

Transcutaneous yellow fever vaccination of subjects with or without atopic dermatitis

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Background: Atopic dermatitis (AD) is a common inflammatory skin disease with a global prevalence ranging from 3% to 20%. Patients with AD have an increased risk for complications after viral infection (eg, herpes simplex virus), and vaccination of patients with AD with live vaccinia virus is contraindicated because of a heightened risk of eczema vaccinatum, a rare but potentially lethal complication associated with smallpox vaccination.

Objective: We sought to develop a better understanding of immunity to cutaneous viral infection in patients with AD.

Methods: In a double-blind randomized study we investigated the safety and immunogenicity of live attenuated yellow fever

virus (YFV) vaccination of nonatopic subjects and patients with AD after standard subcutaneous inoculation or transcutaneous vaccination administered with a bifurcated needle. Viremia, neutralizing antibody, and antiviral T-cell responses were analyzed for up to 30 days after vaccination.

Results: YFV vaccination administered through either route was well tolerated. Subcutaneous vaccination resulted in higher seroconversion rates than transcutaneous vaccination but elicited similar antiviral antibody levels and T-cell responses in both the nonatopic and AD groups. After transcutaneous vaccination, both groups mounted similar neutralizing antibody responses, but patients with AD demonstrated lower antiviral T-cell responses by 30 days after vaccination. Among transcutaneously vaccinated subjects, a significant inverse correlation between baseline IgE levels and the magnitude of antiviral antibody and CD4⁺ T-cell responses was observed. **Conclusions:** YFV vaccination of patients with AD through the transcutaneous route revealed that high baseline IgE levels provide a potential biomarker for predicting reduced virus-specific immune memory after transcutaneous infection with a live virus. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Yellow fever virus, antibody, T-cell memory, IgE, atopic dermatitis

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Atopic dermatitis (AD) is a common inflammatory skin disease¹ that causes substantial morbidity, with costs of therapy, loss of work, and disability estimated at \$1 to \$4 billion per year in the United States.² Clinical observations indicate that patients with AD have more severe cutaneous viral infections, including herpes simplex virus and molluscum contagiosum.³ Moreover, AD is a formal contraindication for smallpox vaccination because of the risk of eczema vaccinatum, a rare but potentially life-threatening disease.^{4,5} On the basis of recommendations that patients with AD or those who have family members with AD not be exposed to vaccinia virus, greater than 30% of US military personnel refrained from smallpox vaccination in 2002.⁶

Little is known about the immune deficit predisposing patients with AD to viral infections, but we anticipated that immune defects might occur in the skin. The goal of this study was to vaccinate patients with AD with an attenuated live virus vaccine to assess clinical and immunologic deficits that might explain difficulties patients have with controlling viral infections. To better understand cutaneous and systemic antiviral immunity of patients with AD, we examined immune responses after vaccination with the US Food and Drug Administration–approved live-attenuated yellow fever-17D (YFV-17D) vaccine. Unlike most live virus vaccines, YFV-17D vaccination/infection

Abbreviations used

AD: Atopic dermatitis
 AE: Adverse event
 LNI: Log neutralizing index
 NT₅₀: Neutralizing titer 50
 YFV: Yellow fever virus

can be performed through either subcutaneous vaccination (thus bypassing the skin) or transcutaneous vaccination performed similarly to traditional smallpox vaccination. Transcutaneous vaccination (referred to as “scarification”) was practiced in Africa during the 1950s, and studies of mass vaccinations totaling greater than 130,000 recipients documented that transcutaneous vaccination with YFV-17D was safe and immunogenic.⁷⁻⁹ Also, from a public health cost perspective, our studies indicate that a single 0.5-mL subcutaneous dose of YFV-17D could be reconstituted in a small volume to provide up to 50 doses when administered through the transcutaneous route. This report documents a randomized, double-blind multicenter study of YFV-17D vaccination administered through either the transcutaneous or subcutaneous routes in patients with AD and nonatopic control subjects.

METHODS**Study design**

The protocol design was a randomized, double-blind, multicenter pilot study performed in the United States (NCT00723489: Immune response to yellow fever vaccination in adults with atopic dermatitis). From September 2008 through March 2011, we enrolled 82 subjects 27 to 43 years of age who were evenly divided into 4 groups: patients with AD and nonatopic control subjects who received subcutaneous or transcutaneous vaccination with YFV-17D. This age range was selected because there had been no cases of viscerotropic disease from YFV-17D vaccination in that age range.¹⁰ Subjects were informed of the risks involved with YFV vaccination, including the risk of YFV-associated viscerotropic disease. Because of the potential risk for YFV-associated viscerotropic disease, enrollment was divided into 3 stages, with interim safety analysis performed by an independent National Institute of Allergy and Infectious Diseases Data and Safety Monitoring Board after each stage. In the first stage enrollment was limited to nonatopic subjects ($n = 10$) and patients with mild AD ($n = 10$). In stage 2 enrollment was limited to nonatopic subjects ($n = 15$) and patients with moderate AD ($n = 15$). In stage 3 enrollment was limited to nonatopic subjects ($n = 15$) and patients with moderate and severe AD ($n = 15$). Subjects were enrolled at 3 sites: Oregon Health & Science University, National Jewish Health, and the University of California San Diego. Subjects were required to be off systemic corticosteroids and other immunosuppressive medications for 30 days and topical corticosteroids/calcineurin inhibitors for 1 week before vaccination. Inhaled steroids were restricted to no more than 440 $\mu\text{g}/\text{d}$ within 6 months before vaccination. Subjects provided written informed consent, and the studies were conducted according to the principles of the Declaration of Helsinki and approved by institutional review boards at each center.

Study groups and vaccinations

Subjects with normal clinical laboratory test results and no history of flavivirus vaccination or infection who had not traveled to Africa or South America were recruited for the study. Subjects with egg allergy or acute hypersensitivity to vaccine components were excluded. Women were required to have a negative pregnancy test result. Subjects were vaccinated with YFV-17D (YF-VAX; Sanofi Pasteur, Swiftwater, Pa) through the standard subcutaneous route or the experimental transcutaneous route on nonlesional

skin on the right or left deltoid or thigh. Transcutaneous vaccination consisted of performing 5 to 15 jabs with a bifurcated needle using 5-fold concentrated vaccine (ie, reconstituted in one fifth standard volume), which is estimated to be equivalent to approximately 1×10^3 plaque-forming units and similar to the lowest effective dose used in previous trials.⁸ By using a double-dummy design, each subject received subcutaneous vaccination on one arm/thigh and transcutaneous vaccination on the contralateral arm/thigh with one inoculum containing YFV-17D and one containing placebo (vaccine diluent). Transcutaneous vaccination sites were covered with an adhesive patch for 2 days, and the site was swabbed at day 3 after vaccination to assess viral shedding.

