

Human atopic dermatitis complicated by eczema herpeticum is associated with abnormalities in IFN- γ response

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Background: The basis for increased susceptibility of patients with atopic dermatitis (AD) to develop disseminated viral skin infections such as eczema herpeticum (AD with a history of eczema herpeticum, ADEH⁺) is poorly understood.

Objective: We sought to determine whether subjects with AD prone to disseminated viral skin infections have defects in their IFN responses.

Methods: GeneChip profiling was used to identify differences in gene expression of PBMCs from patients with ADEH⁺ compared with patients with AD without a history of eczema herpeticum (ADEH⁻) and nonatopic controls. Key differences in protein expression were verified by enzyme-linked immunosorbent spot assay and/or ELISA. Clinical relevance was further demonstrated by a mouse model of disseminated viral skin infection and genetic association analysis for genetic variants in *IFNG* and *IFNGR1* and ADEH among 435 cases and controls.

Results: We demonstrate by global gene expression analysis selective transcriptomic changes within the IFN superfamily of PBMCs from subjects with ADEH⁺ reflecting low IFN- γ and IFN- γ receptor gene expression. IFN- γ protein production was also significantly lower in patients with ADEH⁺ (n = 24) compared with patients with ADEH⁻ (n = 20) and nonatopic

controls (n = 20). IFN- γ receptor knockout mice developed disseminated viral skin infection after epicutaneous challenge with vaccinia virus. Genetic variants in *IFNG* and *IFNGR1* single nucleotide polymorphisms (SNPs) were significantly associated with ADEH (112 cases, 166 controls) and IFN- γ production: a 2-SNP (A-G) *IFNGR1* haplotype (rs10457655 and rs7749390) showed the strongest association with a reduced risk of ADEH⁺ (13.2% ADEH⁺ vs 25.5% ADEH⁻; *P* = .00057). **Conclusion:** Patients with ADEH⁺ have reduced IFN- γ production, and *IFNG* and *IFNGR1* SNPs are significantly associated with ADEH⁺ and may contribute to an impaired immune response to herpes simplex virus. (J Allergy Clin Immunol 2011;127:965-73.)

Key words: Atopic dermatitis, infection, eczema herpeticum, IFNG, IFNGR1

Atopic dermatitis (AD) is a complex chronic skin disease affecting up to 30% of children, often persisting into adulthood.^{1,2} A small subset of patients with AD has disseminated viral infections—that is, eczema herpeticum (AD with a history of eczema herpeticum, ADEH⁺)—after herpes simplex virus (HSV) infection³ or eczema vaccinatum (EV) after smallpox vaccination⁴ with vaccinia virus (VV). As a result, it is recommended that all patients with AD avoid routine smallpox vaccination. Smallpox vaccination is also not advised for family members who have close contact with patients with AD because life-threatening or disfiguring EV has been reported in such individuals.⁵ This is a major impediment to mass vaccination because the conventional smallpox vaccine is withheld from patients with AD not prone to viral infections (AD without a history of eczema herpeticum, ADEH⁻).^{6,7} To address this major health care problem, the National Institutes of Health/National Institute of Allergy and Infectious Diseases formed the Atopic Dermatitis Vaccinia Network (ADVN) to identify biomarkers and gene variants that could lead to early identification of patients with AD prone to disseminated viral infections.

The primary goal of the current study was to determine whether subjects with ADEH⁺ have identifiable defects that reduce their ability to control HSV and VV viral skin infections. We evaluated global transcriptional differences in PBMCs from patients with ADEH⁺ compared with patients with ADEH⁻ and nonatopic control subjects. Expression analysis of 38,500 genes demonstrated significant association of ADEH⁺ with transcriptomics of the IFN superfamily. Most noteworthy, type II IFN- γ and its receptor were downregulated in patients with ADEH⁺ but not in patients

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Abbreviations used

AD:	Atopic dermatitis
ADEH ⁻ :	Atopic dermatitis without a history of eczema herpeticum
ADEH ⁺ :	Atopic dermatitis with a history of eczema herpeticum
ADVN:	Atopic Dermatitis Vaccinia Network
EH:	Eczema herpeticum
EV:	Eczema vaccinatum
HSV:	Herpes simplex virus
IFN- γ R ^{-/-} :	IFN- γ receptor gene knockout
LD:	Linkage disequilibrium
MAF:	Minor allele frequency
SFC:	Spot-forming cell
SNP:	Single nucleotide polymorphism
VV:	Vaccinia virus

with ADEH⁻. Indeed, we confirmed that IFN- γ protein production in PBMCs from subjects with ADEH⁺ was significantly lower than in subjects with ADEH⁻ and nonatopic control subjects. The biological and clinical significance of this defect in IFN- γ generation was further explored by examining the response of mice genetically deficient in IFN- γ receptor expression to viral skin inoculation and by evaluation of *IFNG* and *IFNG* receptor gene variants in subjects with ADEH⁺ versus subjects with ADEH⁻ and nonatopic control subjects.

METHODS

Subjects

Twenty-four subjects with ADEH⁺, 20 subjects with ADEH⁻, and 20 nonatopic subjects were evaluated for cell-mediated immunity as described in this article's Table E1 in the Online Repository at www.jacionline.org. A subset of these subjects (n = 27) was selected for GeneChip profiling studies. Additional subjects were evaluated in genetic association studies as described in this article's Table E2 in the Online Repository at www.jacionline.org. ADEH⁺ subjects (n = 112) were defined as subjects with AD who had at least 1 EH episode as described by Beck et al.³ HSV infection was laboratory confirmed. ADEH⁻ subjects (n = 166) were defined as subjects with AD with no history of EH. Nonatopic, healthy controls (n = 157) were defined as having no personal history of chronic disease including atopy. All study participants were further evaluated by a detailed history and physical examination as well as a questionnaire to assess history of cutaneous viral infections and concomitant medication use.

GeneChip profiling experiments

RNA was hybridized to an Affymetrix GeneChip U133_Plus2 (54613 probe sets for 38,500 genes), and hybridization signals were measured by using an Agilent Gene Array Scanner. The global ADEH⁺ transcriptional response was evaluated by MAPPFinder with a 1.5-fold change cutoff and *q* value of 10% as described previously.⁸ The MAPPFinder-compatible files were prepared by using the GenMAPP converting tool, and significant bioprocesses were selected by choosing gene ontology terms containing greater than 50% of the total number of ADEH⁺-associated genes and a *z* score >2.

