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INVESTIGATIVE REPORT

Malassezia sympodialis Stimulation Differently Affects Gene Expression in Dendritic Cells from Atopic Dermatitis Patients and Healthy Individuals

Susanne GABRIELSSON[1], Eva BUENTKE[1], Agne LIEDÉN[1], Margit SCHMIDT[2], Mauro D'AMATO[3], Maria TENGVALL-LINDER[1] and Annika SCHEYNIUS[1]

[1]*Department of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet and Hospital, Stockholm, Sweden,* [2]*East Carolina University, Department of Biology, Greenville, USA and* [3]*Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden*

It is known that 28–84% of patients with atopic dermatitis exhibit IgE and/or T-cell reactivity to the opportunistic yeast *Malassezia sympodialis*, which can be taken up by immature monocyte-derived dendritic cells (MDDCs), resulting in MDDC maturation. The aim of this study was to investigate whether MDDCs from patients with atopic dermatitis respond differently to *M. sympodialis* compared to MDDCs from healthy individuals. Immature MDDCs were stimulated with *M. sympodialis* and the gene expression profiles were analysed with cDNA arrays containing 406 genes. Our results show that *M. sympodialis* differently affected MDDCs from patients with atopic dermatitis, and more so in severely ill patients, compared with healthy individuals. Six genes were more than fivefold up-regulated in MDDCs from more than one patient with atopic dermatitis, coding for CD54, CD83, IL-8, monocyte-derived chemokine (MDC), BTG1 and IL-1R antagonist. In healthy individuals this was true only for BTG1. Up-regulations of IL-8 and MDC were confirmed at the protein level. Our findings might reflect an increased trafficking and stimulatory capacity in MDDCs from the patients, which is likely to result in a stronger inflammatory response to *M. sympodialis*.

Key words: cDNA array; dendritic cells; Malassezia sympodialis; atopic dermatitis.

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Susanne Gabrielsson, Dept of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet/Hospital, SE-171 76 Stockholm, Sweden. E-mail: Susanne.Gabrielsson@ks.se

The prevalence of IgE-associated allergic diseases has increased dramatically in Westernized countries during the last decades (1). One of these diseases is atopic dermatitis (AD), a chronic relapsing eczema. A family history of atopy is present in about 70% of the AD cases (2) and several candidate genes have been identified, which show association with AD (3, 4).

Malassezia sympodialis, formerly known as *Malassezia furfur* or *Pityrosporum orbiculare/ovale*, is a unicellular lipophilic yeast that is part of the normal microflora on human skin (5). However, this yeast has been shown to act as an allergenic source in patients (6). Eight different species of *Malassezia* have been described, and nine allergens have so far been cloned and sequenced (6). Specific IgE reactivity against *Malassezia* has been reported in 28–84% of patients, depending on selection criteria, but rarely in patients with respiratory allergy or in healthy individuals. Positive skin prick test (SPT) and atopy patch test (APT) reactions to extract from *M. sympodialis* have been shown in patients, and treatment with ketoconazole results in clinical remission and decreased IgE levels (6). Dendritic cells (DCs) are the most potent antigen-presenting cells (APC), able to activate naïve T cells, but also capable of inducing tolerance (7). Cutaneous APCs include $CD1a^+$ Langerhans cells (LCs) and dermal dendritic cells (DDCs). $CD1a^+$ LCs are mainly found in the epidermis, but smaller numbers are seen within the dermis (8). Immature LCs in the skin use several mechanisms for antigen uptake, i.e. macropinocytosis and receptor-mediated endocytosis, via mannose receptors (MR), FcεR, FcγR, DEC-205 and DC-SIGN (7). After antigen uptake, LCs may undergo maturation and migrate to the lymph nodes, where they show loss of their ability to take up antigen and up-regulation of their cell surface MHC class II and accessory molecule expression (9). The role of DCs in the pathogenesis of AD has not been fully elucidated. It has been shown that LCs in AD express large amounts of the co-stimulatory molecule CD86, associated with Th2 stimulation (10), and inflammatory dendritic epidermal cells have been shown to up-regulate co-stimulatory molecules in AD (11). Immature monocyte-derived dendritic cells (MDDCs) from healthy individuals have been shown to internalize whole yeast cells and extract from *M. sympodialis*, at least partly through the MR (12). This process is associated with maturation, production of proinflammatory and immunoregulatory cytokines, which might favour induction of a Th2-type

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immune response, and a capacity to stimulate lymphocyte proliferation (13, 14). *M. sympodialis* can thus act as an allergenic source that elicits specific IgE reactivity and T-cell proliferation in patients with AD (6). The possibility of producing DCs from peripheral blood monocytes (15, 16) has facilitated the studies of DC in recent years.

