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**Plasmacytoid dendritic cells from parent strains of the NZB/W F1 lupus mouse
contribute different characteristics to autoimmune propensity**

Short title: pathogenic characteristics of pDCs

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Abstract

The NZB/W F1 (F1) mice develop severe disease that is similar to human systemic lupus erythematosus. In contrast, each parent strain, NZB or NZW, has limited autoimmunity, suggesting traits of both strains contribute to pathogenesis. Although many of the contributing genes have been identified, the contributing cellular abnormality associated with each parent strain remains unresolved. Given that plasmacytoid dendritic cells (pDCs) are key to the pathogenesis of lupus, we investigated the properties of pDCs from NZB and NZW mice. We found that NZB mouse had higher numbers of pDCs; much of the increase being contributed by a more abundant CD8⁺ pDC subset. This was associated with prolonged survival and stronger proliferation of CD4⁺ T cells. In contrast, NZW pDCs had heightened capacity to produce IFN- α and IFN- λ , and promoted stronger B-cell proliferation upon CpG-stimulation. Thus, our data reveal the different functional and numerical characteristics of pDCs from NZW and NZB mouse.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with highly variable manifestation. There are several animal models of SLE with immunological abnormalities resembling those observed in humans. The NZB/W F1 mouse represents the canonical model of SLE and is generated by crossing the NZB and NZW strains¹. NZB/W F1 mice develop lupus-like disease (including anti-nuclear Ab, glomerulonephritis and proteinuria) with high incidence and severity, whereas the parental strains have limited autoimmunity, suggesting that traits of both NZB and NZW contribute to pathogenesis. This is congruent with the fact that several genes from both NZB and NZW contribute to disease development^{1,2}.

There is abundant evidence that dysregulation of type I IFN contributes to pathogenesis of SLE^{3,4}. As plasmacytoid dendritic cells (pDCs) were major producers of IFN- α , it was speculated for a long time that pDCs were drivers of lupus autoimmunity⁵. Two studies have provided cogent evidence that pDCs are essential for experimental lupus development^{6,7}. They showed that ablation of pDCs (based on BDCA2-diphtheria toxin receptor or E2-2 deficiency) dramatically ameliorated murine lupus, despite the existence of other genetic and lymphocyte abnormalities. The evidence for a role of pDCs in human SLE is also compelling⁸⁻¹⁰. For example, genes in the type I IFN pathway have been found to be associated with SLE susceptibility in genome-wide association studies and a type I IFN

expression signature is commonly found in blood cells of lupus patients. Interestingly, several treatments used in SLE, including corticosteroids, hydroxychloroquine and belimumab, have been linked to pDCs: corticosteroids can directly kill pDCs¹¹, hydroxychloroquine impairs cytokine production by pDCs¹² and BAFF, the target of belimumab, is produced by pDCs¹³. Immune complexes in SLE patient blood can activate pDCs to upregulate type I IFN expression *in vitro* and this is now believed to be the key pathogenic mechanism operative in human SLE. In addition, IFN- λ , a type III IFN that is predominantly produced by DCs including pDCs^{14, 15} has also been implicated in lupus¹⁶. Despite such evidence for the role of pDCs and IFNs in lupus, the cellular contribution of pDCs from the NZW and NZB strains has until now been ignored.

Comparison of pDCs among different laboratory mouse strains revealed that different mouse strains vary in pDC abundance and IFN- α production^{17, 18}. Of note, the higher production of IFN α in response to CpG observed with some mouse strains was not due to an intrinsic higher ability of pDC, rather it reflected the higher abundance of spleen pDCs in the mouse strain¹⁷. We and others had recently found that pDCs from NZB/W F1 mice produce more IFN- α , *in vitro* due to a survival advantage compared to C57BL/6 mice^{19, 20}. However, it is unclear what functional properties of parent strains are and what contribution of these properties is likely to autoimmune propensity of NZB/W F1 mice.

