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 2007;119:464-71.)

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 challenges[5] 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

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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

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 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 $CD4^+CD45R0^+$ 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

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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 $CD4CD45RA^+$ 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