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Patients with atopic dermatitis and history of eczema herpeticum elicit HSV-specific type 2 immune responses

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Capsule summary: An increased type 2 and in parallel decreased type 1 T cell immune response to herpes simplex virus 1 may lead to the clinical phenotype of eczema herpeticum.

Key words: Eczema herpeticum; Allergy; Atopy; Herpes; Influenza; Skin; T cells; T_H1; T_H2; T_C2.

Abbreviations: CCR, chemokine receptor (CC-motif); AD, atopic dermatitis; EH, eczema herpeticum; ADEH, atopic dermatitis with history of eczema herpeticum; gD, glycoprotein D; HA, hemagglutinin; HSV1, herpes simplex virus 1; PBMC, peripheral blood mononuclear cells; SCORAD, SCORing atopic dermatitis.

To the editor

Atopic dermatitis (AD) is an inflammatory, relapsing skin disorder which affects 15-25% of children worldwide and persists in adulthood in around 25% of these cases.¹ About 3-8% of AD patients 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, poly-allergen sensitization and other measures.^{3,4} Additionally, not only skin affecting viral diseases but also respiratory tract infections, such as influenza, are more prevalent in AD patients.^{5,E4} Several studies point to a defect in the antiviral IFN γ response and the skin barrier in AD patients 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 (TCL), 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 TCL generated in the presence of HSV1 and Influenza antigens and peptides revealed a significantly reduced antiviral IFN γ response of AD patients compared to TCL from healthy controls. The significantly reduced IFN γ production by TCL from AD patients grown in the presence of HSV1 protein gD, HSV1 immunodominant peptides or the immunodominant peptides from influenza hemagglutinin (HA) was more pronounced in ADEH+ subjects compared to AD patients without 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 T cell lines generated from ADEH+ patients compared to healthy controls (Fig 1, A and B, *right*).

In order 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 (Fig E1, A and B). 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 literature⁹, were not different from healthy controls. Interestingly, a pronounced increase of CD4⁺CD154⁺CXCR3⁺CCR4⁺CCR6⁺CCR10⁺ polarized cells, mentioned as T_H2⁹, in response to HSV1 was displayed (Fig 1, C). While 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 HA-specific CXCR3⁺CCR4⁺CCR6⁺CCR10⁺T_H cells in AD patients (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 2A). More precise, in AD patients 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 to healthy subjects (Fig 2, A and B, *right*).

Furthermore, we applied HSV1-specific MHC class I tetramers, harboring an immunodominant peptide from UL25.^{E6} In order to gain adequate cells numbers, these were propagated *in vitro* for 14d in presence of the respective peptide prior to the staining (Fig E2, A). 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, while ADEH+ patients bear a substantial amount of T cells (median=10.16% $\pm$ 15.11) expressing IL-4. In parallel, tetramer⁺ IFN γ ⁺ 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 to healthy individuals (Fig 2, C, 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 for the susceptibility of AD patients to HSV1. Applying different techniques, we were able to show differences in the response to viral antigens between healthy donors and patients suffering from atopic dermatitis. Within the patient cohort, the largest differences were consistently detectable within the subgroup of patients with a history of eczema herpeticum. The observed type2-skewed phenotype leads to presumably inappropriate cytokine responses and eventually ineffective expansion.^{E7} Noteworthy, these observed effects were not directly related to a more severe AD disease burden, an increased atopic predisposition, or higher age since the three groups were matched for age, total IgE, severity of AD (SCORAD) and Sx1 positivity (see Fig E3). Our finding of elevated type 2 and reduced type 1 T cell immune response to HSV1, in parallel with the discovery of increased IL-4 levels in specific CD8⁺ cells in patients with AD and in particular ADEH requires further investigation concerning their ability of viral defense. In summary, we demonstrate a type 2 immune profile in virus-specific T cells in AD that is more pronounced in ADEH+ patients which may contribute to a susceptibility to viral infections.

