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Psoriasis is a chronic, inflammatory skin disease caused by a combination of environmental and genetic factors. The Tnip1 gene encodes A20 binding and inhibitor of NF-κB-1 (ABIN-1) protein and is strongly associated with susceptibility to psoriasis in humans. ABIN-1, a widely expressed ubiquitin-binding protein, restricts TNF- and TLR-induced signals. In this study, we report that mice lacking ABIN-1 specifically in dendritic cells (DCs), ABIN-1flCD11c-Cre mice, exhibit perturbed immune homeostasis. ABIN-1-deficient DCs display exaggerated NF-κB and MAPK signaling and produce more IL-23 than do normal cells in response to TLR ligands. Challenge of ABIN-1flCD11c-Cre mice with topical TLR7 ligand leads to greater numbers of Th17 and TCRγδ T cells and exacerbated development of psoriasis. These phenotypes are reversed by DC-specific deletion of the TLR adaptor MyD88. These studies link ABIN-1 with IL-23 and IL-17, and they provide cellular and molecular mechanisms by which ABIN-1 regulates susceptibility to psoriasis.

Cutting Edge: ABIN-1 Protects against Psoriasis by Restricting MyD88 Signals in Dendritic Cells

Joseph A. Callahan,* Gianna E. Hammer,* Alexander Ageides,* Bao H. Duong,* Shigeru Oshima,* Jeffrey North,‡ Rommel Advincula,* Nataliya Shifrin,* Hong-An Truong,† Jonathan Paw,† Julio Barrera,* Anthony DeFranco,‡ Michael D. Rosenblum,‡ Barbara A. Malynn,* and Averil Ma*
investigated whether ABIN-1 expression in DCs may regulate psoriasis susceptibility.

Materials and Methods

Mice

The initial targeting of the *Tnp1* (ABIN-1) gene in C57BL/6N PRX-B6T embryonic stem cells was previously described (11). ABIN-1–targeted embryonic stem cells were transfected with an EF1a-Cre expression construct (20), and colonies were screened for deletion of the neomycin gene and retention of ABIN-1 exons 12–15 flanked by LoxP sites (floxed allele). Genotypes were confirmed both by Southern blot analyses and by PCR (primers: 5′-TTGATTCCCCTTTCGCCATTCCACGACGCAAGAAGGAAAGC-3′, 5′-GGCTCAACAGCAAGAGGAAAAGC-3′, and 5′-ATGGGGTTGGAAGCGATAGGGCATAG-3′). MyD88<sup>−/−</sup> mice were described previously (23). All mouse experiments were approved by the University of California, San Francisco Institutional Care and Use Committee.

Cell preparation and analyses

Cell preparations and flow cytometric analyses were performed as previously described (20). Immunoblots were performed as described (11). Abs to surface markers (BD Biosciences), actin (Calbiochem), phospho-IκBα, IκBα, phospho-Erk, Erk, phospho-JNK, and JNK (Cell Signaling Technologies) were purchased.

Imiquimod treatment and scoring of skin inflammation

Imiquimod (IMQ) treatments were performed largely as previously described (24, 25). Mice received a daily topical dose of 12.5 μg IMQ cream (5%) (Perrigo) or lotion control for 12 consecutive days. Skin inflammation was scored using a previously described scoring system (24). Histological sections were prepared by the University of California, San Francisco Veterinarians Affairs Medical Center Pathology Core.

Results and Discussion

To investigate whether ABIN-1 expression in DCs regulates immune functions in vivo, we generated mice bearing LoxP sites flanking exons 12–15 of ABIN-1 (ABIN-1<sup>−/−</sup>) mice and bred them with CD11c-Cre transgenic mice to create mice lacking ABIN-1 in DCs (Supplemental Fig. 1A) (26). ABIN-1<sup>−/−</sup>CD11c-Cre mice appeared grossly normal for up to 6 mo of age. Conventional DCs (cDCs; CD11chiMHC class II<sup>+</sup>) and plasmacytoid DCs (pDCs; CD11clB220hi) were present in slightly elevated numbers in spleens from ABIN-1<sup>−/−</sup>CD11c-Cre mice (Fig. 1A). These cells expressed relatively normal levels of activation markers, with the exception of minimally elevated levels of CD40 on cDCs and slightly lower CD80 levels on pDCs (Supplemental Fig. 1B, 1C). Thus, ABIN-1 expression in DCs is not required for DC development but modestly regulates DC activation under basal conditions. ABIN-1<sup>−/−</sup>CD11c-Cre mice developed splenomegaly and lymphadenopathy by 3–4 mo of age, accumulating myeloid (CD11b<sup>+</sup>Gr-1<sup>+</sup>) cells and memory phenotype T cells (Fig. 1B–D and data not shown). Thus, ABIN-1 expression in DCs preserves myeloid and lymphoid immune homeostasis.

