

IL-10-producing monocytes differentiate to alternatively activated macrophages and are increased in atopic patients

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Background: Recently the immune regulatory role of T cell-derived IL-10 in allergic disease has been extensively studied. In contrast, there is mounting evidence that IL-10 might also have a role in the perpetuation of allergic inflammation and fibrotic remodeling. It has been reported that alternatively (IL-4) activated macrophages (aaMΦ) produce large quantities of IL-10 and lack IL-12 production.

Objective: Bearing this in mind, we hypothesized whether functionally different properties of IL-10-producing monocytes could be identified.

Methods: Intracellular cytokine expression of IL-10, IL-12, and IL-6 in peripheral blood CD14⁺ monocytes was measured in 19 atopic patients and 18 healthy control subjects by means of flow cytometry. In addition, IL-10-secreting monocytes were sorted by means of flow cytometry. Capabilities of these cells regarding further differentiation, accessory cell capacity, and surface molecule expression were analyzed.

Results: Our data show a dichotomous expression pattern of either IL-10 or IL-12p40/p70 in peripheral blood monocytes after LPS stimulation. Compared with healthy control subjects, the percentage of IL-10-producing monocytes was significantly increased in atopic patients. IL-10-secreting monocytes were isolated by using an IL-10 secretion assay, and functional analysis of these sorted cells revealed that IL-10-secreting monocytes preferentially differentiate into suppressor of cytokine signaling 3 expressing aaMΦ, which perpetuate T_H2 immune response.

Conclusion: Our study shows the existence of an IL-10-producing monocyte subset, which is increased in atopic disease and which might facilitate allergic inflammation and fibrotic remodeling by differentiation into aaMΦ.

Clinical implications: Controlling aaMΦ in T_H2-driven inflammatory processes might be a novel target for intervention strategies. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: IL-10, monocytes, atopy, alternative activation, FACS, suppressor of cytokine signaling 3, CCL18, macrophages, flow cytometry, subset

The focus of recent literature regarding IL-10 has been its immune regulatory role in allergic diseases. Numerous articles describe the importance of IL-10-producing regulatory T cells in inhibiting allergic inflammation.¹⁻³ Expression of IL-10 by antigen-presenting dendritic cells represents an established pathway for induction of antigen-specific tolerance in T cells, including tolerance to allergens.⁴ In contrast to these anti-inflammatory effects of IL-10, the appearance of IL-10 in ongoing allergic inflammation might contribute to disease severity. In human subjects several articles describe the presence of IL-10 mRNA after allergen challenges⁵ in the airways of patients with severe asthma and in the skin of patients with atopic dermatitis.^{6,7} Thus IL-10 might have different effects, depending on the timing and source.⁸

Monocytes are the main producer of IL-10.⁹ In atopic individuals a reduced production of IL-12p40 and IL-12p70 and increased production of IL-10 by PBMCs, as measured by using ELISA,^{7,10} as well as by means of intracellular flow cytometry,¹¹ has been described. Furthermore, it was shown that the A-form polymorphism of the IL-10 promoter is associated with atopic diseases.^{8,12} The A form of the promoter is linked to enhanced functional activity of the promoter and increased specific IgE levels. IL-10 enhances IgE secretion in B lymphocytes, which have already undergone the ε-isotype switch.¹³ In mice it was shown that transgenic overexpression of IL-10 in the lung causes mucus metaplasia, tissue inflammation, and airway remodeling, thus facilitating allergic disease.¹⁴ Furthermore, in a murine model of allergic dermatitis, a critical role of IL-10 for T_H2 inflammatory processes was described.¹⁵ In addition, it was shown that there are fundamental differences regarding exogenous administration and endogenous production of IL-10.¹⁶ Although exogenous administration of IL-10 blocks lung inflammation, endogenous upregulation of IL-10 production promotes T_H2 immune response.¹⁶ Recently, the concept of T_H1/T_H2 responses has been expanded to the antigen-presenting cell level. Macrophages treated with IL-4 acquire an alternative activation phenotype (alternatively

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

D. V. Pechkovsky is a recipient of the Alexander von Humboldt Research Fellowship.

Received for publication April 16, 2006; revised September 10, 2006; accepted for publication September 13, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.09.030

Abbreviations used

aaMΦ:	Alternatively activated macrophages
FI:	Fluorescence intensity
FITC:	Fluorescein isothiocyanate
FSC:	Forward scatter
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
IL-1ra:	IL-1 receptor antagonist
PE:	Phycoerythrin
PMA:	Phorbol 12-myristate 13-acetate
SEB:	Staphylococcus enterotoxin B
SOCS3:	Suppressor of cytokine signaling 3
SSC:	Side scatter

activated macrophages [aaMΦ]), which is characterized by missing IL-12 and exaggerated IL-10 production by these cells.^{17,18} Although studies have investigated subtypes of monocytes regarding the expression of surface molecules, little is known about their differential cytokine expression pattern. However, several studies show in detail the influence of exogenously administered IL-10 on monocyte differentiation.^{19–21} In light of these results, we were interested in the functional properties of IL-10–producing monocytes after LPS stimulation.

