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Tumor Necrosis Factor as an Autocrine and Paracrine Signal Controlling the Macrophage Secretory Response to *Candida albicans*

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We have previously demonstrated that the hyphal form of *Candida albicans* (H-*Candida*), but not the yeast form (Y-*Candida*), acts as a macrophage-stimulating agent. The early response (1 to 3 h) of the macrophage cell line ANA-1 to H-*Candida* results in enhanced tumor necrosis factor (TNF) transcription and production. Here we show that when coincubation times are prolonged (3 to 24 h), Y-*Candida* also exhibits stimulatory properties. This phenomenon has been ascribed to the occurrence of the dimorphic transition, as demonstrated by microscopic evaluation of the cultures and by experiments in which both killed Y-*Candida* and the agerminative strain *C. albicans* PCA-2 failed to induce cytokine production. TNF produced in response to H-*Candida* acts as an autocrine and paracrine signal controlling the macrophage secretory response to *C. albicans*. In fact, addition of anti-TNF polyclonal antibodies to the coculture of ANA-1 macrophages and H-*Candida* results in a marked and time-dependent decrease of TNF transcript levels. Moreover, pretreatment of macrophages with recombinant TNF for 3 h enhances TNF and induces interleukin-1 production in response to both forms of *Candida*, while pretreatment for 18 h renders macrophages refractory to any stimuli. Interestingly, the kinetics of interleukin-1 transcription and secretion in response to H-*Candida* are delayed with respect to those of TNF. Overall, these data indicate that TNF, produced by macrophages in response to H-*Candida*, regulates its own production as well as that of other soluble factors, thus suggesting that this cytokine plays multiple roles in the immune mechanisms involved in *Candida* infection.

Macrophages and monocytes are the principal sources of tumor necrosis factor (TNF) in vivo (2, 29, 30, 32). TNF is produced by these cells in response to a variety of stimuli, including bacterial lipopolysaccharide (LPS) (3, 6–9, 31, 38). Once produced, TNF appears to have multiple regulatory effects, exerting endocrine, paracrine, and autocrine control of inflammatory responses (21, 36). In particular, TNF exerts profound effects on its own principal cell of origin, the macrophage. In addition to enhancing macrophage antitumoral and antimicrobial activities in vitro (4, 13, 18, 20, 27, 28, 43), TNF has been shown to potentiate its own production, as well as that of other cytokines (21). In particular, interleukin-1 (IL-1) activity has been detected in culture supernatants of human mononuclear cells and murine resident peritoneal macrophages upon stimulation with TNF (1, 14).

Using an in vivo experimental model, we have shown that TNF is produced in large amounts during *Candida albicans* infection (40), while in vitro studies have proven that murine splenic macrophages (41) or human blood mononuclear cells respond to *C. albicans* with enhanced TNF production (16). More recently, we have demonstrated that the *C. albicans* hyphal form (H-*Candida*), but not the yeast form (Y-*Candida*), acts as a stimulating signal in the cloned macrophage population ANA-1 (7). In particular, the early response (1 to 3 h of coincubation) of ANA-1 macrophages to H-*Candida* results in increased TNF transcription and production. The extent and kinetics of H-*Candida*-mediated effects are similar to those observed in ANA-1 macrophages exposed to LPS (6). Overall, these findings imply that *C. albicans* is a potent TNF inducer in macrophages, provided that the morphogenetic change to the hyphal form has taken place. In this paper, we demonstrate the role of TNF as a paracrine and autocrine immunomodulator of the macrophage secretory response to *C. albicans*.

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MATERIALS AND METHODS

Reagents. Recombinant mouse TNF-α (rTNF) (4×10^7 U/ml) and a polyclonal rabbit anti-mouse TNF-α antibody (anti-TNF Ab) (1 μl neutralizes 1,000 U of mouse TNF-α bioactivity in the standard L-929 cell cytotoxicity assay) were obtained from Genzyme (Cambridge, Mass.).

Cell lines. ANA-1 murine macrophages, obtained by immortalization of bone marrow cells from C57BL/6 mice with a recombinant retrovirus carrying the v-*raf* and v-*myc* oncogenes (5, 12), were cultured in RPMI 1640 medium supplemented with glutamine (4 mM), gentamicin (50 μg/ml), and 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, Utah) (complete medium). L-929 fibroblast cells (CCL1; American Type Culture Collection, Rockville, Md.) were maintained in complete medium. All reagents were purchased from Flow Laboratories (Rockville, Md.).