DCs are activated by TLR ligands during overt immunizations and infections and may also respond to MyD88-dependent signals under basal conditions (20). We asked whether ABIN-1 preserves immune homeostasis by restricting MyD88-dependent signals in DCs by generating compound ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre mice that lack both ABIN-1 and MyD88 specifically in DCs. Remarkably, in contrast to ABIN-1<sup>−/−</sup>CD11c-Cre mice, the spontaneous accumulation of myeloid cells and activated T lymphocytes observed in ABIN-1<sup>−/−</sup>CD11c-Cre mice was abrogated in ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre mice (Fig. 2, Supplemental Fig. 2A, 2B). Thus, ABIN-1 restricts basal Myd88-dependent intracellular signals in DCs, thereby preserving immune homeostasis in unperturbed mice.

To determine how ABIN-1 restricts MyD88-dependent signals in DCs, we tested the responses of ABIN-1<sup>+/+</sup> and ABIN-1<sup>−/−</sup> bone marrow–derived DCs (BMDCs) to the TLR4 ligand LPS. LPS-stimulated ABIN-1<sup>−/−</sup> BMDCs secreted more TNF, IL-6, IL-12, and IL-23 than did control BMDCs (Supplemental Fig. 2D). This is consistent with ABIN-1 expression in DCs.
a recent report showing that ABIN-1 restricts TLR-induced IL-6 and TNF (12). Importantly, our current study implicates ABIN-1 in restricting IL-23 production by DCs. After LPS stimulation, ABIN-1\(^{2/2}\) BMDCs also exhibited exaggerated NF-\(\kappa\)B, JNK, and p38 (but not ERK) signaling when compared with control BMDCs (Supplemental Fig. 2C). Hence, ABIN-1 regulates TLR responses in DCs by restricting TLR-induced NF-\(\kappa\)B and MAPK signals. Given the genetic linkage of ABIN-1 to psoriasis and the exaggerated production of IL-12 and IL-23 by ABIN-1\(^{2/2}\) DCs, we asked whether ABIN-1 expression in DCs regulates susceptibility to experimental psoriasis. Topical treatment with the TLR7 ligand IMQ can cause a psoriasis-like condition in humans and causes similar lesions in mice. This is now an

**FIGURE 3.** ABIN-1 restricts IMQ responses in DCs and prevents experimental psoriasis. (A) Clinical scores of IMQ-induced skin inflammation in ABIN-1\(^{+/+}\) CD11c-Cre\(^{-}\) and ABIN-1\(^{+/+}\) CD11c-Cre\(^{-}\) (control) mice at indicated days of IMQ treatment. (B–G) H&E-stained sections of back skin of mice of indicated genotypes from areas treated with IMQ. Epithelial layer denoted by brackets and “epi.” Note that skin inflammation in ABIN-1\(^{+/+}\) MyD88\(^{+/+}\) mice is abrogated in ABIN-1\(^{+/+}\) MyD88\(^{+/+}\) mice. All mice are CD11c-Cre\(^{-}\). Error bars represent SD. *\(p<0.05\) by Student t test. Scale bar, 0.1 mm. Data are representative of three independent experiments.

![Cumulative Psoriasis Score](image1)

**FIGURE 4.** ABIN-1 restricts MyD88 signals in DCs to prevent IMQ-induced psoriasis. (A) Clinical skin inflammation scores in the indicated genotypes of mice at the indicated days of IMQ treatment. (B) H&E-stained sections of back skin of mice of indicated genotypes after treatment. Epithelial layer denoted by brackets and “epi.” Note that skin inflammation in ABIN-1\(^{+/+}\) MyD88\(^{+/+}\) mice is abrogated in ABIN-1\(^{+/+}\) MyD88\(^{+/+}\) mice. All mice are CD11c-Cre\(^{-}\). Error bars represent SD. *\(p<0.05\) by Student t test. Scale bar, 0.1 mm. Data are representative of three independent experiments.

