Severe atopic dermatitis is characterized by selective expansion of circulating T_H2/T_C2 and T_H22/T_C22, but not T_H17/T_C17, cells within the skin-homing T-cell population

Tali Czarnowicki, MD,[a] Juana Gonzalez, PhD,[b]* Avner Shemer, MD,[c]* Dana Malajian, BA,[a,e] Hui Xu, MSc,[a] Xiuzhong Zheng, MSc,[a] Saakshi Khattri, MD,[a] Patricia Gilleaudeau, NP,[a] Mary Sullivan-Whalen, NP,[a] Mayte Suárez-Fariñas, PhD,[a,d] James G. Krueger, MD, PhD,[a] and Emma Guttman-Yassky, MD, PhD[a,f] *New York, NY, and Tel Aviv, Israel*

Background: Past studies of blood T-cell phenotyping in patients with atopic dermatitis (AD) have provided controversial results and were mostly performed before the identification of T_H9, T_H17, and T_H22 T-cell populations in human subjects.

Objective: We sought to quantify T_H1, T_H2, T_H9, T_H17, and T_H22 T-cell populations and corresponding $CD8^+$ T-cell subsets in both cutaneous lymphocyte antigen (CLA)–positive and CLA^- T-cell subsets in patients with AD and control subjects.

Methods: We studied 42 adults with severe AD (mean SCORAD score, 65) and 25 healthy subjects using an 11-color flow cytometric antibody panel. Frequencies of IFN-γ–, IL-22–, IL-13–, IL-17–, and IL-9–producing $CD4^+$ and $CD8^+$ T cells were compared in CLA^- and CLA^+ populations.

Results: We measured increased $T_H2/T_C2/IL\text{-}13^+$ and $T_H22/T_C22/IL\text{-}22^+$ populations ($P < .1$) in patients with severe AD versus control subjects, with significant differences in CLA^+ T-cell numbers ($P < .01$). A significantly lower frequency of CLA^+ IFN-γ–producing cells was observed in patients with AD, with no significant differences in CLA^- T-cell numbers. The CLA^+ T_H1/T_H2 and T_C1/T_C2 ratio was highly imbalanced in patients with AD (10 vs 3 [$P = .005$] and 19 vs 7 [$P < .001$], respectively). Positive correlations were found between frequencies of IL-13– and IL-22–producing $CD4^+$ and $CD8^+$ T cells ($r = 0.5$ and 0.8, respectively; $P < .0001$), and frequencies of IL-13–producing CLA^+ cells were also correlated with IgE levels and SCORAD scores. Patients with AD with skin infections had higher $CD4^+$ $IL\text{-}22^+$ and $IL\text{-}17^+$ cell frequencies, which were highly significant among CLA^- cells (IL-22: 3.7 vs 1.7 [$P < .001$] and IL-17: 1.7 vs 0.6 [$P < .001$]), with less significant effects among CLA^+ T cells (IL-22: 11 vs 7.5, $P = .04$).

Conclusions: Severe AD is accompanied by expansion of skin-homing T_H2/T_C2 and T_H22/T_C22 subsets with lower T_H1/T_C1 frequencies. These data create a critical basis for studying alterations in immune activation in adults and pediatric patients with AD. (J Allergy Clin Immunol 2015;136:104-15.)

Key words: *Atopic dermatitis, T cell, cutaneous lymphocyte antigen, IL-13, IL-22, IFN-γ, skin infections*

It is now recognized that "polar" T-cell subsets, including T_H1, T_H2, T_H17, and T_H22 cells, and corresponding $CD8^+$ subsets are activated in acute and chronic atopic dermatitis (AD) lesions.[1-6] Performing intracellular cytokine staining (ICS) in skin lesions to classify the differentiation status of T-cell subsets is limited by the need for relatively large volumes of biopsy specimens to study sufficient T-cell numbers, especially for relatively low-frequency populations, such as T_H17 cells.