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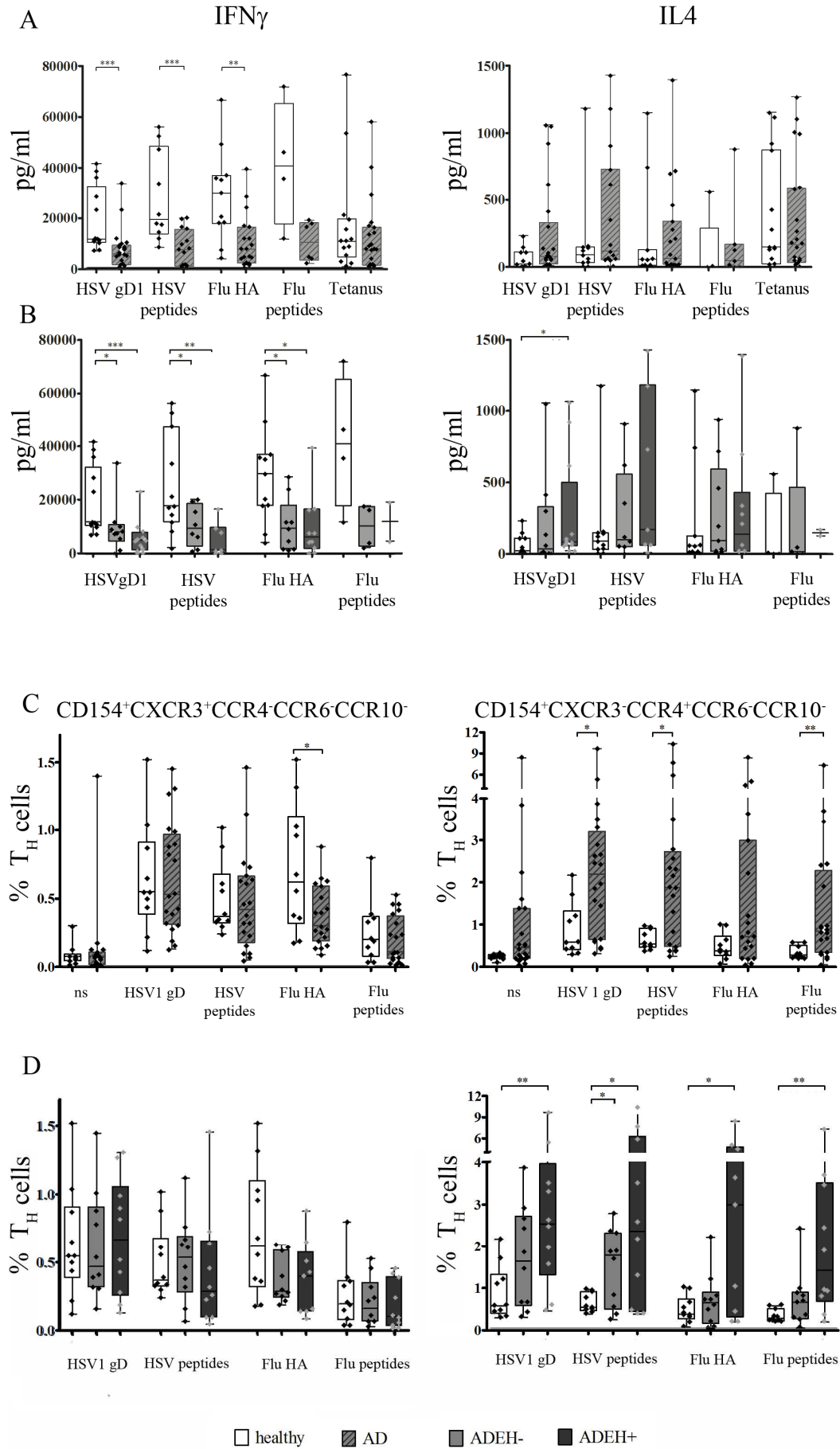
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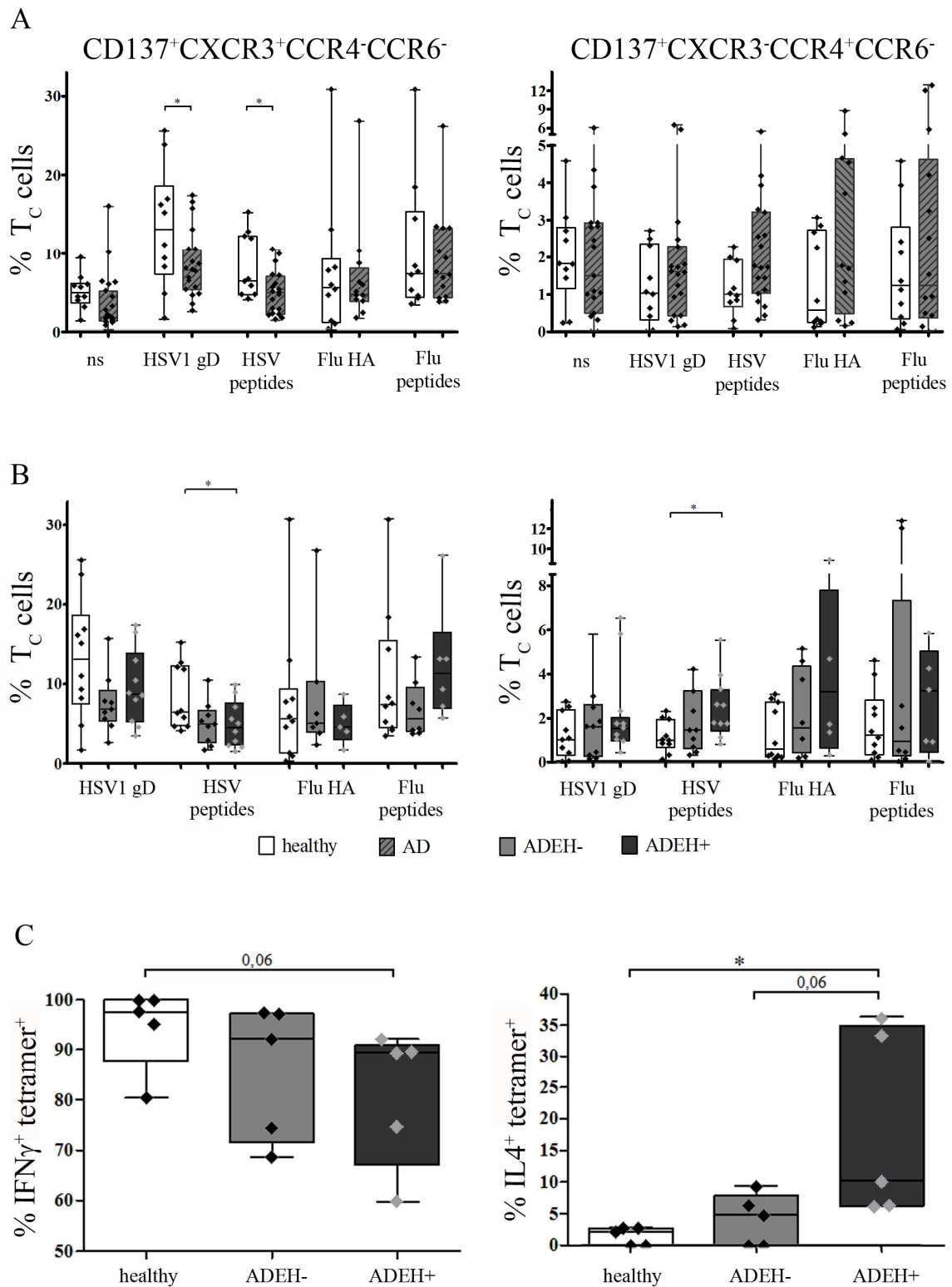
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Figures

