the initial antigenic response to β cell Ags; 2) the propagation of pancreatic islet infiltration, i.e., insulitis; 3) the destruction of insulin-producing β cells; and 4) the regulation of diabetes progression (1–3). Thus, regardless of which of these manifold roles a given β cell-specific CD4+ T cell plays, it must first encounter its cognate Ag as a peptide bound to MHC class II on a host APC because the target β cell does not itself express MHC class II molecules (4). Thus, given the dependence of CD4+ T cells on the host APC, the eventual nature of the T cell response will likely be impacted profoundly by the nature and activation state of the APC. Yet, to date the specific APC subsets involved in providing activating or regulatory signals to islet cell-responsive CD4+ T cells remain unresolved.

Although, genetic deletion of mature B cells renders NOD mice resistant to spontaneous T1DM (5), B cells are not obligate APC for CD4+ T cells in vivo because naïve CD4+ T cells can drive diabetes development without any help from B cells or B cell functions, including Ag presentation (6–10).

Macrophages may play critical roles in T1DM such as Ag presentation or β cell killing (reviewed in Ref. 11). Islet Ag-loaded macrophages can transfer diabetes, whereas silica-mediated inhibition of macrophages reduces diabetes onset (12–15). Moreover, macrophages are among the first cell populations to infiltrate the islets and, when fully activated, can mediate cell cytotoxicity against β cells via the production of IFN-γ, TNF-α, and reactive oxygen intermediates such as NO (16–18).

Like macrophages, dendritic cells (DC) are a major innate immune cell constituent of islet infiltrates. A number of studies suggest distinct and vital roles for DC in the development and progression of T1DM in the NOD mouse. Work by Drexhage and coworkers (19) show that DC are the first leukocytes to infiltrate islets during insulitis, followed only secondarily by macrophages and T cells. In addition, Nikolic et al. (20) have proposed that DC are essential for the retention of lymphocytes in the early pancreatic lesions before the onset of progressive insulitis. Turley et al. (21) found that the activation of diabetogenic CD4+ T cells is triggered via Ag presented on CD11b+CD11c+ DC in the draining pancreatic lymph nodes (PLN). Interestingly, the developmental program of NOD DC is delayed and aberrant, and this may explain, in part, the poor tolerance to self-Ags observed in the NOD mouse (22). Moreover, a subset of DC grown from NOD bone marrow (BM) in vitro by Flt3L, GM-CSF and IL-6 delay the onset of diabetes (23, 24), whereas CD11c+PDCA-1+ plasmacytoid DC (pDC) may dampen the immune response to both self and foreign Ags.
(25) (PDCA-1 is pDC Ag-1). Additionally, recent evidence suggests that pDC act in concert with regulator T (Treg) and/or NK T (NKT) cells and may, in fact, rely on these cells for their maintenance (26–28).

The distinct requisite and temporal requirements for macrophages, CD11bCD11c (myeloid DC (mDC)), CD11cCD8α (lymphoid DC (lyDC)), and pDC in the immunopathogenesis of T1DM and the extent to which each subset of APC can compensate for each other in vivo remains unclear. Herein, we describe the direct assessment of the functional need for macrophages and DC subsets in the development of T1DM in vivo by way of selective subset ablation. Transgenic mice carrying diphtheria toxin (DT) receptor (DTR) under either the control of a truncated human CD11b or mouse CD11c promoters drive expression in macrophages or DC, respectively. When treated with DT, these mice have rapid and specific ablation of either macrophages or DC in vivo (29, 30). We now have produced NOD mice carrying these DTR transgenes. DT treatment of CD11b-DTR/NOD mice leads to the loss of CD11b+F4/80+ macrophages while sparing DC, including CD11b+ subsets. The temporal ablation of macrophages has no discernable impact on either insulitis or diabetes induced by the CD4+ T cells in either spontaneous or transfer diabetes models. Moreover, macrophages are not required for the indirect killing of β cells, as the loss of macrophages in mice with ongoing insulitis progressed to diabetes with identical kinetics as those of untreated mice. In contrast, treatment of CD11c-DTR/NOD mice leads to the complete abrogation of diabetes via the loss of CD11bCD11c+ mDC and/or CD11bCD11cCD8+ lyDC. Both mDC and lyDC subsets appear to compensate for each other vis-à-vis Ag presentation and activation of diabetogenic CD4+ T cells. Paradoxically, the ablation of DC from NOD mice with ongoing T cell-mediated intrapancreatic infiltration developed accelerated insulitis as a result of the specific loss of pDC in the pancreas.

In the absence of pDC, localized production of IDO is lost and a more aggressive phase of insulitis ensues. This is also seen in wild-type mice treated with the IDO antagonist 1-methyl-D-tryptophan (1-MT). Thus, the loss of either pDC or IDO production in the pancreas leads to enhanced insulitis, suggesting that the pace of insulitis in NOD is controlled by pDC or IDO or by pDC via IDO. In addition, the loss of pDC correlated with the relocalization of NKT cells from the pancreas to regional lymph nodes, suggesting that pDC and NKT cells may act in league to regulate insulitis progression.

Materials and Methods

Mice

FVB/N mice carrying the human DTR transgene under control of the CD11c and CD11b promoters obtained from Dr. R. Lang (Divisions of Ophthalmology and Molecular and Developmental Biology, Children’s Hospital, Cincinnati, OH) were backcrossed onto NOD/LtJ mice (originally purchased from The Jackson Laboratory) for 10–12 generations to produce CD11c-DTR/NOD and CD11b-DTR/NOD mice; these mice, if left unmanipulated, develop spontaneous T1DM at 4–6 mo of age, similar to the NOD/LtJ mice in our specific pathogen-free facility breeding colony. Moreover, to ensure the inheritance of NOD idd susceptibility regions we typed N3/N5 generation mice by PCR for microsatellite markers of the diabetic diabetogenic (idd)15 allele. For the study of insulitis, CD11c-DTR/ NOD and CD11b-DTR/NOD mice were crossed to BDC/NOD mice to produce BDC2.5/CD11c-DTR/NOD and BDC2.5/CD11b-DTR/NOD mice; both male and female were used at the ages described in the text to assess insulitis. Both male and female BDC2.5 TCR transgenic mice develop insulitis and diabetes at the same rate and severity (7). For transfer diabetes and BM reconstitutions, BDC2.5/CD11c-DTR/NOD and BDC2.5/ CD11b-DTR/NOD mice were crossed to NOD.scid to fix transgenic TCR expression. Spontaneous diabetes develops at 18–23 days of age in 100% of the BDC2.5/NOD.scid mice of both sexes (7). T cells from either male or female BDC2.5/NOD.scid mice transfer diabetes with >95% incidence to NOD.scid recipients in 8–14 days (7). Our NOD.scid and NOB/LtJ colonies originated from founders purchased from The Jackson Laboratory. Peripheral blood cells from pups were stained with mAb to Vβ4 (BD Pharmingen) or BDC2.5 TCR (31) and CD4 to identify transgenic T cells. DTR transgenes were identified by PCR on tail DNA using primer sequences for DTR-1 (5′-AAAGAAGAGAAGGACAGG-3′) and DTR-2 (5′-AGTAGAATCTCAGGGTCACG-3′). PCR product size was seen as indicated in CD11b-DTR/NOD and BDC2.5/TCR/NOD mice transfer with anti-BDC2.5 TCR clone Ab to fix transgenic TCR expression. In vivo APC ablation

