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

Antonella Torosantucci,[1] Giulia Romagnoli,[1] Paola Chiani,[1] Annarita Stringaro,[2]
Pasqualina Crateri,[2] Sabrina Mariotti,[1] Raffaela Teloni,[1] Giuseppe Arancia,[2]
Antonio Cassone,[1] and Roberto Nisini[1]*

Laboratorio di Batteriologia e Micologia Medica[1] and Laboratorio di Ultrastrutture,[2] Istituto Superiore di Sanità, 00161 Rome, Italy

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 into DCs 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

* Corresponding author. Mailing address: Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. Phone: 39 06 4990 2659. Fax: 39 06 49387112. E-mail: r.nisini@iss.it.

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 (FCS) (HyClone, Logan, Utah) (complete medium).

Fungal cells. The virulent, hyphal conversion-competent *C. albicans* strain BP (serotype A) from the established type collection of the Istituto Superiore di Sanità (Rome, Italy) was used throughout this study. Y and GT forms were obtained as previously described (44). Briefly, Y cells grown in Winge medium for 24 h at 28°C were washed in saline, resuspended at a density of 2×10^6 cells/ml in buffered Lee's medium, and incubated at 28 or 37°C for 60 min. At 28°C, fungal cells remained in the Y form, whereas, at 37°C, >90% of the initial Y cells produced GT. In control experiments, the *C. albicans* mutant strain CA-2, which is unable to convert into the hyphal form, was also used (10). This strain maintains the Y morphology even when it is cultured at 37°C in GT-inducing media. Y and GT cells were harvested by centrifugation, washed twice with phosphate-buffered saline (PBS), and resuspended in PBS at the desired concentration.

Monocyte isolation, *C. albicans* infection, and DC generation. Peripheral blood mononuclear cells were purified from heparinized blood on a density gradient (Lymphoprep; Nycomed Pharma AS, Oslo, Norway) as previously described (33)

and were resuspended in PBS containing 10% FCS. Monocytes were sorted by using anti-CD14-labeled magnetic beads (MACS, Miltenyi, Germany) according to the manufacturer's instructions. Freshly isolated monocytes were resuspended in complete medium containing GM-CSF (200 U/ml) and IL-4 (100 U/ml) for generation of control DCs or with M-CSF for generation of control macrophages and cultured for 5 days. To examine the possible interference of *C. albicans* infection during DC differentiation, monocytes were infected with single-cell suspensions of Y or GT forms, CA-2, or inert latex particles. In preliminary experiments, infection was performed at different fungus/monocyte ratios (1:1, 2:1, 4:1, and 6:1), and then a ratio of 3:1 was used unless indicated otherwise. In some experiments, monocytes were treated with fungi which were previously heat killed by incubation at 80°C for 30 min. *C. albicans* phagocytosis was evaluated in each experiment by staining parallel cultures. Untreated or *C. albicans*-treated monocytes were then cultured with GM-CSF and IL-4 as described above in the presence of amphotericin B (0.62 μg/ml). As indicated below, 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-CXCR4, anti-CCR7, and appropriate isotypic controls. All antibodies were purchased from Pharmingen (San Diego, Calif.). Biotin-conjugated anti-CD1c antibodies were obtained from Cymbus Biotechnology Ltd. (Hampshire, United Kingdom). Human-adsorbed, fluorescein isothiocyanate (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 mg 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) after fixation and permeabilization with Cytofix/Cytoperm (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 CD1, CD11c, CD14, CD123, and CCR7, and the median intensity of fluorescence (MIF) 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 for 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% (vol/vol) glutaraldehyde 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 Ultrotome 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 1×10^6 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 (Perkin-Elmer 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 naïve 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 5×10^4 allogeneic cord blood lymphocytes (CB-Ts) per well. The proliferative response was measured on day 5 by using a 16-h pulse with [^{3}H]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 1×10^6 CB-Ts and 1×10^5 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 4×10^5 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-α, IFN-β, tumor necrosis factor alpha [TNF-α], and transforming growth factor β) 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-Keulz or Bonferroni-Dunn posttests.

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 phagocytose 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 plasmocytoid 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 the 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