![Cumulative Psoriasis Score](image2)

These results demonstrate that ABIN-1 regulates TLR responses in DCs by restricting TLR-induced NF-\(\kappa\)B and MAPK signals. Given the genetic linkage of ABIN-1 to psoriasis and the exaggerated production of IL-12 and IL-23 by ABIN-1\(^{+/+}\) DCs, we asked whether ABIN-1 expression in DCs regulates susceptibility to experimental psoriasis. Topical treatment with the TLR7 ligand IMQ can cause a psoriasis-like condition in humans and causes similar lesions in mice. This is now an...
established mouse model of psoriasis (24, 27). To investigate the functions of radiation-sensitive DCs, we generated radiation chimera bearing hematopoietic stem cells from ABIN-1<sup>fl/fl</sup> CD11c-Cre or ABIN-1<sup>−/−</sup> CD11c-Cre mice. Treatment of mice with IMQ caused markedly increased erythema, scaling, and skin thickening in ABIN-1<sup>−/−</sup> CD11c-Cre chimera, which combine to yield increased composite psoriasis scores in ABIN-1<sup>−/−</sup> CD11c-Cre mice compared with control chimera (Fig. 3A). Histologic examination of skin sections from these mice revealed epidermal hyperplasia, hypogranulosis, hyperkeratosis, and parakeratosis with neutrophils—all stereotypical histologic findings of human psoriasis—in ABIN-1<sup>−/−</sup> CD11c-Cre mice but not in control mice (Fig. 3B–G). Treatment of mice with a topical emollient as control did not lead to significant clinical responses. Hence, ABIN-1 expression in DCs prevents susceptibility to experimental psoriasis.

IMQ-induced psoriasis involves IL-17–dependent production of IL-17 (24). We thus measured the levels of inflammatory cytokines produced by ABIN-1<sup>−/−</sup> BMDCs compared with control BMDCs in response to IMQ. IMQ stimulated higher levels of IL-23, IL-6, IL-12p70, and TNF secretion from ABIN-1<sup>−/−</sup> BMDCs compared with wild-type BMDCs, whereas IL-12p40 levels were similar (Fig. 3H). We next tested the induction of IL-17 expression in IMQ-treated mice. Whereas the total numbers of T cells in skin-draining lymph nodes were similar in ABIN-1<sup>−/−</sup> CD11c-Cre and ABIN-1<sup>−/−</sup> CD11c-Cre chimera, increased numbers and percentages of CD4<sup>+</sup> Th17 cells were observed in ABIN-1<sup>−/−</sup> CD11c-Cre mice (Fig. 3I). Many IL-17–producing T cells in IMQ-treated mice are epidermal TCR<sup>γδ</sup> T cells (28). Consistent with this notion, increased percentages and numbers of TCR<sup>γδ</sup> T cells were noted in draining lymph nodes from ABIN-1<sup>−/−</sup> CD11c-Cre mice (Fig. 3J). In contrast, analyses of the skin-draining lymph nodes from IMQ-treated ABIN-1<sup>−/−</sup> CD11c-Cre mice showed approximately normal Th1 (IFN-γ<sup>+</sup>) cell numbers and no significant numbers of Th2 (IL-4<sup>+</sup>) cells (Fig. 3I and data not shown). Finally, consistent with the role of IL-17 in supporting neutrophil recruitment, ABIN-1<sup>−/−</sup> CD11c-Cre mice contained increased epidermal neutrophil microabscesses (3G). Thus, ABIN-1 expression in DCs restricts IL-23 secretion, Th17 cell differentiation, neutrophilic inflammation, and psoriatic lesions after IMQ treatment.

