

Reduced IFN- γ receptor expression and attenuated IFN- γ response by dendritic cells in patients with atopic dermatitis

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Background: Atopic dermatitis (AD) is characterized by a predominance of T_H2 immune reactions but weaker T_H1 immune responses in acute skin lesions.

Objectives: To evaluate whether enhanced T_H2 immunity in patients with AD might impair T_H1 immune responses by affecting the IFN- γ responsiveness of antigen-presenting cells, we investigated IFN- γ receptor and IL-4 receptor α chain expression, IFN- γ signaling, and the expression of IFN- γ -responsive mediators in dendritic cells (DCs) and their precursors from patients with AD compared with those from healthy subjects.

Methods: Skin biopsy specimens were obtained and both monocytes and monocyte-derived dendritic cells (MoDCs) from patients with AD (n = 86) and control subjects (n = 84) were analyzed by means of flow cytometry, real-time PCR, ELISA, and HPLC.

Results: We observed lower IFN- γ receptor II expression combined with higher IL-4 receptor α chain expression on epidermal DCs, monocytes, and MoDCs from patients with AD. Induction of IFN- γ -inducible factors, such as interferon regulatory factor 1, interferon-inducible protein 10, and indoleamine 2,3-dioxygenase, was attenuated in IFN- γ -pulsed MoDCs from patients with AD. Weaker signal transducer and activator of transcription 1 activation mirrored by lower phosphorylated signal transducer and activator of transcription 1 levels in response to IFN- γ stimulation could be observed in epidermal DCs, monocytes, and MoDCs from patients with AD. **Conclusion:** Impaired IFN- γ signaling together with attenuated IFN- γ responses in DCs and their precursor cells might contribute to the T_H2 bias in patients with AD. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Monocytes, dendritic cells, IFN- γ , IFN- γ receptor, signal transducer and activator of transcription 1, atopic dermatitis

Abbreviations used

AD:	Atopic dermatitis
ADEH:	AD with a history of eczema herpeticum
DC:	Dendritic cell
FITC:	Fluorescein isothiocyanate
IDO:	Indoleamine 2,3-dioxygenase
IFN- γ R:	IFN- γ receptor
IL-4R α :	IL-4 receptor α chain
IP-10:	Interferon-inducible protein 10
IRF-1:	Interferon regulatory factor 1
MoDC:	Monocyte-derived dendritic cell
PE:	Phycoerythrin
p-STAT1:	Phosphorylated signal transducer and activator of transcription 1
STAT1:	Signal transducer and activator of transcription 1

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by dysregulated immune responses that feature increased systemic T_H2 responses combined with T_H2/T_H1 responses in the lesional skin.¹ Moreover, AD is associated with a strong genetic predisposition, cutaneous hyperreactivity to environmental stimuli, and susceptibility to skin infections, which influence the course and severity of AD.² Compared with healthy skin or nonlesional skin of patients with AD, acute skin lesions are marked by increased IL-4, IL-5, and IL-13 mRNA expression but low expression of IFN- γ and IL-12 mRNA, whereas chronic AD skin lesions show increased expression of IL-5, IL-12, and IFN- γ mRNA but lower IL-4 and IL-13 mRNA expression compared with that seen in acute AD skin lesions.^{3,4} In addition to T_H1 and T_H2 cells, T_H17 and T_H22 cells have been described to accumulate in the lesional skin of patients with AD and are supposed to play an important role in the pathogenesis of the disease.^{5,6} However, murine models provide evidence that an excess of T_H2 cytokines in the skin plays a crucial role in the manifestation of the disease.⁷ In this context it is known that IL-4 exerts downregulating effects on the IFN- γ response in patients with AD.⁸ Both cytokines, IFN- γ and IL-4, mediate their biological effects by signaling through specific receptors and thereby promote cell-mediated immune responses.⁹ The IL-4 receptor (IL-4R) complex is composed of the IL-4R α chain, which binds IL-4 with high affinity and associates with either the common γ chain or the IL-13 receptor α 1 subunit.¹⁰ The IFN- γ receptor (IFN- γ R) forms a heterodimer composed of 2 polypeptide chains, the ligand-binding IFN- γ R α chain (IFN- γ RI) and the signal-transducing IFN- γ R β chain (IFN- γ RII).¹¹ Although IFN- γ RI is constitutively expressed at moderate levels on the surface of nearly all cells, IFN- γ RII is expressed at extremely

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low levels, but its expression can be regulated in certain cell types by external stimuli, thus being a critical factor in the determination of IFN- γ responsiveness in these cells.¹² On IFN- γ binding, receptor units form dimers, leading to activation of Janus tyrosine kinase 1 and 2 and recruitment of signal transducer and activator of transcription 1 (STAT1). Phosphorylation of STAT1 at tyrosine 701 induces STAT1 dimerization and translocation to the nucleus, where transcription of IFN- γ -inducible genes is initiated.¹³ IFN- γ production is controlled by several transcription factors, among which interferon regulatory factor 1 (IRF-1) is of special importance.¹⁴ IRF-1 expression is induced by interferons and stimulates the production of IL-12, IL-18, and IL-23, thus favoring T_H1 immunity. Furthermore, IRF-1 binds to the IL-4 promoter and represses IL-4 transcription, thereby inhibiting T_H2 responses.¹⁵