Here we investigate the phenotypes and function of pDCs from parent strains and F1 mice. We revealed that NZB mouse contained a higher proportion of pDCs expressing CD8. Functionally, NZW pDCs had heightened capacity to produce IFN- α to TLR9 stimulation and promoted a stronger CpG-stimulated B-cell proliferation than NZB pDCs. The significance of these differences was discussed in the context of autoimmune lupus.

RESULTS

NZB and NZW mice have different numbers of pDCs

pDCs of different mouse strains may have different properties^{17, 19, 20, 24}. Here we investigated the pDC difference among NZB, NZW and NZB/W F1 mice. We defined pDCs by flow cytometry as Siglec H⁺CD317⁺CD11b⁺CD11c^{int} (Figure 1a). To avoid any interference due to overt autoimmunity, 6-8 week old mice were analyzed. We observed that the numbers of total spleen pDCs of NZB and F1 were higher than of NZW mice ($P < 0.05$; Figure 1b). This difference was even more remarkable for the pDCs with CD8 expression; NZB and F1 CD8⁺ pDCs were 4 times more abundant than NZW CD8⁺ pDCs ($P < 0.01$;

Figure 1b). The proportion of CD4⁺ DCs were similar between 3 strains (Figure 1b). Thus, the increase in pDC numbers in NZB and F1 mice were largely due to the CD8⁺ subset.

To determine whether pDC difference between NZB and NZW mice was cell-intrinsic, we made mixed chimera in which irradiated F1 mice were reconstituted with both NZB and NZW bone marrow (BM) cells that were distinguished by expression of H-K^d by NZB cells. Consistent with the findings above the proportion of CD8⁺ pDCs derived from NZB BM was almost 3 times higher than those originating from NZW BM ($P < 0.01$; Figure 1c). Importantly the capacity of NZB and NZW to reconstitute the CD4⁺ pDC, T and B cell compartments was found to be similar between genotypes.

Enhanced survival of NZB pDCs *in vitro*

Previously, we and others had shown that NZB/W F1 pDCs survived better than C57BL/6 pDCs *in vitro* and *in vivo*^{19, 20, 24}. We decided to extend such studies to the two parent strains. In order of pDC survival, NZB pDCs survived better than NZW pDCs while NZW pDCs survived better than C57BL/6 pDCs (Figure 2a). NZB pDCs survived in significantly greater numbers than NZW pDCs with or without CpG stimulation (Figure 2b). As reported for C57BL/6 mice²⁴, CpG stimulation also enhanced *in vitro* survival of pDCs from NZB and NZW mice (Figure 2b). Survival of NZB/W F1 pDCs was intermediate between NZB and NZW pDCs (Figure 2c). In an attempt to dissect the molecular basis for survival difference of pDCs, we investigated the expression of several molecules that regulate pDC survival. BCL-2 and MCL-1 are anti-apoptotic molecules expressed by pDCs and key to pDC survival²³. However, there was no difference in expression levels of these two molecules by pDCs between the 3 strains (Figure 2d).

We have previously published that pDC survival is dependent on the anti-apoptotic molecule BCL-2^{19, 23}. In light of the differential abundance of the CD8⁺ subset above, we decided to investigate the role of BCL-2 on this subset. As expected, compared with WT, BCL-2^{-/-} mice had greatly reduced numbers of CD8⁺ pDCs (Supplementary figure 1a) and conversely BCL-2 transgenic mice had higher numbers of CD8⁺ pDCs (Supplementary figure 1b). BCL-2 antagonism by ABT-199 treatment also reduced CD8⁺ pDC numbers (Supplementary figure 1c). Bim^{-/-} and Noxa^{-/-} but not Puma^{-/-} mice had increased CD8⁺ pDC numbers (Supplementary figure 1c).