B

Fluorescence intensity
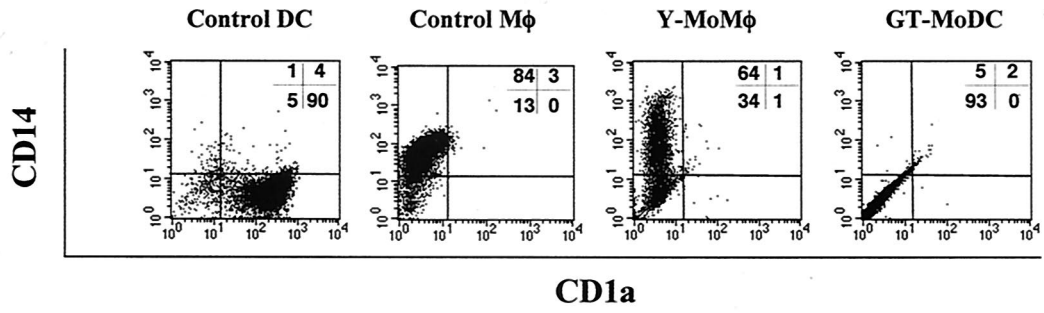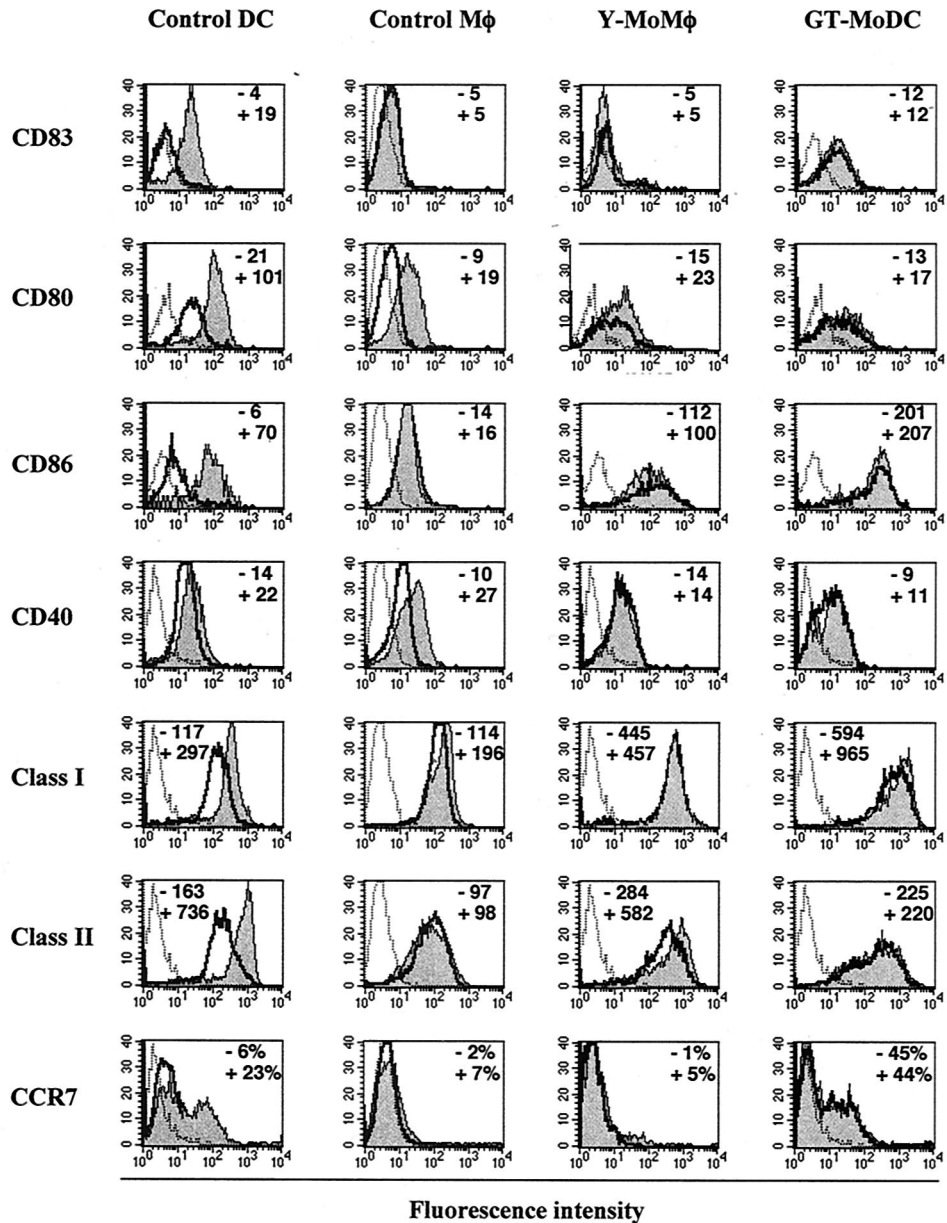
microscopic examination indicated that Y form-treated monocytes did not develop into DCs but rather differentiated into activated macrophage-like cells (Y-MoMφs). In contrast, GT-treated monocytes developed into a subset of mature DCs (GT-MoDCs), a high percentage of which were CCR7$^+$ but were CD1a$^-$ 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 uninfected 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-MoMφs, thus demonstrating the strict morphology 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-MoMφs 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-12p70. Next, we analyzed the cytokines and chemokines produced by cells derived from *C. albicans*-infected monocytes. Interestingly, in contrast to control DCs, Y-MoMφs and GT-MoDCs did not produce IL-12p70 even in response to overnight LPS stimulation, but Y-MoMφs 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-MoMφs 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-MoMφs, or GT-MoDCs, irrespective of LPS treatment. Analysis of IL-10 mRNA in Y-MoMφs 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-MoMφs 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-MoMφs and GT-MoDCs are unable to secrete IL-12p70, 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-MoMφs and GT-MoDCs and not an artifact due to functional exhaustion of these cells (22).

