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

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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 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-1fl CD11c-Cre mice with topical TLR7 ligand leads to greater numbers of Th17 and TCRγδ T cells and exacerbated development of psoriasis lesions. 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.


Psoriasis is a common immune-mediated skin disorder whose complex pathophysiology involves environmental factors, including microbes, as well as host susceptibility factors, including genetically determined immunologic propensities (1, 2). Recent genome-wide association studies have highlighted potential roles of specific proteins in disease pathogenesis, including HLA-C, IL-12b, TNIP1/A20 binding and inhibitor of NF-κB-1 (ABIN-1), TNFAIP3/A20, IL-23α, and IL-23R (3). Some of these polymorphisms are also linked to therapeutic responses of psoriasis patients to specific therapies (4). These findings emphasize the immunological nature of psoriasis and implicate specific immune functions, such as HLA-C–mediated Ag presentation and IL-12/IL-23–dependent innate immune signals. Because innate immune cells such as dendritic cells (DCs) are outstanding APCs, and because IL-12 and IL-23 are secreted by innate immune cells to amplify T cell activation and differentiation events, these genetic clues suggest that aberrant DCs and T cell functions are integral to psoriasis.

TNIP1, which encodes the ABIN-1 protein, is strongly linked to psoriasis in both European (combined genome-wide p value of $1 \times 10^{-20}$) and Chinese (combined genome-wide p value of $3.8 \times 10^{-21}$) populations (5–7). ABIN-1 restricts several NF-κB signaling cascades and regulates cell survival (8–12). In vitro studies suggest that ABIN-1 can bind NEMO/IKKγ and inhibit TNF-induced NF-κB signaling (13). ABIN-1 can also bind ubiquitin chains, and ubiquitin binding by ABIN-1 is important for the ability of ABIN-1 to restrict TNF and TLR signals (11, 12). Global loss or mutation of ABIN-1 leads to either embryonic lethality or spontaneous inflammation and autoimmunity (11, 12, 14). These studies indicated that ABIN-1 plays critical roles in regulating TNF and TLR signals. However, the mechanisms by which ABIN-1 regulates physiological immune homeostasis and psoriasis susceptibility are unknown.

DCs have long been recognized as important cells for triggering immune responses during overt immunizations or infections (15). Recent studies suggest that DCs also preserve immune homeostasis under basal conditions (16–21). DCs may regulate susceptibility to psoriasis by secreting type I IFNs, TNF, or other proinflammatory cytokines, as well as by stimulating skin T cells (22). Given the potential importance of DCs to immune homeostasis in the skin and the potential importance of ABIN-1 polymorphisms to psoriasis, we have...
investigated whether ABIN-1 expression in DCs may regulate psoriasis susceptibility.

**Materials and Methods**

**Mice**

The initial targeting of the Tnip1 (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 EF1α-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 (flanked alleles). Genotypes were confirmed both by Southern blot analyses and by PCR (primers: 5′-TTGATTCCCCTTGCCATCAGC-3′, 5′-CTGACACGCA- GAAGAGGAAAGC-3′, and 5′-ATGGGTGGTACGCGATAGGATAG-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-1β-actin, 1β-actin, 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 Veterans Affairs Medical Center Pathology Core.

**Results and Discussion**

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 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
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\(^{-/-}\) 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\(^{-/-}\) 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 indicated by brackets (B–G). Epidermal hyperplasia (thickening of epidermal layer) is evident in ABIN-1\(^{-/-}\) mice (C, E, G) compared with ABIN-1\(^{+/+}\) mice (B, D, F). In (E), broad areas of hypogranulosis (abnormal loss of purple keratohyaline granules in the skin’s granular layer) are indicated by closed arrows. In (G), neutrophils (blue arrow) and parakeratosis (abnormal retention of nuclei in the outermost layer of skin, green arrow) are shown in Munro’s microabscess (dotted boxed area). Scale bar, 0.1 mm for (D–G). Scale bar, 0.5 mm for (B) and (C). (H) ELISA and multiplex Luminex analyses of cytokine production from BMDCs after treatment with the indicated doses of IMQ. (I) Numbers of CD3\(^+\) and CD4\(^+\) T cells from skin-draining lymph nodes from IMQ-treated mice of indicated genotypes. Distinct CD4\(^+\)IL-17\(^+\), CD4\(^+\)IL-23\(^+\), and CD4\(^+\)IL-4\(^+\) populations were not detected. All mice in (A–G) are CD11c-Cre\(^+\) radiation chimeras. All data are representative of three to seven independent experiments. Error bars represent SD. *p < 0.05 by Student \(t\) test.

**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.
established mouse model of psoriasis (24, 27). To investigate the functions of radiation-sensitive DCs, we generated radiation chimera bearing homopoietic stem cells from ABIN-1f/f CD11c-Cre or ABIN-1f/f CD11c-Cre mice. Treatment of mice with IMQ caused markedly increased erythema, scaling, and skin thickening in ABIN-1f/f CD11c-Cre chimera, which combine to yield increased composite psoriasis scores in ABIN-1f/f 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-1f/f 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−/− 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−/− 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-1f/f CD11c-Cre and ABIN-1f/f CD11c-Cre chimera, increased numbers and percentages of CD4+ Th17 cells were observed in ABIN-1f/f CD11c-Cre mice (Fig. 3I). Many IL-17–producing T cells in IMQ-treated mice are epidermal TCRγ/δ+ T cells (28). Consistent with this notion, increased percentages and numbers of TCRγ/δ+ T cells were noted in draining lymph nodes from ABIN-1f/f CD11c-Cre mice (Fig. 3I). In contrast, analyses of the skin-draining lymph nodes from IMQ-treated ABIN-1−/−CD11c-Cre mice showed approximately normal Th1 (IFN-γ+) cell numbers and no significant numbers of Th2 (IL-4+) cells (Fig. 3I and data not shown). Finally, consistent with the role of IL-17 in supporting neutrophil recruitment, ABIN-1f/f CD11c-Cre mice contained increased epidermal neutrophil microabscesses (Fig. 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 mice generated from ABIN-1f/f MyD88f/f CD11c-Cre compound mutant and control hematopoietic stem cells. Double mutant ABIN-1f/f MyD88f/f CD11c-Cre mice exhibited much less psoriasis than did ABIN-1f/f MyD88f/f CD11c-Cre mice (Fig. 4A). Indeed, ABIN-1f/f MyD88f/f CD11c-Cre mice exhibited similar clinical responses to MyD88f/f CD11c-Cre and wild-type chimera (Fig. 4A, Supplemental Fig. 2E). Histological studies confirmed the reduced inflammation observed in ABIN-1f/f MyD88f/f CD11c-Cre mice compared with ABIN-1f/f 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 NFκB 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-23x and IL-23r, 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.

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Disclosures
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References