

Expression of IL-18 mRNA and secretion of IL-18 are reduced in monocytes from patients with atopic dermatitis

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Background: IL-18 has been found to be an IFN- γ -inducing factor that plays an important role in T_H1 cell activation. Recently, IL-18 has also been found to enhance a T_H2 cellular response in a specific setting.

Objective: The aim of this study was to elucidate the role of monocytes and soluble factors, with special focus on IL-18, in the pathogenesis of atopic dermatitis (AD).

Methods: The release of cytokines from PBMCs and purified monocytes was measured through use of ELISA; mRNA expression was evaluated by RT-PCR. The results from patients with AD were compared with those from healthy controls.

Results: IL-18 secretion was reduced in both unstimulated and lipopolysaccharide-stimulated monocytes from patients with AD. The mRNA expression of IL-18 and IL-1 β -converting enzyme was significantly reduced in unstimulated monocytes from patients with AD ($P < .03$ and $P < .01$, respectively). Patients with AD had an elevated secretion of prostaglandin E₂ (PGE₂) from unstimulated PBMCs ($P < .001$). The anti-PGE₂ antibody reversed the suppressive effect of PGE₂ on IL-18 secretion in unstimulated PBMCs from patients with AD.

Conclusions: Decreased IL-18 production, together with a significantly reduced IL-18 and ICE mRNA expression in unstimulated monocytes and elevated PGE₂ secretion from PBMCs, was associated with the pathogenesis of AD. (J Allergy Clin Immunol 2001;108:607-14.)

Key words: Atopic dermatitis, CD14, IL-1 β , IL-8, IL-10, IL-18, IL-1 β -converting enzyme, LPS, monocyte, PBMC, prostaglandin E₂

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease that is associated with increased serum IgE levels and a family history of atopy.¹ Experimental evidence strongly suggests that patients

Abbreviations used

AD: Atopic dermatitis
GAPDH: Glyceraldehyde-3-phosphate-dehydrogenase
HBSS: Hanks' balanced salt solution
ICE: IL-1 β -converting enzyme
NC: Normal control subject
PGE₂: Prostaglandin E₂

with AD have a skewed immune system, T-cell responses being significantly tilted toward T_H2 lymphocyte activity—that is, the secretion of IL-4, IL-5, and IL-13, leading to IgE-mediated immune reactions—in contrast to T_H1 lymphocyte responses, in which IL-2 and IFN- γ are the predominant cytokines.^{2,3}

T lymphocytes can be influenced by a variety of factors in their development into either T_H1- or T_H2-type cells. A recently discovered cytokine, one that can drive naive T lymphocytes toward a T_H1 profile, is IL-18.⁴ It is produced in activated monocytes as well as in osteoblasts, keratinocytes, dendritic cells, intestinal epithelial cells, and cells of the adrenal cortex.⁴⁻⁹ IL-18 is produced in a precursor form, which, much like IL-1 β , is enzymatically split to its biologically active form by the IL-1 β -converting enzyme (ICE).¹⁰ The activation of T_H1 cells by IL-18 is independent of action by IL-12, which by itself can stimulate T_H1 cells to IFN- γ release.¹¹ However, costimulation with IL-12 and IL-18 enhances IFN- γ production from T_H1 cells.¹² IL-18 has also been demonstrated to augment a T_H1 response in a murine *Leishmania* model.¹³ Furthermore, a T_H2-type reaction with eosinophilia and increased IgE levels was reduced by IL-12 and IL-18 stimulation in antigen-induced airway hyperresponsiveness in mice.¹⁴ However, very recent data suggest that IL-18 can also enhance a T_H2 cellular response in a specific setting.¹⁵⁻¹⁹

Reduced IFN- γ production in patients with AD has been related to an elevated prostaglandin E₂ (PGE₂) and IL-10 secretion from monocytes, which points toward the possibility that an aberrant function of monocytes might be significant in the skewing of the immune system toward a T_H2-type cellular profile.²⁰ Recently, Snijders et al²¹ found that AD was associated with an increased PGE₂/IL-12 production ratio by monocytes, and Jung et al²² reported that IFN- γ deficiency in atopic T cells was not due to a lack of T-cell responsiveness to CD28, IL-2, IL-12, IL-15, or IL-18.²²

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In an attempt to clarify the role of IL-18 in the pathogenesis of AD, we investigated the expression of IL-18, together with that of IL-1 β , IL-8, IL-10, PGE₂, and ICE, in monocytes from patients with AD.

METHODS

Patients and normal control subjects

Blood samples were obtained from a total of 31 patients with AD aged 18 to 50 years (mean age, 32.5 years; 15 men and 16 women) diagnosed according to the criteria of Hanifin and Rajka.¹ Seventeen healthy control subjects aged 25 to 60 years (mean age, 41 years; 11 men and 6 women) were also included. All of the patients had moderate to severe AD, but none was receiving systemic immunosuppressive therapy at the time of the investigation. Informed consent was obtained before inclusion in the study, which was approved by the Ethical Committee of Aarhus County.