The aim of this study was to investigate whether MDDCs generated from patients with AD with serum IgE reactivity, positive APT and SPT reaction to *M. sympodialis* respond differently to this yeast than MDDC from non-atopic healthy individuals. Gene expression profiles of MDDCs from patients with moderate and severe AD and healthy controls, cultured in the presence or absence of *M. sympodialis*, were characterized by the use of a macro array-based approach. Interleukin-8 (IL-8) and monocyte-derived cytokine (MDC) were also measured at the protein level. Our results show that several genes involved in cell migration, adhesion and co-stimulation are differently expressed in MDDCs from patients with AD and healthy individuals, upon stimulation with the yeast *M. sympodialis*.

MATERIALS AND METHODS

Subjects

Four patients with AD (Table I) were recruited from a recent study (17). At the time of sampling they were diagnosed with moderate to severe AD according to SCORAD and had specific IgE to *M. sympodialis* (m70, Pharmacia Diagnostics, Uppsala, Sweden) and had previously (17) proved to be positive for *M. sympodialis* in both skin prick test and atopy patch test (APT). Healthy controls were ImmunoCap-negative to a panel of common allergens (Phadiatop, Pharmacia Diagnostics) and *M. sympodialis*, and had no history of allergy (Table I). The study was approved by the ethics committee of the Karolinska Hospital, and all participating subjects gave their informed consent.

Generation of monocyte-derived dendritic cells (MDDCs)

Peripheral blood (450 ml) supplemented with 15 IE/KY/ml heparin (LEOPharma, Malmö, Sweden) from healthy controls and patients with AD was diluted 1:1 with phosphate-buffered saline (PBS, pH 7.4). Peripheral blood mononuclear cells (PBMC) were obtained by separation on Ficoll Paque (Pharmacia Biotech, Uppsala, Sweden). Serum was collected and stored at $-20^{\circ}C$. $CD14^+$ monocytes were enriched by positive selection using magnetic activated cell sorting (MACS, Miltenyi Biotech, Gladbach, Germany) according to the manufacturer's protocol with minor changes. The $CD14^+$ monocytes were diluted to 4×10^5 cells/ml in complete culture medium (RPMIc) (12) and cultured in 25- or 75-cm^2 culture flasks for 6 days with refeeding on day 3. Immature MDDCs were harvested on day 6 by gentle flushing. Anti-CD14 staining was performed to evaluate the separation efficiency of monocytes and shown to be always >92%. MDDCs on day 6 showed a typical immature phenotype ($CD83^+$ cells <5%) (13).

M. sympodialis

M. sympodialis strain no. 42132 (American Type Culture Collection) was cultured on Dixon solid phase medium, at $37^{\circ}C$ for 4 days (18). The culture was controlled for bacterial contamination and growth of other yeasts using a blood agar plate and a Sabouraud dextrose agar plate. The yeast cells were harvested into sterile water and counted.

Stimulation of immature MDDCs with M. sympodialis

Immature (<5% $CD83^+$) MDDCs (4×10^5 cells/ml) were harvested on day 6 and incubated with or without *M. sympodialis* (five yeast cells per MDDC) in complete culture medium, as described above but with 5% heat-inactivated FCS and 5% autologus serum, for 18 h at $37^{\circ}C$ in 6% CO_2 (13).