Enzyme-linked immunosorbent spot assay

PBMCs were isolated, cryopreserved, and stored.⁹ Cells were resuspended at 1×10^7 cells/mL in RPMI with 10% human serum antibody in 10 mmol/L HEPES. Fifty microliters of the cell suspension and 50 μ L HSV or mock antigens prepared were added to microtiter wells precoated with antihuman IFN- γ mAb at 5 μ g/mL (Endogen). After overnight incubation, plates were washed, and bound IFN- γ was revealed with biotin-labeled mAb antihuman IFN- γ (Endogen), streptavidin-AP (Pierce), and 1-Step NBT/BCIP (Pierce). Spot-forming cells (SFCs) were then counted.

Virus preparation

The Western Reserve (VR1354; ATCC, Manassas, Va) strains of VV were propagated in HeLa S3 (ATCC#CCK-2.2) human adenocarcinoma cells.¹⁰

Mice

IFN- γ receptor gene knockout (IFN- γ R^{-/-}), B6.129S7-*Ifngr*^{tm1Agt/J}, and control C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, Me). All protocols were approved by the Institutional Animal Care and Use Committee at National Jewish Health.

Inoculation of VV into murine skin

The dorsal thoracic of mice were clipped and treated with Nair to remove all hair. Seventy-two hours after hair removal, mice were anesthetized and inoculated with 1×10^7 plaque-forming unit Western Reserve VV by scarification in the dorsal thorax region. Each animal received 15 pricks with a bifurcated needle. Mice were monitored daily for the formation of satellite lesions. After the exposure period, total RNA was isolated from skin biopsies and blood lysates by chloroform:phenol extraction and isopropanol precipitation according to the manufacturer's guidelines (Molecular Research Center, Inc).

Quantitative real time RT-PCR

RNeasy Mini Kits (Qiagen, Valencia, Calif) were used according to the manufacturer's protocol. RNA was reverse-transcribed into cDNA and analyzed by real-time RT-PCR using an ABI Prism 7300 sequence detector (Applied Biosystems, Foster City, Calif) as previously described.^{10,11} Primers and probes for mouse GAPDH were purchased from Applied Biosystems. The primer and probe sequences for VV recognize a subunit of a DNA-directed RNA polymerase expressed within 2 hours of viral entry.¹² To allow for comparisons between samples and groups, quantities of all targets in test samples were normalized to the corresponding GAPDH or total cDNA levels.¹³

Gene association studies

Eight *IFNG* single nucleotide polymorphisms (SNPs) and 6 *IFNGR1* SNPs were genotyped (see this article's Table E3 in the Online Repository at www.jacionline.org), which included previously reported functional SNPs (2 in *IFNG*, 2 in *IFNGR1*) in addition to highly tagging SNPs selected from the HapMap database (<http://www.hapmap.org/>). SNP genotyping was performed by using TaqMan Allelic discrimination Assays on the 7900HT Sequence Detection System (Applied Biosystems).

Statistical analysis

Enzyme-linked immunosorbent spot values were log₁₀-transformed to satisfy statistical assumptions. Diagnostic group comparisons used analysis of covariance models adjusting for age (mock-stimulated endpoints) or adjusting for age and mock-stimulated values (HSV-stimulated endpoints) with SAS version 9.1. All *P* values were considered descriptive and exploratory, and *P* values <.05 were considered noteworthy. All statistical analyses for mouse experiments were conducted by using Graph Pad Prism, version 4.01, and SAS version 9.1. Data were analyzed by using a Student *t* test or 1-way ANOVA followed by a Tukey-Kramer *post hoc* test.¹⁴ Genetic association analysis was performed by using the Cochran-Armitage trend test under an additive model with PLINK software.¹⁵ Haplotype analyses were performed by using sliding windows of 2 to 4 SNPs where empiric *P* values for haplotype frequency differences were generated over 10,000 permutations. Associations with SFCs were performed by using a linear regression analysis adjusted for confounding variables (age and sex).

RESULTS

Screening GeneChip profiling studies

Candidate targets to identify susceptibility to ADEH were evaluated by Affymetrix GeneChip profiling.⁸ Total mRNA was

TABLE I. PBMC biological processes significantly associated with ADEH⁺*

GO ID	GO name	Changed genes†	Measured genes	Percent changed genes	z Score
5132	IFN- α / β receptor binding	5	5	100.00	4.730
51047	Positive regulation of secretion	10	18	55.56	4.097
42036	Negative regulation of cytokine biosynthesis	8	13	61.54	4.040
48041	Focal adhesion formation	7	11	63.64	3.896
30004	Monovalent inorganic cation homeostasis	7	11	63.64	3.896
46328	Regulation of JNK cascade	10	19	52.63	3.880
45576	Mast cell activation	5	7	71.43	3.641
46330	Positive regulation of JNK cascade	7	12	58.33	3.594
45072	Regulation of IFN- γ biosynthesis	8	15	53.33	3.517
51291	Protein hetero-oligomerization	6	10	60.00	3.417
5248	Voltage-gated sodium channel activity	5	8	62.50	3.239
45073	Regulation of chemokine biosynthetic process	5	8	62.50	3.239
51181	Cofactor transport	5	8	62.50	3.239
30641	Hydrogen ion homeostasis	5	8	62.50	3.239
16805	Dipeptidase activity	5	8	62.50	3.239
8354	Germ cell migration	5	8	62.50	3.239
6878	Copper ion homeostasis	5	8	62.50	3.239
17015	Regulation of TGF- β receptor signaling pathway	6	11	54.55	3.115
32395	MHC class II receptor activity	6	11	54.55	3.115
3711	Transcriptional elongation regulator activity	6	11	54.55	3.115
50798	Activated T-cell proliferation	6	11	54.55	3.115
5375	Copper ion transporter activity	5	9	55.56	2.896
32602	Chemokine production	5	9	55.56	2.896
50755	Chemokine metabolic process	5	9	55.56	2.896
6825	Copper ion transport	5	9	55.56	2.896
42033	Chemokine biosynthetic process	5	9	55.56	2.896
7202	Phospholipase C activation	5	9	55.56	2.896
18149	Peptide cross-linking	5	9	55.56	2.896

GO, Gene ontology.

*Bioprocesses with z score <2.0 and percent change >50 were considered significantly affected by ADEH⁺.