Our study shows the existence of an IL-10–secreting monocyte subset that lacks IL-12 production and is increased in patients with atopic disease. To our knowledge, this is the first study analyzing this subset of monocytes by sorting IL-10–secreting monocytes. We demonstrate that IL-10–producing monocytes differentiate into aaMΦ *in vitro*, which might perpetuate T_H2 immune response and favor fibrotic remodeling.

METHODS

Materials

For more information, see the Methods section in the Online Repository at www.jacionline.org.

Subjects

Twenty patients with a known atopic disease (11 female and 9 male patients; mean age, 34 ± 4 years) were included in the study. The diagnosis of atopic disease was established in all patients based on clinical criteria, a positive skin prick test response, and increased total IgE levels (>100 kU/L; mean, 649 ± 281 kU/L). Eight patients had atopic dermatitis, 4 had mild allergic asthma and rhinoconjunctivitis, and 8 had rhinoconjunctivitis alone. Thirteen patients had positive skin prick test responses to *Dermatophagoides pteronyssinus*, and 8 patients disclosed a positive reaction to cat dander extract, 6 to birch pollen extract, and 10 to grass pollen extract. In total, 13 patients were found to be polysensitized. None of the patients had evidence for an alternative diagnosis nor had any received anti-inflammatory treatment with corticosteroids or other immunosuppressive therapy before inclusion in the study. Twenty healthy subjects (12 female and 8 male subjects; mean age, 34 ± 7 years) with normal total IgE levels (<100 kU/L; mean, 42 ± 24 kU/L) who did not receive any current medication served as control subjects. Not all patients could be included in all experiments. All subjects provided their consent after being informed about the nature and purpose of the study. The study was approved by the local ethics committee.

Analysis of intracellular cytokine expression in monocytes from PBMCs

Intracellular cytokine measurement was performed, as recently described, in atopic individuals ($n = 19$) and healthy volunteers ($n = 18$).²²

Sorting of IL-10–secreting monocytes

PBMCs of 20 healthy volunteers were isolated by means of Ficoll-Hypaque separation and isolated with a commercially available IL-10 secretion detection kit according to the recommendations of the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were sorted with a MoFlo cell sorter (Dako Deutschland GmbH, Hamburg, Germany). For further details, see the Methods section in the Online Repository at www.jacionline.org.

Sorting of monocytes, CD4⁺CD45RA⁺ cells, and CD4⁺CD45RO⁺ cells

For more information, see the Methods section in the Online Repository at www.jacionline.org.

Generation of dendritic cells

Isolated monocyte subsets were cultured in 24-well culture plates in the presence of GM-CSF (1000 U/mL) and IL-4 (1000 U/mL). Cells were harvested at day 7. CD1a and CD83 expression of the harvested cells was determined by means of flow cytometry.

Analysis of spontaneous differentiation of monocyte subsets

Isolated monocyte subsets were cultured in 24-well culture plates in RPMI/10% FCS. Cells were harvested after 48 hours of cell culture. Cell-culture supernatants were analyzed for CCL18 production. Cells were immediately analyzed for annexin, CD68, CD16, and CD163 staining by means of flow cytometry, as previously described.²³ In addition, some cells were harvested in TRIzol Reagent (Invitrogen, Carlsbad, Calif) for analysis of CCL18 mRNA, IL-1 receptor antagonist (IL-1ra) mRNA, and suppressor of cytokine signaling 3 (SOCS3) mRNA expression.

Reverse transcription and real-time PCR

For more information, see the Methods section in the Online Repository at www.jacionline.org.

Measurement of T-cell proliferation by means of tritiated thymidine incorporation

Staphylococcus enterotoxin B (SEB)–pulsed IL-10–secreting and non-IL-10–secreting monocytes were cocultured with CD4⁺CD45RA⁺ cells for 3 days. Tritiated thymidine incorporation within the last 18 hours of the experiment was analyzed by means of liquid scintillation spectroscopy with a TopCount (PerkinElmer, Rodgau-Jugesheim, Germany). For further information, see the Methods section in the Online Repository at www.jacionline.org.

T-cell polarization assay

SEB–pulsed IL-10–secreting and non-IL-10–secreting monocytes were cocultured with CD4⁺CD45RA⁺ cells in U-bottom 96-well plates for 5 days (ratio, 1:5). SEB was used as a surrogate to measure antigen-presenting cell function. After the culture period, cells were harvested, washed, and placed in 48-well plates in the presence of IL-2 (20 U/mL). After a further 5-day culture period, cells were harvested and washed again and placed in RPMI/10% FCS containing phorbol 12-myristate 13-acetate (PMA) 10^{-8} mol/L and 10^{-6} mol/L