Mechanisms contributing to the predominance of T_H2 immune reactions in patients with AD are only partially elucidated. Recent findings revealed a preferential apoptosis of circulating memory T_H1 cells, which express high levels of IFN- γ ,^{16,17} as well as a genetically predetermined T_H2 polarization of circulating T cells,¹⁸ which might contribute to the T_H2 bias in patients with AD. Additionally, mast cells, basophils, eosinophils, and natural killer T cells are capable of promoting T_H2 polarization through production of IL-4.^{19,20} Reduced IL-12 expression can be detected in acute AD skin lesions,⁴ implying reduced IFN- γ production and emphasizing further the hypothesis of a diminished T_H1 cell differentiation in patients with this disease. In line with this observation, PBMCs from patients with AD produce less IFN- γ after IL-12 stimulation^{8,21} but display increased IL-4 levels, as well as IL-4R mRNA expression, compared with that seen in healthy subjects.²² Higher expression of IL-4R α can further be observed on monocytes from patients with AD.²³

In the present study we aimed to evaluate whether enhanced T_H2 immunity in patients with AD might impair T_H1 immune responses by affecting the IFN- γ responsiveness of antigen-presenting cells. Therefore we investigated both IFN- γ R and IL-4R α expression, IFN- γ signaling, and the expression of IFN- γ -responsive mediators in monocytes, monocyte-derived dendritic cells (MoDCs), and epidermal dendritic cells (DCs) from patients with AD compared with healthy subjects.

METHODS

Reagents

Unconjugated mAb against IFN- γ R1 was from R&D Systems (Minneapolis, Minn), and mAb against IFN- γ R2 was from PBL Interferon Source (Piscataway, NJ). mAb against indoleamine 2,3-dioxygenase (IDO) was purchased from Oriental Yeast Co, Ltd (Tokyo, Japan). Phycoerythrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-CD14 and anti-IL-4R α (CD124), PE-Cy5-conjugated monoclonal anti-CD1a, and allophycocyanin-Cy7-labeled anti-CD14 were from BD Systems (San Jose, Calif). PE-conjugated mAb against CD1a and langerin were purchased from Beckman Coulter (Fullerton, Calif). Rabbit IgG as an isotype control, FITC-conjugated F(ab')₂ of goat anti-mouse IgG (H+L) (GaM/FITC) and of donkey anti-rabbit IgG (H+L) (DaRab/FITC), normal murine serum, normal goat serum, and normal rabbit serum for blocking purposes were obtained from Jackson ImmunoResearch (West Grove, Pa). Recombinant IFN- γ was purchased from PeproTech (Rocky Hill, NJ). All other reagents were obtained from Sigma-Aldrich (St Louis, Mo) unless otherwise indicated. The trypsin sensitivity of the primary antibodies used for flow cytometric staining of epidermal skin cells was tested.

Patients

After local ethics committee approval and written informed consent of the patient were obtained, peripheral blood and shave biopsy specimens were taken from patients with AD ($n = 86$; age, 34.7 ± 12.9 years; total IgE, 3411.7 ± 6514.5 kU/L; SCORAD score, 44.1 ± 20.2) at the Department of Dermatology and Allergy of the University of Bonn. AD was diagnosed according to the criteria of Hanifin and Rajka.²⁴ Additionally, blood and skin samples were taken from control subjects without AD ($n = 84$).

Preparation of skin cell suspensions

After achievement of local anesthesia, skin shavings from patients with AD or split-thickness skin specimens from healthy donors were taken, and single-cell suspensions were prepared by means of enzyme digestion, as described elsewhere.²⁵

Monocyte isolation and generation of MoDCs

Monocytes were isolated from peripheral blood with a modified density gradient protocol by using Nycoprep (Progen Biotechnik GmbH, Heidelberg, Germany), as described previously.^{26,27} Monocytes were cultured for 6 days in VLE-RPMI 1640 (Biochrom AG, Berlin, Germany) with 1% antibiotics-antimycotics and 10% inactivated FCS, 500 U/mL GM-CSF (Berlex Laboratories, Richmond, Calif), and 500 U/mL IL-4 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and were fed every second day with half of the cytokine dosage to generate MoDCs. MoDCs were stimulated with the indicated IFN- γ concentration in the presence of 500 U/mL GM-CSF and IL-4.

Flow cytometric analysis

Extracellular staining of IL-4R α , IFN- γ R1, and IFN- γ R2 was performed, as reported in detail elsewhere.²⁸ Intracellular staining of phosphorylated signal transducer and activator of transcription 1 (p-STAT1) and STAT1 was conducted as previously described.¹³ Briefly, unstimulated or IFN- γ -pulsed (100 or 1000 U/mL) cells were fixed in 1.5% formaldehyde, permeabilized with ice-cold methanol, and washed twice with staining media (1% BSA in PBS). Cells were resuspended in staining media with 5% (vol/vol) goat serum for blocking. After 20 minutes, primary antibodies against p-STAT1 (Tyr701; Cell Signaling Technology, Danvers, Mass) or STAT1 (Santa Cruz Biotechnology, Santa Cruz, Calif) were added directly into the blocking solution for another 30 minutes. After washing, cells were treated with DaRab/FITC for 30 minutes. Epidermal skin cells and MoDCs were incubated with PE-conjugated anti-CD1a after a second blocking step with rabbit serum. Monocytes were gated physically according to forward- and side-scatter characteristics. Intracellular IDO staining of CD1a⁺ MoDCs was performed as reported in detail elsewhere.²⁹ Stained cells were acquired on a FACSCanto (BD Biosciences, Heidelberg, Germany) and analyzed with FACSDiva (BD Biosciences) or FlowJo (TreeStar, Inc, Ashland, Ore) software. Cell-surface expression of IL-4R α , IFN- γ R1, and IFN- γ R2 and intracellular IDO expression were determined by using the relative fluorescence index $\pm$ SEM as follows:

$$\text{Relative fluorescence index} = \frac{\text{MFI} [\text{receptor}] - \text{MFI} [\text{isotype control}]}{\text{MFI} [\text{isotype control}]},$$

whereas p-STAT1 and STAT1 levels were determined as the percentage of positive cells. Phosphorylation of STAT1 was quantified as the ratio of p-STAT1/STAT1 (IFN- γ treated) relative to p-STAT1/STAT1 (nontreated). Results are shown as box plots.