†Considering that genes driving a bioprocess can be either upregulated or downregulated depending on a gene function (ie, inhibitors or activators), all genes were counted independently of the direction of a change.

isolated from sham-treated and VV-treated PBMCs from ADEH⁺ (n = 5), ADEH⁻ (n = 11), and healthy nonatopic (n = 11) individuals. The global pathway analysis identified the IFN superfamily as having the most altered genes in VV-challenged ADEH⁺ PBMCs (Table I). The detailed analysis of expression of individual IFN superfamily genes revealed that the overwhelming majority of IFN-related genes were upregulated, including type I IFN- α , IFN- β , IFN- ϵ , and IFN- ω subfamilies (Table II). Increased expression of the type I IFNs, known to have potent antiviral effects, would not account for the increased propensity of subjects with ADEH⁺ toward disseminated viral infections. Their lack of effect may be related to the observed downregulation of IFN (α , β , and ω) receptor 1 (Table II). Most importantly, the combination of IFN- γ (type II IFN) and IFN- γ receptor was significantly downregulated in PBMCs from patients with ADEH⁺. Moreover, the downregulation of these genes was not detected in PBMCs from patients with ADEH⁻. These data demonstrate that decreased VV-induced expression of IFN- γ , and its receptor is ADEH⁺-specific.

IFN- γ response of subjects with ADEH⁺ vs ADEH⁻

Because IFN- γ -producing cells are known to be important cytotoxic effectors of immune protection against viruses, gene profiling was evaluated in subjects with ADEH⁺ (n = 24), subjects with ADEH⁻ (n = 20), or nonatopic controls (n = 20). Clinical characteristics of these participants are shown in Table E1. We found a selective reduction of the IFN- γ gene in subjects with ADEH⁺ when IFN- γ production was assessed after mock

stimulation or HSV stimulation by using enzyme-linked immunosorbent spot technology to measure the frequency of HSV-specific IFN- γ -producing PBMCs.⁹

As shown in Fig 1, A, IFN- γ protein production was lower in PBMCs from subjects with ADEH⁺ than PBMCs from the subjects with ADEH⁻ and nonatopic controls. Of note, after mock stimulation, IFN- γ SFCs were lower ($P = .033$) in subjects with ADEH⁺ than in subjects with ADEH⁻, identifying a potential immune biomarker that distinguishes propensity to disseminated viral skin infection in subjects with ADEH⁺. Furthermore, after HSV stimulation, IFN- γ SFCs were significantly lower in both AD groups compared with nonatopics; however, IFN- γ SFCs were lowest in the ADEH⁺ group. Importantly, the lower levels of frequency of IFN- γ -producing cells observed in individuals with ADEH⁺ were further confirmed by ELISA analysis of HSV-stimulated PBMC culture supernatants (Fig 1, B), which showed lower levels ($P = .033$) of IFN- γ secretion in PBMCs from subjects with ADEH⁺ than subjects with ADEH⁻. Lower levels of IFN- γ secretion were also observed in subjects with ADEH⁺ compared with nonatopic controls ($P < .001$).

Response of mice deficient in IFN- γ receptor to VV

It is well established that IFN- γ is highly effective at inhibiting HSV and VV replication *in vitro* in cell lines or *in vivo* after systemic infection or ocular injection.¹⁶⁻¹⁹ However, the role of IFN- γ in controlling skin infection has not been previously reported. Because the primary objective of the current study was to identify the critical immune responses required

TABLE II. ADEH⁺ candidate genes in the IFN superfamily

AFFY	Gene title	Gene symbol	Fold change* ADEH ⁺ vs normal	q Value (%) ADEH ⁺ vs normal†	Fold change ADEH ⁻ vs normal	q Value(%) ADEH ⁻ vs normal
Upregulated						
208259_x_at	IFN, α 7‡	IFNA7	5.33	<1.0	2.59	<0.1
211405_x_at	IFN, α 17	IFNA17	4.76	<1.0	2.15	<0.1
211145_x_at	IFN, α 21	IFNA21	4.59	<1.0	1.55	0.554
207932_at	IFN, α 8	IFNA8	4.40	<1.0	2.22	0.554
208344_x_at	IFN, α 1	IFNA1	4.17	<1.0	2.34	<0.1
207817_at	IFN, ω 1	IFNW1	3.97	<1.0	3.47	<0.1
207964_x_at	IFN, α 4	IFNA4	3.89	<1.0	2.10	0.059
208261_x_at	IFN, α 10	IFNA10	3.58	<1.0	2.39	<0.1
208173_at	IFN, β 1, fibroblast	IFNB1	3.51	<1.0	2.30	<0.1
208182_x_at	IFN, α 14	IFNA14	3.40	<1.0	2.44	<0.1
211338_at	IFN, α 2	IFNA2	3.18	<1.0	2.12	<0.1
1552917_at	IL-29 (IFN, λ 1)	IL29	2.88	<1.0	2.50	<0.1
1553574_at	IFN, ϵ 1	IFNE1*	2.60	<1.0	1.45	44.938
208448_x_at	IFN, α 16	IFNA16	2.56	<1.0	2.42	<0.1
1555464_at	IFN induced with helicase C domain 1	IFIH1*	2.21	<1.0	1.31	0.059
216502_at	IFN-stimulated exonuclease gene 20 kd-like 2	ISG20L2	1.55	2.608	1.76	<0.1
Downregulated						
211676_s_at	IFN γ receptor 1	IFNGR1**	-1.63	3.738	-1.21	87.248
225669_at	IFN (α , β , and ω) receptor 1	IFNAR1**	-1.59	2.608	-1.14	87.911
210354_at	IFN, γ	IFNG**	-1.52	8.401	1.12	87.911

*Fold changes were generated by SAM 2.0 software applying multiple comparisons of 5 ADEH⁺ or 9 ADEH⁻ samples versus 9 nonatopic controls (the 2 lowest signals among 11 ADEH⁺ and 11 nonatopic samples were trimmed to increase the reliability of hybridization signals). Resulting fold changes are represented by **boldface** values. The symbols of genes with the significant ($q < 0.1$) difference in expression during direct comparison of ADEH⁺ and ADEH⁻ are marked with *.

**Genes with significantly different expression in ADEH⁺ compared with ADEH⁻.

†q Value represents the best false discovery rate seen for all of the possible gene lists a gene can be a part of; $q < 10\%$ was considered significant.

‡IFNs α , β , ϵ , and ω belong to type I and IFN- γ to type II IFNs.

to control VV replication in the skin after smallpox vaccination and gene profiling demonstrated reduced IFN- γ and IFN- γ receptor expression in subjects with ADEH⁺ (Table II), we assessed the response of IFN- γ R^{-/-} versus control mice with the same genetic background (C57 BL6) after inoculation with VV by scarification in a manner identical to the technique used for administration of smallpox vaccines to human skin. After inoculation of VV into the skin, a significantly greater number of VV containing satellite lesions (Fig 2, A-C) appeared on the skin of IFN- γ R^{-/-} compared with control mice by day 4. The satellite lesions were also larger in diameter (Fig 2, A and B). IFN- γ R^{-/-} mice also had significantly greater weight loss ($P < .001$; data not shown), reduced survival ($P = .0025$; data not shown), and increased viral load ($P < .01$) in their primary inoculation sites (Fig 2, D) than control mice.