Primary outcome: Neutralizing antibody responses

YFV-specific neutralizing antibody levels were measured by using 2 approaches: the log₁₀ neutralization index (LNI), which refers to a constant serum-varying virus reduction test,¹¹ and the neutralizing titer 50 (NT₅₀), which refers to a constant virus-varying serum reduction test.¹²

Secondary outcome: YFV viremia and RNAemia

Infectious YFV was measured by using a plaque assay, and YFV RNA was isolated from 500 μL of serum obtained at 3 to 4, 5 to 6, 7 to 8, 10 to 11, 13 to 15, and 28 to 35 days after vaccination.¹³ Although quantitative real-time PCR was not performed, this was not necessary to determine the duration of RNAemia.

Secondary outcome: YFV-specific T-cell quantitation

Intracellular cytokine staining was used to measure T-cell responses¹⁴ by using YFV-17D purified by means of ultracentrifugation through 25% glycerol. PBMCs with or without YFV were cultured for 18 hours, with brefeldin A added for the last 6 hours to block cytokine secretion, and cells were stained and analyzed on an LSR Fortessa.

Serum IgE

Total serum IgE levels were measured by using the ImmunoCAP System (Phadia/Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's directions.

Randomization and masking

Subjects were randomized to receive YFV through subcutaneous or transcutaneous administration in a 1:1 ratio. Randomization took place according to a fixed schedule by using a permuted block design. The randomization schedule was stratified by disease classification in study stage 1 (block sizes of 4 and 2) and by disease classification and sex in study stages 2 and 3 (block size of 2). Subjects were randomized through a centralized, automated, Web-based randomization system that distributed subject vaccination route assignments on an individual basis after entry of stratification information by the site study coordinator. Site pharmacy personnel responsible for preparing the vaccine and vaccine administrators were unblinded, but all other study personnel, including the subjects, physicians, and laboratory staff, were blinded to vaccine designation.

Statistical methods

Nonparametric survival curves for the duration of detectable YFV were estimated based on maximum likelihood for interval-censored data, and differences in viral clearance rates between patients with AD and nonatopic control subjects were compared by using generalized log-rank tests.¹⁵ Mean day 30 antibody levels (log₁₀ neutralization index and log₁₀ transformed NT₅₀ levels) and seroconversion rates between patients with AD and nonatopic control subjects were compared by using 2-independent-sample t tests and Fisher exact tests, respectively. Longitudinal cytokine responses

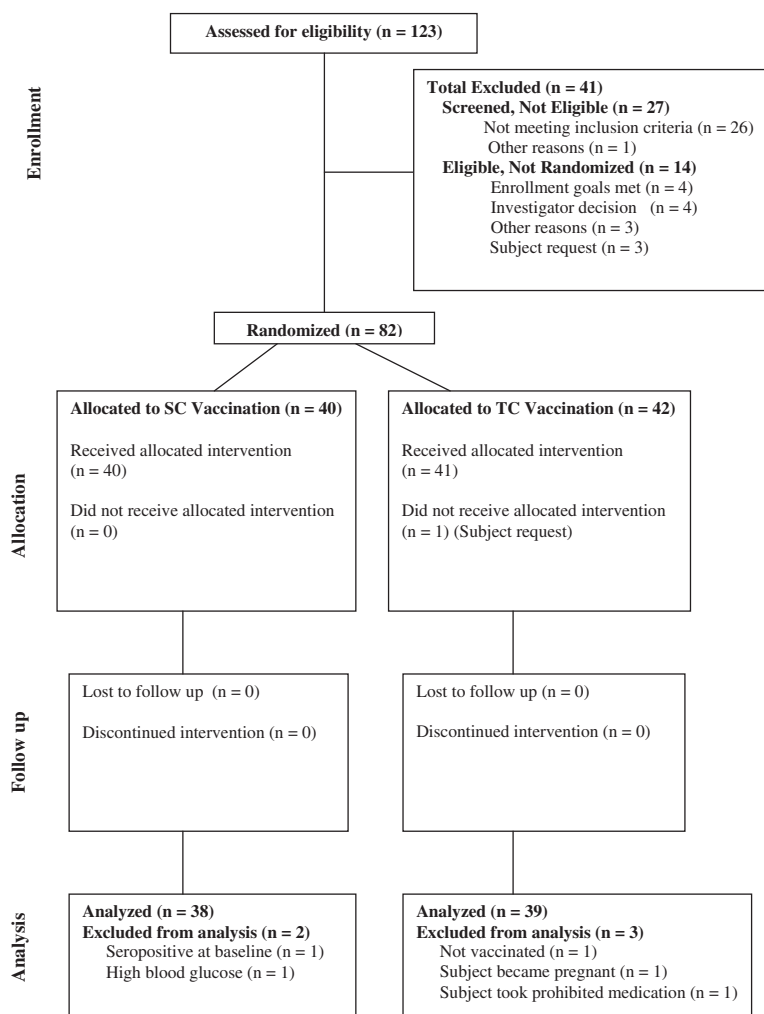


FIG 1. Flow diagram of study subjects participating in the trial. SC, Subcutaneous; TC, transcutaneous.

(log₁₀ transformed IFN- γ ⁺TNF- α ⁺CD4⁺ and IFN- γ ⁺TNF- α ⁺CD8⁺) were modeled by using mixed-effects models with unstructured residual variance-covariance matrices. Study visit, atopic status, and their interaction were included as classification factors and baseline cytokine response as a covariate, and linear contrasts were used to compare differences between patients with AD and nonatopic control subjects at days 14 and 30. Associations between prevaccination log₁₀ transformed total IgE levels and log₁₀ transformed NT₅₀ and cytokine levels were estimated by using Pearson correlations.

RESULTS

Study population, vaccinations, and safety

A total of 123 subjects were screened; 82 were randomized, and 81 received YFV vaccination through the subcutaneous or transcutaneous routes (Fig 1). Safety assessments included vital signs, vaccination-site reactions, adenopathy, hematology, and blood chemistry. There were no serious adverse events (AEs) among 81 vaccinated subjects. AEs occurring in greater than 5% of subjects are listed in Table I. The AE incident rates were generally similar between groups, but headache and fatigue were more common in patients with AD. The primary immunologic and virologic analysis population included 77 subjects (43% male, 83% white, 3% African American, and 14% of “other” race) with a mean age of 33 years. Total IgE levels

at baseline were significantly higher in patients with AD than in nonatopic control subjects ($P = .0001$). Of 81 vaccinated subjects included in the safety analysis, there were 8 (3 with AD and 5 nonatopic subjects) who were nonresponders (ie, negative for YFV RNAemia and viremia and did not seroconvert by LNI or NT₅₀), indicating that they had not been infected by the YFV-17D inoculum. Seven of 8 nonresponders were in the transcutaneous group, suggesting inadequate viral penetration and infection of the skin. All subjects were included in determining seroconversion rates, but because nonresponders had not been infected and did not mount an antiviral immune response, they were excluded from subsequent RNAemia and immunologic analyses.

