

Der p 1 suppresses indoleamine 2, 3-dioxygenase in dendritic cells from house dust mite-sensitive patients with asthma

Kittipong Maneechotesuwan, MD, PhD,^{a,b} Valla Wamanuttajinda, BSc,^b Kanda Kasetsinsombat, MSc,^b Sukit Huabprasert, BA,^b Metha Yaikwawong, MD,^b Peter J. Barnes, DM, FRS,^c and Adisak Wongkajornsilp, MD, PhD^b Bangkok, Thailand, and London, United Kingdom

Background: Indoleamine 2, 3-dioxygenase (IDO), a tryptophan-degrading enzyme in dendritic cells (DCs), mediates an immunosuppressive effect on activated T lymphocytes. However, little is known about the effect of Der p 1 on IDO in human DCs.

Objective: The aim was to investigate the effect of Der p 1 on the expression and activity of IDO in monocyte-derived DCs from house dust mite (HDM)-sensitive patients with asthma.

Methods: Using real-time RT-PCR and HPLC, the expression and activity of IDO were assessed in TNF- α -induced mature DCs from HDM-sensitive and nonatopic patients with asthma in response to Der p 1 exposure *ex vivo*. We also monitored the alteration of IDO activity in Der p 1-pulsed DCs after the cocubation with autologous T cells.

Results: With a reliance on its protease activity, Der p 1 suppressed functional IDO in DCs from HDM-sensitive patients with asthma but enhanced IDO activity in DCs from nonatopic patients with asthma. This suppression was maintained by the reciprocally induced IL-4 from the coculturing autologous HDM-sensitive T cells. Conversely, the upregulation of IDO activity in Der p 1-pulsed DCs was maintained by IFN- γ released from autologous nonatopic T cells and the regulatory T-cell subset. Der p 1 pulsation to sensitive DCs failed to raise regulatory T cells but raised progenitor fractions from cloned HDM-sensitive CD4⁺ cells through direct contact and soluble mediators.

Conclusion: House dust mite-sensitive DCs exposed to Der p 1 downregulated IDO activity and tipped the T_H1/T_H2 cytokine balance toward IL-4, resulting in sustainable IDO suppression. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Der p 1, IDO, dendritic cells, T_H2 cells, asthma

Indoleamine 2, 3-dioxygenase (IDO), a tryptophan-degrading enzyme, has recently emerged as an important T-cell immunomodulator, a tolerance inducer.¹ IDO expression by DCs may suppress T-cell responses and promote tolerance through direct effects on T-cell function, which are mediated by tryptophan depletion, or toxic metabolites.¹ The role of IDO in modulating allergic airway inflammation has more recently been investigated in human beings² and a mouse model of asthma.^{3,4} We have recently shown that IDO expression and activity in sputum cells from house dust mite (HDM)-sensitive patients with asthma were less than those from individuals without asthma.² The reasons for this reduction in IDO activity in asthma are not yet certain, however. In particular, the effect of HDM on IDO activity in DCs from subjects with allergic asthma has not been investigated.

T_H2 clones predominate among allergen-specific T-cell clones from individuals with allergy.⁵ In addition, allergen-specific T-cells from HDM-sensitive individuals produce large amounts of IL-4 after the cocubation with HDM allergen-stimulated DCs.⁶ Furthermore, the major allergen of HDM, *Dermatophagoides pteronyssinus* (Der p 1), may influence allergic responses by suppressing the secretion of IFN- γ , thus promoting a T_H2 response.⁷ In contrast, T cells from nonatopic subjects produced only IL-10 and IFN- γ , but no T_H2 cytokines,⁸ whereas the production of IFN- γ was increased in response to HDM allergen.⁶ This suggests that both IFN- γ and IL-10 might modulate the induction and maintenance of allergen-specific tolerance after allergen exposure in nonatopic subjects. Because of the ability of IL-4 to inhibit and IFN- γ to enhance IDO activity,^{9,10} differential expression of both cytokines between atopic and nonatopic subjects in response to HDM exposure might affect IDO activity in DCs during their interaction with T cells. This may account for the enhancement of the allergic inflammatory response in atopic subjects with asthma but immune tolerance in nonatopic patients with asthma after HDM exposure.

We therefore investigated whether the effects of Der p 1 on the expression and the enzymatic activity of IDO in monocyte-derived DCs from HDM-sensitive subjects with asthma differ from those in nonatopic subjects with asthma after treatment with Der p 1. Alterations of IDO activity from DCs after the coculture with autologous CD4⁺ T cells in both groups of subjects were also monitored. Finally, we determined the effects of IL-4 and IFN- γ released from autologous CD4⁺ T cells on IDO activity in the cocultures through neutralization experiments.

From ^athe Division of Respiratory Disease and Tuberculosis, Department of Medicine, and ^bthe Department of Pharmacology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok; and ^cthe National Heart and Lung Institute, Imperial College, London.

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Reprint requests: Kittipong Maneechotesuwan, MD, PhD, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkokknoi, Bangkok 10700, Thailand. E-mail: sikmn@mahidol.ac.th.

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Abbreviations used

DC: Dendritic cell
HDM: House dust mite
iDC: Immature dendritic cell
IDO: Indoleamine 2, 3-dioxygenase
mDC: Mature dendritic cell
1-MT: 1-Methyl DL-tryptophan
TNFR: TNF- α receptor
UK: United Kingdom

METHODS**Subjects**

Nonatopic and HDM-sensitive subjects with asthma who had been nonsmokers were included in the study. Subjects with asthma had a baseline of $>60\%$ predicted FEV₁ and demonstrated reversibility of FEV₁ 12% or more after 2.5 mg albuterol nebulization and PC₂₀ ≤ 4 mg/mL. HDM sensitivity was documented by positive skin prick test to Der p 1 (Greer Laboratories, Lenoir, NC), the major allergen of *D pteronyssinus*, and by the detection of Der p 1-specific IgE antibodies in the sera (RAST class ≥ 2). Nonatopic subjects with asthma were defined by a negative skin prick test to common allergens (eg, grass pollen, cat dander, *D pteronyssinus*, *Aspergillus fumigatus*) and by undetectable specific IgE antibodies in the sera. All patients provided written informed consent for participation after an approval from the Ethics Committee of Siriraj Hospital.

