Candida albicans Yeast and Germ Tube Forms Interfere Differently with Human Monocyte Differentiation into Dendritic Cells: a Novel Dimorphism-Dependent Mechanism To Escape the Host’s Immune Response

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Received 14 May 2003/Returned for modification 16 July 2003/Accepted 31 October 2003

The ability of Candida albicans to convert from the yeast (Y) form to mycelial forms through germ tube (GT) formation is considered a key feature of the transition of the organism from commensalism to virulence. We show here that human monocytes cultured with granulocyte-macrophage colony-stimulating factor and interleukin-4 (IL-4) after phagocytosis of Y forms did not differentiate into dendritic cells (DCs); they retained CD14, did not acquire CD1a, and were unable to express the maturation markers CD83 and CCR7. Moreover, they did not produce IL-12p70 but secreted IL-10. In addition, they spontaneously expressed high levels of tumor necrosis factor alpha (TNF-α), IL-6, and IL-8 mRNA transcripts and were able to induce proliferation of alloreactive memory but not naïve T lymphocytes. Conversely, monocytes that had phagocytosed GT forms differentiated into mature CD83+ and CCR7+ DCs; however, there was no up-regulation of CD40, CD80, and major histocompatibility complex class II, irrespective of lipopolysaccharide (LPS) treatment. In addition, these cells were unable to produce IL-12 even after LPS stimulation, but they were not functionally exhausted, as shown by their capacity to express TNF-α and IL-8 mRNA transcripts. These cells were able to prime naïve T cells but not to induce their functional polarization into effector cells. These data indicate that phagocytosis of Y and GT forms has profound and distinct effects on the differentiation pathway of monocytes. Thus, the differentiation of human monocytes into DCs appears to be tunable and exploitable by C. albicans to elude immune surveillance.

Dendritic cells (DCs) patrol tissues, phagocytosing pathogens and infected or dying cells (6, 23, 30). Upon exposure to inflammatory mediators and/or pathogens, immature DCs are transformed into strongly migratory and stimulatory mature DCs, which migrate with high efficiency into draining lymph nodes. Here, mature DCs can activate antigen-specific T lymphocytes, ultimately leading to both memory T-cell expansion and differentiation of effector T cells, thus providing immediate protection against pathogens in peripheral tissues (24–26). There seem to be several pathways that generate DCs. A small CD34+ subset of hematopoietic progenitors gives rise to all blood cells, including DCs, while monocytes have the potential to generate both macrophages and DCs (5, 12). In vitro, human monocytes may be directed to differentiate into macrophages by the addition of cytokines, such as macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) plus interleukin-4 (IL-4), respectively (42). Monocytes may also differentiate into DCs in an in vitro model of transendothelial trafficking without addition of exogenous cytokines (38). It is well established that monocytes continuously exit the blood-stream and enter body tissues, and there is in vivo evidence that they may proceed to further differentiate into macrophages or DCs (8, 39). Therefore, monocytes are precursors of killer-scavenger macrophages, as well as professional antigen-presenting DCs. Which differentiation route (leading to either macrophages or DCs) pathogen-phagocytosing monocytes enter is, however, far from being completely defined (21).

It has recently been shown that a prototypical intracellular pathogen, Mycobacterium tuberculosis, subverts the differentiation of infected monocytes into DCs (28). We suggested that the interference with monocyte differentiation into fully competent DCs could contribute to the persistence of this pathogen. In the present study, we analyzed the functional consequences of the interaction of an extracellular pathogen, Candida albicans, with human monocyte differentiation. C. albicans is a human commensal that commonly colonizes mucosal surfaces of the oral cavity, gastrointestinal tract, and vagina without causing symptomatic disease (35). Severe forms of candidiasis may, however, occur in immunocompromised, neutropenic, bone marrow transplant, or AIDS patients, while other milder diseases, such as vulvovaginal candidiasis, are frequent and often relapse in healthy subjects without any sign of defective systemic immunity (4, 17, 19, 29, 43).

Clinical and experimental evidence indicates that antifungal CD4+ T helper 1 (Th1)-mediated responses play a central role in anti-Candida defense. These responses can provide prompt
and effective control of fungal infectivity at both colonization and infection sites through mobilization and gamma interferon (IFN-γ)-mediated activation of natural antifungal effectors and/or can help in generating a protective antibody response (2, 3, 15, 18, 31, 34, 35, 41). However, there is evidence indicating that C. albicans supposedly displays a number of strategies for attenuating or dysregulating local, potentially eradicating T-cell activities (14, 41).

