

Atopic dermatitis complicated by eczema herpeticum is associated with HLA B7 and reduced interferon- γ -producing CD8+ T cells

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Summary

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Conflicts of interest

The authors state no conflict of interest.

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Background The increased susceptibility of patients with atopic dermatitis (AD) to disseminated viral skin infections such as eczema herpeticum (ADEH+) is poorly understood.

Objectives The primary goal of the current study was to determine whether ADEH+ subjects have identifiable defects in cell-mediated immunity that reduce their ability to control viral infections.

Materials and methods In this study, we evaluated cytokine expression by various subsets of peripheral blood mononuclear cells from ADEH+ ($n = 24$) compared with AD without a history of viral infections (ADEH-) ($n = 20$) before and after treatment with herpes simplex virus (HSV).

Results We found that interferon (IFN)- γ expression after HSV treatment was lower in the CD8+ T cells and monocytes from patients with ADEH+ compared with patients who are ADEH- or nonatopic. Given the induction of CD8+ T cells as the result of antigen presentation by human leucocyte antigen (HLA) class I, consistent with the findings described above we also found that the HLA B7 allele was significantly associated with risk of the ADEH+ phenotype (odds ratio = 1.91, $P = 0.02$, 125 ADEH+ and 161 ADEH- subjects).

Conclusions These data suggest that defects in viral-induced IFN- γ from CD8+ T cells contribute to the ADEH+ phenotype.

What's already known about this topic?

- Interferon (IFN)- γ -producing cells are known to be important cytotoxic effectors of immune protection against viruses.
- There have been limited reports of human leucocyte antigen (HLA) associations with risk of atopic dermatitis (AD), but there have been no reports of associations with risk of AD with eczema herpeticum (ADEH+).

What does this study add?

- This study presents evidence that low IFN- γ expression in the CD8+ T-cell subset is associated with increased susceptibility of a subset of patients with AD to develop severe viral infections.
- The association of B7 alleles with EH in AD is significant because HLA B7 is more efficiently induced by IFN- γ than are HLA A locus products.

A subset of patients with atopic dermatitis (AD) suffer from disseminated viral infections, i.e. eczema herpeticum (ADEH+), after herpes simplex virus (HSV) infection¹ or eczema vaccinatum, after exposure to smallpox vaccination with vaccinia virus.² Here, we sought to determine whether ADEH+ subjects have identifiable defects in cell-mediated immunity (CMI) that reduce their ability to control viral infections. The immunoprofiles of peripheral blood T lymphocytes and natural killer (NK) cells pre- and post-HSV stimulation^{3–6} as well as major histocompatibility complex (MHC) genotyping^{7,8} were evaluated as they are known to be involved in viral immune responses.

Materials and methods

ADEH+ subjects were defined as patients with AD who had at least one previous EH episode as physician documented in Beck *et al.*¹ ADEH– subjects were defined as patients with AD with no history of EH. The study was approved by the institutional review boards at all participating centres. Immunoprofiling studies were conducted on a subset of participants including 24 ADEH+, 20 ADEH– and 20 nonatopic subjects. Of the 24 ADEH+, 63% ($n = 15$) experienced an EH episode in the past year, 13% ($n = 3$) in the past 2 years, and 17% ($n = 4$) more than 2 years ago. All the samples were obtained from the recovered stage. Peripheral blood mononuclear cells (PBMC) were stimulated with HSV-1 or mock antigens as previously described.^{9,10} On day 6, cells were stained with fluorescent anti-CD4, CD8, CD14, CD107a, interferon (IFN)- γ , tumour necrosis factor (TNF)- α , interleukin (IL)-10, IL-13 and IL-17 monoclonal antibody and analysed using a FACSCalibur flow cytometer. For NK assays, PBMC were infected with HSV-1 overnight; cells were then stained for CD3, CD16, CD56, CD107a and IFN- γ . Flow values were log₁₀ transformed to satisfy statistical assumptions. Diagnostic group comparisons employed analysis of covariance (ANCOVA) models adjusting for age (mock-stimulated endpoints) or adjusting for age and mock-stimulated values (HSV-stimulated endpoints). P -values < 0.05 were considered significant.

A total of 50 μ L of genomic DNA at 20–80 ng μ L^{–7} was isolated from 125 ADEH+ and 161 ADEH– European-American subjects for HLA typing by the Johns Hopkins University Immunogenetics Laboratory at a low resolution (A, B and C loci) using reverse Sequence Specific Oligonucleotide probe

hybridization assays (One Lambda Inc., Canoga Park, CA, U.S.A.) and high resolution (HLA B7) using Sanger Sequencing. Tests for association in a logistic framework collapsing one and two copies of the allele into a single category (e.g. presence vs. absence of B*07) were restricted to MHC class I alleles with a minor allele frequency $> 5\%$ within each subtype, and significance was estimated using 10 000 permutations. To evaluate the potential for confounding by AD severity, we also tested for association between the Rajka–Langeland score total (mild = 3–4, moderate = 4.5–7.5, and severe = 8–9) using a χ^2 test.

