

Exaggerated IDO1 expression and activity in Langerhans cells from patients with atopic dermatitis upon viral stimulation: a potential predictive biomarker for high risk of Eczema herpeticum

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To cite this article: Staudacher A, Hinz T, Novak N, von Bubnoff D, Bieber T. Exaggerated IDO1 expression and activity in Langerhans cells from patients with atopic dermatitis upon viral stimulation: a potential predictive biomarker for high risk of Eczema herpeticum. *Allergy* 2015; DOI: 10.1111/all.12699.

Keywords

atopic dermatitis; biomarker; Eczema herpeticum; herpes simplex virus; indoleamine 2,3-dioxygenase; Langerhans cells.

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Accepted for publication 17 July 2015

DOI:10.1111/all.12699

Edited by: Hans-Uwe Simon

Abstract

Background: Atopic dermatitis (AD) is a heterogeneous and highly complex disease characterized by an increased microbial colonization. For unknown reasons, a subgroup of patients with AD develops Eczema herpeticum (EH), a severe viral complication due to spreading of herpes simplex virus (HSV). Indoleamine 2,3-dioxygenase (IDO1) is a tryptophan (Trp)-catabolizing enzyme which is assumed to be instrumental in the antibacterial and antiviral defence mechanisms.

Methods: Comparative investigation of the IDO1 expression and activity in freshly isolated monocytes, plasmacytoid DC (pDC) and *in vitro*-generated Langerhans cells (LC) obtained from AD patients with HSV infections and EH and nonatopic controls.

Results: We demonstrate an increase in Trp degradation in the serum of patients during acute EH episodes. Circulating pDC from patients with history of EH display an increased IDO1 expression. An increased Trp degradation is detected in the supernatants of circulating monocytes from AD patients with acute EH. Mature LC from AD patients with history of EH and with acute EH display an increased IDO1 expression and activity, respectively. In LC from patients with history of EH, viral signals induce an exaggerated IDO1 expression and activity.

Conclusion: IDO1 expression and activity in LC seem to be involved in the pathophysiology of EH in AD and could represent a predictive biomarker for patients with risk to develop EH and other viral complications.

Abbreviations

AD, atopic dermatitis; APC, allophycocyanin; DC, dendritic cells; EH, Eczema herpeticum; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSV, herpes simplex virus; hTGF- β 1, human transforming growth factor beta1; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; Kyn, kynurenine; LC, Langerhans cells; LPS, lipopolysaccharide; mAb, monoclonal antibody; pDC, plasmacytoid dendritic cells; PE, phycoerythrin; Poly I:C, polyribinosinic-polyribocytidilic acid; TNF- α , tumour-necrosis factor- α ; Trp, tryptophan.

Atopic dermatitis (AD) is a complex and heterogeneous disease characterized by chronic eczematous skin lesions with a large clinical phenotype requesting adapted therapeutic approaches (1–5). AD is frequently aggravated by superinfections (1, 2). Eczema herpeticum (EH) is an uncommon but severe viral complication due to herpes simplex virus (HSV) type 1 or 2 which can be observed in a subset of patients with AD (6, 7). Recently, a number of risk factors have been identified which may predispose patients with AD to develop EH (8–11). Plasmacytoid dendritic cells (pDCs) have been identified as important components of the antiviral defence (12, 13), but they seem to be underrepresented

in lesional skin of AD (14). Subsets of myeloid dendritic cells (DCs) including epidermal Langerhans cells (LC) are postulated to be involved not only in the resistance towards but also in the spreading of viruses (15–17).

