FcεRI Induces the Tryptophan Degradation Pathway Involved in Regulating T Cell Responses

Dagmar von Bubnoff, Heike Matz, Christine Frahnert, Marie Luise Rao, Daniel Hanau, Henri de la Salle and Thomas Bieber

*J Immunol* 2002; 169:1810-1816; doi: 10.4049/jimmunol.169.4.1810

http://www.jimmunol.org/content/169/4/1810

**References**

This article cites 36 articles, 12 of which you can access for free at: http://www.jimmunol.org/content/169/4/1810.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
FceRI Induces the Tryptophan Degradation Pathway Involved in Regulating T Cell Responses

Dagmar von Bubnoff,1,2 Heike Matz,1 Christine Frahnert, † Marie Luise Rao, ‡ Daniel Hanau, ‡ Henri de la Salle,3* and Thomas Bieber3*}

FceRI is suspected to play a pivotal role in the pathophysiology of atopic disorders such as atopic dermatitis. In search for genes differentially regulated by FceRI on APCs, a differential cDNA bank of receptor-stimulated and unstimulated monocytes was established. By means of suppression subtractive hybridization, we identified kynurenine 3-monooxygenase and subsequently indoleamine 2,3-dioxygenase (IDO) to be overexpressed in FceRI-activated monocytes. IDO is the rate-limiting enzyme in the catabolism of the essential amino acid tryptophan. We show that cross-linking of FceRI on monocytes results in low tryptophan concentrations associated with impaired T cell stimulatory capacity. Importantly, T cell suppression could be prevented by the addition of tryptophan or inhibition of IDO. Moreover, stimulation of T cells by FceRI-activated monocytes was increased compared with T cell stimulation by nonactivated monocytes if exogenous supply of tryptophan was available. We speculate that the expression of IDO by FceRI+ APCs in vivo allows these cells to regulate T cell responses by inhibiting or stimulating T cell proliferation, depending on the metabolic environment. The Journal of Immunology, 2002, 169: 1810–1816.

The high-affinity receptor for IgE (FceRI) is involved in IgE-mediated allergic reactions (1). Besides its constitutive expression on mast cells and basophils, FceRI can be detected on certain APCs of atopic individuals (2, 3). On human monocytes, FceRI has been identified both in donors with atopic diseases such as atopic dermatitis (AD) and in clinically healthy individuals with an atopic family background (4). There, FceRI has been shown to mediate efficient IgE-dependent allergen uptake and presentation to T cells (5).

The processes that activate and promote T cell-dependent inflammation are better known than those that limit, turn off, or prevent T cell proliferation. APCs are thought to mediate these profound effects on T cells where kinetics of regulatory mechanisms seem to be highly important (6, 7). The activation of FceRI almost immediately results in the synthesis and secretion of proinflammatory cytokines that contribute to the establishment of allergic inflammation (8, 9). Recently, new immunomodulatory signals have been described by the engagement of FceRI on human monocytes: the activation of monocytes by FceRI mediates protection of these cells against apoptosis, thereby prolonging the survival of monocytes (10). In contrast, FceRI-mediated IL-10 production by monocytes prevents their differentiation into myeloid dendritic cells (DCs) (11), which are the most potent activators of T cells (12). Apparently, there are differences in the mobilization of defense mechanisms by the engagement of the receptor. To clarify functional significances of FceRI, it is essential to exactly define and investigate donors presenting similar clinical and cellular appearance (atopic inflammatory disease or not; FceRI, CD23 cell surface expression; serum IgE levels).

To identify differentially regulated genes by the engagement of FceRI, suppression subtractive hybridization was conducted of FceRI-stimulated and unstimulated monocytes from a clinically healthy person with an atopic family background. Interestingly, besides sequences coding for proinflammatory cytokines or chemokines, we found one clone whose sequence corresponded to kynurenine 3-monooxygenase, an enzyme involved in the degradation of the amino acid tryptophan. Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme in the catabolism of tryptophan converting this amino acid into kynurenine. Kynurenine is catalyzed by kynurenine 3-monooxygenase into 3-hydroxy-kynurenine and quinolinic acid (13, 14). The induction of cellular IDO is a common antimicrobial defense mechanism for certain cells by depleting tryptophan from intracellular pools or local microenvironments. The restriction of available tryptophan leads to a condition in which cells become starved for tryptophan (15). Recently, IDO production from macrophages and DCs stimulated with IFN-γ or IFN-γ and CD40 ligand (CD40L) has been demonstrated to inhibit T cell proliferation in vitro (16, 17). The functional role of IDO has been demonstrated in vivo where tryptophan depletion in the mammalian placenta is crucial for establishing immune tolerance during pregnancy (18). Because AD is clinically and immunohistologically a cell-mediated hypersensitivity reaction, positive and negative (tolerogenic) FceRI-mediated events may induce immunomodulatory signals that regulate lymphocyte proliferation (19).