Generation of monocyte-derived DCs

PBMCs were isolated from heparinized blood by Isoprep (Robbins Scientific, Sunnyvale, Calif) density gradient centrifugation, and monocytes were enriched by adherence. Briefly, PBMCs were incubated for 60 minutes in RPMI 1640 medium (Invitrogen, Carlsbad, Calif) supplemented with 10% FBS (Biochrom AG, Berlin, Germany) at 37°C. After the nonadherent cells were harvested, the remaining monocytes were incubated in RPMI 1640 supplemented with 10% FBS, 500 U/mL IL-4 (Genzyme, Cambridge, Mass), and 400 U/mL GM-CSF (Xiamen Amoytop Biotech, Fujian, China). Cells were fed with fresh medium every other day. After 7 days of culture, the resulting immature DCs (iDCs) were pulsed with 100 ng/mL Der p 1 (Indoor Biotech, Charlottesville, Va), E-64-pretreated Der p 1 or Bla g 1 (1 μ g/mL; Indoor Biotech, Wilshire, United Kingdom [UK]), or PBS. At the same time, DCs were stimulated with 1000 U/mL TNF- α (a gift from Dr Daniela Maennel, University of Regensburg, Germany) to induce their maturation in the presence of either 1000 U/mL IFN- γ (Genzyme) or 200 μ mol/L 1-methyl DL-tryptophan (1-MT; Sigma, St. Louis, MO). Mature DCs (mDCs) were harvested 48 hours after stimulation for RT-PCR, coculture experiments, or flow-cytometric analysis. For some experiments, 100 μ mol/L tryptophan (Sigma, St Louis, Mo) was added to mature DCs in the final 4 hours to monitor IDO activity clearly in the medium. To inhibit protease activity, Der p 1 was pretreated with an irreversible cysteine protease-specific inhibitor, E-64¹¹ (Peptides International, Louisville, Ky) before incubation with DCs.

Preparation of CD4⁺ T cells and allergen-specific T-cell clones

CD4⁺ T cells were prepared from nonadherent PBMCs by using magnetic sorting (Miltenyi Biotech, Gladbach, Germany). The purity of CD4⁺ T cells after purification as determined by flow cytometry was more than 97%.

To prepare allergen-specific T-cell clones as previously described with some modification,¹² PBMCs (1 $\times 10^7$) from HDM-sensitive patients with asthma were inoculated with either Der p 1 or Bla g 1 for 7 days. Irradiated (30Gy) autologous PBMCs (10⁵), 20 U/mL IL-2 (Genzyme), and either 0.5 μ g/mL Der p 1 or 0.5 μ g/mL Bla g 1 were then added. The culture medium was changed on day 10. On day 14, the CD4⁺ T cells were isolated from the stimulated PBMCs.

Coculture of DCs and autologous CD4⁺ T cells

Der p 1-pulsed or unpulsed DCs were seeded in a 96-well plate at 1×10^4 cells per well. Autologous CD4⁺ lymphocytes were added to make a ratio of DCs to lymphocytes equal to 1:10, and were incubated for 24 or 48 hours. Tryptophan was added to the cocultures in the final 4 hours of incubation.

RNA isolation and real-time quantitative reverse transcriptase polymerase chain reaction analysis

Total RNA was prepared using an RNeasy Mini kit (Qiagen, Valencia, Calif) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA by using the Improm-II Reverse Transcription system (Promega, Madison, Wis). Real-time RT-PCR was performed by using FastStart Universal SYBR Green Master (Roche, Mannheim, Germany) with an ABI PRISM 7900 thermal cycler (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. Primers were purchased from First BASE Laboratory (Singapore): IDO sense: AGT CCG TGA GTT TGT CCT TTC AA, antisense: TTT CAC ACA GGC GTC ATA AGC T; IL-4 sense: AAC AGC CTC ACA GAG CAG AAG AC, antisense: GCC CTG CAG AAG GTT TCC TT; IFN- γ sense: GTG GAG ACC ATC AAG GAA GAC AT, antisense: TGC TTT GCG TTG GAC ATT CA; GATA-3 sense: ACT ACG GAA ACT CGG TCA GG, antisense: CAG GGT AGG GAT CCA TGA AG; T-box expressed in T cells (T-bet) sense: AGG ATT CCG GGA GAA CTT TGA, antisense: TAC TGG TTG GGT AGG AGA GGA GAG TA, and glyceraldehyde-3-phosphate dehydrogenase (GADPH) sense: GAA ATC CCA TCA CCA TCT TCC, antisense: AAA TGA GCC CCA GCC TTC TC. 7300 System SDS software version 1.4.0.25 (Applied Biosystems, Foster City, Calif) was used to analyze relative quantification of the target cDNA according to the $\Delta\Delta$ Ct method.

Fluorescence-activated cell sorting analysis

Flow-cytometric analysis was performed as previously described.¹³ Briefly, dendritic cells were washed and incubated with the corresponding primary monoclonal antibodies for 60 minutes at 4°C, washed twice, and resuspended in PBS containing 1% BSA. Flow cytometric analysis on 20,000 cells was performed by using a FACSort (Becton Dickinson, San Jose, Calif). The primary mAbs used were antibodies raised against human IDO (1:100; Chemicon International Inc, Temecula, Calif), CD80, CD83, CD86 (BD, San Jose, Calif), Jagged-1, IL-6, IL-12, TNF- α , TNF- α receptor 1, TNF- α receptor 2 (R&D Systems, Minneapolis, Minn), DELTA-4 (Abcam, Cambridge, UK), HLA-DR (Santa Cruz Biotech, Santa Cruz, Calif), IL-10, IL-1 β (e-Bioscience, San Diego, Calif), AlexaFluor 647-conjugated anti-forkhead box P3 (FOXP3) (BioLegend, San Diego, Calif), phycoerythrin-Cy5-conjugated anti-CD4, and phycoerythrin-conjugated anti-CD25 mAb (BioLegend, San Diego, Calif). The respective negative controls were matched isotype controls. Data were analyzed using WinMDI version 2.9 (Scripps Research Institute, La Jolla, Calif).

Determination of IDO enzymatic activity

Enzymatic activity of IDO was determined in conditioned media from DCs or the cocultures of DCs and autologous CD4⁺ T cells as previously described.²

Determination of cytokines

Cytokine concentrations (IL-4 and IFN- γ) were determined in the culture supernatants as indicated by using Duo-set ELISA kits (R&D Systems) according to the manufacturer's instructions.

