Original article

Phenotype of atopic dermatitis subjects with a history of eczema herpeticum

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Background: A subset of subjects with atopic dermatitis (AD) are susceptible to serious infections with herpes simplex virus, called eczema herpeticum, or vaccina virus, called eczema vaccinatum.
Objective: This National Institute of Allergy and Infectious Diseases–funded multicenter study was performed to establish a database of clinical information and biologic samples on subjects with AD with and without a history of eczema herpeticum ($ADEH^+$ and $ADEH^-$ subjects, respectively) and healthy control subjects. Careful phenotyping of AD subsets might suggest mechanisms responsible for disseminated viral infections and help identify at-risk individuals.
Methods: We analyzed the data from 901 subjects ($ADEH^+$ subjects, n = 134; $ADEH^-$ subjects, n = 419; healthy control subjects, n = 348) enrolled between May 11, 2006, and September 16, 2008, at 7 US medical centers.
Results: $ADEH^+$ subjects had more severe disease based on scoring systems (Eczema Area and Severity Index and Rajka-Langeland score), body surface area affected, and biomarkers (circulating eosinophil counts and serum IgE, thymus and activation-regulated chemokine, and cutaneous T cell–attracting chemokine) than $ADEH^-$ subjects ($P < .001$). $ADEH^+$ subjects were also more likely to have a history of food allergy (69% vs 40%, $P < .001$) or asthma (64% vs 44%, $P < .001$) and were more commonly sensitized to many common allergens ($P < .001$). Cutaneous infections with *Staphylococcus aureus* or molluscum contagiosum virus were more common in $ADEH^+$ subjects (78% and 8%, respectively) than in $ADEH^-$ subjects (29% and 2%, respectively; $P < .001$).
Conclusion: Subjects with AD in whom eczema herpeticum develops have more severe T_H2-polarized disease with greater allergen sensitization and more commonly have a history of food allergy, asthma, or both. They are also much more likely to experience cutaneous infections with *S aureus* or molluscum contagiosum. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Atopic dermatitis, herpes simplex virus, eczema herpeticum, eczema vaccinatum, biomarkers, Staphylococcus aureus*

The overall objective of the National Institute of Allergy and Infectious Diseases (NIAID)–funded Atopic Dermatitis and Vaccinia Network (ADVN) is to investigate the mechanism or mechanisms responsible for the susceptibility of subjects with atopic dermatitis (AD) to cutaneous viral infections. The most severe example is eczema vaccinatum (EV), which occurs after exposure to the smallpox vaccine.[1] Fortunately, cases of EV have occurred only rarely since the risk of vaccinating high-risk subjects was appreciated. However, 7% to 10% of subjects with AD have difficulty containing other cutaneous viral infections caused by herpes simplex virus (HSV) and molluscum contagiosum virus.[2] The most commonly recognized viral complication in subjects with AD is eczema herpeticum (EH), which is caused by an extensive cutaneous infection with HSV. EH can be complicated by keratoconjunctivitis, viremia and sometimes multiple organ involvement with meningitis and encephalitis.[3] The central hypothesis of the ADVN registry study is that subjects with AD who have had EH ($ADEH^+$) have a unique phenotype that can be recognized by obtaining a careful history and physical examination, by means of serum biomarkers, or both. This information might also be useful to identify subjects with AD who are at risk for EV, the more life-threatening viral complication that would be a concern if variola was weaponized and obligatory smallpox vaccination strategies were deployed.[1] This is the first study to characterize the phenotype and biomarkers of two ethnically diverse American $ADEH^+$ populations and is the most comprehensive

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Abbreviations used

AD: Atopic dermatitis
ADEH$^+$: Atopic dermatitis with a history of eczema herpeticum
ADEH$^-$: Atopic dermatitis without a history of eczema herpeticum
ADVN: Atopic Dermatitis Vaccinia Network
ASC: Animal Study Consortium
CBC: Complete blood count
CSC: Clinical Study Consortium
CTACK (CCL27): Cutaneous T cell–attracting chemokine
CTL: Healthy control
DACI: Dermatology, Allergy, and Clinical Immunology Laboratory
DAIT: Division of Allergy, Immunology, and Transplantation at NIAID branch
EASI: Eczema Area and Severity Index
EH: Eczema herpeticum
EV: Eczema vaccinatum
HSV: Herpes simplex virus
IP-10 (CXCL11): Interferon-inducible protein-10
IV: Ichthyosis vulgaris
JHAAC: Johns Hopkins Asthma and Allergy Center
NIAID: National Institute of Allergy and Infectious Diseases
SDCC: Statistical and data coordinating center
SEA: *Staphylococcus aureus* enterotoxin A
SEB: *Staphylococcus aureus* enterotoxin B
TARC (CCL17): Thymus and activation-regulated chemokine
TSST-1: Toxic shock syndrome toxin-1

study performed to date based on the number of subjects recruited, serum/plasma collected, and detailed disease characterization (including a 29-page case report form).

Most cases of EH are caused by HSV-1. Because HSV-1 seropositivity is high in the general population (approximately 20% of children and approximately 60% of adults), it is unlikely that EH episodes are simply a function of viral exposure.[4] An analysis of ADEH$^+$ subjects examined at a single German University between 1959 and 1986 demonstrates a significant increase in the incidence of this complication, from a rate of 0.6 cases per year to more than 15 cases per year.[5] This increase is not likely explained by the increased prevalence of AD because this would predict a mere doubling or tripling of the cases. Rather, it suggests that AD has evolved into a disease with greater susceptibility to infection which would need to be explained by environmental effects rather than genetic drift. Consequently, there is growing concern that smallpox vaccination would pose a greater problem than would be explained by the increasing AD prevalence data alone.

It has been hypothesized that the increased susceptibility of subjects with AD to EH may be due to their T_H2 predominance and relative T_H1 deficiency. Collectively, this leads to the diminished production of antimicrobial peptides and reduced skin barrier proteins, which is more pronounced in AD subjects with severe, allergen-driven (or extrinsic) disease. To more definitively characterize the epidemiologic, clinical, and laboratory characteristics of African American and European American ADEH$^+$ subjects, we established a registry of ADEH$^+$ subjects, subjects with AD without a history of EH (ADEH$^-$), and healthy control (CTL) subjects and are reporting our findings from 901 subjects who have been recruited at 7 academic centers within the United States.

METHODS

The study was approved by the institutional review boards at 7 US academic centers. Information about the ADVN structure and statistical and data coordinating center (SDCC) and a more detailed study outline can be found in the Method's section of this article's Online Repository at www.jacionline.org.

Standard diagnostic criteria and study procedures

Standard diagnostic criteria were developed for this registry study in which all subjects were between 1 and 80 years of age (see Table E1 in this article's Online Repository at www.jacionline.org). AD was diagnosed based on standard criteria with the additional requirement for subjects less than 4 years of age that the disease needed to be present for at least 6 months before study enrollment to minimize the likelihood of recruiting children with other eczematous disorders that commonly mimic AD.[7] ADEH$^+$ subjects were defined as subjects with AD who had at least 1 EH episode that had a diameter of 5 cm or larger documented either by a physician at an ADVN study site or by an outside provider. HSV infection was confirmed by means of PCR, Tzanck smear, immunofluorescence, and/or culture. ADEH$^-$ subjects were defined as subjects with AD with no history of EH as described by the patient, caregiver, or both. Subjects with AD whose EH history was equivocal were not enrolled. CTL subjects were defined as having no personal or family history of atopic diseases and no personal history of chronic skin or systemic diseases.

All study participants underwent a detailed history, physical examination, disease severity assessment, and blood draw. Disease severity was assessed by using the Rajka-Langeland and Eczema Area and Severity Index (EASI) scoring systems. The EASI is a standardized grading system (score range, 0-72) that assesses erythema, excoriation, lichenification, infiltration, and/or papulation.[8] The Rajka-Langeland score rates extent, course, and itch intensity separately and yields a score from 0 to 9.[9] The Rajka-Langeland score system provides a broad and somewhat historical view of a subject's AD severity, whereas the EASI provides a more sensitive measure of disease severity at the time of enrollment. Blood samples were sent to Quest Diagnostics Laboratory for a complete blood count (CBC) with differential and to the Dermatology, Allergy, and Clinical Immunology Laboratory (DACI) at Johns Hopkins Asthma and Allergy Center (JHAAC) for a serum total IgE measurement, both multiallergen and allergen-specific IgE by the UniCap 250 System (Pharmacia-Upjohn, Uppsala, Sweden). All remaining serum and plasma samples were catalogued, aliquoted and stored at the University of Rochester Medical Center at −80°C.

Biomarker analysis

The DACI laboratory performed the following tests on serum samples from all ADEH$^+$ and ADEH$^-$ subjects: total IgE (kilounits per liter) and multiallergen-specific Phadia ImmunoCAP tests, including food (FX5E), mite-roach (HX2), animal dander (EX2), weed (WX1), grass (GX2), tree (TX3), tree (RTX10), and mold (MX2), and allergen-specific tests, including *Staphylococcus aureus* enterotoxin A (SEA; AM80), *S aureus* enterotoxin B (SEB; BM81), and *S aureus* toxic shock syndrome toxin 1 (TSST-1; RM226). CTL subjects had total IgE levels and a multiallergen RAST called a Phadiatop performed. Total and allergen-specific IgE levels were determined from serum samples by using the UniCap 250 System (Pharmacia-Upjohn); samples were measured in duplicate. The total eosinophil count (cells per cubic millimeter) was calculated from the CBC with differential.