C. albicans. *C. albicans*, serotype A, was isolated from a clinical specimen and was grown at 28°C with mild agitation in low-glucose Winge medium as previously described (23). Under these conditions, the organism grew as an essentially pure Y-*Candida* population. To obtain H-*Candida*, a previously described procedure was employed (35). Briefly, a pure Y-

Candida population was harvested from Winge medium, washed twice in saline, resuspended in complete medium, dispensed in 60-mm-diameter tissue culture plates (2×10^6 cells per ml; 5 ml per plate), and incubated at 37°C in 5% CO_2. After 3 h of incubation, more than 98% of the yeasts had germinated to form H-*Candida* as determined by microscopic examination. Heat-killed Y-*Candida* and heat-killed H-*Candida* were prepared by heating Y- and H-*Candida* at 80°C for 30 min. Only batches that showed no regrowth in Sabouraud broth and no [^{3}H]glucose uptake were employed in the assays. The agerminative strain *C. albicans* PCA-2, a kind gift of D. Kerridge (Department of Biochemistry, University of Cambridge, Cambridge, England), is an echinocandin-resistant mutant of the parental strain 3153 (24). It was maintained in Winge medium at 28°C as detailed previously for *C. albicans* serotype A (23).

All of the *C. albicans* preparations were tested for endotoxin contamination, and only those containing less than 0.05 ng of endotoxins per ml (as assessed by the *Limulus* amebocyte lysate assay) were used.

Experimental protocol. ANA-1 macrophages (10^7 per plate) were coincubated with H-*Candida* or Y-*Candida* at an effector-to-target cell ratio of 1:1 or were exposed to LPS (1 μg/ml) in 60-mm-diameter tissue culture plates at 37°C in 5% CO_2. All experiments were performed in complete medium. The treatment and incubation times varied as detailed in the tables and in the figure legends. The cultures were then employed in biological assays or Northern (RNA) blot analysis.

Microscopic analysis. ANA-1 macrophages and H-*Candida* cocultures were fixed at different times in 10% neutral formalin, dehydrated, and stained with periodic acid-Schiff stain.

TNF assay. The quantitation of TNF activity was performed by a bioassay with L-929 cells, as described previously (34). Briefly, L-929 cells were seeded into 96-well flat-bottom plates (4×10^4 cells per well) and incubated for 24 h at 37°C. The spent medium was then removed and replaced with a test sample or a standard TNF preparation containing actinomycin D (3 μg/ml). After 20 h of incubation at 37°C, the plates were stained with 0.5% crystal violet in 20% methanol for 15 min and washed in tap water. After the plates were dried, the A_{450} was determined by using a Titertek Multiskan plate reader (Flow). All determinations of TNF activity in test samples were compared with those of commercially available TNF preparations with known titers; the results are expressed as units per milliliter.

IL-1 assay. To quantitate the production of IL-1 in ANA-1 macrophage supernatants, a commercially available enzyme-linked immunosorbent assay kit (Intertest-1-αx Mouse; Genzyme) was used. The data were analyzed and calculated by linear regression analysis, using standard concentrations ranging between 15 and 405 pg/ml ($r \geq 0.97$).

RNA extraction and Northern blot analysis. Total cellular RNA was isolated from stimulated and unstimulated ANA-1 cells solubilized with guanidine isothiocyanate as previously described (11). Samples of 10 μg of total RNA were electrophoresed in denaturing conditions, blotted onto nylon membranes (Amersham International, Amersham, United Kingdom), cross-linked by UV irradiation, and heated for 1 h at 60°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate. Filters were prehybridized for 6 h at 37°C in prehybridization buffer containing formamide and denatured salmon sperm DNA (100 μg/ml), and then 10 ng of the specific ^{32}P-labeled probe was added and left for 18 h in hybridization buffer containing dextran sulfate. Filters were washed four times at room temperature for 5 min and four times at 60°C for 30 min in 1× SSC–0.5% sodium dodecyl sulfate and then autoradiographed with Kodak X-AR5 films (Eastman Kodak Co., Rochester, N.Y.) with intensifying screens at −80°C. Probes were labeled by nick translation with a commercial kit (Amersham) as suggested by the manufacturer. The specific activity was always higher than 10^8 cpm/μg. For IL-1α mRNA detection, the 2.0-kb *Bam*HI fragment (from the pBR322 plasmid) was used (22). For TNF detection, the 1.2-kb *Pst*I-*Eco*RI fragment (cloned in from the pUC9 plasmid) was used (10). For β-actin detection, the 2.1-kb *Bam*HI fragment (from the Okayama-Berg pcD-X plasmid) was used (19).

For scanning densitometry, slot blots with twofold serial dilutions (from 5 μg) were made. Membranes were hybridized as described above and exposed to X-ray film. After development, the films were scanned by using an Electrophoresis Data Center (Helena Laboratories, Beaumont, Tex.). The results depicted in Table 2 are arbitrary optical density units obtained by comparing H-*Candida*- or LPS-treated with untreated macrophages.

