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The NF-κB Regulator Bcl-3 Governs Dendritic Cell Antigen Presentation Functions in Adaptive Immunity

Ilaria Tassi,* Estefania Claudio,* Hongshan Wang,* Wanhua Tang,* Hye-lin Ha,* Sun Saret,* Madhu Ramaswamy, ‡ Richard Siegel, † and Ulrich Siebenlist*

Bcl-3 is an atypical member of the IκB family and modulates gene expression via interaction with p50/NF-κB1 or p52/NF-κB2 homodimers. We report in the present study that Bcl-3 is required in dendritic cells (DCs) to assure effective priming of CD4 and CD8 T cells. Lack of Bcl-3 in bone marrow–derived DCs blunted their ability to expand and promote effector functions of T cells upon Ag/adjuvant challenge in vitro and after adoptive transfers in vivo. Importantly, the critical role of Bcl-3 for priming of T cells was exposed upon Ag/adjuvant challenge of mice specifically ablated of Bcl-3 in DCs. Furthermore, Bcl-3 in endogenous DCs was necessary for contact hypersensitivity responses. Bcl-3 modestly aided maturation of DCs, but most consequentially, Bcl-3 promoted their survival, partially inhibiting expression of several antiapoptotic genes. Loss of Bcl-3 accelerated apoptosis of bone marrow–derived DCs during Ag presentation to T cells, and DC survival was markedly impaired in the context of inflammatory conditions in mice specifically lacking Bcl-3 in these cells. Conversely, selective overexpression of Bcl-3 in DCs extended their lifespan in vitro and in vivo, correlating with increased capacity to prime T cells. These results expose a previously unidentified function for Bcl-3 in DC survival and the generation of adaptive immunity. The Journal of Immunology, 2014, 193: 000–000.

Dendritic cells (DCs) are the most potent APCs and are crucial for the initiation of adaptive immune responses. The immunogenicity of DCs is determined by their ability to capture, process, and present Ags, their production of cytokines and other soluble mediators, and their expression of costimulatory molecules, but also by their longevity. Enhanced survival or expansion of DCs can result in autoimmunity (1–3). Upon activation, the lifetime of DCs may need to be strictly regulated to maintain a balanced and functional immune response (4–6). However, how DCs manage to carefully control their own survival, particularly during priming of T cells, is largely unknown.

NF-κB is a master regulator of inflammation, and several NF-κB subunits have been described to control DC functions (7–11). The dimeric NF-κB transcription factors are composed of five variably combined polypeptides that comprise the Rel/NF-κB family (RelA [p65], RelB, c-Rel, p50 [NF-κB1], and p52 [NF-κB2]). Both p50 and p52 lack transactivation domains, and the abundant p50 homodimers have been implicated in inhibition of NF-κB–dependent gene transcription (12, 13). NF-κB activity is regulated by the IκB family proteins, which include the classical members IκBα, IκBβ, and IκBe, the p105/NF-κB1 and p100/NF-κB2 precursors, and the atypical members IκBζ, IκBNS, and Bcl-3. The atypical members modulate transcriptional activities of NF-κB complexes in the nucleus.

Bcl-3 exclusively binds homodimers of p50 or p52 and may convert these homodimers into transactivating complexes owing to transactivation domains present within Bcl-3, yet Bcl-3 may also enhance their inhibitory function. The exact outcome may depend on the particular target gene and cellular context, which also involves the posttranslational modifications of Bcl-3 (12, 14). The specific cellular functions and mechanisms of action of Bcl-3 in biologic contexts remain poorly understood. Nevertheless, much evidence points to profound roles of Bcl-3 in vivo. Its gene is a partner in recurring chromosomal translocations, especially in B cell leukemias (15, 16), and its expression is elevated in various solid tumors (17). Bcl-3 is critical for both innate and adaptive immune responses to pathogens and contributes to immune system development (18–22).

In this study we have explored the role of Bcl-3 in DCs. We discovered that Bcl-3 was required for efficient priming of CD4 and cross-priming of CD8 T cells in vitro and in vivo. Mice specifically ablated for Bcl-3 in CD11c+ cells failed to generate a proper Ag-specific CD4 T cell response and failed to develop a normal CD8-dependent contact hypersensitivity reaction. Mechanistically, Bcl-3 contributed to expression of costimulatory factors on DCs and modestly reduced expression of some T cell inhibitory factors, but most notably it promoted survival of DCs to allow for optimal priming of T cells. Conversely, transgenic (Tg) mice overexpressing Bcl-3 in CD11c+ cells prolonged the lifespan of DCs in vitro and in vivo and enhanced T cell priming. Our findings reveal an unexpected and critical role for Bcl-3 in DCs to assure survival and efficient priming of T cells.