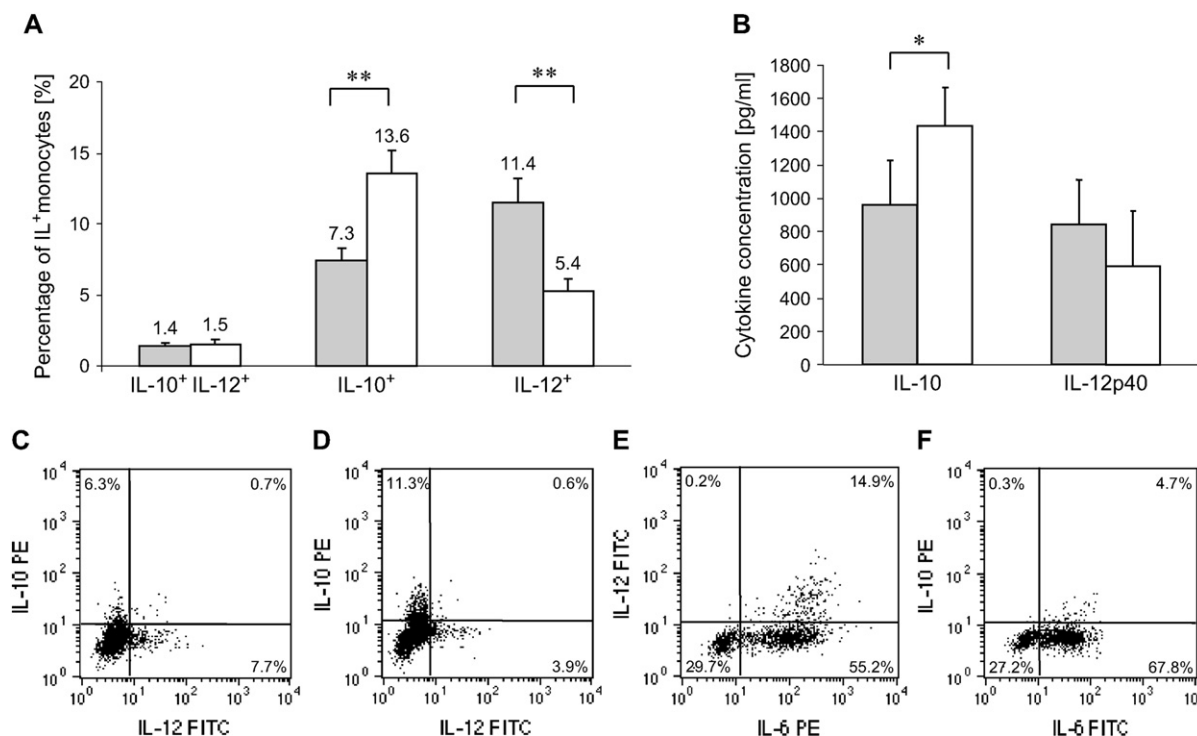


FIG 1. CD14⁺ monocytes from patients with atopic disease produce statistically significantly more IL-10 and less IL-12p40. **A**, Percentage of IL-10⁺ and IL-12p40⁺ monocytes after 12 hours of stimulation with LPS. *Solid bars* indicate control subjects, and *open bars* indicate patients with atopic disease. **B**, Production of IL-10 and IL-12p40 of monocytes stimulated with LPS for 48 hours (* $P < .05$, ** $P < .005$). **C-F**, Flow cytometric analysis of intracellular cytokine expression from a healthy volunteer (**Fig 1, C**) and a patient with atopic disease (**Fig 1, D**) is shown. The distribution of IL-10⁺ and IL-12⁺ production is merely dichotomous. In contrast, IL-6 is expressed in IL-10⁺, as well as in IL-12p40⁺, monocytes (**Fig 1, E and F**).

Ca ionophore. Culture supernatants were harvested after 48 hours of cell culture and analyzed for IFN- γ (Endogen, Bonn, Germany), IL-4, and IL-13 (BD Biosciences, Heidelberg, Germany) by using commercially available ELISA systems.

In addition, CD4⁺CD45RO⁺ cells were also cocultured with IL-10-secreting and non-IL-10-secreting monocytes (ratio, 1:5) for 5 days in 48-well plates. After this culture period, cells were harvested, washed, and placed in RPMI/10% FCS containing PMA 10^{-8} mol/L and 10^{-6} mol/L Ca ionophore. Culture supernatants were harvested after 48 hours of cell culture and analyzed for IFN- γ , IL-4, and IL-13 concentrations.

RESULTS

Measurement of intracellular cytokine production after LPS stimulation reveals different monocyte subsets

In preliminary experiments we found that 12 hours after LPS stimulation was an optimal time point for simultaneous measurement of both cytokines (data not shown). Before any stimulation, the percentage of IL-10- or IL-12p40-expressing cells was less than 1% in all groups. In our hands the percentage of IL12p70⁺ monocytes after 12 hours of LPS stimulation is less than 1% (data not shown), which is in line with reports of other authors.²⁴ Monocytes

were defined by their position in the forward scatter (FSC)/side scatter (SSC) plot and their positive staining for anti-CD14. Analysis of the intracellular cytokine expression of CD14⁺ peripheral blood monocytes revealed 7.3% $\pm$ 1.0% IL-10⁺CD14⁺ monocytes and 11.4% $\pm$ 1.8% IL-12p40⁺CD14⁺ monocytes in healthy control subjects (**Fig 1, A**) after stimulation with LPS for 12 hours. Most (81.9% $\pm$ 1.9%) of the monocytes produced neither IL-10 nor IL-12. There was a statistically significant expansion of the IL-10-producing monocyte subset. In patients with atopic disease, 13.6% $\pm$ 1.6% of the monocytes showed IL-10 staining, but only 7.3% $\pm$ 1.0% of the monocytes from healthy volunteers showed such staining ($P = .004$; **Fig 1, A**). IL-12p40⁺ cells were significantly reduced compared with those seen in healthy volunteers (5.4% $\pm$ 0.9% vs 11.4% $\pm$ 1.8%, respectively; $P = .003$). Simultaneous expression of both cytokines was detected in 1.5% $\pm$ 0.3% of the monocytes from patients with atopic disease and 1.4% $\pm$ 0.2% of the monocytes from healthy volunteers (not statistically significant; **Fig 1, A**). Therefore the distribution of IL-10 and IL-12 production is merely dichotomous (**Fig 1**). The dichotomous staining of monocytes for IL-10 or IL-12p40 is in sharp contrast to the expression pattern of IL-6/IL-10 and IL-6/IL-12p40, respectively (**Fig 1, C-F**).