Measurements of YFV replication

Viremia levels were low (<20 plaque-forming units/mL) and infrequent, with detection of live virus observed between 4 and 7 days after vaccination in only 16% (6/38) and 10% (4/39) of subjects after subcutaneous or transcutaneous immunization, respectively, and the few viremic samples that were identified were equally distributed among patients with AD and nonatopic control subjects. Swabs of transcutaneous vaccination sites were negative for infectious virus by using the plaque assay.

TABLE I. Clinical AEs experienced by greater than 5% of subjects after yellow fever vaccination

AE	SC + AD (n = 19), no. (%)	SC + NA (n = 21), no. (%)	TC + AD (n = 23), no. (%)	TC + NA (n = 19), no. (%)
Fatigue	7 (37)	3 (14)	7 (32)	0 (0)
Back pain	3 (16)	1 (5)	5 (23)	1 (5)
Headache	5 (26)	1 (5)	4 (18)	0 (0)
Arthralgia	4 (21)	3 (14)	2 (9)	0 (0)
Myalgia	4 (21)	3 (14)	2 (9)	0 (0)
Nausea	4 (21)	1 (5)	3 (14)	0 (0)
Injection-site pain	2 (11)	4 (19)	1 (5)	0 (0)
Upper respiratory tract infection	3 (16)	0 (0)	3 (14)	0 (0)
Application-site pain	1 (5)	0 (0)	3 (14)	1 (5)
Lymphadenopathy	1 (5)	0 (0)	3 (14)	1 (5)

Eighty-one subjects received subcutaneous or transcutaneous YFV vaccination. One subject withdrew consent before being vaccinated. Seventy-seven subjects were included in the overall analysis population, including 38 patients with AD and 39 nonatopic control subjects. One subject was excluded because of pre-existing YFV-specific antibody at baseline, 1 subject had high blood glucose levels, 1 subject used a prohibited medication, and despite a negative urine pregnancy test result at the time of vaccination, 1 woman became pregnant with a positive urine test result at 34 days after vaccination, discontinued participation in the study, but was included in the safety analysis. The pregnancy was uneventful, and she gave birth to a healthy child. Safety assessments were made on days 3, 5, 7, 10, 14, and 30 after vaccination. Grading and attribution of AEs followed the criteria set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Vaccine Clinical Trials guidelines, ranging from grade 1 to grade 4 (September 2007). More than 90% of AEs were mild or moderate. All but 3 of 13 grade 3 AEs and each of 3 grade 4 AEs were transient laboratory abnormalities (mostly increased cholesterol levels), none of which were considered relevant to vaccine. Therefore abnormal laboratory values unrelated to vaccination are not included in this table. NA, Nonatopic; SC, subcutaneous; TC, transcutaneous.

Analysis of viral replication by means of RT-PCR was more informative. Although there were 4 subjects who seroconverted without detectable YFV-17D RNA in serum (ie, RNAemia), the other 65 analyzable subjects demonstrated measurable levels of RNAemia of varying duration (Fig 2).

After subcutaneous vaccination, there was no statistically significant difference between duration of YFV-17D RNAemia in patients with AD (SC-AD) and nonatopic subjects (SC-NA; $P = .535$). In contrast, patients with AD (TC-AD) cleared RNAemia significantly faster than healthy control subjects (TC-NA) after transcutaneous vaccination ($P = .034$). This was an unexpected finding and indicates that route of infection (transcutaneous vs subcutaneous) might affect systemic viral replication. From a safety perspective, this also suggests that transcutaneous vaccination of patients with AD with YFV-17D does not represent an increased risk for systemic spread.

Antiviral antibody and T-cell responses after vaccination

Virus-specific neutralizing antibody represents the principal mechanism of protection against YFV.^{10,16} Seroconversion and protection are typically defined by a neutralizing antibody titer of 0.7 LNI or greater, which is roughly estimated to be greater than an NT₅₀ of 1:10.¹² After subcutaneous vaccination, we obtained 97% seroconversion, whereas after transcutaneous vaccination, the overall seroconversion rate was 82% ($P = .059$; Fig 3, A). Lower seroconversion through the transcutaneous route might have been due to technical features of transcutaneous vaccination with a bifurcated needle; during the first stage of

the protocol, transcutaneous vaccination was performed by using a 5-jab technique, resulting in 67% (6/9) seroconversion, whereas in subsequent stages the 15-jab technique was used, resulting in 87% (26/30) seroconversion. There was no significant difference in antibody titers elicited after vaccination by using the 5-jab or 15-jab techniques when measured based on LNI ($P = .986$) or NT₅₀ ($P = .247$) results. Antibody responses, as measured by using LNI (Fig 3, B) or NT₅₀ (Fig 3, C), showed no significant differences among seroconverters, regardless of route (transcutaneous vs subcutaneous) or group designation (SC-AD vs SC-NA or TC-AD vs TC-NA).

Transcutaneous vaccination against YFV has not been previously examined in terms of antiviral T-cell responses. Subcutaneous vaccination with YFV-17D elicits a strong and polyfunctional virus-specific T-cell response^{17,18} and is an excellent model for analyzing the interplay between innate and adaptive immunity. After *in vitro* stimulation of PBMCs with YFV-17D, virus-specific T cells producing 2 antiviral cytokines, IFN- γ and TNF- α , were enumerated by means of intracellular cytokine staining analysis (Fig 4, A). Although the IFN- γ ⁺TNF- α ⁺ T-cell response was greater than the IFN- γ ⁺TNF- α ⁻ T-cell response at day 14, by days 28 to 35, the majority of T cells coexpressed both IFN- γ and TNF- α (Fig 4, A, and data not shown). The total IFN- γ ⁺ T-cell response did not have the same specificity as the IFN- γ ⁺TNF- α ⁺ T-cell response because of higher frequencies of IFN- γ ⁺ T cells at day 0 (ie, before vaccination). However, the trends for the total IFN- γ ⁺ T-cell response (see Fig E1 in this article's Online Repository at www.jacionline.org) were similar to that observed when IFN- γ ⁺TNF- α ⁺ T-cell numbers were measured (Fig 4). YFV-specific CD4⁺ T-cell numbers peaked by 14 days after subcutaneous vaccination (Fig 4, B), but after transcutaneous vaccination, CD4⁺ T-cell responses were lower at 14 days after vaccination and either remained flat or continued to increase by day 30 (Fig 4, C, and see Fig E2 in this article's Online Repository at www.jacionline.org). We found no significant differences in CD4⁺ T-cell responses between the AD and nonatopic groups after subcutaneous vaccination (Fig 4, B, and see Fig E2), whereas YFV-specific CD4⁺ T-cell responses differed significantly at 30 days after transcutaneous vaccination (Fig 4, C; $P = .036$). YFV-specific CD8⁺ T-cell responses peaked by 14 days after subcutaneous vaccination (Fig 4, D) but were delayed after transcutaneous vaccination (Fig 4, E, and see Fig E2). Similar to antiviral CD4⁺ T cells, subcutaneous vaccination resulted in CD8⁺ T-cell responses that were indistinguishable between the AD and nonatopic groups (Fig 4, D), but after transcutaneous vaccination, antiviral CD8⁺ T-cell responses showed a modestly significant difference (Fig 4, E; $P = .045$). Analysis of polyfunctional T cells that express 3 cytokines (ie, IFN- γ ⁺TNF- α ⁺IL-2⁺) provided similar results; there was a significant difference in antiviral CD4⁺ T-cell responses ($P = .043$) and CD8⁺ T-cell responses ($P = .0097$) at 30 days after transcutaneous vaccination but no significant difference between groups at 30 days after subcutaneous vaccination ($P > .50$, data not shown). There was also no significant effect of subject age on the outcome of antiviral T-cell responses at day 14 ($P > .4$) or day 30 ($P > .1$, data not shown). Together, these results demonstrate that when skin is bypassed by subcutaneous vaccination, there is no difference in antiviral T-cell responses between patients with AD and nonatopic control subjects, but if viral infection is initiated in skin through transcutaneous vaccination, patients