Culture and stimulation of PBMC

PBMCs were isolated through use of Lymphoprep (Nycomed, Oslo, Norway). Ten million PBMCs were cultured in 3 mL complete culture medium, which was composed of RPMI-1640 with l-alanyl-l-glutamine (Life Technologies, Paisley, United Kingdom) supplemented with 2% FCS and antibiotics (10 IE/mL penicillin G, 100 μ g/mL streptomycin, and 25 μ g/mL gentamycin). Stimulation of PBMCs was done with LPS in a final concentration of 50 ng/mL (from *Escherichia coli* 055:B5, Sigma, St Louis, Mo) for 24 hours in a humidified cell incubator (37°C; 5% CO₂). Supernatants were harvested in Cryotubes (Nunc, Roskilde, Denmark) and stored at -20°C until determination of IL-1 β , IL-8, IL-10, IL-18, and PGE₂.

Neutralizing antibody assay in PBMC culture

Ten million PBMCs from patients with AD were cultured in 3 mL complete culture medium with or without neutralizing antibodies against PGE₂, IL-10, and IT9302 (the C-terminal peptide from IL-10) for 24 hours in a humidified cell incubator (37°C; 5% CO₂).²³ The polyclonal antibodies for PGE₂ and IL-10 were used at a concentration of 2 μ L/mL (R&D Systems, Abingdon, United Kingdom). The polyclonal antibodies for IL-10 agonist IT9302 were used at a concentration of 4 μ L/mL.²³ Supernatants from cultured PBMCs were harvested and stored at -20°C until determination of IL-18.

Monocyte isolation and culture

PBMCs (5 to 10 $\times$ 10⁶ cells/mL) were incubated in 20 mL RPMI-1640 with l-alanyl-l-glutamine supplemented with 10% FCS and antibiotics in 600-mL tissue culture flasks (Nunc) for 1 hour at 37°C to allow monocytes to adhere to the flask. After the removal of nonadherent cells, 15 mL Hanks' balanced salt solution (HBSS) with 1% FCS was added and kept at -20°C for 3 minutes to detach the adherent cells. The purified monocytes (0.5 $\times$ 10⁶ cells/mL) were cultured in 2 mL complete culture medium in 6-well plates (Nunc), with or without 50 ng/mL LPS, for 1, 2, 3, 6, 12, 18, 24, and 48 hours in a humidified cell incubator. Supernatants were harvested and stored as described above. One million cells per milliliter of monocytes were also incubated in 6-well plates, with or without 50 ng/mL LPS, for 2 and 4 hours and then stored in guanidium thiocyanate buffer for total RNA extraction and RT-PCR.

Cell staining and flow cytometry

To determine the percentage of CD14⁺ cells, we stained PBMCs and isolated monocytes with anti-CD14 antibodies and isotype controls (Dako, Copenhagen, Denmark). One million PBMCs or monocytes in HBSS with 1% FCS were incubated with 20 μ L FITC-conjugated anti-CD14 mAb or isotype control for 30 minutes at 4°C. Cells

were washed twice in HBSS with 1% FCS and fixed in 100 μ L 4% paraformaldehyde-HBSS for 15 minutes at 4°C. The cells were again washed twice and analyzed by flow cytometry (Coulter, Luton, United Kingdom), whereby 10,000 gated cells were analyzed and stored in list-mode files and analyzed with the SYSTEM II software (Coulter).

RNA extraction and RT-PCR

Total RNA was extracted through use of the acid guanidium thiocyanate-phenol-chloroform method.²⁴ The pellet was washed 3 times in 80% ethanol and dissolved in RNase-free water. The amount of extracted RNA was measured with a UV spectrophotometer (Shimadzu, Kyoto, Japan). Reverse transcription of RNA and PCR was performed as previously described, though there were slight modifications.²⁵ Samples (8 μ L) of total RNA were heated to 65°C for 10 minutes and cooled on ice. Reverse transcription of RNA was carried out with a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Copenhagen, Denmark) for 1 hour at 37°C in 15- μ L volumes with 5- μ L bulk first-strand reaction mix (Moloney murine leukemia virus reverse transcriptase, RNAGuard, RNase/DNase-free BSA, dATP, dCTP, dGTP, and dTTP in aqueous buffer), 1 μ L pd(N)₆ primer (random hexadeoxynucleotides at 0.2 μ g/ μ L in aqueous solution), and 1 μ L DTT solution (200 mmol/L aqueous solution) per sample. We made a cDNA dilution series from each cDNA reaction mixture and constructed a dilution curve by using 10-fold dilutions of cDNA. Next, we confirmed that the plot of band intensity and cDNA dilution series was set up within a linear range of the saturation curve for semiquantitative RT-PCR. One microliter of original or diluted cDNA was used for PCR reaction of IL-1 β , IL-8, and IL-10. Original cDNA (the equivalent of 0.1 μ g RNA) was used for the PCR reaction of IL-18 and ICE, because the PCR products of IL-18 and ICE were detected only in a few samples when diluted cDNA was used. PCR was performed with 1 μ L of the reaction mixture, Taq polymerase (0.5 units per reaction; Advanced Biotechnologies, Surrey, United Kingdom), 50 μ mol/L of each pair of gene-specific primers, and 200 μ mol/L dNTP in a reaction buffer (75 mmol/L Tris-HCl, 1.5 mmol/L MgCl₂, 20 mmol/L (NH₄)₂SO₄, 0.01% (v/v) Tween 20; Advanced Biotechnologies).