An interesting and multifaceted fashion of how DC contribute to immune homeostasis is the expression of the enzyme indoleamine 2,3-dioxygenase (IDO) (18–20) which metabolizes the essential amino acid tryptophan (Trp) as the first step in the so-called kynurenine (Kyn) pathway. The degradation of Trp to Kyn can be reached by three enzymes, that is, (i) the initially reported IDO1, (ii) its more recently identified analogous enzyme IDO2 and (iii) the tryptophan 2,3-dioxygenase (TDO). Each Trp-degrading enzyme seems to have distinct tissue localization and may be induced by distinct ways (18): While TDO regulates the systemic Trp level, IDO1 and IDO2 act more locally in the lymph nodes and the tissues. TDO is constitutively expressed in the liver and can be induced by distinct stimuli such as, for example, glucocorticosteroids. IDO2 is expressed in the tissue of liver, kidney, brain and in antigen-presenting cells. IDO1 is variably expressed in myeloid DC, while pDC can express both IDO1 and IDO2. IDO activity has been reported not only in a number of antigen-presenting cells, for example different DC subsets like CD8 α -positive DCs, CD103-positive DCs, CD19+/B220-/CD11c+ DCs and pDCs (21, 22), but also in epidermal LC (23). IDO activity degrades Trp locally, thereby lowering its concentration to an amount critical for T cells (19, 24) or pathogens (25). T cells are either halted in their proliferation or transformed into regulatory T cells (Tregs) (19). There is growing evidence for a role of the IDO1-induced Trp degradation as a broad antimicrobial effector (26, 27) in parasitic infections such as *Toxoplasma gondii* and bacterial infections such as *Listeria monocytogenes* (28) or *Staphylococcus aureus* (29, 30). Most importantly, IDO1 activity has been shown to be crucial in antiviral defence including HSV (27, 31, 32). In contrast, some viruses such as HIV, cytomegalovirus or EBV are also able to impair or exploit IDO1 activity (33, 34). Recently, IDO1 has been shown to be of importance in atopic disorders (35–37). This could be linked not only to the catalytic activity of IDO1 but

also to a signalling but nonenzymatic capability of IDO1 relevant for long-term tolerance (38).

In this study, we were interested to gain more insight into the regulation of IDO1 in antiviral defence on the background of HSV-1 infection in patients with AD. We hypothesized that IDO1 expression and/or function in myeloid DC may be critical for the control of HSV-1 infections in patients with AD at risk for recurrent episodes of EH. We therefore investigated the expression and function of IDO1 in circulating pDC and monocytes as well as in LC-like DC obtained from healthy nonatopic controls and AD patients with and without recurrent HSV-1 infections and from AD patients with history of EH as well as patients presenting with an acute episode of EH.

Material and methods

Recruitment of patients

Fifty-five healthy nonatopic controls and 104 patients with AD were recruited and subdivided into the following groups: healthy nonatopic controls without clinical HSV-1 infections (NA; $n = 33$); healthy nonatopic controls with recurrent HSV-1 infections (NA/HSV; $n = 22$) (recurrent HSV-1 infections have been defined as two or more episodes per year); AD patients without recurrent HSV-1 infections (AD; $n = 34$); AD patients with recurrent HSV-1 infections but without EH (AD/HSV; $n = 37$); AD patients with recurrent episodes of HSV-1 infections and with a history of EH (AD/HSV/EH history; $n = 19$); and AD patients with recurrent episodes of HSV-1 infections and presenting with an episode of acute EH (AD/HSV/EH acute; $n = 14$). Demographics in our collectives are shown on Table 1. The study was approved by the local ethics committee, and written informed consent was obtained from all participants of the study.

Reagents

The following monoclonal antibodies (mAb) were used for flow cytometric analysis. Phycoerythrin (PE)-labelled mon-

Table 1 Demographics of the enrolled controls and patients. Results are given as mean $\pm$ SD

Groups	Age	Sex ratio ♀/♂	Frequency HSV-1 infections	SCORAD	Total IgE	HSV IgG titre
NA ($n = 33$)	30.4 $\pm$ 10.6	21/12	–	–	–	1 : 5929 $\pm$ 9794
NA/HSV ($n = 22$)	32.1 $\pm$ 13	13/9	2.3 $\pm$ 1.3	–	–	1 : 16 959 $\pm$ 11 607
AD ($n = 34$)	29.5 $\pm$ 8.7	20/14	–	34.6 $\pm$ 9.7	1607 $\pm$ 3244	1 : 7644 $\pm$ 11 918
AD/HSV ($n = 37$)	34.3 $\pm$ 11.4	21/16	2.4 $\pm$ 1.6	31.2 $\pm$ 11	1627 $\pm$ 3528	1 : 17 860 $\pm$ 8345
AD/HSV/EH history ($n = 19$)	35.3 $\pm$ 9.9	4/15	1.7 $\pm$ 2.5	39.3 $\pm$ 11	4391 $\pm$ 6667	14 457 $\pm$ 11 743
AD/HSV/EH acute ($n = 14$)	29.2 $\pm$ 8.2	4/10	1.6 $\pm$ 2.9	47.2 $\pm$ 12.2	6245 $\pm$ 9022	14 392 $\pm$ 13 708