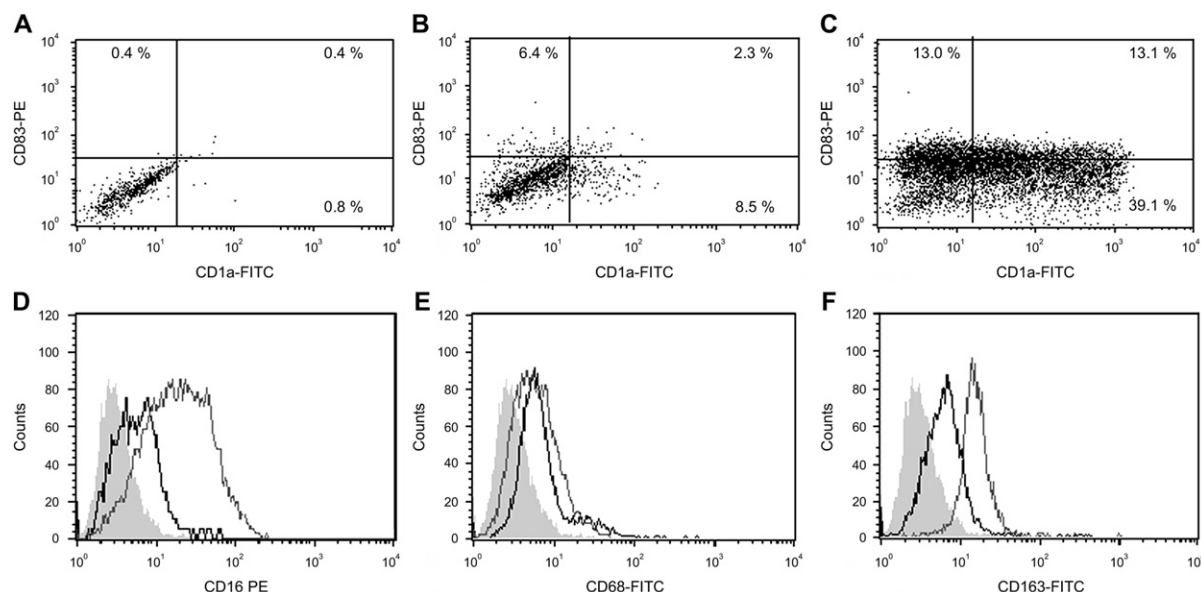


FIG 2. IL-10-secreting monocytes spontaneously express markers of macrophages and alternative activation. **A** and **B** disclose CD83 and CD1a expression of IL-10-secreting cells and non-IL-10-secreting monocytes (**C**) after 7 days of culture in the presence of IL-4 and GM-CSF. In contrast to non-IL-10-secreting monocytes, IL-10-secreting monocytes did not differentiate into dendritic cells (Fig 2, A, vs Fig 2, C). In Fig 2, B, neutralizing IL-10 antibodies were added, which partly restored the capacity of IL-10-secreting cells to differentiate into dendritic cells. **D** through **F** disclose histograms demonstrating the fluorescence intensity of CD16 (Fig 2, D), CD68 (Fig 2, E), or CD163 (Fig 2, F) of IL-10-secreting (gray line) and non-IL-10-secreting monocytes (black line). Control values are shown as gray areas.

Cytokine production of isolated monocytes from patients with atopic disease and control subjects

In vitro cytokine production of monocytes was evaluated by means of ELISA ($n = 20$). IL-10 production of monocytes stimulated for 48 hours with $1 \mu\text{g/mL}$ LPS was statistically significantly increased in atopic patients ($1435 \pm 270 \text{ pg/mL}$) compared with healthy control subjects ($959 \pm 236 \text{ pg/mL}$; $P < .01$; Fig 1, B). Furthermore, monocytes from patients with atopic disease produced less IL-12p40 after LPS stimulation compared with those from healthy control subjects ($593 \pm 332 \text{ pg/mL}$ and $841 \pm 279 \text{ pg/mL}$, respectively), although the difference did not reach statistical significance (Fig 1, B).

Evaluation of the sort procedures for IL-10-producing monocytes

For more information, see the Results section and Fig E1 in the Online Repository at www.jacionline.org.