Primer sequences were 5'-AAA CAG ATG AAG TGC TCC TTC CAG G, 3'-ACC TCT TGT GGT GAA CAA CGA GGT for IL-1 β (expected product size, 388 bp),²⁶ 5'-ACG AAT TCC TAG GAC AAG AGC CAG GAA G, 3'-CTA ACT CTC ACC TGG TGT GAC TTA AGT G for IL-8 (expected product size, 253 bp), 5'-ATG CCC CAA GCT GAG AAC CAA GAC CCA, 3'-ACC CTA TCG ACT GGG TCG GGG AAC TCT for IL-10 (expected product size, 351 bp), 5'-GCT TGA ATC TAA ATT ATC AGT C, 3'-CTT CTA AGT TTA ACG TAG AAT A for IL-18 (expected product size, 342 bp),²⁷ 5'-GGT CCT GAA GGA GAA GAG AA, 3'-TCC GGA CCT ACT ACT AGT GG for ICE (expected product size, 842 bp),²⁷ and 5'-GAG AAT TCG AGT CAA CGG ATT TGG TCG T, 3'-TAC CGT TTA AGG TAC CGT GGC TTA AGC G for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (expected product size, 174 bp), as a control for the RNA isolation and transcription (IL-1 β , IL-18, ICE, DNA Technology A/S, Aarhus, Denmark; IL-8, IL-10, GAPDH, Amersham Pharmacia Biotech).

PCR was performed in a DNA engine thermocycler (MJ Research, Watertown, Mass) for 37 cycles (35 cycles for GAPDH). Amplification was performed with a first cycle of 94°C (5 minutes), 60°C (1 minute), and 72°C (2 minutes); this was followed by 33 or 35 cycles at 93°C (1 minute), 60°C (1 minute), and 72°C (2 minutes). The last cycle was 93°C (1 minute), 60°C (1 minute), and 72°C (10 minutes). The reaction products were visualized by electrophoresis in a 2% agarose gel (NuSieve, BioWhittaker Molecular Applications ApS, Ringevej, Denmark) and by UV light illumination after staining with SYBR Green I nucleic acid gel stain (Molecular Probes, Leiden, The Netherlands).

To analyze semiquantitatively the result of RT-PCR, we scanned the gel images and measured the intensity of the PCR product through use of NIH Image software (National Institutes of Health, Bethesda, Md). We determined the relative intensity of individual bands on a gel image as the ratio of the intensity of cytokine to the intensity of GAPDH.

Measurement of cytokine and PGE₂ productions

The release of IL-1 β , IL-8, IL-10, and IL-18 from purified monocytes and PBMCs was measured by ELISA. Each sample was tested in duplicate. PGE₂ concentration was measured through use of an immunoassay kit with a detection limit of 36.2 pg/mL (R&D Systems). IL-1 β and IL-18 concentrations were measured through use of an ELISA kit with a sensitivity of 31.2 pg/mL (R&D Systems). IL-8 and IL-10 were determined through use of ELISA, as previously described.²⁸

Statistical analysis

Statistical analysis was performed through use of the Wilcoxon signed rank test for paired data and the Mann-Whitney *U* test for unpaired data. The qualitative RT-PCR results were analyzed through use of the Fisher exact probability test. Differences associated with a probability of *P* < .05 were considered statistically significant. The results in our tables and figures are given as means $\pm$ SEMs.

RESULTS

CD14⁺ cell percentage in PBMC and adherent cell populations

The percentages of CD14⁺ cells in PBMCs were 32% $\pm$ 22.6% (mean $\pm$ SD) for the normal control subjects (NCs; *n* = 7) and 32% $\pm$ 13% (mean $\pm$ SD) for the patients with AD (*n* = 5). The purity of CD14⁺ cells in the adherent populations was 83% $\pm$ 1% (mean $\pm$ SD) for the NCs (*n* = 4) and 83% $\pm$ 5% (mean $\pm$ SD) for the patients with AD (*n* = 4). Thus there were no differences in cellular composition between the control and patient groups.