Table I. *Characterization of healthy controls (HC) and patients with atopic dermatitis (AD)*

Subject	Gender	Age (years)	SCORAD[a]	SPT[b]	APT[c]	*M. sympodialis*[d] specific s-IgE (kU/l)	Total s-IgE[e] (kU/l)	Phadiatop[f]	Asthma/rhinitis
HC1	M	45	NA	ND	ND	<0.35	45	Negative	−/−
HC2	M	48	NA	ND	ND	<0.35	15	Negative	−/−
HC3	M	30	NA	ND	ND	<0.35	2	Negative	−/−
AD1	F	31	23	4.8	+	4.8	5300	Positive	+/−
AD2	M	25	28	5	+	14	2400	Positive	+/+
AD3	M	45	54	6.5	+++	2.4	4710	Positive	+/−
AD4	M	33	46	8	++	5.4	1800	Positive	−/+

F, female; M, male; NA, not applicable; ND, not done.

[a]Assessed by SCORAD (34) at the time of monocyte-derived dendritic cell generation.

[b]The skin prick test (SPT) was performed with extract of *M. sympodialis*, 100 μg/ml, evaluated after 15 min and graded as mean diameter (mm) of the wheal (17). A reaction with a mean diameter of ≥3 mm was considered positive.

[c]Atopy patch test (APT) was performed with extract of *M. sympodialis*, 5 mg/ml, on tape-stripped non-lesional skin (17). The test results were scored from 0 to 3+, where 0 = negative reaction, 1+ = erythema, infiltration, possibly papules; 2+ = erythema, infiltration, papules and/or small vesicles and 3+ = erythema, infiltration, papules and large vesicles (35).

[d]ImmunoCap™ m70 (Pharmacia Diagnostics AB), reference range <0.35 kU/l.

[e]ImmunoCap™ (Pharmacia Diagnostics AB), reference range 1.6–122 kU/l.

[f]Phadiatop® (Pharmacia Diagnostics AB), serum IgE to any of 11 common aero-allergens. Reference range <0.35 kU/l.

Flow cytometric analysis

Approximately 5×10^4 MDDCs were incubated for 30 min on ice with either fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mouse monoclonal antibodies (mAbs) diluted in PBS with BSA 0.5% and NaN_3 0.01%. A minimum of 10^4 cells were acquired on a FACSCalibur flow cytometer (Becton Dickinson, Bedford, MA, USA). Cells were considered positive when they had a higher mean fluorescence index (MFI) value than cells incubated with an irrelevant isotype matched antibody. The following FITC- or PE-conjugated mouse mAbs were used for evaluating phenotype: anti-CD14 FITC (Leu-M3, Becton Dickinson) to evaluate the separation efficiency, anti-CD1a PE (T6-RDI, Coulter Hialeah), anti-CD40 FITC (LOB7/6, Serotech Ltd), anti-CD80 FITC and anti-CD83 FITC (L307.4 and HB15e, PharMingen/Becton Dickinson), anti-CD86 FITC (2331, FUN-1, PharMingen), anti-HLA-DR FITC (L243, Becton Dickinson). The isotype-matched controls were mouse IgG_1-PE, IgG_1-FITC, IgG_{2a}-PE and IgG_{2a}-FITC (Becton Dickinson). MDDC not stimulated with *M. sympodialis* were evaluated on day 7, and were shown to be immature ($CD83^+$ cells ranged from 3 to 12%, median 6%); no differences were seen between healthy individuals and patients with AD. As the yeast is highly autofluorescent and interferes with detection, flow cytometry analysis could not be performed on the *M. sympodialis*-stimulated cells (13).

Preparation of total RNA from MDDCs

Non-adherent and semi-adherent cells were collected by thorough flushing with cold PBS. The same numbers of cells were used from stimulated and non-stimulated flasks. The medium was saved for later evaluation of cytokine production and stored at -20°C. Total RNA samples were prepared using a NucleoSpin RNA II Kit (BD Clontech, Palo Alto, CA, USA) according to the manufacturer's protocol. To avoid non-specific degradation of RNA, anti-RNAse (Ambion, Austin, TX, USA) was added to the column during the DNAse treatment. Total RNA yield was calculated by measuring the optical density (OD) at 260 nm and a small aliquot was analysed on an agarose gel (1%), stained with ethidium bromide. The purified amount was usually 4.0–5.0 μg/10^6 cells. The samples were concentrated under vacuum and stored at -80°C for a maximum of 2 days before poly A enrichment.