Serum concentrations of cutaneous T cell–attracting chemokine (CTACK/CCL27), thymus and activation-regulated chemokine (TARC/CCL17), interferon-inducible protein (IP-10/CXCL10), and IFN-β were measured in a subset of ADEH$^+$ and ADEH$^-$ subjects who were age and gender-matched (see below). Each sample was run in duplicate, and the minimum detectable concentration for these ELISA assays (R&D Systems, Minneapolis, Minn) was 1.6, 7.0, 1.7, and 12.5 pg/mL, respectively.

HSV-1 and HSV-2 serology

HSV-1 IgG and HSV-2 IgG antibody testing was performed on serum samples (Quest Diagnostics Laboratory). The reference ranges for the tests are as follows: less than 0.90, negative; 0.90 to 1.10, equivocal; and greater than 1.10, positive.

Statistical analysis

All analyses used the full sample of ADVN registry subjects for the indicated diagnostic groups who completed the ADVN registry protocol by September 16, 2008, unless otherwise specified. Comparisons between the $ADEH^+$ and $ADEH^-$ groups for categorical endpoints were assessed by using the Fisher exact test. These included categorical demographic variables (eg, sex), categorical IgE antibody results (classified based on values >0.35 or <0.35 kU_A/L, the lower limit of detection), self-reported history of asthma or food allergy, and categorical body surface area affected by eczema (>35% or <35%). History of *S aureus* infection was collected as "Any previous infection (Y/N)?" combined with the text entered into the follow-up question indicating specific infections. Similarly, comparisons of categorical end points across the $ADEH^+$, $ADEH^-$, and CTL groups were made by using pairwise Fisher exact tests, including self-reported history of human papilloma virus, molluscum contagiosum skin infections, HSV eye and skin infections, and history of *S aureus* infection. Comparisons across the $ADEH^+$, $ADEH^-$, and CTL groups for continuous endpoints were made with the full sample by using 2-sample *t* tests. These endpoints included allergen-specific IgE values of greater than 0.35 kU_A/L, total IgE levels and eosinophil counts, and disease severity measures. Additionally, correlations of the EASI score with total IgE levels and eosinophil counts were calculated by using Pearson correlation coefficients and presented in scatterplots. Log_{10} transformations of continuous end points were applied when necessary.

A matched sample was generated by subjecting $ADEH^-$ subjects to gender and age (within 5 years) matching with a subset of $ADEH^+$ subjects to adjust for the effects of age and gender on comparisons between $ADEH^+$ and $ADEH^-$ subjects. Relationships between $ADEH^+$ and $ADEH^-$ subjects for continuous end points were then assessed by using paired *t* tests, and binary endpoints were tested with McNemar tests. The correlations between EASI score and CTACK, TARC, and IP-10 levels were calculated by using Pearson correlation coefficients and presented in scatterplots. Correlations between Rajka-Langeland scores and the biomarkers listed above were also computed.

All *P* values reported were considered descriptive. No adjustments for multiple comparisons were made. SAS version 9.1 software (SAS Institute, Inc, Cary, NC) was used for all analyses.

RESULTS

Demographics

A total of 901 subjects were enrolled in the 3 diagnostic groups: $ADEH^+$, $ADEH^-$, and CTL (see Table E2 in this article's Online Repository at www.jacionline.org). Both AD subgroups ($ADEH^+$ and $ADEH^-$) were younger than the CTL group ($P < .001$), and the $ADEH^+$ group was younger than the $ADEH^-$ group ($P < .001$). There was a greater percentage of female subjects in the $ADEH^-$ group (68%, $P < .001$) compared with the $ADEH^+$ (50%) and CTL (54%) groups.

Nearly 50% of $ADEH^+$ subjects had more than 1 episode of EH, and 4.5% reported more than 5 episodes. Ten percent of $ADEH^+$ subjects reported that a first-degree family member also had EH compared with 1% of $ADEH^-$ subjects and 0% of CTL subjects. The vast majority (94%) of $ADEH^+$ subjects had AD before 5 years of age in contrast to only 59% of $ADEH^-$ subjects ($P < .001$). More $ADEH^+$ subjects (58%) answered yes in response to the question, "Do you have keratosis pilaris, hyperlinear palms or ichthyosis?" compared with the $ADEH^-$ group (42%, $P = .005$). Both groups ($ADEH^+$ and $ADEH^-$) reported a similar frequency (4% to 5%) of alopecia areata.

EH and disease severity

Disease severity was significantly greater in $ADEH^+$ subjects compared with that seen in $ADEH^-$ subjects by using several objective measures of AD severity. Both the EASI and Rajka-Langeland scores were higher in $ADEH^+$ subjects, even after adjusting for age ($P < .001$; Fig 1, *A* and *B*). Greater severity among the $ADEH^+$ group was also reflected in serum IgE levels and circulating eosinophil counts (cells per cubic millimeter) compared with both the $ADEH^-$ and CTL groups, and this difference was also unaffected by age adjustment ($P < .001$; Fig 1, *C* and *D*). $ADEH^+$ subjects had greater surface area of involvement, with 32% having 35% or greater body surface area compared with only 9% of $ADEH^-$ subjects ($P < .001$). Not surprisingly, serum IgE levels and eosinophil counts from both $ADEH^+$ and $ADEH^-$ subjects correlated with EASI scores ($r = 0.54$ and 0.48 respectively; $P < .001$, Fig 2) and Rajka-Langeland scores ($r = 0.49$ and 0.41, respectively; $P < .001$; data not shown).

EH and history of atopic disorders

Significantly more $ADEH^+$ subjects (69%) reported a history of food allergy than $ADEH^-$ subjects (40%, $P < .001$; Fig 3, *A*). Remarkably similar findings were observed for asthma, with 64% of $ADEH^+$ subjects reporting a positive history compared with 44% of $ADEH^-$ subjects ($P < .001$; Fig 3, *B*).

EH and allergen sensitization

The fact that total serum IgE values were significantly higher in the $ADEH^+$ group compared with those seen in the $ADEH^-$ group (Fig 1, *C*) suggested that there might be differences in allergen-specific sensitization. To address this, we measured the following in all subjects with AD: multiallergen ImmunoCAP values (food/FX5E, mite/roach mix/HX2, animal dander/EX2, weed/WX1, grass/GX), tree/TX3, tree/RTX10, and mold/MX2) and specific ImmunoCAP values for SEA (AM80), SEB (BM81), and *S aureus* TSST-1 (RM226). The log_{10}-transformed ImmunoCAP values that were greater than -0.4559 (log_{10} of 0.35 kU_A/L) are shown as a Gaussian distribution for all of the ImmunoCAP values that were significantly different between AD subgroups (Fig 4). The animal dander mix ImmunoCAP test, which measures reactivity to cat dander and epithelium, dog dander, and guinea pig, rat, and mouse epithelium, showed significantly greater values in $ADEH^+$ subjects (Log mean ± SD, 1.58 ± 0.88 kU_A/L) than in $ADEH^-$ subjects (0.96 ± 0.91 kU_A/L, $P < .001$; Fig 4, *A*). The food ImmunoCAP test measures the reactivity to 6 food allergens, including egg white, milk, fish, wheat, peanut, and soybean, and values were significantly greater in $ADEH^+$ subjects (1.13 ± 1.04 kU_A/L) than in $ADEH^-$ subjects (0.68 ± 0.98 kU_A/L, $P < .001$; Fig 4, *B*). The mite-cockroach ImmunoCAP test, which measures reactivity to house dust (Hollister Stier), *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blatella germanica*, showed values that were significantly greater in $ADEH^+$ subjects (1.33 ± 0.90 kU_A/L) than in $ADEH^-$ subjects (1.02 ± 0.92 kU_A/L, $P = .006$; Fig 4, *C*). The grass ImmunoCAP test measures reactivity to Bermuda, rye, timothy, Kentucky blue, Johnson grass, and Bahia, and values were significantly greater in $ADEH^+$ subjects (1.13 ± 0.80 kU_A/L) than in $ADEH^-$ subjects (0.91 ± 0.79 kU_A/L, $P = .021$; Fig 4, *D*). The weed mix ImmunoCAP test measures reactivity to common ragweed, mugwort, English plantain, lamb's quarters, and