oclonal antibody (mAb) against CD14 (M5E2) (IgG2a κ), the PE-labelled mAb against CD123 (9F5) (IgG1 κ) and the isotype matched controls were purchased from Becton Dickinson (Heidelberg, Germany). PE-labelled mAb against Langerin (DCGM4) (IgG1) was from Coulter-Immunotech (Marseille, France) and allophycocyanin (APC)-labelled mAb against CD1a (HI149) (IgG1) from BD Biosciences (Heidelberg, Germany). Appropriate isotype controls were used. APC-labelled mAb against BDCA2 (AC144) (IgG1) was from Miltenyi Biotec (Bergisch Gladbach, Germany). 7-amino-actinomycin (7-AAD) was from Sigma (Deisenhofen, Germany). The mAb against IDO1 was a kind gift of Dr. Takikawa (Aichi, Japan). FITC-labelled F(ab')₂ of goat-anti-mouse Ab (GaM/FITC) and mouse serum were from Dianova (Hamburg, Germany). For the generation of LC-like DC, recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) was purchased from Novartis Pharma AG (Nürnberg, Germany), recombinant human interleukin (IL)-4 from Strathmann Biotech GmbH (Hannover, Germany) and recombinant human transforming growth factor beta1 (hTGF- β 1) was from R&D Systems (Wiesbaden-Nordenstadt, Germany).

Maturation of LC was induced with a cytokine cocktail consisting of tumour-necrosis factor-alpha (TNF- α , Sigma-Aldrich, Munich, Germany), IL-1 beta and IL-6 (R&D Systems) as reported previously (39). Stimulation was performed with the TLR3 agonist polyriboinosinic-polyribocytidilic acid (Poly I:C), Sigma (Munich, Germany) and the TLR4 ligand lipopolysaccharide (LPS) (Sigma). Both have been shown to induce IDO1 in myeloid DC (39).

Isolation of cells from peripheral blood

Monocytes were isolated from peripheral blood with a density gradient protocol using Nycoprep™ (Axis-Shield, Oslo, Norway) as reported in details elsewhere (40). CD14 expression was assessed by flow cytometry (FACSCanto™ flow cytometer and FACS Diva software (BD)) and was >90%. PBMC were isolated using density gradient centrifugation with Lymphoprep™ (Axis-Shield, Oslo, Norway) from heparin blood (30 min at 1000 *g*) (39). pDCs were identified by flow cytometry using mAb against CD123 and BDCA2.

Generation of Langerhans cell-like dendritic cells

Langerhans cell-like dendritic cells (LC-like DCs) were generated as reported in details elsewhere (41). Briefly, monocytes were cultured for 7 days in VLE-RPMI medium containing 500 IU/ml human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/ml; 500 IU/ml), human recombinant IL-4 (500U/ml) and natural hTGF- β 1 (10 ng/ml; 25U/ml). Medium was changed every second day adding half of the cytokine dose on day 2 and full cytokine dose on day 4 of culture. For maturation, LC-like DCs were cultured for further 24–48 h with a cocktail of pro-inflammatory cytokines containing 10 ng/ml TNF- α , 10 ng/ml IL-1 β and 5 ng/ml IL-6 (39, 42). For stimulation, the immature cells were cultured for 24–48 h with Poly I:C (25 μ g/ml) or 100 ng/ml LPS.

Flow cytometric analysis

The viable pDC, CD14+ monocytes or LC-like DCs populations were analysed for IDO1 expression by intracellular staining and analysis on a FACSCanto™ flow cytometer (BD) as reported in details elsewhere (39).

Assessment of IDO1 activity in serum and culture supernatants

Analysis of Trp and Kyn in the serum samples and cell supernatants was carried out as reported in details elsewhere (35, 39). IDO1 activity is expressed as the ratio Kyn/Trp $\times$ 1000.