Capacity of IL-10-producing monocytes to differentiate into dendritic cells after incubation in GM-CSF- and IL-4-enriched culture medium is reduced

The capacity of differentiation into dendritic cells after cell culture in the presence of GM-CSF and IL-4 was markedly reduced in the IL-10-producing monocyte subgroup. Only $6.4\% \pm 4.0\%$ of the differentiated cells

derived from IL-10-producing monocytes expressed CD1a, a marker for immature dendritic cells, after 7 days of cell culture in the presence of GM-CSF and IL-4. Moreover, no cells expressed CD83, a marker for mature dendritic cells. In comparison, $52\% \pm 17\%$ of the non-IL-10-producing cells stained positively for CD1a, and $10\% \pm 15\%$ stained positively for CD83 (Fig 2, A-C). Thus IL-10-secreting monocytes did not differentiate into dendritic cells, despite appropriate culture conditions. Additional experiments ($n = 3$) revealed that addition of neutralizing antibodies against IL-10 partly restored this effect (Fig 2, B).

IL-10-secreting monocytes differentiate into aaMΦ

Because several studies suggested that IL-10 favors macrophage differentiation, spontaneous differentiation into distinct macrophage subsets was analyzed. The rate of spontaneous apoptosis was calculated on the basis of measurements of annexin-positive cells. Of all IL-10-producing and non-IL-10-producing monocytes, $20.0\% \pm 4.7\%$ and $19.6\% \pm 5.0\%$, respectively, showed positive annexin staining. There was no statistical difference between the monocyte subsets (data not shown). Both subsets highly expressed the macrophage markers CD16 and CD68 (Fig 2, D and E). However, although both monocyte subsets expressed CD16 and CD68 ($95.4\% \pm 2.7\%$ and $86.2\% \pm 6.5\%$ vs $94.6\% \pm 2.1\%$ and $72.8\% \pm 7.1\%$, respectively) the fluorescence intensity (FI) for CD16 was slightly reduced in the non-IL-10-producing monocytes