A special feature of C. albicans that is likely to play an important role in evasion of the immune response is the morphological transition from a unicellular yeast (Y) form to an elongated, multinucleate hyphal or mycelial form through the critical stage of germ tube (GT) formation (35). Conversion from the Y form to the GT form is strictly associated with virulence, as demonstrated by the fact that several mutants with the hypha-specific genes deleted, as well as wild-type strains unable to grow into the mycelial form, invariably have low systemic pathogenicity (9, 10, 27).

As for other pathogens, the DC system of antigen-presenting cells (APCs) is the critical initiator and modulator of the anti-Candida immune response. Interestingly, phagocytosis of Y or hyphal forms of C. albicans appears to activate divergent functional programs in murine DCs: ingestion of the Y form induces DCs to produce IL-12 and to prime Th1 lymphocytes, whereas ingestion of the hyphal form results in IL-4 production, which favors Th2 cell priming (14). To investigate the interference of extracellular microorganisms with human monocyte differentiation into DCs, we analyzed the influence of Y and GT cell phagocytosis. This ex vivo model could mimic an in vivo environment characterized by growing microorganisms and active monocyte recruitment. This issue was addressed in this study for candidiasis but could be applicable to other pathogens, assuming that interference with the generation of fully active DCs represents a relevant target for evasion of the immune response.

MATERIALS AND METHODS

Reagents. Phytohemagglutinin was obtained from Murex (Dartford, United Kingdom). Recombinant IL-2 was a kind gift from Chiron (Siena, Italy). GM-CSF (Leucomax) was purchased from Sandoz (Basel, Switzerland), and M-CSF and IL-4 were obtained from R&D Systems (Minneapolis, Minn.). Tritiated thymidine was obtained from Amersham (Little Chalfont, United Kingdom). Amphotericin B was purchased from Gibco BRL (Grand Island, N.Y.). Phorbol 12-myristate 13-acetate and ionomycin were obtained from Sigma Chemical Co. (St. Louis, Mo.). The RPMI 1640 (Euroclone Ltd., Wetherby, United Kingdom) used was supplemented with 100 U of kanamycin of per ml, 1 mM glutamine, 1 mM sodium pyruvate, 1% nonessential amino acids, and 10% fetal calf serum.

Thymidine was obtained from Amersham (Little Chalfont, United Kingdom). Human-adsorbed, lipopolysaccharide (LPS) from Escherichia coli (0.1 μg/ml; Sigma Chemical Co.) was added to both infected and noninfected cells for the last 18 h of culture to induce DC maturation.

Antibodies and flow cytometry. We used the following antibodies: anti-HLA class I, anti-HLA class II, anti-CD1a, anti-CD1b, anti-CD3, anti-CD11c, anti-CD14, anti-CD25, anti-CD40, anti-CD80, anti-CD83, anti-CD86, anti-CD123, anti-CCR5, anti-CCR6, anti-CCR7, and appropriate isotypic controls. All antibodies were purchased from Pharmingen (San Diego, Calif.). Biotin-conjugated anti-CD4 antibodies were obtained from Cymbus Biotechnology Ltd. (Compton, United Kingdom). Hymenoptera venom (FITC)-conjugated goat anti-mouse immunoglobulin G and goat anti-mouse immunoglobulin M (Southern Biotechnology Associates, Inc., Birmingham, Ala.) were used as the secondary antibodies. FITC-conjugated streptavidin (Sigma Chemical Co.) was used in association with anti-CD1c. Cells were harvested and washed in PBS containing 1% FCS and 0.1% NaN3 (staining buffer) and were stained by using the antibodies mentioned above or appropriate isotype controls for background determination. After incubation and washing in staining buffer, cells were resuspended in medium suitable for cytometric analysis (FACSFlow; Becton Dickinson, Mountain View, Calif.) supplemented with 5 μg of propidium iodide per ml to eliminate dead cells. Staining of intracellular cytokines in T cells was performed by using phycoerythrin-conjugated rat anti-human IL-4 or IL-10 and FITC-conjugated mouse anti-human IFN-γ in PerCP-conjugate CD3+ cells (PharMingen) used according to the manufacturer’s instructions.