Statistics

Statistical analysis was performed using the parametric *t*-test and nonparametric Mann–Whitney *U*-test. Correlations were calculated with the Pearson test. A *P*-value <0.05 was considered as statistically significant. Data were calculated with SPSS software (SPSS 20.0 and 22.0) for windows (SPSS, Inc, Chicago, IL, USA). Results are shown as means $\pm$ SD or boxplots with medians, 25th and 75th quartiles, minimum and maximum of distribution. Asterisks and circles mark outliers.

Results

Increased tryptophan degradation activity in serum of patients with AD presenting with acute EH

In a first approach, we explored the levels of Trp degradation in the serum of our patients and control individuals. When comparing NA and AD (excluding all individuals with HSV-1 infections and/or EH), there was no significant difference suggesting that AD *per se* is not characterized by an altered Trp degradation (data not shown). Similarly, there was no difference between individuals suffering from recurrent HSV-1 infections and those without, within the group of control or patients with AD. In contrast, a significantly higher level of Trp degradation was found in the serum of AD patients with acute EH when compared to all other groups with HSV infections (Fig. 1). From these experiments, we concluded that there is evidence for an involvement of IDO/TDO expressing cells during acute episodes of EH in patients with AD, raising the question of the source of IDO1 expression and activity in this condition.

IDO1 expression and activity in freshly isolated blood plasmacytoid cells and monocytes

The next set of experiments aimed to identify whether circulating plasmacytoid and myeloid cells may represent a source of the Trp degradation activity described above. Therefore, pDCs were analysed by flow cytometry after intracellular staining. Thereby, the overall IDO1 expression in pDC was low ($\leq$ 2% positive cells) and a significantly (*P* < 0.05) higher

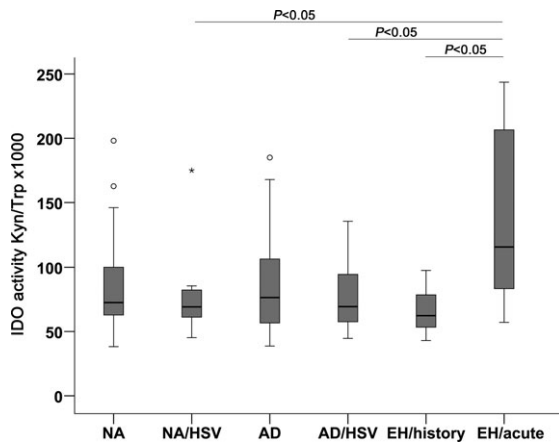


Figure 1 IDO activity in serum of patients and controls. IDO activity expressed as ratio of Kyn and Trp levels $\times 1000$ was detected by HPLC in serum of NA controls ($n = 26$), NA/HSV ($n = 12$), AD ($n = 24$), AD/HSV ($n = 17$), AD/HSV/EH history ($n = 12$); and AD/HSV/EH acute patients ($n = 11$). A P -value < 0.05 was considered statistically significant and is indicated accordingly. Circles and asterisks mark outliers.

expression was observed in AD patients with history of EH (Fig. 2A). Hence, although this finding confirms that pDCs are a source of IDO1, its relevance for the increased Trp degradation activity seen in the serum in the acute EH episode remains unlikely.

Monocytes are considered as the circulating precursor cells for tissue myeloid dendritic cells and important players in the defence against HSV infection. Therefore, we explored the IDO1 expression and function in freshly isolated monocytes. As for pDC, the overall expression was low (range from 2.5 to 5.6%). Although there was a trend for a higher IDO1 expression in monocytes from AD patients with recurrent HSV infections and patients with acute EH, the difference was not statistically significant (data not shown). However, the supernatants of monocytes from AD patients with acute EH had higher IDO1 activity compared with the other groups (Fig. 2B).