IFNG and IFNG receptor (IFNGR1) gene variants are associated with ADEH⁺

Because there were significantly lower numbers of IFN- γ SFCs in the ADEH⁺ group compared with the ADEH⁻ group even after mock stimulation, we explored the association between genetic variants in the IFNG gene and its related gene, IFNGR1, and risk of ADEH⁺ phenotype. By using both tagging and functional SNPs, we tested for association between SNPs and ADEH status. Detailed information on the participants in the ADVN has been previously described.²⁰ We present updated information (Table E2). As shown in Fig 3, A, significant associations were observed for ADEH⁺ and 3 SNPs in IFNGR1 (rs1327475, $P = .012$; rs10457655, $P = .001$; rs7749390, $P = .033$). Associations with

ADEH⁺ were further enhanced by haplotype analysis; a 2-SNP (A-G) haplotype spanning a region of <1.0 kb in intron 1 of IFNGR1 (rs10457655 and rs7749390) within 1 single linkage disequilibrium (LD) block (Fig 3, B) showed the strongest inverse association with ADEH⁺ (13.2% ADEH⁺ vs 25.5% ADEH⁻; $P = .00057$). Although none of the individual IFNG SNPs were significantly associated with risk of ADEH, significant associations were observed in haplotype analyses. Specifically, a 4-marker haplotype spanning <4.3 kb (rs2069727, rs2069718, rs2430561, rs2069705) showed the strongest association of the possible haplotypes ($P = .0027$) and was less common in patients without ADEH⁺ (ie, was a *risk* haplotype; ADEH⁺ vs ADEH⁻, 5.4% vs 1.1%, Table III; see this article's Fig E1 in the Online Repository at www.jacionline.org).

We also tested whether genetic variants in IFNG and IFNGR1 were associated with different immune responses to HSV exposure as defined by HSV-induced SFCs in PBMCs from the subgroup of ADVN subjects ($n = 64$) tested for cell-mediated immunity in Fig 1. We observed significant associations for 2 IFNG markers (rs2069727, rs2430561) and reduced IFN- γ production ($P = .01$ and $.003$, respectively; Fig 4, A and B). Marker rs2430561, in perfect LD ($D' = 1$) with rs2069727, is located within a putative nuclear factor- κ B binding site and has been reported to be associated with low IFN- γ production.²¹⁻²³

We found that 2 functional SNPs in IFNGR1 were associated with an increase in IFN- γ production (rs223471 [-56C/T],²⁴ $P = .026$ [data not shown]; rs7749390,²⁵ $P = .030$; Fig 4, C). The SNP rs7749390 was also significantly associated with a reduced risk of the ADEH⁺ phenotype (35.6% ADEH⁺ vs 45.1% ADEH⁻; $P = .03$; Fig 3, A). Collectively, these data suggest that

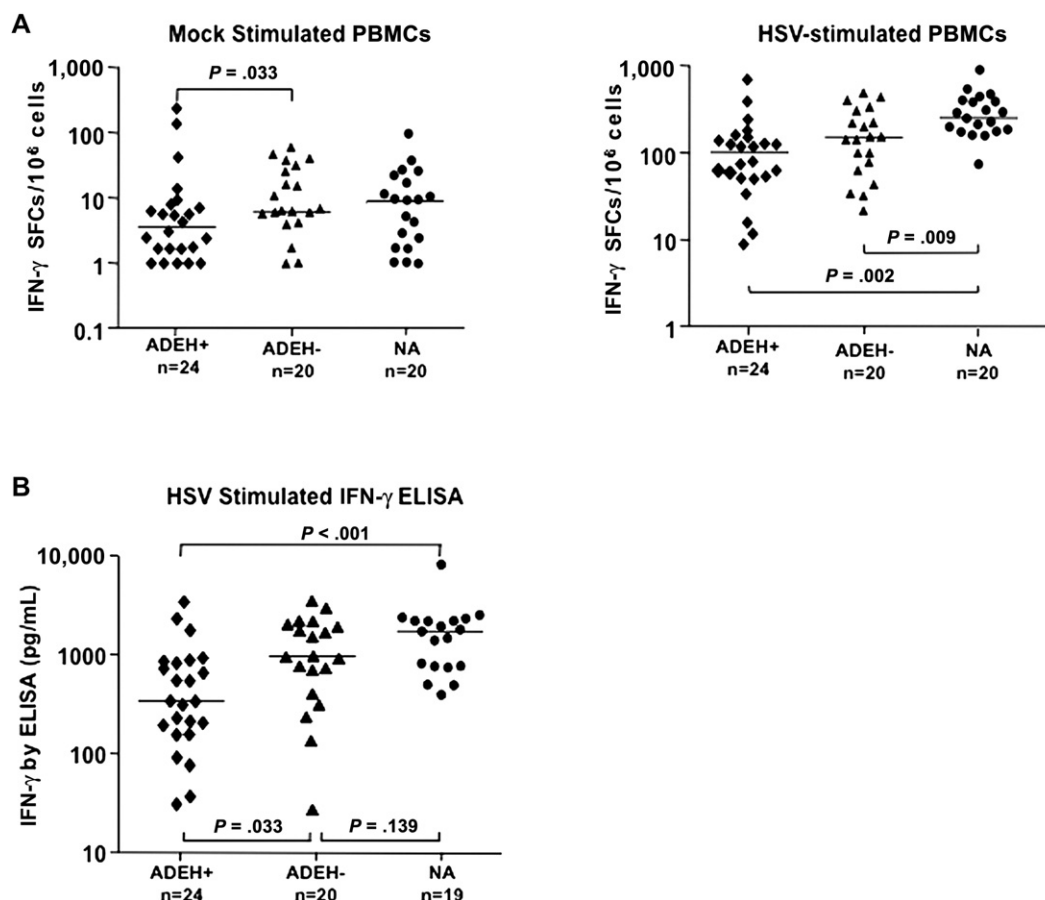


FIG 1. IFN- γ production is decreased in PBMCs from human subjects with ADEH⁺ compared with subjects with ADEH⁻ and nonatopic subjects. **A**, IFN- γ SFCs after mock (left panel) or HSV stimulation (right panel) *ex vivo*. **B**, IFN- γ protein measurements in culture supernatants of HSV-stimulated PBMCs. PBMCs from each subject group were stimulated for 6 days with mock or HSV antigens. Then culture supernatants were removed and analyzed for IFN- γ secretion by ELISA. Data points represent the difference between IFN- γ secretion in HSV versus mock-stimulated PBMCs for each subject; *P* values reflect adjusted (analysis of covariance-derived) comparisons. Horizontal lines represent median values. NA, Nonatopic.

these variants are involved in gene regulation and directly affect the levels of IFN- γ expression.