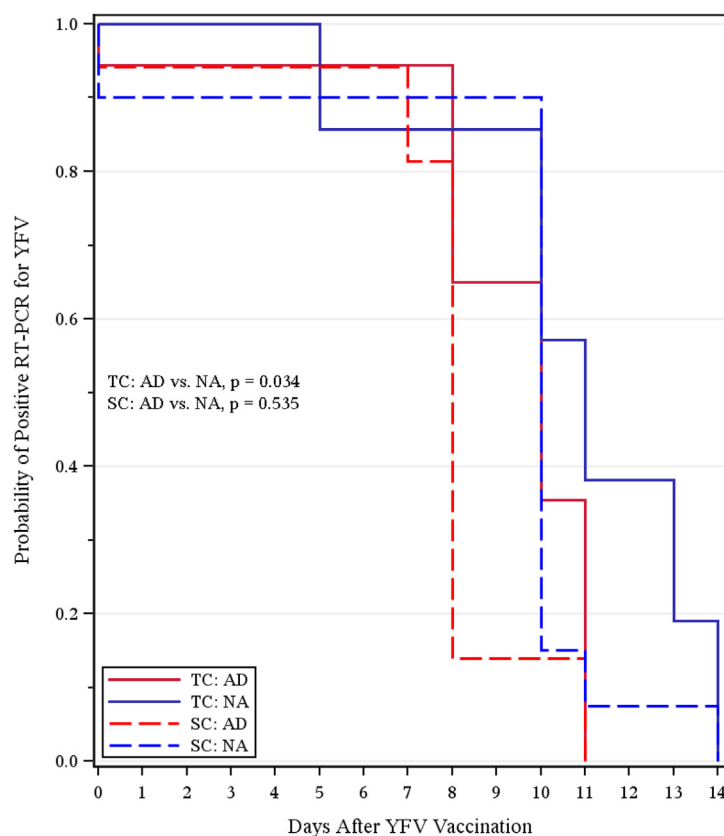


FIG 2. Duration of YFV RNAemia after subcutaneous or transcutaneous vaccination. Kaplan-Meier curves show the proportion of YFV-seroconverted subjects with positive scores for YFV RNA by using RT-PCR during the first 2 weeks after vaccination. The limit of detection was less than 1 plaque-forming unit/mL for serum spiked with purified YFV. NA, Nonatopic; SC, subcutaneous; TC, transcutaneous. TC-AD, n = 18; TC-NA, n = 14; SC-AD, n = 17; and SC-NA, n = 20.

with AD demonstrate significantly lower YFV-specific T-cell responses at later time points.

Association between pre-existing total IgE levels and antiviral immunity

Patients with AD often have increased serum IgE levels, and for some viral infections, the highest IgE levels are associated with more severe disease manifestations.¹⁹ We measured the magnitude of antiviral antibody (Fig 3) and T-cell responses (Fig 4) after YFV vaccination and compared these parameters with total serum IgE levels at baseline (Fig 5 and see Table E1). After subcutaneous vaccination (Fig 5, A), there was no significant correlation between neutralizing antibody responses and pre-existing IgE levels for patients with AD ($P = .12$) or nonatopic control subjects ($P = .88$). After transcutaneous vaccination (Fig 5, B), there was a trend toward a significant inverse correlation between IgE and neutralizing antibody levels in nonatopic subjects (95% CI, -0.82 to 0.04 ; $P = .057$) and a modestly significant inverse correlation between IgE and antibody titers in patients with AD (95% CI, -0.77 to -0.01 ; $P = .041$). The results of Fig 5, B, are consistent with those of Fig 3, C, in which there is a trend toward lower NT₅₀ titers (although not significant, $P = .102$) after transcutaneous vaccination of patients with AD versus nonatopic subjects. When all transcutaneously vaccinated subjects were analyzed together, the inverse correlation became highly significant

(95% CI, -0.75 to -0.25 ; $P < .001$). In contrast, combined analysis of all subjects who received subcutaneous vaccination did not alter the outcome; there was no significant correlation between IgE and antiviral antibody levels after immunization through the subcutaneous route (95% CI, -0.48 to 0.17 ; $P = .32$).

Analysis of antiviral T-cell responses indicated a dichotomy between the 2 arms of the cellular immune response. As with the relationship between IgE and neutralizing antibody responses, YFV-specific CD4⁺ T-cell responses showed a significant inverse correlation with IgE levels after transcutaneous vaccination (95% CI, -0.73 to -0.09 ; $P = .015$; Fig 5, D) but not after subcutaneous vaccination (95% CI, -0.45 to 0.27 ; $P = .58$; Fig 5, C). YFV-specific CD8⁺ T-cell responses showed no significant relationship with baseline IgE titers in the AD or nonatopic groups, regardless of receiving transcutaneous or subcutaneous vaccination (Fig 5, E and F). Together, these results indicate that, unlike CD8⁺ T cells, the CD4⁺ T-cell response elicited by patients with AD was significantly reduced in patients with high baseline IgE levels, but this is found only when YFV-17D infection is initiated in the skin, and this relationship no longer exists when skin is bypassed by subcutaneous vaccination.