IDO1 expression and activity upon maturation of Langerhans cells

The above-reported experiments suggest that, despite the low IDO1 expression, monocytes have been either primed *in vivo* or alternatively have an intrinsic capacity to produce IDO1 when differentiated into DC. Therefore, the IDO1 expression and function were investigated in LC-like DC generated from circulating monocytes of these patients and controls as a surrogate for their tissue LC. After *in vitro* generation, the cells were investigated either as immature LC-like DC (at day 7) or after further 24–48 h culture in the presence or absence of a defined cytokine cocktail known to induce their maturation (39, 42). As shown in Fig. 3A, the IDO1 expression was low in all conditions at day 7, but was significantly increased in mature LC-like DC at day 8. This increase in expression was

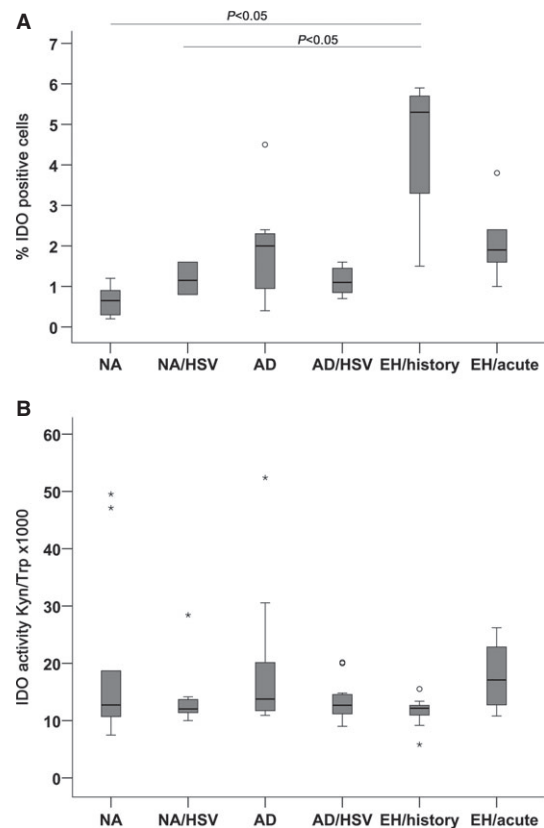


Figure 2 IDO expression in pDC (A) and IDO activity in monocytes (B). (A) IDO expression in pDC as determined by FACS analysis. Significantly higher IDO expression was found in AD/HSV/EH history ($n = 4$) but not in AD/HSV/EH acute patients ($n = 5$) P -value < 0.05 . (B) Increased IDO activity in the supernatants of monocytes from AD patients with acute EH analysed by HPLC. IDO activity is expressed as ratio of Kyn and Trp levels $\times 1000$.

particularly pronounced in cells from patients with history of EH. As expected, the IDO1 activity was also increased in mature LC-like DC, but the highest activity was measured in the supernatants from cells obtained from patients with acute EH (Fig. 3B).

Viral signal induces an exaggerated IDO1 expression and activity in Langerhans cells from AD patients with EH

In a next set of experiments, we subjected immature LC-like DC to stimulation with Poly I:C, mimicking a viral infection. As shown on Fig. 4A, this stimulation leads to an increase in IDO1 expression in these cells from all groups, but significantly more pronounced in AD patients with history of EH. IDO1 activity in the supernatants was significantly higher in patients with acute EH and history of EH (Fig. 4B), but unexpectedly also in nonatopic patients with recurrent HSV infections (data not shown).

To explore whether the phenomenon observed is specific to stimulation mimicking viral infection, we cultured the immature cells for 24 h to 48 h with LPS and measured the