Poly A enrichment, synthesis of radioactive labelled cDNA probes and hybridization to membrane

Radioactively labelled cDNA probes were prepared using the Atlas™ Pure Total RNA labelling system (Clontech) according to the manufacturer's protocol. The probes were hybridized overnight at 68°C to a positively charged nylon membrane containing the arrayed DNA. The used arrays carry cDNA from 406 genes in duplicates, 9 housekeeping genes and controls for genomic contamination and are referred to as haematology/immunology membranes (Clontech). For the complete gene list see www.clontech.com.

Analysis of gene expression

The arrays were visualized by phosphoimaging using Fuji BAS 1800 I IR after 1–3 days of exposure. Image Gauge was used for processing of the scan files and AtlasImage™ 2.0 (Clontech) for final analysis and generation of result sheets. To assess differences in gene expression between filters, stimulated compared to non-stimulated MDDC from each individual, the intensity values for each gene were calculated after subtraction of the background and then normalized to the mean of intensity of four housekeeping genes on the same filter (ubiquitin C, liver glyceraldehyde 3-phosphate dehydrogenase, major histocompatibility complex class I C and cytoplasmic β-actin). Cut-off was set at three times the background level, and ratios between *M. sympodialis*-stimulated and non-stimulated samples were calculated. For values below cut-off, the cut-off value was used for ratio calculations.

Detection of cytokines in supernatant

IL-8 was measured by the cytometric bead assay (CBA, Becton Dickinson, San Diego, CA, USA), according to the manufacturer's instructions. The detection level was 20 pg/ml. MDC was measured in duplicate by ELISA Quantikine (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions, with a detection level of 125 pg/ml.

Statistical analysis

The Mann–Whitney U-test was used to compare differences between patients with AD and healthy controls.

RESULTS

Selection of housekeeping genes

Nine housekeeping genes are spotted on the arrays. Of these, five were expressed in all experiments, namely ubiquitin C (UBC), liver glyceraldehyde 3-phosphate dehydrogenase (GAPDH), major histocompatibility complex class I C (HLA-C), cytoplasmic β-actin (ACTB) and ribosomal protein L13A (RPL13A). To determine which were stable, we used a global sum normalization, where the total intensity of the whole membrane is taken into account (www.clontech.com). Based on this approach, we excluded RPL13A, which showed instability, with ratios from 0.18 to 1.4 in different experiments. HLA-C and ACTB have also been described as being unstable in the maturation process of MDDCs (19, 20), however, we did not observe any relevant change. This was true also for GAPDH, which has been suggested as a stable housekeeping gene in MDDCs (21). Thus, the four housekeeping genes used for normalization were UBC, GAPDH, HLA-C and ACTB. The mean value of their expression was used for normalization.

Gene expression in MDDCs from patients with AD and healthy controls with or without M. sympodialis *stimulation*

Immature MDDCs from four patients with AD and three healthy controls were incubated with or without whole *M. sympodialis* yeast cells for 18 h. The number of expressed genes (cut-off = 3 × background value) varied between 27 and 85 among individuals without stimulation, and between 33 and 99 after stimulation,

with no general differences between patients and controls. Fourteen genes were always expressed above the cut-off value on all arrays, i.e. in both non-stimulated and stimulated samples for both patients and healthy controls. These were: myeloid cell nuclear differentiation antigen (MNDA), glutathione S-transferase, CD81, RNA-binding protein fus, TARC, CD54 (ICAM-1), annexin-II, CD53, CD83, ferritin heavy chain, STAT-6, granulins precursor (GRN, granulin), L-plastin and TR-AP. Generally, genes were more often up-regulateed than down-regulated by *M. sympodialis* in both patients and healthy individuals (Table II). However, no genes were exclusively up-regulated in patients or controls, and we only saw differences at the level of up-regulation. No major differences between patients and controls were observed for the down-regulated genes.