Real time-PCR

RNA was isolated with the NucleoSpin RNA II Kit (Macherey-Nagel GmbH & Co KG, Dürren, Germany), including digestion of genomic DNA, and subjected to cDNA synthesis with TaqMan reverse transcription reagents according to the manufacturer's instructions (Applied Biosystems, Foster City, Calif). The cDNA was amplified with the TaqMan Gene Expression Master Mix (Applied Biosystems), according to the recommendations of the

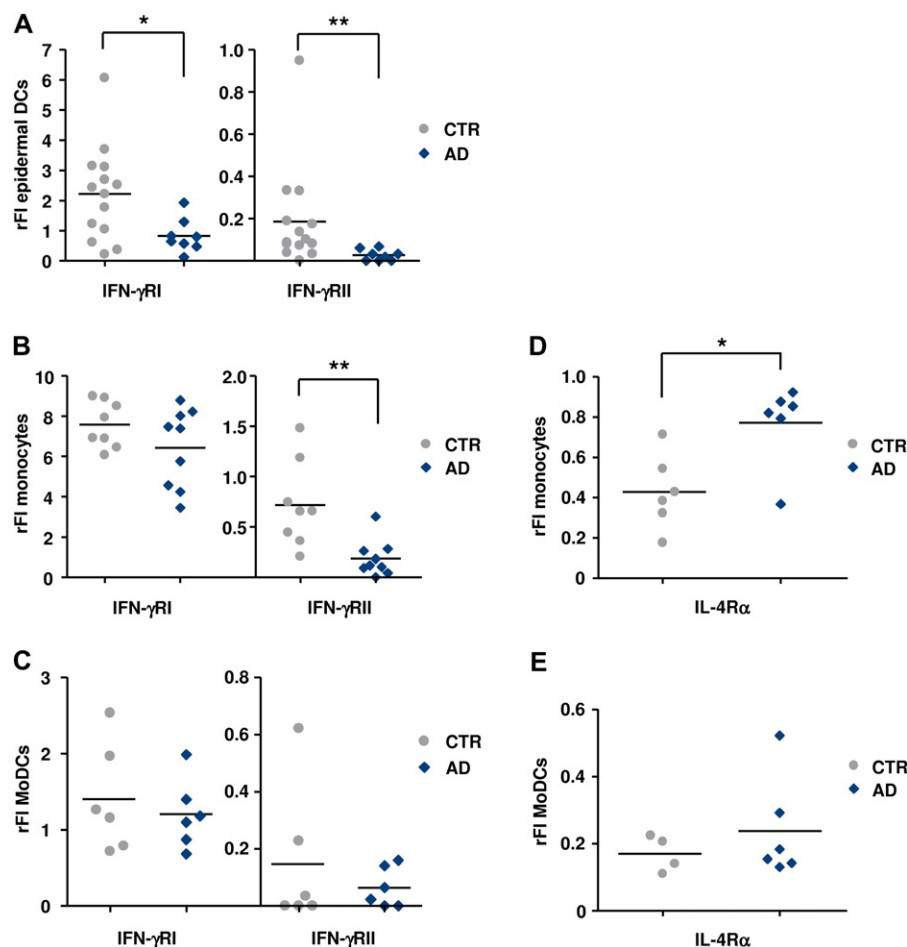


FIG 1. Flow cytometric analysis of cell-surface IFN-γRI and IFN-γRII expression on epidermal skin DCs (A), monocytes (B), and MoDCs (C) and cell-surface IL-4Rα expression on monocytes (D) and MoDCs (E) from control donors (CTR; gray circles) and patients with AD (blue squares) are shown as scatter plots.

manufacturer, in an ABI Prism 7300 Sequence Detection System (Applied Biosystems). Primers for IDO, interferon-inducible protein 10 (IP-10), and IRF-1, as well as endogenous controls (18s), were from Applied Biosystems. Relative quantification and calculation of the range of confidence was performed by using comparative $\Delta\Delta CT$ methods.³⁰ All analyses were conducted in duplicates, and experimental samples were quantitated by using sample dilutions, ensuring that the cycle threshold values were in the linear proportion of the standard curve. Results are given as the fold mRNA induction normalized to the unstimulated control condition and are displayed as box plots.

ELISA

For quantification of IP-10/CXCL10 protein concentration in the cell-culture medium, MoDCs were harvested 18 hours after stimulation with IFN-γ (0, 100, and 1000 U/mL), and culture supernatants were obtained and stored immediately at -70°C . ELISA was conducted according to the manufacturer's instructions (PeproTech, Rocky Hill, NJ). Results are depicted as box plots.

