BCAP promotes lupus-like disease and TLR-induced IFNα production in plasmacytoid dendritic cells

Talyn A. Chu

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Reading Committee:

Jessica A. Hamerman, Chair

Adam Lacy-Hulbert

Daniel B. Stetson

Program Authorized to Offer Degree:

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University of Washington

Abstract

BCAP promotes lupus-like disease and TLR-induced IFNα production in plasmacytoid dendritic cells

Talyn A. Chu

Chair of the Supervisory Committee:
Affiliate Associate Professor Jessica A. Hamerman
Department of Immunology

Systemic lupus erythematosus (SLE) severity correlates with elevated serum levels of type I interferons (IFN), cytokines produced in large quantities by plasmacytoid dendritic cells (pDC) in response to engagement of TLR7 and TLR9 with endocytosed nucleic acids. B cell adaptor for PI3K (BCAP) promoted many aspects of TLR7-driven lupus-like disease including *Isg15 and Ifit1* expression in blood and an immature pDC phenotype associated with higher IFN production. BCAP$^{-/-}$ mice produced significantly less serum IFNα than WT mice after injection of TLR9 agonist, and BCAP promoted TLR7 and TLR9-induced IFNα production specifically in pDC. TLR-induced IFNα production in pDC requires DOCK2-mediated activation of Rac1 leading to activation of IKKα, a mechanism we show was dependent on BCAP. BCAP$^{-/-}$ pDC had decreased actin polymerization, Rac1 activation, and reduced IKKα phosphorylation upon TLR9 stimulation. We show a novel role for BCAP in promoting TLR-induced IFNα production in pDC and in SLE pathogenesis.

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Dedication

To my family and friends, without whom this PhD would have been completed a lot faster.

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Chapter 1

Introduction

IFNα in Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by the loss of tolerance to self-antigens and dysregulated cytokine activities. Patients suffering from SLE have circulating autoantibodies against nuclear proteins and nucleic acids that results in the formation and deposition of immune complexes (IC), which can lead to a diverse array of clinical manifestations. Disease activity and severity is correlated with elevated serum levels of type I interferons (IFN), specifically IFNα (1). Type I interferons (IFN) are a cytokine family that act on all cells to cause an anti-viral state and enhance innate and adaptive immune responses. These cytokines promote natural killer cell cytotoxicity, B cell antibody and cytotoxic T lymphocyte effector responses, expansion of naïve and memory T cells, and maturation of antigen-presenting dendritic cells. The pleiotropic effects of type I IFN make them key regulators in infection, autoimmunity, and cancer. Type I IFN levels are tightly regulated because these cytokines can have toxic effects including blocking proliferation and inducing apoptosis, and because their immune activating properties can have deleterious consequences. Secretion of IFNα is initiated upon detection of viral, foreign, or aberrant autologous DNA and RNA by innate immune cellular sensors.

Several studies have characterized a type I IFN signature in blood samples from SLE patients (1–5). This type I IFN signature, or increased expression of type I IFN genes, is present in most children, ~70-80% of adults suffering from aggressive forms of SLE disease, and ~50% of adults experiencing a milder form of disease. The amount of type I IFN or IFN signature in the blood of SLE patients is correlated with disease severity, pathogenic autoantibodies and clinical manifestations (6, 7) of disease. Therefore, there has been recent interest in blocking type I IFN for therapy of SLE, though trials to date have shown little efficacy, except in patients with a low

IFN signature (8). This suggests that once secreted from cells, the pleiotropic effects of type I IFN may be difficult to block. Therefore, a therapeutic strategy that inhibits IFN production may be more efficacious than blockade of type I IFN protein or receptor.

Though all cells can produce small amounts of type I IFN, plasmacytoid dendritic cells (pDC) produce large amounts of these cytokines and there is increasing evidence that pDC are an important source of IFNα promoting disease in SLE. pDC are a small population of hematopoietic cells whose prime function is swift and robust secretion of type I IFN in response to foreign nucleic acids, such as viral DNA or RNA. In SLE, self nucleic acids acquire access to the pDC endosome and promote TLR signaling. There is increasing evidence suggesting that pDC are important in the etiology and pathogenesis of SLE. First, pDC constitutively express endosomal TLR7 and TLR9, which recognize RNA and DNA respectively, and produce large amounts of IFNα in response to DNA and RNA containing immune complexes in SLE (6, 8). Second, patients suffering from SLE experience a reduction of pDC numbers in the blood, but a higher number of pDC in inflamed skin and tissues. Third, studies show a direct link between pDC and disease progression. These studies showed protection from or reduced severity of lupus-like disease in various mouse models either by depleting pDC using diphtheria toxin, causing pDC deficiency in the absence of IRF8 or E2-2, or diminishing the capacity of pDC to induce TLR7/9 induced IFNα due to lack of Slc15a4 (9–12). Therefore, pDC are crucial mediators for the pathogenesis and progression of SLE disease, and approaches for specifically limiting pDC IFNα production may deliver successful treatment options for patients suffering from SLE. Understanding the mechanisms by which pDC regulate type I IFN production may have important clinical implications for therapeutically regulating IFNα production in SLE patients.

pDC activation occurs upon RNA or DNA recognition by TLR7 or TLR9, respectively, leading to a response that can be dominated by IFNα or inflammatory cytokines. The type of response initiated by pDC is dependent on TLR ligand structure and endosomal compartmentalization. IFNα production by pDC requires the binding of the nucleic acid ligand to TLR7 or TLR9 within the IRF7 activating endosome, whereas inflammatory cytokine induction requires the binding of nucleic acid ligand within the NF-κB activating endosome. The Type I IFN response requires activation of MyD88, phosphorylation of inhibitor of NF-κB kinase-α (IKKα), and subsequent activation and translocation of IRF7 into the nucleus (13). IRAK1, TRAF3, DOCK2, Rac1, AP3, and numerous other signaling molecules are specifically required for IFNα induction in pDC (13–15).

TLR-induced IFNα production in pDC involves dual signaling pathways that converge upon IKKα activation (Figure 1.1). At the plasma membrane, nucleic acid ligand recognition leads to DOCK2-mediated activation of Rac1, which is required for the phosphorylation of IKKα (Figure 1.1A). This pathway occurs independently of TLR-nucleic acid binding. The pathway dependent on TLR-nucleic acid binding initiates in the endosome, where nucleic acids are recognized by TLR7 or TLR9, which activates MyD88 (Figure 1.1B). Both pathways are required for IKKα phosphorylation and subsequent IRF7 activation and IFNα production. DOCK2 activation of Rac1 and phosphorylation of IKKα are required for IFNα, but not inflammatory cytokine, production in pDC. Interestingly, phosphatidylinositol-3 kinase (PI3K) is essential for IRF7 activation and IFNα production but dispensable for the inflammatory cytokine response in pDC (16).

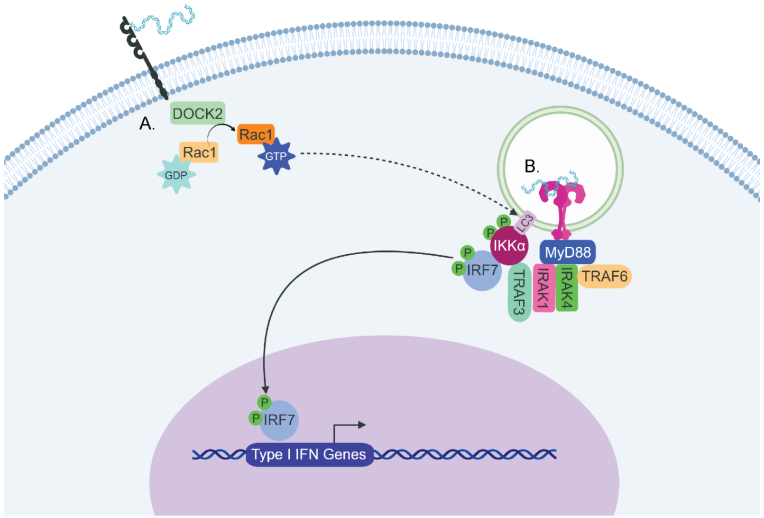


Figure 1.1 *Two signaling pathways required for TLR-induced IFNα induction in pDC.* (A) Nucleic acid recognition at plasma membrane initiates DOCK2 activation of Rac1, which is required for IKKα phosphorylation. This pathway occurs independently of TLR-ligand engagement. (B) In the IRF7 activating endosome, nucleic acid ligand binds to TLR7 or TLR9 to activate MyD88 and activate a signaling cascade that phosphorylates IKKα. (A-B) Both signaling pathways converge upon IKKα phosphorylation and are required for IRF7 activation and translocation into the nucleus and subsequent IFN induction.

