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9 **Plasmacytoid dendritic cells from parent strains of the NZB/W F1 lupus mouse**
10 **contribute different characteristics to autoimmune propensity**
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12 Short title: pathogenic characteristics of pDCs
13
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33 **Abstract**

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

46

47

48 **INTRODUCTION**

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

57 There is abundant evidence that dysregulation of type I IFN contributes to
58 pathogenesis of SLE[3, 4]. As plasmacytoid dendritic cells (pDCs) were major producers of
59 IFN-α, it was speculated for a long time that pDCs were drivers of lupus autoimmunity[5].
60 Two studies have provided cogent evidence that pDCs are essential for experimental lupus
61 development[6, 7]. They showed that ablation of pDCs (based on BDCA2-diphtheria toxin
62 receptor or E2-2 deficiency) dramatically ameliorated murine lupus, despite the existence of
63 other genetic and lymphocyte abnormalities. The evidence for a role of pDCs in human SLE
64 is also compelling[8-10]. For example, genes in the type I IFN pathway have been found to be
65 associated with SLE susceptibility in genome-wide association studies and a type I IFN

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66 expression signature is commonly found in blood cells of lupus patients. Interestingly,
67 several treatments used in SLE, including corticosteroids, hydroxychloroquine and
68 belimumab, have been linked to pDCs: corticosteroids can directly kill pDCs[11],
69 hydroxychloroquine impairs cytokine production by pDCs [12] and BAFF, the target of
70 belimumab, is produced by pDCs[13]. Immune complexes in SLE patient blood can activate
71 pDCs to upregulate type I IFN expression *in vitro* and this is now believed to be the key
72 pathogenic mechanism operative in human SLE. In addition, IFN-λ , a type III IFN that is
73 predominantly produced by DCs including pDCs[14, 15] has also been implicated in lupus[16].
74 Despite such evidence for the role of pDCs and IFNs in lupus, the cellular contribution of
75 pDCs from the NZW and NZB strains has until now been ignored.

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

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

89 **RESULTS**

90 **NZB and NZW mice have different numbers of pDCs**

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

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98 Figure 1b). The proportion of CD4$^+$ DCs were similar between 3 strains (Figure 1b). Thus,
99 the increase in pDC numbers in NZB and F1 mice were largely due to the CD8$^+$ subset.
100 To determine whether pDC difference between NZB and NZW mice was cell-
101 intrinsic, we made mixed chimera in which irradiated F1 mice were reconstituted with both
102 NZB and NZW bone marrow (BM) cells that were distinguished by expression of H-K^d by
103 NZB cells. Consistent with the findings above the proportion of CD8$^+$ pDCs derived from
104 NZB BM was almost 3 times higher than those originating from NZW BM (P<0.01; Figure
105 1c). Importantly the capacity of NZB and NZW to reconstitute the CD4$^+$ pDC, T and B cell
106 compartments was found to be similar between genotypes.

107 **Enhanced survival of NZB pDCs *in vitro***

108 Previously, we and others had shown that NZB/W F1 pDCs survived better than
109 C57BL/6 pDCs *in vitro* and *in vivo*[19, 20, 24]. We decided to extend such studies to the two
110 parent strains. In order of pDC survival, NZB pDCs survived better than NZW pDCs while
111 NZW pDCs survived better than C57BL/6 pDCs (Figure 2a). NZB pDCs survived in
112 significantly greater numbers than NZW pDCs with or without CpG stimulation (Figure 2b).
113 As reported for C57BL/6 mice[24], CpG stimulation also enhanced *in vitro* survival of pDCs
114 from NZB and NZW mice (Figure 2b). Survival of NZB/W F1 pDCs was intermediate
115 between and NZB and NZW pDCs (Figure 2c). In an attempt to dissect the molecular basis
116 for survival difference of pDCs, we investigated the expression of several molecules that
117 regulate pDC survival. BCL-2 and MCL-1 are anti-apoptotic molecules expressed by pDCs
118 and key to pDC survival[23]. However, there was no difference in expression levels of these
119 two molecules by pDCs between the 3 strains (Figure 2d).
120 We have previously published that pDC survival is dependent on the anti-apoptotic
121 molecule BCL-2 [19, 23]. In light of the differential abundance of the CD8$^+$ subset above, we
122 decided to investigate the role of BCL-2 on this subset. As expected, compared with WT,
123 BCL-2-/- mice had greatly reduced numbers of CD8$^+$ pDCs (Supplementary figure 1a) and
124 conversely BCL-2 transgenic mice had higher numbers of CD8$^+$ pDCs (Supplementary figure
125 1b). BCL-2 antagonism by ABT-199 treatment also reduced CD8$^+$ pDC numbers
126 (Supplementary figure 1c). Bim-/- and Noxa-/- but not Puma-/- mice had increased CD8$^+$
127 pDC numbers (Supplementary figure 1c).

