Letter to the Editor

Patients with atopic dermatitis and history of eczema herpeticum elicit herpes simplex virus–specific type 2 immune responses

To the Editor:

Atopic dermatitis (AD) is an inflammatory, relapsing skin disorder that affects 15% to 25% of children worldwide and persists in adulthood in around 25% of these cases.[1] About 3% to 8% of patients with AD seem to have a disturbance in viral clearance, manifesting severe forms of molluscum contagiosum, papilloma virus, and most prominently, the generalized cutaneous infection with herpes simplex virus 1 (HSV1), called eczema herpeticum (EH),[2,E1,E2] which can lead to life-threatening complications.[E3] The occurrence of EH is associated with a more severe AD disease according to disease scoring systems, total serum IgE, polyallergen sensitization, and other measures.[3,4] In addition, not only skin-affecting viral diseases but also respiratory tract infections, such as influenza, are more prevalent in patients with AD.[5,E4] Several studies point to a defect in the antiviral IFN-γ response and the skin barrier in patients with AD with a history of EH (ADEH+).[6-8]

In this study, we aimed to characterize virus-specific T-cell responses in patients suffering from AD with or without history of EH applying state-of-the-art detection systems, namely, measurements of IL-4 and IFN-γ cytokine expression in stimulated T-cell lines (TCLs), direct *ex vivo* enrichment and characterization of virus-specific $CD4^+$ and $CD8^+$ T cells via CD154 (CD40L) or CD137 (4-1BB), respectively, as well as specific detection of virus-specific $CD8^+$ T cells with MHC class I tetramers in combination with intracellular cytokine staining. For a detailed delineation of material and methods, please see this article's Online Repository at www.jacionline.org.

The analysis of the cytokines IL-4 and IFN-γ in supernatants of TCLs generated in the presence of HSV1 and influenza antigens and peptides revealed a significantly reduced antiviral IFN-γ response of patients with AD compared with TCLs from healthy controls. The significantly reduced IFN-γ production by TCLs from patients with AD grown in the presence of HSV1 protein glycoprotein D (gD), HSV1 immunodominant peptides, or the immunodominant peptides from influenza hemagglutinin was more pronounced in ADEH+ subjects than in patients with AD without a history of EH. No differences between the groups were observed when tetanus toxoid was used as a control antigen under similar experimental conditions (Fig 1, *A* and *B*, left). Interestingly, we found the secretion of the type 2 cytokine IL-4 to be significantly elevated in HSV1 gD-stimulated TCLs generated from patients with ADEH+ compared with healthy controls (Fig 1, *A* and *B*, right).

To investigate T cells directly *ex vivo*, we detected the upregulated expression of CD154 (CD40L) on $CD4^+$ cells after stimulation for analyzing virus-specific T cells in combination with a panel of surface markers to define T-cell subpopulations (see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). By this, we observed that the HSV1-specific cell numbers expressing the surface marker panel $CD4^+CD154^+CXCR3^+CCR4^-CCR6^-CCR10^-$, described as T_H1 in literature,[9] were not different from healthy controls. Interestingly, a pronounced increase in $CD4^+CD154^+CXCR3^-CCR4^+CCR6^-CCR10^-$ polarized cells, mentioned as T_H2,[9] in response to HSV1 was displayed (Fig 1, *C*). Although this effect was consistent among all stimuli in ADEH+ patients, it was less pronounced but observable by trend in ADEH− patients (Fig 1, *D*, *right*). The antigen response to influenza displayed differences in both investigated subsets. We detected a significantly reduced frequency of influenza hemagglutinin-specific $CXCR3^+CCR4^-CCR6^-CCR10^-T_H$ cells in patients with AD (Fig 1, *C*, left); however, no differences are apparent in the AD subgroups (Fig 1, *D,* left).

Similar to $CD4^+$ T cells, TCR-stimulated $CD8^+$ T cells can be identified by the expression of a surface marker, namely CD137 (4-1BB) (see Fig E1, *C* and *D*).[E5] Further on, cytotoxic T cells have been subgrouped into T_C subsets, with T_C1 expressing CXCR3,[E13] T_C2 expressing CCR4 but not CXCR3,[E13] and T_C17 expressing CCR6.[E14] In our hands, differences in the expression of these markers between patients and controls were detected specifically after stimulation with HSV1 but not after stimulation with influenza antigens (Fig 2, *A*). More precise, in patients with AD significantly less HSV1 gD and HSV1 peptide-specific cytotoxic T cells expressed the *bona fide* Tc1 marker panel $CD8^+CD137^+CXCR3^+CCR4^-CCR6^-$ (Fig 2, *A*, left). This effect was comparable in ADEH− and ADEH+ subjects (Fig 2, *B*, left). More interestingly, an increase in cells expressing the marker set described for T_C2 T cells ($CD8^+CD137^+CXCR3^-CCR4^+CCR6^-$) was observed in the response of ADEH+ patients to HSV1 peptides compared with healthy subjects (Fig 2, *A* and *B*, right).

Furthermore, we applied HSV1-specific MHC class I tetramers, harboring an immunodominant peptide from UL25.[E6] To gain adequate cells numbers, these were propagated *in vitro* for 14 days in the presence of the respective peptide before the staining (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). Detection of cytokines by intracellular staining revealed that UL25-specific T cells of healthy subjects respond nearly exclusively with IFN-γ and not with IL-4 production, whereas ADEH+ patients bear a substantial amount of T cells (median, 10.16% ± 15.11%) expressing IL-4. In parallel, $tetramer^+$ $IFN\text{-}\gamma^+$ cells were found to be reduced in ADEH+ patients by trend. Patients of the ADEH− group displayed an intermediate phenotype: we found slightly less IFN-γ and more IL-4–producing specific T cells compared with healthy individuals (Fig 2, *C*; for exemplary scatter plots see Fig E2, *B*). Importantly, these findings were observed by trend also directly *ex vivo* without *in vitro* T-cell proliferation (Fig E2, *C*).