Cytokine secretion from PBMCs

The results of cytokine and prostaglandin secretion from PBMCs are shown in Table I. IL-8 secretion was significantly increased after LPS stimulation in patients with AD (*P* < .03), whereas IL-10 and IL-1 β secretion did not differ between the groups. PGE₂ secretion from unstimulated PBMCs of patients with AD was significantly higher than that from unstimulated PBMCs of NCs (*P* < .001). Although the IL-18 secretion was reduced from AD PBMCs both in unstimulated and stimulated cultures, the difference did not reach statistical significance.

IL-18 production from PBMCs in neutralizing antibody assay

To evaluate the influence of IL-10 and PGE₂ on IL-18 production from monocytes, a neutralizing antibody assay was carried out through use of polyclonal antibody for PGE₂, IL-10, and IT9302. Interestingly, IL-18 production from PBMCs of patients with AD was increased in the culture with anti-PGE₂ antibody, the culture with anti-PGE₂ antibody plus anti-IT9302 antibody, and the culture with anti-PGE₂ antibody plus anti IL-10 antibody, in compari-

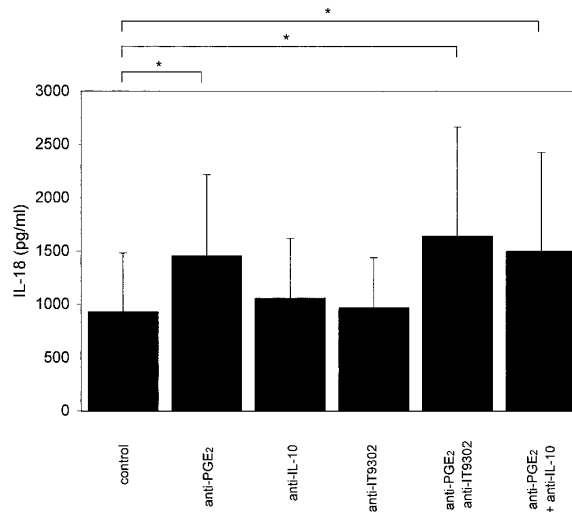
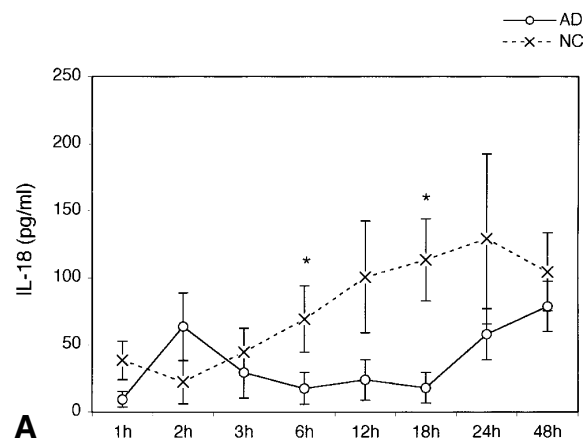
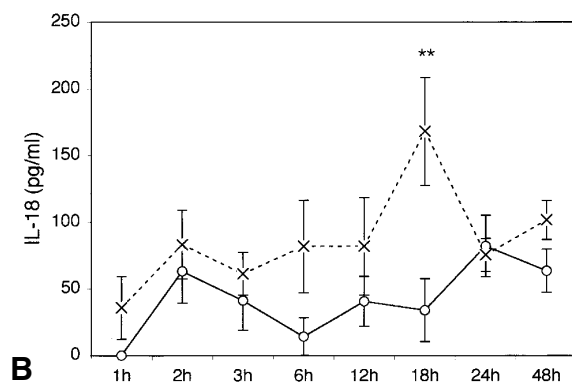


FIG 1. IL-18 production in unstimulated PBMCs from patients with AD (*n* = 8) with neutralizing antibody against PGE₂, IL-10, and IT9302. Results are expressed as means $\pm$ SDs. **P* < .03.



A



B

FIG 2. **A**, IL-18 production in unstimulated monocytes from NCs (*n* = 6) and patients with AD (*n* = 6). **B**, IL-18 production in LPS-stimulated monocytes from NCs (*n* = 6) and patients with AD (*n* = 6). Results are expressed as means $\pm$ SEMs. **P* < .03 and ***P* < .01 for NC vs AD.