128 **NZW pDCs produced more IFN-α and IFN-λ**

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

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131 Therefore, to test the capacity of pDCs isolated from NZW, NZB and F1 mice to produce
132 IFN-α upon CpG stimulation, we measured IFN-α production by intracellular staining and
133 IFN-α secretion by ELISA. The percentage of IFN-α producing pDCs was highest for NZW
134 (4.3%), intermediate for F1 (1.8%) and lowest for NZB (0.8%) (Figure 3a). Consistently with
135 the above findings, culture supernatants of CpG activated pDCs isolated from NZW have the
136 higher concentration of IFN-α, followed by CpG activated NZB/W pDCs, with NZB/W
137 producing the least IFN-α after CpG stimulation (Figure 3b). To dissect early signalling
138 events that might explain the difference in IFNα production, expression of TLR9, IRF7 and
139 IRF3 in pDCs with or without CpG stimulation was evaluated by qRT-PCR. We did not
140 observe a conspicuous difference to explain the higher IFN-α production by NZW pDCs
141 (Supplementary figure 2).
142 In addition, emerging evidence demonstrated that DCs including pDCs also produce
143 abundant amounts of IFN-λ, a type III IFN[14, 15]. We investigated the production of IFN-λ by
144 pDCs from NZW, NZB and F1 mice under CpG stimulation. Similar to Type I IFN, splenic
145 pDCs from NZW mice produced more IFN-λ than NZB pDCs (Figure 3c).
146 **NZW pDCs more potently promoted CpG-stimulated B cell proliferation**
147 Given that induction of autoantibody is a key feature of lupus, we examined how
148 pDCs from parent strains might influence B cells. CTV-labelled naïve B cells from NZB/W
149 F1 mice were cultured with pDCs from either NZB or NZW for various durations. We found
150 that NZW pDCs stimulated a stronger B-cell proliferative response than for NZB pDCs,
151 although both sources of pDCs showed enhanced B-cell proliferation over cultures without
152 pDCs (Figure 4a&b). Analysis of total cohort number that deduces cell survival[27] indicated
153 that pDCs conferred a survival advantage on B cells but there was no difference between the
154 two pDC genotypes (Figure 4b). Analysis of mean division number over the culture duration
155 indicated that NZW pDCs conferred a proliferative advantage on B cells (Figure 4b) i.e. B
156 cells cultured with NZW pDCs divided at a faster rate compared to B cells with NZB pDCs
157 (Figure 4b). Overall, it appears that pDCs from both strains promote B-cell survival, however,
158 NZW pDCs stimulated a stronger CpG-induced proliferative response than did NZB pDCs.
159 Cultures with NZW pDCs also had a higher proportion of $CD138^+$ plasma cells than those
160 with NZB pDCs (16% vs 5%; $P<0.05$; Figure 4c). Thus, NZW pDCs promoted greater B cell
161 proliferation and differentiation than NZB pDCs.
162 **$CD8^+$ pDCs expressed higher levels of class II and stimulated greater $CD4^+$ T cell**
163 **proliferation**