For DC ablation, CD11c-DTR/NOD.scid mice, BDC2.5/CD11c-DTR/ NOD mice, or NOD mice reconstituted with BM from CD11c-DTR/ NOD.scid mice were injected i.p. with 4 mg of DT per gram of body weight (List Biological Laboratories) in PBS. For macrophage ablation, CD11b- DTR/NOD.scid or BDC2.5/CD11b-DTR/NOD.scid mice were injected with 25 mg of DT per gram of body weight. Controls were injected with similar volume of 1× PBS (vehicle). The Ab-mediated depletion of pDC was performed using an anti-mouse PDCA-1 purified Ab (Miltenyi Biotec). Mice were treated i.p. twice, 2 days apart, with 500 μg of Ab per mouse in PBS.

Antibodies

mAbs to CD11c (clones HI3 or N418), CD11b (clone M1/70), CD80 (clone 10-16A1), CD86 (clone GL1), CD25 (clone 7D4), MHC I (clone KH95), MHC II (clone 10-3-6), CD40 (clone 323), Ly6G/Grl (clone AL21), B220 (clone RA3-6B2), CD19, CD209, Foxp3 and F4/80 were purchased from BD Pharmingen or eBioscience. B220, Ly6G, F4/80, CD11c and MOMA-1 were purchased from BioLegend. CD11b and F4/80 Ab were purchased from BioXCell. pDC were identified in multicolor staining using a FACScalibur or an LSR II flow cytometer (BD Biosciences). For the detection of mDC, mice were stained with mAb to CD11b, CD11c, and MHC II. For the detection of pDC, cells were stained with PDCA1-allophycocyanin in combination with CD11b, CD11c, B220, and Ly6G/Grl mAbs. For detection of macrophages, cells were stained with mAbs to CD11b and F4/80 (Caltag Laboratories). Rat IgG2a and rat IgG2b were used as isotype controls for DC. BDC2.5 T cells in different tissues were detected directly using the anti-BDC2.5 mAb (31). Data were analyzed using WinMDI software (version 2.1.4) originally written by J. Trotter (FACS Core Facility, The Scripps Research Institute, La Jolla, CA) or with FlowJo 7.3.1 (http://www.flowjo.com).

BDC2.5 T cell purification

For diabetes transfer studies, BDC2.5 T cells were sort purified from pre-diabetic BDC/NOD or BDC/NOD.scid mice using a FACSVantage flow cytometer (BD Biosciences). Splenocytes were labeled with anti-BDC2.5 TCR clone Ab and anti-CD4. This allowed the isolation of naive BDC2.5 T cells to prevent the contamination of APC populations. Purity was between 95 and 98%, with APC contamination of <0.5%. Sorted cells were washed in 1× HBSS, counted and injected (5 × 106 cells/mice i.p.) 24 h after the first DT treatment.

Preparation of cells from pancreas

Pancreata were removed from mice and disrupted in a glass tissue homogenizer in PBS containing 2% BSA and further dispersing by drawing the suspension with hypodermic needles. The resulting cell homogenate was then filtered through a 40-μm filter basket to remove tissue debris followed by Percoll-gradient centrifugation as described (9).

Quantification of NKT cells using CD1d-tetramers

NKT cells were directly assessed using CD14-tetramers loaded with the NKT superagonist α-galactosylceramide. PE-conjugated CD1d tetramers,
a gift from Dr. M. Kronenberg (La Jolla Institute of Allergy and Immunology, La Jolla, CA), were used as described (33). Control staining used CD1d tetramers without α-galactosylceramide (empty control).

Isolation of islets from the NOD pancreas

Pancreata from NOD mice were removed, placed in siliconized glass containers with chilled PBS, and then put on ice. Tissues were macerated thoroughly with scissors into small pieces. Macerated tissues were transferred to round-bottom tubes and pelleted, by a quick 30-s spin at 400 g. Two milliliters of collagenase (Sigma-Aldrich) per two pancreata were added to the pellets. Digestion was performed in a 37°C water bath by shaking the tubes (10 min) until the mixture became a slurry. Cold PBS containing 5% FBS was added to immediately stop digestion and mixed by inversion. The slurry were washed three times (800 × g, 400 × g, and 250 × g) with 5 ml of ice-cold PBS. The supernatant was discarded each time. The pellet was resuspended in 4 ml of 25% Ficoll. Two milliliters each of 23, 20.5, and 11.5% Ficoll were then carefully layered on top. After centrifugation at 800 × g for 12 min, the interface between the top two interfaces. Islets were washed with 1× PBS, irradiated at 2 g, and dispersed with trypsin/EDTA treatment if used for proliferation studies.

DC purification by magnetic sorting

DC were purified from the spleens of 6- to 8-wk-old NOD mice by a magnetic bead separation method according to the manufacturer’s recommendations (Miltenyi Biotec) with some modifications. Spleens (n = 4) were pooled and digested for 45 min in collagenase D (1 mg/ml; Roche) at 37°C, meshed, filtered, and centrifuged at 300 × g for 10 min. Cells (1 × 10^7) were labeled with 100 μl of anti-mouse PDCA-1 microbeads (Miltenyi Biotec) for 15 min at 4°C. Cells were washed with MACS buffer (PBS and 0.5% BSA containing 2 mM EDTA) and resuspended in buffer (400 μl/10^6 cells). LS columns were prepared by washing with 3 ml of column buffer. Magnetically labeled cells were loaded onto the column and immediately washed three times with 3 ml of MACS buffer. Two consecutive column purification steps were performed to achieve the highest purity. The positive fraction was eluted by placing the column out of the magnetic field. Five milliliters of buffer was applied onto the column and immediately flushed with phosphate-buffered saline (PBS). A similar two-column purification step was performed and CD11c positive cells were eluted using CD11c microbeads. Purity was measured by FACS staining with anti-PDCA-1-allophycocyanin and anti-CD8a-PE Abs in combination with anti-MHC II and anti-Ly6G-FITC Abs.