HPLC

MoDCs were stimulated with IFN-γ (0, 100, and 1000 U/mL) in VLE-RPMI media containing 100 $\mu\text{mol/L}$ tryptophan for 24 hours. Cells were washed with PBS and harvested for intracellular IDO staining. Culture supernatants were collected and stored at -70°C for quantification of tryptophan and kynurenine concentrations by means of HPLC. Sample

preparation and HPLC analysis were performed as reported in detail elsewhere.³¹ Tryptophan degradation was quantified as the ratio of kynurenine to tryptophan expression. Results are given as the ratio of kynurenine/tryptophan (IFN-γ treated) relative to kynurenine/tryptophan (nontreated) and are depicted as box plots.

Statistical analysis

Statistical analysis was performed with SPSS 17.0 software (SPSS, Inc, Chicago, Ill). Quantitative values were compared between different patient groups (2 independent samples) by using the Mann-Whitney U test. Results are shown as means $\pm$ SEMs or box plots referring to sample medians and sample first and third quartiles. Outliers are marked as circles and asterisks. Any P values are 2-sided and subject to a global significance level of less than .05 or less than .005.

RESULTS

Lower IFN-γ receptor but higher IL-4Rα expression on antigen-presenting cells in patients with AD

Data on the expression of immune-regulatory receptors on DCs and DC precursor cells in the skin and blood of patients with AD is rare.³² Therefore we investigated the expression of IFN-γRI and IFN-γRII in the blood and skin of patients with AD compared with healthy control subjects.

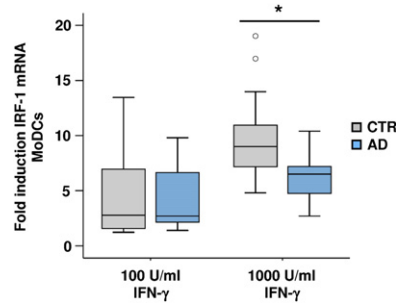


FIG 2. Relative expression of IRF-1 mRNA after IFN- γ stimulation of MoDCs from control subjects (CTR; gray bars, $n = 18$) and patients with AD (blue bars, $n = 11$) is shown.

We observed significantly lower expression of IFN- γ R2 on both epidermal DCs (Fig 1, A) and monocytes (Fig 1, B) from patients with AD. IFN- γ R1 expression on epidermal DCs from patients with AD was significantly lower as well. IFN- γ R2 expression was slightly decreased on MoDCs from patients with AD compared with that seen in control subjects (Fig 1, C), whereas IL-4R α expression was slightly increased on these cells (Fig 1, E). Furthermore, monocytes from patients with AD showed significantly higher cell-surface expression of IL-4R α (Fig 1, D).

Diminished IFN- γ response in MoDCs from patients with AD

To investigate whether the expression of genes known to be regulated by IFN- γ is distinct, we analyzed the expression of mRNA transcripts of IRF-1, IP-10/CXCL10, and IDO in MoDCs from patients with AD in relation to that seen in healthy control subjects.

We observed a significantly lower IRF-1 mRNA expression in MoDCs from patients with AD after stimulation of the cells with high concentrations of IFN- γ (Fig 2). Moreover, stimulation of MoDCs with 1000 U/mL IFN- γ tended to induce a weaker expression of IP-10/CXCL10 mRNA in patients with AD compared with that seen in control subjects ($P = .068$; Fig 3, A). In parallel, a significantly lower IP-10/CXCL10 protein concentration was detectable in supernatants of MoDCs from patients with AD after stimulation of the cells with 1000 U/mL IFN- γ (Fig 3, B). Additionally, a significantly lower expression of IDO mRNA after stimulation of MoDCs with IFN- γ at high doses (Fig 4, A) was detected in patients with AD. In parallel, intracellular IDO protein expression was significantly lower in MoDCs from patients with AD stimulated with low and high doses of IFN- γ (Fig 4, B). This went along with a significantly diminished capacity of these cells to degrade tryptophan into its metabolite kynurenine after stimulation of the cells with both low and high doses of IFN- γ (Fig 4, C). Notably, baseline levels of kynurenine/tryptophan were higher in the group of patients with AD compared with those seen in control subjects (data not shown). However, after stimulation of the cells with IFN- γ , tryptophan degradation relative to the nonstimulated condition was higher in the control group.

Together, these data indicate attenuated induction of the IFN- γ -regulated factors IRF-1, IP-10/CXCL10, and IDO in DCs from patients with AD.