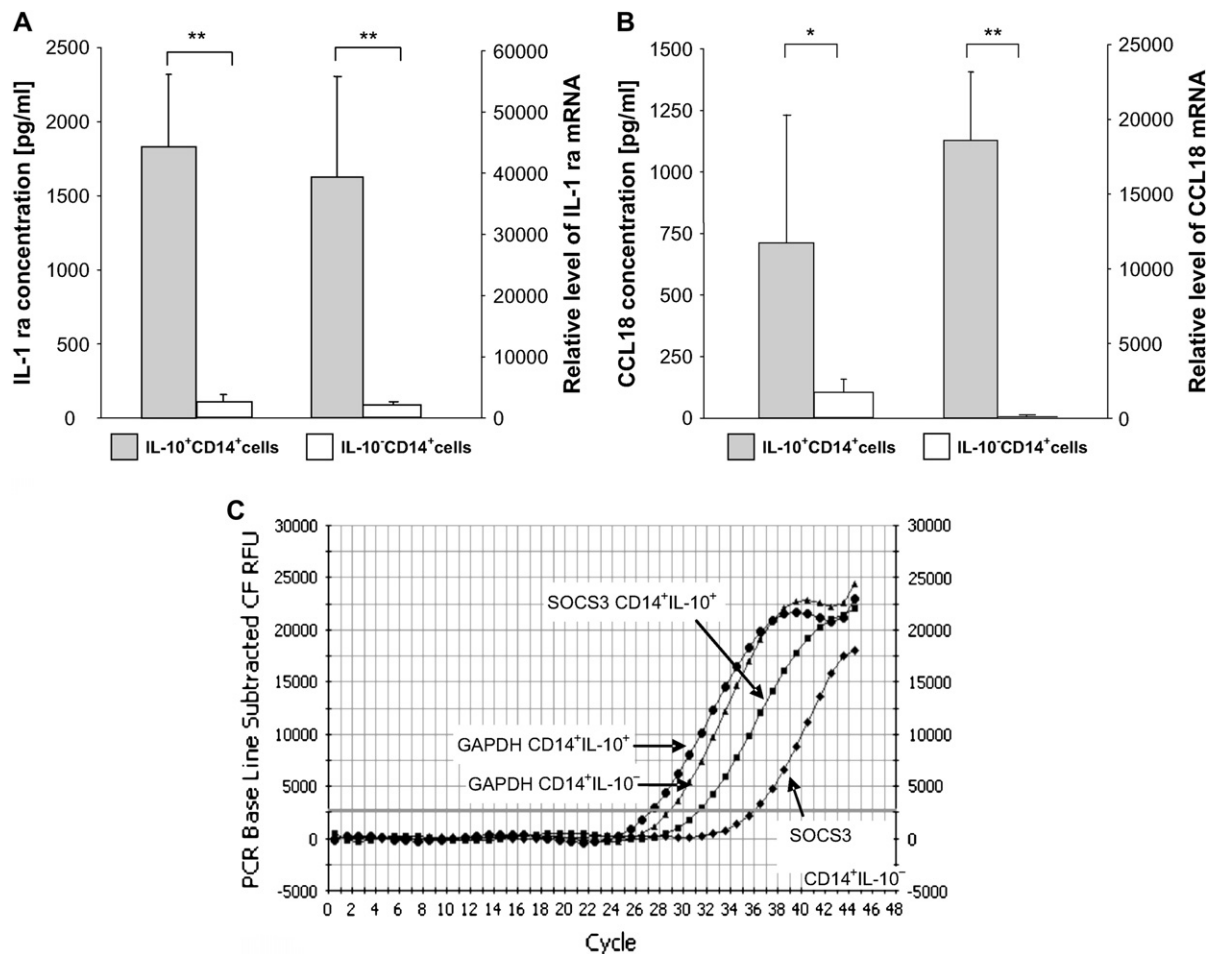


FIG 3. IL-10-secreting monocytes express IL-1ra, CCL18, and SOCS3. IL-10-secreting monocytes produce and express more IL-1ra (**A**) and CCL18 (**B**) than non-IL-10-secreting monocytes after 24 hours of cell culture. *Left bars* depict protein concentrations in conditioned culture medium. *Right bars* depict relative level of IL-1ra mRNA ($n = 10$, $*P < .05$, $**P < .005$). **C** depicts data from RT-PCR with SOCS3-specific oligonucleotide primers.

(236 ± 52 FI vs 321 ± 94 FI), and CD68 expression was statistically significantly increased in the IL-10-producing monocytes ($P = .005$). In addition, there was a reduced expression of HLA-DR in the IL-10-producing monocyte subgroup regarding the percentage of HLA-DR-expressing monocytes ($40.7\% \pm 8.7\%$ vs $61.9\% \pm 14.8\%$, $P = .005$, data not shown). We obtained no statistically significant difference in CD86, CD80, or CD11b expression for both monocyte subsets (data not shown). However, analysis of CD163, a known marker of aaMΦ, revealed a statistically significant difference between both monocyte subsets. Macrophages derived from IL-10-producing monocytes showed a statistically significantly increased expression of the scavenger receptor CD163 ($74.8\% \pm 10.1\%$ vs $50.8\% \pm 7.6\%$, $P = .014$; Fig 2, F). In addition, macrophages derived from IL-10-producing monocytes showed a markedly enhanced expression of CCL18 and IL-1ra mRNA. CCL18 and IL-1ra are known signature cytokines of aaMΦ. Furthermore, analysis of cell-culture supernatants also showed a statistically significant increase

of both proteins in cell-culture supernatants of IL-10-producing monocytes versus non-IL-10-producing monocytes (Fig 3, A and B).

IL-10-secreting monocytes express SOCS3

IL-10-secreting monocytes expressed statistically significantly more SOCS3 mRNA than non-IL-10-secreting monocytes ($n = 5$, 423 ± 96 relative level of SOCS3 mRNA vs 92 ± 18 relative level of SOCS3 mRNA, $P = .002$; Fig 3, C).

IL-10-producing monocyte subset favors T_H2 immune responses in coculture experiments either with CD4CD45RA⁺ or CD4CD45R0⁺ T cells and reduces T-cell proliferation

For further analysis, coculture experiments of both monocyte subsets were performed either with memory CD4 T cells (CD4⁺CD45R0⁺ cells) or with naive T cells (CD4⁺CD45RA⁺ cells). Analysis of T-cell proliferation revealed a decreased proliferation of naive T cells cocultured

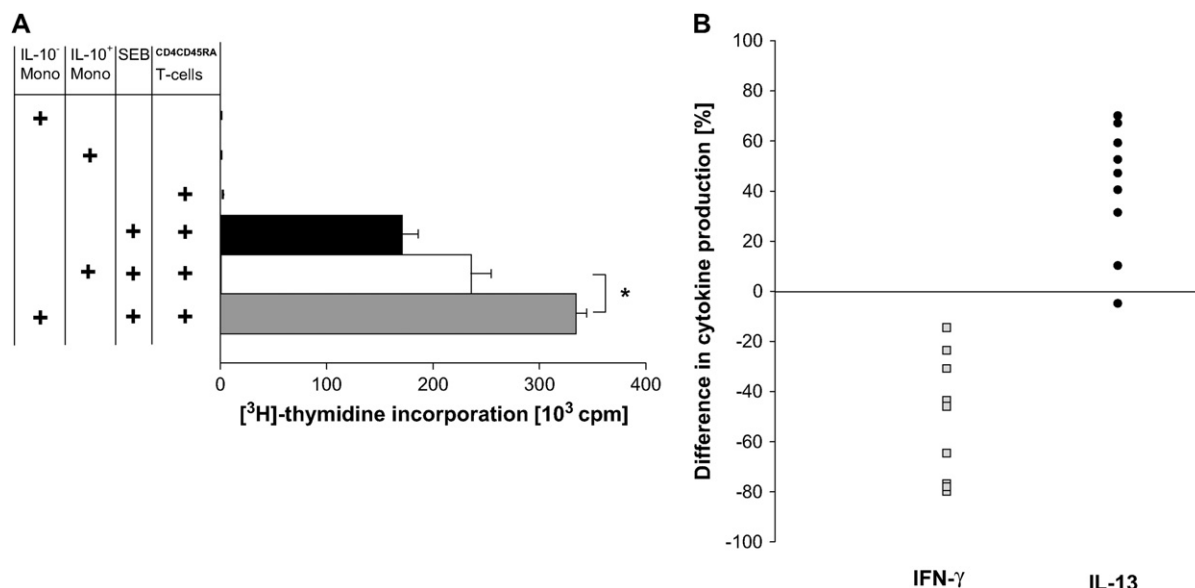


FIG 4. IL-10-secreting monocytes exhibit decreased T-cell stimulatory capacity and favor T_H2 immune responses. **A**, CD4⁺CD45RA⁺ T cells were cocultured with IL-10-secreting monocytes or non-IL-10-secreting monocytes in the presence of SEB. T-cell proliferation was measured on the basis of tritiated thymidine incorporation (***P* < .005). **B**, CD4⁺CD45RA⁺ T cells were cocultured with IL-10-secreting monocytes or non-IL-10-secreting monocytes, and cytokine secretion was analyzed. Data are presented as percentages of the calculated difference between IL-10-secreting monocytes versus non-IL-10-secreting monocytes (*n* = 10). There is a statistically significant reduction in IFN- γ production, as well as an increase in IL-13 production, by T cells stimulated with IL-10-secreting monocytes.