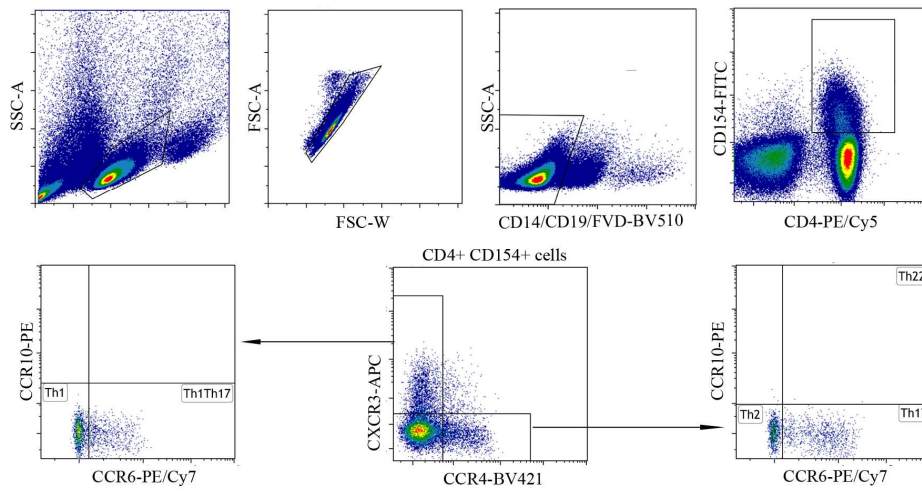
Fig 1. Cytokine production of T cell lines and polarization analysis of virus-specific T_H cells. **A**, T cell lines from AD patients compared to healthy individuals (n=16) were grown in the presence of antigens as indicated on the x-axis. Cytokines were detected by ELISA in cell culture supernatants. Only T cell lines are depicted that showed increased antigen-specific proliferation in re-stimulation testing. **B**, AD patients shown in 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**, AD patients compared to healthy individuals. **D**, AD patients were differentiated into ADEH- and ADEH+.