Materials and Methods

Mice

All mice used were on C57BL/6 backgrounds. OT-I, OT-II, and LPR mice were from Taconic, and IL-10−/− Igax-cre (CD11c-cre) mice were from...
The Jackson Laboratory, Bcl3−/− (18) and Bcl-3 Tg mice have been described (23); and Bcl3GFP/− mice are described in Supplemental Fig. 2. All mice were housed in National Institute of Allergy and Infectious Diseases facilities, and all experiments were done with approval of the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee and in accordance with all relevant institutional guidelines.

Flow cytometry

Samples were stained at 4°C with Alexa Fluor 488–anti-CD11b (M1/70), and PE–anti-PD-L1 (MIH5), PE–anti-class II (MHC-II; M5/114.15.2), PE–anti-MHC-II (M5/114.15.2), allophycocyanin–anti-CD11c (HL3), allophycocyanin–anti-NK1.1 (PK136), allophycocyanin–anti-CD8 (58-6.7), FITC–anti-CD3 (145-2C11), PE–anti-CD86 (B7-2), FITC–anti-CD54 (3E2), PE–anti-CD40 (3/23), PerCP–anti-CD45.2 (104), PerCP–anti-Vo2 TCR (B20.1), and PE-Cy7–anti-IFN-γ (XMG1.2), PE-Cy5–anti-MHC class II (MHC-II; MS/14.15.2), PE–anti-MHC-II (MS/14.15.2), allophycocyanin–anti-CD207 (eBioRMUL.2), PE–anti-Fas ligand (FasL; MFL3), eFluor 450–anti-CD11b (M1/70), and PE–anti-PD-L1 (MH5), PE–anti-CD103 (2E7) (all from eBiosciences); and allophycocyanin–anti-CD8 (53-6.7), allophycocyanin–anti-CD49b (DX5), allophycocyanin–anti-CD25 (PC61), allophycocyanin–Cy7–MHC-II (MS/14.15.2), FITC–anti-CD80 (16-10A1), and FITC–anti-MHC class I (34-1-2s) (all from BioLegend). Dead cells were excluded and FITC–anti-MHC class I (34-1-2s) (all from BioLegend). For propidium iodide annexin V analysis, the Annexin V eFluor 450 apoptosis detection kit was used (Invitrogen). Caspase-3 activation was measured with a NuView 488 caspase-3 assay kit (Biotium, Hayward, CA). Cells were dead excluding an aqua Live/Dead fixable dye (Invitrogen). Stained cells were analyzed on a FACSCanto and data were analyzed with FlowJo software (BD Biosciences).

In vitro priming of T cells

Bone marrow–derived DCs (BMDCs) were generated with GM-CSF for 7–9 d (24). DC yield was monitored with flow cytometry after anti-CD11b and anti-CD11c staining. BMDCs were stimulated with ultrapure LPS (Escherichia coli 0111:B4; List Biological Laboratories). Surface markers were stained for 30 min at 4°C. Caspase-3 activation was measured with a NuView 488 caspase-3 assay kit (Biotium, Hayward, CA). Dead cells were excluded with an aqua Live/Dead fixable dye (Invitrogen). Stained cells were analyzed on a FACSCanto and data were analyzed with FlowJo software (BD Biosciences).