NZW pDCs produced more IFN- α and IFN- λ

pDCs produce abundant amounts of type I IFN^{25, 26}. We and others had found that pDCs from NZB/W F1 mice produce more IFN- α *in vitro*, compared to C57BL/6 mice^{19, 20}.

Therefore, to test the capacity of pDCs isolated from NZW, NZB and F1 mice to produce IFN- α upon CpG stimulation, we measured IFN- α production by intracellular staining and IFN- α secretion by ELISA. The percentage of IFN- α producing pDCs was highest for NZW (4.3%), intermediate for F1 (1.8%) and lowest for NZB (0.8%) (Figure 3a). Consistently with the above findings, culture supernatants of CpG activated pDCs isolated from NZW have the higher concentration of IFN- α , followed by CpG activated NZB/W pDCs, with NZB/W producing the least IFN- α after CpG stimulation (Figure 3b). To dissect early signalling events that might explain the difference in IFN α production, expression of TLR9, IRF7 and IRF3 in pDCs with or without CpG stimulation was evaluated by qRT-PCR. We did not observe a conspicuous difference to explain the higher IFN- α production by NZW pDCs (Supplementary figure 2).

In addition, emerging evidence demonstrated that DCs including pDCs also produce abundant amounts of IFN- λ , a type III IFN^{14, 15}. We investigated the production of IFN- λ by pDCs from NZW, NZB and F1 mice under CpG stimulation. Similar to Type I IFN, splenic pDCs from NZW mice produced more IFN- λ than NZB pDCs (Figure 3c).

NZW pDCs more potently promoted CpG-stimulated B cell proliferation

Given that induction of autoantibody is a key feature of lupus, we examined how pDCs from parent strains might influence B cells. CTV-labelled naïve B cells from NZB/W F1 mice were cultured with pDCs from either NZB or NZW for various durations. We found that NZW pDCs stimulated a stronger B-cell proliferative response than for NZB pDCs, although both sources of pDCs showed enhanced B-cell proliferation over cultures without pDCs (Figure 4a&b). Analysis of total cohort number that deduces cell survival²⁷ indicated that pDCs conferred a survival advantage on B cells but there was no difference between the two pDC genotypes (Figure 4b). Analysis of mean division number over the culture duration indicated that NZW pDCs conferred a proliferative advantage on B cells (Figure 4b) i.e. B cells cultured with NZW pDCs divided at a faster rate compared to B cells with NZB pDCs (Figure 4b). Overall, it appears that pDCs from both strains promote B-cell survival, however, NZW pDCs stimulated a stronger CpG-induced proliferative response than did NZB pDCs. Cultures with NZW pDCs also had a higher proportion of CD138⁺ plasma cells than those with NZB pDCs (16% vs 5%; $P<0.05$; Figure 4c). Thus, NZW pDCs promoted greater B cell proliferation and differentiation than NZB pDCs.

CD8⁺ pDCs expressed higher levels of class II and stimulated greater CD4⁺ T cell proliferation

When spleen pDCs from the three strains were analyzed for expression of molecules that are critical for antigen presentation, we found that NZW pDCs expressed significantly lower levels of MHC class II than NZB/W F1 and NZB pDCs (Figure 5a). Separation of pDCs of three strains into CD8⁺ and CD8⁻ pDCs showed a consistent pattern that CD8⁺ pDCs of all strains expressed more cell surface MHC class II than CD8⁻ pDCs of corresponding mouse strains (Figure 5a). We did not detect a difference in CD86 expression (data not shown).