Fig 2. Polarization of virus-specific T_C cells and intracellular IL-4 and IFN γ staining of HSV1 UL25 MHC class I tetramer⁺ cells. **A** and **B**, Stimulated CD8⁺ cells were detected by CD137 PE magnetic bead enrichment and characterized for CXCR3, CCR4 and CCR6. **A**, AD patients compared to healthy individuals. **B**, AD patients shown in A were differentiated into ADEH- and ADEH+. **C**, Intracellular cytokine staining of healthy, ADEH- and ADEH+ donors for IFN γ and IL-4 in HSV1 UL25 MHC class I tetramer⁺ cells.

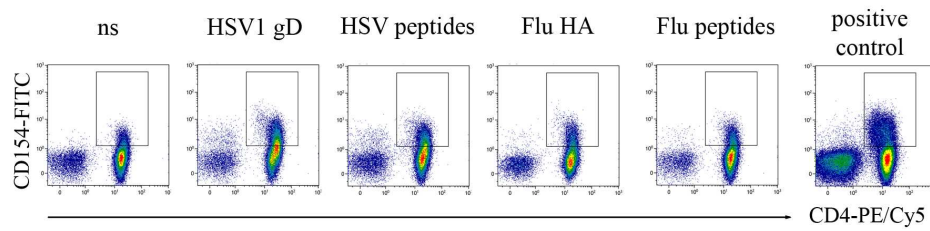




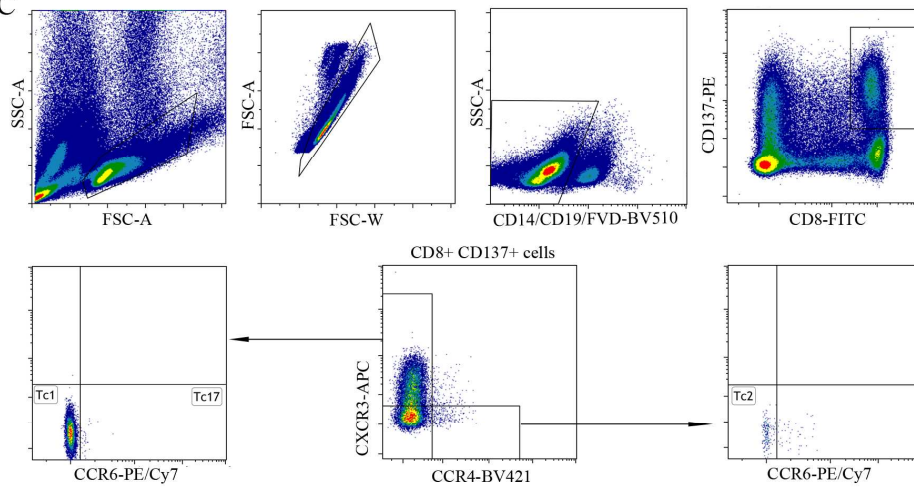
A



B



C



D