Assessment of T-cell proliferation

Der p 1-sensitive or Bla g 1-sensitive CD4⁺ T-cell clones were incubated for 72 hours with Der p 1-pulsed or Bla g 1-pulsed DCs or with LPS-treated mDCs at different ratios for 72 hours. The CD4⁺ T-cell clones (10⁵ cells per well) were also cultured in 50% conditioned media derived from DCs or the cocultures of DCs with their autologous CD4⁺ T cells in the presence of anti-CD3 mAb (1 μ g/mL) for 72 hours. Carboxyfluorescein succinimidyl ester (Invitrogen, Eugene, Ore) staining was performed. Fractions of stained cells were analyzed by using fluorescence-activated cell sorting to calculate the percentage of CD4⁺ T-cell progenitors undergoing division as previously described.¹⁴

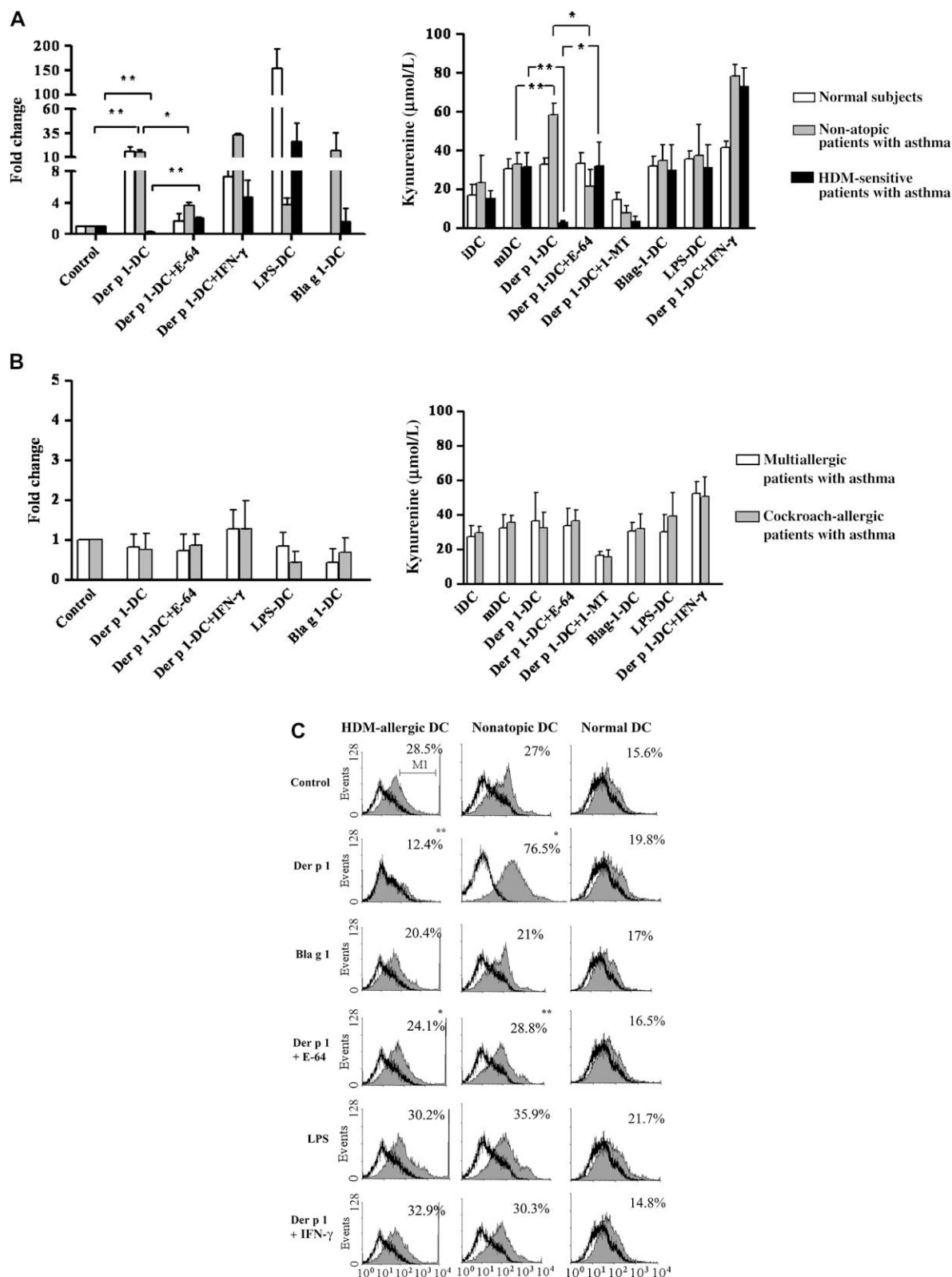


FIG 1. Differential effect of Der p 1 on the expression and activity (**A and B**) of IDO in DCs. Protein levels of IDO (**C and E**), surface molecules, and intracellular cytokines (**D**) in DCs and TNF receptors on monocytes (**F**) were analyzed by fluorescence-activated cell sorting. One representative experiment of 3 is shown. Results are expressed as the means $\pm$ SDs. * $P < .05$; ** $P < .01$.