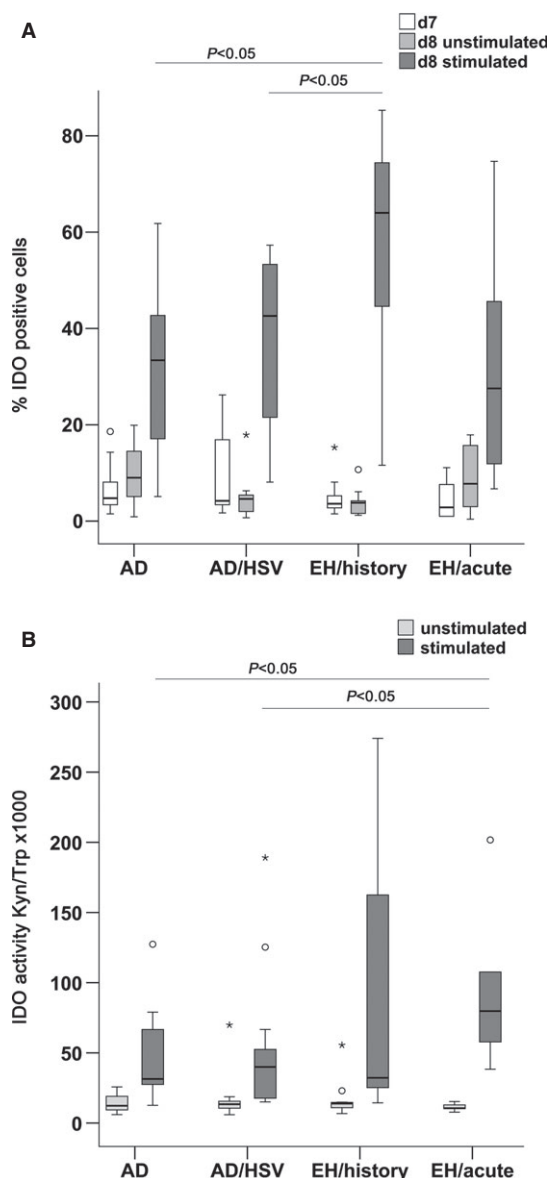


Figure 3 IDO expression (A) and activity (B) in LC-like DC after maturation. (A) Immature LC-like DC were stimulated with 10 ng/ml IL-1 β , 5 ng/ml IL-6 and 10 ng/ml TNF- α for 24 h and analysed by flow cytometry. IDO expression is shown in immature LC on day 7 (white), unstimulated cells on day 8 (light grey) and stimulated LC on day 8 (dark grey). The increase was most pronounced in AD/HSV/EH history patients ($n = 9$), and IDO expression was significantly higher in this group than in the other groups, P -value < 0.05 . (B) IDO activity in unstimulated control LC (light grey) and LC after incubation in 100 μ M Trp medium with the above-mentioned cytokines for 48 h (dark grey).

IDO1 expression and activity. As observed with Poly I:C, LC-like DC expressed an increased level of IDO1 in all groups. However, the increase tended to be higher in AD patients with HSV infections (Fig. 4C). Most importantly, the IDO1 activity in the supernatants was not significantly

increased in AD patients with EH history but only in acute EH (Fig. 4D). The IDO1 activity after Poly I:C or LPS stimulation correlated to the expression of the enzyme for all groups except for the nonatopic controls (see Table S1).

Discussion

The Kyn pathway has been suspected to be operative in a number of distinct diseases including allergic conditions and AD where a decreased IDO1 activity has been reported (43). In the present study, we could not find any significant differences comparing the Trp and Kyn levels of AD patients with those of healthy nonatopic controls. This might be due to the fact that we have carefully phenotyped our patients and focused on a particular group of patients with AD as a representative of atopic disorders. Alternatively, the cause might be attributable to the limited number of selected patients examined herein and the fact that AD is to be considered as a heterogeneous disease with a complex genetic and immunologic background.

EH is a severe viral complication which seems to affect only a subgroup of patients with AD. In this report, we have confirmed that pDCs are a potential source of IDO1 activity, but they do not seem to represent the major origin of the observed increased Trp degradation in our patients with acute episode of EH. This may also be the case in lesional skin of AD where pDCs have been shown to be absent (14). Thus, either hepatic TDO and/or some peripheral sources of IDO1 or IDO2 activity could explain the phenomenon observed in AD patients with acute EH. With this regard, we have shown herein that monocytes and myeloid DC such as LC may represent a significant source of IDO1 activity during EH episodes. However, it remains unclear whether the observed increased IDO1 activity is the cause or the result of the HSV spreading on the skin. If it is the result, it may represent an attempt to control exaggerated HSV infection and/or the damage caused to the tissue by immune cells during the course of this infection. On the other hand, exaggerated IDO1 may also contribute to the severe inflammation typically seen in these patients as this enzyme participates in a delicate balance between immunostimulatory and immunosuppressive signals mediated by DC. As microbial agents need up to 40-fold higher Trp concentrations than T cells, an exaggerated IDO1 activity may in a first step block the pathogens and in a second step during ongoing activation block also T cell proliferation and IFN production. This has been postulated previously (30) and represents a deleterious mechanism for efficient fighting against HSV. Therefore, the overactivation we observed in our LC may correspond to a similar phenomenon described in pDC and other cells which may locally or systemically contribute to inhibit antiviral T cell response (44, 45) in chronic viral infections and might already be initiated during the acute episodes of EH.