Stained cells were analyzed by flow cytometry by using a FACScan cytometer (Becton Dickinson) equipped with CellQuest software (Becton Dickinson). Fluorescence intensity was evaluated by computerized analysis of dot plots or histograms generated by 5,000 viable cells. The percentage of positive cells was calculated for CD1c, CD11c, CD14, CD123, and CCR7, and the median intensity of fluorescence (MFI) was used as a measure of major histocompatibility complex (MHC) class I, MHC class II, CD40, CD80, CD83, and CD86 cell membrane expression.

Transmission electron microscopy. Freshly isolated monocytes, DCs derived from monocytes cultured for 6 days in the presence of GM-CSF and IL-4, monocytes incubated in 1 h with Y or GT forms, or cells derived from monocytes allowed to phagocytose Y or GT forms and then cultured for 6 days with GM-CSF and IL-4 were fixed with 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4). The cells were then rinsed twice in the cacodylate buffer (pH 7.4) for 2 h at room temperature. After three washes in the same buffer, cells were postfixed overnight with 1% (vol/vol) osmium tetroxide in 0.2 M cacodylate buffer (pH 7.4) for 2 h at room temperature. After three washes in the same buffer, cells were postfixed overnight with 1% (vol/vol) osmium tetroxide in 0.2 M cacodylate buffer (pH 7.4) at 4°C. The cells were then rinsed twice in the same buffer and embedded in 2% (wt/vol) Noble agar (Difco Laboratories, Detroit, Mich.) in distilled water. Dehydration was carried out with a graded acetone series and embedding in epoxy resin (TAAB 812 resin; TAAB Laboratories Equipment Limited, Aldermaston, Berkshire, United Kingdom) by using routine procedures. Ultrathin sections, obtained with an LKB Ultratome Nova ultramicrotome, were stained with uranyl acetate and lead citrate and examined with a Philips 208 transmission electron microscope (FEI Company, Eindhoven, The Netherlands) (11).

Reverse transcription-PCR. Poly(A)+ RNA was extracted from 1 × 10⁸ cells by using a Micro-FastTrack 2.0 kit (Invitrogen, San Diego, Calif.) according to the manufacturer’s instructions and was reverse transcribed as previously described (46). Aliquots of cDNA that yielded equivalent amounts of β-actin amplified products were used for semiquantitative evaluation of cytokine gene expression. A PCR was performed by using a 20-μl mixture in a thermal cycler (Cycler 9600; Perkin-Elmer, Norwalk, Conn.). Cytokine-specific primer pairs were synthesized by Gibco-BRL on the basis of previously published sequences (7, 45), and amplification conditions were chosen accordingly. The PCR
products were visualized by agarose gel electrophoresis and ethidium bromide staining and were identified on the basis of their expected molecular sizes.

**Stimulation of naive T cells.** To test the T-lymphocyte stimulation capacity, decreasing numbers of immature DCs, mature DCs, and cells derived from monocytes infected with Y or GT forms were tested for the capacity to induce proliferation of 5 x 10^5 allogeneic cord blood lymphocytes (CB-Ts) per well. The proliferative response was measured on day 5 by using a 16-h pulse with [3H]thymidine (32). To examine the T-lymphocyte polarization capacity, immature DCs, mature DCs, and cells derived from Y form- or GT-treated monocytes were cultured for 6 days with allogeneic CB-Ts. In detail, duplicate cocultures of 1 x 10^6 CB-Ts and 1 x 10^5 APCs were placed in 12-well plates in RPMI supplemented with 10% FCS. T cells were then stimulated with 10^-7 M phorbol 12-myristate 13-acetate and 0.5 μg of ionomycin per ml for 5 h in the presence of brefeldin at a concentration of 2 μg/ml for the last 2 h. For cytokine determination in the supernatants, parallel cultures were stimulated in the absence of brefeldin. Brefeldin-treated cells were washed with staining buffer and stained with PerCp-conjugated anti-CD3 antibody to identify the population of T lymphocytes, and then they were fixed and permeabilized with Cytofix/Cytoperm. Phycoerythrin-labeled anti-IL-4 or -IL-10 antibodies and FITC-labeled anti-IFN-γ antibodies were used for intracellular cytokine staining.

**Cytokine determination.** Culture supernatants from cultures containing 4 x 10^6 cells/ml were collected 18 h after infection of monocytes with Y or GT forms. Supernatants of cells derived from C. albicans-infected or noninfected monocytes were collected after 6 days of culture before and after LPS stimulation. Supernatants of CB-Ts were obtained as described above. The supernatants were frozen until they were used. Secretion of cytokines (IL-10, IL-12p70, IFN-γ) was determined by using commercially available kits (R&D) according to the manufacturer’s instructions, and the results were expressed in picograms per milliliter.