Antigen presentation function of GT-MoDCs or Y-MoMφs. We next investigated whether differentiation of infected monocytes into GT-MoDCs or Y-MoMφs 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 naïve CB-Ts as responder cells.

Both GT-MoDCs and Y-MoMφs 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 control mature DCs. Y-MoMφs 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-MoMφs, 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-MoMφs 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-MoMφs and GT-MoDCs. (A) Dot plots showing the surface expression of CD14 and CD1a of control DCs, control macrophages (control Mφs), Y-MoMφs, and GT-MoDCs. The numbers indicate the percentages of cells in the four quadrants. (B) Histograms of control DCs, control macrophages, Y-MoMφs, 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-MoMφs, 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-MoMφs and GT-MoDCs express levels of activation markers and responsiveness to LPS different from those of the control DC

Marker	Control DCs MIF[a]			Y-MoMφs MIF[a]			GT-MoDCs MIF[a]		
	Without LPS	With LPS	% Expression compared to control DCs with LPS[b]	Without LPS	With LPS	% Expression compared to control DCs with LPS[b]	Without LPS	With LPS	% Expression compared to control DCs with LPS[b]
MHC class I	166.0 (406–49)	242.5 (461–100) [74.1 ± 22][c]		324.6 (567–105)	349.4 (620–120) [8.7 ± 2][d]	147 ± 28	363.8 (640–140)	502.3 (937–156) [29.3 ± 11]	199.4 ± 73[e]
MHC class II	221.3 (632–55)	489.7 (820–189) [278.2 ± 93]		210.5 (286–93)	339.9 (588–140) [58.2 ± 16]	64.1 ± 18	190.2 (381–64)	206.8 (410–82) [17.0 ± 9][d]	43.7 ± 9[e]
CD40	21.4 (28–15)	39.4 (56–23) [79.3 ± 9]		21.1 (28–17)	20.2 (27–15) [−3.7 ± 7][d]	58.1 ± 24[e]	16.3 (20–12)	20.3 (24–14) [25.6 ± 10][d]	57.3 ± 20[e]
CD80	14.1 (21–9)	55.4 (97–30) [278.9 ± 34]		11.8 (17–6)	18.4 (22–13) [65.8 ± 17][d]	36.8 ± 11[e]	8.4 (13–3)	10.6 (17–6) [33.5 ± 13][d]	19.6 ± 5[e]

[a] The values are means (ranges) of the MIF measured in four independent experiments.
[b] The values are means ± standard errors measured in four independent experiments.
[c] The values in brackets are the percent increases after LPS treatment and are means ± standard errors.
[d] Increases in the MIF after LPS treatment were significantly different from the increases measured for LPS-treated control DCs.
[e] 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.

mined by an enzyme-linked immunosorbent assay) were not synthesized by CB-Ts irrespective of the allogeneic stimulator APCs (data not shown). These data suggest that cells derived from *C. albicans*-infected monocytes are functionally unable to expand T cells with an effector function.

DISCUSSION

In this paper we demonstrate for the fist time that the Y and GT forms of *C. albicans* interfere with the process of monocyte differentiation into DCs, leading to distinct populations of cells, which, however, have the common feature of hampered APC function.

As a consequence of commensalism or chronic infection, encounters of the microorganism with immature DCs in tissue are continuous, and there is subsequent DC maturation and migration to lymph nodes. Tissue DCs must be renewed, and this should occur in the presence of a growing infectious agent. Monocytes are logical precursors for this renewal. This issue is of particular interest since monocytes sense microorganisms through an array of receptors, which transduce signals potentially conflicting with the differentiation program (20, 48). In this context, the microbial invader could exploit the monocyte differentiation pathway to DCs to its own advantage. In this study we addressed this issue using *C. albicans*. This fungus commensally inhabits the mucosal surface of healthy subjects but is prone to invade the mucosal tissues under various circumstances. To proceed to tissue invasion, *C. albicans* characteristically changes its habit of growth from Y forms to hyphal forms. This morphological change has long been suspected to imply interference with host immunity. Thus, we allowed phagocytosis of Y or GT forms of *C. albicans* by isolated human monocytes and then induced differentiation into DCs. We found that the phagocytosis of Y cells inhibited the differentiation of monocytes into DCs and forced differentiation into macrophages instead. In fact, Y-MoMφs retained CD14 and were characterized by high levels of CD86 and MHC class I and II expression, but they did not up-regulate CD80 and express CD1a, CD83, and CCR7 molecules even after LPS treatment. Interestingly, the lack of CCR7 expression made these cells unable to respond to a chemokine, CCL19 (MIP-3β, ELC), that induces cell migration into the T-cell-rich paracortical area of lymph nodes (13), and indicated that Y-MoMφs are potential resident tissue cells. Although Y-MoMφs were capable of inducing mixed-leukocyte reaction responses of lymphocytes from adults, they did not induce T-cell priming of naïve CB-Ts. Y-MoMφs secreted TNF-α but not IL-12, even with LPS stimulation. A distinct feature of the Y form-monocyte interaction was the stimulation of high levels of IL-10 secretion and IL-6 gene expression compared to the levels observed upon interaction with GT cells. Both these cytokines have been reported to interfere with the generation of monocyte-derived DCs, skewing monocyte differentiation toward the macrophage phenotype (1, 8). Thus, it is conceivable that Y forms inhibit the generation of potent APCs, such as DCs, through the autocrine effect of these cytokines on differentiating monocytes.