Many past studies have detected increases or decreases in T_H1 or T_H2 T-cell subsets in peripheral blood of patients with AD, but these studies have been done inconsistently across children and adults, different phenotypes of AD (intrinsic vs extrinsic), a variety of background treatments, and different ethnic populations.[7-15] Furthermore, most of these studies were done before human T_H9, T_H17, and T_H22 T-cell populations were characterized.

Given that a major T-cell axis is usually expressed in circulating T cells of patients with AD, even more power to detect disease-related changes comes from the focus on T-cell subsets that bear cutaneous lymphocyte antigen (CLA). CLA^+ T cells are specialized for skin homing and represent the main population in AD lesions.[16-19]

Few studies have examined polar T-cell subsets in CLA^+ versus CLA^- T cells of patients with AD and control subjects.[20-23] Although some T_H2 expansion is seen in healthy subjects in CLA^+ T cells, additional expansion of this T-cell subset is seen in patients with AD.[20-22] Interestingly, one study detected

From [a]the Laboratory for Investigative Dermatology, [b]the Translational Technology Core Laboratory, and [d]the Center for Clinical and Translational Science, Rockefeller University, New York; [c]the Department of Dermatology, Tel-Hashomer Hospital, Tel Aviv; [e]Columbia University College of Physicians and Surgeons, New York; and [f]the Dermatology Department at the Icahn School of Medicine at Mount Sinai, New York.

*These authors contributed equally to this work.

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Corresponding author: Tali Czarnowicki, MD, Laboratory for Investigative Dermatology, Rockefeller University, 1230 York Ave, New York, NY 10065. E-mail: tczarnowic01@rockefeller.edu.

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Abbreviations used
AD: Atopic dermatitis
CLA: Cutaneous lymphocyte antigen
ICS: Intracellular cytokine staining
MFI: Median fluorescence intensity
PMA: Phorbol 12-myristate 13-acetate

a decrease in the T_H1 (interferon-producing) population from skin-homing T cells of patients with AD, but this decrease was very similar to T_H1 levels measured in skin-homing cells from healthy control subjects.[22] It is currently unclear whether patients with AD have a relative deficiency of T_H1 or T_C1 T cells within the overall T-cell population or within the skin-homing subset.[8,20-26]

Finally, immunopathogenic models have implicated T_H17 and T_H22 T cells in specific elements of the AD phenotype. Very little ICS data are available on IL-17,[27,28] IL-22,[1] and IL-9,[29] and practically no data are available on CLA^+ versus CLA^- cells in patients with AD.

In this study we sought to simultaneously measure the frequencies of T_H1, T_H2, T_H9, T_H17, and T_H22 populations, as well as corresponding $CD8^+$ T cells, in both CLA^+ and CLA^- subsets in a sizable number of European American patients with moderate-to-severe AD who were not receiving active treatment. Results have been correlated with IgE levels, disease severity (by SCORAD score), and infection history. We found extremely high expansion of T_H2 and T_H22 cell frequencies within CLA^+ T cells and significant decreases in CLA^+ T_H1/T_C1 T-cell frequencies but no increased frequency of IL-17–producing $CD4^+$ or $CD8^+$ subsets, whether CLA^+ or CLA^-.

METHODS

Patients' characteristics and blood samples

Blood was obtained from 42 patients (18 female and 24 male patients; age, 18-74 years; mean age, 42 years) with moderate-to-severe AD and 25 control subjects (12 female and 13 male subjects; age, 19-66 years; mean age, 39 years) under an institutional review board–approved protocol. No age ($P = .2$, Student t test), ethnicity ($P = .8$, Fisher exact test), or sex ($P = .8$, Fisher exact test) disparities were observed between the groups. Patients were stratified into extrinsic (n = 25) and intrinsic (n = 15; IgE was unavailable for 2 patients) and moderate (n = 18) and severe (n = 24) AD groups. Serum IgE levels of 200 kU/L or greater and less than 200 kU/L were used to define extrinsic or intrinsic AD status, respectively (intrinsic: range, 4-182 kU/L; mean, 67.3 kU/L; extrinsic: range, 216-50,000 kU/L; mean, 9,941 kU/L [reference range, 0-200 kU/L]). The SCORAD score was used to evaluate disease severity. Patients were classified as having moderate (SCORAD score, <60; range, 32-60; mean, 51) or severe (SCORAD, ≥60; range, 62-97; mean, 76) AD. Each patient was interviewed regarding history of skin infections. Medical records, including prescriptions of antibacterial and antiviral medications, were reviewed; 26 and 13 patients with AD had a history of bacterial and viral skin infections, respectively (11 patients had both), whereas control subjects had no skin infections (demographic and laboratory data are summarized in Table I and see Table E1 in this article's Online Repository at www.jacionline.org).