In this study, we show that IDO and kynurenine 3-monooxygenase are overexpressed in FceRI-activated monocytes. Functionally, this effect seems to be time dependent starting after ~24 h of activation. Then, these cells acquire the ability to suppress T cell proliferation in vitro by degradation of tryptophan. T cell suppression can be prevented by pharmacological inhibition of IDO or
supplementation of tryptophan. In comparison with nonactivated atopic monocytes, our studies imply an increase of T cell proliferation with FceRI-activated monocytes in vitro under nonlimiting conditions for tryptophan. This T cell stimulatory capacity of atopic monocytes may mirror the pathophysiological condition of atopic patients in vivo (20).

Materials and Methods

Cell samples

All blood samples were obtained after informed consent from volunteers in accordance with the local ethics committee. For the generation of a FceRI-specific cDNA library, monocytes were isolated by continuous flow centrifugation leukophoresis and centrifugal cell elutriation as previously described (21). Monocytes were determined to be >95% pure by cell surface markers. For the following experiments, clinically healthy atopics were determined by their positive atopic family background, i.e., family members were known to have a history of atopic diseases such as allergic rhinitis, allergic asthma, or AD (n = 4; referred to as atopics). They disclosed IgE levels >100 kU/L in the serum and were determined as positive for FcεRI, when FcεRI was expressed on >15% of peripheral monocytes. Nonatopics were healthy individuals without any atopic background (n = 4; referred to as nonatopics); the serum IgE levels of nonatopics were <100 kU/L and FcεRI surface expression was <10%.

Flow cytometry

Surface labeling for FcεRI, CD23, and CD40 was performed as reported previously (22). Results are expressed as the percentage of positive cells compared with the isotype control.

Stimulation of cells

Cells were incubated for 1 h with 4 µg/ml human (h)IgE or 10 µg/ml anti-FcεRI mAb (Ab’s); 22E7 (Dr. J. Kochan, Department of Autoimmune Diseases, Hoffmann-LaRoche, Nutley, NJ) or an isotype-matched control mAb (mIgG1) at 37°C. After washing with culture medium, 20 µg/ml rabbit anti-IgE or 5 µg/ml goat anti-mouse IgG (GaMIGG) were added for the duration of the culture. For RT-PCR, cells were stimulated in the presence of IFN-γ (1000 U/mL). Cells were incubated in culture flasks or six-well plates (Nalge Nunc International, Roskilde, Denmark) at a density of 1 × 10^6 cells per milliliter unless otherwise indicated.

Subtractive cDNA library

Monocytes were selectively stimulated by cross-linking FcεRI with hIgE and rabbit anti-human IgE as indicated above for 4 h. Polyadenylated mRNA was purified using the Oligotex mRNA kit from Qiagen (Courtaboeuf, France) from 50 × 10^6 stimulated (testor population) and 150 × 10^6 unstimulated (driver population) monocytes. One and 6.4 µg of poly(A)^+ mRNA from stimulated and unstimulated monocytes, respectively, were reverse transcribed and processed by the use of PCR-Select cDNA Subtraction kit (Clontech Laboratories, Burlingame, CA), which is based on the method of suppression subtractive hybridization.

Cloning and analysis of the subtracted cDNA

Products from the subtracted cDNA were digested with the RsraI restriction enzyme and inserted into the EcorV restriction site of pKS cloning vector (Stratagene, Amsterdam, The Netherlands). Plasmid DNAs were prepared (Flexiprep extraction kit; Amersham Pharmacia, Orsay, France), DNA sequencing reactions were performed using the BigDye terminator sequencing kit (PE Applied Biosystems, Fullerton, CA). Nucleic acid homology searches were performed using the basic local alignment search tool program at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, MD).

RT-PCR

RT-PCR was done as described previously (3). Specific primer sequences for each gene were as follows: human β-actin forward (5’-GAGCGG GAAATCTGCTGGTGACATT-3’) and reverse (5’-GATGGAGCTTGGAA GATGTTCGTCCTG-3’) (240 bp); human tryptase forward (5’-CTCCCT CATTCACCCCGGAG-3’) and reverse (5’-GGATCCACGGTCACTG GAAGTTCCTGTGAGCTGGTG-3’) (520 bp); human kynurenine 3-monooxygenase forward (5’-ACGCTCGTCGACGGTTGGCCCAGTAAG-3’) and reverse (5’-TGGACTTCCCATCCAC CCCCAACCAGTAAAT-3’) (400 bp); human IFD0 forward (5’-CTCCCTGGTCTCCTTACGTCG-3’) and reverse (5’-GAAATCTGCTGGTGACATT-3’) (430 bp). Amplification was performed on a PerkinElmer GeneAmp PCR System 9600 thermocycler (PE Applied Biosystems). The PCR cycle numbers were 30 for trypstat and 25 for kynurenine 3-monooxygenase and IDO. β-Actin was used to normalize specific PCR amplifications. PCR fragments were separated on 1% agarose gels and visualized using ethidium bromide staining. Identity of RT-PCR products was confirmed by direct sequencing.