RESULTS

Kinetic experiments were performed to assess whether ANA-1 macrophages could differentially modulate TNF or IL-1 mRNA levels in response to *C. albicans* in its two morphogenetic forms. LPS was included as a positive control. Cytokine mRNA levels were monitored in cells exposed to H-*Candida*, Y-*Candida*, or LPS for 3, 6, 9, and 24 h (Fig. 1). Untreated ANA-1 macrophages constitutively expressed detectable levels of TNF after 3 and 6 h of incubation. When the incubation time was increased to 9 h, TNF mRNA levels decreased, and TNF mRNA disappeared after 24 h. Exposure of macrophages to H-*Candida* significantly enhanced TNF mRNA levels. The maximal level was observed at 3 h, and TNF mRNA gradually decreased after 6 to 9 h and then returned to undetectable levels after 24 h. As expected (7), macrophages exposed for 3 h to Y-*Candida* did not differ from controls in TNF-specific transcript levels. Surprisingly, we observed a substantial increase of TNF mRNA levels in macrophages exposed to Y-*Candida* for 6 and 9 h. After 24 h, such levels partially decreased but remained higher than those observed in control macrophages. The pattern of TNF mRNA levels observed in ANA-1 macrophages exposed to LPS closely resembled that of H-*Candida*-treated macrophages. A 3-h treatment resulted in maximal levels of TNF transcripts; these levels gradually decreased with increasing time in culture. Analysis of IL-1-specific transcripts (Fig. 1) revealed that ANA-1 macrophages did not constitutively express detectable levels of specific IL-1 mRNA. Exposure of macrophages to H-*Candida* resulted in detectable IL-1 mRNA levels after 6 h; maximal levels were reached at 9 h. Y-*Candida* also induced IL-1 mRNA transcripts, which reached maximal levels after 9 h of coincubation. LPS induced IL-1 mRNA transcripts after 3 h; such levels remained high after 6 and 9 h of treatment and were barely detectable after 24 h.

Table 1 shows the kinetics of TNF and IL-1 biological activities in culture supernatants from ANA-1 macrophages exposed to *C. albicans* or LPS. Untreated macrophages produced low or undetectable levels of TNF activity. Exposure of macrophages to H-*Candida* resulted in the rapid production of TNF, which reached maximal levels after 3 h and gradually decreased at later times. The pattern of TNF production by macrophages incubated with Y-*Candida* also showed a time-dependent increase, with a maximal level at 6 h. In all experimental groups, a gradual reduction of TNF levels was observed at 9 and 24 h. While low levels of IL-1 secretion were

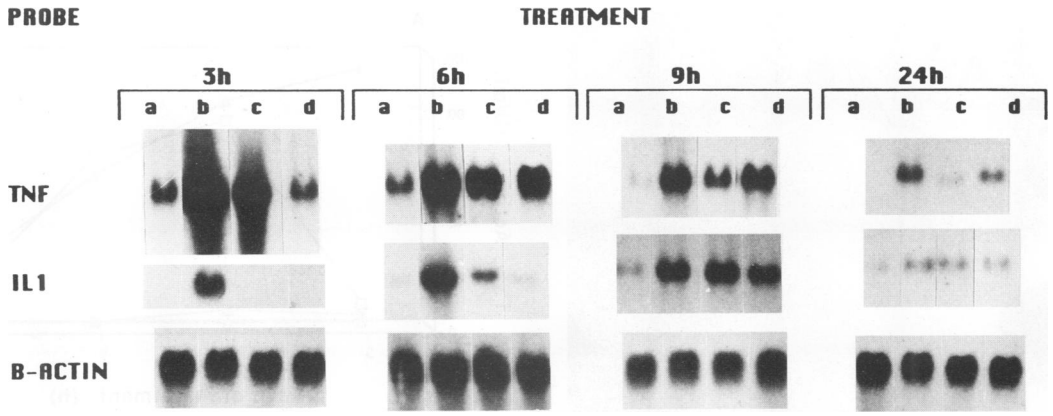

FIG. 1. Kinetics of TNF- and IL-1-specific mRNA levels in ANA-1 macrophages exposed to *C. albicans* or LPS. ANA-1 macrophages were untreated (lanes a) or exposed to LPS (lanes b), H-*Candida* (lanes c), or Y-*Candida* (lanes d) for the indicated times. Northern blot analysis on total cellular RNA then was performed as described in Materials and Methods. The results of one representative experiment of three performed are shown.

found in untreated macrophages, macrophages exposed to H-*Candida* showed a time-dependent increase in the secretion of IL-1, which reached a maximum at 9 h and decreased within 24 h. Macrophages treated with Y-*Candida* secreted IL-1, although to a lesser extent. Moreover, ANA-1 macrophages produced IL-1 and TNF in a time-dependent manner when stimulated with LPS.