On the other hand, monocytes that had been exposed to GT cells developed into mature DCs, but these DCs had phenotypic and functional characteristics that were atypical com-
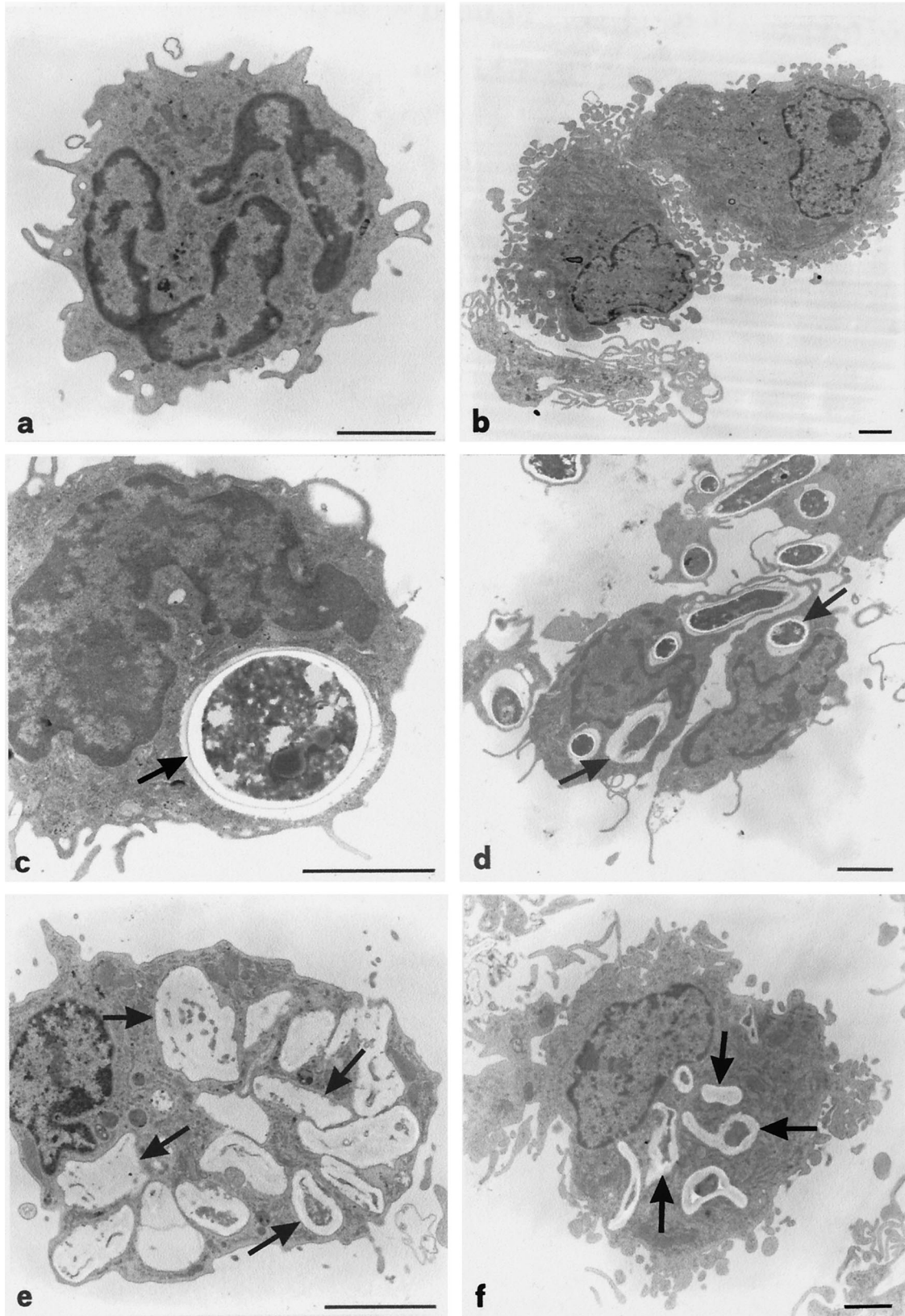
FIG. 2. Transmission electron microscopy reveals that monocytes phagocytosing *C. albicans* Y forms or GTs are induced to differentiate into morphologically distinct cell populations. (a) Control monocyte. (b) Cells cultured for 6 days in the presence of GM-CSF and IL-4, showing the typical DC morphology. (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.
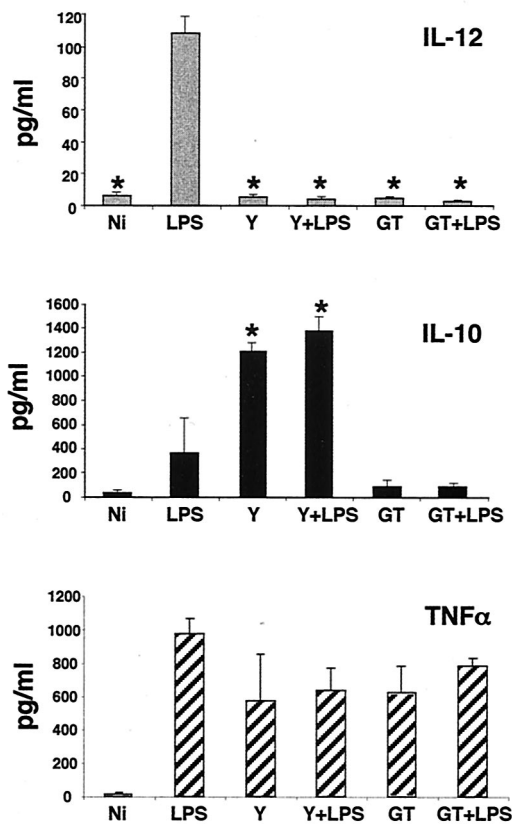
FIG. 3. Cells derived from *C. albicans*-phagocytosing monocytes are unable to produce IL-12p70: IL-12p70, IL-10, and TNF-α secretion in supernatants of cells derived from monocytes infected with Y forms or GTs. Ni, noninfected control DCs; LPS, control DCs stimulated overnight with LPS; Y+LPS and GT+LPS, cells derived from monocytes infected with Y forms and GTs, respectively, 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.