As NZB and NZW mice have different MHC-I and -II, we could not easily compare T cell stimulation using the same T cell source. Therefore, we compare the functional ability of CD8⁺ versus CD8⁻ pDC subsets from the same genetic background (C57BL/6). Even without stimulation, CD8⁺ pDCs from C57BL/6 mice expressed more MHC-II (Figure 5b). Accordingly, we found that CD8⁺ pDCs stimulated stronger antigen-specific proliferation of CD4⁺ T cells (OT-II) but not CD8⁺ T cells (OT-I) (Figure 5c). Thus, CD8⁺ pDCs and CD8⁻ pDCs are functionally distinct, at least *in vitro*.

pDC depletion does not alter Foxp3⁺ Treg abundance

It has recently been reported that NZW mice have fewer Foxp3⁺CD25⁺ regulatory T cells (Treg)²⁸, a finding we confirmed here (Figure 6a). As we have shown above that NZB mice have increased numbers of pDCs, especially CD8⁺ pDCs, we investigated whether removal of pDCs in NZB would affect Treg numbers. Hence, we backcrossed Siglec H-diphtheria toxin receptor transgenic BALB/c mice onto a NZB background over 8 generations. Treatment with diphtheria toxin virtually ablated Siglec H⁺ cells in the spleen (Figure 6b), blood and BM (not shown). However, depletion of pDCs over 7 days did not result in any significant changes in Treg abundance in spleens (Figure 6c) and peripheral lymph nodes (not shown). Indeed, the lower proportion of Treg in the NZW strain is cell intrinsic as the lower proportions were recapitulated in mixed BM irradiation chimeras (Figure 6d), thus supporting the lack of involvement by pDC genotype.

DISCUSSION

pDCs and their product type I IFN have long been associated with lupus autoimmunity. Two recent reports have cogently confirmed the pivotal role of pDCs whereby genetic or pharmacological ablation of pDCs prevents lupus development in murine models^{6, 7}. Even though the genes involved in producing the lupus phenotype in NZB/W F1 mice have been known for twenty years, the cellular contributions of the parent strains remain undefined, although various abnormalities have been noted in T and B cells²⁸⁻³¹.

The most pronounced difference in the pDCs of NZB contained higher proportion of CD8⁺ fraction. Although CD8⁺ pDCs are often described as a subset³², questions remain as to the stability of such a subset given that CD8 expression by pDCs is readily induced by activation^{33, 34} (Supplementary figure 3). Our observation that NZW pDCs and NZB pDCs maintained their CD8 identity when they co-exist in competitive chimera F1 mice would suggest that CD8 expression is not merely a feature endowed by the environment. Despite CD8⁺ pDCs being reported as more potent inducer of Treg cell differentiation than, we did not observe an overt change in Treg *in vivo* upon pDC depletion³³. Thus, an essential role for pDCs to maintain Treg homeostasis remains to be established.

Prolonging DC life span by manipulation of BCL-2 regulated apoptosis pathway can lead to autoimmunity^{35, 36}. Relative to non-autoimmune prone C57BL/6 mice, pDCs from NZB/W F1 mice survival better *in vitro*^{19, 20}. In this study, we found that NZB pDCs survived longer *in vitro* than NZW pDCs. Whereas the shorter survival of C57BL/6 pDCs can be explained by low expression of BCL-2, a key pro-survival molecule for pDCs¹⁹, the molecular basis for the inferior survival of NZW pDCs relative to NZB pDCs is less clear as both BCL-2 and MCL-1 were normally expressed. We propose that differential pDC survival may have some implications in lupus. For example, we showed previously that pDCs with high BCL-2 expression lived longer and became resistant to glucocorticoid-mediated killing but remained sensitive to BCL-2 antagonist¹⁹.

pDCs are major producers of IFN- α ⁵. It has been noted that different mouse strains vary in capacity of IFN- α production^{17, 18}. We and others had recently found that pDCs from NZB/W F1 mice produce more IFN- α *in vitro*, compared to C57BL/6 mice^{19, 20}. Here we revealed further that between two parent strains, NZW pDCs are potent producer of IFN- α than NZB. IFN- α and - β production by pDCs relies on the signalling through TLR and the transcription factors IRF-3 and IRF-7³⁷. Despite the observation that NZW pDCs and NZB pDCs greatly differ in capacity to produce IFN- α , we found equivalent expression of IRF-3, IRF-7 and TLR9 in pDCs from each strain, suggesting that other factors are involved in controlling the extent of IFN- α expression upon CpG stimulation. Adding to complexity of IFN contribution to lupus, IFN- λ , also produced by pDCs^{4, 15} has also been implicated in lupus¹⁶. Here we showed that pattern of IFN- λ expression by pDCs from three mouse strains was closely similar to pattern of IFN- α expression. Regulation of Type I IFN and Type III expression by pDCs as well as other myeloid cells are rather complicated³⁸. The stimulating ligands and transcriptional factors required for two types of IFNs showed subtle differences³⁸.