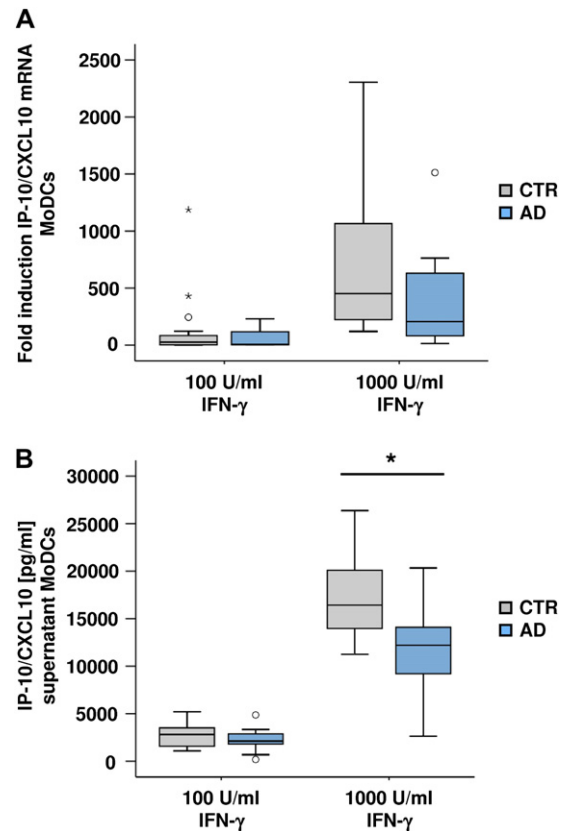


FIG 3. Relative expression of IP-10/CXCL10 mRNA after IFN- γ stimulation of MoDCs from control subjects (CTR; gray bars, $n = 18$) and patients with AD (blue bars, $n = 11$) is shown (A). IP-10/CXCL10 concentration in the cell-culture supernatant of IFN- γ -pulsed MoDCs ($n = 11$) was measured by means of ELISA (B). IP-10/CXCL10 concentration in the cell-culture supernatant of unstimulated MoDCs was less than the detection minimum.

Attenuated IFN- γ signaling in patients with AD

To determine whether a lower IFN- γ R2 expression in patients with AD could alter IFN- γ -mediated signal transduction, we examined the phosphorylation of the signaling protein STAT1 in epidermal skin DCs, monocytes, and MoDCs pulsed with high and low doses of IFN- γ .

Stimulation of epidermal skin cells with IFN- γ induced a weaker p-STAT1/STAT1 ratio in response to IFN- γ treatment relative to the unstimulated control condition (Fig 5, A). Monocytes from patients with AD revealed lower p-STAT1/STAT1 levels after stimulation with IFN- γ (Fig 5, B). After stimulation of MoDCs with IFN- γ , we observed reduced levels of p-STAT1/STAT1 in the group of patients with AD as well (Fig 5, C). In general, we observed a significantly lower constitutive expression of STAT1 in epidermal DCs from patients with AD (data not shown), which could in part result in a lower p-STAT1/STAT1 ratio after stimulation of the cells with IFN- γ . A lower constitutive STAT1 level could not be observed in monocytes and MoDCs from patients with AD.

DISCUSSION

In this study we observed significantly lower expression of IFN- γ R1 and IFN- γ R2 on epidermal skin DCs, as well as significantly lower IFN- γ R2 expression on monocytes from