“Add-back” of mDC and pDC to CD11c-DTR mice treated with DT

Myeloid, lymphoid, and plasmacytoid DC subsets were purified by the combination of magnetic bead and fluorocytometric cell sorting. mDC were defined as CD11b<sup>+</sup>CD11c<sup>-</sup>C<sup>-</sup>Fl<sup>-</sup>, LyDC as CD11b<sup>+</sup>CD11c<sup>-</sup>C<sup>-</sup>Fl<sup>+</sup>, and pDC as Ly6G<sup>-</sup>PDCA<sup>-</sup>-1<sup>-</sup>. Mice were treated with DT on day −1 and then every 5 days thereafter. On day 0 all mice were given 5 × 10<sup>4</sup>-sort-purified T cells from 2- to 3-wk-old BDC/NOD.scid.i, in vivo: In vivo, on day 0 and every 5 days thereafter recipients were either given 4 × 10<sup>6</sup> sort-purified mDC or 4 × 10<sup>5</sup> pDC from DT-resistant wild-type NOD mice or CD11c-DTR/NOD mice. Diabetes was assessed by standard blood glucose measurements using a one-step glucometer. Diabetes incidence is cumulative on macrophages (CD11b-DTR) or the simian DTR on dendritic cells (CD11c-DTR) (29, 30, 38). In general, most mice are resistant to DT when given in nanogram per gram of body weight concentrations. However, when individual mouse cells express a DT receptor they are rapidly and specifically ablated upon systemic DT treatment (29, 30, 38). We backcross bred the CD11b-DTR and CD11c-DTR transgenics onto the NOD genetic background for 12 generations and fixed the NOD susceptibility alleles by use of the “speed congenic” technique at N3-N5 (39).

Using CD11b-DTR transgenic NOD (CD11b-DTR/NOD) mice, we found that a single i.p. treatment with 25 ng of DT per gram of body weight led to near complete ablation of CD11b<sup>-</sup>F<sub>480</sub>-<sup>-</sup> macrophages from the spleen and lymph nodes, including from the PLN and pancreas of transgenic NOD mice (Fig. 1). The ablation of macrophages was significant (p < 0.001) both in percentile (Fig. 1a) and absolute number terms (Fig. 1b). The specific ablation of CD11b<sup>-</sup>F<sub>480</sub>-<sup>-</sup> macrophages from CD11b-DTR/NOD mice lasted from 3 to 5 days. We found that treating NOD mice every 3 days maintained near-complete macrophage deletion for periods of up to 2–3 wk. Importantly, DT treatment of CD11b-DTR/NOD mice spared CD11b<sup>-</sup>CD11c<sup>-</sup> DC in all lymphoid organs and the pancreas (Figs. 1 and 2a). Why CD11b<sup>-</sup>CD11c<sup>-</sup> DC are spared remains unclear; however, characterization of the CD11b promoter region used to drive DTR gene expression revealed transcriptional activity in macrophages and neutrophils but not in the myeloid precursors from which mDC develop (40).
were given 5 leaving mice with only DC as APC. On day 0, these recipients and every third day thereafter to ablate host macrophages, thereby with DT (25 ng per gram of body weight i.p.) on day PBS-treated mice (open bars) is shown. DT treatment largely spares total DC and specific subsets. Macrophages were defined as CD11b (Panc.). Statistically significant ablation of macrophages from spleen, PLN and pancreas of CD11b-DTR mice treated with DT (filled bars) compared with , Specificity of DT-mediated ablation was revealed by analysis of absolute numbers of DC subsets and macrophages from spleen (Spl.), PLN, and pancreas (Panc.). Statistically significant ablation of macrophages from spleen, PLN and pancreas of CD11b-DTR mice treated with DT (filled bars) compared with PBS-treated mice (open bars) is shown. DT treatment largely spares total DC and specific subsets. Macrophages were defined as CD11b "F4/80" , total DC as CD11c "I-A<sup>+</sup>"; mDC/lyDC as CD11c "CD11b"; and pDC as CD11c<sup>lo</sup>PDCA-1<sup>+</sup>. Values are means (± SD) of four independent experiments. *** p < 0.001; ** p < 0.01; and * p < 0.05, as determined by Student’s t test.

**FIGURE 1.** Specific macrophage ablation in DT-treated CD11b-DTR/NOD mice. *a,* Flow cytometric analysis reveals ablation of macrophages in spleen (Spl.), PLN, and pancreas (Panc.) of 6- to 8-wk-old CD11b-DTR/NOD mice treated with DT. DT treatment, (bottom panels), PBS treatment (top panels), spleen (left panels), PLN (middle panels) and pancreas (right panels) cells were analyzed 48 h after in vivo treatment. Macrophages were identified by anti-F4/80 and anti-CD11b mAb, size gated, and analyzed by flow cytometry. Confirmation of the retention of normal CD11b<sup>+</sup>CD11c<sup>+</sup> and CD11c<sup>low</sup> DC subsets were performed using anti-CD11b and CD11c mAbs. A total of 10<sup>5</sup> cells were collected per sample; dot plots were normalized to equal numbers of viable gated cells and were displayed with high cell numbers to allow for the visualization of rare cell subsets and to improve statistical analysis. The percentage of cells in each analysis gate is shown relative to total viable cells. Values and dot plots are representative of seven independent experiments. **b,** Specificity of DT-mediated ablation was revealed by analysis of absolute numbers of DC subsets and macrophages from spleen (Spl.), PLN, and pancreas (Panc.). Ablation of macrophages has no effect on spontaneous diabetes in NOD mice lacking macrophages

Having established that DT-treatment ablated only macrophages in CD11b-DTR/NOD mice, we undertook a set of diabetes transfer studies using CD11b-DTR/NOD.scid mice as recipients of diabetogenic CD4<sup>+</sup> T cells. CD11b-DTR/NOD.scid mice were treated with DT (25 ng per gram of body weight i.p.) on day −1, day 3, and every third day thereafter to ablate host macrophages, thereby leaving mice with only DC as APC. On day 0, these recipients were given 5 × 10<sup>4</sup> naïve, sort-purified BDC2.5 T cells. By days 10–14 both the macrophage-ablated and control mice were diabetic (Fig. 2b). We confirmed that CD11b<sup>+</sup>F4/80<sup>+</sup> macrophages were depleted and that the mice were “macrophage-free” at the time of diabetes onset (Fig. 2a). Histological studies showed little remaining islet mass and few remaining intraislet leukocytes in either DT or control mice at the time of overt diabetes; thus, the pancreata from diabetic mice were not enumerated for leukocyte numbers. Therefore, the in vivo ablation of macrophages had no effect on the tempo and penetrance of diabetes by T cell transfer.