Results

Our immune profiling studies revealed a statistically significant difference in HSV CMI responses ($P < 0.05$) between ADEH+ and nonatopic controls for CD8+ IFN- γ + T cells. Detailed dot plots (Fig. 1a; left panel) show that ADEH+, but not ADEH–, subjects had lower percentages of HSV-specific CD8+ IFN- γ + T cells ($P = 0.025$) than nonatopic subjects. Figure 1b (right panel) shows that the CD14+ IFN- γ + percentage was lower ($P = 0.035$) in HSV-stimulated CD14+ cells of ADEH+ compared with ADEH– subjects.

No differences were observed in the CD4+ IFN- γ + or the NK expression of IFN- γ after HSV stimulation (see Fig. S1). We also evaluated T-helper (Th) 2 and Th17 T cells to determine whether these cell types were different in ADEH+ vs. ADEH– subjects. No significant differences were observed between ADEH+ and ADEH– subjects for the percentage of IL-10+, IL-13+ or IL-17+ CD4+ or CD8+ T cells after mock or HSV stimulation. However, the ratio of IFN- γ – to IL-10-producing cells ($P = 0.031$) as well as the ratio of TNF- α to IL-10-producing cells ($P = 0.017$) were significantly lower in CD14+ cells after HSV-stimulation in the ADEH+ samples.

Because we observed a defect in IFN- γ generation in the CD14+ antigen presenting cells, we hypothesized that this could be explained by MHC-associated variants. Of the 19 alleles tested (Table 1), only B*07 was significantly associated with ADEH+ [odds ratio (OR) = 1.91, 95% confidence intervals (CI): 1.10–3.31, permutation P -value = 0.0285]; 30% of patients who were ADEH+ carried at least one copy of the B*07 allele, in contrast to 18.6% of patients who were ADEH–. High-resolution sequencing of the B*07 allele showed that the most common allele was B*07:ANVB (i.e. 0702/0761; $n = 66$),

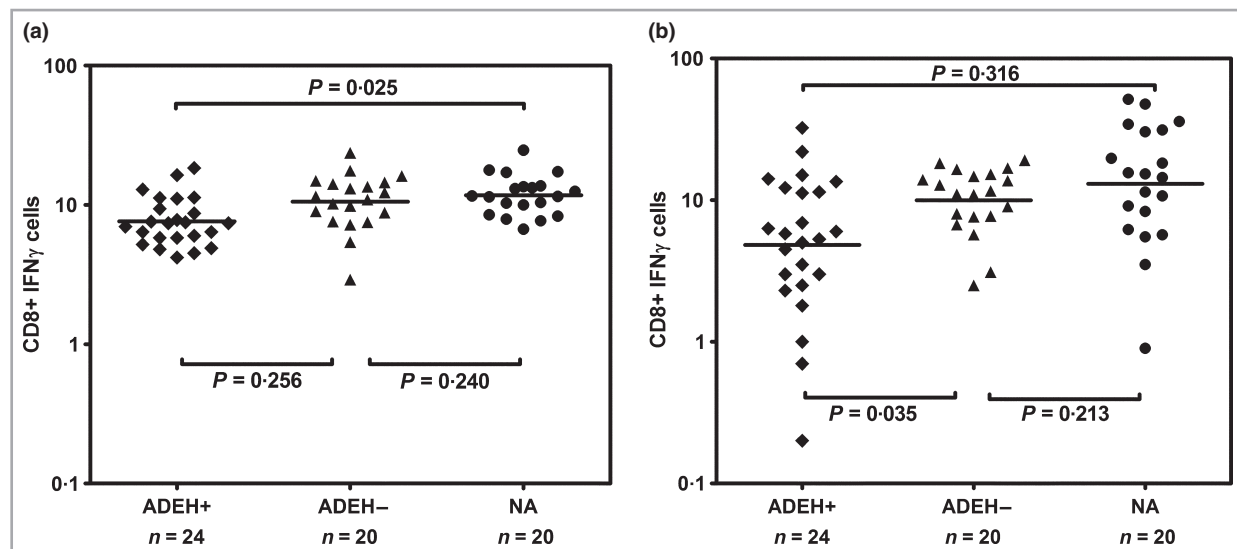


Fig 1. Low IFN- γ expression in ADEH+ subjects as assessed by flow cytometry. IFN- γ expression is shown for CD8 $^{+}$ cells (left panel, a) and CD14 $^{+}$ cells (right panel, b) assessed by intracellular staining and flow cytometry as described in Materials and methods. P-values reflect robust ANCOVA comparisons (adjusted for age and mock stimulated value). Horizontal lines represent geometric means. Values represent percentage of IFN- γ + cells among the parent cell population, i.e. CD8 $^{+}$ IFN- γ + out of total CD8 $^{+}$ and CD14 $^{+}$ IFN- γ + out of total CD14 $^{+}$ cells. ADEH, patients with atopic dermatitis with/without a history of eczema herpeticum; IFN, interferon; NA, nonatopic.