In vivo T cell priming

CD45.1 mice were injected i.v. with 5 × 106 CFSE-labeled CD45.2 OT-II T cells, previously isolated by negative or positive selection (CD4+ T cell isolation kits, Miltenyi Biotec). One day later, mice were injected i.p. with 1 × 107 OVA-loaded (100 μg/ml) and LPS (100 ng/ml)-stimulated BMDCs (described above). Three days later, splenocytes were isolated and OT-II proliferation (CFSE dilution) was measured with flow cytometry gated on CD4+CD8−. CD45.2 wild-type (WT) and Bcl3−/− mice were injected i.v. with 5 × 106 CFSE-labeled CD45.1+ OT-I T cells, previously isolated by negative selection (CD8+ T cell isolation kit, Miltenyi Biotec). One day later, mice were injected intradermally with 1 μg OVA. Cells were isolated from spleens and draining lymph nodes 3 d later and OT-I proliferation (CFSE dilution) was measured with flow cytometry, gated on CD8+CD45.1+ cells. Bcl-3ΔΔ DC (and control) mice were injected with 5 × 106 CFSE-labeled OT-II cells and 1 d later with 1 μg OVA and 30 μg LPS s.c. Three days later, cells from draining lymph nodes were analyzed with flow cytometry for OT-II proliferation (CFSE dilution) after gating on CD4+Vα2 TCR+ cells.

Contact hypersensitivity and skin DC isolation

Mice were sensitized on shaved bellies with 25 μl 10 μg/ml oxazolone (Sigma-Aldrich, 1.5 olive oil/acetone solution) for 2 consecutive days. Five days later, both sides of ears were challenged with 5 μl 10 μg/ml oxazolone or solvent control. Ear thickness was measured at time of challenge with oxazolone on ears and during the course of the next 4 consecutive days.

To extract skin DCs from mouse ears, ears were split in two parts (dorsal and ventral) and incubated for 30 min at 37°C in PBS containing 2.5 mg/ml dispase II (Roche) to allow separation of dermal and epidermal sheets. The separated epidermal and dermal sheets were incubated for 1 h at 37°C with a solution of RPMI 1640 containing 2.5 mg/ml Liberase (Roche) to obtain homogeneous cell suspensions.

DC apoptosis

BMDCs (3 × 105/well) of 24-well plate were incubated with 3 μg/ml OVA, stimulated with LPS (100 ng/ml) overnight, washed, and cocultured with 1.5 × 105 OT-II cells per well (24-well plate) for 72 h. Apoptosis of BMDCs was analyzed with flow cytometry after staining for CD11c, annexin V/7-aminocoumarin D (7-AAD) or caspase-3, and gating on CD11c+ T cells. WT and Bcl3−/− mice were injected i.v. with 30 μg LPS or PBS. After 48 h, splenocytes were isolated, stained for CD11c, and MHC-II was assessed with flow cytometry. Absolute numbers of CD11c+ MHC-II+ T cells in the spleen were determined using CountBright absolute counting beads (Invitrogen). In situ apoptosis of splenic DCs was determined with TUNEL assay (Histoserv) and quantitated by counting in 0.63-mm2 areas.

Western analysis

BMDCs were stimulated for various times with LPS (100 ng/ml), and nuclear and cytoplasmic fractions were prepared by a nuclear extraction–protein extraction reagent kit (Pierce). Proteins from cell lysates were separated by standard SDS-PAGE and analyzed by immunoblotting with Abs specific for p65 (CT; Millipore), p50 (sc-1141), actin (sc-7783), and Lamin B (sc-6216) (all from Santa Cruz Biotechnology).

Statistical analysis

Data were recorded as the means ± SEM. Differences between groups were analyzed by unpaired, two-tailed Student t tests. A p value ≤ 0.05 was considered significant (Prism; GraphPad Software). For multiple comparisons, data were analyzed by the one-way ANOVA followed by a Bonferroni multiple comparisons test. The numbers of independent data points (n) for each experiment are stated in the figure legends.

Results

Bcl-3 promotes DC-mediated priming of CD4 T cells

BMDCs from WT and Bcl-3–deficient (Bcl3−/−) animals were loaded with OVA, stimulated with LPS, and cocultured with CFSE-labeled OT-II CD4 T cells for 3 d (lack of Bcl-3 did not affect generation of BMDCs; Supplemental Fig. 1A). Bcl3−/− BMDCs were much less effective in inducing proliferation of T cells than WT BMDCs (Fig. 1A). This was not due to a defect in the 3-d reaction or in the spleen in the spleen when Bcl-3 was absent; Supplemental Fig. 1B). We also observed diminished induction of CD25, IL-2, and IFN-γ when T cells were primed by Bcl3−/− BMDCs (Fig. 1D).