Kynurenine assay

The measurement of kynurenine levels in the media provides evidence of tryptophan degradation and functional IDO activity. Monocytes were cultured for 24 h at a density of 1 × 10^6 cells/well in 96-well flat-bottom plates. The cells were washed and resuspended in HBSS containing 100 µM tryptophan (Sigma-Aldrich, St. Louis, MO) for an additional 4 h. Supernatants were harvested thereafter for quantification of tryptophan and kynurenine by HPLC. For measurements of tryptophan and kynurenine in monocyte and T cell cocultures, normal culture medium supernatants were collected 24 h after the addition of T cells to monocytes.

HPLC

Samples were deproteinized by the addition of 250 µl 5% sulfosalicylic acid to 250 µl cell culture supernatant (23). After incubation for 10 min at room temperature samples were centrifuged at 13,000 × g for 10 min at 8°C. Tryptophan and l-kynurenine were assayed by HPLC with UV detection (UV detector SPD-10A at 254 nm; Shimadzu, Duisburg, Germany).

Proliferation assays

T cell and monocyte isolates from peripheral blood of atopic and nonatopic donors were done as described (13). Monocytes were stimulated with hIgE/anti-IgE for 24 h or left untreated and seeded in 96-well flat-bottom plates (1 × 10^6 cells/well; 200 µl). After 24 h, medium was replaced and purified autologous T cells (2 × 10^6 cells/well) were added along with anti-CD3 mAb (100 ng/ml; clone UCH1; Beckman Coulter, Marseille, France) in the presence or absence of different concentrations of the IDO inhibitor 1-methyl-L-tryptophan (1-MT; 250, 500, and 1000 µM; Aldrich Chemicals, Milwaukee, WI). To determine the role of IFN-γ and IL-10, antihuman mAb (500 ng/ml) and anti-IL-10 mAb (10 µg/ml) or an equivalent isotype control were added as indicated at the same time with the T cells. Tryptophan (1 µg/ml) equal to 1× normal tryptophan concentration of the medium; Sigma-Aldrich) was added to cocultures after 24, 48, and 72 h as indicated. Seventy-two hours later, T cell proliferation was measured using overnight incubation with [3H]thymidine (0.5 µCi/well). Apoptotic and nonviable monocytes were determined using FITC-labeled annexin V and propidium iodide (Roche Molecular Biochemicals, Mannheim, Germany). In all conditions (stimulated and unstimulated), monocyte viability was >90% after 48 h.

To study T cell proliferation without monocytes, anti-CD3 mAb was immobilized onto 96 flat-bottom tissue culture wells (10 µg/ml, 100 µl well, according to the manufacturer’s protocol). Monocytes were activated overnight. The 24 h and the medium was replaced. After an additional 24 h of incubation, supernatants were collected. Sheep anti-mouse IgG magnetic beads (Dynabeads M-280; Dynal Biotech, Oslo, Norway) that were bound to mouse anti-IL-10 mAb (3 µg/10^5 beads) were added to the supernatants (2 × 10^6 beads/ml) according to the manufacturer’s protocol. After 2 h of incubation at 4°C with gentle mixing, IL-10 was depleted by applying a magnet at least six times. Supernatants were collected for IL-10 by ELISA and T cells were stimulated in IL-10-depleted monocyte supernatants.

Endotoxin levels in culture preparations were determined using a Limulus amebocyte lysate assay (BioWhittaker, Walkersville, MD) by an independent laboratory and were <0.025 IU/ml in all media.

Detection of cytokines

For ELISA, culture supernatants were centrifuged twice at 4°C followed by immediate storage at −70°C. IL-10 production from FcεRI-activated (IgE/anti-IgE) and nonactivated monocytes was assayed following culture of monocytes (1 × 10^6 cells per well; 200 µl) for 24 h, replacement of medium, and incubation for another 24 h. For quantification of IFN-γ in cocultures, supernatants were harvested 24 h after the addition of T cells. ELISA were conducted in triplicate according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

IFI-γ bioassay

The human colonic adenocarcinoma cell line HT-29 (kindly provided by Prof. N. Koch, Department of Zoophysiology, University of Bonn, Bonn, Germany) was used to determine bioactive IFN-γ 24 h after neutralization of IFN-γ in cocultures. IFN-γ induces the de novo synthesis of MHC class
II molecules in a concentration-dependent manner on the cell surface of HT-29 cells (24). MHC class I molecules, which are constitutively expressed on HT-29 cells, are sensitively up-regulated upon low doses of IFN-γ (25). As a dose-response curve and positive control, increasing amounts of human rIFN-γ was added in the following concentrations: 10, 100, 250, 500, 1000, and 2000 pg/ml. As negative control, the cells were incubated with normal culture medium. All experiments were in triplicate.