Table 1 Summary of allelic tests for association for all MHC class I alleles with an allele frequency > 5% and EH status. Presence/absence of specific allele, estimated OR and 95% CI for ADEH+ vs. ADEH-, asymptotic P-value, and permutation on P-value along with its 95% CI for those alleles with asymptotic P-value < 0.05 are presented

	ADEH- (n)		ADEH+ (n)		OR (95% CI)	Asymptotic P-value	Permutation P-value (95% CI)
Allele	-	+	-	+			
A*01	114	47	83	42	1.23 (0.74–2.03)	0.425	
A*02	75	86	63	62	0.86 (0.54–1.37)	0.522	
A*03	126	35	96	29	1.09 (0.62–1.90)	0.769	
A*11	140	21	111	14	0.84 (0.41–1.73)	0.637	
A*24	142	19	103	22	1.60 (0.82–3.10)	0.168	
B*07	131	30	87	38	1.91 (1.1–3.31)	0.021	0.0285 (0.025–0.032)
B*07:ANVB	133	27	87	35	1.98 (1.12–3.50)	0.019	0.0195 (0.017–0.022)
B*08	118	43	100	25	0.69 (0.39–1.20)	0.187	
B*18	147	14	112	13	1.22 (0.55–2.70)	0.625	
B*27	145	16	109	16	1.33 (0.64–2.78)	0.447	
B*35	138	23	110	15	0.82 (0.41–1.64)	0.573	
B*44	105	56	92	33	0.67 (0.40–1.12)	0.13	
B*51	144	17	107	18	1.42 (0.70–2.89)	0.327	
B*62	143	18	101	24	1.89 (0.97–3.66)	0.06	
C*04	131	30	104	21	0.88 (0.48–1.63)	0.688	
C*05	127	34	104	21	0.75 (0.41–1.38)	0.359	
C*06	143	18	111	14	1.00 (0.48–2.10)	0.996	
C*07	73	88	59	66	0.93 (0.58–1.48)	0.755	
C*10	140	21	104	21	1.35 (0.70–2.59)	0.374	
C*12	144	17	109	16	1.24 (0.60–2.57)	0.557	

AD, atopic dermatitis; ADEH+, patients with AD with (+) or without (–) a history of EH; CI, confidence interval; EH, eczema herpeticum; MHC, major histocompatibility complex; OR, odds ratio.

followed by B*07:EH (i.e. 0705/0706; $n = 3$) and lastly B*07:04 ($n = 1$). Analysis restricted to the B*07:ANVB allele marginally strengthened the association signal (OR = 1.98,

95% CI = 1.12–3.50; permutation $P = 0.0195$). There is no association between AD severity and the B*07 allele ($P = 0.353$).

Discussion

We demonstrate that low IFN- γ expression in the CD8⁺ T-cell subset is associated with the ADEH⁺ phenotype. This is an important observation as IFN- γ -producing cells are known to be important cytotoxic effectors of immune protection against viruses.⁶ IFN- γ plays a crucial role in the initiation and propagation of the antiviral cell-mediated defence. The specificity of this finding is supported by our finding that other subsets of PBMCs known to be altered in viral immune responses, including Th2, Th17 and NK cells, were not abnormally expressed in ADEH⁺ subjects.

We also observed a novel association between specific HLA class I alleles and risk of ADEH⁺. Global statistical tests revealed significant associations between ADEH status and the HLA B7 locus. Specifically, the B*07:ANVB allele was 1.7 times more common in patients who were ADEH⁺ (29%) compared with those who were ADEH⁻ (17%). The association of B7 alleles has significance because HLA B7 is more efficiently induced by IFN- γ than are HLA A locus products.⁸ Furthermore, vaccinia virus CD8⁺ T cell epitopes have been reported to be restricted by HLA B7.⁸ The propensity of patients who are ADEH⁺ to develop disseminated skin infections may be due to a combination of skin barrier defects facilitating skin viral penetration^{7,11} and a defective systemic antiviral immune response. A clear understanding of these risk factors may improve our ability to identify patients at greatest risk for ADEH⁺.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publishers' website:

Fig S1. Pairwise comparisons of HSV-stimulated endpoints between ADEH⁺, ADEH⁻ and NA groups. Measurements of cytokine expression in mononuclear cell subsets were assessed by intracellular staining and flow cytometry as described in Materials and methods. Differences between groups were obtained by robust ANCOVA, adjusting for age and mock stimulated value. Point estimates of differences and 95% confidence intervals were calculated on the log₁₀ scale, then back-transformed and plotted on the original scale. (A difference of 0.0 on the log₁₀ scale is identical to a ratio of 1.0 on the original scale. Points to the left of the vertical reference line at $x = 1.0$ in the left panel indicate ADEH⁺ lower than ADEH⁻.) ADEH, patients with AD with (+) or without (–) a history of eczema herpeticum; HSV, herpes simplex virus; NA, nonatopic

