Increased endocytic activity in monocyte-derived dendritic cells in patients with psoriasis vulgaris

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Background & objectives: The understanding of the pathogenesis of psoriasis vulgaris has focused on T cell mediated immune disorder for many years. Recent studies provide evidence that dendritic cells may be of major importance as regulatory cells driving the psoriasis tissue reaction, and they are one of the therapeutic targets. In order to further characterize the role of dendritic cells in psoriasis, this study was designed to assess the differentiation of dendritic cells from monocytes (MoDC), the expression of phagocytosis related receptors by MoDC, their endocytic activity for fluorescent beads and lucifer yellow as well as their superoxide generation in patients with psoriasis.

Methods: Twenty eight patients with psoriasis vulgaris and 12 healthy controls were included in the study. MoDC were obtained by culturing monocytes with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) for 5 days. Cell surface expression of CD1a, CD14, CD40, CD80, CD83, CD86, HLA-DR, mannose receptor (MR) and Fcγ receptor II (CD32) by MoDC and their endocytosis of dextran and lucifer yellow were analyzed by flow cytometry. Zymosan ingestion was measured to access the phagocytosis of MoDC.

Results: Differentiation of monocytes to dendritic cells was upregulated in patients manifested as significantly increased expression of CD40, CD80, CD86 and HLA-DR compared with that in healthy controls (P<0.01). Expression of MR and Fcγ receptor II (CD32) by MoDC was significantly increased in patients with psoriasis as well (P<0.01). Endocytosis of dextran but not lucifer yellow in patients was significantly higher than controls (P<0.01), and significantly enhanced phagocytosis by increasing zymosan ingestion was also observed (P<0.01) in patients. Taken together, endocytic and phagocytic activity of MoDC in psoriasis was increased than normal persons.

Interpretation & conclusion: Enhanced activity of dendritic cells binding and capturing foreign antigens for subsequent antigen presentation and the initiation of immune responses in psoriasis may contribute to the pathogenesis of the disease. The upregulated expression of MR and the enhanced endocytic activity of DC might be an explanation for the absence of skin infection observed in psoriasis.

Key words Endocytosis - monocyte-derived dendritic cell - phagocytosis - psoriasis
Psoriasis is a common inflammatory skin disorder with a genetic susceptibility and an immunology based aetiology. It is characterized by epidermal hyperplasia with cellular infiltration of lymphocytes, monocytes and neutrophils. Activation of T cells in the pathogenesis of psoriasis has been confirmed in the past two decades, and in 1990 the potential significance of dendritic cell/T cell interaction has been pointed out. Additionally, effective antipsoriatic agents such as cyclosporin and FK506 have been shown to suppress either T cells, antigen-presenting cells or both. Topical use of calcipotriol, a vitamin D₃ analogue was found to be helpful for psoriasis and has been proved to suppress the ability of Langerhans cells (LC) to stimulate antigen-dependent T cell proliferation. Our study on the mechanisms of fumaric acid esters in treating psoriasis established the target as dendritic cells. Therefore, apart from the commonly accepted concept that psoriasis is a T cell mediated immune disorder, the role of antigen-presenting cells, particularly dendritic cells, as initiator of the disease as well as the recruitment of disease specific T cells in the immunopathogenesis of psoriasis has gained more attention.

Dendritic cells (DC) are potent antigen-presenting cells specialized for the initiation of primary immune responses. Epidermal and dermal dendritic cells have been shown to be increased in number in lesional psoriatic skin and to possess increased antigen presenting activities. These cells are immature dendritic cells possessing similar phenotypes as monocyte-derived dendritic cells (MoDC). In epidermis and dermis, most cells that can be found in psoriasis can produce factors that are required for the differentiation of monocytes to DC, for example, granulocyte macrophage-colony stimulating factor (GM-CSF) can be produced by fibroblasts and interleukin-4 (IL-4) by mast cells. Therefore, the increase in number was thought to be a result of an enhanced influx of monocytes from peripheral blood with subsequent differentiation into dendritic cells. In view of the paucity of information concerning the antigen uptake activity of dendritic cells in psoriasis, which is a characteristic and main function of immature dendritic cells, we developed in vitro MoDC to investigate the expression of phagocytosis related receptors, their phagocytotic activity for fluorescent beads and lucifer yellow as well as their superoxide generation ability.