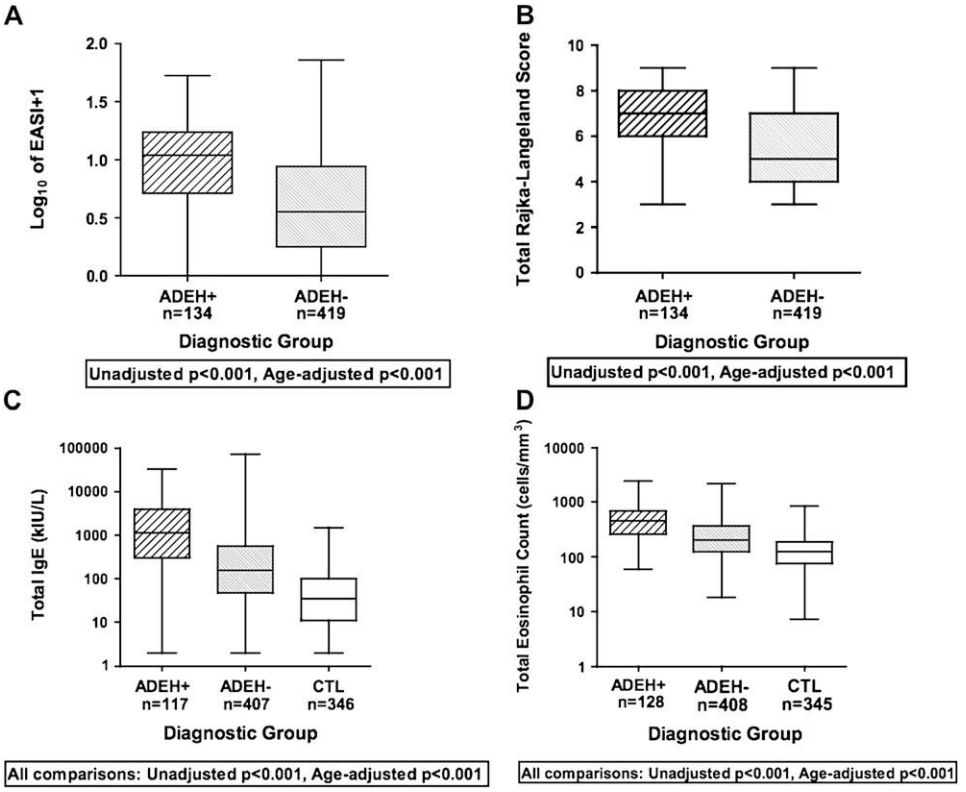

FIG 1. Box plots of EASI **(A)** and Rajka-Langeland **(B)** severity scores and serum total IgE levels **(C)** and total eosinophil counts **(D)**. The statistics are reported for all data points (as shown in these graphs), as well as for age-adjusted cohorts.

Russian thistle, and values were significantly greater in ADEH$^+$ subjects (0.71 ± 0.70 kU$_A$/L) than in ADEH$^-$ subjects (0.52 ± 0.64 kU$_A$/L, $P = .029$; Fig 4, *E*). The mold mix ImmunoCAP test measures reactivity to *Penicillium notatum*, *Cladosporium herbarum* (Hormodendrum), *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*/*Alternaria tenuis*, and *Helminthosporium halodes*, and values were significantly greater in ADEH$^+$ subjects (0.68 ± 0.60 kU$_A$/L) than in ADEH$^-$ subjects (0.51 ± 0.56 kU$_A$/L, $P = .047$; Fig 4, *F*). By using this analytic approach, there were no differences between ADEH$^+$ and ADEH$^-$ subjects for the 2 tree ImmunoCAP tests (TX3 and RTX10) or the *S aureus* toxin (SEA, SEB, and TSST-1)–specific ImmunoCAP tests (data not shown).

We also performed descriptive analyses of each of the ImmunoCAP measurements as a binary trait, with values reported as the proportion less than or equal to 0.35 kU$_A$/L (or negative) shown in the left aspect of each graph (Fig 4). The percentage of ADEH$^+$ subjects with a negative ImmunoCAP result was significantly less than that for ADEH$^-$ subjects for all ImmunoCAP tests performed except grass (GX2; Fig 4, *D*). Although not shown, when using this statistical approach, the *S aureus*–specific ImmunoCAP results (SEA [AM80], SEB [BM81], and TSST-1 [RM226]) were positive in a greater proportion of ADEH$^+$ subjects compared with ADEH$^-$ subjects ($P < .001$).

Serum IgE and Phadiatop results for the CTL population

As shown in Fig 1, *C*, CTL subjects had a mean total IgE level of 36.4 ± 1.2 kU/L, which was significantly ($P < .001$) lower than those seen in both the ADEH$^+$ (1041.5 ± 83.6 kU/L) and ADEH$^-$ (175.3 ± 7.6 kU/L) populations with and without age adjustment. The Phadiatop test was the only RAST assay performed on the CTL group and measures 15 common allergens, covering weeds, grasses, trees, epidermals, mites, and molds, with results reported in kilounits of antibody per liter. The Phadiatop result was positive (>0.35 kU$_A$/L) in 165 (48%) of 346 CTL subjects, with a mean ± SD value in those with positive results of 10.6 ± 17.6 kU$_A$/L.

EH and history of cutaneous infections

ADEH$^+$ subjects more frequently reported a history of cutaneous infections with *S aureus* (78%) and molluscum contagiosum (8%) than either the ADEH$^-$ (29% and 2%, respectively) or CTL (1% and 0%, respectively) populations (Fig 5, *B* and *D*). Human papilloma virus infections were more frequent in both AD subgroups compared with the CTL group, but there was no difference between ADEH$^+$ and ADEH$^-$ subjects (Fig 5, *C*). Approximately 1 year after initiating the registry study, we added a question to the case report form to evaluate subjects' histories of HSV ocular infections. We found that significantly more ADEH$^+$ subjects (16%, $P < .001$) reported a history of ocular infections compared with ADEH$^-$ (1%) and CTL (0%) subjects (Fig 5, *A*). We reviewed subjects' dental histories, focusing on gingivitis, periodontal disease, extractions, root canals, and the number of cavities, and found no significant difference among our 3 groups based on any of these parameters of oral health and hygiene.

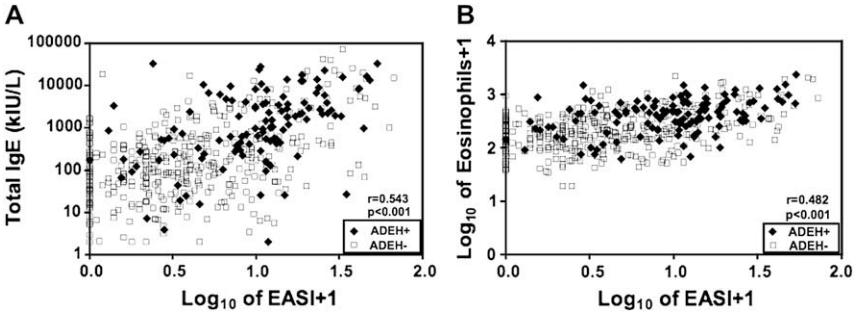

FIG 2. Correlations between the log_{10}-transformed EASI scores and serum IgE levels **(A)** and log_{10}-transformed total eosinophil counts **(B)** in subjects with AD. Fig 2, *A*, n = 117 for $ADEH^+$ subjects *(solid diamonds)* and n = 407 for $ADEH^-$ subjects *(open squares)*. Fig 2, *B*, n = 128 for $ADEH^+$ subjects *(solid diamonds)* and n = 408 for $ADEH^-$ subjects *(open squares)*.

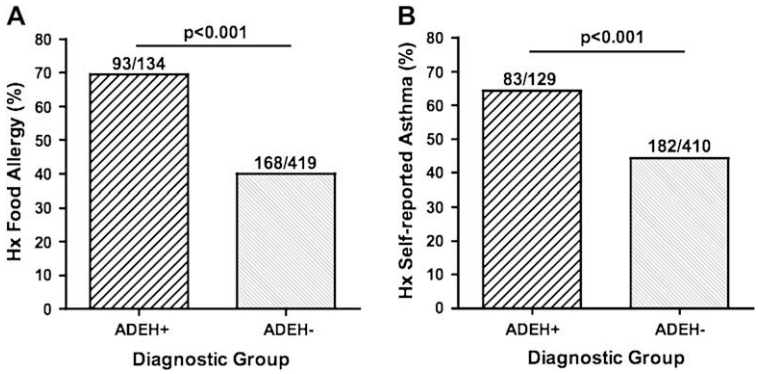

FIG 3. Percentage of subjects with AD or caregivers who self-report a history of or current food allergy or asthma.

EH and HSV serology

A higher proportion of the $ADEH^+$ group had seropositive results for HSV-1 (92.9%) than either $ADEH^-$ (52.1%, $P < .001$) or CTL (54.2%, $P < .001$) subjects. HSV-1 positivity was slightly greater for $ADEH^+$ subjects with more than 1 episode (95.5%) when compared with subjects with 1 episode (81.8%), but this did not reach statistical significance ($P = .178$). The $ADEH^+$ group had a lower proportion of HSV-2–seropositive subjects (8.8%) than either the $ADEH^-$ (36.3%, $P < .001$) or CTL (31.3%, $P < .001$) groups, which likely reflects the differences in the mean age of these groups. For overall HSV status, the $ADEH^+$ group had a higher proportion of subjects that were seropositive to both HSV-1 or HSV-2 (94.7%) than either the $ADEH^-$ (65.9%, $P < .001$) or CTL (66.4%, $P < .001$) groups (see Table E3 in this article's Online Repository at www.jacionline.org). The $ADEH^-$ and CTL groups were not statistically different from each other. Six $ADEH^+$ subjects were not seropositive for either HSV-1 or HSV-2.