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 in response to LPS stimulation.

The inability of GT-MoDCs, as well as Y-MoMφs, 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-MoMφs 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 naïve 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 inefficacious 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-MoMφ 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-MoMφs, 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 antimycotic 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
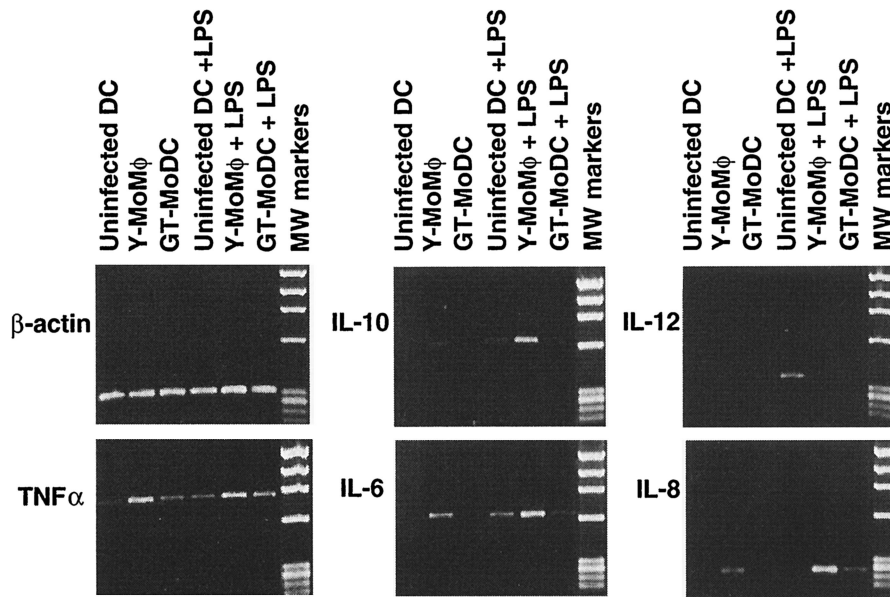
FIG. 4. Cells derived from Y form- or GT-phagocytosing monocytes are not exhausted and display different patterns of mRNA cytokine expression: reverse transcription-PCR analysis of cells derived from monocytes infected with Y forms or GTs stimulated or not stimulated with LPS in the last 18 h of culture. The data are representative of three experiments. MW, molecular weight.
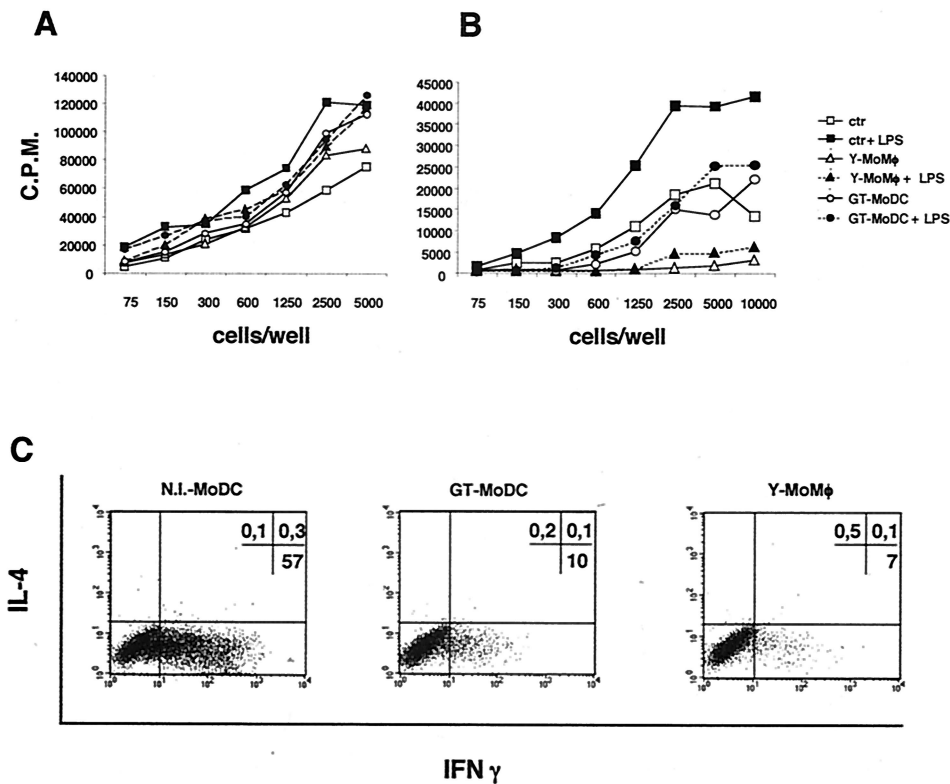

FIG. 5. Cells derived from *C. albicans*-phagocytosing monocytes have an impaired antigen presentation function. (A and B) Proliferative response of T lymphocytes from adult peripheral blood (A) or cord blood (B) stimulated with allogeneic DCs (ctr) or with cells derived from monocytes phagocytosing *C. albicans* Y forms or GTs. Control DCs, Y-MoMφs, 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.

CD80, CD40, and MHC class II, and there is no IL-12 production. These cells were able to prime naïve T cells but were not able to induce functional polarization of these T cells into effector cells.

The different modulatory effects exerted by Y and GT forms of *C. albicans* on differentiating monocytes may be a key phenomenon that contributes to an explanation of the intriguing paradox that distinguishes this fungus, as represented by its persistence in some body compartments as a commensal yeast or as pathogen hyphae despite the presence of vigorous cellular and humoral systemic immune responses.

