INVESTIGATIVE REPORT

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

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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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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), FccR, FcyR, 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 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), FccR, FcyR, 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
immune response, and a capacity to stimulate lympho-
cyte 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 differ-
cently 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°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 (RPMI) (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°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% autologous serum, for 18 h at 37°C in
6% CO2 (13).

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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (years)</th>
<th>SCORADa</th>
<th>SPTb</th>
<th>APTc</th>
<th>M. sympodialisd specific s-IgE (kU/l)</th>
<th>Total s-IgEe (kU/l)</th>
<th>Phadiatopf</th>
<th>Asthma/rhinitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC1</td>
<td>M</td>
<td>45</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.35</td>
<td>45</td>
<td>Negative</td>
<td>−/−</td>
</tr>
<tr>
<td>HC2</td>
<td>M</td>
<td>48</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.35</td>
<td>15</td>
<td>Negative</td>
<td>−/−</td>
</tr>
<tr>
<td>HC3</td>
<td>M</td>
<td>30</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.35</td>
<td>2</td>
<td>Negative</td>
<td>−/−</td>
</tr>
<tr>
<td>AD1</td>
<td>F</td>
<td>31</td>
<td>23</td>
<td>4.8</td>
<td>+</td>
<td>4.8</td>
<td>5300</td>
<td>Positive</td>
<td>+/−</td>
</tr>
<tr>
<td>AD2</td>
<td>M</td>
<td>25</td>
<td>28</td>
<td>5</td>
<td>+</td>
<td>14</td>
<td>2400</td>
<td>Positive</td>
<td>+/−</td>
</tr>
<tr>
<td>AD3</td>
<td>M</td>
<td>45</td>
<td>54</td>
<td>6.5</td>
<td>+++</td>
<td>2.4</td>
<td>4710</td>
<td>Positive</td>
<td>+/−</td>
</tr>
<tr>
<td>AD4</td>
<td>M</td>
<td>33</td>
<td>46</td>
<td>8</td>
<td>++</td>
<td>5.4</td>
<td>1800</td>
<td>Positive</td>
<td>+/−</td>
</tr>
</tbody>
</table>

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

aAssessed by SCORAD (34) at the time of monocyte-derived dendritic cell generation.

bThe 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.

cAtopy 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).

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culture medium, as described above but with 5% heat-
inactivated FCS and 5% autologous serum, for 18 h at 37°C in
6% CO2 (13).
Flow cytometric analysis

Approximately $5 \times 10^4$ 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 NaN₃ 0.01%. A minimum of $10^6$ 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 (LOB76, 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 IgG₁-PE, IgG₁-FITC, IgG₂a-PE and IgG₂a-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 instructions. 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%). The purified amount was usually measured 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 measured 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 measured.