Phosphoinositide 3-kinase (PI3K) and B cell adaptor for PI3K (BCAP)

BCAP was initially identified as a B cell expressed adaptor protein that is capable of recruiting PI3-kinase (PI3K) through its four YxxM motifs (Figure 1.2A) (17). After B-cell receptor (BCR) ligation, BCAP is phosphorylated on its YxxM tyrosines allowing the phospho-YxxM motifs to bind the regulatory p85 subunit of PI3K, which contains two SH2 domains that bind to phosphotyrosines within YxxM regions (Figure 1.2A-B) (18). This binding between p85 and the phospho-YxxM motifs leads to conformational changes that increase the catalytic activity of the p110 PI3K subunit, and induces recruitment of PI3K to the plasma membrane where it can access its lipid substrate PI(4,5)P_2. PI3K catalyzes the generation of PI(3,4,5)P_3 that then turns on downstream signaling through PDK1 and Akt. BCAP is expressed in various lymphocyte and myeloid cell populations such as B cells, T cells, monocytes, macrophages, and DC (18–22). Our lab has studied BCAP signaling in macrophages, and shown that BCAP is required for linking TLR to downstream activation of the PI3K pathway, thereby inhibiting inflammatory cytokine production (Figure 1.2C) (18, 19).

mRNA encoding BCAP is expressed abundantly in mouse pDC (Immgen.org), and we could identify high expression of BCAP protein in pDC by intracellular staining. We found in macrophages that BCAP associates with two signaling proteins known to mediate IFNα production in pDC, the p85 subunit of class I PI3K and the Rac activator DOCK2 (Ni and Hamerman, unpublished data). Because of the link between BCAP and TLR signaling in macrophages and the importance of PI3K in pDC IFNα production, we investigated the role of BCAP in regulating the TLR-induced IFNα production in pDC and in TLR7-driven lupus-like disease.

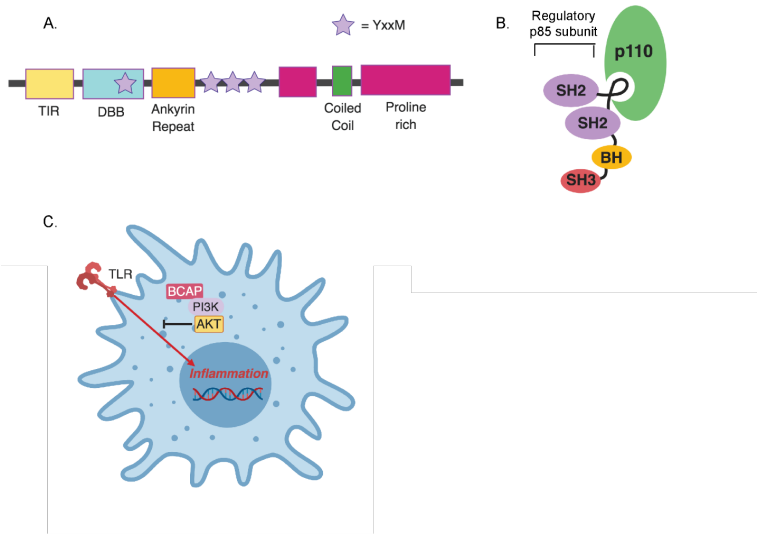


Figure 1.2 *Representation of BCAP and PI3K and their role in regulating TLR signaling in macrophages*. (A) Schematic of BCAP: various protein domains found within 811-amino acid structure of BCAP and four YxxM motifs which upon tyrosine phosphorylation can bind to the regulatory subunit of PI3K. (B) PI3K consists of p85 regulatory subunit, which contains two SH2 domains that can bind to phosphotyrosines within YxxM regions, and p110 catalytic subunit. (C) BCAP negatively regulates TLR-induced inflammatory cytokine production in macrophages through PI3K.

Chapter 2:

BCAP promotes TLR7-driven lupus like disease

Introduction

Toll-like receptors (TLR) are a family of pattern recognition receptors that detect components on a large variety of pathogens ranging from bacteria to fungi to viruses. Pathogen associated molecular pattern (PAMP) categories include lipids (detected by TLR2/TLR1, TLR2/TLR6, and TLR4), proteins (detected by TLR5), and nucleic acids (detected by TLR3, TLR7, TLR8, and TLR9) (23). TLR recognition of PAMPs is critical for initiating a proper innate immune response through secretion of inflammatory cytokines such as IL-6, TNFα, and IFNα. The inflammatory environment created by TLR signaling is also important for mounting an enhanced adaptive response against pathogens. Given the inflammatory nature of TLR-ligand activation, regulation of TLR stimulation is crucial for maintaining a healthy immune environment. Unregulated TLR activation triggered either by circulating PAMPS in chronic infections, by an increase in host derived self-antigens, or by a defect in TLR regulation can lead to sterile inflammation or autoimmunity.

Dysregulated recognition of self-nucleic acids through endosomal TLR7 and TLR9 is important in autoantibody production and subsequent lupus pathogenesis (8). Increased *Tlr7* gene dosage through spontaneous Yaa mutation or through transgene insertion induces systemic autoimmunity and immune activation (24). TLR7.1 mice express 8-16 fold higher TLR7 genomic DNA and mRNA than wildtype mice. These mice were generated by injecting excised BAC construct RP23-139P21, so only *TLR7* gene was expressed, into C57BL/6 zygotes (24). TLR7.1 transgenic mice exhibit various disease symptoms associated with lupus such as early lethality, lymphocyte activation, myeloid cell expansion, splenomegaly, autoantibody production, and mild kidney disease (24). A major effect of nucleic acid recognition within endosomal TLR7 or TLR9

is type I IFN induction, an inflammatory cytokine known to enhance innate and adaptive immune properties. pDC are a specialized producer of IFNα, that have been shown to have an increased IFN mRNA signature in blood of TLR7.1 mice (25). Also, when IFNα receptor (IFNAR) is genetically depleted in TLR7.1 overexpressing mice, aspects of lupus-like disease is ameliorated (25), suggesting that IFNα production by pDC may be important for promoting lupus like disease in TLR7.1 model. Given our interest in studying the role of BCAP in regulating TLR-induced IFNα production in pDC we decided to evaluate the contribution of BCAP to pathogenesis of TLR7-driven lupus-like disease. Therefore, we crossed the TLR7.1 transgene into the $BCAP^{-/-}$ background and analyzed disease at 10-13 weeks of age.

Results

BCAP promotes lupus-like disease

TLR7.1/BCAP$^{-/-}$ mice developed significantly less severe lupus like-disease compared to TLR7.1 mice, with a significant reduction of splenomegaly (Figure 2.1) and of CD11b^{+}CD11c^{+} myeloid expansion in the blood of TLR7.1/BCAP$^{-/-}$ compared to TLR7.1 mice (Figure 2.2) (24). We also found a reduced frequency of activated/effector CD44^{+}CD62L^{-} and recently activated CD69^{+} populations in both CD4^{+} and CD8^{+} T cell subsets in TLR7.1/BCAP$^{-/-}$ mice (Figure 2.3). We also observed a significant reduction in CD95^{+}GL7^{+} germinal center B cells (Figure 2.4A) and CD23^{+}CD21^{low} follicular B cells in TLR7.1/BCAP$^{-/-}$ mice with an increase of CD23^{-}CD21^{High} marginal zone B cells (Figure 2.4B). TLR7.1/BCAP$^{-/-}$ mice produced significantly less IgM, IgG and IgG2c anti-RNA autoantibodies (Figure 2.4C). As disease progresses, TLR7.1 mice develop immune complex deposition in the kidney and mild kidney pathology (Figure 2.5), which was reduced in TLR7.1/BCAP$^{-/-}$ mice. Overall, our data show that BCAP is a critical regulator in lupus-like disease progression in TLR7.1 mice.

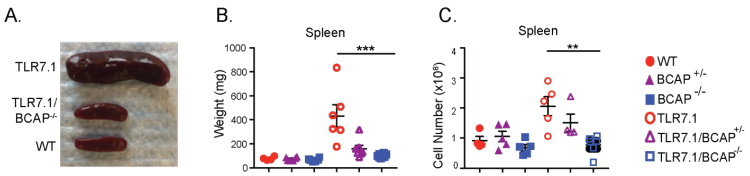


Figure 2.1 *BCAP promotes splenomegaly in TLR7.1 mice.* (A-C) Spleens from male and female 10-13 week old mice were analyzed for size (A) weight (B) and cell number (C). Each symbol represents an individual mouse (n=4-9 mice per group) from 3 independent experiments, mean +/- SEM are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Kruskal Wallis with Dunn's multiple comparison posttest.