**Ablation of macrophages has no effect on spontaneous diabetes**

To assess the role of macrophages on spontaneous diabetes, we treated 10-day old BDC2.5/CD11b-DTR/NOD.scid mice with 25 ng/g DT i.p. and repeated this treatment every 3 days thereafter. By initiating the treatment on day 10 all of the recipients were free of insulitis, as previous studies indicated that insulitis begins on or after day 13 in these animals (7). We found that DT treatment of BDC2.5/CD11b-DTR/NOD.scid mice resulted in near-complete ablation of macrophages from these animals while sparing the CD11b<sup>+</sup>CD11c<sup>+</sup> and plasmacytoid DC subsets (Fig. 3a). DT-treated BDC2.5/NOD.scid mice developed spontaneous insulitis (Fig. 3b) and diabetes (Fig. 3c) indistinguishable from that of BDC2.5/CD11b-DTR/NOD.scid mice treated with the PBS control. Taken together with the transfer studies, we concluded that macrophages were not required for either the activation of CD4<sup>+</sup> T cells in vivo or the indirect killing of β cells.

Specific subsets of dendritic cells but not macrophages are ablated in CD11c<sup>-</sup>DTR NOD mice

Both insulitis and diabetes progressed normally in macrophage-ablated mice where the sole remaining APC were DC; we therefore decided to directly assess the requirement for DC in T1DM pathogenesis. However, because NOD mice have altered DC development (22), we decided to first determine the fine specificity of DC and the subset expression of the DTR in the NOD mouse. Using multiple series of seven-color flow cytometry, we subdivided DC and macrophages into 18 distinct subsets based on 12 defined surface markers (Fig. 4). Specifically, as shown in Fig. 4a, by using CD11b and CD11c we defined four major splenic subsets
Specific ablation of macrophages had no affect on the incidence of diabetes transfer by naive CD4⁺ BDC2.5 T cells. Flow cytometric analysis of spleen (Spl.) and PLN leukocytes from DT-treated or control CD11b-DTR/NOD.scid recipient mice at the time of diabetes onset. Representative dot plots for macrophages (right panels) and DC (left panels) for DT-treated mice (bottom panels) and PBS control mice (top panels). A total of 10⁶ cells were collected per sample; dot plots were normalized to equal numbers of viable gated cells and were displayed with high cell numbers to allow for visualization of rare cell subsets and to improve statistical analysis. The percentage of cells in each analysis gate is shown relative to total viable cells. Dot plots are representative of six independent mice. b, CD11b-DTR/NOD.scid mice were treated with 25 ng/g DT, i.p. (●) or with PBS control (○) on day −1 and then every 3 days thereafter. On day 0 all mice were given naive T cells from 2- to 3-wk old BDC/NOD.scid mice i.v. DT-treated, n = 12; vehicle control, n = 6.

FIGURE 3. No alteration in severity of insulitis or spontaneous diabetes in macrophage-ablated BDC2.5/CD11b-DTR/NOD.scid mice. BDC2.5/CD11b-DTR/NOD.scid mice were treated with DT or with PBS from 10 days of age every 3 days i.p. On day 15 or 16 splenocytes (Spl.) were harvested for analysis by flow cytometry (a) and pancreata were fixed and sectioned for histopathology (b). a, Splenocytes from mice treated with DT were depleted of F4/80⁺CD11b⁺ macrophages as compared with PBS controls. A total of 10⁶ cells were collected per sample; dot plots were normalized to equal numbers of viable gated cells and were displayed with high cell numbers to allow for visualization of rare cell subsets and to improve statistical analysis. The percentage of cells in each analysis gate is shown relative to total viable cells. b, Spontaneous insulitis development was assessed on 15- or 16-day-old BDC2.5/CD11b-DTR/NOD.scid mice, three mice per group. Peri-insulitis (open bars), moderate insulitis (gray bars), and severe insulitis (filled bars) are the cumulative incidences of insulitis from total islets scored per group. c, BDC2.5/CD11b-DTR/NOD.scid mice were treated with DT (●), vehicle control (○), or left untreated (□) on day 10 and then every 3 days thereafter. DT-treated mice, n = 11; vehicle control, n = 6, untreated, n = 10.

FIGURE 2. Specific ablation of macrophages had no affect on the incidence of diabetes transfer by naive CD4⁺ BDC2.5 T cells. a, Flow cytometric analysis of spleen (Spl.) and PLN leukocytes from DT-treated or control CD11b-DTR/NOD.scid recipient mice at the time of diabetes onset. Representative dot plots for macrophages (right panels) and DC (left panels) for DT-treated mice (bottom panels) and PBS control mice (top panels). A total of 10⁶ cells were collected per sample; dot plots were normalized to equal numbers of viable gated cells and were displayed with high cell numbers to allow for visualization of rare cell subsets and to improve statistical analysis. The percentage of cells in each analysis gate is shown relative to total viable cells. Dot plots are representative of six independent mice. b, CD11b-DTR/NOD.scid mice were treated with 25 ng/g DT, i.p. (●) or with PBS control (○) on day −1 and then every 3 days thereafter. On day 0 all mice were given naive T cells from 2- to 3-wk old BDC/NOD.scid mice i.v. DT-treated, n = 12; vehicle control, n = 6.