REFERENCES

1. **Allavena, P., L. Piemonti, D. Longoni, S. Bernasconi, A. Stoppacciaro, L. Ruco, and A. Mantovani.** 1998. IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. Eur. J. Immunol. **28:**359–369.
2. **Ausiello, C. M., F. Urbani, S. Gessani, G. C. Spagnoli, M. J. Gomez, and A. Cassone.** 1993. Cytokine gene expression in human peripheral blood mononuclear cells stimulated by mannoprotein constituents from *Candida albicans.* Infect. Immun. **61:**4105–4111.
3. **Bistoni, F., E. Cenci, A. Mencacci, E. Schiaffella, P. Mosci, P. Puccetti, and L. Romani.** 1993. Mucosal and systemic T helper cell function after intragastric colonization of adult mice with *Candida albicans.* J. Infect. Dis. **168:**1449–1457.
4. **Cassone, A., F. De Bernardis, C. M. Ausiello, M. J. Gomez, M. Boccanera, R. La Valle, and A. Torosantucci.** 1998. Immunogenic and protective *Candida albicans* constituents. Res. Immunol **149:**289–299. (Discussion, **149:**504.)
5. **Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, I. Durand, M. Cella, A. Lanzavecchia, and J. Banchereau.** 1997. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor alpha. II. Functional analysis. Blood **90:**1458–1470.
6. **Cella, M., F. Sallusto, and A. Lanzavecchia.** 1997. Origin, maturation and antigen presenting function of dendritic cells. Curr. Opin. Immunol. **9:**10–16.
7. **Chiani, P., C. Bromuro, and A. Torosantucci.** 2000. Defective induction of interleukin-12 in human monocytes by germ-tube forms of *Candida albicans.* Infect. Immun. **68:**5628–5634.
8. **Chomarat, P., J. Banchereau, J. Davoust, and A. K. Palucka.** 2000. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat. Immunol. **1:**510–514.
9. **Corner, B. E., and P. T. Magee.** 1997. *Candida* pathogenesis: unravelling the threads of infection. Curr. Biol. **7:**R691–R694.
10. **De Bernardis, F., D. Adriani, R. Lorenzini, E. Pontieri, G. Carruba, and A. Cassone.** 1993. Filamentous growth and elevated vaginopathic potential of a nongerminative variant of *Candida albicans* expressing low virulence in systemic infection. Infect. Immun. **61:**1500–1508.
11. **De Bernardis, F., A. Molinari, M. Boccanera, A. Stringaro, R. Robert, J. M. Senet, G. Arancia, and A. Cassone.** 1994. Modulation of cell surface-associated mannoprotein antigen expression in experimental candidal vaginitis. Infect. Immun. **62:**509–519.
12. **de Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y. J. Liu, S. Lebecque, and C. Caux.** 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J. Immunol. **160:**1666–1676.
13. **Dieu, M. C., B. Vanbervliet, A. Vicari, J. M. Bridon, E. Oldham, S. Ait-Yahia, F. Briere, A. Zlotnik, S. Lebecque, and C. Caux.** 1998. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J. Exp. Med. **188:**373–386.
14. **d'Ostiani, C. F., G. Del Sero, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, and L. Romani.** 2000. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans.* Implications for initiation of T helper cell immunity in vitro and in vivo. J. Exp. Med. **191:**1661–1674.
15. **Farah, C. S., S. Elahi, G. Pang, T. Gotjamanos, G. J. Seymour, R. L. Clancy, and R. B. Ashman.** 2001. T cells augment monocyte and neutrophil function in host resistance against oropharyngeal candidiasis. Infect. Immun. **69:**6110–6118.
16. **Fattorossi, A., R. Nisini, J. G. Pizzolo, and R. D'Amelio.** 1989. New, simple flow cytometry technique to discriminate between internalized and membrane-bound particles in phagocytosis. Cytometry **10:**320–325.
17. **Fidel, P. L., Jr.** 1998. Vaginal candidiasis: review and role of local mucosal immunity. AIDS Patient Care STDS **12:**359–366.
18. **Fidel, P. L., Jr., and J. D. Sobel.** 1998. Protective immunity in experimental *Candida* vaginitis. Res. Immunol. **149:**361–373. (Discussion, **149:**510–512.)
19. **Gottlieb, G. S., A. P. Limaye, Y. C. Chen, and W. C. Van Voorhis.** 2001. *Candida dubliniensis* fungemia in a solid organ transplant patient: case report and review of the literature. Med. Mycol. **39:**483–485.
20. **Janeway, C. A., Jr., and R. Medzhitov.** 2002. Innate immune recognition. Annu. Rev. Immunol. **20:**197–216.