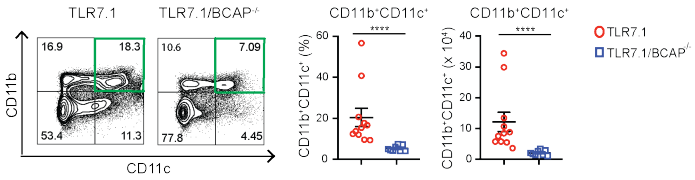


Figure 2.2 *BCAP promotes myeloid cell expansion in blood*. Blood from male and female 10-13 week old mice were analyzed for percent and cell number of myeloid ($CD11b^+CD11c^+$) cell expansion. Each symbol represents an individual mouse (n=8-11 mice per group) from 3 independent experiments, mean +/- SEM are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Mann-Whitney test.

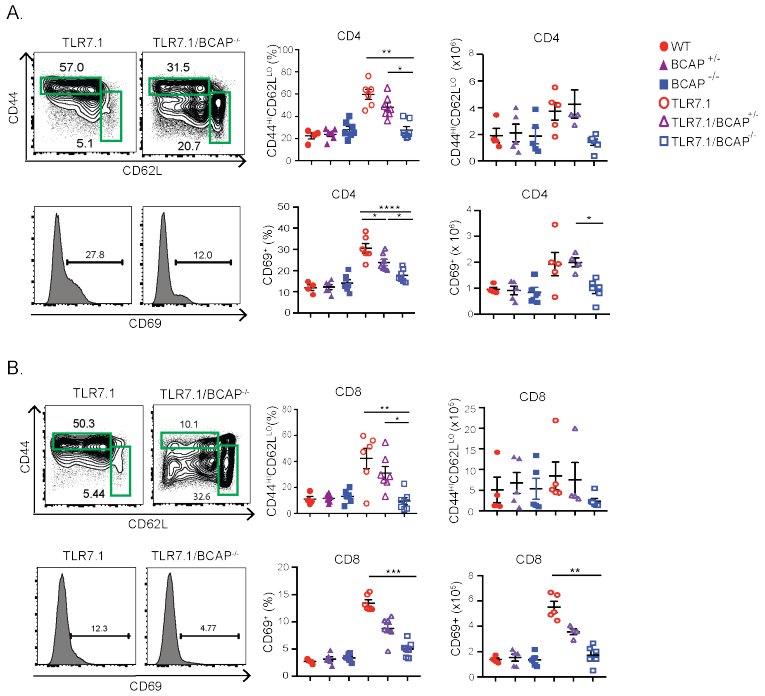


Figure 2.3 *BCAP promotes increased activation of effector and early activated T cells*. Spleens from male and female 10-13 week old mice were analyzed. (A) Percent of activated effector $CD4^+TCR\beta^+$ T cells (gated as ($CD44^+CD62L^-$) and early activated $CD4^+TCR\beta^+$ T cells (gated as $CD69^+$). (B) Percent of activated effector $CD8^+TCR\beta^+$ T cells (gated as $CD44^+CD62L^-$) and early activated $CD8^+TCR\beta^+$ T cells (gated as $CD69^+$). Each symbol represents an individual mouse (n=4-9 mice per group) from 3 independent experiments, mean +/- SEM are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Kruskal Wallis with Dunn's multiple comparison posttest.

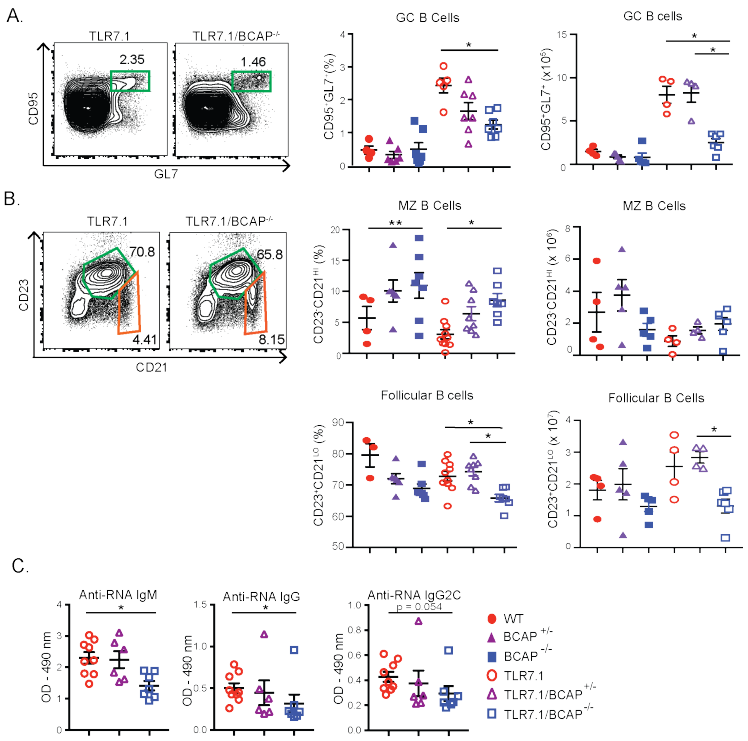
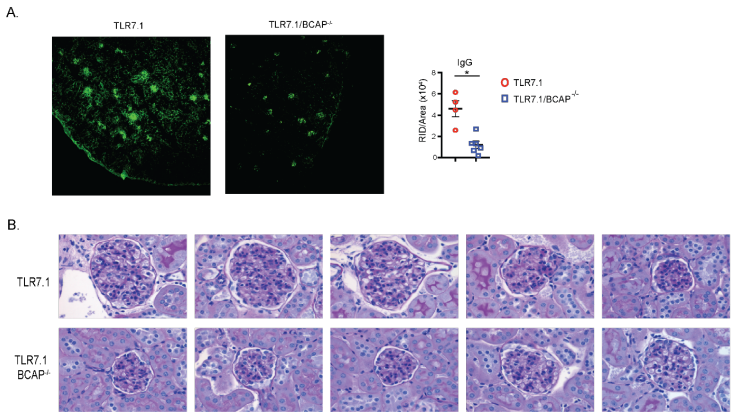


Figure 2.4 *BCAP promotes germinal center B cells and anti-RNA autoantibody production.* Spleens from male and female 10-13 week old mice were analyzed. (A) Percent and cell number of TCRβ$^-$B220$^+$ cells with a germinal center phenotype (gated as CD95$^+$GL7$^+$). (B) Percent and cell number of TCRβ$^-$B220$^+$ cells with a marginal zone and follicular phenotype (gated as CD23$^-$CD21^{HI} and CD23$^+$CD21^{LO}). (C) Serum anti-RNA antibodies of indicated isotypes. Each symbol represents an individual mouse (n=3-9 mice per group) from 3 independent experiments, mean +/- SEM are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Kruskal Wallis with Dunn's multiple comparison posttest.



	TLR7.1	TLR7.1/ BCAP-/-
Glomerulonephritis	4/5	2/5
Increased Glomeruli Size	3/5	1/5
Increased Glomeruli Cellularity	4/5	2/5

Figure 2.5 *BCAP promotes kidney pathology in TLR7.1 mice.* (A) Representative IgG staining of kidney sections with quantitation. (B) Kidney sections stained for PAS and analyzed for kidney pathology. (A) Each symbol represents an individual mouse (n=4-6 mice per group) from 3 independent experiments, mean +/- SEM are shown. *p < 0.05, Mann-Whitney test.

BCAP regulates TLR-induced IFNα responses

Dysregulation of IFNα secretion in response to innate recognition of nuclear antigens contributes to SLE pathogenesis (6). The amount of type I IFN or IFN signature in the blood of SLE patients correlates with disease severity, pathogenic autoantibodies, and clinical manifestations of disease. TLR7.1 mice have a type I IFN signature (24, 25), and TLR7.1 pDC constitutively express type I IFN mRNA (25). To determine a role for pDC in TLR7.1 mice, we have performed a pDC depletion experiment in this model (Figure 2.6). 5-6 week old TLR7.1 and TLR7.1/BDCA2-DTR^{+} mice were treated with diphtheria toxin every 48 hours for 3 weeks. Mice were euthanized 24 hours after last DT treatment, spleens were harvested, and cellular immune activation was quantified by flow cytometry. We showed that TLR7.1/BDCA2-DTR^{+} mice had significantly reduced splenomegaly (Figure 2.6B) and expression of early activation marker CD69^{+} in both CD4^{+}TCRβ^{+} and CD8^{+}TCRβ^{+} T cells (Figure 2.6C-D). Interestingly, there was no difference in activated/effector CD44^{+}CD62L^{-} CD4^{+}TCRβ^{+} T cells, but there was a significant reduction in activated CD44^{+}CD62L^{-} CD8^{+}TCRβ^{+} T cells in TLR7.1/BDCA2-DTR^{+} mice in comparison with TLR7.1 mice. There was also a trend towards a reduction in germinal center B cells (gated as TCRβ^{-}B220^{+}CD95^{+}GL7^{+}) in TLR7.1/BDCA2-DTR^{+} mice (Figure 2.6G), showing an important role for pDC early in TLR7.1 disease.