The total number of genes which were up-regulated

Table II. *Genes up-regulated or down-regulated by* M. sympodialis

Gene	GenBank accession no.	Subjects/Folds up- or down-regulation						
		HC1	HC2	HC3	AD1	AD2	AD3	AD4
Genes mainly up-regulated								
IL-3R-β and IL-5R-β subunit	M59941	-	−1.2	+2.1	+1.1	+2.6	+2.3	+1.6
EBI2, EBV-induced G-protein-coupled receptor 2	L08177	+1.3	+1.2	-	-	-	-	+2.5
LFA-1, integrin β2	M15395	1.0	-	1.0	-	**+5.4**	−1.6	−1.4
CCR9, CC chemokine receptor type 9	Y12815	-	-	-	-	-	-	+2.1
NPM1, nucleophosmin 1	M23613	+2.0	+1.9	-	+2.6	+1.3	+1.3	+1.0
IRF1, interferon regulatory factor 1	X14454	-	+1.4	+3.3	-	+1.7	+1.7	+3.4
CD81	M33680	+2.0	+1.3	+2.1	+1.1	+4.3	+1.5	+1.2
BTG1	X61123	+1.3	**+7.0**	**+15**	**+6.2**	+4.3	**+21**	**+22**
Eosinophil lysophospholipase	L01664	-	+2.7	+1.6	-	-	-	+3.0
IL-1RA	M63099	+1.2	+2.3	+1.8	+2	+1.7	**+6.9**	**+9.6**
MDC	U83171	+3.6	+4.2	**+8.3**	-	1.0	**+24**	**+46**
IL-8	Y00787	+2.9	+2.1	**+13**	**+24**	+2.0	**+9.5**	**+60**
MIP 1α, macrophage inflammatory protein	M23452	+1.7	+2.8	+1.4	-	-	+2.4	+4.7
MIP 1β	J04130	-	-	-	-	-	-	+3.3
MIG, γ-interferon-induced monokine	X72755	-	-	-	-	-	-	+4.3
TARC, thymus- and activation-regulated chemokine	D43767	+2.8	+2.2	**+5.0**	+3.1	+3.2	+2.9	+4.7
MIP 3β	U77180	-	-	**+5.5**	-	-	-	+4.8
CD54, ICAM-1	J03132	+2.4	+2.5	+3.9	**+5.5**	**+7.0**	**+5.5**	**+6.2**
CD58, LFA-3	Y00636	+2.0	+1.6	-	+4.3	-	+3.5	+2.5
CD9	M38690	+1.5	+1.0	-	-	-	-	+2.8
CD83	Z11697	+2.5	+2.5	**+5.8**	+2.9	+3.6	**+6.2**	**+12**
HOX-A5, homeobox protein	M26679	+1.4	-	+4.8	-	−0.7	-	**+5.3**
PDGF receptor β-like tumour suppressor	D37965	−1.1	+1.9	+2.8	+2.6	+1.7	+1.3	+2.5
Ferritin heavy chain	M97164	+1.4	+2.7	+2.9	+1.7	+1.6	+2.8	+2.9
LCP-1, L-plastin, lymphocyte cytosolic protein 1	M22300	+1.0	+1.6	+2.6	+2.6	+3.0	+1.6	+3.6
LSP1, lymphocyte-specific protein	M33552	-	+1.1	+1.5	-	-	-	+2.8
Hypothetical protein KIAA0053	D29642	-	+1.1	+3.4	-	-	-	+3.0
GLVR1, leukaemia virus receptor 1	L20859	−1.4	+1.1	-	-	-	+1.6	+2.3
Genes mainly down-regulated								
fli-1 proto-oncogene	M93255	−2.4	−2.7	-	-	-	−1.9	−3.2
BTK, Bruton's tyrosine kinase	U10087;X58957	-	-	1.0	-	-	-	−2.6
T-cell-specific rantes protein precursor	M21121	-	-	-	**−18**	-	-	-
CD23, FcεRII	M15059	−3.4	-	-	-	-	-	−2.1
CD53	M37033	−1.1	−2.1	−2.2	−1.4	+1.4	+1.2	−1.3
CD11a	Y00796	+2.9	-	-	**−22**	-	-	-
MNDA, myeloid cell nuclear differentiation antigen	M81750	−2.0	−1.6	−1.9	1.0	1.0	−2.4	**−5.9**
RAC2, ras-related C3 botulinum toxin substrate	M64595; M29871	−2.6	−1.1	−1.9	-	−1.3	+1.1	−1.2
31-kDa transforming protein (transcription factor pu 1)	X52065	−2.2	−1.1	−2.0	−1.1	-	−1.1	−1.4
RGC1, rho-GAP haematopoietic protein C1	X78817	−2.5	−2.7	−4.3	−1.2	−1.3	−2.0	**−5.0**
SLP-76, 76-kDa tyrosine phosphoprotein	U20158	-	−1.2		-	-	−1.3	−3.4
GRN, granulin	M75161	−1.4	1.0	+1.2	−2.6	−1.2	+1.3	+1.4