HSV-1 or HSV-2 status was also treated as a binary trait and compared in the $ADEH^+$ and $ADEH^-$ groups by using 51 age- and sex-matched pairs and the McNemar test (see Table E4 in this article's Online Repository at www.jacionline.org). There was significant discordance ($P < .0001$) between $ADEH^+$ and $ADEH^-$ members of the pairs with respect to HSV status (including HSV-1, HSV-2, and both HSV-1 and HSV-2).

EH and biomarkers

Little is known about the effect of age and sex on serum levels of CTACK (CCL27) and TARC (CCL17). Therefore we evaluated only age-matched (within 5 years) and gender-matched samples from the AD subgroups. We found that serum levels (mean ± SD) of CTACK (CCL27) were significantly increased in the $ADEH^+$ compared with $ADEH^-$ subjects (1233.0 ± 2298.9 vs 595.2 ± 310.5 pg/mL, respectively; $P = .019$; Fig 6, *A*). Similarly, serum TARC (CCL17) levels were increased in $ADEH^+$ subjects (3211.5 ± 5741.2 vs 805.5 ± 806.4 pg/mL, respectively; $P = .019$; Fig 6, *B*). CTACK and TARC values correlated with measures of AD severity, including EASI ($P < .001$; Fig 6, *C* and *D*) and Rajka-Langeland scores (data not shown). We noted no differences in serum levels of IP-10 (CXCL10; n = 13 per group) and IFN-β (n = 46 per group; see Fig E1 in this article's Online Repository at www.jacionline.org). Only serum IP-10 levels weakly correlated with AD severity, as assessed based on either EASI ($r = 0.22$, $P = .04$) or Rajka-Langeland ($r = 0.24$, $P = .02$) scores (see Fig E1).

DISCUSSION

This is the largest study to date and the only study conducted in the United States to comprehensively characterize subjects with AD who have EH. Our data suggests that $ADEH^+$ subjects have an enhanced susceptibility for infections with microbes that commonly affect the skin and eye. Not surprisingly, almost half of the $ADEH^+$ subjects had a specific IgE to one or more of the *S aureus* toxins (SEA, SEB, or TSST-1) compared with one fifth of the $ADEH^-$ group. This was consistent with our observation that $ADEH^+$ subjects had a higher prevalence of *S aureus* skin infections than $ADEH^-$ subjects. In general, $ADEH^+$ subjects were polysensitized and mounted greater IgE responses per allergen

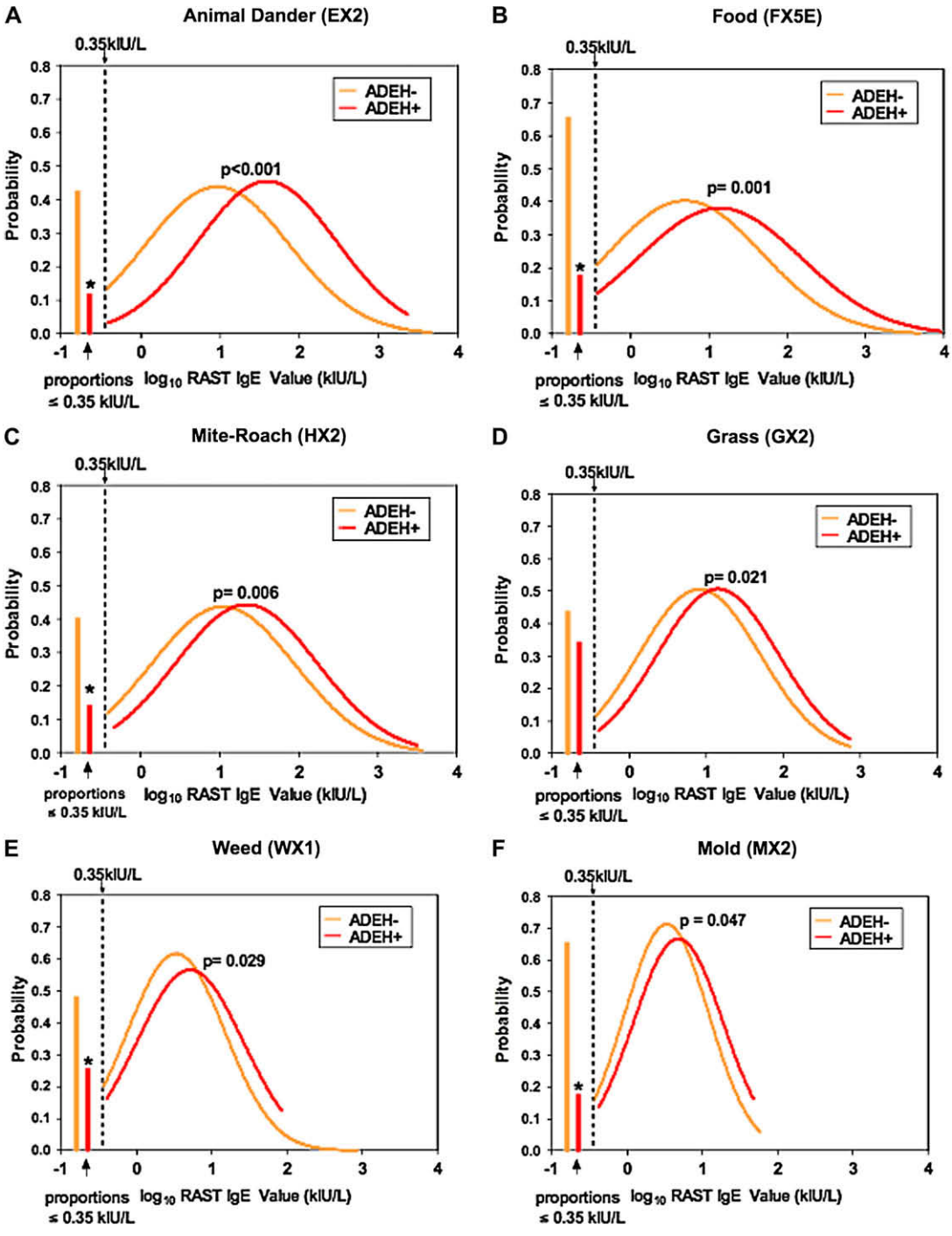

FIG 4. Six allergen-specific ImmunoCAP tests performed on subjects with AD were depicted as a Gaussian distribution with ADEH$^+$ *(red)* curves shifted to the right compared with ADEH$^-$ *(orange)* subjects are shown in **A** through **E**. On the *far left* of each graph is the proportion of ADEH$^-$ *(orange)* and ADEH$^+$ *(red)* subjects with values of 0.35 kU_A/L or less. When ImmunoCAP results (including SEA, SEB, and TSST-1; data not shown) were treated as a binary trait, a greater proportion of ADEH$^+$ subjects had positive results than ADEH$^-$ subjects with the exception of grass (Fig 4, *D*). *$P < .001$ for ADEH$^+$ vs ADEH$^-$ subjects on binary outcome ($\leq$0.35 or >0.35 kU_A/L).

than ADEH$^-$ subjects, which were also reflected in their total IgE levels and the fact that they commonly had other atopic diseases. Based on a current hypothesis that argues that allergen sensitization in subjects with AD occurs primarily through the skin and is enhanced by epidermal barrier defects, our findings strongly implicate epidermal barrier and innate immune defects as risk factors for EH.[10,11] Our study also found that ADEH$^+$ subjects have more severe disease characterized by earlier age of onset. We have strengthened the evidence that subjects with EH have more T_H2-polarized disease (or less T_H1 cytokines) by

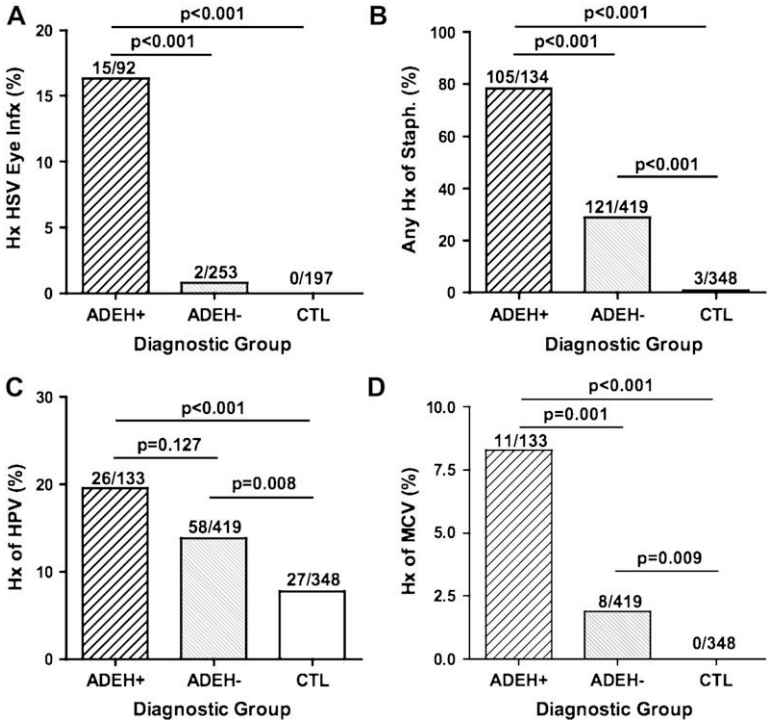

FIG 5. Percentage of subjects (ADEH$^+$, ADEH$^-$, and CTL subjects) who self-report a history of ocular infections with HSV **(A)**, or skin infections with *S aureus* **(B)**, human papilloma virus *(HPV)* **(C)**, or molluscum contagiosum virus *(MCV)* **(D)**.

demonstrating their serum levels of the T_H2 chemokine TARC/CCL17 are higher and their peripheral eosinophilia is greater. The greater T_H2 polarity noted in ADEH$^+$ subjects was also reflected in their greater allergen sensitization.