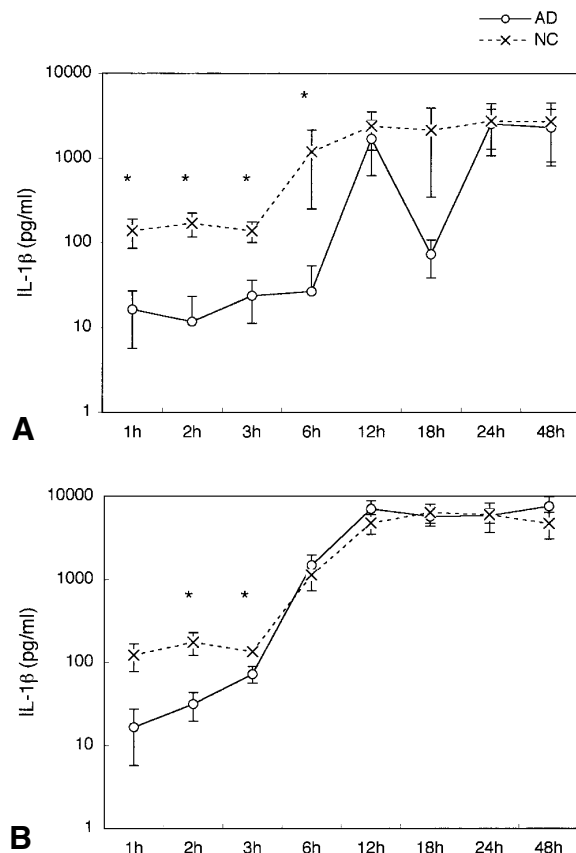


FIG 3. A, IL-1 β production in unstimulated monocytes from NCs (n = 6) and patients with AD (n = 6). B, IL-1 β production in LPS-stimulated monocytes from NCs (n = 6) and patients with AD (n = 6). Results are expressed as means $\pm$ SEMs. * P < .05 for NC vs AD.

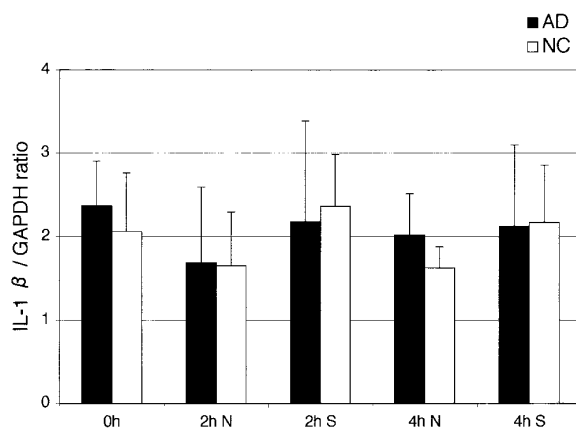


FIG 4. Comparison of levels of mRNA expression for IL-1 β in monocytes from NCs (n = 6; open columns) and patients with AD (n = 6 to 9; closed columns). Isolated monocytes were incubated for 0, 2, and 4 hours in stimulation with (S) or without (N) LPS. IL-1 β mRNA expression was analyzed by RT-PCR. Relative intensity of IL-1 β mRNA was determined as described in the Methods section. Results are expressed as means $\pm$ SEMs.

son with the culture without neutralizing antibody (P < .03; Fig 1). However, anti IL-10 and anti-IT9302 antibodies did not affect IL-18 production from AD PBMCs.

Cytokine secretion from purified monocytes

To investigate the kinetics of cytokine secretion from monocytes, adherent cells were cultured with or without LPS (50 ng/mL). We measured the secretion of IL-1 β , IL-8, IL-10, and IL-18 at 8 different time points from monocytes in both groups by ELISA. There were no significant differences between NCs and patients with AD regarding IL-8 and IL-10 secretion, including secretion from stimulated monocytes and unstimulated monocytes (data not shown). The IL-18 secretion from AD monocytes was, however, lower than that from NC monocytes at almost all time points. The reduction reached statistical significance at 6 hours in unstimulated culture (P < .03) and at 18 hours in both unstimulated and stimulated monocyte cultures (P < .03 and P < .05, respectively; Fig 2, A and B). The doubling of IL-18 secretion, which was evident in LPS-stimulated PBMC cultures, was not evident in the cultures of purified monocytes. Furthermore, the IL-18 level in purified monocyte cultures was lower than that observed in the PBMC cultures, most likely because of the approximately 3- to 5-fold lower number of monocytes in the cultures. The IL-1 β production from AD monocytes was also lower than that from NC monocytes at the early phase of culture. The reduction reached statistical significance at 1 and 6 hours in unstimulated cultures (P < .05) and at 2 and 3 hours in both unstimulated and stimulated monocyte cultures (P < .05; Fig 3, A and B).