FACS analysis was performed after cultivation of these cells for 72 h. MHC class I-specific mAb W6/32 (mIgG2b, hybridoma culture supernatant), HLA-DR-specific mAb L243 (mIgG2a, hybridoma culture supernatant), and an isotype control Ab were used to analyze cellular fluorescence.

IL-10 bioassay

Ba/F3 cells, an IL-3-dependent pro-B cell line cotransfected with hIL-10R, were used to determine biologically active IL-10 after supernatant depletion of IL-10 from activated monocytes. A standard curve with hIL-10, ranging from 10 to 100,000 pg/ml per well was prepared. Positive (10 ng/ml IL-3) and negative (culture medium) controls were included. Proliferation was done in 96-well plates in triplicate (final volume, 100 µl; 5 x 10³ cells per well). After 72 h, [³H]thymidine (0.5 µCi/well) was added for another 24 h.

Data analysis

Data are mean ± SD. For T cell proliferation assays, different numbers of experiments were performed per experimental unit (donor). To account for the variable number of observations per experimental unit, the dependency of T cell proliferation on the experimental conditions was analyzed using general linear modeling techniques as implemented in the SAS procedure Genmod (SAS Institute, Cary, NC). Values of p < 0.05 are reported as statistically significant.

Results

Kynurenine 3-monooxygenase is detected by differential display

First, FcεRI expression on monocytes of the atopic donor of whom the differential cDNA bank was established from has been determined by flow cytometry. Monocytes revealed 72% FcεRI expression compared with the isotype control; the low-affinity receptor for IgE, CD23, was found to be expressed on <10% of monocytes and CD40 expression was >90%. The cells were stimulated for 4 h with IgE/rabbit anti-IgE or left untreated. A differential cDNA bank was constructed. About 100 clones were sequenced and analyzed for homology in the GenBank and European Molecular Biology Laboratory databases. One cDNA clone was found to correspond to human kynurenine 3-monooxygenase encoding mRNA, an enzyme involved in the degradation of tryptophan along the kynurenine pathway. The overexpression of this gene in FcεRI-stimulated monocytes was verified by specific PCR run in the tester (FcεRI-stimulated) and driver (unstimulated) population (data not shown).

IDO and kynurenine 3-monooxygenase overexpression in FcεRI-stimulated monocytes

To confirm the above results, four additional clinically healthy individuals with an atopic family background and four nonatopics were investigated. Monocyte surface morphology, mRNA overexpression of IDO, the rate-limiting enzyme in the tryptophan degrading pathway, and kynurenine 3-monooxygenase transcript expression after FcεRI cross-linking were determined. Flow cytometric analysis for FcεRI, FcεRII (CD23), and CD40 of freshly isolated atopic and nonatopic monocytes (n = 4; >90% CD14 positive) was performed (Fig. 1). These two groups of donors were chosen because they differed significantly only in their FcεRI surface expression. Basophil cell contamination was ruled out by specific RT-PCR for tryptophan (data not shown).

IDO and kynurenine 3-monooxygenase gene transcription after monocyte stimulation with IgE/anti-IgE, 22E7 (anti-FcεRIα)/goat anti-mouse IgG, mlgG1/GaMlgG, and IFN-γ was analyzed by RT-PCR and compared with unstimulated cells. Total RNA was prepared after 4 and 24 h of incubation, converted into cDNA, and subjected to PCR. In atopic monocytes after 24 h of induction, IDO and kynurenine 3-monooxygenase transcripts were significantly overexpressed in FcεRI-stimulated cells as compared with the isotype control and unstimulated monocytes (Fig. 2). At 4 h of induction, ~10 additional cycles each were necessary to obtain the same levels of RT-PCR products corresponding to IDO and kynurenine 3-monooxygenase (data not shown). In nonatopic FcεRI-activated and nonactivated monocytes, no specific transcripts were seen applying the above PCR conditions for 4 h (data not shown). At 24 h of induction, low levels of kynurenine 3-monooxygenase transcripts could be detected in nonatopic nonactivated and activated monocytes, which was comparable to nonactivated atopic monocytes. This baseline expression of kynurenine 3-monooxygenase presumably results from the activation of cells by adherence onto plastic and/or during isolation procedures. In contrast, IFN-γ induced similar kynurenine 3-monooxygenase transcript band intensities in atopic and nonatopic monocytes at 4 h (data not shown) and 24 h. At 24 h of IFN-γ stimulation, atopics showed an enhanced IDO band intensity over nonatopics.