The demographics of the subgroups (ADEH$^+$, ADEH$^-$, and CTL) revealed significant differences in age and sex (see Table E2). Therefore, where appropriate, we adjusted for age and gender in our analysis (eg, EASI, RL, total IgE level, total eosinophil count, and serum biomarkers). All diagnostic groups had the same age restrictions (1-80 years), although the ADEH$^+$ subjects were significantly younger than the ADEH$^-$ and CTL subjects ($P <$ .001, see Table E2). This is likely the consequence of two factors. The first is that EH episodes typically occur early in life, and therefore it was easier to find the necessary documentation of EH if the subject had experienced this complication more recently (see Table E1). The second factor was that the ADVN genetics study that followed the registry study restricted the age of the ADEH$^-$ and CTL groups to $\geq$ 18 years to provide greater assurance that these populations had been exposed to HSV and to minimize the possibility that the difference between ADEH$^+$ and ADEH$^-$ subjects simply reflected viral exposure. Most ADVN sites tried to enroll subjects in both the registry and genetic studies, and that would result in older subjects in the ADEH$^-$ and CTL groups for both studies (see Table E2). We believe that having recruited older ADEH$^-$ subjects was a strength in that their likelihood of being misclassified would be diminished because most episodes of EH occur within the first 3 decades of life.[5] There were no restrictions on sex, and the ADEH$^+$ group was equally represented by male and female subjects. This result is consistent with previous reports showing no gender bias in EH.[3] It is unclear why ADEH$^-$ subjects were more commonly female ($P <$ .001, see Table E2). The differences in ethnicity and race observed are likely due to the restrictions placed on the ADEH$^-$ and CTL groups that were enacted approximately 1 year after commencement of registry recruitment to ensure that these 2 groups would also qualify for the ADVN genetics study. For both studies, the ADEH$^-$ and CTL subjects had to self-report as non-Hispanic and either African American or European American to meet enrollment criteria. These restrictions were put in place because the ADVN genetics study focused its initial analysis on these ethnic and racial groups to allow for smaller sample sizes while maintaining power to detect differences between ADEH$^+$ and ADEH$^-$ populations.

Our results show that EH recurs in about half of subjects, which is more than was noted in previous publications that reported recurrence in 13% to 16% of cases.[3,5] The mean age of our ADEH$^+$ subjects is comparable with that seen in previous publications, and therefore this is unlikely to explain the difference. About 95% of ADEH$^+$ subjects had a positive serology for HSV-1, HSV-2, or both (see Table E3). The majority (91%) of ADEH$^+$ subjects had positive results for HSV-1, with only 9% having positive results for HSV-2, confirming that most EH episodes are caused by HSV-1. This degree of HSV-1 seroprevalence is markedly higher than seen in the most recent US National Health and Nutrition Examination Survey data, in which the seroprevalence in the 20- to 30-year-old age group is only 52%.[4] This also suggests that EH is not likely due to a diminished immunoglobulin response to HSV and is consistent with a prospective study demonstrating similar T-cell and immunoglobulin responses to a diphtheria–tetanus toxoid immunization in atopic compared with nonatopic subjects.[6] In contrast, only about half of ADEH$^-$ and CTL subjects had positive results for HSV-1, although 31% to 36% had positive results for HSV-2, which likely reflects their older age (36.0 and 38.4 years, respectively). Only 6

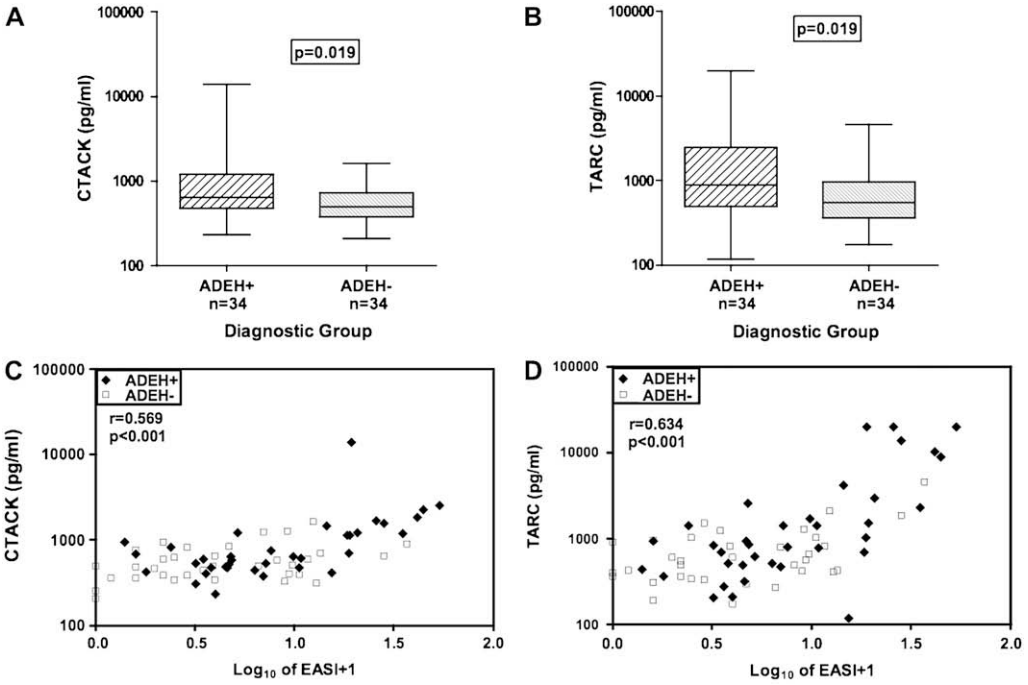

FIG 6. Box plots of serum CTACK (CCL27; **A**) and TARC (CCL17; **B**) levels in age- and sex-matched ADEH$^+$ and ADEH$^-$ cohorts. Correlations between the $\log_{10}$-transformed EASI scores and the serum levels of CTACK **(C)** and TARC **(D)** in subjects with AD are also shown (n = 34 for ADEH$^+$ subjects *[solid diamonds]* and n = 34 for ADEH$^-$ subjects *[open squares]*).

ADEH$^+$ subjects had negative serology to both HSV-1 and HSV-2. Whether this indicates that these subjects have been misclassified as ADEH$^+$ subjects or the fact that some subjects were enrolled during their first EH episode and therefore their IgG response had not yet developed is not known.

One of the more remarkable findings was that ADEH$^+$ subjects also had other cutaneous infections, such as those caused by *S aureus* (78% vs 29%, ADEH$^+$ vs ADEH$^-$ subjects; $P < .001$), molluscum contagiosum (8% vs 2%, $P = .001$), and a history of HSV infection of the eye (16% vs 0.8%, $P < .001$; Fig 5). However, they did not have a greater incidence of dental infections (gingivitis or periodontitis) or skin infections with human papilloma virus. The frequency of *S aureus* infections in ADEH$^+$ subjects is much higher than the 30% previously reported in patients with AD and observed in our ADEH$^-$ subgroup.[12] This work suggests that some global defect in cutaneous immune responses to microbes might be present in subjects with a history of EH that is relevant for both viral and bacterial infections of the skin and possibly the eyes. Interestingly, this susceptibility to *S aureus* infections was also reflected in the frequency of positive ImmunoCAP test results to specific staphylococcal toxins (SEA: 43% vs 19%, ADEH$^+$ vs ADEH$^-$ subjects [$P = .001$]; SEB: 43% vs 20% [$P = .001$]; and TSST-1: 44% vs 21% [$P = .001$]).

Another signature of the ADEH$^+$ subgroup was the breadth and magnitude of their allergen responsiveness. Subjects with AD underwent 8 ImmunoCAP tests to animal dander, food, mite-cockroach, grass, weed, mold, and tree in addition to the *S aureus* toxins. When ImmunoCAP results were evaluated as Gaussian curves, ADEH$^+$ subjects had greater reactivity to 6 of the 8 allergen-specific ImmunoCAP tests compared with ADEH$^-$ subjects (Fig 4). When ImmunoCAP results were analyzed as binary traits (positive vs negative), all allergens were more frequently positive in ADEH$^+$ subjects, except grass (Fig 4). The most significant differences were observed for food and perennial allergens (animal dander and mite-cockroach), suggesting that these allergens would be most predictive of ADEH$^+$ subjects. Importantly, the greater reactivity to food allergens observed in ADEH$^+$ subjects corroborates self-reported histories of food allergy, which were higher in this group (Fig 3). Although this is the most extensive assessment of allergen sensitization in ADEH$^+$ subjects, a smaller study by Peng et al[13] showed similar findings for 5 allergen-specific RASTs.