**Statistical analysis.** Data were analyzed by using the SPSS program (SPSS Inc., Chicago, Ill.). The statistical significance of differences between groups of data with a normal distribution was determined by the analysis of variance test with Student-Newman-Keuls or Bonferroni-Dunn posttests.

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**RESULTS**

**Phenotypic and morphological characterization of cells derived from C. albicans Y form- or GT-treated monocytes.** Phagocytosis of Y cells of C. albicans by human monocytes occurs irrespective of complement or immunoglobulin opsonization (16). Elongated hyphal forms have been described as refractory to complete phagocytosis (47), although they are as sensitive as Y cells to killing by phagocytes (7). Thus, to investigate the functional consequences of phagocytosis of the different forms of C. albicans by human monocytes, we used Y cells and GTs, short hyphal filaments that represent the early stage of hyphal development (35). Freshly isolated monocytes were allowed to phagocyte live Y or GT cells during overnight coincubation. In all cases more than 95% of the monocytes contained at least one fungal cell, and frequently monocytes contained up to five fungal cells (mean, 2.4 C. albicans cells/monocyte), as determined by light microscopy. Microscopic analysis also showed that Y forms and GTs were internalized equally by human monocytes. To test whether phagocytosis of C. albicans influences the differentiation of monocytes into DCs, Candida-treated cells were cultured in the presence of GM-CSF and IL-4. Some of the cells were cultured with LPS for the last 18 h of incubation, a stimulation procedure that is routinely used in vitro to induce phenotypic and functional maturation of control DCs.

The majority of cells derived from Y form-phagocytosing monocytes retained CD14 and did not acquire CD1a or CD83, and the percentage of cells expressing CCR7, even after LPS treatment, was comparable to the percentage of control macrophages and immature control DCs (Fig. 1). On the other hand, MHC class I was constitutively expressed in cells derived from Y form-phagocytosing monocytes, and the MIF was not significantly different from that of LPS-treated control DCs or macrophages (Fig. 1 and Table 1). However, MHC class I was not up-regulated after LPS treatment; in fact, as shown in Table 1, the mean percent increase in MHC class I expression after LPS treatment (8.7% ± 2%) was significantly lower than that of control DCs (74.1% ± 2%). MHC class II molecules were expressed and up-regulated after LPS treatment. On the other hand, CD80 and CD40 were expressed as they were in control macrophages, but they were not up-regulated as they were in control DCs after LPS treatment. CD86 was inducible by LPS treatment and was expressed at levels comparable to those in LPS-treated control DCs.

GT-treated monocytes had a quite different phenotype after 6 days of culture with GM-CSF and IL-4. These cells lost CD14 but did not acquire expression of molecules belonging to the CD1 family (Fig. 1A). They spontaneously expressed CD83; in addition, CCR7 was detected in more than 45% of the cells, and there were no changes in the intensity of expression or in the percentage of positive cells following LPS treatment (Fig. 1B). Interestingly, MHC class I was constitutively highly expressed and up-regulated after LPS treatment. In fact, as shown in Table 1, MHC class I was expressed and up-regulated in these cells with an intensity significantly higher than that in LPS-treated control DCs (the mean MIF was 199.4% of that of control mature DCs). On the other hand, the intensities of MHC class II, CD40, and CD80 expression were significantly lower than those in control DCs (the MIFs were 43.7, 57.3, and 19.6% of the control DC values, respectively). In addition, these surface molecules were significantly less up-regulated after LPS treatment compared to the molecules in control DCs (Table 1). CD86 showed the same MHC class I expression characteristics and was constitutively highly expressed and up-regulated in the majority of cells after LPS treatment. The chemokine receptors CCR5 and CXCR4 were not expressed (data not shown).

Lastly, cells derived from both Y form- and GT-phagocytosing monocytes were CD11c positive and CD123 negative (data not shown), indicating that they had a myeloid origin and eliminating the possibility of C. albicans-mediated selection of plasmacytoid DC precursors.