To investigate priming in vivo, CD45.1 mice were injected with CFSE-labeled CD45.2 OT-II cells, then with LPS-stimulated and OVA-pulsed WT or Bcl3−/− BMDCs, and proliferation of splenic CD45.2 CD4 OT-II cells was monitored 3 d later. Compared to...
WT, Bcl3−/− BMDCs induced significantly less OT-II proliferation in vivo (Fig. 1E). Of note, Bcl3−/− BMDCs migrated to the spleen as efficiently as did WT BMDCs (Fig. 1F). Therefore, expression of Bcl-3 in BMDCs is required for efficient priming of CD4 T cells in vitro and in vivo.

**Bcl-3 promotes DC-mediated cross-priming of CD8 T cells**

To investigate cross-priming of CD8 T cells, we cultured LPS-stimulated and OVA-pulsed WT or Bcl3−/− BMDCs together with CFSE-labeled OT-I CD8 T cells. Both proliferation of T cells (Fig. 2A) and levels of IL-2 (Fig. 2B) were significantly reduced when BMDCs lacked Bcl-3. To assess cross-priming in vivo, WT and Bcl3−/− mice were injected with CFSE-labeled CD8 OT-I cells, OVA was administered 1 d later, and proliferation of OT-I CD8 T cells was monitored in spleen and draining lymph node 3 d later (Fig. 2C). Cross-priming in Bcl3−/− compared with WT mice resulted in reduced proliferation of T cells in both spleen and lymph node. Therefore, efficient cross-priming of CD8 T cells requires Bcl-3 expression in DCs in vitro and in vivo (see also below).

**Ablation of Bcl-3 in CD11c+ cells impairs priming of T cells in mice**

To investigate the role of Bcl-3 in endogenous Ag-presenting DCs we made use of mice in which DCs lacked Bcl-3. We generated...
conditional Bcl-3 knockout mice (Bcl3fl/fl) and deleted Bcl-3 in DCs with CD11c-driven Cre (CD11c-Cre;Bcl3fl/fl; hereafter referred to as Bcl-3-D-DC) (Supplemental Fig. 2A, 2B). We confirmed that BMDCs generated from Bcl-3-D-DC mice were defective in priming CD4 T cells in vitro (Supplemental Fig. 2C) and that loss of Bcl-3 in CD11c+ cells did not alter the total numbers of DCs, CD4, CD8, or B cells in spleens (Supplemental Fig. 2D).

CFSE-labeled OT-II CD4 cells were injected into Bcl3fl/fl (WT) and Bcl-3-D-DC mice, followed by challenge with OVA and LPS, and proliferation of OT-II cells in draining lymph nodes was measured 3 d later. OT-II proliferation was significantly reduced in Bcl-3-D-DC compared with WT mice (Fig. 3A). Bcl-3 was thus critical within endogenous CD11c+ cells to properly prime CD4 T cells. To assess migration of DCs from skin to lymph node, we painted shaved bellies of WT and Bcl-3-D-DC mice with FITC, injected LPS s.c., and enumerated FITC-labeled CD11c+ cells in draining lymph nodes 18 h later (Fig. 3B). Loss of Bcl-3 did not appear to affect migration.

To further explore the role of Bcl-3 in DCs in cross-priming in vivo, we employed a CD8-dependent contact hypersensitivity model. Bcl-3-D-DC and WT mice were sensitized ventrally to the hapten oxazolone, rechallenged on ears 5 d later, and ear thickness was measured as a readout of inflammation at time of challenge and during the course of the next 4 consecutive days. Bcl-3-D-DC mice had notably reduced ear thickening compared with WT mice (Fig. 3C). To exclude the possibility that the observed reduction in contact hypersensitivity was not due to the lack of one or more DC subtypes in the skin, we analyzed the skin DC subsets in ears of WT and Bcl32/2 mice (25, 26). We did not find any significant