Induction of functionally active IDO upon FcεRI ligation on monocytes

To measure functionally active IDO, HPLC was done to determine the breakdown product of tryptophan and kynurenine. Monocytes from atopics and nonatopics were activated for 24 h with IgE/anti-IgE or IFN-γ (1000 U/ml) or were left untreated. Cells were washed and resuspended in HBSS containing 100 µM tryptophan.

FIGURE 1. Flow cytometric analysis of monocytes from atopic and nonatopic donors. Mean percentages of FcεRI, CD23, and CD40 surface expression of four atopic and four nonatopic donors ± SD.

FIGURE 2. FcεRI-inducible IDO and kynurenine 3-monooxygenase transcripts in atopic monocytes. RT-PCR of IDO and kynurenine 3-monooxygenase gene transcript expression from unstimulated and stimulated monocytes. Results are representative for all atopic and nonatopic donors.
and incubated at 37°C for an additional 4 h. Supernatants were harvested and analyzed by HPLC for the presence of kynurenine (Table I). In nonatopic monocytes kynurenine was detected significantly only upon stimulation with IFN-γ. In all but one of the atopic donors, kynurenine was significantly detected after FcεRI stimulation and each donor responded with the induction of IDO upon IFN-γ stimulation.

Suppression of T cell proliferation and its prevention by inhibition of IDO and supplementation with tryptophan

Next we addressed the question of whether the degradation of tryptophan by FcεRI-activated atopic monocytes results in functional abrogation of T cell proliferation. If so, the suppression should be prevented by pharmacologic inhibition of IDO or supplementation of tryptophan. 1-MT has been shown to be a competitive inhibitor for IDO. Atopic monocytes were FcεRI activated or left untreated for 24 h and the medium was replaced. Autologous T cells were added in the presence of different concentrations of 1-MT (Fig. 3, A and C). Alternatively, cocultures were supplemented with 1 μg/10 μl of tryptophan (which corresponds to 1× the tryptophan concentration found in normal medium) 24, 48, and 72 h after T cell addition. FcεRI-activated atopic monocytes functionally suppressed T cell proliferation, which could be prevented in a dose-dependent manner by the addition of 1-MT. Atopic nonactivated monocytes did not show this T cell-suppressive effect. The supplementation of l-tryptophan to atopic activated monocytes and T cells not only prevented T cell suppression but in addition increased T cell proliferation compared with cultures with nonactivated monocytes. In contrast, nonatopic monocytes did not show this suppressive T cell effect (Fig. 3, B and D). To rule out an immunostimulatory influence of 1-MT, T cell proliferation without monocytes was done in different concentrations of 1-MT but no effect of inhibitor was observed throughout the range of concentrations used (data not shown).

Catabolism of tryptophan in atopic FcεRI-activated cocultures

To confirm functional IDO activity in atopic activated (IgE/anti-IgE and 22E7/GaMIgG) monocyte cocultures with T cells, culture supernatants were harvested for tryptophan and kynurenine 24 h after the addition of T cells to stimulated or unstimulated atopic monocytes (Fig. 4). Mean tryptophan levels in the culture medium of stimulated (IgE/anti-IgE and 22E7/GaMIgG) monocytes of three atopic cocultures decreased ~37% of the initial amount of tryptophan in the culture medium after 24 h, whereas this amount was ~7% for unstimulated monocytes with T cells. IDO activity was detected in three of three cocultures with atopic FcεRI-activated monocytes and each donor responded with the induction of IDO supplementation of tryptophan. 1-MT has been shown to be a competitive inhibitor for IDO or supplementation of tryptophan. If so, the suppression should be prevented by pharmacologic inhibition of IDO or suppression but in addition increased T cell proliferation compared with cultures with nonactivated monocytes. In contrast, nonatopic monocytes did not show this suppressive T cell effect (Fig. 3, B and D). To rule out an immunostimulatory influence of 1-MT, T cell proliferation without monocytes was done in different concentrations of 1-MT but no effect of inhibitor was observed throughout the range of concentrations used (data not shown).