Values represent ratios of intensities between *M. sympodialis*-stimulated and non-stimulated monocyte-derived dendritic cells after subtraction of background and normalization with the mean of four housekeeping genes (ubiquitin C, GAPDH, HLA-C, β-actin). All genes with an increase or decrease larger than twofold seen in at least one individual are shown. When one of the values was below cut-off, the value was set to the cut-off value for calculations. Positive and negative values indicate up-regulation and down-regulation, respectively. A dash (-) corresponds to values below cut-off (3 × background) on both membranes (stimulated and non-stimulated). Genes up- or down-regulated more than five times are shown in bold. HC, Healthy control; AD, atopic dermatitis.

by a factor of two or more in at least one patient was 27, while the corresponding number was 21 in healthy individuals (Table II), suggestive of a different stimulation by *M. sympodialis* in patients. Four genes were up-regulated in all patients and all healthy individuals (IL-8, TARC, CD54 and CD83). Down-regulation by a factor of two or more after *M. sympodialis* stimulation was seen for eight genes in any of the patients, and for seven genes in normal individuals. In some instances we observed de novo expression after *M. sympodialis* stimulation, although only in the case of IL-8 was this seen in all individuals.

Large up-regulations, more than fivefold increase in more than one individual, were detected for six genes in patients; the chemokine IL-8, BTG1, the adhesion molecule CD54, the maturation factor CD83, the MDC precursor, and IL-1R antagonist (Table II). In healthy individuals this was true only for BTG1. We saw a tendency for these molecules to be more highly up-regulated in the two patients with AD with the highest SCORAD index (patients 3 and 4), compared with the two patients with lower SCORAD (patients 1 and 2) and the healthy individuals. However, the variation between individuals was great. The only statistically significant difference between patients with AD and healthy individuals was seen for CD54 (Table II), with a higher up-regulation in patients with AD ($p < 0.5$).

Protein expression

To verify the major differences seen in the array experiments, protein levels in culture supernatants were measured in AD1, AD3, AD4 and HC1 and HC2. The CBA inflammation assay was used for confirmation of array results for IL-8, and MDC was tested by ELISA. MDC was increased on gene level 24 and 46 times in AD3 and AD4, respectively, and on protein level 6 and 4.4 times. MDC was not detected for AD1. When the healthy individuals 1 and 2 were compared the gene results were 3.6 and 4.2, and the corresponding protein increases were 3 and 1.6 times, respectively. The very strong up-regulation of IL-8 mRNA in patients 1 and 4 (24 and 60 times) was reflected by a 585-fold and 826-fold up-regulation on protein level. AD3, which showed a moderate (9.5-fold) up-regulation, had a 65-fold up-regulation on protein level, and the healthy HC1 and HC2 (2.1-fold and 2.9-fold) showed 85- and 56-fold up-regulation on protein level. The absolute values for IL-8 in the supernatants after stimulation with *M. sympodialis* were 30 ng/ml and 31 ng/ml, respectively, for HC1 and HC2; and 102, 64 and 107 ng/ml, respectively, for AD1, AD3 and AD4.

DISCUSSION

This study indicates that MDDCs from patients with AD with IgE reactivity and positive in vivo reactivity against *M. sympodialis* have a different gene expression after stimulation with *M. sympodialis* compared with healthy individuals. This suggests that there are differences in the MDDC population in patients and controls. The difference could either lie in the uptake/activation by the yeast, or in the maturation process which follows. A differential expression of Ig receptors or MRs could result in different uptake, which might explain at least part of the differences seen here. In our experiments we added 5% autologous serum at the same time as *M. sympodialis* to the MDDCs. The autologous serum potentially contains IgG subclasses, IgE and other factors that may facilitate antigen uptake and stimulate the cells. Langerhans' cells in the skin of patients with AD have an increased capacity to capture antigens/allergens because of their increased expression of receptors for IgE on their cell surface (21). In addition, allergen-mediated activation through FcεRI is likely to stimulate cytokine secretion by MDDCs (22).