Cytokine mRNA expression in monocytes from NCs and patients with AD

To reveal mRNA expression of cytokines from monocytes, isolated monocytes were incubated with or without LPS for 2 and 4 hours. We semiquantitatively studied mRNA expression of IL-1 β , IL-8, IL-10, IL-18, and ICE through use of diluted cDNA samples. However, we did not detect PCR products of IL-10, IL-18, and ICE in short-term cultured monocytes from the 2 groups. This lack of PCR products was likely due to very small amounts of mRNA in short-term cultures rather than to a technical problem, because we could detect PCR products of IL-10, IL-18, and ICE in a few samples (data not shown). However, there were no significant differences between NCs and patients with AD in mRNA expression of either IL-1 β (Fig 4) or IL-8 (data not shown).

Because the expression of IL-18 and ICE mRNA could not be semiquantitatively elucidated in monocytes from both groups, we qualitatively analyzed IL-18 and ICE mRNA expression. Both IL-18 and ICE mRNA were constitutively expressed in monocytes unstimulated for 0 hours from NCs (100% positive), whereas only 50% and 62.5% of monocytes unstimulated for 0 hours from patients with AD expressed IL-18 and ICE mRNA, respectively (n = 6; Fig 5); these differences did not reach statistical significance (P = .069 and P = .15, respectively). However, when we determined the mRNA expression

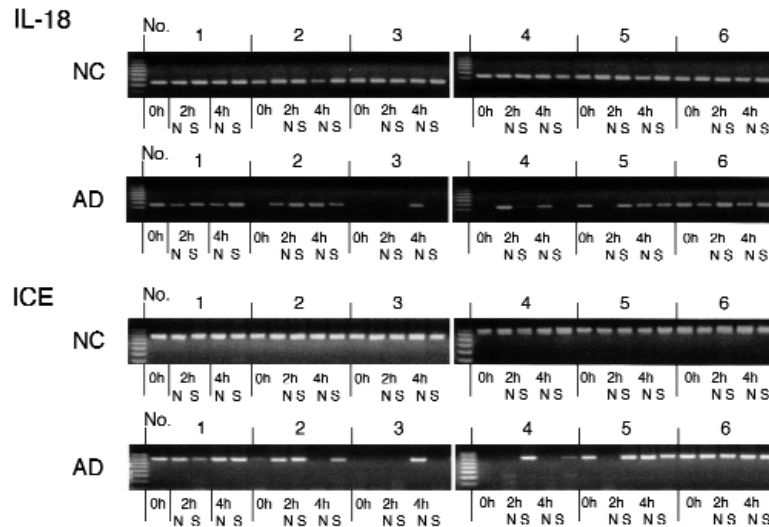


FIG 5. IL-18 and ICE mRNA expression in monocytes from NCs and patients with AD was qualitatively analyzed by RT-PCR. Isolated monocytes were incubated for 0, 2, and 4 hours in stimulation with (S) or without (N) LPS. Original cDNA (the equivalent of 0.1 μ g RNA) from monocytes was used for PCR reaction. GAPDH mRNA expression was also confirmed (data not shown).

TABLE I. Cytokine production in PBMCs from normal control subjects and patients with AD

Group	IL-8 (ng/mL)		IL-10 (pg/mL)		IL-18 (pg/mL)		PGE ₂ (pg/mL)		IL-1 β (pg/mL)	
	(-)	LPS	(-)	LPS	(-)	LPS	(-)	LPS	(-)	LPS
NC (n = 8)	2.14 $\pm$ 1.26	413.63 $\pm$ 46.73	8.00 $\pm$ 8.00	2147.5 $\pm$ 281.09	532.5 $\pm$ 136.49	1095 $\pm$ 289.66	16.75 $\pm$ 8.21	47,325 $\pm$ 5,069.90	0	4897.5 $\pm$ 2876.6
AD (n = 8)	1.66 $\pm$ 0.40	985.63 $\pm$ 241.96*	36.25 $\pm$ 14.36	1825 $\pm$ 304.65	298.5 $\pm$ 112.62	643.75 $\pm$ 211.35	103 $\pm$ 8.61**	36,971.3 $\pm$ 6,989.72	0	4231.3 $\pm$ 3667

Ten million PBMCs were stimulated with LPS (50 ng/mL) or without LPS (-) for 24 hours. Culture supernatants were harvested, and concentrations of secreted cytokine and PGE₂ were determined by ELISA. Results are indicated as means $\pm$ SEMs.

* $P < .03$ vs IL-8 concentration in NC.

** $P < .001$ vs PGE₂ concentration in NC.

in all unstimulated monocyte cultures from 0-, 2-, and 4-hour culture periods, we observed a significantly reduced IL-18 and ICE mRNA expression in comparison with NCs ($P < .03$ and $P < .01$, respectively; Table II). LPS-stimulated monocytes in short-term cultures did lead to an upregulation of mRNA for both IL-18 and ICE (Table II).