Catabolism of tryptophan in atopic FcεRI-activated cocultures

To confirm functional IDO activity in atopic activated (IgE/anti-IgE and 22E7/GaMIgG) monocyte cocultures with T cells, culture supernatants were harvested for tryptophan and kynurenine 24 h after the addition of T cells to stimulated or unstimulated atopic monocytes (Fig. 4). Mean tryptophan levels in the culture medium of stimulated (IgE/anti-IgE and 22E7/GaMIgG) monocytes of three atopic cocultures decreased ~37% of the initial amount of tryptophan in the culture medium after 24 h, whereas this amount was ~7% for unstimulated monocytes with T cells. IDO activity was detected in three of three cocultures with atopic FcεRI-activated monocytes and each donor responded with the induction of IDO supplementation of tryptophan. 1-MT has been shown to be a competitive inhibitor for IDO or supplementation of tryptophan. If so, the suppression should be prevented by pharmacologic inhibition of IDO or suppression but in addition increased T cell proliferation compared with cultures with nonactivated monocytes. In contrast, nonatopic monocytes did not show this suppressive T cell effect (Fig. 3, B and D). To rule out an immunostimulatory influence of 1-MT, T cell proliferation without monocytes was done in different concentrations of 1-MT but no effect of inhibitor was observed throughout the range of concentrations used (data not shown).

Catabolism of tryptophan in atopic FcεRI-activated cocultures

To confirm functional IDO activity in atopic activated (IgE/anti-IgE and 22E7/GaMIgG) monocyte cocultures with T cells, culture supernatants were harvested for tryptophan and kynurenine 24 h after the addition of T cells to stimulated or unstimulated atopic monocytes (Fig. 4). Mean tryptophan levels in the culture medium of stimulated (IgE/anti-IgE and 22E7/GaMIgG) monocytes of three atopic cocultures decreased ~37% of the initial amount of tryptophan in the culture medium after 24 h, whereas this amount was ~7% for unstimulated monocytes with T cells. IDO activity was detected in three of three cocultures with atopic FcεRI-activated monocytes and each donor responded with the induction of IDO supplementation of tryptophan. 1-MT has been shown to be a competitive inhibitor for IDO or supplementation of tryptophan. If so, the suppression should be prevented by pharmacologic inhibition of IDO or suppression but in addition increased T cell proliferation compared with cultures with nonactivated monocytes. In contrast, nonatopic monocytes did not show this suppressive T cell effect (Fig. 3, B and D). To rule out an immunostimulatory influence of 1-MT, T cell proliferation without monocytes was done in different concentrations of 1-MT but no effect of inhibitor was observed throughout the range of concentrations used (data not shown).

Catabolism of tryptophan in atopic FcεRI-activated cocultures

To confirm functional IDO activity in atopic activated (IgE/anti-IgE and 22E7/GaMIgG) monocyte cocultures with T cells, culture supernatants were harvested for tryptophan and kynurenine 24 h after the addition of T cells to stimulated or unstimulated atopic monocytes (Fig. 4). Mean tryptophan levels in the culture medium of stimulated (IgE/anti-IgE and 22E7/GaMIgG) monocytes of three atopic cocultures decreased ~37% of the initial amount of tryptophan in the culture medium after 24 h, whereas this amount was ~7% for unstimulated monocytes with T cells. IDO activity was detected in three of three cocultures with atopic FcεRI-activated monocytes and each donor responded with the induction of IDO supplementation of tryptophan. 1-MT has been shown to be a competitive inhibitor for IDO or supplementation of tryptophan. If so, the suppression should be prevented by pharmacologic inhibition of IDO or suppression but in addition increased T cell proliferation compared with cultures with nonactivated monocytes. In contrast, nonatopic monocytes did not show this suppressive T cell effect (Fig. 3, B and D). To rule out an immunostimulatory influence of 1-MT, T cell proliferation without monocytes was done in different concentrations of 1-MT but no effect of inhibitor was observed throughout the range of concentrations used (data not shown).

### Table I. IDO activity from purified, 24-h FcεRI-activated monocytes

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Atopics</th>
<th>Nonatopics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
<td>Donor 2</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgE/anti-IgE</td>
<td>15</td>
<td>52</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>39</td>
<td>30</td>
</tr>
</tbody>
</table>

FIGURE 3. Proliferation of T cells with stimulated and nonstimulated atopic and nonatopic monocytes. A and C, Inhibition of T cell proliferation by 24 h FcεRI stimulated atopic monocytes. Data in A and C represent one donor each. Autologous T cells (2 × 10⁵) were added to 24-h activated (■) and nonactivated (□) atopic monocytes (1 × 10⁵) along with or without anti-CD3 mAb (□) after replacement of the medium. T cells were added in the presence or absence of various concentrations of the IDO inhibitor 1-MT. Alternatively, cocultures were supplemented with tryptophan (1 μg/10 μl) 24 and 48 h after T cell addition. Seventy-two hours later, T cell proliferation was measured. Data are expressed as the mean ± SD of data from three experiments per donor. Each experiment was done in triplicate (* p < 0.001). Data are representative of four atopic donors. B and D, T cell stimulation in vitro with anti-CD3 mAb and autologous nonatopic monocytes. Data in both B and D represent one donor. The experimental design was the same as in A and C. By general linear modeling analysis, no effects of nonatopic monocytes on T cell proliferation were found applying these experimental conditions.

