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 2014;133:439-47.)

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

Atopic dermatitis (AD) is a common inflammatory skin disease[1] 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

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Abbreviations used
AD: Atopic dermatitis
AE: Adverse event
LNI: Log neutralizing index
NT_{50}: 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 μg/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 $\log_{10}$ neutralization index (LNI), which refers to a constant serum-varying virus reduction test,[11] and the neutralizing titer 50 (NT_{50}), 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 responses[14] 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 ($\log_{10}$ neutralization index and $\log_{10}$ transformed NT_{50} 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