Ankyrin repeat domain 1 regulates innate immune responses against herpes simplex virus 1: A potential role in eczema herpeticum

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Background: Atopic dermatitis (AD) is a common inflammatory skin disease. A subset of patients with AD are susceptible to disseminated herpes simplex virus (HSV) infection, a complication termed eczema herpeticum (ADEH+). The immune mechanisms causing ADEH+ remain elusive. Using RNA sequencing, we recently found that ankyrin repeat domain 1 *(ANKRD1)* was significantly induced in human PBMCs upon HSV-1 stimulation, and its induction in patients with ADEH+ was significantly reduced compared with that seen in AD patients without a history of eczema herpeticum (ADEH−).

Objective: We sought to validate *ANKRD1* gene expression in nonatopic (NA) subjects, patients with ADEH−, and patients with ADEH+ and to delineate the biological function of *ANKRD1* and the signaling pathway or pathways involved.

Methods: Purification of human PBMCs, monocytes, B cells, dendritic cells, T cells, and natural killer cells; RNA extraction and quantitative RT-PCR; small interfering RNA technique; co-immunoprecipitation; and Western blot assays were used.

Results: *ANKRD1* expression was significantly reduced in PBMCs from patients with ADEH+ after HSV-1 stimulation compared with PBMCs from patients with ADEH−. We found that the induction of *ANKRD1* by HSV-1 and multiple pattern recognition receptor agonists are mediated by inflammatory cytokines. Silencing *ANKRD1* gene expression in antigen-presenting cells led to increased viral load and reduced *IFNB1* and *IL29* production. Using co-immunoprecipitation methods, we demonstrated that ANKRD1 formed protein complexes with interferon regulatory factor (IRF) 3 and IRF7, which are important transcription factors regulating signaling transduction of pattern recognition receptors. Overexpression of ANKRD1 enhanced the IRF3-mediated signaling pathways.

Conclusion: *ANKRD1* is involved in IRF3-mediated antiviral innate immune signaling pathways. Its reduced expression in patients with ADEH+ might contribute to the pathogenesis of ADEH+. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

Key words: Herpes simplex virus, ankyrin repeat domain 1, innate immunity, atopic dermatitis, eczema herpeticum, interferon regulatory 3, nuclear factor κB1, IFN-β1, IL-29

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Atopic dermatitis (AD) is the most common chronic skin inflammatory disease worldwide, affecting up to 25% of children and 10% of adults.[1] A subset of patients with AD are susceptible to disseminated skin viral infection, including herpes simplex virus (HSV), molluscum contagiosum, and vaccinia virus.[2,3] The most common viral complication in patients with AD is eczema herpeticum (atopic dermatitis with a history of eczema herpeticum [ADEH+]), which is mainly caused by HSV-1.[2] Patients with ADEH+ are invaluable resources for mechanistic investigations of the interplay between host immune defense and HSV-1 in human subjects. Based on this perspective, the National Institute of Allergy and Infectious Diseases funded the Atopic Dermatitis Research Network to investigate ADEH+ with comprehensive mechanistic studies.

Previously, we reported a study comparing transcriptomes of PBMCs from patients with atopic dermatitis without a history of eczema herpeticum (ADEH−) versus patients with ADEH+.[4] This study found that ankyrin repeat domain 1 *(ANKRD1)* was one of the most downregulated genes in ADEH+.[4] *ANKRD1*, which is also known as c-193 and cardiac ankyrin repeat protein, is a pleiotropic protein containing 4 ankyrin repeat domains; a nuclear localization signal; a sequence rich in proline, glutamic acid, serine, and threonine; and multiple phosphorylation consensus sites.[5] ANKRD1 was discovered as a nuclear DNA-binding protein induced by inflammatory cytokines in human dermal vascular endothelial cells.[5] Subsequently, ANKRD1 was found to be expressed abundantly in cardiac myocytes and skeletal muscle tissues, where they localized to the I band of sarcomere through binding to titin and myopalladin.[6-8] ANKRD1 can also enter the nuclei of cardiac myocytes to regulate cardiomyogenesis as a transcriptional regulator.[9]

Abbreviations used
- AD: Atopic dermatitis
- ADEH+: Atopic dermatitis with a history of eczema herpeticum
- ADEH−: Atopic dermatitis without a history of eczema herpeticum
- ANKRD1: Ankyrin repeat domain 1
- APC: Antigen-presenting cell
- HA: Hemagglutinin
- HCV: Hepatitis C virus
- HPV: Human papillomavirus
- HSV: Herpes simplex virus
- IRF: Interferon regulatory factor
- MOI: Multiplicity of infection
- NA: Nonatopic
- NF-κB: Nuclear factor κB
- NK: Natural killer
- Poly dA:dT: Poly(deoxyadenylic-deoxythymidylic) acid sodium salt
- Poly I:C-LMW: Polyinosinic-polycytidylic acid, low molecular weight
- PRR: Pattern recognition receptor
- qRT-PCR: Quantitative RT-PCR
- siRNA: Small interfering RNA
- TLR: Toll-like receptor

Upregulation and gene mutations of ANKRD1 are associated with cardiomyopathy.[10-12] Additionally, induction of ANKRD1 has been found in patients with ovarian cancer, injured podocytes, cutaneous wounds, TGF-β–induced mouse mammary epithelial cells, hepatitis C–infected hepatocytes, and papilloma virus–infected keratinocytes.[13-18] Although *ANKRD1* has been studied extensively, its induction by HSV-1 in human immune cells and the biological significance of this response have not been investigated.