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 $= 3 \times$ 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 (MDA), 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-regulated 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>
<thead>
<tr>
<th>Genes mainly up-regulated</th>
<th>Subjects/Folds up- or down-regulation</th>
<th>HC1</th>
<th>HC2</th>
<th>HC3</th>
<th>AD1</th>
<th>AD2</th>
<th>AD3</th>
<th>AD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3R-β and IL-5R-β subunit</td>
<td>M59941</td>
<td>-1.2</td>
<td>+2.1</td>
<td>+1.1</td>
<td>+2.6</td>
<td>+1.1</td>
<td>+2.3</td>
<td>+1.6</td>
</tr>
<tr>
<td>EB2, EBV-induced G-protein-coupled receptor 2</td>
<td>L08177</td>
<td>+1.3</td>
<td>+1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+2.5</td>
</tr>
<tr>
<td>LFA-1, integrin β2</td>
<td>M15395</td>
<td>1.0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>+5.4</td>
<td>-1.6</td>
<td>-1.4</td>
</tr>
<tr>
<td>CCR9, CC chemokine receptor type 9</td>
<td>Y12815</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+2.1</td>
</tr>
<tr>
<td>NPM1, nucleophosmin 1</td>
<td>M23613</td>
<td>+2.0</td>
<td>+1.9</td>
<td>-</td>
<td>+2.6</td>
<td>+1.3</td>
<td>+1.3</td>
<td>+1.0</td>
</tr>
<tr>
<td>IRF1, interferon regulatory factor 1</td>
<td>X14454</td>
<td>-</td>
<td>+1.4</td>
<td>+3.3</td>
<td>-</td>
<td>+1.7</td>
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<td>CD81</td>
<td>M36850</td>
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<td>+1.3</td>
<td>+2.1</td>
<td>+1.1</td>
<td>+4.3</td>
<td>+1.5</td>
<td>+1.2</td>
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<td>BTG1</td>
<td>X61123</td>
<td>+1.3</td>
<td>+7.0</td>
<td>+15</td>
<td>+6.2</td>
<td>+4.3</td>
<td>+21</td>
<td>+22</td>
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<tr>
<td>Eosinophil lysophospholipase</td>
<td>L01664</td>
<td>-</td>
<td>+2.7</td>
<td>+1.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+3.0</td>
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<tr>
<td>IL-1RA</td>
<td>M63099</td>
<td>+1.2</td>
<td>+2.3</td>
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<td>+2</td>
<td>+1.7</td>
<td>+6.9</td>
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<tr>
<td>MDC</td>
<td>U33171</td>
<td>+3.6</td>
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<td>IL-8</td>
<td>Y00787</td>
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<td>+2.1</td>
<td>+13</td>
<td>+24</td>
<td>+2.0</td>
<td>+9.5</td>
<td>+60</td>
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<tr>
<td>MIP 1α, macrophage inflammatory protein</td>
<td>M23452</td>
<td>+1.7</td>
<td>+2.8</td>
<td>+1.4</td>
<td>-</td>
<td>-</td>
<td>+2.4</td>
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<tr>
<td>MIP 1β</td>
<td>J04130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+3.3</td>
</tr>
<tr>
<td>MIG, γ-interferon-induced monokine</td>
<td>Z72575</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+4.3</td>
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<tr>
<td>TARC, thymus- and activation-regulated chemokine</td>
<td>D43767</td>
<td>+2.8</td>
<td>+2.2</td>
<td>+5.0</td>
<td>+3.1</td>
<td>+3.2</td>
<td>+2.9</td>
<td>+4.7</td>
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<tr>
<td>MIP 3β</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+4.8</td>
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<tr>
<td>CD54, ICAM-1</td>
<td>J03132</td>
<td>+2.4</td>
<td>+2.5</td>
<td>+3.9</td>
<td>+5.5</td>
<td>+7.0</td>
<td>+5.5</td>
<td>+6.2</td>
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<tr>
<td>CD58, LFA-3</td>
<td>Y00636</td>
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<td>+1.6</td>
<td>-</td>
<td>+4.3</td>
<td>-</td>
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<td>CD9</td>
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<td>Z16979</td>
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<td>+2.5</td>
<td>+5.8</td>
<td>+2.9</td>
<td>+3.6</td>
<td>+6.2</td>
<td>+12</td>
</tr>
<tr>
<td>HOX-A5, homeobox protein</td>
<td>M26679</td>
<td>+1.4</td>
<td>-</td>
<td>+4.8</td>
<td>-</td>
<td>+0.7</td>
<td>-</td>
<td>+5.3</td>
</tr>
<tr>
<td>PDGF receptor β-like tumour suppressor</td>
<td>D37965</td>
<td>-1.1</td>
<td>+1.9</td>
<td>+2.8</td>
<td>+2.6</td>
<td>+1.7</td>
<td>+1.3</td>
<td>+2.5</td>
</tr>
<tr>
<td>Ferritin heavy chain</td>
<td>M97169</td>
<td>+4.1</td>
<td>+2.9</td>
<td>+1.7</td>
<td>+1.6</td>
<td>+2.8</td>
<td>+1.5</td>
<td>+2.3</td>
</tr>
<tr>
<td>LCP-1, L-plastin, lymphocyte cytosolic protein 1</td>
<td>M22300</td>
<td>+1.0</td>
<td>+1.6</td>
<td>+2.6</td>
<td>+2.6</td>
<td>+3.0</td>
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<td>LSP1, lymphocyte-specific protein</td>
<td>M33552</td>
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<td>+1.5</td>
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<tr>
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<tr>
<td>GLYR1, leukemia virus receptor 1</td>
<td>L20859</td>
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<td>+1.1</td>
<td>-</td>
<td>-</td>
<td>+1.6</td>
<td>+2.3</td>
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<th>Genes mainly down-regulated</th>
<th>Subjects/Folds up- or down-regulation</th>
<th>HC1</th>
<th>HC2</th>
<th>HC3</th>
<th>AD1</th>
<th>AD2</th>
<th>AD3</th>
<th>AD4</th>
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<td>fli-1 proto-oncogene</td>
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<td>BTK, Bruton's tyrosine kinase</td>
<td>U100087;X58957</td>
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<td>-</td>
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<td>T-cell-specific rantes protein precursor</td>
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<tr>
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<td>MND4, myeloid cell nuclear differentiation antigen</td>
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<td>M65495; M29871</td>
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<tr>
<td>RGC1, rho-GAP haematopoietic protein C1</td>
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<td>SLP-76, 76-kDa tyrosine phosphoprotein</td>
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</table>

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.

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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. The absolute values
for IL-8 in the supernatants after stimulation with
*M. sympodialis* were 30 ng/ml and 31 ng/ml, respec-
tively, 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 FceRI 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
antigens/allergens because of their increased expression
of receptors for IgE on their cell surface (21). In
addition, allergen-mediated activation through FceRI is
likely to stimulate cytokine secretion by MDDCs (22).

MDC is produced by T cells but also by macro-
phages 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-1\( \beta \) protein expression in cultures with MDDCs stimulated with *M. sympodialis* from healthy controls (13), no significant gene expression of IL-1\( \beta \) 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.

### ACKNOWLEDGEMENTS

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