of non-T and non-B cells: CD11b⁺CD11c⁻ macrophages (region 1); CD11b⁺CD11c⁺ high DC (region 2); CD11b⁺CD11c⁺ high DC (region 3); and CD11b⁻CD11c⁻ low DC (region 4). After gating on these regions we assessed the expression of other common myeloid and lymphoid markers of DC to produce the fine specificity of DC depicted in Fig. 4b. Surprisingly, many NOD DC express markers of macrophages (F4/80 and MOMA-1) and a small subset of macrophages expresses the DC marker CD209. The cells in region 3 and a subset of region 2, region 2a, were quite similar in many subset markers with the major difference lying in the levels of PDCA-1 and CD19; the cells in region 3 were uniformly positive for the expression CD19 and PDCA-1 whereas the cells in 2a were negative for both. These cells are similar to those described by others and may represent the immature DC subsets attributed to NOD mice (22, 32). Upon the treatment of CD11c-DTR/NOD mice with 4 ng of DT per gram of body weight, all of the major subsets of DC were lost from the spleens of NOD mice as shown in Fig. 4a and by the fine subset analysis in Fig. 4b as denoted by the red text. Over 90% of DC were lost 48 h after treatment, with most subsets losing 95–97%. The macrophage populations did not show >5–8% cell loss. Often, the remaining cells in a region were of a markedly different surface phenotype; for example, the remaining CD11c⁻ cell population region 4 after treatment were largely negative for the pDC markers PDCA-1 and 120G8 (Fig. 4c). The effective depletion of DC did not correlate with the overall expression patterns of the DTR. The level of DTR expression, as inferred by the linked GFP expression, varied widely over each subset, with the highest levels of expression seen in regions 3 and 2a and the lowest level in region 4 as shown in Fig. 4d. Nonetheless, region 4 cells were as effectively depleted as the cells in the other DC compartments. In addition to the spleen, we determined that treatment of the CD11c-DTR/NOD mice with DT led to the ablation of CD11c⁺ DC from the lymph nodes, including the PLN, as well as from the pancreas of these transgenic NOD mice (Fig. 5). The ablation of DC was near complete for both of the CD11b⁺CD11c⁺ subsets (mDC and lyDC) as well as for the CD11b⁺CD11c⁻PDCA-1⁻Ly6G⁻ pDC subset. This was true both in percentiles (Fig. 5a) and absolute cell number (Fig. 5b) measures. Treatment of CD11c-DTR mice spared CD11b⁺ macrophages and neutrophils (Fig. 5a) in all of these tissues.

It has been previously reported that multiple injections of DT in CD11c-DTR transgenic mice on other genetic backgrounds led to...
death (30). We found this to be equally true for the CD11c-DTR mice on the NOD background. DT treatment of CD11c-DTR/NOD.scid and BDC2.5/CD11c-DTR/NOD.scid mice induced an acute and large spike in the production of IFN-γ/H9253 and TNF-α/H9251 with subsequent cachexia and death (data not shown), thereby making the direct study of T cell-DC interactions using “off-the-shelf” CD11c-DTR/NOD.scid mice problematic. Like others, we circumvented this problem by reconstituting wild-type NOD mice with CD11c-DTR/NOD.scid BM, suggesting that the toxicity resided with nonhemopoietic cells (41). Therefore, all subsequent experiments with the CD11c-DTR mice were performed on radiation BM chimeras. We irradiated 4- to 6-wk-old NOD mice with 950 rad of whole body gamma irradiation and then reconstituted them with CD11c-DTR/NOD.scid BM cells. After 5–7 wk these mice are fully reconstituted, lacking both the B and T cell lineages consistent with the use of scid BM donors. In addition, CD11c+ peripheral blood cells expressed the transgenic DTR as observed by following the surrogate GFP marker and by RT-PCR for the DTR in PBMC (data not shown). Importantly, when BDC2.5 T cells were transferred into these reconstituted mice no toxicity was observed after DC ablation.

We treated CD11c-DTR BM-reconstituted NOD mice with DT on day −1, day 4, and every fifth day thereafter. Sort-purified naive BDC2.5 T cells were transferred, i.p., on day 0. As depicted in Fig. 6a, recipient mice given BDC2.5 T cells and treated with DT to ablate DC failed to develop insulitis whereas PBS-treated mice did. These results indicate that DC were required for development of insulitis. They also indicated that the BM chimeras had been fully reconstituted, because had residual DT-resistant DC persisted DT treatment would have had no effect on insulitis.

**FIGURE 4.** Multiple subset analysis reveals distinct DC ablation in DT-treated CD11c-DTR mice. Splenocytes from CD11c-DTR/NOD mice treated 48 h earlier with DT were analyzed by multicolor flow cytometry. Using a series of seven-color stains of 10^6 splenocytes, we were able to determine the fine subset analysis of NOD spleen monocyte/macrophage and DC subsets. a, Splenocytes were gated on live cells and then subgated on non-T cells (exclusion of CD3ε+ and non-B cells (exclusion of B220+ and surface IgM+). Based on CD11b and CD11c expression, the remaining cells were separated into five distinct subsets of macrophages/monocytes (Region 1) or dendritic cells (Region 2 and 2a–4). Upon treatment with DT, CD11c-DTR mice lost significant levels of DC but not macrophages from all DC regions. Absolute cell numbers per compartment from 10^8 collected cells, PBS vs DT-treated: Region 1, blue, 28,820 vs 27,636; Region 2, green, 17,129 vs 891; Region 3, brown, 10,385 vs 654; Region 4, red, 11,536 vs 151. b, Fine subset analysis of each region defines multiple subsets of macrophages and DC. Subsets were established based on differential expression of surface markers. The percentage in each subset was based on the total live gated cell population. Surface markers that do not vary in a given subset are depicted in bold above each color-coded region. Subsets depicted in red were depleted to >90% by DT treatment. c, Flow cytometric analysis of CD11b/CD11c+ (Region 4) revealed that the majority of the cells in this subset were positive for both PDCA-1 and 120G8, both markers for pDC. After treatment with DT the remaining cells lacked PDCA-1 and 120G8. d, Subset analysis using the coexpressed surrogate GFP reveals that all CD11c+ subsets express the DTR. Cells from Region 2, mostly mDC, express intermediate levels of DTR whereas the pDC-rich fractions in Region 3 and Region 4 express high or low levels of DTR, respectively. The highest expression was observed in Region 2a in a population of CD11bhighCD11clow/PDCA-1− cells.
which was clearly not the case. Not surprisingly, given the lack of insulitis the ablation of DC led to a complete absence of diabetes (Fig. 6b). These data suggest that DC were required for CD4+ T cells to transfer diabetes and, therefore, likely acted as the primary APC in vivo. Moreover, these data suggest that in the absence of functioning DC the resident macrophages failed to restore the lost APC function, at least to naive CD4+ T cells.