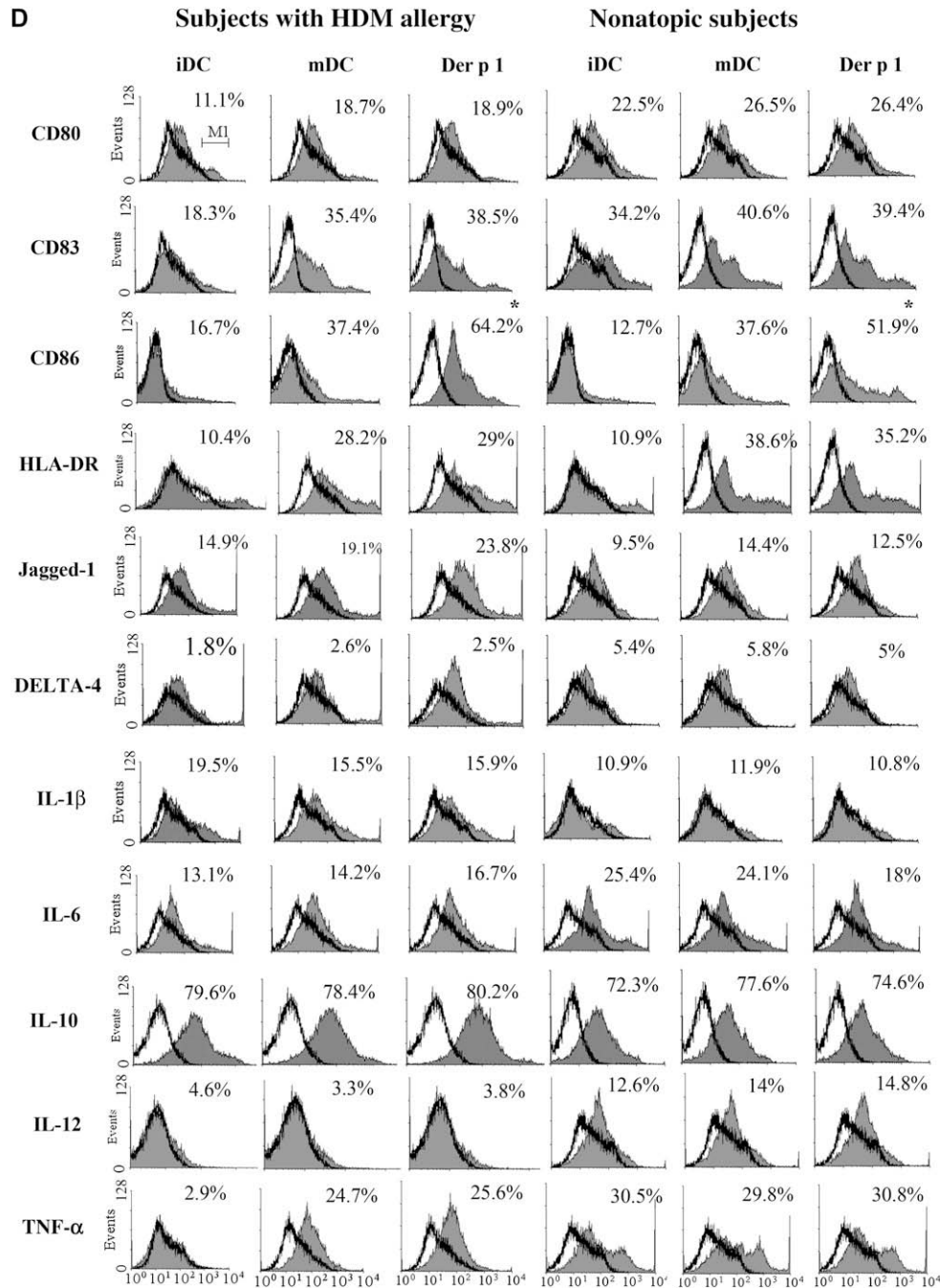


FIG 1. (Continued)

Statistical analysis

Means $\pm$ SDs of at least 3 independent experiments are shown. The Student paired *t* test was used to determine the significance of values in within-subject groups and the unpaired *t* test in between-subject groups.

RESULTS

Expression and function of IDO in DCs only from HDM-sensitive patients with asthma were suppressed with Der p 1

To study the effects of Der p 1 on IDO, immature DCs from HDM-sensitive patients with asthma, nonatopic patients with asthma, and healthy subjects were loaded with Der p 1 or Bla g

1 in the presence of TNF- α for maturation induction. In HDM-sensitive subjects with asthma, pulsation of DCs with Der p 1 significantly suppressed IDO mRNA ($P = .008$; Fig 1, A, left panel), protein expression ($P = .001$; Fig 1, C), and its bioactivity ($P = .005$; Fig 1, A, right panel). However, Der p 1 could not override IFN- γ -induced IDO transcription and its enzymatic activity in DCs from HDM-sensitive patients with asthma. In contrast, the pulse of Der p 1 to DCs from nonatopic patients with asthma dramatically enhanced mRNA ($P = .009$), protein expression ($P = .02$), and enzymatic activity of IDO ($P = .007$), whereas in healthy subjects, cockroach-sensitive patients, and patients with multiple allergies, only the increase in IDO mRNA was observed

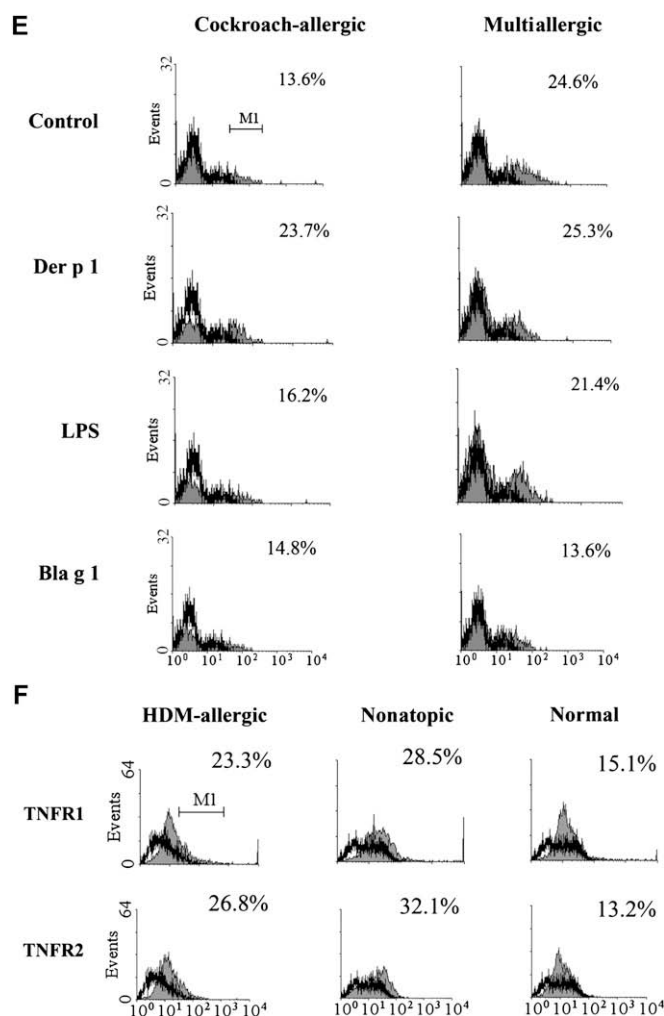


FIG 1. (Continued)

after Der p 1 pulsation (Fig 1, B and E). Der p 1-induced IDO activity in all conditions could be suppressed with a specific IDO inhibitor 1-MT. Bla g 1 pulsation or LPS treatment also induced IDO transcription but no significant differences in kynurenine levels in the respective conditioned media from HDM-sensitive, nonatopic, and cockroach-sensitive subjects with asthma. These data clearly indicated that Der p 1 differentially modulated the expression of functional IDO among subjects with asthma, depending on HDM sensitivity in nature.

We investigated whether Der p 1 effects were mediated through its protease activity. Pretreatment of Der p 1 by a specific protease inhibitor E-64 before the pulsation could prevent the modulation of IDO expression (Fig 1, A, left panel) and activity ($P = .025$, $P = .017$, respectively; Fig 1, A, right panel) in DCs from HDM-sensitive and nonatopic patients with asthma. Finally, we determined the effects of Der p 1 on costimulatory molecules and cytokine from DCs of these 2 groups with asthma. As shown in previous studies,^{6,15} Der p 1 exposure to HDM-allergic and nonatopic DCs did not raise the expression of CD80, CD83, and HLA-DR, whereas upregulation of CD86 expression was observed in HDM-sensitive DCs and nonatopic DCs exposed to Der p 1. Jagged-1 protein expression was increased only in Der p 1-pulsed DCs from HDM-sensitive patients with asthma and unchanged in those from nonatopic patients with asthma. No differences in IL-6,

IL-1 β , IL-10, IL-12, TNF- α , and DELTA-4 were observed in both groups with asthma (Fig 1, D). The possibility that the differential effects of Der p 1 on IDO expression could be the result of differences in maturation of DCs from monocytes was excluded by the observation that monocytes from HDM-sensitive and nonatopic patients with asthma had comparable expression of TNF- α receptors (TNFR1 and TNFR2; Fig 1, F).