TLR7.1/BCAP$^{-/-}$ mice had reduced mRNA for the ISGs *Ifit1* and *Isg15* in the blood (Figure 2.7A), suggesting BCAP may be important in pDC activation. We found that TLR7.1/BCAP$^{-/-}$ mice had a reduced population of MHCII^{LO} pDC compared to TLR7.1 mice (Figure 2.7B), a population that has been associated with higher IFNα production (26, 27), consistent with a role for BCAP in pDC function during chronic TLR7 signaling. To examine the role of BCAP in regulating TLR-driven IFNα production in vivo in a non-transgenic model, we injected WT and

BCAP$^{-/-}$ mice with TLR9 agonists complexed with DOTAP, which induce IFNα in vivo in a pDC-dependent manner (28). BCAP$^{-/-}$ mice produced significantly less serum IFNα (Figure 2.8A), but comparable quantities of IL-6, after injection of CpG-A and CpG-C (Figure 2.8B), consistent with a pDC IFNα defect. The decreased *Ifit1* and *Isg15* expression and decreased percentage of immature MHCII^{LO} pDC in TLR7.1/BCAP$^{-/-}$ mice, and the reduced IFNα in serum after TLR9 agonist injection in BCAP$^{-/-}$ mice led us to hypothesize BCAP promotes TLR-induced IFNα in pDC.

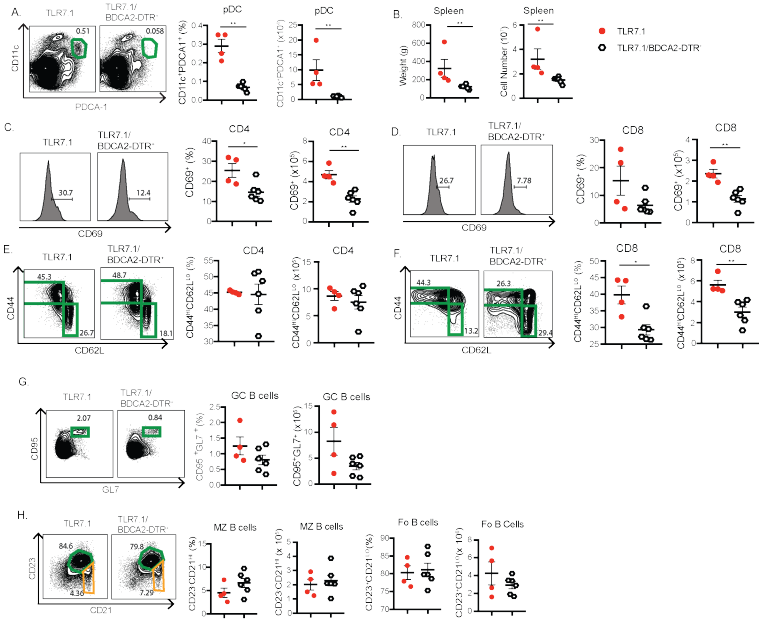


Figure 2.6 *pDC promote early lupus-like disease in TLR7.1 model.* 5-6 week old TLR7.1 and TLR7.1/BDCA2-DTR^{+} mice were treated with 10 ng/g body weight diphtheria toxin every 48 hours for 3 weeks to deplete pDC (A). Mice were euthanized 24 hours after last DT treatment, spleens were harvested and weighed to quantify splenomegaly (B). pDC depletion (A) and cellular immune activation was quantified by flow cytometry (C-H). Each symbol represents cells from an individual mouse (n=4-6 mice per group), mean +/- SEM are shown. *p < 0.05, Mann-Whitney test.

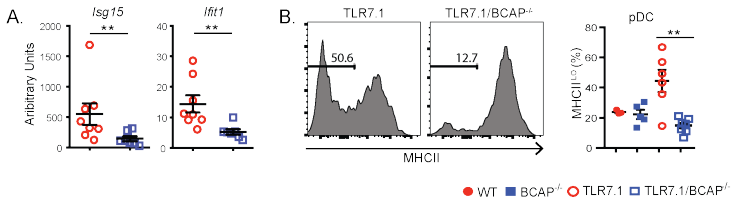


Figure 2.7 *BCAP promotes Isg15 and Ifit1 expression and immature pDC phenotype in TLR7.1 mice.* (A) Isg15 and Ifit1 mRNA levels in whole blood measured by qPCR. (B) pDC were gated as CD11b^{-}PDCA-1^{+}CD11c^{+} cells. Each symbol represents an individual mouse (n=4-8 per group) from 3 independent experiments, mean +/- SEM are shown. **p < 0.01, (A) Mann-Whitney test, (B) Kruskal Wallis with Dunn's multiple comparison posttest.

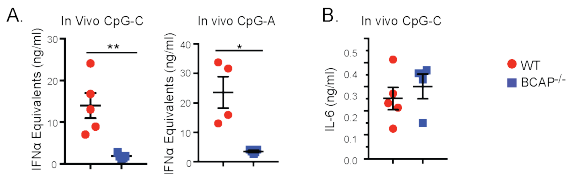


Figure 2.8 *BCAP promotes IFNα production upon TLR9 agonist injection*. Mice were injected with (A-B) DOTAP-CpG-C or (A) DOTAP-CpG-A mixture and serum was collected 3 hours post injection. (A) IFNα was detected by ISRE reporter assay and (B) IL-6 was quantified by ELISA. Each symbol represents an individual mouse (n=4-6 per group) from 3 independent experiments, mean +/- SEM are shown. **p < 0.01, ***p < 0.001, Mann-Whitney test.

Discussion

Here, we show BCAP promotes TLR7-driven lupus-like disease in vivo and TLR-induced IFNα production in mice that is likely mediated by pDC (28). We found that TLR7.1/BCAP$^{-/-}$ mice have reduced disease, but whether this alleviation of disease is dependent specifically on expression of BCAP in pDC, or in other cells such as B cells, activated T cells, or other myeloid cells requires further investigation (20, 21, 29). There is reason to believe the role of BCAP in promoting pDC IFNα expression could be significant for disease reduction. First, transient pDC depletion ameliorated TLR7.1 disease (Figure 2.6). Second, others have shown protection from or reduced severity of lupus-like disease in various mouse models either by acutely or constitutively reducing pDC number or diminishing the capacity of pDC to induce TLR7/9 induced IFNα (9, 11, 12). Third, all B cell responses are not lacking in TLR7.1/BCAP$^{-/-}$ mice consistent with prior reports (17, 30). TLR7.1/BCAP$^{-/-}$ mice have more germinal center B cells than WT mice without the TLR7.1 transgene, and TLR7.1/BCAP$^{-/-}$ mice produce anti-RNA IgM and IgG, suggesting their B cell-T cell interactions are at least partially intact. Together, this suggests that disease reduction in TLR7.1/BCAP$^{-/-}$ mice is attributed to something more than just a B cell defect. Our results point to a reduction of pDC IFNα production as one part of the protection of TLR7.1/BCAP$^{-/-}$ mice from lupus-like disease. However, we do not rule out a contribution of other pDC functions as well as defects in other innate or adaptive immune cells that may depend upon BCAP (17–22, 30, 31).

Chapter 3:

BCAP promotes TLR-induced IFNα in pDC

Introduction

Plasmacytoid dendritic cells (pDC) are important mediators of IFNα and inflammatory cytokine secretion. pDC activation occurs upon RNA or DNA recognition by TLR7 or TLR9, respectively, leading to a response that can be dominated by IFNα, inflammatory cytokines or a mixture of these cytokines. The cytokine response initiated by pDC is dependent on the TLR-ligand entering and being retained within the proper endosomal compartment (Figure 3.1A-B). For pDC to induce inflammatory cytokines and DC maturation the TLR-ligand must enter the NF-κB activating lysosomal compartment and signal through MyD88 to predominately activate NF-κB (Figure 3.1B).

Nucleic acid-induced IFNα production in pDC requires two signaling pathways that converge upon IKKα activation (Figure 3.1A). The first pathway begins at the cell surface where nucleic acid recognition initiates a cascade leading to DOCK2-mediated activation of Rac1, required for phosphorylation and activation of IKKα (15, 32). The second pathway initiates in the endosome where nucleic acid-TLR engagement leads to a signaling cascade where MyD88 complexes with TNF receptor-associated factor 6 (TRAF6), IL-1 receptor-associated kinase 4 (IRAK4), IL-1 receptor associated kinase 1 (IRAK1), TNF receptor-associated factor 3 (TRAF3), and IFN regulatory 7 (IRF7) (Figure 3.1A). IRF7 is an essential regulator of type I IFN in pDC, and IFNα induction requires IRF7 to translocate into the nucleus upon IRF7 phosphorylation by activated IKKα. Therefore, both the cell surface and endosomal pathways are required for IKKα phosphorylation and subsequent IRF7 activation and IFNα production.