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164 When spleen pDCs from the three strains were analyzed for expression of molecules
165 that are critical for antigen presentation, we found that NZW pDCs expressed significantly
166 lower levels of MHC class II than NZB/W F1 and NZB pDCs (Figure 5a). Separation of
167 pDCs of three strains into $CD8^+$ and $CD8^-$ pDCs showed a consistent pattern that $CD8^+$ pDCs
168 of all strains expressed more cell surface MHC class II than $CD8^-$ pDCs of corresponding
169 mouse strains (Figure 5a). We did not detect a difference in CD86 expression (data not
170 shown).
171 As NZB and NZW mice have different MHC-I and -II, we could not easily compare T
172 cell stimulation using the same T cell source. Therefore, we compare the functional ability of
173 $CD8^+$ versus $CD8^-$ pDC subsets from the same genetic background (C57BL/6). Even without
174 stimulation, $CD8^+$ pDCs from C57BL/6 mice expressed more MHC-II (Figure 5b).
175 Accordingly, we found that $CD8^+$ pDCs stimulated stronger antigen-specific proliferation of
176 $CD4^+$ T cells (OT-II) but not $CD8^+$ T cells (OT-I) (Figure 5c). Thus, $CD8^+$ pDCs and $CD8^-$
177 pDCs are functionally distinct, at least *in vitro*.
178 **pDC depletion does not alter $Foxp3^+$ Treg abundance**
179 It has recently been reported that NZW mice have fewer $Foxp3^+CD25^+$ regulatory T
180 cells (Treg)[28], a finding we confirmed here (Figure 6a). As we have shown above that NZB
181 mice have increased numbers of pDCs, especially $CD8^+$ pDCs, we investigated whether
182 removal of pDCs in NZB would affect Treg numbers. Hence, we backcrossed Siglec H-
183 diphtheria toxin receptor transgenic BALB/c mice onto a NZB background over 8
184 generations. Treatment with diphtheria toxin virtually ablated Siglec H^+ cells in the spleen
185 (Figure 6b), blood and BM (not shown). However, depletion of pDCs over 7 days did not
186 result in any significant changes in Treg abundance in spleens (Figure 6c) and peripheral
187 lymph nodes (not shown). Indeed, the lower proportion of Treg in the NZW strain is cell
188 intrinsic as the lower proportions were recapitulated in mixed BM irradiation chimeras
189 (Figure 6d), thus supporting the lack of involvement by pDC genotype.
190 **DISCUSSION**
191
192 pDCs and their product type I IFN have long been associated with lupus
193 autoimmunity. Two recent reports have cogently confirmed the pivotal role of pDCs whereby
194 genetic or pharmacological ablation of pDCs prevents lupus development in murine models[6,
195 7]. Even though the genes involved in producing the lupus phenotype in NZB/W F1 mice have
196 been known for twenty years, the cellular contributions of the parent strains remain undefined,
197 although various abnormalities have been noted in T and B cells[28-31].

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198 The most pronounced difference in the pDCs of NZB contained higher proportion of
199 CD8$^+$ fraction. Although CD8$^+$ pDCs are often described as a subset[32], questions remain as to
200 the stability of such a subset given that CD8 expression by pDCs is readily induced by
201 activation[33, 34] (Supplementary figure 3). Our observation that NZW pDCs and NZB pDCs
202 maintained their CD8 identity when they co-exist in competitive chimera F1 mice would
203 suggest that CD8 expression is not merely a feature endowed by the environment. Despite
204 CD8$^+$ pDCs being reported as more potent inducer of Treg cell differentiation than, we did
205 not observe an overt change in Treg *in vivo* upon pDC depletion[33]. Thus, an essential role for
206 pDCs to maintain Treg homeostasis remains to be established.

