

Short communication

Characterization of monocyte subtypes in the allergic form of atopic eczema/dermatitis syndrome

Background: Monocyte (Mo) subsets exhibiting distinct phenotypic and functional properties identified in peripheral blood are assumed to be under the control of soluble factors from their surrounding micromilieu. Atopic eczema/dermatitis syndrome (AEDS) is accompanied by humoral and cellular alterations among which an increased expression of the high-affinity receptor for IgE (*FcεRI*) on antigen presenting cells, like Mo, could be found. Therefore we analyzed the assembly of circulating Mo populations and their *FcεRI* surface expression during the course of AEDS.

Methods: Blood samples were taken from AEDS patients before and after topical treatment as well as from psoriasis patients and healthy control donors. Detailed analysis of Mo subsets was done by flow cytometry. Meticulous clinical scoring included quantification of the surface damage using the eczema area and severity index (EASI score) as well as evaluation of the serum level of thymus- and activation-regulated chemokine (TARC); this was done before and after 2 weeks of topical treatment with tacrolimus ointment 0.1%.

Results: During the exacerbation phase of AEDS, patients harboured a different assembly of Mo subtypes from normal nonatopic individuals and patients with psoriasis with a significant increased population of $CD14^{+}CD64^{-}CD16^{+}$ Mo. Clinical improvement led to a significant decrease of this subpopulation in favor of $CD14^{+}CD64^{+}CD16^{-}$ Mo, leading to a composition of Mo subsets similar to the state found in healthy donors. Interestingly, *FcεRI* expression was confined to the $CD14^{+}CD64^{+}CD16^{-}$ Mo subpopulation and the percentage of this *FcεRI*⁺ Mo subset increased significantly in the peripheral blood after topical treatment.

Conclusion: Our data provide for the first time clear evidence that fluctuations of Mo subsets in AEDS might reflect qualitatively and quantitatively distinct contributions of Mo subsets to the development of AEDS.

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Monocytes (Mo) are members of the mononuclear phagocyte system, which is important for defence against pathogenetic organisms and tumor surveillance, and exerts immunoregulatory functions like accessory activities and cytokine production (1–3). In past years it has been reported that Mo consist of further cellular subtypes that are heterogeneous in their phenotype and function (4–7, 8, 9). Recently, the picture of Mo subsets in the peripheral blood has been summarized by Grage-Griebenow (10–13). Four major Mo types have been characterized:

1. $CD14^{+}CD64^{+}CD16^{-}$ cells of monocytic origin, known to play a role as professional phagocytes
2. $CD14^{+}CD64^{+}CD16^{+}$ Mo, which display a high expression of CD86 and HLA-DR and induce an Th1-type immune response (10, 13)
3. $CD14^{+}CD64^{-}CD16^{+}$ intermediate Mo which are assumed to represent precursors of macrophages and display the highest expression of costimulatory molecules, leading to an enormous stimulatory capacity toward autologous and allogeneic T cells (10)
4. The lineage-negative $CD14^{-}CD64^{-}CD16^{-}$ plasmacytoid Mo which express the IL-3R (CD123) (10), and secrete interferon (IFN)- α .

In contrast to myeloid subsets, the plasmacytoid Mo favor Th2 type T-cell responses and might be precursor cells of DC2 (10). Changes in the phenotype and function of Mo subsets in peripheral blood might not only be influenced by

Abbreviations: 7AAD: 7-aminoactinomycin-D; AEDS: atopic eczema/dermatitis syndrome; EASI: eczema area and severity index; *FcεRI*: high-affinity receptor for IgE; Mo: monocyte(s); NA: non-atopic donors; PSO: psoriasis; TARC: thymus- and activation-regulated chemokine.

physiological and pathophysiological conditions but also by the state of disease and different treatment forms (14). Therefore we explored the composition of these Mo subsets in patients with atopic eczema/dermatitis syndrome (AEDS) with regard to the severity of the disease and response to successful topical treatment.

Material and methods

Patients and diagnostic criteria

The following participants were recruited: 18 patients (12 women and 6 men) with moderate to severe allergic/extrinsic form of AEDS (age 38.16 ± 11.23 ; from 22 to 60 years); 10 patients with type II psoriasis without atopic diseases (age 47.5 ± 2.8 ; from 36 to 62 years); 10 healthy volunteers (NA), without atopic diseases or a positive family history for atopy (age 42.2 ± 12.9 ; from 24 to 63 years). The local ethics committee approved the protocol and we obtained written informed consent from all patients.