Der p 1-reduced IDO activity in DCs from HDM-sensitive patients with asthma was associated with increasing T-cell proliferation

We investigated whether the differential effects of Der p 1 on IDO activity modulated the proliferation of Der p 1 and Bla g 1-specific T-cell clones. The proliferation of Der p 1-specific CD4⁺ T cells was significantly increased when cultured with Der p 1-pulsed DCs from HDM-sensitive subjects with asthma ($P = .009$ and $P = .001$ at the ratio of DCs to T cells (DC:T) of 1:50 and 1:5, respectively; Fig 2, A, upper panel), whereas LPS-treated DCs and Bla g 1-pulsed DCs did not significantly alter T-cell proliferation. In contrast, Der p 1-pulsed DCs from nonatopic subjects with asthma markedly attenuated proliferation of Der p 1-specific T cells ($P = .02$ and $P = .03$ at DC:T of 1:50 and 1:5, respectively), and this could be dramatically reversed by 1-MT treatment ($P = .01$ and $P = .003$ at DC:T of 1:50 and 1:5, respectively).

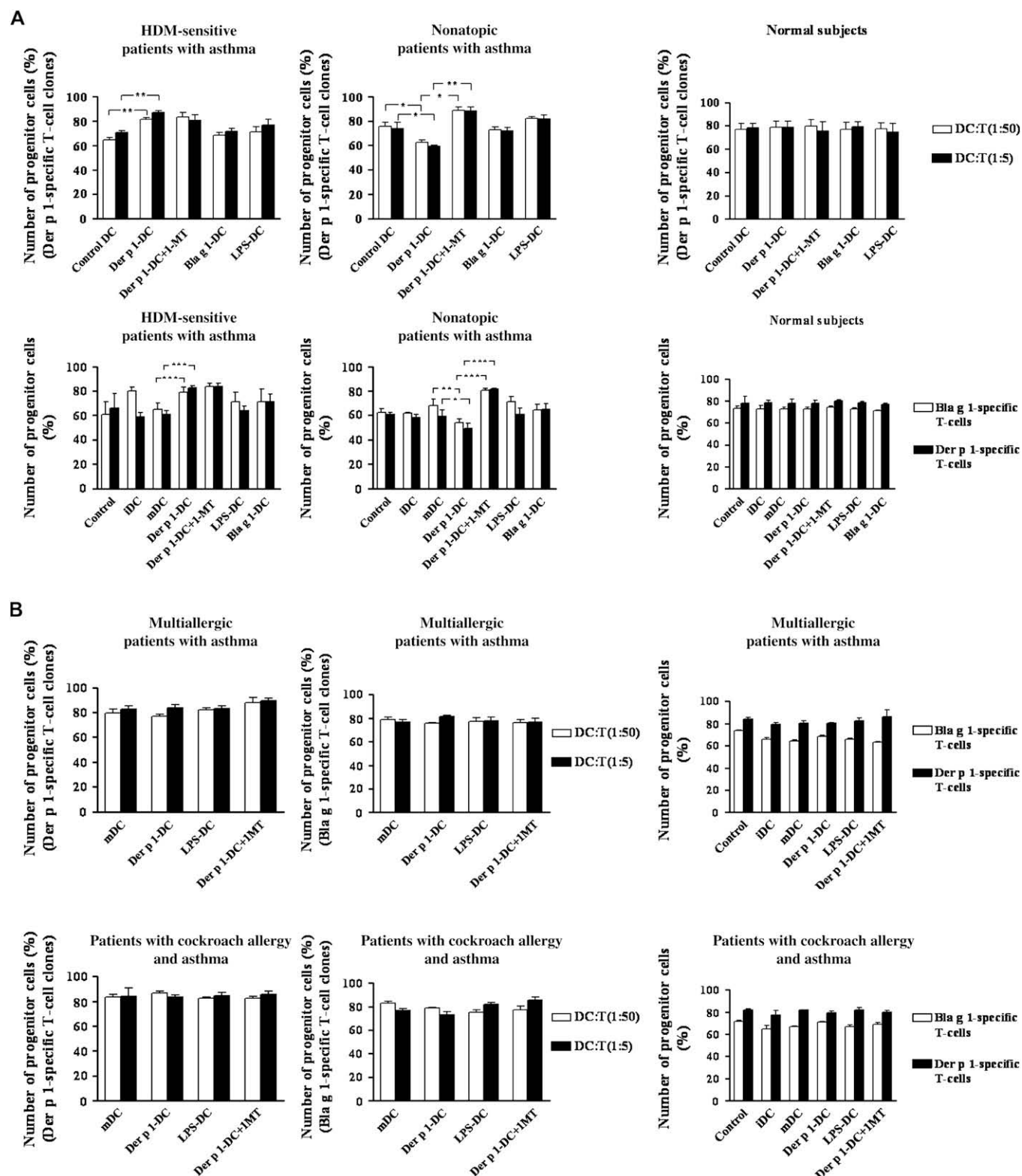


FIG 2. Proliferative response of allergen-specific T-cell clones to Der p 1-pulsed DCs or their conditioned medium from HDM-sensitive, nonatopic patients with asthma and normal subjects (A), and to Der p 1-pulsed DCs or their conditioned medium from multiallergic subjects and subjects with cockroach allergy (B). Means $\pm$ SDs of 3 independent experiments are shown.