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

217 pDCs are major producers of IFN-α[5]. It has been noted that different mouse strains
218 vary in capacity of IFN-α production[17, 18]. We and others had recently found that pDCs from
219 NZB/W F1 mice produce more IFN-α *in vitro*, compared to C57BL/6 mice[19, 20]. Here we
220 revealed further that between two parent strains, NZW pDCs are potent producer of IFN-α
221 than NZB. IFN-α and -β production by pDCs relies on the signalling through TLR and the
222 transcription factors IRF-3 and IRF-7[37]. Despite the observation that NZW pDCs and NZB
223 pDCs greatly differ in capacity to produce IFN-α, we found equivalent expression of IRF-3,
224 IRF-7 and TLR9 in pDCs from each strain, suggesting that other factors are involved in
225 controlling the extent of IFN-α expression upon CpG stimulation. Adding to complexity of
226 IFN contribution to lupus, IFN-λ, also produced by pDCs [4, 15] has also been implicated in
227 lupus[16]. Here we showed that pattern of IFN-λ expression by pDCs from three mouse strains
228 was closely similar to pattern of IFN-α expression. Regulation of Type 1IFN and Type III
229 expression by pDCs as well as other myeloid cells are rather complicated[38]. The stimulating
230 ligands and transcriptional factors required for two types of IFNs showed subtle differences[38].

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231 Lupus is characterized by production of autoantibodies by B cells. pDCs, either via
232 IFNα dependent or independent fashion, promote B-cell proliferation and plasma cell
233 differentiation[39-41]. Perhaps due to higher production of IFN-α and other cytokines, B cells
234 cultured with NZW pDCs undergo a stronger CpG-stimulated proliferative response,
235 compared to cultures without pDCs or NZB pDCs. Although CD70 costimulation by pDCs
236 has been demonstrated as a mechanism for pDCs to promote B cell response[41], we did not
237 detect a difference in CD70 expression between NZB and NZW pDCs (data not shown).
238 In some circumstances, Tregs are critical for maintenance of immune tolerance.
239 Homeostasis of Treg has been shown to be influenced by various types of DC[42]. Loss of DC
240 led to a loss of Treg, and the DC-dependent loss in Treg led to a heightened inflammatory
241 response[43]. In the context of autoimmunity of NZB/W mice, NZW mice have been shown to
242 have Treg deficiency[28]. Thus, the marked difference in $CD8^+$ pDCs between NZW and NZB
243 mice prompted us to examine the contribution of pDCs to Treg homeostasis. We found that
244 depletion of pDCs in NZB mice did not alter the abundance of Tregs, suggesting that
245 difference in pDCs between NZW and NZB mice does not explain the Treg defect in NZW
246 mice.
247 As mouse pDCs is known to be critical for disease onset in NZB/W F1 mice[6, 7] and
248 we revealed here that pDCs of parent strains differ in immune functions *in vitro*, it still leaves
249 a question whether individual functional characteristics of pDCs from parent strains
250 contribute to autoimmune propensity of NZB/W F1 mouse. By pDC transfer, we showed that
251 NZW pDCs indeed promote development of autoimmune B-cell responses in F1 mice
252 (Supplementary figure 4). Together our study provides the cellular pDC basis for the
253 contributions of each parent strain to the pathogenesis of NZB/W F1 lupus. It would be of
254 interest to determine whether these abnormal characteristics of pDCs identified in the mouse
255 model of lupus are also linked to human lupus.
256

257 **METHODS**

258 **Mice.** NZB, NZW, NZB/W F1, vav-BCL-2–transgenic mice, BCL-2$^{-/-}$ mice, Bim$^{-/-}$, Puma$^{-/-}$,
259 Noxa$^{-/-}$mice, OT-I and OT-II on C57BL/6 background and C57BL/6 mice were bred under
260 specific pathogen-free conditions in the animal facility of the Walter and Eliza Hall Institute
261 of Medical Research (WEHI). Siglec H-DTR–Tg NZB mice were derived by backcrossing
262 Siglec H-DTR transgenic mice on BALB/C background that were originally generated and
263 bred at Nanyang Technological University[21]. Experiments were performed according to the

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264 guidelines of the Institute's Animal Ethics Committee (Ethic numbers #2014.023 and
265 2016.014).