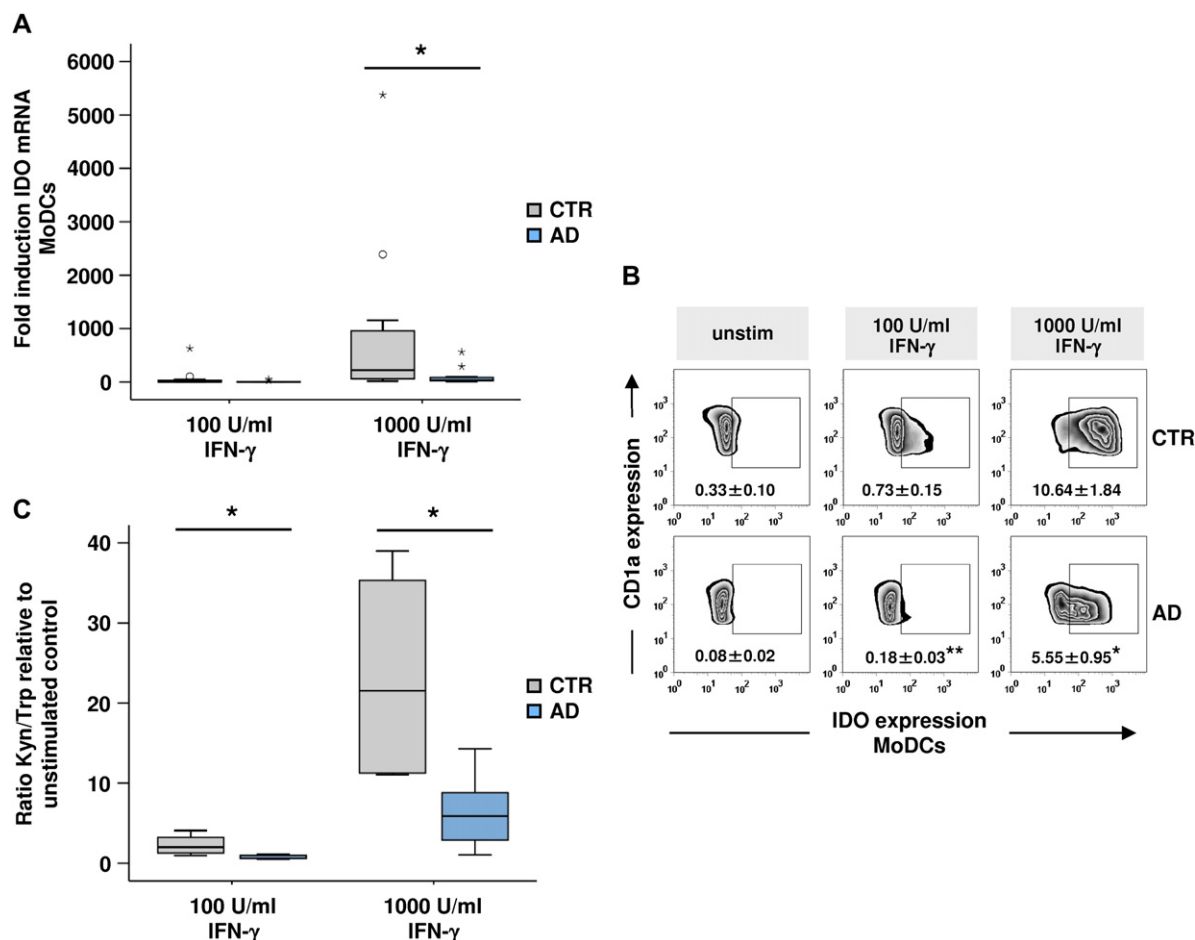


FIG 4. Relative expression of IDO mRNA after IFN- γ stimulation of MoDCs from control subjects (CTR; gray bars, $n = 18$) and patients with AD (blue bars, $n = 11$) is shown (A). Flow cytometric analysis of intracellular IDO expression in IFN- γ -pulsed MoDCs of CTR subjects (top) and patients with AD (bottom) is shown as density blots (B). Representative blots of 6 (CTR) and 9 (AD) experiments are shown. HPLC analysis of MoDC cell culture supernatants quantifying tryptophan (*trp*) degradation into its metabolite kynurenine (*kyn*) in CTR donors (gray line, $n = 4$) and patients with AD (blue line, $n = 9$) is shown (C).

patients with AD, which went along with higher IL-4R α expression on monocytes from patients with AD. We observed attenuated induction of IFN- γ -inducible factors, such as IRF-1, IP-10/CXCL10, and IDO in MoDCs, as well as weaker STAT1 activation mirrored by lower p-STAT1 levels, in patients with AD in response to IFN- γ stimulation.

Because IFN- γ RII represents the limiting factor for IFN- γ responsiveness, low IFN- γ RII cell-surface expression results in low expression of active or functional IFN- γ R.³³ Low expression of functional IFN- γ R might represent an important regulator of attenuated IFN- γ signaling in monocytes, MoDCs, and epidermal DCs from patients with AD. In this regard a recent study reports lower IFN- γ and IFN- γ receptor gene expression in PBMCs from patients with AD with a history of eczema herpeticum (ADEH) compared with that seen in patients without ADEH and nonatopic control subjects.³² Furthermore, single nucleotide polymorphisms in the *IFNG* and *IFNGR1* genes were significantly associated with ADEH, which might impair the antiviral response and increase the risk of ADEH. In addition, it is very likely that other cofactors, such as characteristic cytokines of the current micromilieu, might play a role in modified IFN- γ signaling in patients with

AD. In particular, the T_H2-polarizing cytokine IL-4, which is well known for its antagonistic relationship to IFN- γ ,³⁴ might negatively affect the induction of IFN- γ -inducible genes in patients with AD, and this might be of critical immunologic importance in view of the higher IL-4R α expression of monocytes from patients with AD observed here. In this regard previous studies revealed downregulated IFN- γ -inducible gene expression of IRF-1 mRNA in monocytes and macrophages by IL-4.³⁵ IRF-1 is crucial for the production of IFN- γ and binds to the IL-4 promoter, thus repressing IL-4 transcription while supporting T_H1 immunity.¹⁵ Therefore we suppose that reduced IRF-1 expression in patients with AD might favor T_H2 immune reactions while attenuating T_H1 responses. Moreover, genetic variations in the *IRF1* gene have been identified that might affect the development of atopy, potentially by altered transcription factor binding in the proximal promoter of the *IRF1* gene, changing *IRF1* gene expression and IFN- γ regulation.¹⁴ This is in accordance with other observations demonstrating that mice deficient in *IRF1* (*IRF1*^{-/-}) have a profound, gene dose-dependent decrease in IFN- γ production in case of *Leishmania major* infection, a disease known to provoke T_H1 immune responses in normal mice.³⁶ In addition, T cells

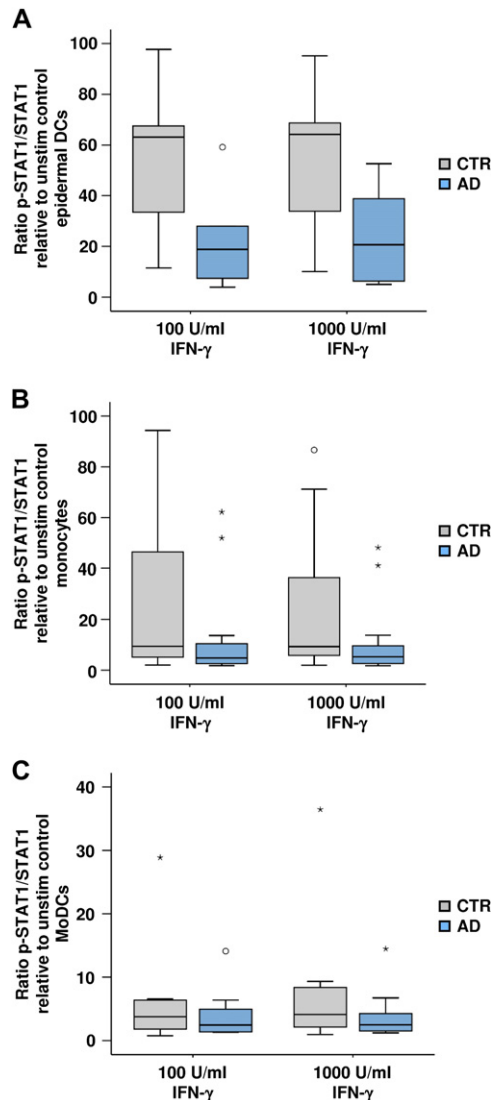


FIG 5. Epidermal skin DCs (n = 6, **A**), monocytes (n = 15 and 16, respectively; **B**), and MoDCs (n = 10, **C**) from CTR donors (gray bars) and patients with AD (blue bars) were treated with the indicated IFN- γ concentrations and analyzed for intracellular STAT1 and p-STAT1 levels by means of flow cytometry.