Transmission electron microscopy confirmed the morphological differentiation of nonphagocytosing monocytes into DCs after 6 days of culture in the presence of GM-CSF and IL-4 (Fig. 2a and b). Each DC had a polarized nucleus and the characteristic dendritic cytoplasmic processes extending from the cell body. Monocytes treated with C. albicans for 1 h completely phagocytosed both Y and GT forms (Fig. 2c and d). Analysis of cells derived from monocytes after phagocytosis of Y forms revealed a well-preserved ultrastructure and numerous digested C. albicans cells inside the cytoplasm. However, these cells did not have the morphological characteristics of DCs (Fig. 2e). On the other hand, most of the cells derived from monocytes after phagocytosis of GT forms had a well-preserved ultrastructure and a typical DC morphology characterized by dendritic cytoplasmic processes extending from the cell body, despite the presence of numerous internalized and digested C. albicans residues (Fig. 2f).

Altogether, the pattern of expression of surface markers and
A

<table>
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<tr>
<th>CD14</th>
<th>Control DC</th>
<th>Control Mφ</th>
<th>Y-MoMφ</th>
<th>GT-MoDC</th>
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<td>1.4</td>
<td>590</td>
<td>843</td>
<td>641</td>
<td>930</td>
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CD1a

B

<table>
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<th>CD83</th>
<th>Control DC</th>
<th>Control Mφ</th>
<th>Y-MoMφ</th>
<th>GT-MoDC</th>
</tr>
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<tbody>
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<td>-4</td>
<td>+19</td>
<td>-5</td>
<td>+5</td>
<td>-12</td>
</tr>
<tr>
<td>CD80</td>
<td>-21</td>
<td>+101</td>
<td>-9</td>
<td>+19</td>
</tr>
<tr>
<td>CD86</td>
<td>-6</td>
<td>+70</td>
<td>-14</td>
<td>+16</td>
</tr>
<tr>
<td>CD40</td>
<td>-14</td>
<td>+22</td>
<td>-10</td>
<td>+27</td>
</tr>
<tr>
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<td>+297</td>
<td>-114</td>
<td>+196</td>
</tr>
<tr>
<td>Class II</td>
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<td>+736</td>
<td>-97</td>
<td>+98</td>
</tr>
<tr>
<td>CCR7</td>
<td>-6%</td>
<td>+23%</td>
<td>-2%</td>
<td>+7%</td>
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</table>

Fluorescence intensity
microscopic examination indicated that Y form-treated monocytes did not develop into DCs but rather differentiated into activated macrophage-like cells (Y-MoMøs). In contrast, GT-treated monocytes developed into a subset of mature DCs (GT-MoDCs), a high percentage of which were CCR7+ but were CD1a− and exhibited expression of CD80, CD40, and MHC class II molecules at levels that were lower than the levels in mature DCs derived from an uninjected control.

In some experiments, we used a mutant strain of C. albicans (CA-2) that is unable to form hyphae and maintains the Y-form even when it is cultured at 37°C in GT-inducing media (11). Monocytes treated with CA-2 cells grown under GT-inducing conditions, as well as non-GT-inducing conditions, did not differentiate into DCs but rather developed into Y-MoMøs, thus demonstrating the strict morphological dependence of the interference with monocyte differentiation. Monocytes infected with heat-killed C. albicans Y or GT forms developed into cells with the same surface marker expression observed with living fungi. However, expression of the maturation-associated CD83 molecules in heat-killed GT-MoDCs was observed only after LPS treatment, suggesting that heat-killed fungal cells are a less potent maturation stimulus than living fungal cells. We also observed that monocytes cultured in the presence of GM-CSF and IL-4 after phagocytosis of inert latex particles developed into immature DCs, like control monocytes, confirming that phagocytosis per se does not interfere with monocyte differentiation into DCs (28). Infection of monocytes at fungus/monocyte ratios of ≥1:1 resulted in immature DCs that were phenotypically indistinguishable from uninfected controls, while infection at ratios of ≥5:1 was associated with progressively higher cell mortality. All these effects were observed by using monocytes isolated from many different donors, and in each experiment all phenotypic characteristics of GT-MoDCs and Y-MoMøs were highly reproducible. Therefore, a defined number of fungi is required in order to observe these effects, which appear independent of the donor tested.