Myeloid DC restore in vitro Ag presentation and in vivo diabetes development to DT-treated CD11c-DTR mice

Having established that the temporal ablation of DC abrogated the development of diabetes, we wished to determine the specific DC subset involved. To this end, we performed a set of in vitro and in vivo experiments with DT-treated CD11c-DTR mice. Using magnetic bead columns and cell sorting, we purified CD11b+CD11c-CD8a-M (mDC), CD11b+CD11c-CD8a+ (lyDC), and PDCA-1+Ly6G+ (pDC) mice as depicted in Fig. 7a. We then used varying numbers of each subset of DC to present a fixed concentration (0.25 μM) of mimic peptide to naive BDC2.5 T cells in culture. As depicted in Fig. 7, a and c, mDC and lyDC were highly effective and comparable APC of peptide Ag to naive BDC2.5 T cells, with 1000–3000 cells sufficient to provide maximal stimulation in a 3-day culture. However, pDC failed to stimulate BDC2.5 T cell proliferation even when >10^5 pDC were added to each culture (Fig. 7b).

We next used DT-treated CD11c-DTR/NOD.scid mice as a source of APC. CD11c-DTR/NOD.scid mice were treated with DT and 24 h later spleens from treated mice were used as APC for in vitro presentation of an antigenic mimic peptide to naive BDC2.5 T cells. Splenocytes from DT-treated mice failed to present antigenic mimic peptide or whole islet cells to naive BDC2.5 T cells, indicating that DT-mediated depletion was functionally complete. Splenic APC from DT-treated CD11c-DTR/NOD.scid mice failed to present either peptide or whole islet cells to naive BDC2.5 T cells over a wide range of Ag concentrations while APC from PBS-treated mice did, suggesting: 1) that DC were the primary APC necessary for diabetes development, and 2) that the presence of myeloid DC is essential for Ag presentation by APC.
The addition of mDC back to DT-treated CD11c-DTR/NOD.scid mice restores Ag presentation and diabetes development. a, Purification of myeloid, lymphoid, and plasmacytoid DC subsets. Subsets were purified as described in Materials and Methods. b, In vitro Ag presentation assay reveals that purified mDC (●) but not purified pDC (○) present peptide Ag to BDC2.5 T cells. Sort-purified naive BDC2.5 T cells were cultured with varying concentrations of purified DC populations in presence of mimic (0.25 µM) peptide for 72 h. c, In vitro Ag presentation assay reveal that both purified CD8α− mDC (●) and purified CD8α− lymphoid DC (□) present peptide Ag equally well to BDC2.5 T cells. No significant difference was observed in Ag-presenting capability between CD8α+ and CD8α− DC populations. Data are representative of three independent experiments. d, CD11c-DTR/NOD.scid mice were treated with DT and 24 h later splenocytes were harvested for in vitro Ag presentation experiments. A total of 1 × 10^6 DT-treated CD11c-DTR splenocytes alone (○) or supplemented with either 1 × 10^6 mDC (●) or 1 × 10^5 pDC (○) were used to present mimic peptide to sort-purified BDC2.5 T cells in a standard 72-h proliferation culture. As a positive control, 1 × 10^6 PBS-treated CD11c-DTR splenocytes were used as APC (●). e, CD11c-DTR/NOD.scid mice were treated with DT and 24 h later splenocytes were harvested for in vitro Ag presentation experiments. A total of 1 × 10^6 DT-treated CD11c-DTR splenocytes supplemented with either 1 × 10^6 mDC (●) or 1 × 10^5 pDC (○) were used to present irradiated NOD.scid islet cells to sort-purified BDC2.5 T cells in a standard 72-h proliferation culture. f, CD11c-DTR/NOD.scid BM was used to reconstitute irradiated NOD mice. After full reconstitution, mice were treated with DT on day −1 and then every 5 days thereafter and reconstituted with BDC2.5 T cells as described in Materials and Methods. In addition, on day 0 and every 5 days thereafter, recipients were either mDC or pDC from DT-resistant wild-type NOD mice. Insulitis as assessed 7 days post-transfer from mice receiving mDC, pDC, or no additional DC subsets. Peri-insulitis (open bars), Moderate insulitis (gray bars), and severe insulitis (filled bars). g, After full reconstitution, mice were treated with DT on day −1 and then every 5 days thereafter. On day 0 all mice were given T cells from 2- to 3-wk-old BDC/NOD.scid,CD11c−/−CD8a−/−CD11b−/−CD11c−/−ERTR-9 mice. Insulitis incidence is cumulative of two independent experiments.
period of intraislet inflammation after initial activation but before overt diabetes.

This critical stage, termed insulitis, progresses from a relatively benign peri-insulitis stage, where the islets are surrounded by infiltrating leukocytes but show no frank damage or loss in glucose-stimulated insulin production, to a more aggressive insulitis with marked intraislet infiltration and β cell destruction. The BDC2.5 TCR transgenic mice have well-defined kinetics of insulitis (7, 37). These mice generally are insulitis-free for the first 13–16 days of life and then show an accelerating rise in insulitis. In the absence of other T cell subsets these mice develop diabetes (7, 9, 10). If, however, the BDC2.5 T cells exist in an environment alongside other T cell subsets, especially Treg cells or NKT cells, the period of insulitis is extended and the progression to frank diabetes is markedly delayed or in some cases halted (9). Thus, the BDC2.5/CD11c-DTR/NOD mice allowed us to investigate the role that DC had on the tempo and severity of insulitis in a Treg cell-rich environment. To this end, we treated BDC2.5/CD11c-DTR/NOD mice with either DT or PBS control on day 24 of age, when most BDC2.5/NOD mice have at least ongoing peri-insulitis but little severe insulitis and no frank diabetes. We then harvested the pancreata from these mice 7 days later for histological examination. Standard H&E staining of pancreatic sections revealed a marked acceleration in the severity of insulitis in mice lacking DC (Fig. 8a) but not in those with DC (Fig. 8b). We found a substantial increase in the accumulation of moderate to severely infiltrated islets in DC-depleted mice (Fig. 8c). Interestingly, this accelerated insulitis was accompanied by a statistically significant loss of both intrapancreatic pDC and NKT cells (Fig. 8d). The loss of NKT cells cannot be attributed to direct DT-mediated ablation, because NKT cells remain largely unchanged in the spleen and non-draining secondary lymphoid compartments. The numbers of invasive BDC within the islets increased, but their overall numbers within the pancreas do not, suggesting that the diabetogenic T cells merely relocated from outside to within the islet tissue (Fig. 8d). Additionally, there was little change in the numbers of Treg (CD4⁺CD25⁺Foxp3⁺) in the pancreas (Fig. 8d).