The most up-regulated genes were not the same in all patients, but we saw a tendency towards a higher up-regulation of more genes in the patients with the highest SCORAD value. Most of the molecules encoded by the genes we found differently expressed in patients are known to be involved in cell–cell adhesion, co-stimulation and chemo-attraction, and in the pathogenesis of AD. This gains further relevance in that our analysis focused at the level of the DC, the cell type that is responsible for uptake and processing of the yeast, and provides differentiation and stimulatory signals to T cells.

The largest differences between patients with AD and healthy individuals were seen for mRNA coding for proinflammatory molecules. Our data show IL-8 up-regulation in three of four patients with AD, at the mRNA level and this was reflected by higher IL-8 protein levels in the supernatant. IL-8 is a chemokine produced by PBMCs and LCs, which attracts neutrophils, $CD45RA^+$ and $CD45RO^+$ T cells to the site of inflammation, leading to an enhanced inflammatory response. IL-8 has been shown to be up-regulated in PBMC from patients with AD (23). Our results suggest that this IL-8 may partly derive from activated DC.

MDC is produced by T cells but also by macrophages and DCs, which have been proposed as a major source for MDC in the skin; and MDC is highly detected in the serum of patients with AD (24). MDC attracts CC chemokine receptor 4-positive ($CCR4^+$) cells, e.g. Th2 cells, and is up-regulated on $CD4^+$ cells from patients with AD (25). Our results show that MDC is more up-regulated in the two patients with the most severe AD than in patients with moderate AD or healthy controls, and this was verified on the protein level. This suggests a difference in the ability to attract inflammatory cells to the site of allergen entry. This

might also facilitate the NK – DC interaction in *M. sympodialis* APT-positive skin from patients with AD (26).

B-cell translocation gene (BTG) 1 has been shown to be expressed in cultured peripheral blood lymphocytes and macrophages (27). It has an anti-proliferative effect on many cells, and is induced in macrophages by platelet-activating factor and prostaglandin E2 (28), a known Th2-inducing factor. BTG1 was up-regulated more than 20-fold in the two patients with the most severe AD. However, it was also highly increased in healthy individuals and the patients with moderate AD, why a possible role in AD needs to be further investigated.

CD83 is a commonly used maturation marker for DC (29). CD83 is up-regulated together with MHC class II and co-stimulatory molecules, and has been shown to be involved in DC-mediated T-cell proliferation (30). CD83 surface expression has been shown to be differently regulated in patients with AD and controls (31). The highest increases observed in this study after *Malassezia* stimulation were seen for the patients with AD with the highest SCORAD values, and might reflect a difference in the maturation level.

IL-1 receptor antagonist (IL-1RA) was also up-regulated in the patients with severe AD compared with the other individuals. IL-1RA can inhibit the effects of IL-1, a cytokine that has been shown to be of major importance for DC activation and migration in the skin. IL-1 has also been reported to be one of the first substances in a chain of events leading to recruitment of CLA^+ T cells to the skin (32). In contrast to earlier results showing increase in IL-1β protein expression in cultures with MDDCs stimulated with *M. sympodialis* from healthy controls (13), no significant gene expression of IL-1β mRNA was detected here. This might be due to different kinetics of mRNA and protein expression.

CD54, which was up-regulated more than fivefold in all patients but not in healthy individuals, facilitate the recruitment of bone marrow-derived inflammatory cells from the circulation into the skin (33). This could lead to a more efficient interaction between DC and T cells, and thus, a stronger stimulation of allergen-specific T cells.

In conclusion, we report here that MDDC from patients with AD with IgE reactivity to *M. sympodialis*, might show a different response to this yeast than healthy individuals. This is typified by an overproduction of proinflammatory molecules and is likely to contribute to the initiation as well as deterioration of the allergic state, supported by our finding that the highest up-regulations were seen in patients with the most severe AD. Part of the differences seen here could be due to differential expression of Ig receptors or MRs in patients with AD, which could result in different uptake of the yeast. More understanding of the regulation mechanisms underlying AD will certainly help to improve future therapeutic strategies.

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