Cells derived from C. albicans Y form- or GT-treated monocytes are unable to secrete IL-12/70. Next, we analyzed the cytokines and chemokines produced by cells derived from C. albicans-infected monocytes. Interestingly, in contrast to control DCs, Y-MoMøs and GT-MoDCs did not produce IL-12/70 even in response to overnight LPS stimulation, but Y-MoMøs secreted substantial amounts of IL-10 and expressed higher levels of IL-6 mRNA than control DCs expressed (Fig. 3 and 4). However, both Y-MoMøs and GT-MoDCs synthesized TNF-α and expressed TNF-α mRNA. No synthesis of IFN-α or IFN-β (data not shown) was detected in the supernatants of DCs, Y-MoMøs, or GT-MoDCs, irrespective of LPS treatment. Analysis of IL-10 mRNA in Y-MoMøs after 6 days of culture revealed enhanced expression after stimulation with LPS, thus suggesting that these cells, if properly stimulated, may undergo subsequent waves of IL-10 production. Lastly, reverse transcription-PCR analysis showed that Y-MoMøs expressed higher levels of IL-6 and IL-8 mRNA molecules than LPS-treated control DCs and GT-MoDCs expressed (Fig. 4). Together, data from cytokine secretion and cytokine-chemokine gene expression experiments indicate that both Y-MoMøs and GT-MoDCs are unable to secrete IL-12/70, an important Th1-inducing cytokine. Moreover, the capacity to secrete and/or to express mRNAs for other cytokines and chemokines, spontaneously or after LPS treatment, indicates that the inability to secrete IL-12 is a distinctive feature of Y-MoMøs and GT-MoDCs and not an artifact due to functional exhaustion of these cells (22).

Antigen presentation function of GT-MoDCs or Y-MoMøs. We next investigated whether differentiation of infected monocytes into GT-MoDCs or Y-MoMøs was associated with alteration of APC functions. The properties of these APCs were studied and compared to those of LPS-matured DCs in a mixed-leukocyte reaction assay by using allogeneic peripheral blood lymphocytes from adult donors or naive CB-Ts as responder cells.

Both GT-MoDCs and Y-MoMøs were as able as control DCs to trigger proliferation of T lymphocytes obtained from adult donors (Fig. 5A). In contrast, GT-MoDCs were capable of inducing T-cell priming, but with reduced antigen presentation efficacy and efficiency compared to the antigen presentation efficacy and efficiency of mature DCs. Y-MoMøs were found to be even less efficient than GT-MoDCs in inducing CB-T priming according to macrophage-like differentiation (Fig. 5B). In addition, CB-Ts were activated when they were stimulated with control mature DCs or GT-MoDCs but not when they were stimulated with Y-MoMøs, as assessed by membrane expression of the activation markers CD25 and MHC class II (data not shown). As shown in Fig. 5C, CB-Ts stimulated with allogeneic Y-MoMøs or GT-MoDCs did not produce IL-4. In addition, an extremely low percentage of these lymphocytes produced IFN-γ compared to the percentage of CB-Ts stimulated with mature control DCs that produced IFN-γ, IL-10 (as detected by fluorescence-activated cell sorting analysis) and transforming growth factor β (as deter-

FIG. 1. C. albicans Y form-phagocytosing monocytes do not differentiate into DCs, while C. albicans GT-phagocytosing monocytes differentiate into atypical mature DCs. Human monocytes from a healthy donor were allowed to phagocytose C. albicans Y or GT forms and then induced to differentiate into DCs during a 6-day culture in the presence of GM-CSF and IL-4. DCs derived from noninfected monocytes derived from the same donor and macrophages derived from monocytes cultured for 6 days with M-CSF were used to compare the phenotypes of Y-MoMøs and GT-MoDCs. (A) Dot plots showing the surface expression of CD14 and CD1a of control DCs, control macrophages (control Møs), Y-MoMøs, and GT-MoDCs. The numbers indicate the percentages of cells in the four quadrants. (B) Histograms of control DCs, control macrophages, Y-MoMøs, and GT-MoDCs that were treated (shaded histograms) or were not treated (unshaded histograms) with LPS. The results obtained with isotypic controls are indicated by dotted lines. The numbers indicate the mean intensities of fluorescence with (positive values) and without (negative values) LPS treatment of cells stained with anti-CD83, anti-CD80, anti-CD86, anti-CD40, or anti-MHC class I and II. For CCR7 staining the percentages of positive cells with (positive values) and without (negative values) LPS treatment are shown. The data for control DCs, Y-MoMøs, and GT-MoDCs are from one experiment that was representative of five independent experiments; the data for control macrophages are representative of the data obtained in two independent experiments.
Table 1. Y-MoMs and GT-MoDCs express levels of activation markers and responsiveness to LPS different from those of the control DCs.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Control DCs</th>
<th>Y-MoMs</th>
<th>GT-MoDCs</th>
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<tbody>
<tr>
<td></td>
<td>Without LPS</td>
<td>With LPS</td>
<td>Without LPS</td>
</tr>
<tr>
<td>MHC class I</td>
<td>166.0 (406–49)</td>
<td>242.5 (461–100)</td>
<td>[74.1 ± 22] †</td>
</tr>
<tr>
<td>MHC class II</td>
<td>22.1 (632–55)</td>
<td>489.7 (820–189)</td>
<td>[278.2 ± 93]</td>
</tr>
<tr>
<td>CD80</td>
<td>14.1 (21–9)</td>
<td>55.4 (97–30)</td>
<td>[278.9 ± 34]</td>
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</table>

The values are means (ranges) of the MIF measured in four independent experiments.