The most unexpected finding is the exaggerated IDO1 profile in LC-like DC from AD patients with history of EH when the cells are exposed to viral stimulation, while after exposure to bacterial signals, only AD patients with acute EH show a significant increase in IDO1 activity. This

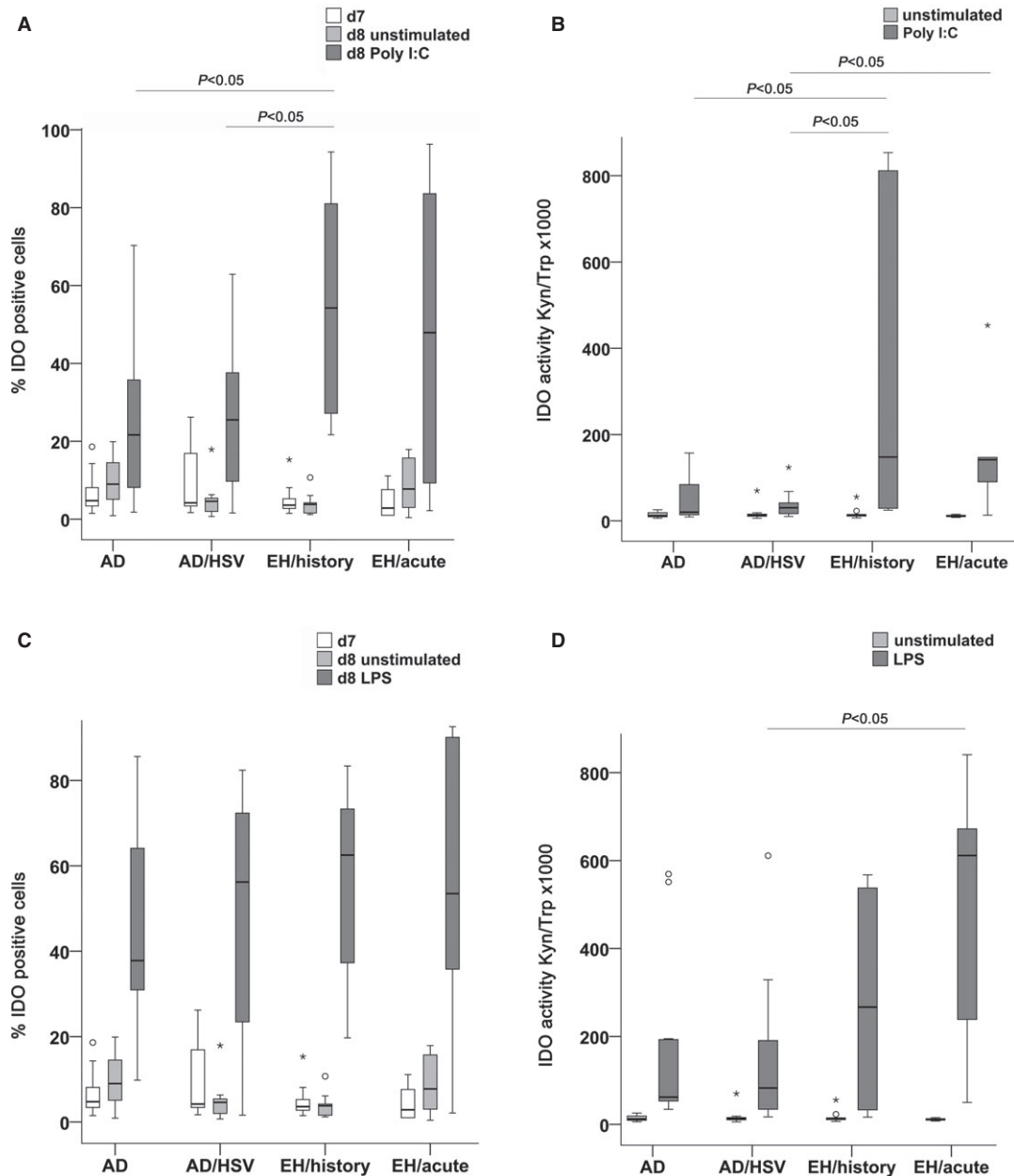


Figure 4 IDO expression and activity after stimulation with microbial stimuli. The IDO expression (A and C) and activity (B and D) was measured in LC upon stimulation with either Poly I:C (A and B) or LPS (C and D). (A) IDO expression in immature d7 cells (white), unstimulated cells on d8 (light grey) and after stimulation with Poly I:C (dark grey). Significant differences between the stimulated cells ($P < 0.05$) are marked appropriately. (B) IDO activity in supernatants of control LC

(light grey) and LC stimulated with Poly I:C (dark grey) incubated in 100 μ M Trp medium. A P -value < 0.05 is noted accordingly as statistically significant. (C) IDO expression in immature LC-like DC (d7 white), unstimulated d8 cells (light grey) and cells stimulated with LPS (dark grey) as analysed by flow cytometry. (D) IDO activity (Kyn/Trp $\times 1000$) in supernatants of LC after stimulation with LPS (dark grey) in comparison with unstimulated controls (light grey).

strongly argues for a specific predisposition in DC from these individuals when challenged by viral signals. Several mutually nonexcluding explanations can be considered. Firstly, this predisposition is genetically determined and would explain the increased vulnerability of a subgroup of patients with AD to HSV. Indeed, a number of single nucleotide polymor-

phisms in the gene encoding IDO1 have been described in relationship with infectious conditions (46, 47). Secondly, the overactivation of LC-like DC in these patients could be the result of an *in vivo* priming of the monocytes for an increased sensitivity to microbial signals. However, based on the fact that most of the patients with history of EH had their

episodes from several months up to several years before the time point we collected the blood samples, this would not be compatible with the limited half-life time of circulating monocytes which does not exceed a few days. Thirdly, another explanation may be related to a kind of 'memory' of previous episodes preparing the DC for subsequent EH episodes. This 'memory' could be explained by an epigenetic regulation of the IDO1 gene in myeloid lineage as it has been described in rheumatoid arthritis (48) and would potentially offer the opportunity to act therapeutically using agents able to modulate the methylation status of the responsible genes.