DISCUSSION

In the current study, we investigated cytokine production and cytokine mRNA expression in monocytes from NCs and patients with AD, focusing especially on IL-18. The production of IL-8 was used as a measure of monocyte activation, and the production of IL-10, as well as that of PGE₂, which is able to inhibit IFN- γ production from T cells, was also determined.

Our results demonstrated that in unstimulated monocytes from patients with AD, IL-18 production was partially reduced (Fig 2, A) and the expression of IL-18 mRNA significantly reduced during short-term culturing (Fig 5 and Table II). We also found that mRNA expression of ICE, which is the enzyme converting pro-IL-18

TABLE II. Cytokine mRNA expression in monocytes from normal control subjects and patients with AD

Group	IL-18		ICE		GAPDH	
	(-)	LPS	(-)	LPS	(-)	LPS
NC	18/18*	12/12	18/18	12/12	18/18	12/12
AD	17/23**	10/14	15/23***	11/14	23/23	14/14

One million monocytes were incubated with or without LPS (50 ng/mL) for 0, 2, and 4 hours. Cells were harvested and total RNA was extracted for RT-PCR. Original cDNA (equivalent of 0.1 μ g RNA) was used for PCR reaction. (-), Cells were cultured without LPS for 0, 2, or 4 hours; LPS, cells were cultured with LPS for 2 or 4 hours.

*Eighteen of 18 samples were positive.

** $P < .03$ vs positive IL-18 mRNA in NCs.

*** $P < .01$ vs positive ICE mRNA in NCs.

into its biologically active form, was reduced in unstimulated monocytes from patients with AD (Fig 5 and Table II). The reduced IL-18 production in unstimulated monocytes from patients with AD was consistent with both decreased IL-18 mRNA and reduced ICE mRNA expression during short-term culturing. Although there was no

significant difference between unstimulated monocytes from controls and those from patients with AD in mRNA expression of IL-1 β (which was semiquantitatively analyzed), the production of IL-1 β was reduced in the early phase of culturing of unstimulated AD monocytes (Fig 3, A and Fig 4). This might be associated with reduced ICE mRNA expression during short-term culturing (Fig 5 and Table II). In unstimulated PBMCs from patients with AD, decreased IL-18 production was observed; however, there was no statistically significant difference between controls and patients with AD. With respect to IL-10 production, there was no significant difference; however, augmented PGE₂ production from unstimulated PBMCs from AD was seen (Table I). Therefore, we carried out a neutralizing antibody assay using antibodies against PGE₂, IL-10, and IT9302 (Fig 1). We then confirmed that PGE₂ suppressed IL-18 production in unstimulated PBMCs from the results of the neutralizing antibody assay. However, we do not know whether PGE₂ can inhibit the expression of ICE mRNA and IL-18 mRNA. Reznikov et al²⁹ have reported that IL-18 suppressed IL-1 β -induced PGE₂ production, the suppressive effect of IL-18 on PGE₂ production being mediated by IFN- γ .

Meanwhile, IL-18 production was partially decreased in LPS-stimulated monocytes from patients with AD (Fig 2, B); the mRNA expression of IL-18 and ICE was somewhat decreased, but the difference was not statistically significant (Fig 5 and Table II). In addition, IL-1 β production was reduced in the early phase in LPS-stimulated monocytes from patients with AD (Fig 3, B); however, IL-1 β mRNA was expressed at the same level as in monocytes from NCs (Fig 4). In LPS-stimulated PBMCs from patients with AD, reduced IL-18 secretion was observed; however, the difference did not reach statistical significance. With respect to IL-1 β , IL-10, and PGE₂ production, there were no significant differences; however, augmented IL-8 production in LPS-stimulated PBMCs from AD was observed (Table I).

In our experiment, we used LPS as stimulus for PBMC and monocyte culture because LPS can stimulate CD14⁺ cells, which are monocytes, and we wished to observe the functions of monocyte, especially cytokine secretion. However, LPS cannot induce IFN- γ production from monocytes. Indeed, we confirmed that no IFN- γ production was detected in LPS-stimulated PBMCs for 24 hours. Thus we could not simultaneously compare IFN- γ production with IL-18 production from either PBMCs or monocytes. In general, IFN- γ production was reduced in AD PBMCs after stimulation with PHA or phorbol 12-myristate 13-acetate.^{2,3} Moreover, Snijders et al²¹ concluded that AD was associated with an increased ratio of PGE₂/IL-12 production by monocytes. Jung et al²² have reported that IFN- γ deficiency in atopic T cells is not due to a lack of T-cell responsiveness to CD28, IL-12, IL-2, IL-15, or IL-18.²² Although we cannot conclude that reduced IL-18 production from monocytes was able to contribute to the IFN- γ deficiency in atopic T cells, it can nevertheless be concluded that the decreased IL-18 and IL-1 β production and reduced ICE mRNA expression in monocytes

from patients with AD are important factors in the pathogenesis of AD.