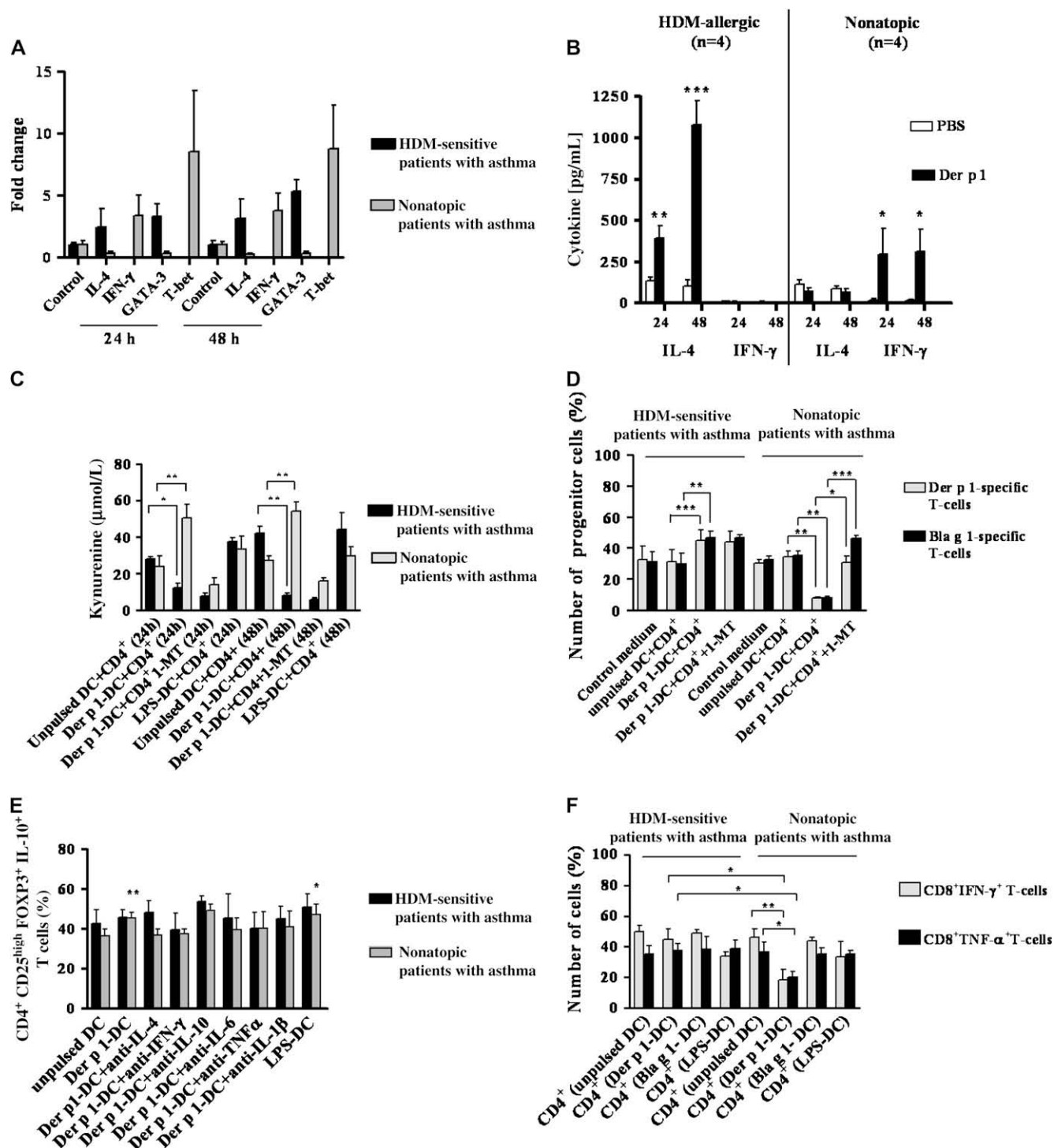


FIG 3. T_H1/T_H2 cytokine profiles (**A and B**) maintained IDO activity (**C**) and clonal T-cell proliferation (**D**) in HDM-sensitive and nonatopic DCs. An increase in regulatory T-cell subset (**E**) and its activity (**F**) was observed in the nonatopic condition. Means $\pm$ SDs of 3 independent experiments are shown.

However, when Bla g 1-specific T cells were applied instead of Der p 1-specific T cells for the coculture with Der p 1-pulsed DCs irrespective of 1-MT pretreatment, the proliferative response was comparable to the level originally induced by DCs without pulsation (data not shown). This suggested that the ability of Der p 1-pulsed DCs to modulate the proliferative response required the interaction with the relevant allergen-sensitive T cells.

The question arose whether the resulting DC-conditioned media with known IDO activity (Fig 1, A, right panel) would also modulate T-cell proliferation. Fresh Der p 1-specific and Bla g 1-specific T lymphocytes were suspended in the conditioned medium and activated with mitogen. Culture medium of Der p 1-pulsed DCs from HDM-sensitive subjects with asthma markedly enhanced the proliferation of both Der p 1-specific and Bla g

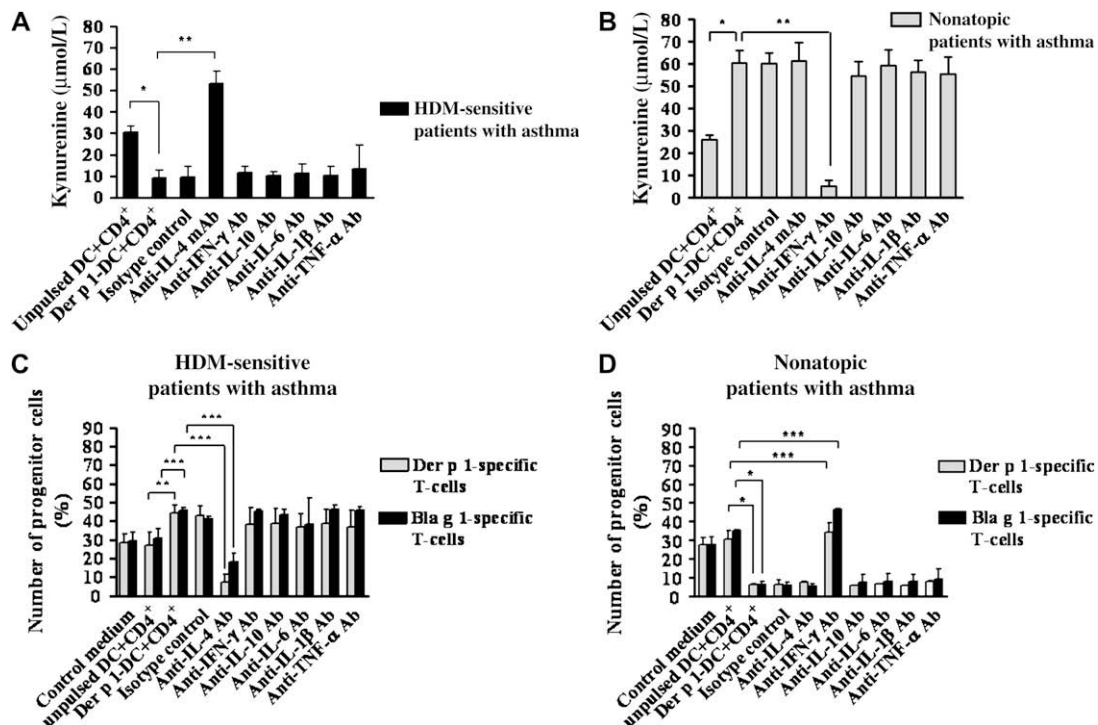


FIG 4. Neutralization study using mAbs revealed the regulation of IDO activity in DCs was mediated through IL-4 and IFN- γ (A and B) with a corresponding action on clonal T-cell proliferation (C and D). Means $\pm$ SDs of 3 independent experiments are shown.