FIGURE 4. Degraded tryptophan in coculture experiments with stimulated atopic monocytes and T cells. Atopic monocytes were left untreated or were activated with IgE/anti-IgE or 22E7/GaMIgG for 24 h. The cells were washed and incubated with autologous T cells (2 × 10⁵) for an additional 24 h in the presence of anti-CD3 mAb (100 ng/ml). Culture supernatants were harvested and measured for the concentration of tryptophan remaining. Data represent mean values of three atopic donors in triplicate ± SD. Repeated measurement analysis of variance displayed a dependency of tryptophan degradation for stimulated monocytes (*, p < 0.05).
monocytes (kynurenine with IgE/anti-IgE- and 22E7/GaMIgG-stimulated monocytes: mean, 11 ± 1.4 and 7 ± 4 μM, respectively), confirming the presence of functional IDO. In unstimulated monocytes with T cells mean kynurenine levels were 0.7 ± 0.4 μM.

T cell suppression is sustained in activated atopic monocyte cocultures using anti-IFN-γ and anti-IL-10 Ab

From previous studies it was known that IFN-γ is an inducer of IDO in monocytes (27). In macrophages, even low amounts of IFN-γ act in concert with CD40L from activated T cells to induce IDO, whereas CD40L by itself does not seem to have an effect on the degradation of tryptophan (18). Therefore, in our cocultures, we had to rule out that IFN-γ was the main inducer of IDO in monocytes. In addition, IL-10 was reported to be induced by FcεRI ligation on monocytes, a cytokine which is known to suppress T cell proliferation (28). First, we determined the amount of IFN-γ produced from cocultures of activated and nonactivated atopic and nonatopic monocytes 24 h after the addition of T cells. Moderate levels of IFN-γ were detected in atopic activated and nonactivated cocultures (1133 ± 115 and 700 ± 400 pg/ml, respectively). Lower levels were present in nonactivated and nonactivated cocultures (85 ± 37 and 69 ± 28 pg/ml, respectively). IL-10 production was determined from monocytes after 24 h of activation or nonactivation, medium replacement, and an additional 24 h of incubation. Atopic activated monocytes produced ~151 ± 46 pg/ml IL-10, whereas nonactivated atopics produced 57 ± 30 pg/ml. Nonatopic activated monocytes produced 102 ± 31 pg/ml and nonactivated nonatopics produced 30 ± 40 pg/ml. To determine the role of IFN-γ and IL-10, neutralizing mAbs against IFN-γ and IL-10 as well as a control mlgG1 Ab were added to atopic activated cocultures (Fig. 5). Without inhibitor of IDO (0 μM 1-MT), the suppression of T cell proliferation by activated monocytes was not affected in the presence of either anti-IFN-γ or IL-10 mAbs. IFN-γ activity was not detected 24 h after the addition of T cells, when neutralizing anti-IFN-γ mAb was added to cocultures as determined by the IFN-γ bioassay (data not shown). In the presence of 1000 μM 1-MT, T cell proliferation was high in all conditions. These results imply that in our cocultures the concentrations of IFN-γ and IL-10 do not seem to influence IDO expression. The supplementation of these cultures with tryptophan prevented T cell suppression in these conditions (data not shown).

**Suppression of T cell proliferation in 24-h FcεRI-activated, IL-10-depleted supernatants of atopic monocytes**

It cannot be excluded that the amount of anti-IFN-γ Ab and anti-IL-10 Ab in our coculture experiments could not have been enough to neutralize these cytokines. Therefore, T cell proliferation was studied without the influence of IFN-γ from T cells and IL-10 from activated monocytes (Table II). Cultures of 24 h stimulated and nonstimulated atopic and nonatopic monocytes were washed and incubated for a additional 24 h. Supernatants were collected and depleted of IL-10 by magnetic isolation (>90% of depletion in stimulated and nonstimulated atopics as controlled by ELISA corresponding to 10 ± 4 and 7 ± 3 pg/ml, respectively; in nonatopics, IL-10 was below the limit of detection). T cell proliferation induced by surface-bound anti-CD3 mAb was significantly inhibited in IL-10-depleted atopic FcεRI-activated supernatants, whereas the addition of tryptophan to these supernatants prevented the T cell suppressive effect. In contrast, atopic nonactivated monocytic IL-10-depleted supernatants supported T cell proliferation. In nonatopics, applying these conditions, no influence of T cell proliferation on culture conditions was seen. In theory, these results might indicate an unanticipated immunostimulatory role for tryptophan itself. To exclude this possibility, T cell proliferation was determined in normal medium with and without the addition of tryptophan by surface-bound anti-CD3 mAb. Applying these conditions, no additional proliferative effect was observed by supplementing tryptophan (data not shown). With a sensitive IL-10 bioassay, we could demonstrate that the amounts of IL-10 after IL-10 supernatant depletion were below the linear range of the dose-response curve and thus biologically inactive (data not shown).
addition, exogenously added IL-10 in concentrations of 10, 30, and 100 pg/ml did not suppress T cell proliferation in control medium with surface-bound anti-CD3 (data not shown). These results directly implicate the T cell suppressive effect of 24-h FcεRI-activated and 24-h cultivated monocytes by degrading tryptophan from external supplies.