DISCUSSION

The goal of this study was to develop a better understanding of the immune responses of patients with AD after cutaneous viral

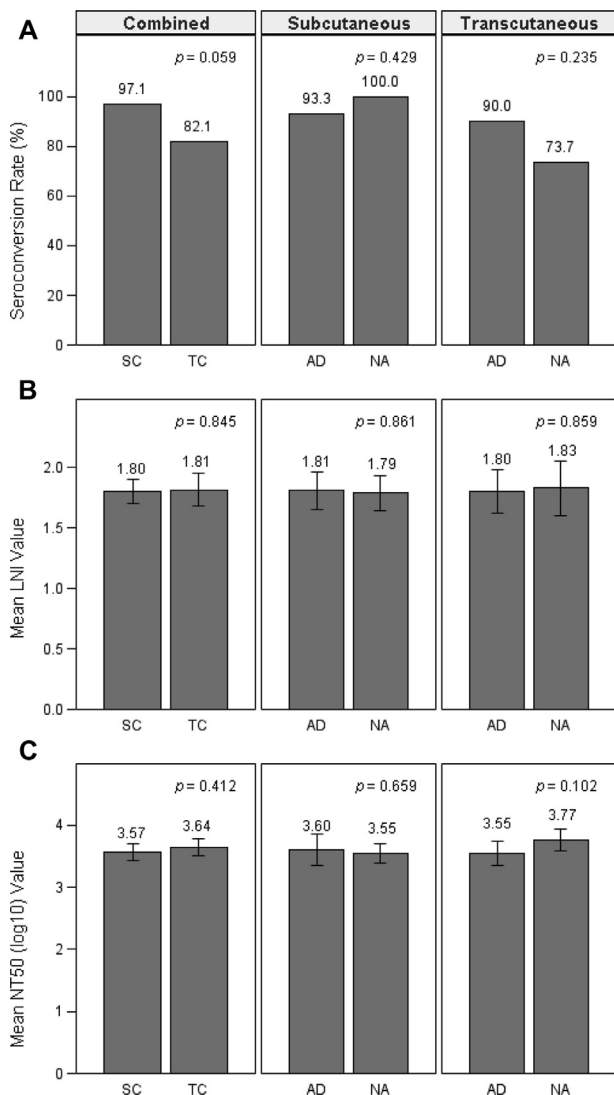


FIG 3. Induction of neutralizing YFV-specific antibody responses after subcutaneous or transcutaneous vaccination. **A**, At 30 days after YFV vaccination, seroconversion rates were determined for the different vaccine groups and defined as a YFV-specific neutralizing titer of 10 or greater. **B**, Levels of YFV neutralizing antibody among seropositive subjects were measured based on the LNI in which serum is held constant and different amounts of virus are neutralized. **C**, Levels of YFV neutralizing antibody among seropositive subjects were measured by determining the serum dilution required to neutralize 50% of viral plaques (held constant at 50-100 YFV plaques). Error bars represent 95% CIs. NA, Nonatopic; SC, subcutaneous; TC, transcutaneous.

infection. No significant differences in antiviral responses were observed between patients with mild versus those with moderate-to-severe AD (data not shown). In contrast, comparisons between transcutaneous and subcutaneous vaccination with a live virus vaccine (YFV-17D) revealed differences in the kinetics/magnitude of antiviral T-cell responses, and patients with AD with higher baseline IgE levels were more likely to have lower antiviral antibody and CD4⁺ T-cell responses. Further studies will be needed to determine whether IgE might serve as a biomarker of potential immunogenicity in response to transcutaneous/transdermal vaccination strategies in other patient populations.

Although patients with AD have higher total serum IgE levels than nonatopic control subjects, it does not appear that they have a substantial deficit in innate or early adaptive immune responsiveness to YFV infection of the skin because patients with AD cleared YFV RNAemia more rapidly than their nonatopic counterparts (Fig 2). Virus-specific IgE levels were not measured in this study, and it is unknown whether nonspecific total IgE is involved with immune regulation in this model. More likely, it simply represents an indicator of immune responsiveness to transcutaneous YFV vaccination. Moreover, there is little known about IgE levels in the skin, and more studies are needed to learn whether cutaneous IgE levels are increased in patients with AD. However, the rapid clearance of viremia by patients with AD is an important observation because a small vaccination study performed in cynomolgus macaques using recombinant strains of YFV-17D expressing other flavivirus genes (eg, JEV envelope) showed that 3 of 3 animals that received chimerivax-JEV transcutaneously with a prototype microenhancer array abrasion device had higher and more prolonged viremia than animals that received standard subcutaneous vaccination.²⁰ Fortunately, our data in 39 human subjects indicate that after nonrecombinant YFV-17D vaccination, there was no evidence for increased viremia after transcutaneous vaccination. Likewise, another clinical study involving intradermal YFV vaccination found no major difference in viral RNA levels at 5 days after vaccination in comparison with subcutaneous vaccination,²¹ further suggesting that this might be a safe approach to vaccine delivery. However, larger clinical studies will be needed to determine the safety of transcutaneous versus subcutaneous vaccination against yellow fever.

In terms of vaccine-induced cellular immunity, the initial antiviral CD4⁺ and CD8⁺ T-cell responses of patients with AD measured at 14 days after infection were not significantly lower than in nonatopic control subjects, but responses were significantly lower at 1 month after vaccination in the transcutaneously vaccinated cohort (Fig 4). Whether lower T-cell responses in patients with AD observed at later time points are due to weaker antiviral immune memory or to lower antigenic load resulting in more rapid memory T-cell contraction is a question that will require further study. We do not believe that genetics or overt immunosuppression are factors in the lower immune responses elicited by patients with AD after transcutaneous vaccination because patients with AD mount normal antiviral immune responses when vaccinated through the subcutaneous route. In any case, the strong inverse relationship between neutralizing antibody responses and antiviral CD4⁺ T-cell responses with baseline IgE levels represents an intriguing finding, especially because it is only observed during transcutaneous infection and is not found in cases in which the skin is bypassed by subcutaneous injection/infection (Fig 5).

This study represents the first immunologic and virologic examination of transcutaneous YFV vaccination performed in more than 40 years. Although our study is not powered to identify rare AEs,^{22,23} prior studies involving more than 130,000 vaccinees indicate that transcutaneous vaccination with YFV-17D is safe, with no serious AEs recorded.⁷⁻⁹ Using a 15-jab approach to transcutaneous vaccination, we obtained 87% seroconversion with neutralizing antibody levels well above the LNI level of 0.7, which is required for protective immunity. It is unclear whether transcutaneous vaccination will provide a