The values are means ± standard errors measured in four independent experiments.

Increases in the MIF after LPS treatment were significantly different from the increases measured for LPS-treated control DCs.

The MIF after LPS treatment were significantly different from the MIF of LPS-treated control DCs. Differences were considered significant when the P value was <0.0167, as determined by analysis of variance with the Bonferroni-Dunn posttest.

In this study, we addressed this concept using C. albicans. This fungus is known to cause chronic infection, which often results in the development of chronic immune responses. The phagocytosis of C. albicans by isolated monocytes leads to the development of monocyte-derived macrophages (MDMs), which are capable of inducing a T-cell response against the fungus. However, the MDMs are unable to respond to a chemokine, CCL19 (MIP-3α), that induces cell migration into the T-cell-rich paracortical area of lymph nodes. This study suggests that the phagocytosis of C. albicans by MDMs results in the differentiation of monocytes into macrophages, which are capable of inducing a T-cell response against the fungus. This process is essential for the immune response against C. albicans, as it allows the fungus to be eliminated from the host.

In conclusion, the phagocytosis of C. albicans by monocytes leads to the development of MDMs, which are capable of inducing a T-cell response against the fungus. This process is essential for the immune response against C. albicans, as it allows the fungus to be eliminated from the host.
FIG. 2. Transmission electron microscopy reveals that monocytes phagocytosing C. albicans Y forms or GTs are induced to differentiate into morphologically distinct cell populations. (a) Control monocyte. (b) Cells cultured for 6 days in the presence of GM-CSF and IL-4, showing the typical DC morphology. (c) Interaction between monocytes and Y forms of C. albicans after incubation for 1 h. The arrow indicates a completely internalized Y cell. (d) Interaction between monocytes and GT forms of C. albicans after incubation for 1 h. The arrows indicate internalized GTs. (e) After 6 days of incubation with the Y forms, numerous digested cells or remnants of fungal cells (arrows) were observed inside monocytes having a macrophage morphology. (f) After 6 days of incubation with the GT forms, the presence of numerous internalized and digested fungal cells (arrows) did not interfere with the morphological differentiation of monocytes into DCs. Bars = 2 μm.
pared to those of LPS-treated mature DCs. GT-MoDCs had phenotypic markers of mature DCs but lacked expression of molecules belonging to the CD1 family and exhibited reduced expression of CD80 and MHC class II molecules. Moreover, they did not produce IL-12, nor did they release this cytokine expression of CD80 and MHC class II molecules. Moreover, GT-MoDCs express mRNA for TNF-α, IL-10, and IL-12, which were further stimulated with LPS overnight. An asterisk indicates that the value is significantly different from the value for control DCs stimulated with LPS, as calculated by using analysis of variance and the Student-Newman-Keuls posttest. The results are the means of three independent experiments; the error bars indicate standard deviations.

The inability of GT-MoDCs, as well as Y-MoM spéc, to secrete IL-12 could in theory be ascribed to functional exhaustion (22) caused by stimulation of the cells by fungal products during the 6-day culture period. However, the observation that GT-MoDCs express mRNA for TNF-α and the observation that Y-MoM spéc express mRNA for TNF-α, IL-10, IL-6, and IL-8 spontaneously or after LPS treatment clearly indicate that the inability to secrete IL-12 was not a consequence of exhaustion.

Interestingly, although GT-MoDCs were able to prime naive T cells, they could not induce functional polarization of naïve T lymphocytes. The phenotype and functional characteristics of these cells are identical to those of mature DCs that differentiate from M. tuberculosis-infected monocytes, as recently described (28). The existence of monocyte receptors that bind structurally and immunologically different ligands that, however, have common molecular patterns (pathogen-associated molecular patterns) has been clearly established (20, 48). The indistinguishable features of GT-MoDCs and M. tuberculosis-infected monocytes imply that there is a predefined, alternative pathway of monocyte differentiation, which may involve signaling through membrane- or phagosome-associated pathogen-associated molecular pattern receptors that can be exploited by different microorganisms irrespective of the taxonomic or genomic divergence and extra- or intracellular growth habitat. In particular, a number of constituents of the microbial cell wall conveying mannan or glucan moieties could be shared by C. albicans and M. tuberculosis and could be involved in driving monocyte differentiation (20, 48).