Material & Methods

Culture media and reagents: The standard medium for cell culture was RPMI 1640 (BioConcept, Umkirch, Germany) supplemented with 2mL-glutamin (BioProducts, Heidelberg, Germany), 100U/ml penicillin and 100 µg/ml streptomycin (Seromed, Berlin, Germany). For dendritic cell culture 800U/ml GM-CSF (Leucomax®, Novartis Pharma, Nürnberg, Germany) and 10 ng/ml IL-4 (Schering-Plough, Kenilworth, USA) were added to the supplemented medium.

Phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)- conjugated monoclonal antibodies against the epitope CD1a, CD14, CD40, CD80, CD83, CD86, HLA-DR, mannose receptor (MR/CD206) and FcγRI, II, III (CD64, CD32, CD16) as well as their respective isotype controls (IgG1) were from Coulter-Imunotech (Krefeld, Germany). FITC-dextran (molecular weight 40,000 Da) and lucifer yellow dipotassium salt were purchased from Sigma, USA.

Complete buffer was constitutive of phosphate buffered saline (PBS) containing 0.25 per cent (wt/vol) bovine serum albumin (Sigma), 0.1 per cent (wt/vol) glucose, 0.9 mmol/l CaCl₂ and 0.5 mmol/l MgCl₂ and stored in aliquots at -20°C. Opsonized zymosan particles (C3b-zymosan) were prepared by incubation of boiled zymosan A (Sigma) in pooled fresh human serum for 2 h at 37°C. After being washed five times with Ca/Mg-free PBS, opsonized zymosan particles were diluted to a concentration of 2.5 mg/ml in complete buffer and stored in aliquots at -20°C. Cytochrome C (horse heart type III, Sigma) was dissolved in complete buffer as a concentration of 1 mg/ml. Cytochalasin B was also purchased from Sigma. All the patients visiting the two hospitals as detailed below with a diagnosis of psoriasis vulgaris from year 2000 to 2003 were included in the study. But only those with no history of medication in the past two months, no past history of systemic diseases and at the same time would like to take part in the study were enrolled in the study.

Patients: Twenty-eight patients (all the patients visiting two hospitals as detailed below with a diagnosis of psoriasis vulgaris from year 2000 to 2003
were included in the study. But only those with no history of medication in the past two months, no past history of systemic diseases and at the same time would like to take part in the study was enrolled). (17 males and 11 females), with psoriasis vulgaris and 12 healthy controls (6 males and 6 females; randomly chosen from our staff members who were healthy) were investigated. Patients were selected randomly from out-patients department of Dermatology at Sir Run Run Shaw Hospital and the Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, People’s Republic of China, during 2000-2003. None of the patients was given any form of systemic treatment for at least two months prior to inclusion in the study, nor with topical treatment in the past two weeks. No history of chronic systemic diseases was recorded and no sign of infection was observed in these patients when enrolled in the study. Four patients were first onset cases and 24 were relapsed cases with an average psoriasis area and severity index (PASI) of 9.8. The type of psoriasis was guttate (3 cases) and plaque (25 cases). The mean age of the patients was 43.3 ± 14.8 yr (ranged from 18 to 68 yr) and controls 34.0 ± 8.9 yr (ranged from 18 to 49 yr). The study protocols were approved by the ethics committees of Zhejiang University.