Taken together, this is the first study indicating that a type 2 response by virus-specific T cells could be part of a complex pathology of EH and the susceptibility of patients with AD to HSV1. Applying different techniques, we were able to show differences in the response to viral antigens between healthy donors and patients suffering from AD. Within the patient cohort, the largest differences were consistently detectable within the subgroup of patients with a history of EH. The observed type 2–skewed phenotype leads to presumably inappropriate cytokine responses and eventually ineffective expansion.[E7] Noteworthy,

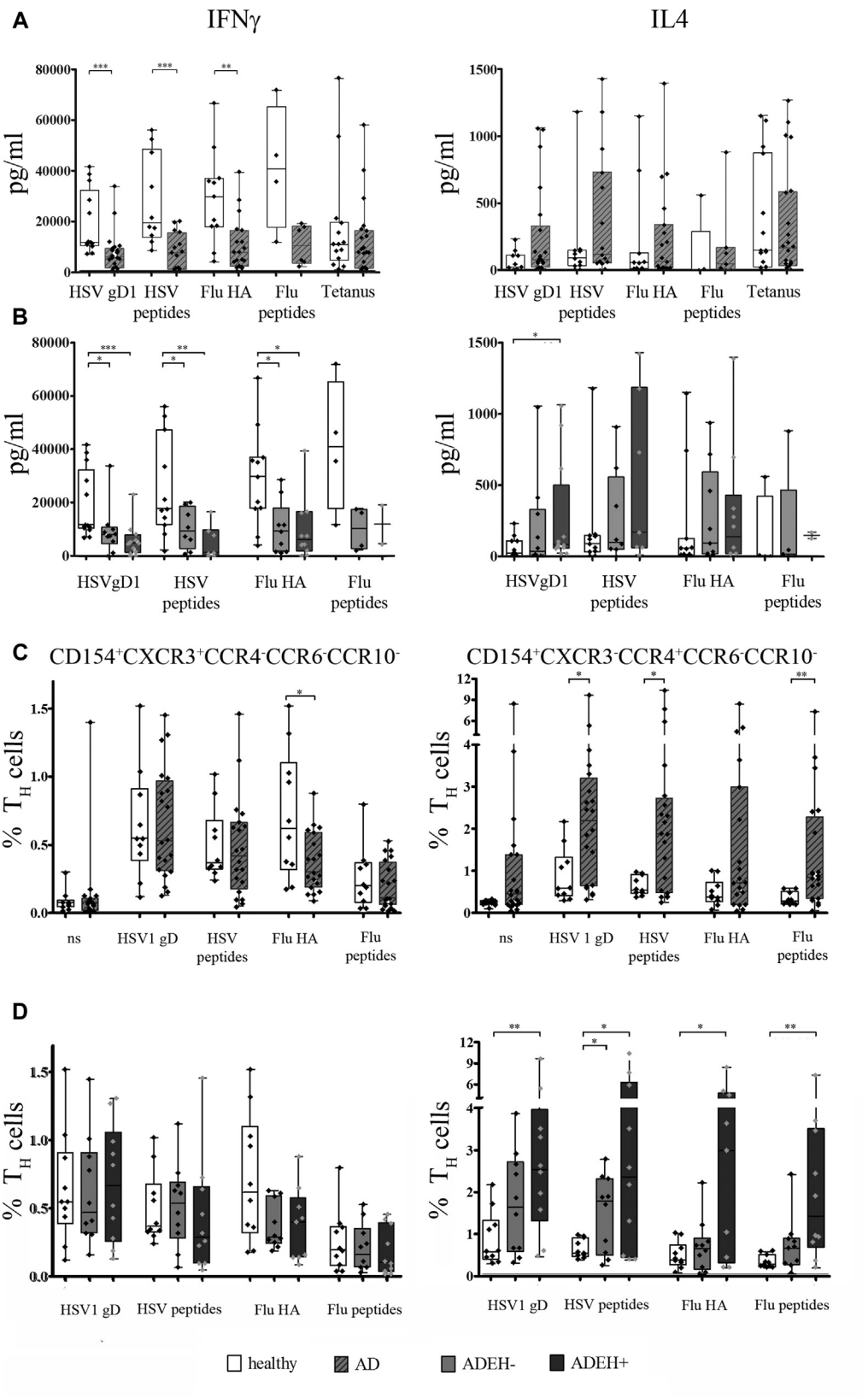

FIG 1. Cytokine production of TCLs and polarization analysis of virus-specific T_H cells. **A,** TCLs from patients with AD compared with healthy individuals (n = 16) were grown in the presence of antigens as indicated on the x-axis. Cytokines were detected by ELISA in cell culture supernatants. Only those TCLs are depicted that showed increased antigen-specific proliferation in restimulation testing. **B,** Patients with AD shown in Fig 1, *A*, were differentiated into ADEH− and ADEH+. **C** and **D,** Stimulated $CD4^+$ cells were detected by CD154 magnetic bead enrichment and characterized for CXCR3, CCR4, CCR6, and CCR10. **C,** Patients with AD compared with healthy individuals. **D,** Patients with AD were differentiated into ADEH− and ADEH+. *Flu*, Influenza.