AEDS was diagnosed according to the criteria of Hanifin and Rajka (15) and the revised nomenclature for allergy (16) was used. This study only included patients with the allergic form of AEDS, with moderate-to-high total serum IgE levels (> 150 kU/l) in combination with positive *in-vitro* screening for aeroallergens and food allergens (e.g. CAP-RAST), and/or positive prick test results for standard aeroallergens and food allergens, and/or allergic rhinitis/asthma. Due to the restrictive criteria for classifying nonallergic AEDS patients, the number of these patients was too low, and only patients with allergic AEDS were included in the follow-up study. Patients on systemic or local glucocorticosteroid therapy were excluded. Disease severity was scored according to the eczema area and severity index (EASI) criteria (17) and the disease activity was evaluated by measuring serum levels of thymus- and activation-regulated chemokine (TARC) (18). Patients treated the affected skin area with tacrolimus ointment 0.1% twice a day for 2 weeks.

Reagents

The monoclonal antibody (mAb) 22E7 (a kind gift from Dr J.P. Kochan and Dr R. Chizzonite, Roche Inc., Nutley, NJ) recognizes the FcεRI α-subunit. The mAb CDw123 (IL-3R) clone 9F5, FITC-labeled F (ab')₂ fragments of goat antimouse were purchased from Dianova, Hamburg, Germany, and PE-labeled mAb against CD14 (M5E2), CD4 (RPA-T4), mAbs against CD56 (Leu 19), HLA-DR (L243) and mouse IgG1-PE or FITC (MOPC-21) from BD (Mountain View, CA). The monoclonal Ab against CD16-PE (3G8) and CD64-PE were from Serotec, Oxford, UK. mAb against CD16-PerCP (3G8) and HLA-DR-PerCP (Immu-357), IgG-PerCP (679.1Mc7), and the antibody against CD203c (97A6) PE were from Immunotech (Marseille, Cedex, France). All other chemicals were from Sigma unless otherwise indicated.

Analysis of monocyte subtypes in the peripheral blood

To evaluate quantitative differences between Mo subtypes, peripheral blood from the donors was drawn into tubes containing EDTA. Samples were subsequently analyzed as described previously (12). In parallel, peripheral blood mononuclear cells (PBMC) and enriched Mo were isolated as described elsewhere (19). Monocytes were isolated from peripheral blood with a modified density gradient protocol after using Nycoprep (Nycomed, Oslo, Norway). Briefly,

red blood cells were separated from plasma by sedimentation from whole EDTA blood with 1/10 (w/v) 6% Dextran 500 in 0.9% NaCl. Plasma was gently layered over Nycoprep and centrifuged for 20 min at 600 g. After separation, the interphase was collected and washed four times with 0.9% NaCl + 0.13% EDTA + 1% BSA. The purity of the enriched Mo evaluated by flow cytometry by CD14, CD19, CD3, CD56 and CD203c staining was $> 90\%$ and contained quantitatively the same amount of Mo subsets found in the whole-blood sample and in PBMC.

In order to reduce putative contamination with other cell types, enriched Mo were used for the flow cytometric analyses of all experiments presented here. Contamination from natural killer (NK) cells was excluded by CD56, and from basophils by CD203c flow cytometric staining.

Flow cytometric analysis

Labeling was performed as previously reported (19, 20). For quantitative evaluation, two-dimensional dot-plot analysis was performed and the Mo populations were gated out manually by a combination of forward- and side-scatter (FSC/SSC) and F12/F13 (CD14/7AAD or CD123/7AAD) gates. The mean fluorescence intensities (MFI) were determined by Cell-Quest software (Becton Dickinson) as described elsewhere (19).

ELISA assay

Serum samples were collected in separator tubes and allowed to clot for 30 min before centrifugation for 10 min at about 1.000 g. Then they were stored at -20°C . Enzyme-linked immunoassays (ELISA) were done according to the manufacturer's instructions (R & D Systems, Wiesbaden, Germany).

Statistical analysis

Statistical analysis using Wilcoxon's test was performed with SPSS 9.0 for WINDOWS (SPSS, Chicago, IL). Calculated values shown are mean $\pm$ standard error of mean (SEM).