1-specific T cells over those with control conditioned medium ($P = .0003$ and $P = .0005$, respectively; Fig 2, A, lower panel) or with unpulsed DC-conditioned medium ($P < .0001$ for both T-cell clones). In contrast, a significant reduction of proliferation was observed after incubating Der p 1- and Bla g 1-specific T-cell clones with Der p 1-pulsed DC-conditioned medium from nonatopic patients with asthma ($P = .01$ and $P = .004$, respectively). The addition of the specific IDO inhibitor 1-MT fully restored the proliferation of both allergen-specific T-cell clones ($P < .0001$; Fig 2, A, lower panel), suggesting that IDO compromised T-cell proliferation. Der p 1-modulating IDO effects on T-cell proliferation were specific because Bla g 1-pulsed DCs and LPS-treated DCs including their conditioned media had comparable activity to control-conditioned medium, and the presence of Der p 1-pulsed DCs or their culture conditioned media from cockroach-sensitive or multiallergic patients with asthma did not significantly modulate the proliferative response of T cells (Fig 2, B).

Reduced IDO activity in the cocultures resulted from Der p 1-modulating T_H1/T_H2 balance toward the latter

Because Der p 1-pulsed DCs from HDM-sensitive individuals could tip the balance of T_H1/T_H2 cytokines in $CD4^+$ T cells in favor of IL-4,⁶ whereas IDO transcription and its activity could be suppressed by IL-4,^{9,16} we investigated whether IL-4 was involved in the maintenance of Der p 1-induced IDO suppression in DCs during the cocubation with autologous $CD4^+$ T cells. To this end, the mRNA levels of both GATA-3 and T-bet were measured in the cocultured autologous $CD4^+$ T cells. In HDM-sensitive patients with asthma, the cocultured $CD4^+$ T cells markedly upregulated GATA-3 and simultaneously downregulated T-bet starting from the first 24 hours (Fig 3, A), leading to

strikingly increasing IL-4 production in parallel with strikingly decreasing IFN- γ production. In contrast, the presence of Der p 1-pulsed DCs from nonatopic patients with asthma induced the reverse conditions.

We further assessed IDO activity in supernatants harvested from the cocultures. In HDM-sensitive patients with asthma, the IDO activity in the coculture conditioned medium with Der p 1 pulsation was much lower than that with unpulsed condition ($P = .01$ for 24 hours, $P = .009$ for 48 hours; Fig 3, C). The coculture-conditioned medium induced significantly higher proliferation of Der p 1-specific and Bla g 1-specific T lymphocytes ($P = .0002$ and $P = .0098$, respectively; Fig 3, D). In contrast, in the coculture-conditioned medium with Der p 1 pulsation from nonatopic patients with asthma, kynurenine levels were significantly increased at 24 hours and 48 hours of the coculture ($P = .008$ and $P = .006$, respectively; Fig 3, C) in association with marked suppression of Der p 1-specific and Bla g 1-specific T-cell proliferation ($P = .0059$ and $P = .0043$, respectively), and this could be dramatically reversed by 1-MT treatment ($P = .01$ and $P = .0001$, respectively). In addition, an exposure of autologous T cells to Der p 1-pulsed DCs from nonatopic patients with asthma led to the upregulation of the $CD4^+CD25^{high}$ FOXP3⁺IL-10⁺ subset ($P = .004$; Fig 3, E) with immunosuppressive activity toward $CD8^+$ T cells demonstrated regarding TNF- α and IFN- γ production (Fig 3, F).

In the light of Der p 1-pulsed DC-regulating T_H1/T_H2 cytokine patterns in our previous findings and others implicating IL-4 as a suppressor^{9,16} and IFN- γ as an inducer of IDO,^{17,18} we speculated that IL-4 from HDM-sensitive asthmatic T cells and IFN- γ from nonatopic asthmatic T cells might mediate IDO suppression and induction, respectively. Consistent with this idea, neutralizing antibodies against IL-4 but not IFN- γ reduced suppression of

tryptophan-degrading activity in Der p 1-pulsed DC coculture-conditioned medium from HDM-sensitive subjects with asthma (Fig 4, A) whereas sequestration of IFN- γ in Der p 1-pulsed DC cocultures from nonatopic patients with asthma reduced induction of IDO activity (Fig 4, B). Furthermore, Der p 1-specific and Bla g 1-specific T cells activated in the presence of the IL-4 blocking antibody-conditioned cocultures from HDM-sensitive patients with asthma had low proliferative responses ($P < .0001$; Fig 4, C), whereas these T-cell clones stimulated in the presence of the neutralizing anti-IFN- γ antibody containing coculture medium from nonatopic patients with asthma actively proliferated ($P = .01$, $P = .0008$, respectively; Fig 4, D). The possibility that there was an additional signal from Der p 1-pulsed DC-produced cytokines that might act in concert with IL-4 or IFN- γ was excluded because none of neutralizing antibodies against DC-derived cytokines affected kynurenine levels and T-cell proliferation in the coculture-conditioned medium.

DISCUSSION

This study has demonstrated for the first time that Der p 1 differentially modulates IDO function in TNF- α -induced maturing DCs from HDM-sensitive and nonatopic patients with asthma. Der p 1 was clearly capable of suppressing IDO production and its activity while markedly enhancing the expression and activity of IDO in HDM-sensitive and nonatopic asthmatic-derived DCs, respectively. In addition, the resulting decrease or increase in IDO activity subsequently increased or decreased Der p 1-specific T-cell proliferation. Both effects were mediated through the exposure of the allergen-specific T cells to either Der p 1-pulsed DCs or to the DC culture microenvironments. A complete reversal of Der p 1-pulsed DC-induced suppression of T-cell proliferation by the addition of 1-MT confirmed the major role of IDO in the regulation of Der p 1-specific T-cell expansion. Furthermore, the initial IDO activities in Der p 1-pulsed DCs from HDM-sensitive and nonatopic patients with asthma were maintained through the coculture with the respective autologous CD4⁺ T cells by IL-4 and IFN- γ as determined by the neutralization studies. In addition, high IDO activity in Der p 1-pulsed DCs of nonatopic patients with asthma after the coculture with CD4⁺ T cells was a result of increasing regulatory T-cell subset among CD4⁺ T cells. All data demonstrated that the capability of Der p 1 to regulate IDO activity differentially in DCs from HDM-sensitive patients with asthma and nonatopic patients with asthma was influenced by the interaction with CD4⁺ T cells (Fig 5). These observations may account for the difference in IDO regulation by DCs *in vivo* during initial allergen exposure and subsequently during their interaction with T cells.