In mice, IL-18 has paradoxical dual functions. One function is the induction of IFN- γ production as a T_H1 response in combination with IL-12^{11,12,14}; the other function is the enhancement of allergic reactions, such as IgE and T_H2 cytokine production, through IL-13 production from natural killer and T cells in combination with IL-2.¹⁵⁻¹⁹ We do not know the serum IL-18 levels in our patients with AD; however, Tanaka et al³⁰ recently noted that serum IL-18 levels were elevated in patients with acute asthma and that these elevated IL-18 levels in acute asthma quickly decreased after treatment.³⁰ They also found that serum IL-18 levels were significantly correlated with serum-soluble IL-2 receptor levels. In addition, Yoshizawa et al³¹ reported that significantly higher serum IL-18 levels were found in patients with AD than in control subjects and that serum IL-18 levels were correlated with disease severity, with the number of eosinophils in peripheral blood, and with serum-soluble IL-2 receptor levels. However, Tanaka et al³⁰ and Yoshizawa et al³¹ did not describe what type of cell secreted large amounts of IL-18. We too have been unable to answer this question clearly, whereas it can be considered that high amounts of serum IL-18 from unknown cells suppressed IL-18 production in monocytes from patients with AD as if in a negative-feedback pathway. Intriguingly, Habu et al³² have indicated that NC/Nga mice that were used in a mouse model of AD produced much lower amounts of IL-18 after *in vivo* LPS stimulation than either C57BL mice or BALB mice.³² This is in line with our findings, which included reduced IL-18 production in AD monocytes.

Many observers have reported a reduced production of IL-1 from monocytes in patients with AD.³³⁻³⁵ A decreased IL-1 β production was paralleled by reduced T-cell proliferation after mitogen stimulation.³⁴ In contrast, Thstrup-Pedersen et al³⁶ demonstrated an increased IL-1 production, which might enhance T_H2-type responses, in purified monocytes from patients with AD.³⁶

We also confirmed an increased secretion of IL-8 from PBMCs, but we did not observe an increased IL-8 secretion and mRNA expression from purified monocytes. Kimata et al³⁷ have documented an augmented IL-8 concentration in sera from patients with AD, and enhanced intracellular IL-8 in eosinophils from patients with AD has been demonstrated.³⁸ Recently, Hatano et al³⁹ reported that an increased mRNA of IL-8 was observed in PBMCs from patients with AD.

Our results, together with previous evidence, suggest that elevated IL-8 might contribute to a recruitment of T cells into skin inflammation of AD. We also found a significant secretion of PGE₂, as has been observed by others.^{20,40-42} In contrast, IL-10 mRNA expression and secretion did not seem to be changed in blood mononuclear cells from our patients with AD, which is in line with other observations,²¹ though increased levels have been observed.⁴³ The observed differences underline the fact that patients with AD exhibit a wide range of disease

activity, reflecting differences in immune activity at the time of the investigation. Furthermore, different experimental settings might lead to differences in results.

It could be argued that our observations reflect only a partial reduction of IL-18 in patients with AD, which suggests that many mediators can be changed during this disease. It is a question not just of IL-18 but of a group of soluble factors that are differentially upregulated or downregulated in this disorder, as shown here for IL-8 and PGE₂.

The reduction of IL-18 mRNA expression and secretion from mononuclear cells of patients with AD could be due to at least 3 factors. First, PGE₂ suppressed IL-18 production in unstimulated PBMCs. Second, a recent observation showed how histamine is able to induce IL-18 secretion from human mononuclear cells.⁴⁴ This is directly relevant to our observations, inasmuch as histamine is increased in patients with AD. Thus, the monocytes are likely to be depleted in vivo of significant amounts of IL-18, which explains the relative loss of IL-18 from the cells. Third, it might be possible to explain that large amounts of serum IL-18 abrogated IL-18 production in AD monocytes.

This study documents that the recently discovered mediator IL-18 might play a role in the skewing of the immune system of patients with AD. In addition, monocyte function, especially production of soluble factors, is aberrant in AD. Monocytes, macrophages, and dendritic cells play a pivotal role in antigen presentation and determination of T_H1/T_H2 differentiation; these monocyte-derived cells are extremely important in innate immunity. The complexity of AD is not to be underestimated, as outlined in the most recent review of this disease.⁴⁵ Not only do we need to know more about the T-lymphocyte system, which provides acquired immunity in patients with AD, and about its development; we also need to know more about the monocyte system, which provides innate immunity in patients with AD. Here we have described one factor, IL-18, that could participate in the skewing of the immune system in patients with AD.

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