The greater allergen sensitization observed in ADEH$^+$ subjects likely reflects greater T_H2 polarity. To address this, we evaluated several T_H2 biomarkers, including total IgE level, eosinophil count, and TARC/CCL17 level (Figs 1 and 6).[14,15] TARC is a T_H2 chemokine that binds to CCR4, which is highly expressed on skin-homing lymphocytes. Subjects with AD express high levels of TARC in lesional skin, and serum levels can reach the nanogram per milliliter range, as was the case in our subjects.[16,17] CTACK/CCL27 plays a role in the homeostatic migration of memory T cells to the skin, but CTACK is not selective for a T-cell subset because serum levels are increased in both subjects with AD and subjects with psoriasis. But CTACK levels have only been shown to correlate with disease severity in subjects with AD, as was the case in our subjects (Fig 6).[18,19] Levels of all 3 T_H2 biomarkers were increased in ADEH$^+$ subjects compared with those seen in ADEH$^-$ subjects ($P \leq .02$), firmly establishing the importance of T_H2 cytokines as a risk factor for widespread HSV infections in subjects with AD. Wollenberg et al[3] demonstrated that high IgE levels were a risk factor for EH among 45 ADEH$^+$ subjects. Furthermore, the strong correlation between total IgE level, eosinophilia, and TARC level with disease severity suggests that

the degree of T_H2 polarization is an important predictor of AD disease activity.

We found a history of food allergy and asthma was more frequently elicited from $ADEH^+$ subjects (69.4% and 64.3%, respectively) than from $ADEH^-$ subjects (40.1% and 44.4%, respectively; $P < .001$; Fig 3). Wollenberg et al[3] noted a similar trend with greater reports of asthma and hay fever in subjects with EH, but these were not statistically different from results in their control AD population. The food allergy prevalence of our subjects with AD (40% to 69%) was higher than in previous reports, which estimate IgE-mediated food allergy prevalence in children with moderate-to-severe AD to be about 30%.[20] It is important to note that historical accounts of food allergy significantly overestimate the true prevalence, sometimes by as much as 2- to 3-fold.[21] Nevertheless, we had about 75% concordance with a history of food allergy and food FX5E ImmunoCAP results (as a binary trait). Asthma prevalence in this ADVN group is also higher than in the general US population, where US prevalence estimates from 1995 were 5.7% with slightly greater values for children than adults and higher among African Americans compared with European Americans. Asthma prevalence in children with AD is estimated to be about 25% to 30%, which is less than what we observed in the ADVN AD subgroups.[22] This difference might reflect a recall bias or might suggest that the subjects with AD recruited from tertiary referral centers have more severe disease that is more frequently complicated by reactive airways. In summary, $ADEH^+$ subjects are more likely to have other atopic diseases than $ADEH^-$ subjects.

To evaluate whether EH susceptibility could be related to a relative reduction in T_H1-associated cytokines, we measured IFN-β and the interferon-induced chemokine IP-10 in age- and gender-matched serum samples (see Fig E1). IFN-β values were highly variable, but no difference was observed between the AD subgroups. Similarly, there was no difference in IP-10 levels between the AD subgroups. Peng et al[13] found that IFN-β levels were reduced in $ADEH^+$ subjects compared with those seen in $ADEH^-$ subjects using a similar sample size. They did not find any differences in serum IFN-α or IFN-γ levels between the AD subgroups. These findings would suggest that the T-cell defect in $ADEH^+$ subjects is primarily the enhanced expression of T_H2 cytokines and not diminished T_H1 cytokines. T_H2 cytokines are thought to be permissive to microbial invasion on the basis of their inhibitory actions on antimicrobial proteins, epidermal barrier proteins, and cell-mediated immunity.[23-31]

Our study confirmed and extended the finding that EH develops in subjects with AD with greater disease severity. We found that the vast majority (94%) of $ADEH^+$ subjects had AD before 5 years of age. Multiple markers of AD severity, including biomarkers (total IgE levels, peripheral eosinophil counts, and TARC and CTACK levels), 2 well-accepted clinical scoring systems (EASI and Rajka and Langeland), and body surface area affected, were all significantly greater in $ADEH^+$ subjects. Most of the biomarkers are thought to dynamically reflect disease severity with the exception of total IgE, which, because of its long half-life, is reflective of chronic changes in disease severity. Importantly, these observations were evident even after controlling for age and gender (total IgE levels, eosinophil counts, and TARC and CTACK levels). Two previous publications have noted the association with early age of onset and IgE levels.[3,13] Peng et al[13] demonstrated that subjects with AD with a history of EH had a slightly increased severity score by using the SCORAD assessment ($P < .05$). In our study we found that a number of the biomarkers, such as TARC levels, CTACK levels, total IgE levels, eosinophil counts, and IP-10 levels, correlated significantly with EASI scores and are listed in order of the strength of this correlation (Figs 2 and 6 and Fig E1).

Landmark studies have demonstrated that subjects with AD, particularly those with more severe disease, might have a loss-of-function mutation in the filaggrin gene, as has been observed in patients with ichthyosis vulgaris (IV).[32] For this reason, we asked subjects or their caregivers if they had a history of any of the features found in subjects with both IV and AD (eg, keratosis pilaris, hyperlinear palms, or ichthyosis). More $ADEH^+$ subjects (58%) reported having 1 or more of these features than $ADEH^-$ subjects (42%, $P < .005$). Recent studies suggest that IV, diagnosed based on ichthyotic changes on the anterior tibial region, can be observed in up to 32% of subjects with AD.[33] Although keratosis pilaris and hyperlinear palms are less specific for IV, they are more commonly observed in subjects with AD/IV (53% and 81%, respectively) than in subjects with AD without IV (28% and 43%, $P < .001$).[33]

Finally, we measured serum total IgE levels and performed a multiallergen ImmunoCAP assay called the Phadiatop test on our CTL group to provide some measure of the allergen sensitization and T_H2 polarity of this group that had no personal or family history of atopic disorders (see Table E1). Although total IgE levels were within age-specific normal values and substantially lower than the values seen in both AD subgroups ($P < .001$), 48% of CTL subjects had a positive Phadiatop result. We did not perform a Phadiatop test on subjects with AD, and therefore we cannot make direct comparisons with other registry groups. This percentage was higher than that reported in a large Italian and Swiss population in which the prevalence of positive Phadiatop results ranged from 24% to 29%, respectively.[34,35] Nevertheless, the National Health and Nutrition Examination Survey III demonstrated that more than 50% of the population has a positive skin test response to at least 1 allergen.[36] Our findings agree with previous literature suggesting that total serum IgE levels are a more sensitive screening assay for atopic diseases in adults than the Phadiatop test.[37]

In conclusion, we have found that subjects with AD who are susceptible to EH are characterized by more severe disease, early age of onset, more frequent history of other atopic disorders, greater T_H2 polarity, allergen sensitization to many common allergens, and more frequent skin infections with other microbes. Collectively, this provides a reasonable snapshot of the at-risk subject with AD and might help identify individuals who are at greatest risk for more life-threatening infections with vaccinia (EV) or variola (smallpox). One of the most profound findings is the remarkably high rate of skin infections with *S aureus* reported by $ADEH^+$ subjects. Further work is warranted to identify additional biomarkers that can be assessed rapidly and that will be both sensitive and specific for $ADEH^+$ subjects.

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Clinical implications: AD subjects with a history of EH have greater allergen sensitization and more frequent history of skin infections with other microbes than AD subjects who have not had EH.

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METHODS

ADVN structure

The ADVN is composed of 3 distinct groups supported by 3 separate National Institutes of Health contracts: the Clinical Study Consortium (CSC; contract NO1 AI 40029), the Statistical and Data Coordinating Center (SDCC; contract NO1 AI 40033), and the Animal Study Consortium (ASC; contract NO1 AI 40030). These groups work closely together to achieve the common goal of the ADVN, which is to investigate the mechanism or mechanisms responsible for the susceptibility of subjects with AD to viral infections. The central hypothesis of the CSC studies is that subjects with AD who are susceptible to generalized cutaneous infections with HSV or who have complications after smallpox (or possibly other live-attenuated vaccinations [yellow fever vaccine]) might have common immunologic defects that could be reflected in serum biomarkers or phenotypes identified by means of an extensive history and physical examination. The role of the ASC is to characterize mouse models of EV and define cellular and molecular immune mechanisms that give rise to EV. The SDCC is managed by Rho, Inc, under the leadership of Drs Susan Lieff and Gloria David and helps with development of clinical protocols; coordination of local institutional review board and Division of Allergy, Immunology, and Transplantation at the NIAID branch (DAIT) protocol and consent form approval; coordination of study activities; study-specific training; data collection; maintenance of a clinical database; sample tracking; statistical data analysis; and coordination of activities of both the ASC and CSC. For example, regulatory documents (informed consents and advertisements) were submitted to the SDCC followed by the DAIT division of the NIAID for review before local institutional review board submission. Institutional review board approvals were forwarded to the SDCC for central tracking of all regulatory documentation. Each site had a site-initiation visit before enrolling a subject, as well as an interim monitoring visit shortly after the start of enrollment and at least once a year thereafter to ensure data quality assurance and quality control.