The plasmacytoid DC regulate the tempo of insulitis

The accelerated insulitis in DT-treated BDC2.5/CD11c-DTR/NOD mice suggested that DC actively regulated the tempo and severity of established insulitis in vivo. To directly address the role of CD11b⁺CD11c⁺ (mDC and lyDC) and pDC in this regulation, we injected 24-day-old BDC2.5/CD11c-DTR/NOD mice with pDC or CD11b⁺CD11c⁺ DC from either wild-type (DT-resistant) NOD or CD11c-DTR/NOD (DT-sensitive) mice and then treated the recipients with DT to destroy the endogenous DC subsets. Seven days later mice were assessed for severity of insulitis. The

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**FIGURE 8.** Ablation of DC exacerbates pathology of ongoing insulitis and alters the absolute numbers of intrapancreatic NKT cells and pDC. a and b, Treatment of BDC2.5/CD11c-DTR/NOD mice with DT accelerated the severity of insulitis as revealed by H&E staining of formalin-fixed pancreas (a) while PBS-treated controls had moderate insulitis and peri-insulitis (b). Shown are representative photomicrographs from one of 11 DT-treated or six PBS-treated mice. From 20 to 25 islets were examined per mouse. c, Enumeration of insulitis score reveals that DT-treated mice from panel a had significantly exacerbated moderate and severe insulitis. Green, No insulitis (None); blue, peri-insulitis (Peri); purple, moderate insulitis (Mod); red, severe insulitis (Severe). Mean values for each group are in black bar, SD in colored bars. A total of 448 DT-treated islets and 234 vehicle control islets were scored. d, Absolute numbers of pDC, mDC, NKT cells (CD1d-Tet⁺), Treg (CD4⁺CD25⁺Foxp3⁺), and BDC2.5 (anti-BDC2.5⁺) T cells from pancreas, PLN and spleen of BDC2.5/CD11c-DTR/NOD mice treated 7 days prior with DT (filled bar) or vehicle control (open bar) revealed significant loss of pDC and NKT cells from pancreas. Shown are cumulative mean and SD of three independent experiments. e, Twenty-four-day-old BDC2.5/CD11c-DTR/NOD mice were given mDC from either CD11c-DTR/NOD (DTR) or wild-type (WT) NOD mice and then treated with DT; 7 days later insulitis was measured. No significant difference was observed between mice given DTR or WT mDC (p = 0.1336, severe; p = 0.4066, moderate). f, Twenty-four-day-old BDC2.5/CD11c-DTR/NOD mice were given pDC from either CD11c-DTR/NOD mice (filled bars) or wild-type NOD (stippled bars) mice and subsequently treated with DT. The restoration of wild-type DT-resistant pDC led to an increase in intrapancreatic pDC and NKT cells. Shown are cumulative mean and SD of three independent experiments. g, Twenty-four-day-old BDC2.5/CD11c-DTR/NOD mice were given pDC from either CD11c-DTR/NOD mice (DTR → DTR) or wild-type NOD (WT → DTR) and subsequently treated with DT. At 31 days of age, mice were analyzed for insulitis severity as before. Green, No insulitis (None); blue, peri-insulitis (Peri); purple, moderate insulitis (Mod); red, severe insulitis (Severe). Mean values for each group are shown in black bar, SD in colored bars; five mice per group.
addition of CD11b<sup>+</sup>CD11c<sup>+</sup> DC made no significant modulation in the frequency or severity of insulitis during the 7 days of this assay (Fig. 8e). However, the presence of supplemental DT-resistant, wild-type pDC inhibited the progression of insulitis, with most islets remaining either free of infiltration or with peri-insulitis, even though the endogenous (DT-sensitive) mDC and pDC were destroyed by the DT treatment (Fig. 8f, WT → DTR). Moreover, the resistant pDC repopulated the pancreas at the same time insulitis abated (Fig. 8g). Likewise, restoring pDC correlated with the return of NKT cells to the pancreas (Fig. 8g), suggesting a relationship between the presence of pDC and NKT cells and reduced inflammation. In contrast, DT treatment of mice that received DT-sensitive pDC from CD11c-DTR/NOD pDC developed heavily infiltrated islets (Fig. 8f, DTR → DTR). In fact, there was no significant difference between the DT-treated BDC2.5/CD11c-DTR/NOD mice depicted in Fig. 8e and those that received supplemental CD11c-DTR/NOD pDC in Fig. 8f. There was also no concomitant increase in pancreatic NKT numbers as well (Fig. 8g). Thus, the presence of pDC appeared to quiet the intrapancreatic inflammatory response and decelerate the tempo of insulitis in vivo. In addition, this reduced insulitis correlated with the accumulation of NKT cells within the pancreas.

**IDO mediates insulitis regulation**

How pDC control the tempo of insulitis is not known. However, it is known that pDC produce the T cell inhibitor IDO when stimulated by either type 1 or type 2 IFNs (43, 44). Recently, we showed that IFN-γ produced by NKT cells was critical for the regulation of BDC2.5 T cells in vivo (9). If pDC regulate the tempo of insulitis and do so by producing IDO, perhaps upon stimulation by...
NKT cells the inhibition of IDO should lead to a more aggressive insulitis, even in the presence of endogenous pDC. To test the role that IDO and pDC play in controlling the severity of established insulitis lesions, we treated 24-day-old BDC2.5/NOD mice with either anti-PDCA-1 Ab to deplete pDC or 1-MT, a potent antagonist of IDO activity, and then assessed the severity of insulitis 7 days later. We observed that the treatment of BDC2.5/NOD mice with anti-PDCA-1 ablated the pDC population within the PLN as revealed by both anti-PDCA-1 and Ly6G staining and by Ly6G and B220 staining of CD11c+ DC cells; the latter stain is not affected by residual mAb to PDCA-1. The total numbers of moderately or severely infiltrated islets was uniformly increased in all of our treated mice (Fig. 9d; anti-PDCA-1 → WT) when compared with the PBS-treated controls (Fig. 9d; PBS → DTR). We confirmed the selective and complete depletion of pDC from these mice by examining the pancreatic lymph nodes from treated mice.