Discussion

In this study, we established a differential cDNA bank of FcεRI-stimulated and unstimulated monocytes from a clinically healthy person with an atopic family background and high FcεRI expression on monocytes. The results were extended to four additional atopic donors of the same clinical criteria and serum parameters (high FcεRI and CD40; low CD23 monocyte surface expression; serum IgE levels >100 kU/L) and compared with donors without any atopic disease or atopic family background (low FcεRI and CD23; high CD40 monocyte surface expression; serum IgE levels <100 kU/L). 1) We found IDO and kynurenine 3-monooxygenase to be overexpressed in FcεRI-activated monocytes from atopic donors; 2) we demonstrated the functionality and specificity of IDO expression in FcεRI-stimulated atopic monocytes, which results in the inhibition of T cell proliferation, whereas 3) external supplies of tryptophan to atopic activated cocultures result in an increase of T cell proliferation; and 4) we showed finally that IL-10-depleted supernatants of 24-h FcεRI-activated and 24-h cultivated atopic monocytes do not support T cell proliferation as a result of reduced tryptophan levels.

FcεRI plays a central role in allergic diseases such as atopic eczema/dermatitis syndrome and allergic asthma (29, 30). In both conditions, immunohistochemical analysis is characterized by an inflammatory T cell infiltrate. Kinetics of APC activation may exert not only activatory functions on T cells but also negative or tolerogenic signals that reduce lymphocyte proliferation. In line with this complex interplay of cellular and metabolic factors, we found, unexpectedly, the involvement of FcεRI in the tryptophan degradation pathway. IDO, the rate-limiting enzyme in the catabolism of tryptophan, is a highly conserved enzyme which has been shown to be widely distributed preferably in tissues that are involved in immune defense or immunoprivilege (31). IDO production by FcεRI-activated monocytes could contribute to self-limitation of immune responses. This is in line with our observation that only atopic activated monocytes, which had been washed 24 h after stimulation to eliminate proinflammatory cytokines (32, 33) or supernatants thereof, mediate this T cell suppressive effect in vitro. In addition, 4 h after FcεRI cross-linking of atopic monocytes no kynurenine was detected in culture supernatants by HPLC (data not shown), whereas these levels increased significantly 24 h after stimulation. The inhibition of IDO with increasing concentrations of the IDO inhibitor 1-MT resulted in the proportional increase in T cell proliferation (data not shown). In addition, by using an anti-IL-10 mAb in our FcεRI-activated cocultures, we still observed inhibition of T cell proliferation. The same was true when we neutralized both IFN-γ and IL-10. Our data were confirmed by the fact that IL-10-depleted, FcεRI-activated monocyte supernatants suppressed T cell proliferation, whereas this effect could be prevented by the addition of tryptophan.

Interestingly, our experimental data further demonstrate an increase in T cell proliferation with FcεRI-activated monocytes if tryptophan is in excess. However, this effect was pronounced only when FcεRI surface expression was high (>50%) and therefore not statistically significant. An explanation could be that, in situations where tryptophan concentrations are not limiting, proinflammatory cytokines from activated monocytes meet optimal conditions to further accelerate T cell proliferation. However, the supplementation of tryptophan does not influence the expression of costimulatory molecules on monocytes (data not shown).

In this study we provide the first evidence that FcεRI induces the degradation of tryptophan upon aggregation. The observation from our proliferation studies suggests that FcεRI+ APCs communicate with T cells by regulating tryptophan concentrations in defined metabolic compartments. T cells could respond with either activation or arrest, depending on the level of tryptophan they find.

Acknowledgments

We thank Drs. Susanne Koch, Elisabeth Geiger, and Thomas Tüting (Department of Dermatology, University of Bonn) for helpful discussions and critical reading of the manuscript. We are grateful to Dr. Rolf Fimmers (Department of Medical Biometry and Computer Science, University of Bonn) for assisting in statistical analysis.

References