21. **Johansson, U., J. Ivanyi, and M. Londei.** 2001. Inhibition of IL-12 production in human dendritic cells matured in the presence of bacillus Calmette-Guerin or lipoarabinomannan. Immunol. Lett. **77:**63–66.
22. **Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto.** 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. Nat. Immunol. **1:**311–316.
23. **Lanzavecchia, A.** 1999. Dendritic cell maturation and generation of immune responses. Haematologica **84**(Suppl. 4)**:**23–25.
24. **Lanzavecchia, A., and F. Sallusto.** 2001. The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics. Curr. Opin. Immunol. **13:**291–298.
25. **Lanzavecchia, A., and F. Sallusto.** 2001. Regulation of T cell immunity by dendritic cells. Cell **106:**263–266.
26. **Lauvau, G., S. Vijh, P. Kong, T. Horng, K. Kerksiek, N. Serbina, R. A. Tuma, and E. G. Pamer.** 2001. Priming of memory but not effector CD8 T cells by a killed bacterial vaccine. Science **294:**1735–1739.
27. **Lo, H. J., J. R. Kohler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G. R. Fink.** 1997. Nonfilamentous *C. albicans* mutants are avirulent. Cell **90:**939–949.
28. **Mariotti, S., R. Teloni, E. Iona, L. Fattorini, F. Giannoni, G. Romagnoli, G. Orefici, and R. Nisini.** 2002. *Mycobacterium tuberculosis* subverts the differentiation of human monocyte into dendritic cell. Eur. J. Immunol. **32:**3050–3058.
29. **Marques, S. A., A. M. Robles, A. M. Tortorano, M. A. Tuculet, R. Negroni, and R. P. Mendes.** 2000. Mycoses associated with AIDS in the Third World. Med. Mycol. **38:**269–279.
30. **Mellman, I., and R. M. Steinman.** 2001. Dendritic cells: specialized and regulated antigen processing machines. Cell **106:**255–258.
31. **Mencacci, A., E. Cenci, F. Bistoni, A. Bacci, G. Del Sero, C. Montagnoli, C. Fe d'Ostiani, and L. Romani.** 1998. Specific and non-specific immunity to *Candida albicans:* a lesson from genetically modified animals. Res. Immunol. **149:**352–361. (Discussion, **149:**517–519.)
32. **Nisini, R., A. Fattorossi, C. Ferlini, and R. D'Amelio.** 1996. One cause for the apparent inability of human T cell clones to function as professional superantigen-presenting cells is autoactivation. Eur. J. Immunol. **26:**797–803.
33. **Nisini, R., M. Paroli, D. Accapezzato, F. Bonino, F. Rosina, T. Santantonio, F. Sallusto, A. Amoroso, M. Houghton, and V. Barnaba.** 1997. Human CD4+ T-cell response to hepatitis delta virus: identification of multiple epitopes and characterization of T-helper cytokine profiles. J. Virol. **71:**2241–2251.
34. **Nisini, R., G. Romagnoli, M. J. Gomez, R. La Valle, A. Torosantucci, S. Mariotti, R. Teloni, and A. Cassone.** 2001. Antigenic properties and processing requirements of 65-kilodalton mannoprotein, a major antigen target of anti-*Candida* human T-cell response, as disclosed by specific human T-cell clones. Infect. Immun. **69:**3728–3736.
35. **Odds, F. C.** 1988. *Candida* and candidosis, vol. 2. Ballière-Tindall, London, United Kingdom.
36. **Ponton, J., A. Marot-Leblond, P. A. Ezkurra, B. Barturen, R. Robert, and J. M. Senet.** 1993. Characterization of *Candida albicans* cell wall antigens with monoclonal antibodies. Infect. Immun. **61:**4842–4847.
37. **Poulain, D., V. Hopwood, and A. Vernes.** 1985. Antigenic variability of *Candida albicans.* Crit. Rev. Microbiol. **12:**223–270.
38. **Randolph, G. J., S. Beaulieu, S. Lebecque, R. M. Steinman, and W. A. Muller.** 1998. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science **282:**480–483.
39. **Randolph, G. J., K. Inaba, D. F. Robbiani, R. M. Steinman, and W. A. Muller.** 1999. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity **11:**753–761.
40. **Rescigno, M., and P. Borrow.** 2001. The host-pathogen interaction: new themes from dendritic cell biology. Cell **106:**267–270.
41. **Romani, L.** 2000. Innate and adaptive immunity in *Candida albicans* infections and saprophytism. J. Leukoc. Biol. **68:**175–179.
42. **Sallusto, F., and A. Lanzavecchia.** 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J. Exp. Med. **179:**1109–1118.
43. **Stoiber, H., L. Kacani, C. Speth, R. Wurzner, and M. P. Dierich.** 2001. The supportive role of complement in HIV pathogenesis. Immunol. Rev. **180:**168–176.
44. **Torosantucci, A., M. Boccanera, I. Casalinuovo, G. Pellegrini, and A. Cas-**