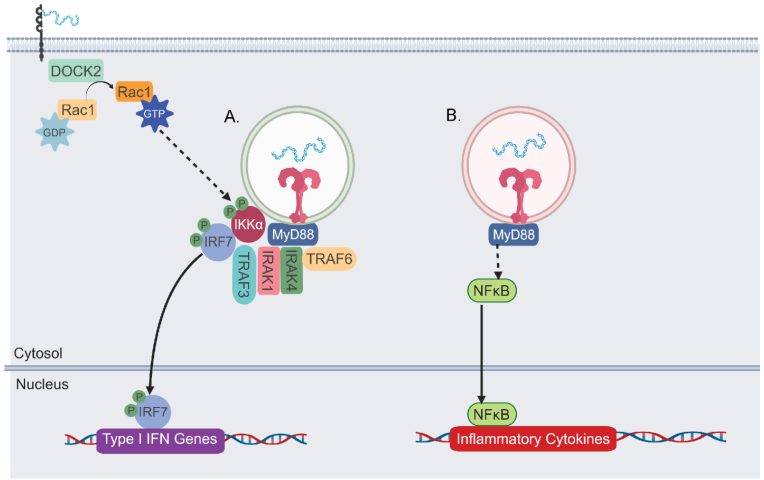


Figure 3.1 *Model of TLR-induced cytokine regulation in pDC.* Nucleic acid engagement with TLR7 or TLR9 located within endosomal compartments leads to cytokine production in pDC. (A) IFNα induction in pDC requires: 1) IKKα activation by DOCK2 mediated activation of Rac1 and 2) binding of nucleic acid ligand to TLR within the IRF7 activating endosome, and subsequent IKKα phosphorylation of IRF7 and translocation of IRF7 into the nucleus. (B) TLR-induced inflammatory cytokine production in pDC requires the nucleic acid ligand to enter and signal through the NF-κB activating endosome.

Results

BCAP-deficient pDC produce less IFNα when stimulated via TLR7 and TLR9

We confirmed that BCAP does not affect pDC development or homeostasis, because BCAP$^{-/-}$ mice had similar numbers and phenotype of pDC in the BM and spleen (Figure 3.2). pDC generated from Flt3L-cultured BCAP$^{-/-}$ BM had similar frequency, but reduced total number compared to WT BM (Figure 3.2A). To assess whether BCAP promotes TLR-induced IFNα production specifically in pDC, we first used pDC grown from BM with Flt3L. BCAP$^{-/-}$ pDC secreted significantly less IFNα in response to CpG-C and R848 (Figure 3.3A). This result was not due to decreased DNA internalization as there was no difference in CpG uptake in WT vs BCAP$^{-/-}$ pDC (Figure 3.4). Similar results were seen with pDC harvested directly ex vivo with CpG-C or CpG-A (Figure 3.3B). Thus, BCAP$^{-/-}$ pDC had an impaired ability to induce TLR9 and TLR7-driven IFNα.

In SLE, immune complexes (IC) of autoantibodies and self-nucleic acids are internalized by Fc receptors to activate endosomal TLR signaling and IFNα secretion. Therefore, we stimulated purified pDC with DNA-containing IC. BCAP$^{-/-}$ pDC produced significantly less IFNα when stimulated with IC (Figure 3.3C). Similar results were obtained when pDC were stimulated with the RNA virus Vesicular stomatitis virus (VSV), an activator of TLR7 signaling (Figure 3.3D). Thus, BCAP selectively promoted TLR-induced IFNα activation in pDC.

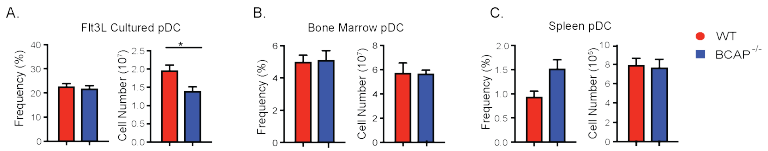


Figure 3.2 *BCAP does not affect pDC development or homeostasis.* pDC cell number and frequency were gated for CD11b$^-$MHCII$^-$CD11c$^+$PDCA-1$^+$Siglec-H$^+$ and quantified by flow cytometry. pDC were (A) grown up in culture media containing Flt3L from BM culture (B) harvested directly ex vivo from BM or (C) harvested directly ex vivo from spleen digested in collagenase mixture. Mean +/- SEM shown (n > 5). *p < 0.05, unpaired Student's t-test.

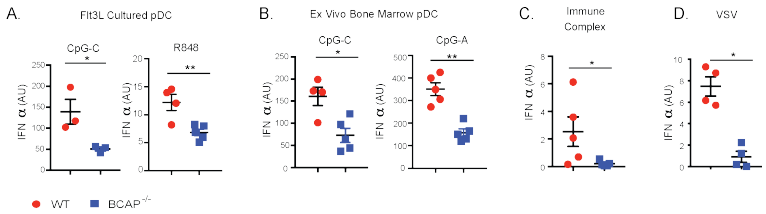


Figure 3.3 *BCAP-deficient pDC produce less IFNα.* (A, C, D) pDC grown in Flt3L-containing media from whole BM and (B) mature pDC harvested directly ex vivo from BM were stimulated with TLR9 agonists CpG-C, CpG-A, and TLR7 agonist R848 (A,B), DNA-containing IC (C), or VSV (D). IFNα was quantified by ISRE reporter assay. Each symbol represents cells from an individual mouse (n=3-5 per group), mean +/- SEM are shown. Representative of 3 independent experiments. *p < 0.05, **p < 0.01, unpaired Student's t-test (A-B), Mann-Whitney (C,D). In all cases IFNα and IL-6 secretion were not detectable from unstimulated pDC.

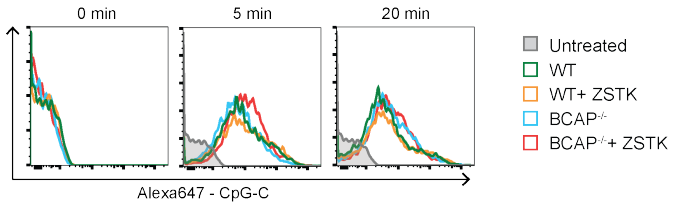


Figure 3.4 *BCAP and PI3K do not regulate ligand uptake.* Kinetics of Alexa647-labeled CpG-C internalization by Flt3L cultured pDC (gated as $CD11c^{+}PDCA\text{-}1^{+}Siglec\text{-}H^{+}$). Internalization was distinguished from cell surface binding with controls treated at 4°C and controls that were washed with dilute acid to remove any surface-bound CpG-C (not shown). These controls showed that A647 signal shown was from internalized CpG-C.

BCAP does not regulate ligand endosomal trafficking or inflammatory cytokine production

To determine whether BCAP regulates IFNα production in pDC by reducing localization, shuttling, or retention of the TLR9 and TLR7 ligands within the IRF7 activating endosome, we stimulated BM derived Flt3L cultured pDC with dual labeled FITC-CpGC-Alexa647 (Figure 3.5A) TLR9 ligand for various time points. We measured the ratio of ligand in the non-acidic IRF7 activating endosome vs the acidic NF-κB activating endosome by quantifying the ratio of FITC to Alexa647 signal (Figure 3.5B). The FITC MFI signal will quench (MFI signal will decrease) if shifted into an acidic environment while the Alexa647 MFI signal will remain constant (Figure 3.5B), so if BCAP promotes ligand retention in the IRF7 activating endosome, then WT pDC will have an increased ratio of FITC/Alexa647 compared to $BCAP^{-/-}$ pDC. Or if the absence of BCAP traffics the ligand away from the IRF7 endosome or directly into the NF-κB endosome, then $BCAP^{-/-}$ pDC will have a reduced ratio of FITC/Alexa647 signal from very early time points. We saw no difference in the ratio of FITC/Alexa647 signals at various time points between WT and $BCAP^{-/-}$ pDC (Figure 3.5C), suggesting that BCAP does not regulate ligand endosomal retention, shuttling, or trafficking. Furthermore, pDC induction of inflammatory cytokines requires ligand entering and activating the NF-κB endosome. To test whether BCAP regulates inflammatory cytokine induction, BM derived Flt3L cultured pDC (Figure 3.6A) and pDC harvested directly ex vivo from BM (Figure 3.6B) were stimulated with CpG-C for 12-15 hours and IL-6 levels were detected by ELISA. No significant difference of IL-6 was detected from WT vs $BCAP^{-/-}$ pDC (Figure 3.6), suggesting BCAP does not regulate the ability of CpG-C to enter the NF-κB activating endosome. Also, no difference in inflammatory cytokine was observed between WT vs BCAP-/- mice when injected with DOTAP-CpG-C (Figure 2.8B).