Overview of ADVN registry and biomarker study

The purpose of the ADVN registry and biomarker study is to establish a cohort of well-characterized subjects with AD (with and without a history of viral infections, such as EH, EV, and molluscum contagiosum) and nonatopic CTL subjects to determine whether the biomarkers reflective of disease severity or T_H cell polarity predict susceptibility to cutaneous viral infections. A comprehensive database of clinical and diagnostic information was obtained from each subject to determine whether other clinical associations can be made between these measurements and viral susceptibility. The registry study also provides for a repository of biologic samples (serum and plasma). Subjects enrolled in the registry study also provide a pool of subjects for enrollment in other ADVN protocols, ensuring that the same diagnostic criteria are used and the same data elements are collected for all subjects enrolled in any ADVN study.

Exclusion criteria

Subjects were excluded if they had any systemic illness other than an atopic disease, such as autoimmune diseases, immunodeficiencies, active systemic malignancies (excluding nonmelanoma skin cancer), and any skin diseases that might compromise the skin barrier. $ADEH^-$ and CTL subjects were excluded if they did not self-report their ethnicity and race as either non-Hispanic African American or non-Hispanic European American after a 06.07 amendment was approved by all local institutional review boards. This limitation was imposed to enable more of the registry subjects to qualify for the ADVN genetics study, in which the comparison groups ($ADEH^+$, $ADEH^-$, and CTL subjects) were similarly restricted in ethnicity and race, which provides greater power to detect genetic differences between diagnostic groups. Because $ADEH^+$ subjects were very difficult to find, we only imposed this racial and ethnic restriction for the genetics study and not the registry study.

Human subjects

A certificate of confidentiality was obtained from the National Institutes of Health to ensure that subject confidentiality was maintained by all ADVN investigators and their associates. The study was approved by the institutional review boards at National Jewish Health, the University of California San Diego, Children's Hospital of Boston, the University of Rochester Medical Center, Oregon Health and Science University, Johns Hopkins Medical Institution, and Children's Memorial Hospital, and all subjects provided written informed consent before participation in these studies.

Study procedures

All study participants were evaluated with a detailed history (by the coordinator) and physical examination and disease severity assessments (by the physician/investigator) to assess personal and family histories of allergic diseases, other dermatologic diseases, cutaneous infections, treatment and medication history, evidence of immunodeficiency, and AD severity.

Each subject provided a blood sample as part of their enrollment into the registry study. Subjects younger than 2 years of age and with a weight of greater than or equal to 6 kg had up to 12 mL of blood drawn. Subjects younger than 2 years of age with a weight of less than 6 kg had up to 9 mL of blood drawn. Subjects 2 years of age and older had 30 mL of blood drawn. Blood sample information was entered into the Web-based sample tracking system called RhoLAB. This system monitored the shipment and receipt of biologic samples to the central JHAAC laboratory. One purple top was sent directly to Quest Diagnostics Laboratory for a CBC with differential. All remaining blood collected was processed for serum and/or plasma separation, placed in aliquots, and stored at $-20^{\circ}C$ or less at the ADVN site and shipped monthly to JHAAC, where the samples were acknowledged in the RhoLAB system and then entered into the JHAAC Biotracking System. Each ADVN site signed a master material transfer agreement, and an electronic material transfer agreement letter was signed by the sending and receiving principal investigator for each shipment received at JHAAC.

One serum sample was sent to the DACI at JHAAC for a serum total IgE measurement, multiallergen RASTs, and individual or mixed allergen RASTs. All remaining serum and plasma samples were then transferred on a monthly basis to URMC for long-term storage at $-80^{\circ}C$ and targeted biomarker analysis. The SDCC performed query checks on all data entries between RhoEDC, RhoLAB, and central laboratory data to ensure all systems matched and were accurate. Any discrepancies resulted in a query to the site, central laboratory, or both.

Data management

All phenotype data were captured during the study by visits using a standardized case report form and were then transferred into an electronic data capture system. The RhoEDC system is an Internet-based remote data entry system developed by Rho, Inc, in which a browser is used to key data into electronic case report forms. This system ensures that clinical data are not stored on the computers at an ADVN site. At the end of each "page," data are submitted to the ADVN secure Web server by using secure sockets link 128-byte public key encryption methodology and stored in the study's "operational database." The database is backed up nightly, and backup tapes are saved in a secure offsite location. Multivariate data validation tests are performed, and monthly query spreadsheets are issued to sites for resolution.

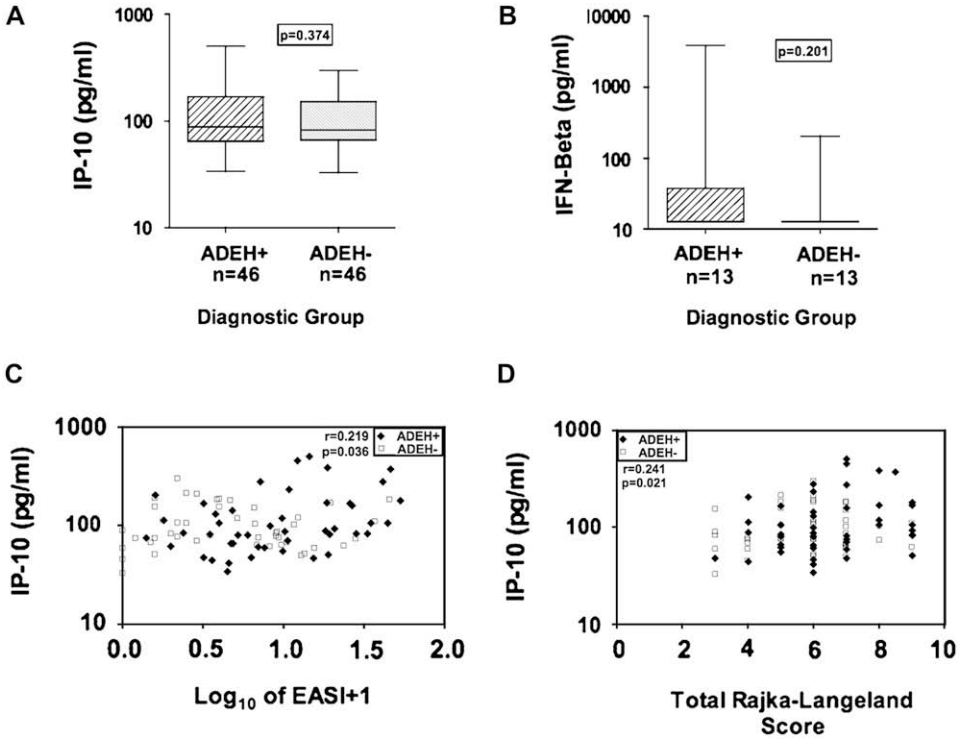

FIG E1. Box plots of serum IP-10 (CXCL10; **A**) and IFN-β **(B)** levels in age- and gender-matched $ADEH^+$ and $ADEH^-$ cohorts. The correlation between the log_{10}-transformed EASI scores and the serum levels of IP-10 are shown **(C)**. Correlation between Rajka-Langeland scores and serum IP-10 levels are also shown **(D)**. Fig E1, *C* and *D*, n = 46 for $ADEH^+$ subjects *(solid diamonds)* and n = 46 for $ADEH^-$ subjects *(open squares)*. IFN-β levels did not correlate with either AD severity scoring system (data not shown).

TABLE E1. ADVN – Standardized diagnostic criteria

Term	Diagnosis Requirements
Active Atopic Dermatitis (AD)	• Subjects *must have* within the last 3 months according to medical records, or based on a careful and credible history (provided by the subject, caregiver, parent, or guardian), or by physical exam by an ADVN investigator:
	1. Pruritus
	2. Eczema (acute, subacute, chronic)
	a. Typical morphology and age-specific patterns*
	b. Chronic or relapsing history
	• Most subjects *will have* (seen in most cases, adding support to the diagnosis):
	1. Early age at onset
	2. Atopy
	a. Personal and/or family history
	b. IgE reactivity
	3. Xerosis
	• Subjects *may have* (these clinical associations help to suggest the diagnosis of AD but are too nonspecific for defining or detecting AD for research or epidemiological studies):
	1. Atypical vascular responses (e.g., facial pallor, white dermographism, delayed blanch response)
	2. Keratosis pilaris/hyperlinear palms/ichthyosis
	3. Ocular/periorbital changes
	4. Other regional findings (e.g., perioral changes/periauricular lesions)
	5. Perifollicular accentuation/lichenification/prurigo lesions
	*Patterns include: (1) facial, neck, and extensor involvement in infants and children; (2) current or prior flexural lesions in any age group; and (3) sparing groin and axillary regions.
Inactive Atopic Dermatitis (AD)	• Absence of active Atopic Dermatitis in the last 12 months (as defined above). Recruiting children with other eczematous disorders that mimic AD.
Atopic Dermatitis and *active* Eczema Herpeticum or a *history of* Eczema Herpeticum (ADEH+)	• Subjects *must have* either a definitive diagnosis of AD1 (as described above) which can be active or inactive at the time of enrollment. In children less than 4 yrs of age the disease must be present for at least six months to minimize the likelihood of recruiting children with other eczematous disorders that mimic AD or
	• A minimum of one EH episode documented by at least one of the following:
	1. ADVN Investigator's clinical exam (or another physician affiliated with the same Academic Center as the ADVN Investigator) consistent with EH (vesicles, pustules, erosions, or crusts) that has involved an area that has a diameter greater than or equal to 5cm in size. The size of the eruption can be verified by direct documentation of eruption or estimated from the anatomic sites involved at the time of the eruption (based on the clinical note). Additionally, the size requirement can also be verified by a credible history obtained from the subject, caregiver, parent, or guardian's description of the extent of the lesions. Oral mucosal involvement should not be included in size determination to exclude cases of primary herpes infection with oral involvement.
	2. Clinical exam by a referring outside provider with a documented exam consistent with EH (vesicles, pustules, erosions, or crusts) that has an involved area diameter greater than 5cm in size AND one of the following:
	a) Documentation of a positive test for HSV confirmed by culture,
	b) Documentation of a positive test for HSV confirmed by PCR,
	c) Documentation of a positive test for HSV confirmed by immunofluorescence,
	d) Documentation of a positive test for HSV confirmed by Tzanck smear, or
	e) Documentation by providers' notes or subjects' (guardians') account that skin lesions responded rapidly to oral or intravenous antiviral agents (valacyclovir, famcyclovir or acyclovir). Rapid response will be defined as crusting of vesicles and no new vesicles within 24 to 48 of initiation of antiviral agents. If subject was also on oral antibiotics this criteria (e) cannot be used to fulfill the diagnosis of ADEH+.
	The size of the eruption can be verified by direct documentation of eruption size or estimated from the anatomic sites involved at the time of the eruption (based on the clinical note). Additionally, the size requirement can also be verified by a credible history obtained from the subject's description of the extent of the lesions. Oral mucosal involvement should not be included in size determination to exclude cases of primary herpes infection with oral involvement.