**FIGURE 2.** Bcl-3 is required for efficient DC-mediated cross-priming in vitro and in vivo. (A) WT and Bcl32/2 BMDCs were stimulated with LPS (100 ng/ml) overnight, pulsed with OVA (100 μg/ml) for 3 h, and cocultured with CFSE-labeled OT-I T cells for 72 h. T cells were analyzed by flow cytometry after staining and gating for CD8. Representative FACS plots and proliferation and division indices (FloJo) are shown; mean ± SEM; n = 5/group. (B) WT and Bcl32/2 BMDCs were treated as in (A) and IL-2 production in supernatants was analyzed with CBA; mean ± SEM; n = 5/group. (C) CFSE-labeled CD45.1 OT-I T cells (53106) were injected i.v. into CD45.2 WT and Bcl32/2 mice. Twenty-four hours later animals were injected intradermally with OVA (1 μg). Cells were isolated from spleens and draining lymph nodes 72 h later, stained and gated for CD45.1 and CD8, and analyzed by flow cytometry. Data are shown as in (A), with n = 10 mice/group based on two experiments. *p < 0.05, **p < 0.01.
The numbers of CD11c+MHC-II+FITC+ DCs were obtained using CountBright absolute counting beads. Data are shown as means with FITC solution and injected s.c. with LPS (30 μg). Division indices are shown; mean ± SEM; n = 8 mice/group based on two experiments. Shaved abdomens of WT and Bcl-3-Δ-DC mice were painted with FITC solution and injected s.c. with LPS (30 μg). Inguinal lymph nodes were obtained after 18 h, stained for CD11c and MHC-II, and absolute numbers of CD11c+MHC-II+FITC+ DCs were obtained using CountBright absolute counting beads. Data are shown as means ± SEM; n = 4 mice/group. WT and Bcl-3-Δ-DC mice were sensitized with oxazolone applied to shaved abdominal skin on 2 consecutive days. Five days later mice were challenged on ears and ear swelling was measured blindly at indicated time points after challenge, presented as mean of increase between LPS-stimulated WT and Bcl-3-Δ-DC mice. Twenty-four hours later animals were injected s.c. with OVA (1 μg) and LPS (30 μg). Inguinal lymph nodes were obtained after 18 h, stained for CD11c and MHC-II, and absolute numbers of CD11c+MHC-II+FITC+ DCs were obtained using CountBright absolute counting beads. Data are shown as means ± SEM; n = 4 mice/group. An additional experiment yielded similar data. *p < 0.05.

FIGURE 3. Selective ablation of Bcl-3 in CD11c+ cells impairs CD4 and CD8 responses in mice. (A) CFSE-labeled OT-II T cells (5 × 10⁶) were injected i.v. into WT and Bcl-3-Δ-DC mice. Twenty-four hours later animals were injected s.c. with OVA (1 μg) and LPS (30 μg). Cells were isolated from draining lymph nodes 72 h later, stained and gated for Vα2 TCR and CD4, and analyzed by flow cytometry. Representative FACS plots and proliferation and division indices are shown; mean ± SEM; n = 8 mice/group based on two experiments. (B) Shaved abdomens of WT and Bcl-3-Δ-DC mice were painted with FITC solution and injected s.c. with LPS (30 μg). Inguinal lymph nodes were obtained after 18 h, stained for CD11c and MHC-II, and absolute numbers of CD11c+MHC-II+FITC+ DCs were obtained using CountBright absolute counting beads. Data are shown as means ± SEM; n = 4 mice/group. (C) WT and Bcl-3-Δ-DC mice were sensitized with oxazolone applied to shaved abdominal skin on 2 consecutive days. Five days later mice were challenged on ears and ear swelling was measured blindly at indicated time points after challenge, presented as mean of increase ± SEM in thickness over basal level of solvent only–treated ears; n = 5 mice/group. An additional experiment yielded similar data. *p < 0.05.

differences in the skin DC populations between WT and Bcl3−/− mice (Supplemental Fig. 2E). These findings implicate Bcl-3 in endogenous DCs for both priming of CD4 and cross-priming of CD8 T cells.

Bcl-3 contributes to BMDC maturation

Efficient priming requires engagement of T cells with Ag-bound MHC-II, with costimulatory ligands on DCs and stimulation by DC-produced cytokines (27). Lack of Bcl-3 in BMDCs partially reduced LPS-induced increases in expression of the costimulatory proteins CD80 and CD86 (Fig. 4A). LPS-induced levels of MHC-II were also somewhat lower, whereas those of PD-L1 were enhanced. However, addition of PD-L1 blocking Abs to cocultures failed to improve priming (not shown). Expression levels of MHC class I, CD40 and ICAM-1 were not noticeably different in the absence of Bcl-3 (Fig. 4A).