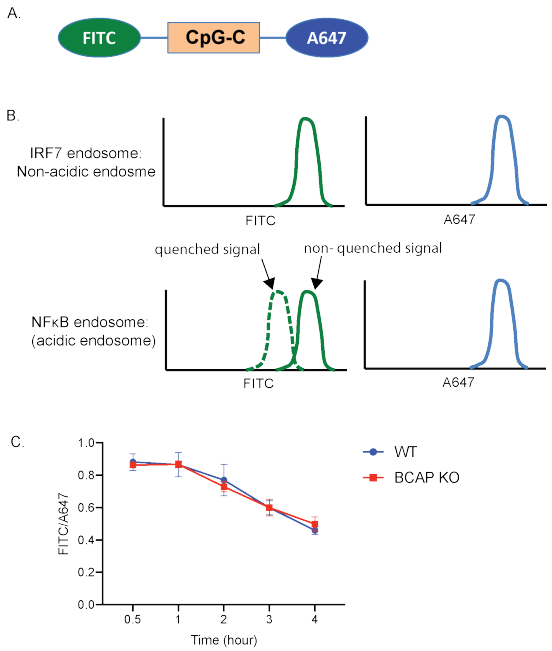


Figure 3.5 *BCAP-/- pDC have similar rate of ligand trafficking within endosomes*. (A) Model of dual labeled TLR9 ligand FITC-CpGC-A647. (B) Representation of dual ligand trafficking assay. FITC MFI signal (green histogram) quenches in an acidic environment while A647 MFI signal (blue histogram) remains constant. If BCAP regulates endosomal retention and more ligand enters IRF7 endosome (non-acidic endosome) then FITC/A647 ratio will be increased/closer to one. If more ligand enters the NF-κB endosome (acidic endosome) then ratio of FITC/A647 will be reduced. (C) pDC grown in Flt3L-containing media from whole BM were stimulated with dual labeled TLR9 agonist FITC-CpGC-A647. MFI was quantified by flow cytometry. (C) Data show Mean +/- SEM, representative of 3 independent experiments.

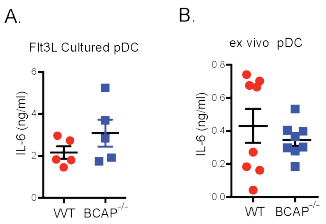


Figure 3.6 *BCAP-/- pDC have similar levels of IL-6 upon nucleic acid stimulation.* (A) pDC grown in Flt3L-containing media from whole BM and (B) mature pDC harvested directly ex vivo from BM were stimulated with TLR9 agonist CpG-C for 12-15 hours. IL-6 was quantified by ELISA. Each symbol represents cells from an individual mouse (n=5-7 per group), mean +/- SEM are shown. Representative of 3 independent experiment. (A) Student's t-test (B) Mann-Whitney test.

BCAP associates with DOCK2

DOCK2 activation of Rac1 and phosphorylation of IKKα are required for IFNα, but not inflammatory cytokine, production in pDC (15). Because BCAP$^{-/-}$ pDC had a similar phenotype to *Dock2*$^{-/-}$ pDC, we hypothesized BCAP regulates TLR-induced IFNα production through DOCK2 activation of Rac1. Importantly, BCAP$^{-/-}$ mice showed no evidence of described *Dock2* genomic mutations (33) (not shown) and BCAP$^{-/-}$ pDC have normal DOCK2 expression (Figure 3.7A). Furthermore, our proteomics data in macrophages suggested a potential interaction between BCAP and DOCK2 (Ni and Hamerman, unpublished observations). We confirmed that BCAP and DOCK2 interact using co-transfection studies and found that BCAP binds to DOCK2 through its N-terminal domain (Figure 3.7B-C).

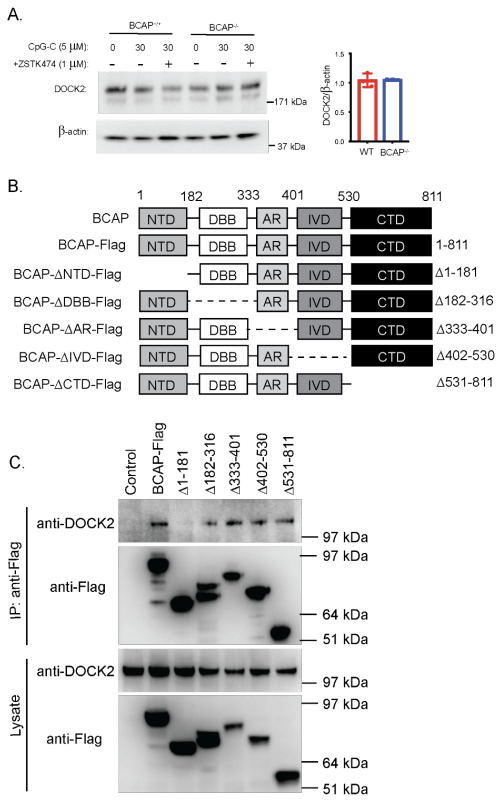


Figure 3.7 *DOCK2 associates with N-terminal domain of BCAP*. (A) Sorted pDC from culture were rested overnight and stimulated with CpG-C (5 μM) in the presence or absence of PI3K inhibitor ZSTK474 (1 μM). Lysates were blotted for DOCK2 or β-actin. (B) Representation of BCAP deletion mutants. NTD: N-terminal domain; DBB: Dof/Bank/BCAP; AR: Ankyrin Repeats; IVD: intervening domain; CTD: C-terminal domain. (C) To determine whether BCAP associates with DOCK2, 293T cells were transfected with DOCK2 and empty control or mutant BCAP-FLAG tagged vectors. Cells were lysed and immunoprecipitated with anti-FLAG antibody. IP and lysates were blotted for DOCK2 or FLAG.

BCAP and PI3K promote Rac1 and IKKα activation

In pDC, DOCK2 activates Rac1, a member of the Rho family GTPases that regulates cytoskeleton organization including actin polymerization (15). BCAP$^{-/-}$ pDC had reduced CpG-induced actin polymerization (Figure 3.8A), an indirect readout for Rac1 activation. Inhibiting class I PI3K in WT pDC phenocopied BCAP-deficiency. We directly tested whether BCAP activates Rac1 by quantifying the active GTP-bound form of Rac1 after CpG-C stimulation. BCAP$^{-/-}$ pDC had reduced CpG-induced Rac1 activation (Figure 3.8B), showing that BCAP is upstream of CpG-induced Rac1 activation. Preliminary data shows that inhibiting class I PI3K in WT pDC reduced Rac1 activation to similar levels seen in BCAP$^{-/-}$ pDC, suggesting BCAP and PI3K may be required for Rac1 activation (Figure 3.8C). Nucleic acid-induced IFNα production in pDC requires both DOCK2-mediated Rac1 activation and MyD88 activation for the phosphorylation and activation of IKKα. Consistent with reduced Rac1 activation, BCAP$^{-/-}$ pDC and WT pDC previously treated with PI3K inhibitor ZSTK474 had reduced IKKα phosphorylation in response to CpG-C stimulation (Figure 3.7D-E).