**Generation of dendritic cells and cell culture:**
T lymphocyte-depleted peripheral blood mononuclear cells (PBMC) were obtained by Ficoll/Paque (Biochem, Berlin, Germany) density gradient centrifugation of ethylenediamine tetraacetic acid (EDTA)-anticoagulated venous blood from psoriasis patients and healthy controls after obtaining informed consent. Monocytes were enriched by plating PBMC on Falcon tissue culture plates (Becton Dickinson, Heidelberg, Germany) and allowed to adhere for 2 h. The non-adherent cells were removed, and the adherent cells cultured for 5 days at 1.5-2.0 × 10⁶/ml in 6-well flat bottom tissue-culture plates at 37°C in a humidified atmosphere with 5 per cent CO₂ in standard medium together with 800 U/ml GM-CSF and 10 ng/ml IL-4 to generate immature [CD1a⁺, CD14⁻, CD83⁺ by (FACS) flow cytometry determination] inflammatory-type MoDC. Differentiation of dendritic cells was evaluated daily using an inverted microscope by observing the morphologic change of the cells. Medium was changed after 3 days of culture. Viability of obtained MoDC was determined by trypan blue exclusion test.

**Flow cytometric analysis:** CD1a, CD14, CD40, CD80, CD83, CD86, HLA-DR, CD206 and FcγRI, II, III (CD64, CD32, CD16) expression on GM-CSF and IL-4 induced MoDC were assessed by single colour flow cytometry. Analysis was performed using an EPICS-XL flow cytometer and system II software (Beckman Coulter, USA). Briefly, cells were harvested after 5 days of culture and resuspended in PBS. Staining was performed on ice for 30 min in darkness (by adding mouse Abs to the cells and afterwards put them in an ice box and keep them in refrigerators for 30 min) using the mouse antibodies described above and their corresponding PE- or FITC- labeled isotype control antibodies to determine the background staining. At least 1x10⁴ viable MoDC per sample, gated according to forward and side scatter characteristics, were analyzed. The results were expressed as percentage of positive cells or as fluorescence intensity (FI), calculated according to the formula: FI=mean fluorescence (sample)-mean fluorescence (control).

Dead cells were gated out after staining with 0.5 µg/ml propidium iodide solution.

**Fluid phase and mannose receptor-mediated endocytosis:** Mannose receptor mediated endocytosis was measured as the cellular uptake of FITC-dextran and quantified by flow cytometry¹⁰. Approximately 2x10⁵ cells per sample were incubated in medium containing 1mg/ml FITC-dextran for 0, 60 and 120 min. After incubation, cells were washed with PBS and fixed in cold 1 per cent formalin. At least 1x10⁴ cells per sample were used to analyze the uptake of FITC-dextran by the cells. Fluid phase endocytosis was measured as the cellular uptake of 1 mg/ml lucifer yellow dipotassium salt and quantified by flow cytometry with the same steps for dextran.

**Superoxide anion generation:** The generation of superoxide anion radicals from MoDC was measured by the superoxide-dismutase-inhibitable reduction of cytochrome C using the method described by Mrowietz et al¹¹. Briefly, 250 µl zymosan-containing complete buffer was added to 500 µl cytochrome C solution (1mg/ml complete buffer). MoDC suspended in complete buffer (4 x 10⁶/ml) were preincubated.
with 5 µg/ml cytochalasin B for 5 min at 37°C in water bath. The reaction was stopped by rapid cooling in iced water, the cells were spun down, and the amount of reduced cytochrome C was determined in the supernatant at 450 and 492 nm versus standards of oxidized and totally reduced cytochrome C using a double-beam spectrophotometer (Lamotta, USA). Results were expressed as the differences between zymosan stimulated and spontaneous (complete buffer alone) superoxide anion generation.

Statistical analysis: Statistical differences between the two groups were evaluated using the unpaired Students’ t-test. P<0.05 was considered significant.