In the current study we first confirmed our previous RNA sequencing results using an increased number of subjects, demonstrating that *ANKRD1* transcripts were significantly downregulated in HSV-1–stimulated PBMCs from patients with ADEH+ compared with PBMCs from patients with ADEH−. We then explored regulation of *ANKRD1* expression in PBMCs, its cell source, and its biological function in host antiviral responses. The results of our study support a functional role for reduced *ANKRD1* expression in ADEH+ pathogenesis.

METHODS

Human subjects

Subjects ranging in age from 6 to 65 years participated in the study. They included 21 control subjects without atopy (nonatopic [NA] subjects), 19 patients with ADEH−, and 20 patients with ADEH+. The groups were stratified based on age and sex. None of the patients with ADEH+ had acute HSV-1 infection. All human subjects were examined for serum HSV-1 IgG and HSV-2 IgG values. Demographic characteristics of the 60 subjects are shown in Table E1 in this article's Online Repository at www.jacionline.org. An additional 10 healthy adults were recruited to participate in the mechanistic investigation. The institutional review board at National Jewish Health approved the study, and all subjects provided written informed consent to participate.

Virus, pattern recognition receptor agonists, recombinant cytokines, neutralization antibodies, and plasmids

HSV-1 virus stock (VR-733) was purchased from the ATCC (Manassas, Va). The pattern recognition receptor (PRR) agonists CpG (ODN2395); CL264 (catalog no. tlrl-c264e); LPS (catalog no. tlrl-b5lps); Pam3CSK4 (catalog no. tlrl-pms); poly(deoxyadenylic-deoxythymidylic) acid sodium salt (Poly dA:dT; catalog no. tlrl-patc); polyinosinic-polycytidylic acid, low molecular weight (PolyI:C-LMW; catalog no. tlrl-picwlv); polyI:C–Toll-like receptor (TLR) 3 agonist (catalog no. tlrl-picw); and SS40 (catalog no. tlrl-lrna40) were purchased from InvivoGen (San Diego, Calif). Recombinant human TNF-α (catalog no. 210-TA-020/CF), IL-1β (catalog no. 201-LB-005), IFN-γ, IL-4, IL-22, GM-CSF, monoclonal mouse IgG_1 clone 11711 (catalog no. MAB002), human IL-1β/IL-1F2 antibody (MAB601), human TNF-αR1/TNFRSF1A antibody (MAB625), and anti-human IFN-γR1 antibody (MAB6731) were bought from R&D Systems (Minneapolis, Minn). Recombinant human IFN-α (catalog no. 11101-1) and mouse anti-human IFN-α (catalog no. 21112-1) were purchased from PBL Biomedical Laboratories (Piscataway, NJ). Myc-DDK1–tagged RELA (RC220780), Myc-DDK1–tagged NFKB1 (RC208384), Myc-DDK1–tagged interferon regulatory factor (IRF) 7 (RC217934), Myc-DDK1–tagged ANKRD1 (RC205609) and Myc-DDK1–tagged STING were purchased from OriGene (Rockville, Md). Dr Hong-Bing Shu (Wuhan University, Wuhan, China) kindly provided pCMV-flag-IRF3 and pCMV-flag-MyD88. PRK-neo-HA-ANKRD1 was generated in our laboratory by means of insertion of an encoding cDNA fragment in frame into the PRK-neo-HA vector (a kind gift from Dr Hong-Bing Shu).

PBMC isolation and purification of different cell types

Human PBMCs were isolated by using Ficoll-Hypaque density gradient centrifugation of heparinized venous blood from donors. PBMCs were subjected to sequential isolation of T cells, natural killer (NK) cells, and monocytes by using anti-CD3, anti-CD56, and anti-CD14 microbeads, according to the manufacturer's guidelines (Miltenyi Biotec, San Diego, Calif). Then the rest of the cells were further separated using a human B Cell Isolation Kit II purchased from Miltenyi Biotec to obtain B cells and dendritic cells. In some experiments we used antigen-presenting cells (APCs; ie, a mix of B cells, dendritic cells, and monocytes) isolated from PBMCs by depletion of T and NK cells.

Cell treatment

PBMCs and other cell types isolated from PBMCs were maintained in RPMI 1640 supplemented with 10% FBS, penicillin (50 IU/mL), and streptomycin (50 μg/mL). For 21 NA subjects, 20 patients with ADEH+, and 19 patients with ADEH−, one million PBMCs in 200 μL of culture media were stimulated with sham or HSV-1 at a multiplicity of infection (MOI) of 0.1 for 21 hours. For mechanistic studies, PBMCs and other types of cells were suspended in complete RPMI 1640 at 1×10^6 cells/mL, seeded in 96-well plates, and stimulated with HSV-1 at an MOI of 0, 0.01, and 0.1 and various PRR agonists and cytokines for 24 hours. For experiments with neutralizing antibodies, PBMCs were first incubated with antibody at 5 μg/mL for 2 hours and then exposed to medium or the TLR9 agonist CpG ODN 2395 at 50 or 200 μmol/L for an additional 24 hours. Cells were harvested at the end of treatment for RNA extraction and real-time PCR.

Small interfering RNA silencing experiment

ANKRD1 and negative nontargeting scrambled small interfering RNA (siRNA) duplexes were purchased from Dhamarcon (Lafayette, Colo). The ON-TARGETplus Non-targeting pool sequences are as follows: 5′-UGGUUUACAUGUCGACUAA-3′, 5′-UGGUUUACAUGUUGUGUGA-3′, 5′-UGGUUUA CAUGUUUUCHGA-3′, and 5′-UGGUUUACAU GUUUUCCUA-3′. The ON-TARGETplus *ANKRD1* siRNA SMARTpool target sequences are as follows: 5′-CUACAAGACCUCUCGCAUA-3′,