Lupus is characterized by production of autoantibodies by B cells. pDCs, either via IFN α dependent or independent fashion, promote B-cell proliferation and plasma cell differentiation³⁹⁻⁴¹. Perhaps due to higher production of IFN- α and other cytokines, B cells cultured with NZW pDCs undergo a stronger CpG-stimulated proliferative response, compared to cultures without pDCs or NZB pDCs. Although CD70 costimulation by pDCs has been demonstrated as a mechanism for pDCs to promote B cell response⁴¹, we did not detect a difference in CD70 expression between NZB and NZW pDCs (data not shown).

In some circumstances, Tregs are critical for maintenance of immune tolerance. Homeostasis of Treg has been shown to be influenced by various types of DC⁴². Loss of DC led to a loss of Treg, and the DC-dependent loss in Treg led to a heightened inflammatory response⁴³. In the context of autoimmunity of NZB/W mice, NZW mice have been shown to have Treg deficiency²⁸. Thus, the marked difference in CD8⁺ pDCs between NZW and NZB mice prompted us to examine the contribution of pDCs to Treg homeostasis. We found that depletion of pDCs in NZB mice did not alter the abundance of Tregs, suggesting that difference in pDCs between NZW and NZB mice does not explain the Treg defect in NZW mice.

As mouse pDCs is known to be critical for disease onset in NZB/W F1 mice^{6, 7} and we revealed here that pDCs of parent strains differ in immune functions *in vitro*, it still leaves a question whether individual functional characteristics of pDCs from parent strains contribute to autoimmune propensity of NZB/W F1 mouse. By pDC transfer, we showed that NZW pDCs indeed promote development of autoimmune B-cell responses in F1 mice (Supplementary figure 4). Together our study provides the cellular pDC basis for the contributions of each parent strain to the pathogenesis of NZB/W F1 lupus. It would be of interest to determine whether these abnormal characteristics of pDCs identified in the mouse model of lupus are also linked to human lupus.

METHODS

Mice. NZB, NZW, NZB/W F1, vav-BCL-2–transgenic mice, BCL-2^{-/-} mice, Bim^{-/-}, Puma^{-/-}, Noxa^{-/-} mice, OT-I and OT-II on C57BL/6 background and C57BL/6 mice were bred under specific pathogen-free conditions in the animal facility of the Walter and Eliza Hall Institute of Medical Research (WEHI). Siglec H-DTR–Tg NZB mice were derived by backcrossing Siglec H-DTR transgenic mice on BALB/C background that were originally generated and bred at Nanyang Technological University²¹. Experiments were performed according to the

guidelines of the Institute's Animal Ethics Committee (Ethic numbers #2014.023 and 2016.014).

Flow cytometry and antibodies. Single-cell suspensions were stained: CD11c (HL3), Siglec H (eBio440c), CD317 (eBio927), Foxp3 (FJK-16s) (eBioscience, San Diego, CA), CD11b (M1/70), MHC class II I-A/I-E (M5/114.15.2), Ly6C (AL-21), Ly6G (IA8), CD19 (ID3), CD3 (KT3), CD4 (RM4-5), CD8 (53.67), CD25 (PC61), CD138 (281-2), B220 (RA3-6B2, Biolegend), Fc R1 α (MAR-1), CD45.1 (A20), CD45.2 (104) and H-2K^d (clone SF1-1.1). All Abs were from BD Biosciences (San Jose, CA), except stated otherwise. Analysis was performed on a BD LSRFortessa and FACSverse and sorting was done on BD FACSAria and BD Influx. Data were analyzed using FlowJo software (Tree Star, Ashland, OR).