Stimulation of blood cell populations for cytokine responses

Whole blood was incubated with phorbol 12-myristate 13-acetate (PMA; 25 ng/mL) plus ionomycin (2 μg/mL) in the presence of brefeldin A (10 μg/mL) for 4 hours at 37°C to induce cytokine responses. After stimulation, red blood cells were lysed with FACS Lysing solution to obtain leukocytes (see the Methods section in this article's Online Repository at www.jacionline.org).

Cell-surface and intracellular staining on PBMCs and stimulated CD4 T cells in blood

Stimulated and nonstimulated blood cells were stained for fluorochrome-labeled antibodies to cell-surface markers (CD3, CD4, CD69, CLA, CCR4, and HLA-DR) and then permeabilized with FACS/perm to stain for cytokines, including IL-13, IL-22, IL-9, IFN-γ, and IL-17 (see the Methods section in this article's Online Repository).

Statistical analysis

Data were analyzed by using ANOVA, followed by contrast in cases of more than 2 groups or more than 1 factor. IgE values were $\log_{10}$ transformed before analysis. Variables were correlated by using Pearson correlation. Unsupervised clustering of variables was carried out with the average agglomeration strategy.

Multivariate scores (combining ≥2 variables) were obtained by using multivariate U-statistics[30] available in the mustat package in R software. This statistical approach can be considered a multivariate version of the Mann-Whitney test, with the objective of comparing groups (eg, patients vs control subjects) with respect to a selected subset of cytokines simultaneously. Because IFN-γ has different polarity than IL-22 and IL-17, μ scores were calculated with IFN-γ considered as a negative weight and IL-22 and IL-17 considered as positive weights.

Adjusted and nonadjusted P values appear in Table E2 in this article's Online Repository at www.jacionline.org.

RESULTS

ICS and flow cytometric analysis were used to measure IFN-γ–, IL-13–, IL-22–, IL-17A–, and IL-9–producing cell frequencies after being activated with PMA/ionomycin, defining T_H1/T_C1, T_H2/T_C2, T_H22/T_C22, T_H17/T_C17, and T_H9/T_C9 subsets in $CD4^+$ and $CD8^+$ T cells, respectively, in a cohort of 42 patients with AD and 25 control subjects. *Ex vivo* cell activation is required to detect cytokine production because less than 1% of nonstimulated cells produce cytokines.

We found that overall percentages of peripheral blood T_H1/T_C1, T_H22/T_C22, T_H17/T_C17, and T_H9/T_C9 T cells did not differ significantly between groups, irrespective of severity and IgE status (Table II). However, T_H2/T_C2 T cells showed higher frequencies in patients with severe and extrinsic AD, corresponding to the known role of IL-13 in IgE production.[31,32]

We then studied cytokine production according to skin-homing (CLA^+) $CD4^+$ and $CD8^+$ T-cell subsets.

$CD4^+$ and $CD8^+$ CLA^+ T-cell frequencies are expanded in patients with AD

Compared with control subjects, skin-homing CLA^+ $CD4^+$ and $CD8^+$ T-cell frequencies were expanded in patients with AD (5.4% vs 3% and 4.2% vs 2.4% respectively; $P = .03$), with particularly significant differences seen in patients with severe AD (CD4: 7.4% vs 3.3%, $P = .01$; CD8: 5.2% vs 2.4%, $P = .0008$). Similar results were obtained when accounting for total lymphocyte numbers (see Table E3 in this article's Online Repository at www.jacionline.org).