266 **Flow cytometry and antibodies.** Single-cell suspensions were stained: CD11c (HL3), Siglec
267 H (eBio440c), CD317 (eBio927), Foxp3 (FJK-16s) (eBioscience, San Diego, CA), CD11b
268 (M1/70), MHC class II I-A/I-E (M5/114.15.2), Ly6C (AL-21), Ly6G (IA8), CD19 (ID3),
269 CD3 (KT3), CD4 (RM4-5), CD8 (53.67), CD25 (PC61), CD138 (281-2), B220 (RA3-6B2,
270 Biolegend), Fc R1α (MAR-1), CD45.1 (A20), CD45.2 (104) and H-2K^d (clone SF1-1.1). All
271 Abs were from BD Biosciences (San Jose, CA), except stated otherwise. Analysis was
272 performed on a BD LSRFortessa and FACSverse and sorting was done on BD FACSAria and
273 BD Influx. Data were analyzed using FlowJo software (Tree Star, Ashland, OR).

274 **IFN assay.** Detection of IFN-α and IFN-α-producing cells has been described previously[19].
275 For measurement of mouse IFN levels, culture supernatants were harvested, and the
276 concentrations of IFN were analyzed by enzyme-linked immunosorbent assay (ELISA). For
277 IFN-α detection, the capture antibody was RMMA-1 (PBL Interferon Source). Polyclonal
278 rabbit anti-IFN-α (PBL Assay Science) was used for detection, followed by peroxidase-
279 conjugated anti-rabbit antibody. For IFN-λ detection, monoclonal rat anti-IFN-λ2/3 antibody
280 (clone 244716) was used for capture and biotinylated monoclonal rat anti-IFN-λ abs (both
281 from R&D Systems), followed by peroxidase-conjugated streptavidin. Cytokine
282 concentrations were interpolated from a standard curve. For intracellular staining of IFN-α,
283 cells were stimulated with CpG for various times, adding Golgi Stop 4 hours before harvest.
284 Cells were then stained for cell surface markers, fixed and then FITC conjugated anti-IFN-α
285 Abs (BD bioscience).

286 **B-cell isolation and cell culture.** B-cell isolation and stimulation has been described
287 previously[22]. Small dense cells from spleen cells of NZB/W F1 mice were collected from
288 the 65–80% interface on Pecoll gradient and B cells purified via negative selection using
289 magnetic bead B-cell isolation kits (Miltenyi Biotec, Auburn, CA). For division-tracking
290 experiments, cells were labelled with CTV. B cells were typically >95% B220$^+$, CD19$^+$,
291 IgM$^+$ and IgD$^+$ as determined by flow cytometry. B cells were cultured in B-cell medium
292 comprising RPMI 1640 medium (Gibco BRL) plus 10% FCS. B cells (50000 cells/well)
293 were stimulated with 3 μM CpG DNA oligonucleotide CpG-1668, fully phosphorothioated
294 (sequence, 5′-TCCATGACGTTCCTGATGCT-3′; Geneworks, Adelaide, South Australia,
295 Australia). Spleen pDCs (50000 cells/well) from NZB or NZW mice were included in half
296 of B-cell culture. To determine absolute cell numbers, the number of live cells per culture

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

299 **Detection of cell bounded autoantibody and CD138$^+$ B cells by FACS.** The procedure for
300 detection of anti-erythrocyte autoantibodies by flow cytometry was described previously[19].
301 Erythrocyte-bound autoantibodies were detected by incubation of extensively washed red
302 blood cells with biotinylated goat anti-mouse IgG and then streptavidin-PE. The geometric
303 mean fluorescence intensity was calculated by subtracting the background with streptavidin-
304 PE only. B220^{low}CD138$^+$ cells were detected after incubating spleen cells with anti-CD138,
305 CD19 and B220 Abs.