IFN assay. Detection of IFN- α and IFN- α -producing cells has been described previously¹⁹. For measurement of mouse IFN levels, culture supernatants were harvested, and the concentrations of IFN were analyzed by enzyme-linked immunosorbent assay (ELISA). For IFN- α detection, the capture antibody was RMMA-1 (PBL Interferon Source). Polyclonal rabbit anti-IFN- α (PBL Assay Science) was used for detection, followed by peroxidase-conjugated anti-rabbit antibody. For IFN- λ detection, monoclonal rat anti-IFN- λ 2/3 antibody (clone 244716) was used for capture and biotinylated monoclonal rat anti-IFN- λ abs (both from R&D Systems), followed by peroxidase-conjugated streptavidin. Cytokine concentrations were interpolated from a standard curve. For intracellular staining of IFN- α , cells were stimulated with CpG for various times, adding Golgi Stop 4 hours before harvest. Cells were then stained for cell surface markers, fixed and then FITC conjugated anti-IFN- α Abs (BD bioscience).

B-cell isolation and cell culture. B-cell isolation and stimulation has been described previously²². Small dense cells from spleen cells of NZB/W F1 mice were collected from the 65–80% interface on Percoll gradient and B cells purified via negative selection using magnetic bead B-cell isolation kits (Miltenyi Biotec, Auburn, CA). For division-tracking experiments, cells were labelled with CTV. B cells were typically >95% B220⁺, CD19⁺, IgM⁺ and IgD⁺ as determined by flow cytometry. B cells were cultured in B-cell medium comprising RPMI 1640 medium (Gibco BRL) plus 10% FCS. B cells (50000 cells/well) were stimulated with 3 μ M CpG DNA oligonucleotide CpG-1668, fully phosphorothioated (sequence, 5'-TCCATGACGTTTCCTGATGCT-3'; Geneworks, Adelaide, South Australia, Australia). Spleen pDCs (50000 cells/well) from NZB or NZW mice were included in half of B-cell culture. To determine absolute cell numbers, the number of live cells per culture

was estimated by reference to a known number of Calibrite beads (Becton Dickinson, San Jose, CA), added directly to cell culture before collecting.

Detection of cell bounded autoantibody and CD138⁺ B cells by FACS. The procedure for detection of anti-erythrocyte autoantibodies by flow cytometry was described previously¹⁹. Erythrocyte-bound autoantibodies were detected by incubation of extensively washed red blood cells with biotinylated goat anti-mouse IgG and then streptavidin-PE. The geometric mean fluorescence intensity was calculated by subtracting the background with streptavidin-PE only. B220^{low}CD138⁺ cells were detected after incubating spleen cells with anti-CD138, CD19 and B220 Abs.

DC survival assays. Cell survival assays were performed in the following 2 ways: 1) DC-enriched spleen cells were cultured at $1-2 \times 10^5$ in 200 μ L medium in flat-bottomed 96-well plates. Upon harvesting, cells were stained for cell surface markers. Cell survival was measured by flow cytometry with PE- or APC-conjugated fluorescence-activated cell sorting (FACS) calibration beads (CaliBrite; BD Biosciences) and propidium iodide (PI) to enumerate viable cells. 2) DC subsets were purified by FACS sorting (95% pure). Purified cell subsets were cultured at $1-2 \times 10^4$ in 200 μ L medium in flat-bottomed 96-well plates. Surviving cells were enumerated by flow cytometry.