WT and Bcl3−/− BMDCs were stimulated with LPS overnight to measure protein levels of IL-6, IL-12p70, TNF-α, and IL-10. Levels of IL-10 were modestly increased in Bcl3−/− BMDCs, compared with WT BMDCs (especially caspase-4 and -12, NF-κB1, and RIPK1, but also including FasL and Bax) (Fig. 5A). We then investigated whether Bcl-3 might promote survival in DCs, especially since the life expectancy of DCs may be critical for efficient priming of T cells (1, 2). We therefore assessed the survival of BMDCs in standard 3-d coculture experiments with annexin V and 7-AAD staining (Fig. 5B, 5C) or caspase-3 activation (Fig. 5D). Bcl3−/− BMDCs survived significantly less well than did WT BMDCs, evident by day 2, and they exhibited increased caspase-3 activation, indicating a role for Bcl-3 in preventing premature apoptosis. Of note, the absence of Bcl-3 in BMDCs did not affect the cytoplasmic and nuclear levels of p50 and p65 during a time course of stimulation with LPS (Supplemental Fig. 3B). This was expected, as Bcl-3 is not known to affect the activation/nuclear translocation of NF-κB; instead, it affects the transcriptional activity of these complexes.

To assess survival of DCs in vivo, we injected WT, Bcl3−/−, and Bcl-3-Δ-DC mice i.v. with LPS and monitored numbers of CD11c+MHC-II+ splenic DCs at days 0 and 2. DCs undergo rapid activation-induced cell death under these conditions (29, 30). We observed an even more pronounced reduction in splenic DCs lacking Bcl-3 (Fig. 5E, 5F). Splenic sections from LPS-treated Bcl-3-Δ-DC and WT mice were stained for TUNEL+ (apoptotic) cells; their numbers were significantly higher in Bcl-3-Δ-DC than in WT mice (Fig. 5G). These data implicate Bcl-3 in the lower chamber mattered for priming of T cells (Fig. 4D). This suggests that soluble factors were not primarily responsible for defective priming.

Bcl-3 promotes survival of DCs in vitro and in vivo

LPS-activated WT and Bcl3−/− BMDCs were transcriptionally profiled with PCR arrays. Expression of several proapoptotic genes was increased in LPS-stimulated Bcl3−/−, compared with WT BMDCs (especially caspase-4 and -12, NF-κB1, and RIPK1, but also including FasL and Bax) (Fig. 5A). We then investigated whether Bcl-3 might promote survival in DCs, especially since the life expectancy of DCs may be critical for efficient priming of T cells (1, 2). We therefore assessed the survival of BMDCs in standard 3-d coculture experiments with annexin V and 7-AAD staining (Fig. 5B, 5C) or caspase-3 activation (Fig. 5D). Bcl3−/− BMDCs survived significantly less well than did WT BMDCs, evident by day 2, and they exhibited increased caspase-3 activation, indicating a role for Bcl-3 in preventing premature apoptosis. Of note, the absence of Bcl-3 in BMDCs did not affect the cytoplasmic and nuclear levels of p50 and p65 during a time course of stimulation with LPS (Supplemental Fig. 3B). This was expected, as Bcl-3 is not known to affect the activation/nuclear translocation of NF-κB; instead, it affects the transcriptional activity of these complexes.

To test survival of DCs in vivo, we injected WT, Bcl3−/−, and Bcl-3-Δ-DC mice i.v. with LPS and monitored numbers of CD11c+MHC-II+ splenic DCs at days 0 and 2. DCs undergo rapid activation-induced cell death under these conditions (29, 30). We observed an even more pronounced reduction in splenic DCs lacking Bcl-3 (Fig. 5E, 5F). Splenic sections from LPS-treated Bcl-3-Δ-DC and WT mice were stained for TUNEL+ (apoptotic) cells; their numbers were significantly higher in Bcl-3-Δ-DC than in WT mice (Fig. 5G). These data implicate Bcl-3 in
survival of DCs in vitro and in vivo, suggesting a mechanism by which Bcl-3 promoted T cell priming.