from mice lacking IRF-1 do not mount T_H1 responses and instead undergo T_H2 differentiation.³⁷

The lower mRNA expression of IP-10/CXCL10 by MoDCs from patients with AD is in line with previous studies, which revealed reduced IP-10/CXCL10 expression in IFN- γ -stimulated keratinocytes from patients with AD compared with that seen in healthy donors and patients with psoriasis.³⁸ IP-10/CXCL10 acts as a chemoattractant for T_H1 cells and monocytes and natural killer T cells expressing the chemokine receptor CXCR3.^{39,40} Decreased IP-10/CXCL10 levels, as we observed in supernatants of IFN- γ -pulsed MoDCs from patients with AD, might therefore result in reduced recruitment of CXCR3⁺ T_H1 cells to the skin *in vivo*, impeding T_H1 immune responses through diminished IFN- γ expression.

In addition to lower IDO mRNA expression in response to IFN- γ , we observed significantly lower intracellular IDO protein expression in MoDCs from patients with AD. In parallel,

significantly diminished degradation of tryptophan into its metabolite kynurenine was detectable. IDO expression in immune cells, including DCs, is regulated and mainly induced by cytokines, such as IFN- γ .^{41,42} STAT1 and IRF-1 were shown to act cooperatively to mediate this process.⁴³ Mice lacking either IFN- γ or IRF-1 are deficient in IDO expression during infection.⁴⁴ This observation is in line with our data, which imply that weaker IDO expression of MoDCs from patients with AD might result secondarily from lower STAT1 phosphorylation, as well as lower IRF-1 expression, in response to IFN- γ stimulation.

Moreover, reduced phosphorylation of STAT1 after IFN- γ stimulation of monocytes and epidermal DCs from patients with AD indicates that attenuated IFN- γ -mediated downstream signaling might be a reason for the lower induction of IFN- γ -responsive elements in patients with AD. Previous studies provided evidence that PBMCs from atopic subjects display lower STAT1 phosphorylation, as well as lower STAT1 baseline expression, than seen in nonatopic subjects.⁴⁵

It is of note that attenuated IRF-1, IP-10/CXCL10, and IDO mRNA expression in MoDCs was only detectable after application of higher doses of IFN- γ , whereas stimulation of the cells with low doses of IFN- γ did not lead to any significantly different expression. This supports our hypothesis of an interaction of the antagonizing cytokines IL-4 and IFN- γ in the cellular micro-milieu as an important comodulator of IFN- γ signaling in MoDCs.

Taken together, our findings provide evidence for an attenuated IFN- γ responsiveness in patients with AD mediated by lower IFN- γ R expression, as well as attenuated IFN- γ signaling of DCs and their precursor cells, which might sustain the T_H2 predominance in acute flares while impairing T_H1 immunity in this disease. We propose a model of interactive immune regulation in the skin of patients with AD, where an excess of IL-4 caused by the increased T_H2 response and the absence of counterregulatory IFN- γ in the acute phase might impair T_H1 immunity by affecting the IFN- γ responsiveness of antigen-presenting cells.

Clinical implications: Our findings might open new perspectives for the design of therapeutic strategies aimed at restoring modified T_H1 responses in patients with AD.

REFERENCES

- Ong PY, Leung DY. Immune dysregulation in atopic dermatitis. *Curr Allergy Asthma Rep* 2006;6:384-9.
- Maintz L, Novak N. Getting more and more complex: the pathophysiology of atopic eczema. *Eur J Dermatol* 2007;17:267-83.
- Hamid Q, Boguniewicz M, Leung DY. Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
- Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DY. *In vivo* expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 1996;98:225-31.
- Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 2008;128:2625-30.
- Nogales KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing Th17 T cells. *J Allergy Clin Immunol* 2009;123:1244-52.
- Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. *J Invest Dermatol* 2001;117:977-83.
- Lester MR, Hofer MF, Gately M, Trumble A, Leung DY. Down-regulating effects of IL-4 and IL-10 on the IFN- γ response in atopic dermatitis. *J Immunol* 1995;154:6174-81.
- Krause CD, Pestka S. Historical developments in the research of interferon receptors. *Cytokine Growth Factor Rev* 2007;18:473-82.