sone. 1990. Differences in the antigenic expression of immunomodulatory mannoprotein constituents on yeast and mycelial forms of *Candida albicans*. J. Gen. Microbiol. **136:**1421–1428.

45. **Torosantucci, A., P. Chiani, and A. Cassone.** 2000. Differential chemokine response of human monocytes to yeast and hyphal forms of *Candida albicans* and its relation to the beta-1,6 glucan of the fungal cell wall. J. Leukoc. Biol. **68:**923–932.

46. **Torosantucci, A., P. Chiani, I. Quinti, C. M. Ausiello, I. Mezzaroma, and A. Cassone.** 1997. Responsiveness of human polymorphonuclear cells (PMNL) to stimulation by a mannoprotein fraction (MP-F2) of *Candida albicans*; enhanced production of IL-6 and tumour necrosis factor-alpha (TNF-alpha) by MP-F2-stimulated PMNL from HIV-infected subjects. Clin. Exp. Immunol. **107:**451–457.

47. **Vazquez-Torres, A., and E. Balish.** 1997. Macrophages in resistance to candidiasis. Microbiol. Mol. Biol. Rev. **61:**170–192.

48. **Visintin, A., A. Mazzoni, J. H. Spitzer, D. H. Wyllie, S. K. Dower, and D. M. Segal.** 2001. Regulation of Toll-like receptors in human monocytes and dendritic cells. J. Immunol. **166:**249–255.

Editor: S. H. E. Kaufmann