Surface staining for FasL confirmed increased, albeit still low, expression levels in Bcl3<sup>−/−</sup> BMDCs (Supplemental Fig. 3C). Because FasL may impair priming due to Fas-mediated apoptosis of T cells and/or BMDCs (1, 2, 31), we added the Fas/Fc-soluble inhibitor to cocultures. However, limited Fas engagement on naive T cells during activation may promote proliferation, whereas only extensive engagement may favor apoptosis (32, 33). Consistent with this, Fas/Fc significantly reduced proliferation of T cells primed by WT BMDCs and further reduced poor proliferation when primed by Bcl3<sup>−/−</sup> BMDCs; however, the difference between WT and Bcl3<sup>−/−</sup> BMDCs was no longer as apparent in the presence of Fas/Fc (Supplemental Fig. 3D). To assess apoptosis of cocultured T cells, induced via Fas or otherwise, we measured caspase-3 activation and stained for live/dead cells (Supplemental Fig. 3E, 3F). We observed a limited increase in apoptosis of OT-II cells when primed by Bcl3<sup>−/−</sup> compared with WT BMDCs. We furthermore tested Fas-deficient CD4 OT-II T cells (from OT-II/LPR crosses). These cells proliferated less well than did Fas-sufficient OT-II cells, consistent with the noted positive role of Fas. However, the difference in proliferation of these cells when primed by WT compared with Bcl3<sup>−/−</sup> BMDCs was no longer as marked (Supplemental Fig. 3G). Therefore, increased expression...
of FasL on BMDCs appears to have moderately increased T cell apoptosis, but was unlikely to be primarily responsible for impaired priming by Bcl3−/− BMDCs.

Overexpression of Bcl-3 in DCs promotes survival and T cell priming

BMDCs were generated from Bcl3 Tg mice that express the transgene in DCs only (CD11c-Cre–mediated removal of loxP-flanked stop cassette) (23). When WT and Bcl3−/− BMDCs were used in standard coculture experiments, the Tg BMDCs caused a significant increase in T cell proliferation above that of WT BMDCs (Fig. 6A). Importantly, Tg BMDCs also exhibited significantly improved survival in these cocultures (Fig. 6B). To assess survival of DCs in vivo, we injected WT and Tg mice i.v. with LPS and monitored CD11chighMHC-II+ splenic DCs at days 0 and 2. As discussed, LPS caused a drastic reduction of DCs in WT mice after 2 d, whereas significantly more DCs remained at that time in mice expressing the Bcl3 Tg in DCs (Fig. 6C). Thus, survival of DCs was extended by increased Bcl-3 levels and correlated with improved T cell priming.

Discussion

The present study explores the function of the NF-κB regulator Bcl-3 in Ag-presenting DCs. We discovered that Bcl-3 plays a surprisingly critical role in DC-mediated priming of CD4 and cross-priming of CD8 T cells. We demonstrate this with BMDCs and, importantly, with Ag-challenged mice specifically ablated of Bcl-3 in DCs. Thus, Bcl-3 was essential within the DC population normally present in animals to initiate an appropriate adaptive.
immune response. Bcl-3 contributed in several ways: it promoted expression of some coactivators associated with DC maturation, and it delimited expression of some potentially negative mediators of T cell activation. However, these contributions were relatively minor and the most consequential function of Bcl-3 was to prevent the premature demise of DCs. Loss of Bcl-3 shortened the lifetime of activated DCs both in vitro and in vivo. In contrast, overexpression of Bcl-3 increased their lifetime in vitro and in vivo, correlating with increased priming. Our findings suggest that activated Bcl-3–deficient DCs failed to survive long enough to assure efficient continuous priming of T cells, thereby compromising the development of an adequate adaptive immune response in vivo.

DC maturation signals invariably activate NF-κB, and prior studies have suggested roles for various Rel subunits in this process, including expression of cytokines (8–10, 34). Bcl-3 interacts with homodimers of p50/NF-κB1 and p52/NF-κB2 (14). NF-κB2 and NF-κB1 have been suggested to act as negative regulators of DC functions, based on studies with BMDCs and/or mice lacking these proteins. Ablation of NF-κB2 increased expression of some costimulatory molecules, postulated to be due to loss of the RelB inhibitor p100/NF-κB2 (35). Ablation of NF-κB1 increased expression of some inflammatory cytokines, especially TNF-α (7), but also of some maturation markers, and it enhanced IFN-γ production by cocultured T cells (36). It was furthermore suggested that loss of inhibitory p50 homodimers might be primarily responsible for these changes. However, these interpretations are confounded by the fact that NK-κB1 and NF-κB2 encode the IkB-like precursors p105 and p100 as well as the processed p50 and p52 subunits, which form NF-κB heterodimers in addition to homodimers. The different forms have distinct activities and it is unclear which are critical for DC-mediated priming. Prior studies have suggested that Bcl-3 enforces inhibitory activities of p50 homodimers (37), and based on the above-cited studies, Bcl-3 would then be expected to inhibit DC functions. However, contrary to this notion, we found that Bcl-3 was dispensable for efficient Ag-specific priming of both CD4 and CD8 T cells, as demonstrated both in vitro and in mice conditionally ablated for Bcl-3 in Ag-presenting DCs, including a CD8 T cell–dependent contact hypersensitivity model. Rather than enforcing inhibitory functions of p50 homodimers, Bcl-3 may reverse these functions, at least for some genes, which is conceivable given that Bcl-3 contains transactivation domains. Additionally, Bcl-3 may execute important functions via p52 homodimers.