DISCUSSION

Using immunologic, genomic, and genetic approaches, we demonstrate that low IFN- γ expression contributes to the increased susceptibility of a subset of patients with AD to develop severe viral infections. Global gene expression profiling of VV-stimulated PBMCs from subjects with ADEH⁺, subjects with ADEH⁻, and healthy nonatopic subjects revealed a significant association of genes that code for IFNs with the ADEH⁺ phenotype (Table I). Further analysis of the IFN family revealed significant (*q* value < 0.1) associations of genes that code for type I and II IFNs, with an aberrant response to stimulation with VV (Table II). Interestingly, gene expression of *type I IFNs* was significantly increased in subjects with ADEH⁺, suggesting an abortive effort to mount an antiviral response in these patients. This may be a result of the observed downregulation of IFN- α , IFN- β , and IFN- ω receptor 1. In contrast, *type II IFN- γ* was significantly downregulated. Although type I IFNs have a direct antiviral effect *in vitro*, which can be reproduced *in vivo* at pharmacologic doses, IFN- γ

plays a crucial role in the initiation and propagation of the antiviral cell-mediated defense. *In vivo* studies in mice have demonstrated a nonredundant synergistic effect of type I and type II IFNs to mount an effective host antiviral response.²⁶

Importantly, our gene profiling studies also revealed a significant downregulation of the IFN- γ receptor gene in subjects with ADEH⁺, but not in patients with ADEH⁻. Low IFN- γ receptor gene expression in combination with low IFN- γ expression would be expected to reinforce the poor antiviral response in these patients. The biological significance of this observation was supported by our finding of disseminated VV infection after scarification of VV into the skin of IFN- γ receptor knockout mice. The clinical phenotype in these IFN- γ receptor knockout mice of multiple VV-induced satellite lesions (Fig 2), increased viremia, and reduced survival is similar to clinical outcomes of EV in human beings.^{4,5}

Because there was significantly less IFN- γ secretion (Fig 1, B) and there were lower numbers of IFN- γ SFCs after HSV stimulation of PBMCs in the ADEH⁺ group compared with the ADEH⁻ group, even after mock stimulation (Fig 1, A), we tested whether genetic variants in the gene encoding *IFNG* and its receptor, *IFNGR1*, were associated with risk of ADEH⁺ and

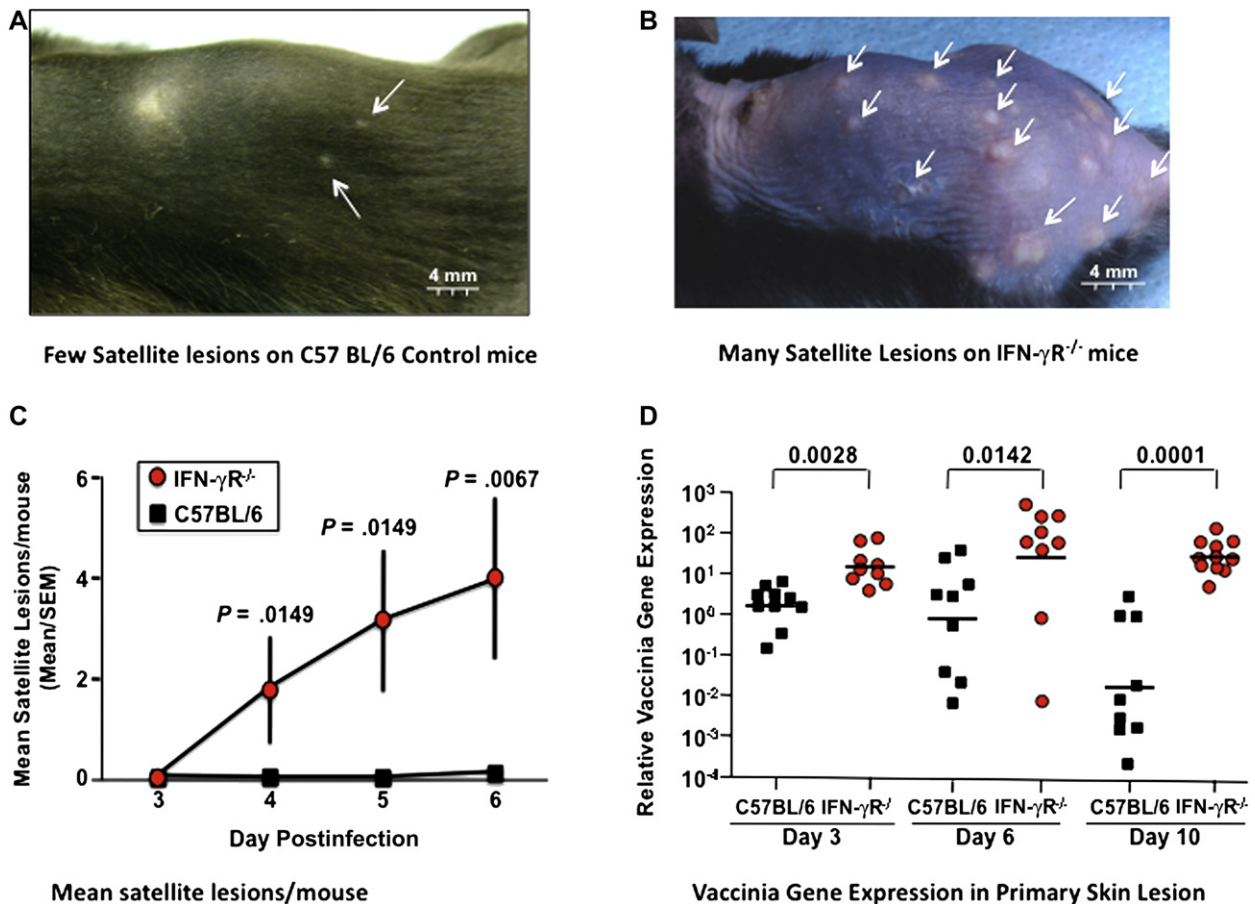


FIG 2. Disseminated viral skin infection in IFN- γ receptor knockout mice after inoculation with VV. Clinical appearances of satellite lesions are shown after epicutaneous inoculation of C57 BL/6 control mice (**A**) and IFN- γ R^{-/-} mice (**B**) with VV. **C**, The mean number of satellite lesions was significantly greater in IFN- γ R^{-/-} mice than control mice. **D**, Data points of relative vaccinia gene expression (measured by real-time PCR) was significantly greater at all time points in IFN- γ R^{-/-} mice than control mice.