When we treated BDC2.5/CD11c-DTR/NOD mice with DT, we found, as above, that the severity of insulitis increased (Fig. 9d; DT → DTR) as compared with controls (PBS → DTR). When we tested pancreatic sections from these mice for the presence or absence of localized IDO production, depletion of DC led to a marked reduction in localized IDO as revealed by anti-IDO immunohistochemistry (Fig. 9c). Thus, in the absence of DC the localized production of IDO wanes and insulitis increases in severity. This was similarly true if we retained the resident DC populations but merely inhibited the action of the locally produced IDO. Although other cells can make IDO, notably mast cells, the localized production of IDO is lost or reduced in the absence of DC, suggesting that DC either are the source of IDO or regulate its local production in the pancreas. We treated 24-day-old BDC2.5/NOD mice for 1 wk with 1-MT in their drinking water. As depicted in Fig. 9d (1MT → WT), BDC/NOD mice showed accelerated insulitis consistent with inhibition of IDO in vivo. Taken together, these findings clearly link IDO with the tempo of insulitis and are consistent with pDC as the source of regulatory IDO. Moreover, we can conclude that the tempo of insulitis in NOD mice is regulated by both pDC and IDO to the same extent and with similar kinetics in vivo.

Discussion

For autoreactive CD4+ T cells, the recognition of the target self-Ag is indirect, as most autoimmune target cells do not express MHC class II; this is certainly true for β cell Ag recognition. Therefore, the ultimate outcome of CD4+ T cell-mediated autoimmune rests upon the nature and function of the APC. APC that rapidly stimulate Ag-specific T cells by providing target Ag presentation by MHC class II, cognate TCR stimulation, secondary signals, and proinflammatory cytokines favor an autoimmune response. Likewise, APC that present self-Ags in a noninflammatory manner or produce soluble mediators that dampen T cell responsiveness will disfavor autoimmune regulation and favor immune regulation (45, 46).

Yet, to date the individual, nonoverlapping roles that APC subsets have in T1DM have remained largely unresolved. Although B cells may play a role as APC (7, 47, 48), their role is clearly not obligatory because many diabetogenic T cells, from TCR transgenic mice as well as independently isolated clones or lines, do not require B cells for their native in vivo function (6–10, 49). Moreover, both macrophages and DC are the first and principal leukocytes to infiltrate the pancreatic islets, and many of these cells are natural tissue inhabitants of the normal functioning islet (19). Therefore, understanding the role of macrophages and DC in T1DM seems preeminent and critical.

We chose to undertake a series of experiments that allowed us to physically and functionally ablate macrophages and DC from NOD mice in vivo to directly ascertain the function and necessity of individual APC subsets on the development, regulation, and progression of T1DM. Using a complementary pair of transgenic mice that drive the expression of the DTR on the surface of CD11b+ or CD11c+ leukocytes, we are able to specifically control the ablation of either macrophages or DC.

We find that the temporal ablation of CD11b+ F4/80+ macrophages from NOD mice had no preclusive effect on the function of CD4+ T cells in both transfer and spontaneous models of T1DM. Moreover, the absence of macrophages in mice that develop diabetes suggests that macrophages are not obligatory participants in the destruction of β cells, as suggested in earlier nonablative studies (15).

In contrast, the ablation of mDC dramatically forestalls the development of insulin and diabetes. These data force two important conclusions: 1) in the absence of mDC, CD4+ T cells cannot transfer T1DM to NOD mice; and 2) in the absence of DC, macrophages cannot substitute as the primary in vivo APC. Furthermore, we find that by restoring the myeloid DC (CD11b+ CD11c+) subset in DC-ablated mice we could restore both Ag stimulation of and diabetes transfer by CD4+ T cells. It should be noted that the ablation of DC subsets in CD11c-DTR/NOD mice does expose the NOD macrophage to dead and dying DC and cell debris. It is formally possible that this exposure might alter the maturation and function of the host macrophage. We find, however, no differences in the numbers and constituency of the various macrophage/monoocyte subsets in the CD11c-DTR/NOD mice treated with DT (Fig. 4). Moreover, the add back of functional DC to these mice speaks against a globally suppressive milieu in these animals or a specific "suppressive" macrophage function in these animals.

If the NOD mice have ongoing peri-insulitis or preclinical disease, the ablation of DC hastens insulitis development both in terms of severity and the numbers of islets involved. These data suggested that DC may also provide a natural check on the forward motion of insulitis. We found that the largest population of DC in the pancreas of these animals, the pDC subset (CD11c+PDCA-1 "Ly6G"), acts to reduce inflammation and insulitis, because restoring pDC to DC-depleted mice reestablishes a more temperate form of insulitis. Although we have not directly linked a particular DC subset to the localized production of IDO within the islets, recent data suggest that pDC or CD19+ DC subset(s) can produce IDO upon stimulation by type I or type II IFNs (50–52). We did, however, find a direct link between localized IDO production and the tempo and severity of insulitis that paralleled the presence of pDC. In the absence or inhibition of IDO or the loss of pDC, insulitis flourished. In their presence insulitis was largely confined to the perivascular spaces outside the islet mass. To date, we have not been able to treat BDC2.5/NOD mice for a sufficient time with either anti-PDCA-1 or 1-MT to drive these mice to frank diabetes. These are largely due to technical limitations, especially with the xenogenic nature of the anti-PDCA-1 Ab.

It is proposed that pDC and NKT cells cooperate in immune regulation and that both cell types play vital roles in limiting autoimmunity, including in T1DM (26). We have recently shown that the accumulation of NKT cells in the pancreas coincides with active regulation of BDC2.5 effector T cells (9). Additionally, the NKT cells need to make IFN-γ to assert this regulatory function (9). The target of the NKT cell-produced IFN-γ remains elusive; yet, the observations that pDC express high levels of IFN-γ receptor and that pDC can directly activate NKT cells (27) lead us to...
suspect that pDC are the target and that NKT cells and pDC act in concert to regulate insulitis. Several lines of evidence support this conjecture: 1) both NKT cells and pDC exist in the pancreas in high numbers and the ablation of one subset (pDC) leads to the relocation of the other (NKT); 2) the restoration of pDC to mice with ongoing insulitis attenuates the rate of insulitis and returns NKT cells to the pancreas; 3) NKT cells can make IFN-γ rapidly and without the requirement for de novo RNA synthesis; 4) IFN-γ itself is a potent inducer of the T cell inhibitor IDO from pDC (33); and 5) antagonism of IDO action via the treatment of NOD mice with 1-MT experiments, and we thank Dr. Mellor for the use of the rabbit anti-mouse IDO antiserum. We thank Drs. David Hildeman and Joerg 1-MT experiments, and we thank Dr. Mellor for the use of the rabbit anti-mouse IDO antiserum. We thank Drs. David Hildeman and Joerg

References

Disclosures