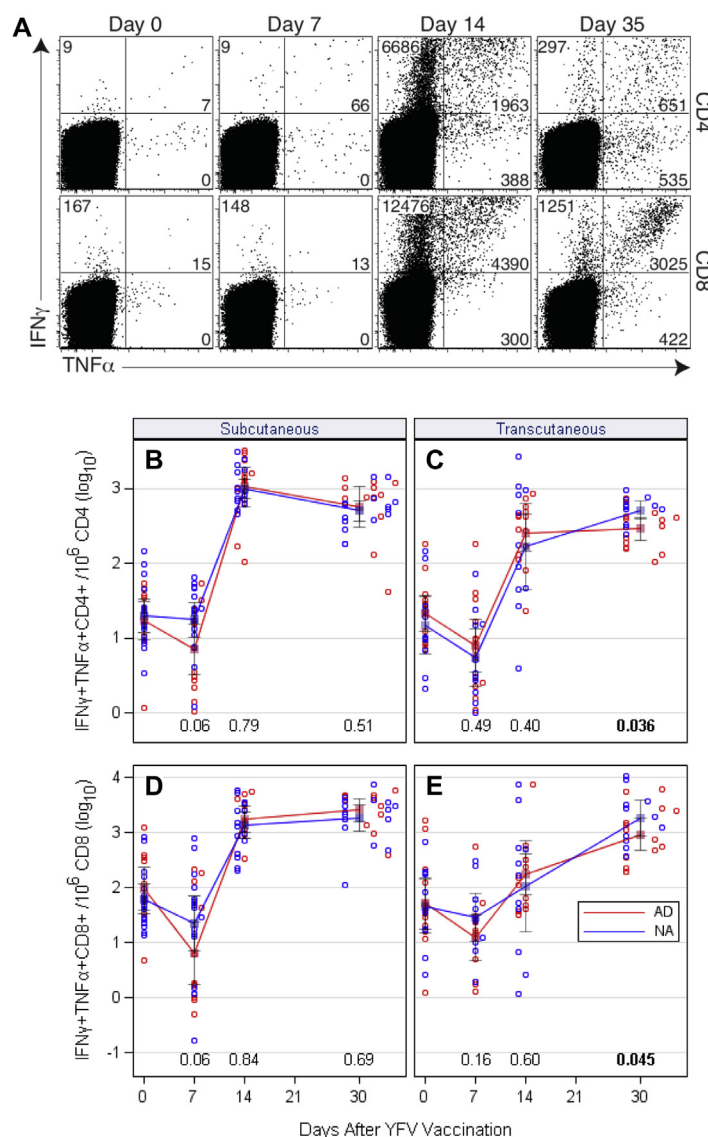


FIG 4. Kinetics and magnitude of vaccine-induced YFV-specific CD4⁺ and CD8⁺ T-cell responses. The frequency of virus-specific T cells was measured by stimulating PBMCs with purified YFV and measuring the frequency of IFN- γ ⁺TNF- α ⁺ T cells at each time point by using intracellular cytokine staining analysis. **A**, Representative flow cytometric dot plot showing the virus-specific CD4⁺ and CD8⁺ T-cell response after YFV vaccination. The dot plots were pregated on CD4⁺CD8⁻ and CD4⁺CD8⁺ T cells and show the number of events per 10⁶ CD4⁺ or CD8⁺ cells after background subtraction (medium alone). **B-E**, The kinetics and magnitude of YFV-specific CD4⁺ T cells (Fig 4, **B** and **C**) or CD8⁺ T cells (Fig 4, **D** and **E**) were measured in subjects vaccinated through the subcutaneous route (Fig 4, **B** and **D**) or the transcutaneous route (Fig 4, **C** and **E**). The numbers indicate measured *P* values at each time point, and error bars represent 95% CIs. NA, Nonatopic.

safety benefit over subcutaneous vaccination, but it might provide a mechanism for decreasing vaccine cost and increasing supplies in times of vaccine shortage because a single subcutaneous dose of YFV-17D can provide approximately 50 doses when delivered through transcutaneous inoculation. This is key because YFV immunization campaigns can be hampered by vaccine shortages, and intradermal YFV vaccination has also been shown to work well with a one-fifth dose typically used for subcutaneous vaccination.²¹ There is growing interest in developing intradermal vaccination strategies,²⁴⁻²⁷ and several studies have targeted the skin to enhance vaccine immunogenicity.²⁸⁻³⁰ However, these protocols did not specifically include patients

with skin diseases. Considering that AD is a common health problem, these patients, as well as those with other skin diseases, should be evaluated during early-stage trials involving cutaneous delivery. In addition, biomarkers, such as total IgE, might prove useful to identify population subsets that might respond differently to cutaneous infection or vaccination.

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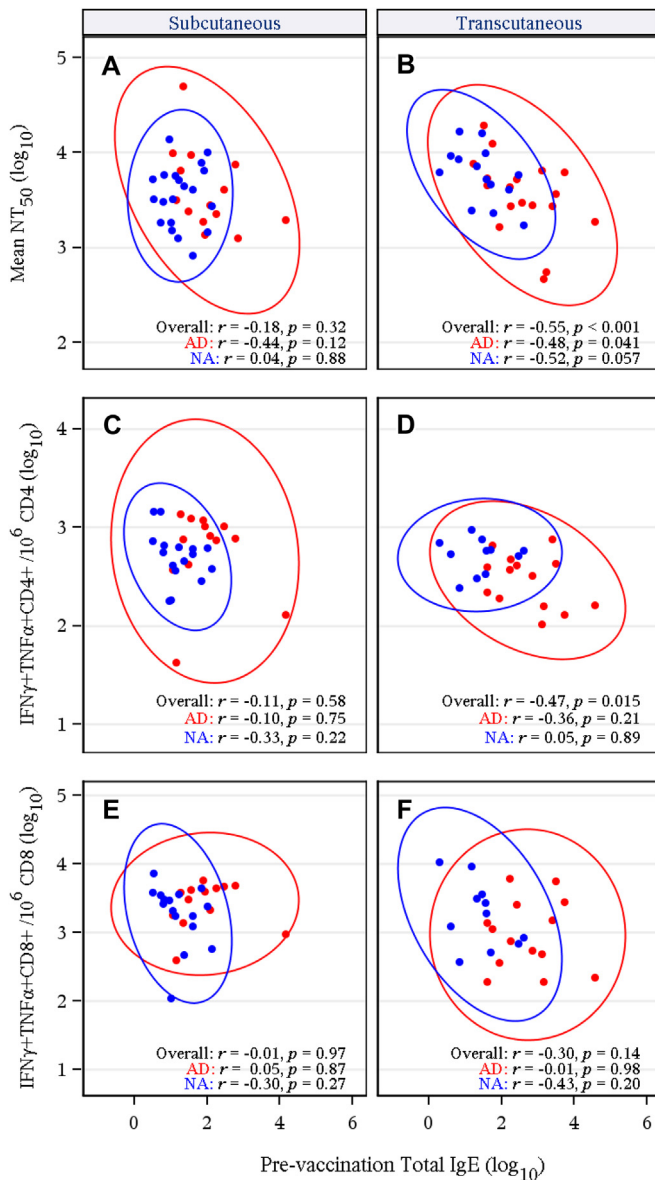


FIG 5. Comparison between baseline total IgE levels and YFV-induced immune responses. Total serum IgE levels obtained at baseline were compared with subsequent YFV-induced immune responses at 30 days after vaccination and included YFV-specific neutralizing titers (NT_{50} ; **A** and **B**), YFV-specific CD4^+ T-cell responses (**C** and **D**), and YFV-specific CD8^+ T-cell responses (**E** and **F**) in subjects vaccinated through the subcutaneous route (Fig 5, **A**, **C**, and **E**) or the transcutaneous route (Fig 5, **B**, **D**, and **F**). Red symbols represent patients with AD, blue symbols represent nonatopic (NA) subjects, and red or blue ellipses represent 95% CIs.