Results

Viability and phenotype of MoDC: After 5 days of culture with GM-CSF/IL-4 the viability of MoDC both of patients and control group was greater than 90 percent as judged by trypan blue exclusion test. A similar morphology of cells manifested as non-adherent, clustered and protruding veils was found using an inverted microscope. Differentiation of MoDC analyzed by flow cytometry showed DC with a typical immature phenotype (CD1a-positive, CD14-negative, CD80-positive, CD83-negative, HLA-DR-positive). Expression of CD1a, CD14, CD40, CD80, CD86 and HLA-DR of both controls and patients was analyzed. The results showed that DC from patients expressed higher levels of CD40, CD80, CD86 and HLA-DR than control-DC (P<0.05). DC from both patients and controls appeared positive for CD1a expression and negative for CD14 and CD83 expression (Fig. 1).

Enhanced expression of CD206 and FcγRII on MoDC of psoriasis patients: Antigen uptake of MoDC can be mediated via phagocytosis related receptors including mannose receptor (MR/CD206) and Fcγ receptors (FcγRI, FcγRII, FcγRIII). We found MoDC of patients expressed significantly enhanced level of CD206 and FcγRII (CD32) when compared with healthy controls (FI: 210.59 ± 22.83 vs 144.52 ± 35.24 and 29.46 ± 4.10 vs 20.60 ± 6.02, P<0.01), whereas no significant difference was found in FcγRI and FcγRIII expression between patients and controls (FI: 2.49 ± 0.91 vs. 2.32 ± 0.83 and 32.36 ± 7.13 vs 34.76 ± 5.87) (Fig. 2).

Discussion

Though the role of T cells in the pathogenesis of psoriasis has been established for more than 20 yr, but this view has been modified with increases in newly defined dendritic cell subsets identified in psoriasis lesions12. Recent evidence showed that psoriasis might be caused by (auto)antigen(s) with dendritic cells driving the pathogenetic process13,14. By using Affymetrix GeneChips technique, Zhou et al15 identified markers of dendritic cell activation in uninvolved psoriatic skin. CD83 was expressed at a high level on immune-competent, activated and mature DC16, and in psoriasis skin CD83+ DC was thought to be derived from activated Langerhan’s cells in situ15. With immunohistochemistry study, a small but significant subpopulation of CD83+ DC was found in the upper dermis of psoriasis with the most common distribution pattern as cluster with mononuclear lymphoid cells, indicating that activated and mature CD83+ DC may play a role in the immune response in psoriasis. This provided in vivo support for the concept that CD83+ DCs give signals for direct intralesional T cell activation16. Further, dendritic cells are proved to be target cells of effective antipsoriatic drugs such as calcitriol and its analogues1 as well as fumaric acid.
Fig. 1. Cell surface molecule expression of monocyte-derived dendritic cells (MoDC). Columns represent binding of individual monoclonal antibodies (MAbs) to cultured cells (mean value ± SD) observed in healthy controls (n=12) and patients with psoriasis (n=28). *P<0.05 compared to controls.

Fig. 2. Expression of phagocytosis related receptors by MoDC generated in patients with psoriasis and health controls. Red line, representative of 28 patients; black line, representative of 12 controls; FI, fluorescence intensity. One representative histogram is shown.
esters. Therefore, dendritic cells seem to be of major importance as regulatory cells driving the psoriatic tissue reaction. As dendritic cells do not proliferate in situ, precursors such as monocytes or CD34+ myeloid progenitor cells are recruited from the peripheral blood differentiating in the tissue into immunologically active cells. GM-CSF/IL-4 induced monocyte-derived dendritic cells represent so called immature dendritic cells, which reach at sites of inflammation. In this study, we demonstrated that MoDC of psoriasis patients expressed higher levels of CD40, CD80, CD86 and HLA-DR, which are molecules involved in antigen presenting. This gave further evidence that DC play a role in psoriasis.