with SEB-pulsed IL-10-producing monocytes compared with that seen in naive T cells cocultured with SEB-pulsed non-IL-10-producing monocytes (*P* = .002; Fig 4, A).

Cytokine production of primed naive T cells was analyzed after PMA/Ca ionophore stimulation at day 12. Naive T cells cocultured with IL-10-producing monocytes showed a decreased production of IFN- γ compared with that seen in T cells cocultured with non-IL-10-producing monocytes (7168 ± 2580 vs $11,080 \pm 2160$ pg/mL, *P* = .004) combined with an increased production of IL-4 (35 ± 18 vs 11 ± 6 pg/mL, *P* = .024) and IL-13 (1357 ± 411 vs 600 ± 185 pg/mL, *P* = .004; Fig 4, B).

Similar differences as naive T cells regarding T_H1/T_H2 cytokine production disclosed memory T cells cocultured with both monocyte subsets for 5 days followed by stimulation with PMA/Ca ionophore. Memory T cells cultured in the presence of IL-10-producing monocytes produced statistically significantly more IL-13 than memory T cells cocultured with non-IL-10-producing monocytes (103 ± 37 vs 82 ± 38.3 pg/mL, *P* = .039) and less IFN- γ (1903 ± 609 pg/mL vs 2939 ± 727 pg/mL, *P* = .0019, data not shown).

DISCUSSION

In contrast to the generally recognized anti-inflammatory effects of IL-10, recent reports suggested a critical role of IL-10 in T_H2 responses in murine models of allergic lung inflammation and atopic dermatitis.^{14,15} Our study shows

the existence of a monocyte subset lacking IL-12p40 production combined with high production of IL-10, which is increased in patients with atopic disease. IL-10-secreting monocytes were not capable of differentiating into dendritic cells but differentiated into macrophages disclosing markers of alternative activation and SOCS3 while promoting T_H2 immune response. Our data suggest that the influence of IL-10 on macrophage differentiation might be one mechanism by which autocrine IL-10 production facilitates T_H2 inflammation and fibrotic remodeling.

Our results show a dichotomous pattern of IL-10 and IL-12p40 expression in peripheral blood monocytes in contrast to a simultaneous expression pattern for IL-6 and IL-10 or IL-6 and IL-12p40, respectively. Many studies suggested a contradictory regulation of IL-10 and IL-12 production.²⁴⁻²⁶ Elenkov et al²⁵ described an increase in IL-10 production and a decrease in IL-12 production in detail after stimulation of monocytes with histamine. Comparable results have been documented for prostaglandin E₂ and IgE.^{26,27} Conversely, increased IL-12 production combined with reduced IL-10 production has been observed after preincubation of monocytes with IFN- γ .²⁴ In addition, both cytokines inhibit the production of each other.²⁴ We are the first to show the existence of distinct IL-10- and IL-12p40-producing monocyte subsets at the single-cell level.

We observed a higher percentage of IL-10-producing monocytes and a reduced percentage of IL-12p40⁺ monocytes in atopic patients compared with percentages seen in healthy control subjects. These results were consistent with those obtained from cell-culture supernatants by

means of ELISA, where monocytes from atopic patients showed an increase in IL-10 production and a decrease in IL-12p40 production after stimulation with LPS. The mechanism of directing cell cytokine production by an initial stimulus followed by a second major stimulus is well known. Several factors could be involved in such a priming process of monocytes favoring IL-10 production. Novak et al²⁷ have shown that IgE contact promotes IL-10 production after LPS stimulation. However, it is presumable that several other factors, such as histamine²⁵ or microbial products,²⁸ are also involved in the priming of monocytes from atopic individuals favoring IL-10 production, including the described IL-10 promoter polymorphism.^{8,12}

Our findings suggest that IL-10 and IL-12p40 might be marker cytokines for functionally different monocyte subtypes. Sorting IL-10-secreting monocytes by means of flow cytometry allowed us to elucidate functional and differentiation properties of this monocyte subset. Control experiments showed that IL-10-secreting monocytes isolated by using this method did indeed produce IL-10, in contrast to negatively sorted cells. IL-10 mRNA signal was also markedly upregulated in IL-10-secreting cells when measured by means of quantitative RT-PCR. Further control experiments showed that the sorted monocyte subtypes did not differ in the percentage of apoptotic or necrotic cells after 48 hours of cell culture. However, after 48 hours of cell culture, we observed several functional differences between both monocyte subsets. IL-10-secreting monocytes spontaneously differentiated into cells that strongly expressed the macrophage markers CD16 and CD68. However, when IL-10-secreting monocytes were cultured in GM-CSF- and IL-4-enriched medium, they were not able to differentiate into dendritic cells in contrast to IL-10[−] monocytes, which easily differentiated. Withdrawal of autocrine-produced IL-10 by neutralizing anti-IL-10 antibodies partly restored the capacity of IL-10-secreting monocytes to differentiate into dendritic cells. These results are in line with previously published data, which showed that stimulation with IL-10 blocks the differentiation of monocytes into dendritic cells^{19,21} while promoting macrophage differentiation.^{19,20}