Whatever the reasons for the observed exaggerated IDO1 activity of LC in AD patients with EH may be, this feature seems to be specific for this subgroup of patients with AD. Hence, it may represent an interesting biomarker for the identification of patients at risk to develop severe viral complications such as EH but possibly also eczema vaccinatum, a life-threatening condition occurring in patients with AD in the context of small pox vaccination.

In conclusion, we have shown for the first time that AD patients with EH are characterized by an exaggerated IDO1 activity in myeloid DC secondary to viral signals. This phenomenon may represent a possible explanation for the lack of efficient antiviral defence seen in these patients and could potentially be considered as an interesting predictive

biomarker for patients at risk to develop severe viral complications.

Acknowledgments

We thank Dr. K. Iwamoto, Dr. M. Brenk, Dr. M. Reckmeyer, H. Wilms and K. Brendes (all Department of Dermatology and Allergy, University of Bonn) for excellent assistance. This work was supported in part by the NIH/NIAID contract N01 AI40029 and by the Christine-Kühne Center of Allergy Research and Education (CK-CARE).

Conflicts of interest

The authors have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation between the percentage of IDO expression in LC-like DC and the IDO activity measured as ratio Kyn/Trp. A *P*-value <0.05, as calculated by two-tailed probability, was considered statistically significant and noted with an asterisk.

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Supplemental material

Correlation between the percentage of IDO expression in LC-like DC and the IDO activity measured as ratio Kyn/Trp. A p-value < 0.05, as calculated by two-tailed probability, was considered statistically significant and noted with an asterisk.

Group	Stimulation with Poly I:C	Stimulation with LPS
NA	0.97 *	0.06
NA HSV	- 0.12	0.65 *
AD	0.96 *	0.71 *
AD HSV	0.43	0.61 *
AD EH his	0.79 *	0.67 *
AD acute EH	0.90 *	0.77