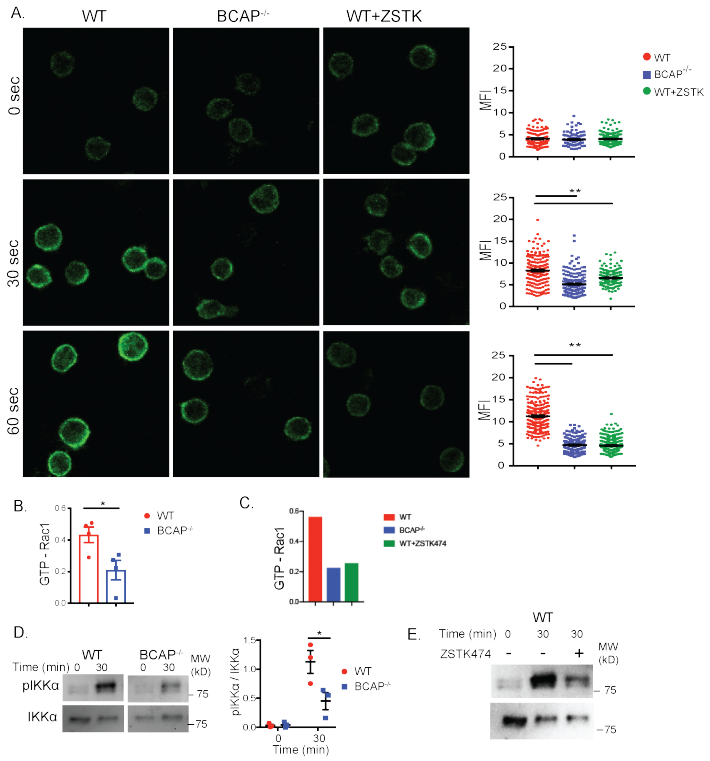


Figure 3.8 *BCAP and PI3K promote actin polymerization, Rac1 activation, and IKKα phosphorylation.* pDC grown from BM in Flt3L were sorted and stimulated with CpG-C. (A) Actin polymerization was visualized by phalloidin staining followed by confocal microscopy and quantification of MFI per cell. Each symbol represents an individual cell analyzed in one experiment (n=100-200 per group) and is representative of 3 experiments. Where indicated, (C, E) pDC were pre-treated with ZSTK474 for 30 min before CpG-C. (B-C) Quantification of Rac1 activation measured by GTP-bound Rac1. Each symbol represents an independent experiment (n=4), and mean +/- SEM is shown. (D-E) Phosphorylation of IKKα was quantified by western blot, panels shown are from the same exposure cropped to exclude other treatment groups. Representative blot and quantification of 3 independent experiments is shown as mean +/- SEM. *p < 0.05, **p < 0.01 (A) One-way ANOVA with Tukey's multiple comparison posttest, (B,D) unpaired Student's t-test.

Discussion

We found that BCAP promotes TLR-induced IFNα production in pDC and that BCAP$^{-/-}$ pDC have reduced F-actin polymerization, Rac1 activation, phosphorylation of IKKα, and IFNα secretion upon stimulation with nucleic acids. We propose that BCAP regulates IFNα production upstream of DOCK2-Rac1 signaling, which are independent of nucleic acid engagement of TLR7 and TLR9 (15) (Figure 3.9). The mechanism by which nucleic acid recognition activates DOCK2 and Rac1 remains unknown. Receptor for advanced glycation end-products (RAGE) can bind and internalize nucleic acids for delivery to TLR7/9 containing endosomes, and can activate Rac1, thus RAGE may be one receptor activating this pathway (34–36). The mechanism by which Rac1 leads to IKKα activation also remains undefined, however there is evidence that Rac-mediated production of ROS may control IKKα phosphorylation (37). Additionally, ROS and the PI3K product PIP3 are required for conjugation of LC3 to LC3-associated phagosomes (LAPosomes). In macrophages after nucleic acid stimulation, LC3 is recruited to LAPosomes to anchor IKKα and bring the kinase to IRF7, where it is phosphorylated and translocated into the nucleus to transcribe type I IFN genes (38). We propose that BCAP, both through direct binding to DOCK2 and through induction of class I PI3K activity, participates in activation of DOCK2 and Rac1, and subsequent downstream signals leading to IKKα phosphorylation. This is supported by studies showing CpG DNA-induced PI3K activation occurs independently of nucleic acid engagement of TLR (39). Further studies are required to determine if BCAP mediates ROS production via Rac1 upon nucleic acid recognition in pDC, and any potential role for BCAP in parts of this signaling pathway downstream of MyD88, which were not investigated in this study.

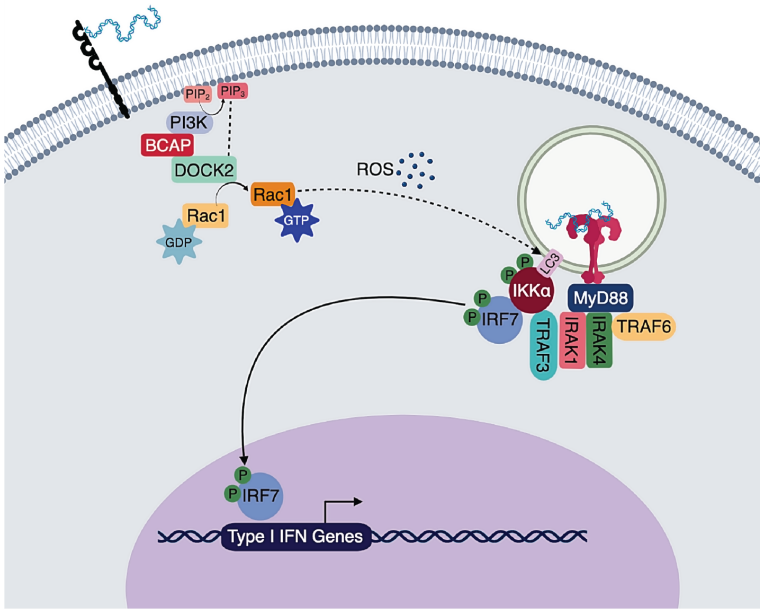


Figure 3.9 *Model of BCAP function in TLR-induced IFNα production.* We show that BCAP regulated IFNα production upstream of DOCK2-Rac1 signaling, a pathway independent of nucleic acid engagement of TLR leading to phosphorylation of IKKα. We propose BCAP is activated and phosphorylated downstream of cell surface receptors that engage nucleic acids. BCAP, when phosphorylated, recruits and activates class I PI3K, which generate PIP3. DOCK2 can bind to BCAP and to PIP3 (40), thus we propose that together PIP3 and BCAP recruit and activate DOCK2 leading to activation of Rac1 and Rac2, which can promote both actin polymerization (15) and ROS production (41). ROS may allow for generation of the LAPosome by recruiting LC3 to the nucleic acid containing endosome, allowing for docking and phosphorylation of IKKα (38). IRF7 phosphorylation and translocation to the nucleus, leading to transcription of genes incoding IFNα. Model was generated with BioRender.

Chapter 4:

Materials and Methods

Mice

All mice were bred and housed at the Benaroya Research Institute vivarium. BCAP$^{-/-}$ mice containing a disrupted *Pik3ap1* gene were backcrossed >9 generations to C57BL/6 mice (18, 29, 31). TLR7.1 transgenic mice, created using a BAC construct, contain 8-16 copies of the *Tlr7* gene and 8-16 fold higher *Tlr7* mRNA levels above wild type mice were provided by S. Bolland (NIH) (24). TLR7.1 mice were crossed to BCAP$^{-/-}$ mice and BDCA2-DTR transgenic mice (42). All experiments were performed under approved Institutional Animal Care and Use Committees protocols.

pDC depletion

TLR7.1 or TLR7.1/BDCA2-DTR mice at 5-6 weeks of age were treated with diphtheria toxin (DT) every 48 hours for 3 weeks. Mice were harvested at 8-9 weeks and effects of pDC depletion on lupus pathogenesis was analyzed by quantifying splenomegaly and immune cell activation by flow cytometry. BDCA-2 is a C-type lectin expressed exclusively in humans but contains conserved target sequences for pDC-specific transcription factor E2-2 that overlaps across species. E2-2 interacts with the BDCA-2 promoter and BDCA-2 DTR mice treated with DT have transient pDC specific (5).

Phenotyping lupus-like disease in TLR7.1 mice

Male and female mice were harvested at 10-13 wk of age. Blood and spleen leukocyte expansion and activation was measured by flow cytometry.

Spleen digestion

Spleens were placed in digest mix (1.6 mg/ml Worthington type IV collagenase in 10% FCS, 10 mM HEPES in RPMI) containing DNAse I (8 mg/ml), cut into tiny pieces, and incubated at 37°C

for 25 minutes. 800 µl of cell dissociation buffer was added, spleens were then incubated for an additional 5 minutes. Mixture was quenched with ice cold digest mix. Spleens were then filtered through a 40 µm filter.

IgG staining of kidneys
Frozen kidney cryosections (5 µm) were stained with anti-IgG-FITC. Raw integrated density/area was calculated via ImageJ. Paraffin sections were stained with H&E and PAS and were analyzed for kidney pathology in a blinded manner.

Anti-RNA antibody ELISA
Serum anti-RNA Abs were quantified by anti-RNA ELISA as in (43).

pDC
Bone marrow (BM) cells were cultured in pDC media (RPMI,10%FCS, P/S, L-glutamate, NEAA, Hepes, Sodium Pyruvate, β-mercaptoethanol) containing 200 ng/ml Flt3L for 7 days and purified by sorting as $CD11b^{-}MHCII^{-}CD11c^{+}PDCA1^{+}$. pDC harvested directly ex vivo from BM were enriched by MACS depletion followed by sorting.

pDC TLR-ligand stimulation
30,000-50,000 purified pDC per well were plated in 96-well plates in pDC media with 200 ng/ml Flt3L. Cells were rested for 2 hours and then stimulated for 12-15 hours: R848 (1 µg/ml), CpG-A ODN1585 (2 µM), CpG-C ODN2395 (2-5 µM) (Invivogen, IDT).