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Clinical implications: Clinical trials involving vaccination through the skin should include patients with AD, especially because this group can differ significantly in terms of immune responses to skin-associated vaccine delivery.

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Transcutaneous yellow fever vaccination of subjects with or without atopic dermatitis

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Table E1. Baseline Total IgE, NT50, and day 30 IFN γ +TNF α +CD4+/106 CD4+, IFN γ +TNF α +CD8+/106 CD8+ counts. Geometric mean counts for each diagnostic group by route are listed in red. N.D. denotes Not Determined.

Figure legends

Figure E1. Vaccine-induced IFN γ + YFV-specific CD4+ and CD8+ T cell responses. The kinetics and magnitude of YFV-specific CD4+ T cells (A and B) or CD8+ T cells (C and D) were measured in subjects vaccinated by the subcutaneous route (A and C) or the transcutaneous route (B and D). The frequency of virus-specific T cells was measured by stimulating PBMC with purified YFV and measuring the frequency of total IFN γ + T cells. Error bars represent 95% confidence intervals. Numbers at bottom of each panel denote *p* values of the comparisons between AD and NA at days 7, 14, and 30.

Figure E2. Effect of vaccination route on YFV-specific CD4+ and CD8+ T cell responses. The kinetics and magnitude of YFV-specific CD4+ T cells (A) or CD8+ T cells (B) were measured in subjects vaccinated by the subcutaneous (SC) and transcutaneous (TC) routes. The frequency of virus-specific T cells was measured by stimulating PBMC with purified YFV and measuring the frequency of total IFN γ +TNF α +. Numbers at bottom of each panel denote *p* values of the comparisons between SC and TC for AD (red) and NA (blue) subjects at days 14 and 30.

Slifka *et al.* Online Repository**Figure E1. Vaccine-induced IFN γ ⁺ YFV-specific CD4⁺ and CD8⁺ T cell responses.**

The kinetics and magnitude of YFV-specific CD4⁺ T cells (A and B) or CD8⁺ T cells (C and D) were measured in subjects vaccinated by the subcutaneous route (A and C) or the transcutaneous route (B and D). The frequency of virus-specific T cells was measured by stimulating PBMC with purified YFV and measuring the frequency of total IFN γ ⁺ T cells. Error bars represent 95% confidence intervals.

Numbers at bottom of each panel denote *p* values of the comparisons between AD and NA at days 7, 14, and 30.

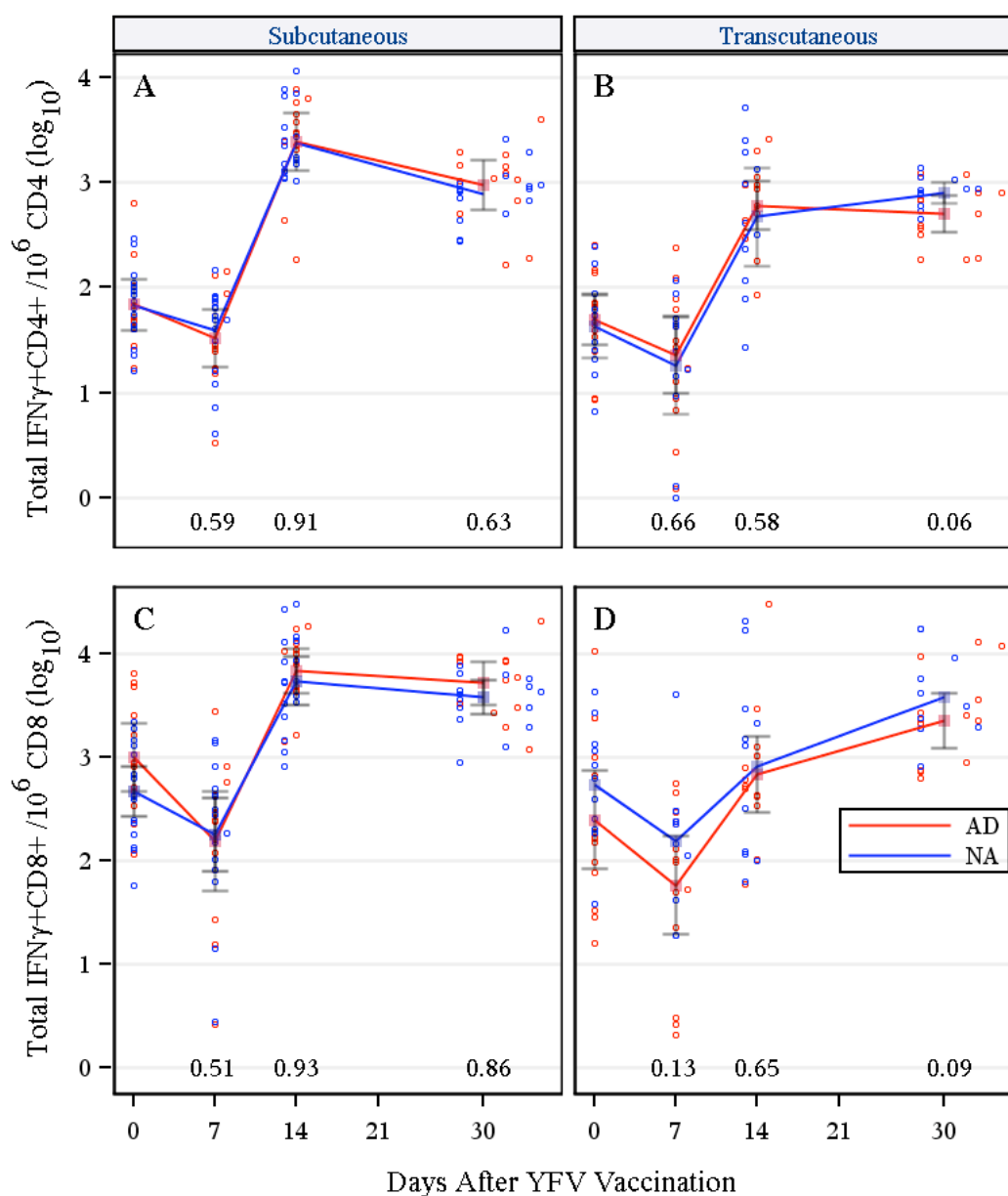


Figure E2. Effect of vaccination route on YFV-specific CD4⁺ and CD8⁺ T cell responses.

The kinetics and magnitude of YFV-specific CD4⁺ T cells (A) or CD8⁺ T cells (B) were measured in subjects vaccinated by the subcutaneous (SC) and transcutaneous (TC) routes. The frequency of virus-specific T cells was measured by stimulating PBMC with purified YFV and measuring the frequency of total IFN γ ⁺TNF α ⁺. Numbers at bottom of each panel denote *p* values of the comparisons between SC and TC for AD (red) and NA (blue) subjects at days 14 and 30.