To determine whether ABIN-1–dependent regulation of MyD88-dependent signals in DCs was integral to disease pathogenesis, we tested the IMQ responses of ABIN-1<sup>−/−</sup> BMDCs compared with wild-type BMDCs, and control hematopoietic stem cells. Double mutant ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre compound mutant ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre mice exhibited much less psoriasis than did ABIN-1<sup>−/−</sup> CD11c-Cre mice (Fig. 4A). Indeed, ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre mice exhibited similar clinical responses to MyD88<sup>−/−</sup> CD11c-Cre and wild-type chimera (Fig. 4A, Supplemental Fig. 2E). Histological studies confirmed the reduced inflammation observed in ABIN-1<sup>−/−</sup>MyD88<sup>−/−</sup> CD11c-Cre mice compared with ABIN-1<sup>−/−</sup> CD11c-Cre mice (Fig. 4B). Thus, ABIN-1–dependent regulation of MyD88-dependent signals in DCs regulates susceptibility to experimental psoriasis.

Our findings indicate that ABIN-1 restricts MyD88–dependent signals in DCs. ABIN-1 expression in DCs restricts TLR-induced NF-κB and JNK signals, thereby limiting DC expression of IL-23 and other cytokines. IL-23 supports the accumulation of IL-17– and IL-22–producing T cells. IL-17 induces epidermal neutrophil infiltration, and IL-22 alters keratinocyte proliferation and differentiation. Thus, exaggerated IL-23 expression likely leads to characteristic dermal lesions of psoriasis (29). Our studies mechanistically link ABIN-1, a major psoriasis susceptibility gene, with IL-23<sub>x</sub> and IL-23<sub>r</sub>, two other major psoriasis susceptibility genes. This linkage suggests that ABIN-1 and IL-23–dependent inflammation may be part of a common dominant pathophysiological pathway leading to psoriasis. These cellular and molecular insights into how ABIN-1 prevents psoriasis provide mechanistic insights for the genetic suggestions about psoriasis pathophysiology. Moreover, mice bearing ABIN-1 mutations should be extremely valuable models for studying psoriasis pathophysiology and treatment.

Acknowledgments
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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Figure 1. Generation of loxP flanked ABIN-1 (tnip1) allele.

(A) Schematic diagram of gene targeting strategy for generating ABIN-1FL mice. PCR confirmation of germline transmission (using tail DNA) of in vitro Cre-mediated deletion of loxP sites, generating ABIN-1FL allele. (B, C) Flow cytometric analyses of expression of indicated maturation markers on conventional (B) and plasmacytoid (C) DCs. Data represent 4-10 CD11c-Cre+ mice age 1.5-6 months. Error bars represent SEM. * indicates p< 0.05 by Student's t-test.

Supplemental Figure 2. ABIN-1 restriction of proinflammatory MyD88 signals

(A-B) Flow cytometrically determined percentage of memory phenotype (CD44hi) T cells within (A) CD4+ and (B) CD8+ lymph node cells of indicated mice. (C) Immunoblot and densitometry of analyses of NFκB and MAP kinase signaling activity in ABIN−/− and control ABIN+/+ BMDCs treated with 10 ng/ml LPS. pIκBα: IκBα ratios and MAPK phosphorylation ratios determined by densitometry and shown beside corresponding immunoblots. Actin protein levels shown below as loading controls. (D) ELISA or multiplex Luminex analyses of cytokine production from ABIN−/− and ABIN−/+/+ BMDCs after treatment with the indicated doses of LPS. (E) Clinical skin inflammation scores in the indicated genotypes of mice at the indicated days of imiquimod treatment. All mice in (A-B, E) are 3-4 months old and CD11c-Cre+. Data are representative of 3 independent analyses, including 6 pairs of Tnip1 FL/FL Myd88 +/+ and Tnip1 FL/FL Myd88 FL/FL mice. Significant differences were determined using 1-way ANOVA. Error bars represent SEM. * indicates p< 0.05 by Student's t-test.
Supplemental figure 1

A

trnp1 (ABIN-1) gene

Targeting Construct

Targeted Allele

Floxed (FL) Allele

Null Allele

B = BamHI
Bg = Bgl II

= PCR primer

= Probe

<table>
<thead>
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<th>Targeting Construct</th>
<th>size (primers)</th>
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<td>Wildtype</td>
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<tr>
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<td>272 bp</td>
</tr>
<tr>
<td>Null</td>
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B

C

Relative MFI

Relative MFI

*