To understand how Bcl-3 may aid DCs in effective priming of T cells, we investigated Ag uptake, processing, maturation, and cytokine expression. LPS-induced expression of MHC-II and the costimulators CD80 and CD86 was modestly reduced in Bcl-3–deficient BMDCs, and expression of the potential inhibitors PD-L1, FasL, and IL-10 was somewhat increased. However, blocking or eliminating these inhibitors failed to overcome defective T cell priming by Bcl-3–deficient BMDCs, and in the case of FasL, appeared to result in an only moderate reversal. This argues against significant roles for these inhibitors, although in vivo contributions cannot be ruled out. In contrast, loss of Bcl-3 markedly increased apoptosis in DCs, which we demonstrated in BMDCs in vitro as well as in DCs in vivo. Prior reports have suggested that the lifespan of DCs significantly affects the magnitude of immune responses to Ags and is critical for the balance between tolerance and inflammation (1, 4–6). Extending the lifetime of DCs, such as via overexpression of antiapoptotic or elimination of apoptotic proteins enhanced Ag-specific T cell responses and/or led to autoimmunity and inflammation; conversely, elimination of the antiapoptotic regulator Bcl-XL shortened DC survival in vivo and blunted an Ag-specific T cell response (38). Therefore, the observed premature apoptosis of Bcl-3–deficient DCs may be the primary reason for impaired priming of T cells. In line with this interpretation, overexpression of Bcl-3 increased survival of DCs in vitro and in vivo and enhanced priming of T cells. The Bcl-3–mediated survival of activated and Ag-loaded DCs may increase their chances to encounter cognate T cells and, additionally, may aid activation of these cells via prolonged interaction.

How may Bcl-3 assure adequate survival of DCs? Transcriptional profiling indicated elevated expression of several proapoptotic genes in Bcl-3–deficient BMDCs, including Bax, caspase-12 and -4, RipK1, and NF-κB1. Loss of NF-κB1 has been suggested to prolong the lifespan of DCs (36), and it is conceivable that Bcl-3 may act in part by limiting expression of this protein, not just by modulating its functions. RPK1 is involved in apoptosis and necrosis, and pretreatment of BMDCs with the inhibitor of necrosis Nec-1 did not rescue T cell priming (data not shown) (39). Bcl-3 appears to modulate the expression of multiple proteins and it may be the combination of these changes that impacts the physiology of DCs.

The ability of DCs to prime T cells has also recently been linked to a major shift in metabolic programming from oxidative phosphorylation to aerobic glycolysis (40). A similar switch occurs in highly proliferative and stressed tumor cells and activated lymphocytes. It may be in the context of metabolic shifts and stress conditions that Bcl-3 is required to carefully control the adequate survival of activated DCs, thereby assuring an effective adaptive immune response.

FIGURE 6. Bcl-3 overexpression promotes T cell priming and DC survival. (A) WT and Tg BMDCs were treated and cocultured with CFSE-labeled OT-II cells and T cells analyzed as in Fig. 1A (standard conditions and analysis). Data are shown as means ± SEM; n = 3/group. (B) WT and Tg BMDCs were treated and cocultured as in (A), then cells were stained for CD11c, annexin V, and 7-AAD and analyzed by flow cytometry after gating on CD11c. Data are shown as mean of percentage live DCs ± SEM; n = 5/group. *p < 0.05, **p < 0.01, ***p < 0.0001.
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Disclosures
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References