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*J Immunol* published online 19 June 2013
http://www.jimmunol.org/content/early/2013/06/17/jimmunol.1203335

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/06/17/jimmunol.1203335.DC1

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

The Journal of Immunology, 2013, 191: 000–000.

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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 (floxed allele). Genotypes were confirmed both by Southern blot analyses and by PCR (primers: 5′-TTGATTCCCCTTGGCCATCCAGC-3′, 5′-CCCTCAACAGCCAGAGGAAAAGC-3′, and 5′-ATGGGTTGAGGCATAGGGATAG-3′). MyD88+ 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-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 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-1fl/fl) mice and bred them with CD11c-Cre transgenic mice to create mice lacking ABIN-1 in DCs (Supplemental Fig. 1A) (26). ABIN-1fl/fl CD11c-Cre mice appeared grossly normal for up to 6 mo of age. Conventional DCs (cDCs; CD11c+MHC class II+ and plasmacytoid DCs (pDCs; CD11cB220+) were present in slightly elevated numbers in spleens from ABIN-1fl/fl 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-1fl/fl CD11c-Cre mice developed splenomegaly and lymphadenopathy by 3–4 mo of age, accumulating myeloid (CD11b+Gr-1+) and lymphoid immune 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-1fl/flMyD88fl/fl CD11c-Cre mice that lack both ABIN-1 and MyD88 specifically in DCs. Remarkably, in contrast to ABIN-1fl/fl CD11c-Cre mice, the spontaneous accumulation of myeloid cells and activated T lymphocytes observed in ABIN-1flox/−CD11c−Cre mice was abrogated in ABIN-1fl/flMyD88fl/fl 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+/+ and ABIN-1+/− bone marrow–derived BMDCs to the TLR4 ligand LPS. LPS-stimulated ABIN-1+/+ 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-κB, 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-κB 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 model for studying psoriasis. The ability of IMQ to induce skin inflammation is dependent on TLR7 signaling (13). In vivo, IMQ is rapidly metabolized; hence, topical treatment with IMQ resulted in the development of a psoriatic-like phenotype within 2 weeks (14). This model is widely used to study the pathogenesis of psoriasis and to develop therapeutic interventions for psoriasis.

**FIGURE 3.** ABIN-1 restricts IMQ responses in DCs and prevents experimental psoriasis. (A) Clinical scores of IMQ-induced skin inflammation in ABIN-1^{−/−} 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 (epi) is 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). (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. Numbers of Th17 (CD4+IL-17+), TCRγδ+ (CD3+GL-3+), and Th1 (CD4+IFN-γ+) T cells from skin-draining lymph nodes from IMQ-treated mice. Distinct Th2 (CD4+IL-4+) populations were not detected. All mice in (A)–(G) and (I) 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.
established mouse model of psoriasis (24, 27). To investigate the function of radiation-sensitive DCs, we generated radi- ation chimera bearing hematopoietic stem cells from ABIN-1fl/fl CD11c-Cre or ABIN-1flox/flox CD11c-Cre mice. Treatment of mice with IMQ caused markedly increased erythema, scaling, and skin thickening in ABIN-1flox/flox CD11c-Cre chimera, which combine to yield increased composite psoriasis scores in ABIN-1flox/flox 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 ste- reotypical histologic findings of human psoriasis—in ABIN-1flox/flox 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 expres-

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 character-

Acknowledgments

We thank Sandra Huling and Ivy Hish at the University of California, San Francisco Veterans Affairs Medical Center Pathology Core for excellent assis-

Disclosures

The authors have no financial conflicts of interest.

References