In vitro T cell proliferation. Single-cell suspensions from spleens of C57BL/6 mice, OT-I and OT-II mice were enriched using FACS sorting. Enriched CD4⁺ T cells and CD8⁺ T cells (purity >95%) were labelled with CellTrace Violet (CTV; Life Technologies, Grand Island, NY) and cultured with enriched pDCs (purity > 95%) with or without soluble OVA Ag (OVA protein 0.1 and 0.5 mg/mL for 3 days. Proliferation was evaluated by dilution of CTV.

Protein Isolation and Western Blot Analysis. Detection of anti-apoptotic BCL-2 and MCL-1 proteins by Western blot analysis has been described previously²³. Primary antibodies used in this study were mouse anti-BCL-2 mAb (cat. no. sc-7382 from Santa Cruz), rat anti-MCL-1 mAb (clone 19C4-15, kindly provided by David Huang at WEHI), and goat anti- β -actin HRP (cat. no. sc-1616 HRP from Santa Cruz). Secondary antibodies used were goat anti-mouse-IgG1-HRP (cat. no. 1070-05) or goat anti-rat-Ig-HRP (cat. no. 3010-05; both from Southern Biotech).

pDC transfer. pDCs from spleens and bone marrows of female NZB and NZW mice were purified by positive selection with PE-conjugated anti-Siglec H Ab and followed anti-PE beads (Miltenyi Biotec, Auburn, CA). Female NZB/W F1 mice were given three weekly intravenous injections of 10^6 purified pDCs. Cell bound autoantibodies of individual F1 mice

before transfer and post transfer (1 week after last transfer) were evaluated. Spleen B cells and B220^{lo}CD138⁺ cells were evaluated 1 week after last transfer.

***In vivo* pDC depletion and evaluation of Treg cells.** For pDC ablation, Siglec H-DTR NZB mice were injected i.p. with DT (0.2 µg/mouse) every 2 day for 6 days. Spleens, blood and bones were harvested 1 day after the last doses. pDC depletion and spleen T cells compartment was evaluated.

qRT-PCR methods and primers. RNAs were isolated using RNeasy Plus Mini Kit according to the manufacturer's recommendations. cDNAs were synthesized from total RNAs with using the 5x iScript cDNA Synthesis Kit (Biorad) following the manufacturer's instructions. Amplification was performed with SYBR green master mix (Biolabs) on a Biorad CFX 384 using the indicated primers (Supplementary table 1).

Statistical analysis. Mean and SEM values were calculated with Graphpad Prism Software. *P*-values for two group comparison were calculated by two-tailed unpaired t test. For multiple group comparisons, *P*-values were generated by an ANOVA analysis followed by multiple comparisons. Presented data are from a single experiment of 3 or 4 repeated experiments. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, *n.s.*= not statistically significant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Figure legends:

Figure 1. NZB pDCs contain a larger CD8⁺ cohort. Spleen cells were prepared from female age matched NZB (n=13), NZW (n=13) and NZB/W F1 mice (n=16). pDC number and phenotype were evaluated. **(a)** Density plots show the proportion of pDCs in total spleen cells [upper] and the expression of CD4 and CD8 in gated pDCs for each strain. **(b)** Number and percentage of total pDCs, CD8⁺ pDCs and CD4⁺ pDCs are shown. **(c)** pDC composition in irradiated NZB/W F1 chimera mice (n=4) reconstituted with NZB BM cells (H-Kd⁺) and NZW BM cells (H-Kd⁻). **P*<0.05, ***P*<0.01. Data are from one of 3 or 4 repeated experiments.

Figure 2. NZB pDCs have superior survival *in vitro*. **(a)** Purified spleen pDCs from NZB, NZW and B6 mice were cultured (1000 cells/well of triplicates) overnight. Viable pDCs were enumerated. **(b)** Purified spleen pDCs from NZB and NZW mice were cultured (10000 cells/well of triplicates) ±CpG over 1-3 days. **(c)** Purified spleen pDCs from NZB, NZW and NZB/W mice were cultured (10000 cells/well of triplicates) overnight. Bar graphs show the mean number and SD of viable pDCs. **P*<0.05, ** *P*<0.01 and *** *P*<0.001. Data shown are from a single representative experiment of 2 or 3 experiments. **(d)** Western blot analysis was performed on sorted pDCs to assess the amounts of MCL-1, BCL-2, and β-actin (loading control) proteins.