10. Dickensheets HL, Donnelly RP. Inhibition of IL-4-inducible gene expression in human monocytes by type I and type II interferons. *J Leukoc Biol* 1999;65:307-12.
11. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* 2004;75:163-89.
12. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu Rev Immunol* 1997;15:563-91.
13. Krutzik PO, Nolan GP. Intracellular phospho-protein staining techniques for flow cytometry: monitoring single cell signaling events. *Cytometry* 2003;55:61-70.
14. Schedel M, Pinto LA, Schaub B, Rosenstiel P, Cherkasov D, Cameron L, et al. IRF-1 gene variations influence IgE regulation and atopy. *Am J Respir Crit Care Med* 2008;177:613-21.
15. Elser B, Lohoff M, Kock S, Giaisi M, Kirchhoff S, Krammer PH, et al. IFN-gamma represses IL-4 expression via IRF-1 and IRF-2. *Immunity* 2002;17:703-12.
16. Akdis M, Trautmann A, Klunker S, Daigle I, Kucuksezzer UC, Deglmann W, et al. T helper (Th) 2 predominance in atopic diseases is due to preferential apoptosis of circulating memory/effector Th1 cells. *FASEB J* 2003;17:1026-35.
17. Akkoc T, de Koning PJ, Ruckert B, Barlan I, Akdis M, Akdis CA. Increased activation-induced cell death of high IFN-gamma-producing T(H)1 cells as a mechanism of T(H)2 predominance in atopic diseases. *J Allergy Clin Immunol* 2008;121:652-8.
18. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009;129:543-52.
19. Werfel T. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J Invest Dermatol* 2009;129:1878-91.
20. Simon D, Kozlowski E, Simon H. Natural killer T cells expressing IFN-gamma and IL-4 in lesional skin of atopic eczema. *Allergy* 2009;64:1681-4.
21. Matsui E, Kaneko H, Teramoto T, Fukao T, Inoue R, Kasahara K, et al. Reduced IFN-gamma production in response to IL-12 stimulation and/or reduced IL-12 production in atopic patients. *Clin Exp Allergy* 2000;30:1250-6.
22. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DY. Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. *J Invest Dermatol* 1992;99:403-8.
23. Novak N, Kruse S, Kraft S, Geiger E, Kluken H, Fimmers R, et al. Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002;119:870-5.
24. Hanifin JM. Diagnostic criteria for atopic dermatitis: consider the context. *Arch Dermatol* 1999;135:1551.
25. Santegoets SJ, Gibbs S, Kroeze K, van de Ven R, Scheper RJ, Borrebaeck CA, et al. Transcriptional profiling of human skin-resident Langerhans cells and CD1a+ dermal dendritic cells: differential activation states suggest distinct functions. *J Leukoc Biol* 2008;84:143-51.
26. Kraft S, Novak N, Katoh N, Bieber T, Rupec RA. Aggregation of the high-affinity IgE receptor Fc(epsilon)RI on human monocytes and dendritic cells induces NF-kappaB activation. *J Invest Dermatol* 2002;118:830-7.
27. Novak N, Kraft S, Haberstok J, Geiger E, Allam P, Bieber T. A reducing microenvironment leads to the generation of FcepsilonRIhigh inflammatory dendritic epidermal cells (IDEC). *J Invest Dermatol* 2002;119:842-9.
28. Novak N, Valenta R, Bohle B, Laffer S, Haberstok J, Kraft S, et al. FcepsilonRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. *J Allergy Clin Immunol* 2004;113:949-57.
29. Scheler M, Wenzel J, Tuting T, Takikawa O, Bieber T, von Bubnoff D. Indoleamine 2,3-dioxygenase (IDO): the antagonist of type I interferon-driven skin inflammation? *Am J Pathol* 2007;171:1936-43.
30. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008;3:1101-8.
31. von Bubnoff D, Matz H, Frahnert C, Rao ML, Hanau D, De La SH, et al. FcepsilonRI induces the tryptophan degradation pathway involved in regulating T cell responses. *J Immunol* 2002;169:1810-6.
32. Leung DY, Gao PS, Grigoryev DN, Rafaels NM, Streib JE, Howell MD, et al. Human atopic dermatitis complicated by eczema herpeticum is associated with abnormalities in IFN-gamma response. *J Allergy Clin Immunol* 2011;127:965-73.
33. Bernabei P, Coccia EM, Rigamonti L, Bosticardo M, Forni G, Pestka S, et al. Interferon-gamma receptor 2 expression as the deciding factor in human T, B, and myeloid cell proliferation or death. *J Leukoc Biol* 2001;70:950-60.
34. Paludan SR. Interleukin-4 and interferon-gamma: the quintessence of a mutual antagonistic relationship. *Scand J Immunol* 1998;48:459-68.
35. Coccia EM, Stellacci E, Marziali G, Weiss G, Battistini A. IFN-gamma and IL-4 differentially regulate inducible NO synthase gene expression through IRF-1 modulation. *Int Immunol* 2000;12:977-85.
36. Lohoff M, Ferrick D, Mittrucker HW, Duncan GS, Bischof S, Rollinghoff M, et al. Interferon regulatory factor-1 is required for a T helper 1 immune response in vivo. *Immunity* 1997;6:681-9.
37. Taki S, Sato T, Ogasawara K, Fukuda T, Sato M, Hida S, et al. Multistage regulation of Th1-type immune responses by the transcription factor IRF-1. *Immunity* 1997;6:673-9.
38. Giustizieri ML, Mascia F, Frezzolini A, De PO, Chinni LM, Giannetti A, et al. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J Allergy Clin Immunol* 2001;107:871-7.
39. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998;187:129-34.
40. Pease JE, Williams TJ. Chemokines and their receptors in allergic disease. *J Allergy Clin Immunol* 2006;118:305-18.
41. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004;4:762-74.
42. Xu H, Zhang GX, Ciric B, Rostami A. IDO: a double-edged sword for T(H)1/T(H)2 regulation. *Immunol Lett* 2008;121:1-6.
43. Chon SY, Hassanain HH, Gupta SL. Cooperative role of interferon regulatory factor 1 and p91 (STAT1) response elements in interferon-gamma-inducible expression of human indoleamine 2,3-dioxygenase gene. *J Biol Chem* 1996;271:17247-52.
44. Silva NM, Rodrigues CV, Santoro MM, Reis LF, Alvarez-Leite JI, Gazzinelli RT. Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infect Immun* 2002;70:859-68.
45. Horr B, Borck H, Thurmond R, Grosch S, Diel F. STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. *Int Immunopharmacol* 2006;6:1577-85.