(*Continued*)

TABLE E1. *(Continued)*

Term	Diagnosis Requirements
Atopic Dermatitis with no history of Eczema Herpeticum (ADEH-)	Subjects *must have*:
	1. Either a definitive diagnosis of AD1 (as defined above) which can be active or inactive at the time of enrollment. (In children less than 4 yrs of age the disease must be present for at least six months to minimize the likelihood of recruiting children with other eczematous disorders that mimic AD).
	2. No history of EH as determined by subject, caregiver, parent, or guardian interview. Subjects will be provided with a description of the clinical manifestations of EH and will be shown representative photos. The description will be as follows:
	''Eczema herpeticum, or EH, represents widespread herpes simplex infection of the skin in subjects with atopic dermatitis. Eczema herpeticum is also known as Kaposi's Varicelliform Eruption. The rash develops as clusters of small fluid-filled blisters which appear on abnormal or even apparently normal or unaffected skin. The rash then spreads over the following 7 to 10 days, and small fluid-filled blisters evolve into discrete ''punched-out'' small sores. Typically, subjects will have a low grade fever, feel a little tired, and sometimes have swollen lymph nodes or glands. The rash usually lasts 2 to 6 weeks. The usual treatment for EH is an oral antiviral pill (such as acyclovir or Zovirax [5 times per day], valacyclovir or Valtrex [2 times per day], or famciclovir or Famvir [2 times per day]). The first episode of EH typically develops in subjects under the age of 30 years. About 80% of subjects will experience more than one episode of EH.''
Non-Atopic Control (CTL)	Subjects *must have*:
	1. No personal or family history or current manifestations of food allergy, AD, asthma, or allergic rhinitis.
	2. No history of or clinical evidence of a chronic, inflammatory skin disease as determined through subject interview using the following questions.
	a. Has a doctor ever diagnosed you (or your child) as having: Eczema (Atopic Dermatitis)___ Asthma___ Hayfever___ Food allergy in infancy___?
	b. Do you (or your child) currently have eczema (an itchy skin condition)? Yes No Unknown
	c. Do you (or your child) have or have you (or your child) ever had itchy rashes in the folds of your elbows or knees? Yes No Unknown
	d. Did you (or your child) have itchy red rashes on your cheeks and neck as a baby? Yes No Unknown
	e. Have you (or your child) ever had itchy rashes that come and go over time, but usually last more than 2 weeks? Yes No Unknown
	f. Have you (or your child) ever seen a doctor for skin problems? Yes No Unknown
	g. Have you (or your child) ever been given a prescription for skin lotion, cream, or ointment? Yes No Unknown

TABLE E2. ADVN registry study demographics

End point	Statistic	ADEH$^+$ subjects	ADEH$^-$ subjects	CTL subjects	Comparison	*P* value
Age (y)	No.	134	419	348	Overall test	<.001
	Mean (SD)	21.54 (20.4)	32.84 (15.3)	38.34 (12.4)	ADEH$^+$ vs ADEH$^-$	<.001
	Median	11.9	31.1	37	ADEH$^+$ vs CTL	<.001
	Minimum-maximum	1-80.7	1.2-73.6	12.4-78.1	ADEH$^-$ vs CTL	<.001
Female	No. (%)	67 (50)	286 (68.3)	189 (54.3)	Overall test	<.001
Male	No. (%)	67 (50)	133 (31.7)	159 (45.7)	ADEH$^+$ vs ADEH$^-$	<.001
					ADEH$^+$ vs CTL	.416
					ADEH$^-$ vs CTL	<.001
Hispanic or Latino	No. (%)	7 (5.2)	4 (1.0)	4 (1.2)	Overall test	.002
Not Hispanic or Latino	No. (%)	127 (94.8)	415 (99.0)	344 (98.8)	ADEH$^+$ vs ADEH$^-$	.006
					ADEH$^+$ vs CTL	.013
					ADEH$^-$ vs CTL	.999
African American	No. (%)	21 (15.7)	192 (45.8)	161 (46.3)	Overall test	<.001
European American	No. (%)	99 (73.9)	217 (51.8)	186 (53.4)	ADEH$^+$ vs ADEH$^-$	<.001
Other	No. (%)	14 (10.4)	10 (2.4)	1 (0.3)	ADEH$^+$ vs CTL	<.001
					ADEH$^-$ vs CTL	.051

TABLE E3. ADVN registry HSV serology results

	ADEH$^+$ subjects	ADEH$^-$ subjects	CTL subjects	Comparison	*P* value
Age (y)					
No.	113	355	336	Overall test	<.001
Mean (SD)	22.7 (20.9)	36 (13.1)	38.4 (12.2)	ADEH$^+$ vs ADEH$^-$	<.001
Median	14.2	33.9	37.2	ADEH$^+$ vs CTL	<.001
Minimum-maximum	1.0-80.7	3.3-73.6	18.5-78.1	ADEH$^-$ vs CTL	.026
Sex, no. (%)					
Female	57 (50.4)	255 (71.8)	183 (54.5)	Overall test	<.001
Male	56 (49.6)	100 (28.2)	153 (45.5)	ADEH$^+$ vs ADEH$^-$	<.001
				ADEH$^+$ vs CTL	.513
				ADEH$^-$ vs CTL	<.001
Ethnicity, no. (%)					
Hispanic or Latino	2 (1.8)	2 (0.6)	3 (0.9)	Overall test	.485
Not Hispanic or Latino	111 (98.2)	353 (99.4)	333 (99.1)	ADEH$^+$ vs ADEH$^-$	.247
				ADEH$^+$ vs CTL	.604
				ADEH$^-$ vs CTL	.678
Race, no. (%)					
African American	19 (16.8)	166 (46.8)	158 (47.0)	Overall test	<.001
White	91 (80.5)	188 (53.0)	178 (53.0)	ADEH$^+$ vs ADEH$^-$	<.001
Other	3 (2.7)	1 (0.3)	0 (0.0)	ADEH$^+$ vs CTL	<.001
				ADEH$^-$ vs CTL	.622
HSV-1, no. (%)					
Negative	8 (7.1)	170 (47.9)	154 (45.8)	Overall test	<.001
Positive	105 (92.9)	185 (52.1)	182 (54.2)	ADEH$^+$ vs ADEH$^-$	<.001
				ADEH$^+$ vs CTL	<.001
				ADEH$^-$ vs CTL	.594
HSV-2, no. (%)					
Negative	103 (91.2)	226 (63.7)	231 (68.7)	Overall test	<.001
Positive	10 (8.8)	129 (36.3)	105 (31.3)	ADEH$^+$ vs ADEH$^-$	<.001
				ADEH$^+$ vs CTL	<.001
				ADEH$^-$ vs CTL	0.172
Overall HSV status, no. (%)					
Negative	6 (5.3)	121 (34.1)	113 (33.6)	Overall test	<.001
Positive	107 (94.7)	234 (65.9)	223 (66.4)	ADEH$^+$ vs ADEH$^-$	<.001
				ADEH$^+$ vs CTL	<.001
				ADEH$^-$ vs CTL	.936

TABLE E4. ADVN registry HSV serology as a binary trait

End point	Group	Control: negative	Control: positive	*P* value*
HSV-1 titer	Case: negative	0 (0.0%)	3 (11.5%)	<.001
	Case: positive	26 (100.0%)	23 (88.5%)	
HSV-2 titer	Case: negative	33 (86.8%)	12 (85.7%)	.090
	Case: positive	5 (13.2%)	2 (14.3%)	
Overall HSV status	Case: negative	0 (0.0%)	1 (3.1%)	<.001
	Case: positive	20 (100.0%)	31 (96.9%)	

*P values result from the McNemar test of agreement.