Figure 3. NZW pDCs are potent IFN- α producers. (a) Spleen cells were stimulated with CpG for 4 hrs and then performed intracellular staining for IFN- α . FACS plots show IFN- α production by gated pDCs. Scatter plots show percentage of IFN- α producing pDCs from individual age-matched NZB (n=9), NZW (n=6) and F1 (n=8) female mice. Data were pooled from 3 experiments. (b) pDCs were purified and stimulated for overnight. IFN- α levels in culture supernatants of 3 individual mice each group were shown. (c) pDCs were purified from 3 or 4 individual female mice and stimulated with CpG for overnight. IFN- λ levels in culture supernatants of individual mice each group were shown. * $P < 0.05$, ** $P < 0.01$. Two (IFN- λ) to three (IFN- α) experiments were performed with similar results.

Figure 4. NZW pDCs are more potent to enhance CpG-stimulated B cell response *in vitro*. CTV-labelled naïve B cells from NZB/W mice were cultured with pDCs from either NZB or NZW for various times. B-cell proliferation and CD138 expression was evaluated. (a) CTV profile of B cell proliferation either cultured with pDCs from NZB (thin solid line), From NZW (thick solid line) or without pDCs (filled), upon stimulation with CpG (3 μ M). (b) Quantitative data of total B cell number, total cohort number and mean division number over time. (c) Representative plot of expression of CD138 on B cells under different culture conditions harvested at 68 hrs.

Figure 5. CD8⁺ pDCs stimulate a stronger proliferation of CD4⁺ T cells. (a) Spleen pDCs from three strains (6-7 individual mice per strain) were analysed for expression of MHC class II by total pDCs (upper panel) and separated pDCs based on CD8 expression (lower panel). Histograms show MHC class II expression. Bar graphs show MFI of MHC class II. *** $P < 0.001$, **** $P < 0.0001$. (b) Bar graphs show MFI of MHC class II on spleen CD8⁺ and CD8⁻ pDCs from 4 individual C57BL/6 mice. ** $P < 0.01$, *** $P < 0.001$. C. CD8⁺ and CD8⁻ pDCs were sorted from C57BL/6 mice. Purified OT-I and OT-II T cells were purified and labelled with CTV. T cells (50000/well) were then co-cultured with pDCs (10000/well) with soluble OVA for 3 days. Bar graphs show mean number and SD of proliferating T cells.

Figure 6. pDC depletion does not reduce Treg abundance in NZB mice. (a) Spleen cells from NZB, NZW and NZB/W F1 mice (4 each) were analysed for T cells. FACS plots show total spleen cells, spleen T cells and Spleen CD4⁺ T cells respectively. Bar graphs show the

mean and SEM of 4 individual mice. $**P<0.01$, $***P<0.001$. Three repeated experiments were performed with similar results. B. Siglec H-DTR NZB mice were injected with DT 200ng/dose/mouse every 2 day for 6 days. 1 day after the last dose, organs were harvested. **(b)** FACS plots show spleen DC composition of DT treated (n=3) and untreated mice (n=4). Bar graph shows the number of spleen pDCs. **(c)** FACS plots show expression of CD25 and Foxp3 on gated CD4⁺ T cells. Bar graph the percentage of Tregs. $***P<0.001$. Two experiments were performed. **(d)** Treg cell numbers in 4 irradiated NZB/W F1 chimera mice reconstituted with NZB BM cells (H-Kd⁺) and NZW BM cells (H-Kd⁻). $***P<0.001$. Data are from one of 2 repeated experiments.

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