Since DC are the most potent antigen-presenting cells in vitro and in vivo with a key role in the initiation of the immune responses and immature DCs are very efficient in their antigen uptake capacity, we addressed the question whether the phagocytic function of DC which represents for the capacity of antigen uptake changed in psoriasis. It is well known that DCs use a variety of mechanisms, including both receptor-mediated and fluid endocytosis (macropinosis) and

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**Fig. 3.** Endocytic activity of MoDC from patients with psoriasis and health controls. Endocytosis was evaluated as uptake of 1 mg/ml FITC-dextran (A) or 1 mg/ml lucifer yellow (B) and was measured using (FACS) flow cytometry. Results are expressed as mean ± SD fluorescence intensity (number of patients=28, number of controls=12). *P<0.01.
phagocytosis as well. Mannose receptor (MR, CD206) is a surface 175 kDa C-type lectin containing eight carbohydrate recognition domains with broad specificity for sugars18 and functions as an endocytic receptor primary expressed on macrophages and dendritic cells. It efficiently mediates internalization of mannosylated glycoproteins or in receptor-mediated facilitated antigen presentation20,21. In a mouse study, zymosan uptake by Langerhans cells, which are immature DCs in the skin, was verified to be mediated by mannose receptor22. Wollenberg et al22 identified MR expression on MoDC and uptake of dextran FITC by MoDC by flow cytometric analysis in psoriatic lesions but not in normal skins, the MR expressed on CD1a/CD206+ epidermal dendritic cells was functional in terms of uptake of mannosylated antigens23. Fcγ receptors are a family of membrane glycoproteins expressed on haematopoietic cells24. They are functionally considered as antigen receptors due to the very high affinity of FcγRII (CD32) and FcγRIII (CD16) to immune complexes25. In this study, expression of antigen uptake molecules (MR and Fcγ RI, II, III), receptor-mediated antigen uptake of MoDC in psoriasis have been analyzed. We demonstrated that patients with psoriasis showed markedly enhanced expression of two antigen uptake receptors (MR and CD32) and receptor-mediated antigen uptake (FITC-dextran) of DCs. Cytochrome C reduction of MoDC was found to be significantly higher in patients than in healthy controls. Therefore phagocytic activity of MoDC in psoriasis was increased as compared with normal persons, and the enhanced phagocytosis may be associated with the upregulation of MR and FcγR. This indicated a potent activity in dendritic cell binding and capturing foreign antigens for subsequent antigen presentation and the initiation of immune responses in psoriasis. Unlike other inflammatory skin diseases, skin infection is rather rare in psoriasis. This phenomenon could be explained by several findings. For examples, polymorphonuclear cells from psoriasis patients showed enhanced phagocytosis of bacteria26. Fraktalkine, a chemokine with properties of both chemoattractants and adhesion molecules, was found to have a higher mRNA expression in psoriatic lesions than in non-lesional skin27, and the increased expression of fractalkine was observed in dermal blood vessels and dermal dendrocytes in the papillary dermis of psoriatic tissues28. Increased levels of granulysin in T cells and dermal dendrocytes in psoriatic plagues also provide an explanation for relative immunity of psoriatic plagues against both Gram-positive and Gram-negative bacterial infection29. Recently characterized cutaneous antimicrobial proteins extracted from psoriatic scale including S100-protein psoriasin, human beta-defensin, RNase 7, lysozyme and human neutrophil defensin 1-3 provide further evidence of exemplified infection in psoriatic skin30. Because MR is able to bind to carbohydrate ligands found on many types of potential pathogens, it is assumed to act as a pattern recognition receptor playing a role in innate immune functions such as phagocytosis31. The clinical relevance of MR expression in skin was demonstrated by MR-mediated interaction and uptake of Malassezia furfur yeast cells by immature MoDC in patients of atopic dermatitis32. Therefore, the upregulated expression of MR and the enhanced endocytic activity of MoDC found in the present study might be contributing to the defense of psoriatic skin against infection due to colonization with mannose-rich pathogens, such as fungi and bacteria.

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References


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