306 **DC survival assays.** Cell survival assays were performed in the following 2 ways: 1) DC-
307 enriched spleen cells were cultured at 1–2 x10^5 in 200 μL medium in flat-bottomed 96-well
308 plates. Upon harvesting, cells were stained for cell surface markers. Cell survival was
309 measured by flow cytometry with PE- or APC-conjugated fluorescence-activated cell sorting
310 (FACS) calibration beads (CaliBrite; BD Biosciences) and propidium iodide (PI) to
311 enumerate viable cells. 2) DC subsets were purified by FACS sorting (95% pure). Purified
312 cell subsets were cultured at 1–2x10^4 in 200 μL medium in flat-bottomed 96-well plates.
313 Surviving cells were enumerated by flow cytometry.

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

319 **Protein Isolation and Western Blot Analysis.** Detection of anti- apoptotic BCL-2 and
320 MCL-1 proteins by Western blot analysis has been described previously[23]. Primary
321 antibodies used in this study were mouse anti-BCL-2 mAb (cat. no. sc-7382 from Santa
322 Cruz), rat anti-MCL-1 mAb (clone 19C4-15, kindly provided by David Huang at WEHI), and
323 goat anti–β-actin HRP (cat. no. sc-1616 HRP from Santa Cruz). Secondary antibodies used
324 were goat anti-mouse-IgG1-HRP (cat. no. 1070-05) or goat anti-rat-Ig-HRP (cat. no. 3010-
325 05; both from Southern Biotech).

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

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330 before transfer and post transfer (1 week after last transfer) were evaluated. Spleen B cells
331 and B220^{lo}CD138^{+} cells were evaluated 1 week after last transfer.
332 ***In vivo* pDC depletion and evaluation of Treg cells.** For pDC ablation, Siglec H-DTR
333 NZB mice were injected i.p. with DT (0.2 μg/mouse) every 2 day for 6 days. Spleens,
334 blood and bones were harvested 1 day after the last doses. pDC depletion and spleen T
335 cells compartment was evaluated.
336 **qRT-PCR methods and primers.** RNAs were isolated using RNeasy Plus Mini Kit
337 according to the manufacturer's recommendations. cDNAs were synthesized from total
338 RNAs with using the 5x iScritpt cDNA Synthesis Kit (Biorad) following the manufacturer's
339 instructions. Amplification was performed with SYBR green master mix (Biolabs) on a
340 Biorad CFX 384 using the indicated primers (Supplementary table 1).
341 **Statistical analysis.** Mean and SEM values were calculated with Graphpad Prism Software.
342 *P*-values for two group comparison were calculated by two-tailed unpaired t test. For
343 multiple group comparisons, *P*-values were generated by an ANOVA analysis followed by
344 multiple comparisons. Presented data are from a single experiment of 3 or 4 repeated
345 experiments. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, *n.s.*= not statistically
346 significant.

347

348

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356

357 **CONFLICT OF INTEREST**

358 The authors declare that there is no conflict of interest.

359

360

361 **REFERENCES**

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474 Figure legends:
475 **Figure 1. NZB pDCs contain a larger CD8^{+} cohort.** Spleen cells were prepared from
476 female age matched NZB (n=13), NZW (n=13) and NZB/W F1 mice (n=16). pDC number
477 and phenotype were evaluated. **(a)** Density plots show the proportion of pDCs in total spleen
478 cells [upper] and the expression of CD4 and CD8 in gated pDCs for each strain. **(b)** Number
479 and percentage of total pDCs, CD8^{+} pDCs and CD4^{+} pDCs are shown. **(c)** pDC composition
480 in irradiated NZB/W F1 chimera mice (n=4) reconstituted with NZB BM cells (H-Kd^{+}) and
481 NZW BM cells (H-Kd^{-}). *P<0.05, **P<0.01. Data are from one of 3 or 4 repeated
482 experiments.
483
484 **Figure 2. NZB pDCs have superior survival *in vitro*. (a)** Purified spleen pDCs from NZB,
485 NZW and B6 mice were cultured (1000 cells/well of triplicates) overnight. Viable pDCs were
486 numerated. **(b)** Purified spleen pDCs from NZB and NZW mice were cultured (10000
487 cells/well of triplicates) ±CpG over 1-3 days. **(c)** Purified spleen pDCs from NZB, NZW and
488 NZB/W mice were cultured (10000 cells/well of triplicates) overnight. Bar graphs show the
489 mean number and SD of viable pDCs. *P<0.05, ** P<0.01 and *** P<0.001. Data shown are
490 from a single representative experiment of 2 or 3 experiments. **(d)** Western blot analysis was
491 performed on sorted pDCs to assess the amounts of MCL-1, BCL-2, and β-actin (loading
492 control) proteins.