contributed to the reduced production of IFN- γ . In this study, we observed significant associations for *IFNGR1* SNPs (rs1327475, rs10457655, and rs7749390) and risk of ADEH⁺ phenotype, and this association was strengthened by haplotype analysis. The strongest association was for a protective haplotype AG (rs10457655 and rs7749390; $P = 5.7 \times 10^{-4}$) that reduced risk of ADEH⁺ phenotype by over half (odds ratio, 0.44) and was relatively common in this European American sample, with a prevalence of 13.2% in patients with ADEH⁺ compared with 25.5% in patients with ADEH⁻. Interestingly, this haplotype includes the functional SNPs rs7749390, a SNP located on the exon/intron splicing site of *IFNGR1*.²⁵ This SNP has been associated with increased levels of IFN- γ production and reduced risk of ADEH⁺, suggesting that this SNP may protect against ADEH⁺ by regulating IFN- γ secretion after exposure to HSV. It is possible that this SNP breaks a consensus splicing site sequence, resulting in the intron remaining in mature mRNA and subsequently the production of aberrant proteins.²⁷ Although there are several other known *IFNGR1* mutations (V61E, Y66C, C77F) that have been associated with partial or complete *IFNGR1* deficiency,²⁸ we did not genotype those because of low minor allele frequencies ([MAFs] <5%) and the limited power in the existing sample to detect associations; however, future studies in an expanded dataset should include these known variants. In addition, we observed

associations for ADEH⁻ and 2 *IFNGR1* SNPs (rs1327475 and rs10457655; Fig 3, A), but the associations were stronger for ADEH⁺, suggesting that these SNPs may confer an increased susceptibility of patients with AD to develop disseminated viral skin infections (ADEH⁺).

Significant associations were also observed for *IFNG* variants and risk of ADEH. In particular, a 4-SNP haplotype showed the strongest association with increased risk of ADEH ($P = .0027$). Of interest, this haplotype includes 2 SNPs (rs2069727 and rs2430561; *IFNG*+874T/A) that were significantly associated with reduced IFN- γ production *ex vivo* in response to HSV exposure. SNP rs2430561 has previously been associated with low IFN- γ production in psoriasis vulgaris²¹ and tuberculosis²² as well as risk of atopic asthma, AD, and allergic rhinitis.²³ Although none of the individual SNPs were associated with ADEH *per se*, the significant associations observed between disease and haplotypes composed of these SNPs suggest that as yet untyped variants within or around the 4-SNP haplotype locus are causally related to risk of ADEH. In this study, we selected 8 *IFNG* SNPs, which included 2 previously described functional variants in *IFNG* (rs2069709, rs2430561) and 6 tagging SNPs spanning a 13.1-kb region on chromosome 12q14 (Fig E1).

Although the genetics portion of the current study was not designed to interrogate the mechanisms by which the *IFNG* and

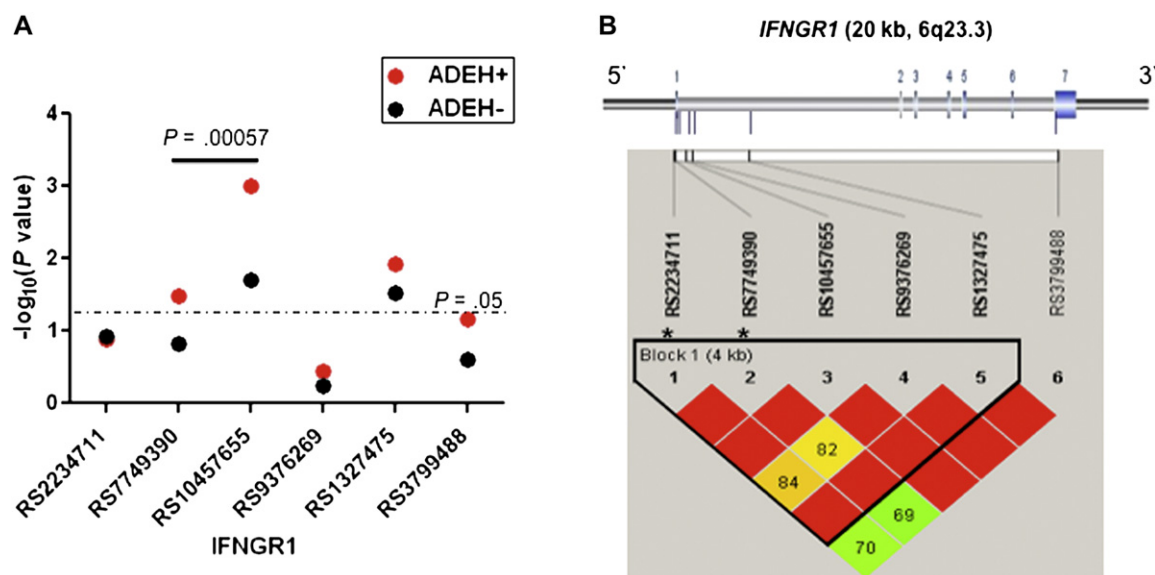


FIG 3. Summary of genetic associations for 6 *IFNGR1* SNPs. **A**, The $\log_{10}$ -transformed *P* values for *IFNGR1* SNPs and ADEH⁺ and ADEH⁻. The dashed gray line represents a *P* value of .05. The thick line represents a 2-SNP haplotype. **B**, Gene structure and pattern of LD (*D'*) in European American healthy controls, with red to green reflecting higher to lower *D'* values. Seven exons (blue) in *IFNGR1* were presented (upper panel). *Functional SNPs.

TABLE III. Strongest association of *IFNG* haplotype with ADEH⁺ among European Americans

rs2069727*	rs2069718	rs2069716	rs2430561*	Haplotype ADEH ⁺	Frequency ADEH ⁻	<i>P</i> value
C	G	C	A	0.05	0.04	.60
C	G	T	A	0.36	0.40	.337
T	G	T	A	0.05	0.01	.0027
T	A	T	T	0.41	0.39	.664
T	G	T	T	0.12	0.15	.332

OMNIBUS, *P* = .033

*Marker associated with IFN- γ production.