Interference with APC function might be instrumental for C. albicans in driving T-cell responses (40), favoring its persistence on the host’s mucosae or in infectious foci. The capacity to modify its form of growth from the Y form to hyphal cells through GT formation may provide C. albicans with an additional tool to modulate potentially dangerous host reactivity (7, 14, 45). The inability of T lymphocytes primed by GT-MoDCs to secrete cytokines may represent a distinctive characteristic of an ineffectual T-cell response promoted by C. albicans GTs to escape eradication by cellular immune responses. Moreover, GT formation and hyphal growth in C. albicans is associated with significant antigenic variations, with loss of immunodominant epitopes that are abundantly expressed on the surface of Y cells and with acquisition of hypha-specific antigens (11, 36, 37). Most preformed host responses, which presumably are targeted against the commensalism-associated Y form, may be ineffective against the hyphal form. Thus, interference with DC generation by GTs may also specifically impair expansion of hypha-specific T lymphocytes.

Our data revealed a defined threshold for the C. albicans/monocyte ratio capable of interfering with monocyte differentiation. In fact, fungus/monocyte ratios of <2:1 did not result in GT-MoDC or Y-MoM spéc differentiation. It is thus reasonable to hypothesize that increased fungal multiplication, due to a transient breakdown of immunological surveillance or to variation in local conditions caused by hormonal modifications, could trigger the beginning of a vicious circle leading to uncontrolled local fungal growth. A persistent inflammation results in a high demand for new tissue DCs, which could be replaced by monocytes differentiating in loco. The presence of replicating fungi would give raise to GT-MoDCs or Y-MoM spéc, which in turn would allow only limited expansion of T cells unable to secrete IFN-γ or to help macrophages in limiting the local fungal growth. In this view, the efficacy of chemotherapy could reside not only in direct killing of C. albicans but also in the reduction of the fungal load required to interfere with the differentiation of monocytes, thus allowing the maturation of DCs capable of expanding IFN-γ-secreting T cells. In this light, it is interesting that most highly efficacious antymycotic agents are candidastatic rather than candidacidal.

In conclusion, we demonstrated that human monocytes after phagocytosis of Y forms do not differentiate into DCs but rather develop into macrophage-like cells capable of inducing proliferation of alloreactive memory but not naïve T lymphocytes. Conversely, monocytes after phagocytosis of GTs differentiate into mature DCs; however, there is no up-regulation of
FIG. 4. Cells derived from Y form- or GT-phagocytosing monocytes are not exhausted and display different patterns of mRNA cytokine expression: reverse transcription-PCR analysis of cells derived from monocytes infected with Y forms or GTs stimulated or not stimulated with LPS in the last 18 h of culture. The data are representative of three experiments. MW, molecular weight.

FIG. 5. Cells derived from \textit{C. albicans}-phagocytosing monocytes have an impaired antigen presentation function. (A and B) Proliferative response of T lymphocytes from adult peripheral blood (A) or cord blood (B) stimulated with allogeneic DCs (ctr) or with cells derived from monocytes phagocytosing \textit{C. albicans} Y forms or GTs. Control DCs, Y-MoMs, and GT-MoDCs were obtained by culturing monocytes from the same healthy donor and were used both with LPS treatment (+ LPS) and without LPS treatment in the last 18 h of the 6-day differentiation culture in the presence of GM-CSF and IL-4. The data are from one experiment that was representative of four experiments. (C) Flow cytometric analysis of intracellular cytokine accumulation in CB-Ts after 6 days of coculture with allogeneic APCs. The intracellular cytokine accumulation was detected in CD3-positive cells after 6 days of culture with allogeneic noninfected mature DCs (N.I.) or cells derived from Y form- or GT-infected monocytes. Dot plots show data obtained in experiments in which all APCs were treated overnight with LPS and then extensively washed before incubation with CB-Ts. The numbers in the panels indicate the percentages of cells in the corresponding quadrants. The data are from one experiment that was representative of four experiments.
FLOW CYTOMETRY


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