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493

494 **Figure 3. NZW pDCs are potent IFN-α producers. (a)** Spleen cells were stimulated with
495 CpG for 4 hrs and then performed intracellular staining for IFN-α. FACS plots show IFN-α
496 production by gated pDCs. Scatter plots show percentage of IFN-α producing pDCs from
497 individual age-matched NZB (n=9), NZW (n=6) and F1 (n=8) female mice. Data were
498 pooled from 3 experiments. **(b)** pDCs were purified and stimulated for overnight. IFN-α
499 levels in culture supernatants of 3 individual mice each group were shown. **(c)** pDCs were
500 purified from 3 or 4 individual female mice and stimulated with CpG for overnight. IFN-λ
501 levels in culture supernatants of individual mice each group were shown. * $P<0.05$,
502 ** $P<0.01$. Two (IFN-λ) to three (IFN-α) experiments were performed with similar results.
503

504 **Figure 4. NZW pDCs are more potent to enhance CpG-stimulated B cell response *in***
505 ***vitro*.** CTV-labelled naïve B cells from NZB/W mice were cultured with pDCs from either
506 NZB or NZW for various times. B-cell proliferation and CD138 expression was evaluated. **(a)**
507 CTV profile of B cell proliferation either cultured with pDCs from NZB (thin solid line),
508 From NZW (thick solid line) or without pDCs (filled), upon stimulation with CpG (3 μM). **(b)**
509 Quantitative data of total B cell number, total cohort number and mean division number over
510 time. **(c)** Representative plot of expression of CD138 on B cells under different culture
511 conditions harvested at 68 hrs.
512

513 **Figure 5. $CD8^+$ pDCs stimulate a stronger proliferation of $CD4^+$ T cells. (a)** Spleen pDCs
514 from three strains (6-7 individual mice per strain) were analysed for expression of MHC class
515 II by total pDCs (upper panel) and separated pDCs based on CD8 expression (lower panel).
516 Histograms show MHC class II expression. Bar graphs show MFI of MHC class II.
517 *** $P<0.001$, **** $P<0.0001$. **(b)** Bar graphs show MFI of MHC class II on spleen $CD8^+$ and
518 $CD8^-$ pDCs from 4 individual C57BL/6 mice. ** $P<0.01$, *** $P<0.001$. C. $CD8^+$ and $CD8^-$
519 pDCs were sorted from C57BL/6 mice. Purified OT-I and OT-II T cells were purified and
520 labelled with CTV. T cells (50000/well) were then co-cultured with pDCs (10000/well) with
521 soluble OVA for 3 days. Bar graphs show mean number and SD of proliferating T cells.
522

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

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526 mean and SEM of 4 individual mice. **P<0.01, ***P<0.001. Three repeated experiments
527 were performed with similar results. B. Siglec H-DTR NZB mice were injected with DT
528 200ng/dose/mouse every 2 day for 6 days. 1 day after the last dose, organs were harvested.
529 **(b)** FACS plots show spleen DC composition of DT treated (n=3) and untreated mice
530 (n=4). Bar graph shows the number of spleen pDCs. **(c)** FACS plots show expression of
531 CD25 and Foxp3 on gated $CD4^+$ T cells. Bar graph the percentage of Tregs. ***P<0.001.
532 Two experiments were performed. **(d)** Treg cell numbers in 4 irradiated NZB/W F1 chimera
533 mice reconstituted with NZB BM cells ($H\text{-}Kd^+$) and NZW BM cells ($H\text{-}Kd^-$). ***P<0.001.
534 Data are from one of 2 repeated experiments.
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