Results

Comparing the composition of Mo subsets in allergic AEDS patients and healthy control donors, a significantly higher fraction of $\text{CD14}^{+} \text{CD64}^{-} \text{CD16}^{+}$ Mo could be detected in the acute phase of AEDS. Additionally, the $\text{CD14}^{+} \text{CD64}^{+} \text{CD16}^{-}$ Mo subset was significantly lower in contrast to healthy control donors in the exacerbation phase of allergic AEDS. The number of $\text{CD14}^{-} \text{CD16}^{-} \text{CD64}^{-}$ Mo was slightly, but not significantly decreased in the AEDS population, and no significant differences of the $\text{CD14}^{+} \text{CD64}^{+} \text{CD16}^{+}$ Mo subpopulation could be found in both groups (Fig. 1).

In order to verify whether the composition of Mo subsets was specific for the allergic form of AEDS or a phenomenon of inflammatory skin diseases in general, Mo subsets of patients in the exacerbation phase of psoriasis (PSO) were evaluated. In contrast to patients with allergic AEDS, the $\text{CD14}^{+} \text{CD64}^{-} \text{CD16}^{+}$ Mo fraction and the $\text{CD14}^{+} \text{CD64}^{+} \text{CD16}^{-}$ Mo fraction of

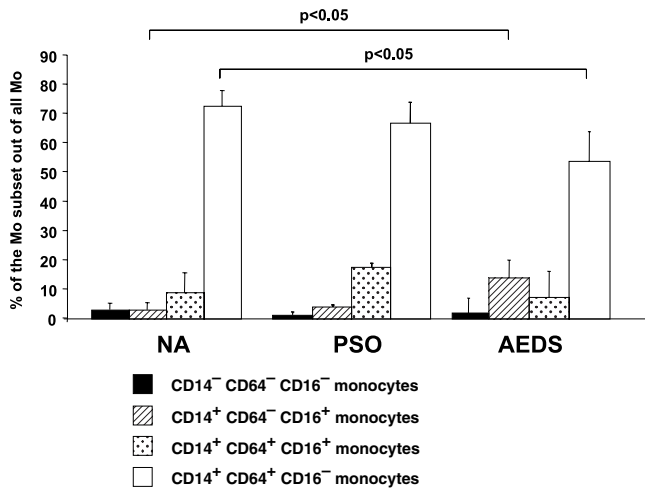


Figure 1. Distinct assembly of monocyte subsets in patients with acute AEDS. Quantitative analysis of monocyte (Mo) subsets of healthy nonatopic donors (NA; $n = 10$), patients in the exacerbation phase of psoriasis (PSO; $n = 10$) and patients in the acute phase of AEDS (AEDS; $n = 18$). The percentage of the CD14⁺ CD64⁻ CD16⁺ monocyte subset is significantly elevated in the acute phase of AEDS, whereas the amount of CD14⁺ CD64⁺ CD16⁻ monocytes is significantly decreased at this state, in contrast to nonatopic donors or patients with psoriasis.

the PSO patients did not differ significantly from healthy, nonatopic donors (Fig. 1).

Phenotypic analysis of enriched Mo of all patients revealed that the surface expression of the high-affinity receptor for IgE (FcεRI) was limited to the CD14⁺ CD64⁺ CD16⁻ Mo subtype in all donors (data not shown).

After 2 weeks of topical treatment with tacrolimus ointment (0.1%) an improvement of 75–99% of the treated skin areas could be achieved. The EASI score and serum TARC (which has been shown to correlate highly with the activity and surface damage of allergic AEDS) quantified severity of the disease. TARC serum levels decreased significantly from 968 ± 118 pg/ml to 728.7 ± 50.4 pg/ml; $P < 0.05$; $n = 18$; EASI score decreased significantly from 18.7 ± 2.1 to 9.5 ± 2.2 ; $P < 0.01$; $n = 18$.

In contrast, there were no significant changes in blood parameters like eosinophil count ($10.1 \pm 3.2\%$ before and $6.4 \pm 4.6\%$ after treatment), IgE serum levels (1922.0 ± 1983 kU/l before and 2115.7 ± 2015 kU/l after), and lactate dehydrogenase (LDH) serum levels (204.6 ± 55.8 U/l before and 206.4 ± 5.9 U/l after treatment).