Our data clearly demonstrate for the first time that IL-10-secreting monocytes differentiate *in vitro* into macrophages, which disclose a phenotype of alternative activation. Macrophages derived from IL-10-secreting monocytes highly expressed CD163 in contrast to cells derived from non-IL-10-secreting monocytes. Furthermore, macrophages derived from IL-10-secreting monocytes expressed a hundred-fold more CCL18 mRNA, as well as IL-1ra mRNA, and produced significantly more CCL18 and IL-1ra protein than cells derived from non-IL-10-secreting monocytes. CD163, CCL18, and IL-1ra are well-known markers of aaMΦ.^{17,18} Furthermore, because it is a key feature of aaMΦ to be capable of augmenting T_H2 immune responses and inhibiting T-cell proliferation,^{17,18} we also tested the functional capabilities of macrophages derived from IL-10-secreting monocytes in coculture systems with T cells. In both naive and memory T cells after coculture

with IL-10-secreting monocytes, T cell-derived IFN-γ production was decreased, whereas IL-13 and IL-4 production was increased. Therefore macrophages derived from IL-10-secreting cells enhanced T_H2 immune response. In addition, T-cell proliferation on stimulation with SEB-pulsed IL-10-secreting monocytes was significantly reduced. The contribution of aaMΦ to allergen presentation to T cells and initiation of the allergic inflammation might be negligible if compared with that of dendritic cells. However, macrophages might play a role in the maintenance of allergic inflammation, as well as in fibrotic remodeling. Interestingly, it has recently been shown that the production of IL-10 enables aaMΦ to inhibit the generation of classically activated macrophages. Our data strongly suggest that endogenous IL-10 production by monocytes has a crucial role in the differentiation of monocytes. The fate of a single monocyte is determined by the presence or absence of IL-10 production. In addition, factors that prime IL-10 production by monocytes will favor the evolution of aaMΦ and inhibit the generation of dendritic cells. Our data clearly show that cells derived from IL-10-secreting monocytes disclose markers and functional properties of aaMΦ.

Several studies suggest a fundamental role for macrophages in allergic inflammation by contributing to the local recruitment of effector cells regulating cellular homeostasis in tissues.^{29–31} Macrophages are capable of promoting T_H2 cytokine responses^{29,30} and reinforce allergic inflammation.³¹ Furthermore, it has been published that in patients with atopic dermatitis, CCL18 production within the dermis is induced, and increased serum CCL18 concentrations were detected in these patients,³² and in a primate asthma model, CCL18 was one of the genes that was highly induced.³³ Additionally, we have shown that IL-10-producing monocytes express more SOCS3 than non-IL-10-producing monocytes. In line with this finding, Arakawa et al³⁴ reported that levels of mRNA for SOCS3 were increased in PBMCs without any stimulation from patients with atopic dermatitis compared with levels seen in healthy control subjects. In a mouse model it was shown that SOCS3 is essential for the onset and maintenance of allergic inflammation.³⁵ In this study a strong correlation between SOCS3 expression and the pathology of asthma and atopic dermatitis, as well as serum IgE levels, in allergic human patients were observed.

We investigated the role of aaMΦ in pulmonary fibrosis, another T_H2-driven disease. In patients with pulmonary fibrosis,³⁶ we observed a dramatic shift of the macrophage phenotype toward alternative activation, with a marked upregulation of CCL18 and IL-1ra production. This macrophage phenotype could be induced by stimulation with IL-10. Other studies have shown an increase in macrophage-derived IL-10 production of patients with interstitial lung diseases associated with pulmonary fibrosis.³⁷ On the background of these findings, we propose a general role of phagocyte-derived IL-10 in the maintenance of T_H2-driven immune processes mediated by the autocrine influence on differentiation of aaMΦ.

In conclusion, our study demonstrates the existence of a distinct monocyte subset with regard to cytokine

production. We show that there is an IL-10-secreting monocyte subset that lacks IL-12 production, and this is increased in atopic individuals. We demonstrate that IL-10-secreting monocytes differentiate into SOCS3-expressing aaMΦ *in vitro*. SOCS3 expression might be a mechanism by which these cells are forced to the Th₂-associated macrophage phenotype of aaMΦ. These cells are capable of promoting Th₂ immune responses while inhibiting T-cell proliferation. Our data point toward a crucial role of endogenously produced IL-10 in monocyte differentiation and provide evidence for a mechanism by which an increase in the subset of IL-10-producing monocytes might maintain allergic inflammation and favor fibrotic remodeling.

We thank Klaus Geiger, Maria Follow, Rita Rzepka, and Sofia Kamenker for skillful technical assistance.

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