IFNGR functional variants are associated with ADEH and altered IFN- γ production, this constitutes an obvious next step. We acknowledge that other variants in these 2 genes may also contribute to risk of ADEH. As indicated, we purposely did not genotype SNPs of low MAF given our current limitations of power; however, it is recognized that a considerable proportion of heritable disease risk in complex traits is in fact associated with rare variants¹² (usually defined as those with a frequency of less than 5% to 1% or lower²⁹). Testing for association between rare variants and rare traits is inherently problematic given the challenges of patient recruitment for a robustly powered discovery sample and the further difficulties of access to similarly robust replication samples. On the basis of our registry enrollment, we estimate that ADEH affects less than 3% of subjects with AD³ and can possibly be defined as a rare trait. Rare diseases are typically characterized as being highly penetrant and caused by polymorphisms of large effect that are often rare. Rare variants of large effects, combined with multiple other rare mutations, together can explain a large proportion of the genetic basis for complex diseases.³⁰ On the other hand, there is evidence that common alleles with modest to large effects underlie the risk for certain rare but complex traits (ie, cleft palate³¹). Our own recent studies demonstrated that a relatively uncommon null mutation in the gene encoding filaggrin (*FLG*; R501X) was 3 times more prevalent in patients with

ADEH⁺ than patients with ADEH⁻ (24% vs 8%, respectively), and the relative risk for ADEH⁺ disease was nearly doubled (odds ratio, 11.8 vs 6.2; *P* = .0008). Thus, although we recognize the potential for association between ADEH and as yet untyped markers in *IFNG* and *IFNGR1*, we believe our tagging approach combined with a focus on known functional variants is a comprehensive first step for detecting causal loci of large effects in ADEH.

Although the genetic association studies including *IFNG* and *IFNGR1* SNPs support our primary observation in this study—that IFN- γ responses are diminished in both murine and human conditions associated with disseminated viral infection—it will ultimately be critical to seek replication of these associations in independent populations of patients with ADEH⁺. The primary sample used for the tests for association with risk of ADEH⁺ was the group of European American patients and healthy controls from the National Institute of Allergy and Infectious Diseases-supported ADVN. We cannot speculate at this time whether similar associations would be observed in populations of different ancestry. However, our studies on associations between *IFNG* and *IFNGR1* variants and IFN- γ production were conducted in both European American and African American subjects, and we observed no difference in the levels of IFN- γ between these 2 racial groups (see this article's Fig E2 in the Online

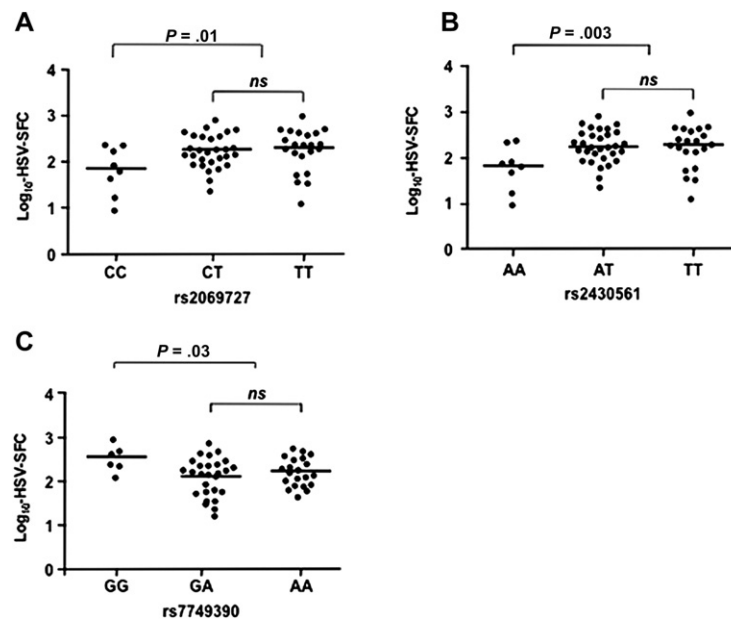


FIG 4. Association of *IFNG* and *IFNGR1* SNPs with IFN- γ production as determined by the log₁₀-transformed mean SFCs/10⁶ cells. *IFNG* SNPs rs2069727 CC genotype and rs2430561 AA were significantly associated with reduced IFN- γ production ($P = .01$ [CC vs CT + TT] and $P = .003$ [AA vs AT + TT], respectively, **A** and **B**). *IFNGR1* SNP rs7749390 GG genotype was significantly associated with increased IFN- γ production ($P = .03$ [GG vs GA + AA]), **C**). *ns*, Not significant.

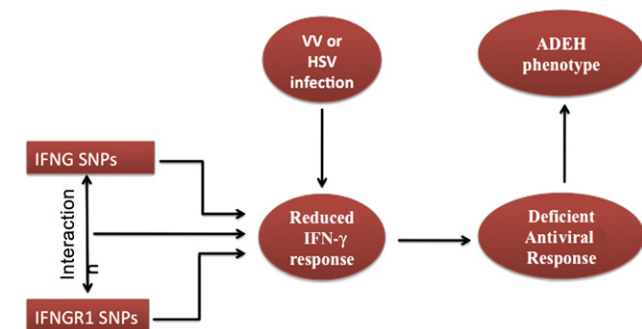


FIG 5. Overview of impaired IFN response leading to AD complicated by eczema herpeticum.

Repository at www.jacionline.org). We believe these findings suggest that the relevance of the functional *IFNG* (+874T-A) and *IFNGR1* (-56C/T and +95T/C) SNPs in regulating IFN- γ production is not ethnic-specific or race-specific and subsequently contributes to a reduced risk of ADEH.

In summary, we used several different approaches to demonstrate a low IFN- γ expression in patients with ADEH⁺ as well as association between *IFNG* and *IFNGR1* SNPs and the ADEH⁺ phenotype and IFN- γ production in a multicenter case-control study (Fig 5). Thus, our data demonstrate, for the first time, that *IFNG* and *IFNGR1* receptor variants may impair the antiviral response against viruses such as HSV and vaccinia and increase the risk of ADEH. The propensity of this small subset of patients with AD to develop disseminated skin infections may be a result of a combination of skin barrier defects facilitating skin viral penetration (reflected in their increased association with filaggrin null mutations) and a defective IFN- γ systemic immune response. Of note, skin barrier defects are common in AD, even in subjects with

ADEH⁻. These skin barrier defects can relate to genetic null mutations or local T_H2-mediated downregulation of the innate immune response.³² Of note, in this study we failed to find an association between enhanced T_H2 systemic immune responses and ADEH (Table II). However, the current study found that IFN- γ protein production was significantly lower in subjects with ADEH⁺ but not subjects with ADEH⁻, suggesting that a defective systemic IFN- γ immune response that fails to control viral replication plays a key role in the pathogenesis of ADEH. A defect in T_H1 cytokines in ADEH may also contribute to the severe eczema and increased atopy associated with this form of AD.^{3,33} A clear understanding of these risk factors may improve our ability to identify patients at greatest risk for ADEH⁺ and ultimately lead to early intervention to prevent this devastating complication of AD.