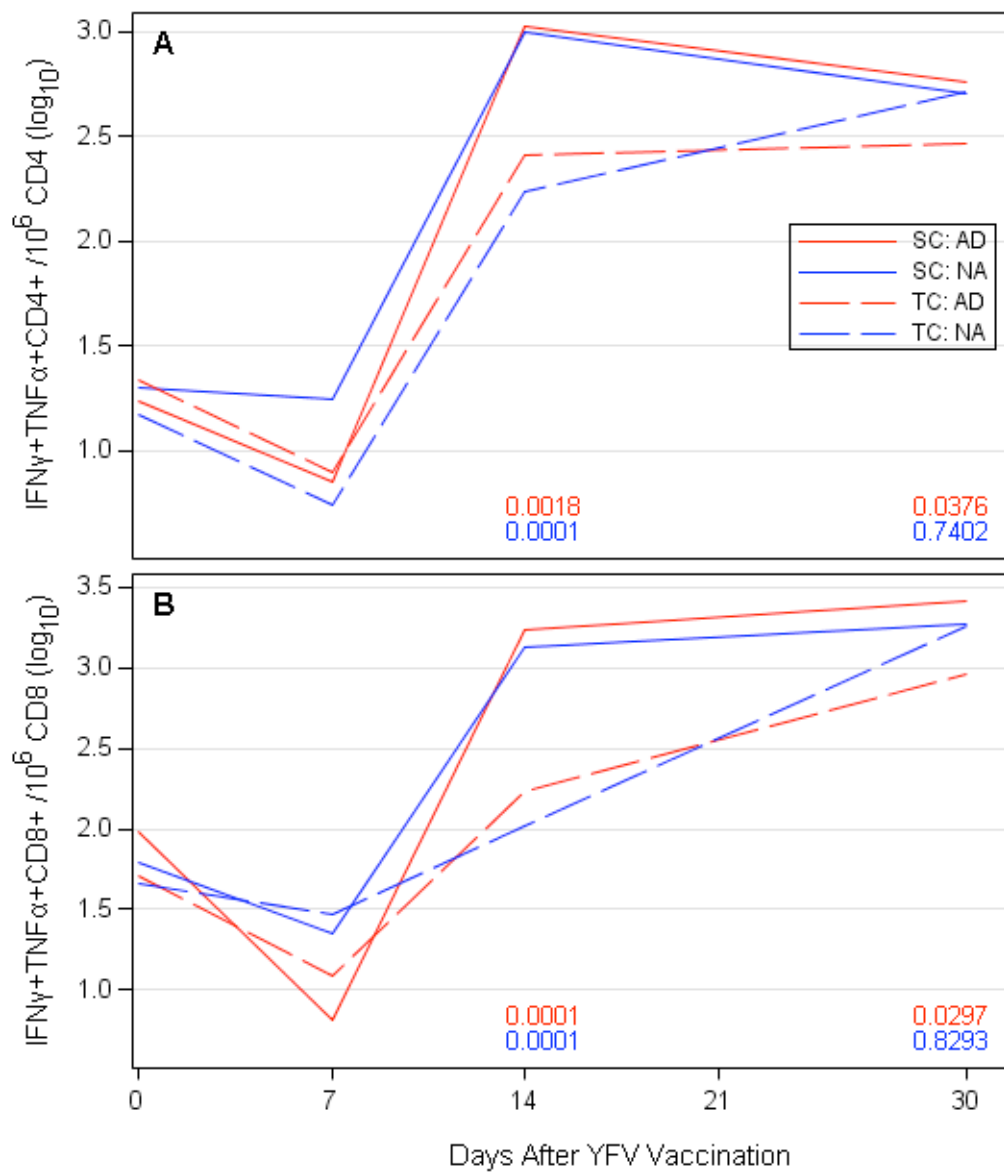


Table E1. Baseline Total IgE, NT₅₀, and day 30 IFN γ ⁺TNF α ⁺CD4⁺/10⁶ CD4⁺, IFN γ ⁺TNF α ⁺CD8⁺/10⁶ CD8⁺ counts. Geometric mean counts for each diagnostic group by route are listed in red. N.D. denotes Not Determined.

Subject	Route	Diagnostic Group	Total IgE	NT ₅₀	IFN γ ⁺ TNF α ⁺ CD4 ⁺ / 10 ⁶ CD4 ⁺	IFN γ ⁺ TNF α ⁺ CD8 ⁺ / 10 ⁶ CD8 ⁺
1	SC	AD	11	9894	372	1788
2			14	3161	42	396
3			19	6437	1354	3900
4			23	49703	753	1399
5			31	2411	421	3035
6			37	9419	1233	4267
7			78	1858	1189	5886
8			86	1369	1023	3938
9			120	2788	826	2162
10			181	2263	747	4449
11			295	4060	1035	4759
12			610	7516	777	4872
13			695	1257	N.D.	N.D.
14			14160	1931	130	958
			105	4002	569	2600
15	NA	NA	3	5283	723	3871
16			3	3200	1438	7333
17			5	1836	1435	3518
18			6	3039	563	2625
19			7	5889	651	3025
20			9	13873	179	2972
21			10	1845	181	111
22			11	1529	N.D.	N.D.
23			12	3205	412	2102
24			14	5734	362	1751
25			16	1250	N.D.	N.D.
26			17	5177	630	3681
27			23	4445	451	479
28			40	822	536	1757
29			40	4096	611	1232
30			70	7758	283	4425
31			85	6422	N.D.	N.D.
32			102	10127	618	2389
33			104	1448	N.D.	N.D.
34			133	2757	377	588
			19	3504	502	1873

Subject	Route	Diagnostic Group	Total IgE	NT ₅₀	IFN γ ⁺ TNF α ⁺ CD4 ⁺ / 10 ⁶ CD4 ⁺	IFN γ ⁺ TNF α ⁺ CD8 ⁺ / 10 ⁶ CD8 ⁺
35	TC	AD	16	7659	N.D.	N.D.
36			33	19297	N.D.	N.D.
37			40	5360	220	189
38			41	4483	397	1385
39			57	12406	653	1142
40			89	1664	191	359
41			172	4378	374	6193
42			174	2729	474	761
43			267	5267	407	2541
44			367	2985	N.D.	N.D.
45			701	2762	320	542
46			1260	6464	103	484
47			1463	467	158	194
48			1662	550	N.D.	N.D.
49			2473	2718	749	1527
50			3133	3660	426	5607
51			5480	6207	130	2781
52			37340	1881	162	220
			391	3532	288	926
					N.D.	N.D.
53	NA	NA	2	6196	702	10651
54			4	9283	531	1230
55			6	8564	N.D.	N.D.
56			7	16854	245	369
57			15	2474	955	9353
58			21	7242	305	3135
59			28	16010	760	3680
60			36	9816	333	2689
61			39	5214	587	1894
62			51	4609	588	515
63			58	2330	N.D.	N.D.
64			158	4051	N.D.	N.D.
65			294	5835	519	696
66			396	1733	578	853
			29	5840	516	1839