Our key finding is that the IDO regulatory action is unique to Der p 1, but not other antigens (ie, Bla g 1), depending on HDM sensitivity. This action could be inhibited by E-64 and therefore requires cysteine protease activity of Der p 1. This indicates that the described effects are intrinsic to DCs from patients with asthma with sensitivity to Der p 1 that are handled differently from those sensitive to Bla g 1. The IDO modulatory actions of Der p 1 are selective because Der p 1 pulsation did not alter the expression of other molecules of mDCs such as maturation-associated receptors, activation markers, and cytokines. We excluded the possibility that the differential effects of Der p 1 could result from the differences in maturation of DCs from monocytes by the observation of comparable expression of TNF- α receptors on monocytes of both HDM-sensitive and nonatopic patients with asthma.

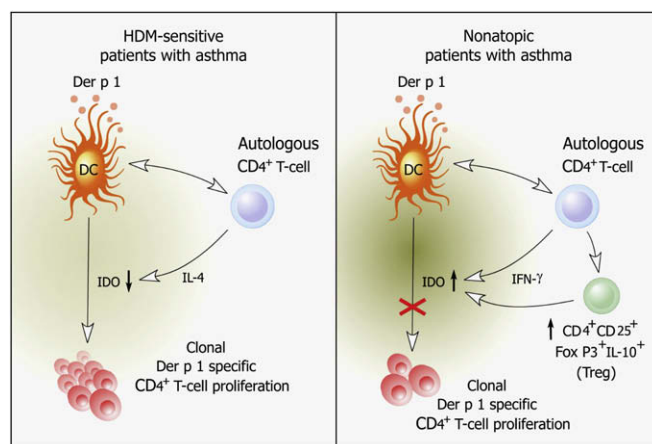


FIG 5. Differential regulation of IDO by Der p 1 in DCs based on HDM sensitivity was mediated through IL-4, IFN- γ , and the regulatory T-cell subset.

Our T-cell proliferation assays showed that the effects of Der p 1 on mitogen-induced proliferation of HDM-specific T cells were mediated via both DC-to-T-cell contact and culture microenvironments. The conditioned medium from nonatopic DCs alone could suppress the proliferation of all T-cell clones, whereas that of Der p 1-sensitive DCs could not suppress any T-cell clone. Therefore, the suppressive mediator had already been released to the medium. Because only the exposure of HDM-sensitive DCs to the Der p 1-sensitive T cells could exempt their proliferation from the suppression, but not in other conditions of cell-cell interactions, the generation of the suppressive mediator possibly required the matching of signal 1, the classic interaction between antigen-coupled HLA on DCs and the TCR on T cells. Although the decrease in proliferation of HDM-specific T cells during the coculture with Der p 1-pulsed DCs from nonatopic patients with asthma is in contrast with the previous study,⁶ this suppression could be completely reversed by inhibiting IDO activity and therefore provided convincing evidence for the major role of IDO in this DC-T-cell interaction. These different observations might be explained by the fact that the responsiveness of IDO to Der p 1 exposure is possibly different or that the IDO levels in our TNF- α -induced mature DCs with Der p 1 pulsation from nonatopic patients with asthma might be markedly higher than those in Der p 1-pulsed DCs without TNF- α stimulation in the previous study.⁶ Interestingly, the proliferation of Bla g 1-specific T cells was not affected by the interaction with Der p 1-pulsed DCs, but their proliferation was modulated by the DC culture-conditioned medium. This suggested that IDO activity in the microenvironments regulated clonal expansion of allergen-specific T cells. We further postulate that the inability of Der p 1-pulsed DCs to regulate proliferation of Bla g 1-specific T cells might be a result of the mismatch in signal 1; the accompanying signal 2 cytotoxic T lymphocyte-associated protein 4 (CTLA-4) could not induce a reverse signal to B7 on DCs, a known upregulatory pathway for IDO.^{19,20} However, the further study is required to investigate the mechanism for this unresponsiveness.

Indoleamine 2, 3-dioxygenase downregulates effector T-cell functions, and the regulatory T-cell subset was increasing.²¹⁻²⁵ The coculture of autologous Der p 1-pulsed DCs and nonatopic asthmatic T cells induced an increase in the CD4⁺CD25^{high}Foxp3⁺IL-10⁺ subset. Consistent with previous studies,^{26,27} the regulatory T-cell subset was able to suppress CD8⁺ T-cell cytokine

responses by inhibiting TNF- α and IFN- γ production. Because the acquisition of suppressive function of regulatory T cells requires the expression of CTLA-4²⁸ and CTLA-4 signaling could upregulate IDO expression in DCs,^{19,20} these data led us to postulate that the induction of regulatory T cells with suppressive activity might be involved in the maintenance of immune tolerance in non-atopic patients with asthma in response to Der p 1 exposure.

The lung has 2 resident cell types, epithelial cells and myeloid DCs, that constitutively express IDO protein, and 2 others, the plasmacytoid DC and eosinophil, that can be extensively recruited during allergic inflammation. It appears that the function of IDO in eosinophils is either stimulatory²⁹ or inhibitory^{3,30,31} depending on target cell, stimulus, and accompanying specific model. In contrast with the recent animal model,³² our *in vitro* data suggested that the function of IDO was inhibitory because the suppression of Der p 1-specific T-cell proliferation mediated by mDC from nonatopic patients with asthma was completely reversed by the IDO inhibitor 1-MT. We speculated that the differences in our findings and previous findings might be a result of the interspecies variation. On the basis of this study and the previous report,²⁹ it is possible that mDCs and eosinophils might cooperate in fully inducing and maintaining allergic immune response in HDM-sensitive patients with asthma via 2 different IDO functions.

Taken together, all data suggest that in HDM-sensitive subjects with asthma, the loss of tolerogenicity of Der p 1-pulsed DCs that occurred at first after allergen exposure and persisted during the interaction with T cells might help to promote allergic immune response. On the other hand, upregulation of IDO activity in nonatopic subjects with asthma may be a negative regulatory mechanism for T-cell unresponsiveness to Der p 1 exposure.

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Clinical implications: IDO suppression can be responsible for Der p 1-driven airway inflammation in HDM-sensitized patients with asthma.

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