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Clinical implications: Subjects with AD prone to disseminated viral skin infections have defects in their IFN responses.

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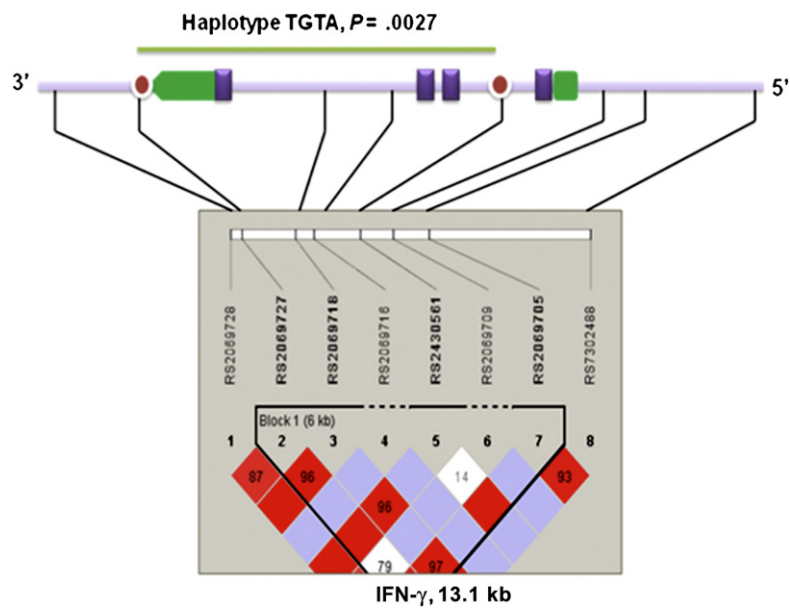


FIG E1. Gene structure and LD estimation for 8 *IFNG* SNPs in a European American population. The *top panel* is the *IFNG* structure containing 4 exons (*purple*) spanning a region of 13.1 kb. (*Filled circles* represent the SNPs associated with IFN- γ production). *Diamonds without a number* correspond to $D' = 1$; *shading* represents LD with a *red to white gradient* reflecting higher to lower D' values.

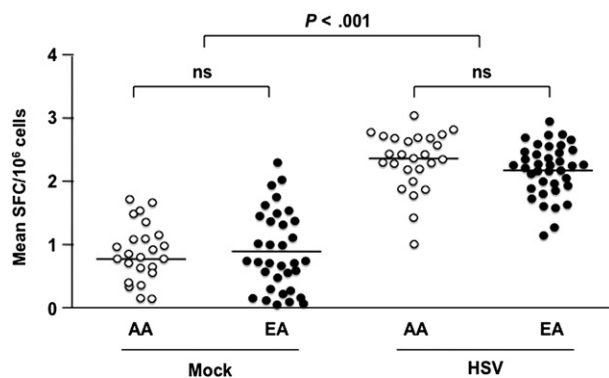


FIG E2. Mean levels of the log₁₀-transformed SFC/10⁶ cells in mock-treated and HSV-treated PBMCs from European Americans (EA) and African Americans (AA). *ns*, Not significant.

TABLE E1. Clinical characteristics of participants in cell-mediated immunity studies

	ADEH ⁺	ADEH ⁻	NA
Age (y)			
n	24	20	20
Mean (SD)	21.65 (15.42)	42.3 (10.35)	40.55 (10.50)
Median	17.7	44.3	38.25
(Minimum, maximum)	(1.62,59.2)	(22.2, 54.7)	(27.3, 56)
Female n (%)	11 (45.8)	15 (75.0)	12 (60.0)
Male n (%)	13 (54.2)	5 (25.0)	9 (45.0)
African American n (%)	5 (20.8)	10 (50.0)	11 (55.0)
Caucasian n (%)	19 (79.2)	10 (50.0)	9 (45.0)

NA, Nonatopic.

TABLE E2. Clinical characteristics of participants in genetics association studies of *IFNG* SNPs

Characteristic	European American		
	ADEH ⁺	ADEH ⁻	NA
Sample size	112	166	157
Males, n (%)	58 (53.7)	49 (29.7)	63 (40.1)
Age (y), mean (SD)	22.9 (22.1)	38.4 (14.6)	36.5 (13.2)
Total IgE, mean* (95% CI)	2083 (1399-3100)	366.7 (261-516)	55.5 (44-69)
EASI, mean† (95% CI)	7.4 (6.0-9.1)	3.5 (2.9-4.3)	ND

EASI, Eczema Area and Severity Index; NA, nonatopic; ND, not done.

*Geometric mean serum levels of total IgE.

†EASI determined by the percentage of eczema area on a 7-point ordinal scale: 0, <10%; 1, 10% to 29%; 3, 30% to 49%; 4, 50% to 69%; 5, 70% to 89%; and 6, 90% to 100%. and *IFNGR1* in the European American healthy controls (n = 157).

TABLE E3. SNP position, allele, role, and MAF of *IFNG* and *IFNGR1* in the European American nonatopic controls (n = 157)

SNP	SNP position	Distance from previous SNP	Alleles	Location	MAF
<i>IFNG</i> (chr12q14)					
rs7302488	66847146		G/T	Promoter	0.23
rs2069705	66841278	5,868	C/T	Promoter	0.36
rs2069709	66839970	1,308	G/T	Promoter	0.02
rs2430561	66838787	1,183	A/T	Intron	0.36
rs2069716	66837082	1,705	A/G	Intron	0.05
rs2069718	66836429	653	C/T	Intron	0.48
rs2069727	66834490	1,939	A/G	Downstream	0.34
rs2069728	66834051	439	A/G	Downstream	0.15
<i>IFNGR1</i> (chr6q23.3)					
rs2234711	137582213		C/T	Promoter	0.44
rs7749390	137582063	150	A/G	Intron (boundary)	0.44
rs10457655	137581503	560	A/G	Intron	0.23
rs9376269	137581198	305	C/G	Intron	0.20
rs1327475	137578148	3,050	C/T	Intron	0.14
rs3799488	137561473	16,675	C/T	Intron (boundary)	0.07