In vivo CpG-ODN
10 µg CpG-A ODN1585 or CpG-C ODN2395 in 100 µl of PBS was mixed with 20 µg DOTAP (Avanti) in 100 µl PBS in a polystyrene tube at room temperature for 10 min, then 200 µl DOTAP-CpG mixture/mouse was injected i.v. Serum was collected at 3 hours post injection.

Immune complex formation
Immune complexes were formed by combining CG50 DNA (30 ng/ml) with anti-DNA mAb PA4 (10 µg/ml) for 1 hour at 37°C (44), then added to pDC for 12-15 hours. pDC were treated with VSV (MOI 10) for 12-15 hours. Alexa647-labled CpG-C (IDT) was incubated with Flt3L-cultured pDC for indicated times and internalization was measured by flow cytometry.

Cytokine quantification
Type I IFN was quantified by incubating supernatant or serum with L929-ISRE reporter cells for 6 hours with rmIFNα standard (PBL), followed by luminescence measurement using Bright-Glo reagent (Promega). IL-6 was quantified by ELISA (eBioscience).

Ligand uptake assay
Flt3L cultured pDC (gated as $CD11c^{+}PDCA\text{-}1^{+}SiglecH^{+}$) were stimulated with Alexa647-labeled CpG-C at different time points. Internalization was distinguished from cell surface binding with controls treated at 4°C and controls that were washed with dilute acid to remove any surface-bound CpG-C (not shown).

Endosome trafficking quenching assay
Flt3L cultured pDC (gated as $CD11c^{+}PDCA\text{-}1^{+}SiglecH^{+}$) were stimulated with dual labeled FITC-CpGC-Alexa647 for different time points. Cells were washed and analyzed by flow cytometry.

Quantitative RT-PCR

Blood RNA was generated using RNeasy protect animal tubes and blood kit (Qiagen). cDNA was synthesized using Qiagen reagents with random hexamers and oligo(dT) primers. Quantitative PCR was performed using SYBR Green reagents (TaKaRa Bio) on a 7500 fast real-time PCR system (Applied Biosystems). Primers were as in (25).

Immunoblotting and co-IP

Sorted pDC from Flt3L culture were rested overnight and stimulated with CpG-C (1-5 μM). Cells were lysed by RIPA buffer (Thermo Sci) and run on SDS-Page. Phosphorylation of IKKα (Ser176/180; 2697; Cell Signaling Technology) was analyzed by immunoblot. For co-IP, HEK-293 T cells were transfected with DOCK2 and BCAP FLAG-tagged vectors. Cells were lysed and immunoprecipitated with anti-FLAG (M2, Sigma). IP and lysates were blotted for DOCK2 and FLAG.

Rac1 activation and actin remodeling assay

Sorted pDC were rested for 3 hours in low serum medium containing 200 ng/ml Flt3L, then stimulated with 1 μM CpG-C. In some cases, pDC were pretreated for 30 min with 1 μM ZSTK474 (Tocris). Rac1 activation in cell lysates was measured by Rac-1 GLISA kit (Cytoskeleton, Inc.). For actin polymerization, cells were fixed with 4% PFA, permeabilized with 0.1% Triton-X100, 3% BSA in PBS, and stained with A488-Phalloidin (Cytoskeleton, Inc.) for 30 min, and analyzed by fluorescence microscopy. MFI for each cell was analyzed using Image J.

Statistics

Statistical analysis was performed with Prism 8 (Graphpad) as specified in figure legends.

Chapter 5

Concluding Remarks

Discussion

In this study, we detailed how BCAP positively regulates type I IFN induction in pDC via DOCK2 activation of Rac1 leading to IKKα phosphorylation. This mechanism initiates at the plasma membrane and occurs independently of TLR engagement with nucleic acid ligand. DOCK2-Rac1 signaling is critical for IKKα phosphorylation and is required for IRF7 phosphorylation and translocation into the nucleus, and subsequent IFNα production in pDC. While we demonstrated a clear role for BCAP in promoting type I IFN induction in pDC, there is increasing evidence that pDC capable of performing additional functions other than IFN production, such as inflammatory cytokine production, antigen presentation, and induction of T and B cell differentiation (45–48). Whether BCAP plays a role in regulating these pDC functions remains to be seen.

We have also investigated the mechanisms by which BCAP promotes lupus-like disease in TLR7.1 mice. We showed significant disease reduction in TLR7.1/BCAP$^{-/-}$ mice. While we do not rule out the possibility that the culmination of loss of BCAP function in various cell types plays a role in ameliorating disease, we contend that there is good reason to believe that pDC IFNα production promotes lupus-like disease in TLR7.1 mice. First, we show that pDC are important in disease pathogenesis in the TLR7.1 model by transiently depleting pDC and reducing aspects of lupus-like disease such as splenomegaly, early activated CD69^{+} CD4 and CD8 T cells, and effector/memory CD8 T cells (Figure 2.6). Second, the similarity in disease reduction phenotypes with BCAP-deficiency and pDC-deficiency is striking. In the study by Sisirak et al., E2-2 deficiency causing pDC deficiency in the related TLR7.6 model led to reduced CD44^{hi}CD45RB^{lo} activated/effector CD4 T cells that was somewhat less than the reduction in CD44^{hi}CD62^{lo} activated/effector CD4 T cells we found in TLR7.1/BCAP$^{-/-}$ mice. Both TLR7.1/BCAP$^{-/-}$ and TLR7.6 Tcf4$^{flox/+}$ Itgax-Cre^{+} mice had a reduction of splenomegaly, myeloid expansion, serum anti-RNA antibodies, IgG deposition in the kidneys, and kidney pathology. TLR7.1/BCAP$^{-/-}$ mice had a more severe reduction of splenomegaly and activated T cells than TLR7.Tg Tcf4$^{flox/+}$ Itgax-Cre^{+} mice, and this may be explained by the differences in age between the mice analyzed, in addition to the function of BCAP

in non-pDC. We quantified early stages of disease pathogenesis at 10-13 weeks in the TLR7.1 mice, whereas Sisirak et al. quantified disease from animals ranging from 30-50 weeks of age in the TLR7.6 Tcf4$^{flox/+}$ Itgax-Cre^{+} mice (11). Additionally, 3 week transient depletion of pDC in the BxSB model that includes TLR7 duplication (12) caused reduced splenomegaly, activated CD4 and CD8 T cells, autoantibodies, and kidney pathology. Thus, although BCAP may function in several cell types to promote lupus-like disease, it is plausible that its role in pDC is sufficient in a TLR7-driven model.

There is increasing evidence that pDC secretion of IFNα contributes to pathogenesis of systemic autoimmune diseases such as SLE and Sjogren's disease. Our data show a novel role for BCAP in regulating IFNα production in pDC through DOCK2 activation of Rac1. Understanding the molecular mechanisms that regulate IFNα production while leaving the NF-kB dependent inflammatory cytokine response intact could reveal novel ways to selectively target the IFNα pathway. This could prove to be significant for maintaining antigen presentation and regulatory aspects of pDC in interferon driven diseases. Despite the inconclusive results of anti-IFN/IFNAR trials, we would still argue that studying the role of IFNα in lupus disease progression is important. First, several currently used effective therapeutics for SLE treatment have been shown to have anti-IFN activity. Glucocorticoids have been shown to reduce the IFN signature when used in pulse dose regimens and may inhibit IFN-I signaling (49) in the myeloid compartment (50). Hydroxychloriquine also ameliorates lupus symptoms and is known to inhibit TLR7/9-induction of IFNα and TNF by inhibiting acidification of endosomes and also may prevent activation of pDC (51). Second, antibodies against IFNα in SLE patients were in some cases associated with reduced disease activity (52). Third, kinetics and disease severity may play a large role in whether anti-IFNα treatment is successful. Early anti-IFNα and pDC depletion treatment in mice were shown to reduce lupus like symptoms, while later anti-IFNα and pDC depletion treatment showed little effect (53, 54). Though anti-IFNα and IFNAR trials have failed to date, many clinicians believe subsets of patients may benefit from these therapies. More studies are required to determine whether ablation of pDC or IFNα production is a viable therapeutic option

or whether anti-IFNα/IFNAR is effective for specific patients. Therefore, we strongly believe the available data suggests understanding the molecular mechanisms leading to type I IFN production is important in SLE pathogenesis.

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