Parallel to the significant improvement in the extent of surface damage, the percentage of CD14⁺ CD64⁻ CD16⁺ Mo decreased significantly to a level typical for healthy people, and even the percentage of CD14⁺ CD64⁺ CD16⁻ Mo increased significantly (Fig. 2). The percentage of FcεRI bearing CD14⁺ CD64⁺ CD16⁻ Mo increased from $12.4 \pm 9.7\%$ to $25.7 \pm 10.3\%$

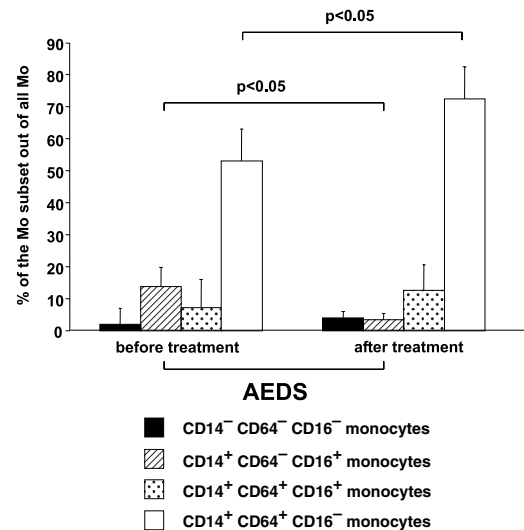


Figure 2. Alteration of the composition of monocyte subsets in response to topical treatment and improvement of the surface damage. Assembly of monocyte subsets of patients with AEDS ($n = 18$) before (left side of the graph) and after successful topical treatment with tacrolimus 0.1% ointment (right side of the graph). The percentage of CD14⁺ CD64⁻ CD16⁺ monocytes decreased in favor of an increase of CD14⁺ CD64⁺ CD16⁻ monocytes, leading to an assembly of monocyte subsets similar to that found in healthy donors.

($P < 0.05$; $n = 18$), but no significant changes in the CD14⁺ CD64⁺ CD16⁺ Mo subset was observed.

Discussion

In recent years it has been proposed that Mo consist of a heterogeneous population of subsets, which exert different defense mechanisms of the immune system and display distinct phenotypic and functional properties. It could be shown that some disease-related conditions or the acute inflammatory environment could lead to an up-regulation of some of these Mo subsets (21). Recent investigations have shown that Mo of the allergic form of AEDS display numerous immunological differences, such as abnormal AMP-phosphodiesterase (PDE) activity, enhanced prostaglandin E₂ production and increased cellular cytotoxicity (22, 23), in combination with profound phenotypic alterations (Novak et al. in press).

In contrast, the pathophysiological background of the nonallergic form of AEDS is almost a “black box”. Therefore, it was important for us to distinguish strictly between both subtypes of AEDS and to focus on the allergic form of this disease in our study. Modulation of the amount of soluble factors, like TARC serum level with disease activity, and EASI score for allergic AEDS, is clearly documented in our study (18). In AEDS, changes in CD16, major histocompatibility complex (MHC) class II and CD4 expression have been described; these

alterations might result from changes in the activation and differentiation of these cells (21, 24). In relation to these observations, we found significant changes in the assembly of Mo subtypes at different stages of the allergic form of AEDS. The increase of CD14⁺ CD64⁺ CD16⁺ Mo in the exacerbation phase might mirror imbalances in the immune system, causing alterations in the microenvironment of the peripheral blood and the release of soluble factors. CD14⁺ CD64⁺ CD16⁺ Mo might contribute to exacerbation of the disease by their high stimulatory potential toward T cells in the acute phase of AEDS (10, 11, 13). These observations could be explained by changes in serum levels of factors that regulate expression of IgG receptors (such as macrophage-colony stimulating factor (M-CSF), granulocyte M-CSF (GM-CSF), and IFN- γ (25, 26)) during the exacerbation phase of AEDS and after successful topical treatment.

On the other hand, Mo subsets like the Fc ϵ RI-bearing CD14⁺ CD64⁺ CD16⁺ Mo might be recruited into the epidermis by chemotactic signals in the exacerbation phase of the disease, differentiating into Fc ϵ RI + DC (27, Novak et al. in press) and therefore quantitatively decrease in the peripheral blood at this state. Improvement of the disease might lead to a reduction of soluble

factors that recruit these cells to the site of inflammation, i.e. epidermal skin lesions and an enhancement of the number of these cells in the peripheral blood.

It has been clearly shown that tacrolimus exerts immunoregulatory functions on antigen-presenting cells (APCs) *in vitro* and *in vivo*. It induces a significant reduction of Fc ϵ RI and IgG receptor-bearing, inflammatory dendritic epidermal cells (IDEC) in skin lesions of AEDS patients treated with topical tacrolimus ointment. This mode of action could be an important link to the mechanisms that cause the alterations in Mo subtypes observed in our study (28, 29).

Improvement of the pathologic imbalance of the assembly of Mo subsets in AEDS might indicate improvement of the disease, thereby providing a useful tool in future therapeutic approaches.

Acknowledgments

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