Microscopic examination of the cultures revealed that incubation times of ≥3 h resulted in morphogenetic transition to the hyphal form of the yeasts which either had been ingested or had escaped macrophage ingestion (Fig. 2). In particular, cocultures incubated for 3 h (Fig. 2A) and 9 h (Fig. 2B) showed hyphae within and outside the macrophages, whose morphology was altered according to whether the microorganisms were growing intracellularly.

To investigate the potential involvement of the dimorphic changes in the kinetic patterns described above, we exposed ANA-1 macrophages to the agerminative strain PCA-2, to killed Y-*Candida*, and to killed H-*Candida*. Production of TNF and IL-1 was measured after 3, 6, 9, and 24 h of incubation. We observed that killed H-*Candida* was effective in inducing both TNF (Fig. 3A) and IL-1 (Fig. 3B) in a time-dependent manner similar to that observed upon exposure to viable hyphae (Table 1). In contrast, unlike viable Y-*Candida*, neither killed Y-*Candida* nor PCA-2 induced significant levels of TNF (Fig. 3A) or IL-1 (Fig. 3B) activity at any of the time points tested.

To establish the effect of pretreatment with rTNF on the above-described phenomenon, cytokine production was assessed as biological activity or as specific mRNA levels in macrophages pretreated for 3 and 18 h with rTNF (250 U/ml), washed, and then exposed to H-*Candida*, Y-*Candida*, or LPS for 3 h. As shown in Fig. 4, exogenous addition of rTNF affected macrophage secretory responses. The effect was strictly dependent upon the pretreatment time employed. In particular, a 3-h pretreatment resulted in consistent enhancement of TNF (Fig. 4A) and IL-1 (Fig. 4B) production in response to all stimuli tested. In contrast, 18 h of pretreatment with rTNF rendered macrophages unable to respond to any stimuli. Molecular analysis confirmed the pattern of results observed at the biological level. In fact, Fig. 5 shows that neither 3 nor 18 h of rTNF pretreatment modified the basal levels of TNF and IL-1 transcripts. The 3-h pretreatment drastically enhanced TNF and IL-1 mRNA levels in macrophages exposed to LPS, H-*Candida*, or Y-*Candida*. In contrast, 18 h of rTNF pretreatment down-regulated levels of both transcripts similarly in all experimental groups.

The role of endogenously produced TNF in the secretory activity of macrophages exposed to LPS or H-*Candida* was also investigated. For this purpose, an anti-TNF Ab and normal rabbit serum (unrelated Ab) were added to the cultures of ANA-1 macrophages exposed to H-*Candida* or LPS. After 3 and 6 h of incubation, quantitative evaluation of transcript levels in RNA samples was performed by slot blot analysis. As shown in Table 2, the presence of the anti-TNF Ab resulted in a marked decrease of TNF mRNA levels in macrophages exposed to H-*Candida* or LPS for 6 h. In contrast, no differences were observed in parallel cultures exposed to the anti-TNF Ab with H-*Candida* or LPS for 3 h.

TABLE 1. Kinetics of TNF and IL-1 secretion by ANA-1 macrophages exposed to *C. albicans* or LPS

Treatment[a]	TNF (U/ml)[b]				IL-1 (pg/ml)[b]			
	3 h	6 h	9 h	24 h	3 h	6 h	9 h	24 h
None	ND[c]	2 ± 0.5	4 ± 2	ND	1.1 ± 0.3	8.4 ± 0.6	11.3 ± 0.7	1.4 ± 0.2
H-*Candida*	138 ± 12	95 ± 15	40 ± 6	20 ± 8	8.2 ± 0.5	20.5 ± 0.7	31.2 ± 0.4	4.5 ± 0.7
Y-*Candida*	4 ± 4	86 ± 15	55 ± 8	14 ± 6	2.3 ± 0.4	13.1 ± 0.5	26.5 ± 0.8	3.9 ± 0.9
LPS	155 ± 25	125 ± 20	94 ± 14	30 ± 10	38.7 ± 0.8	48.3 ± 0.9	28.5 ± 0.4	1.6 ± 0.7

[a] ANA-1 macrophages were untreated or incubated in the presence of H-*Candida*, Y-*Candida*, or LPS for the indicated times. At each time point, cell-free supernatants were harvested and assessed for TNF and IL-1 activities as described in Materials and Methods.

[b] Data represent the means ± standard errors of the means of values from four separate experiments. The value from each experiment was the mean of triplicate determinations.

[c] ND, not detectable.

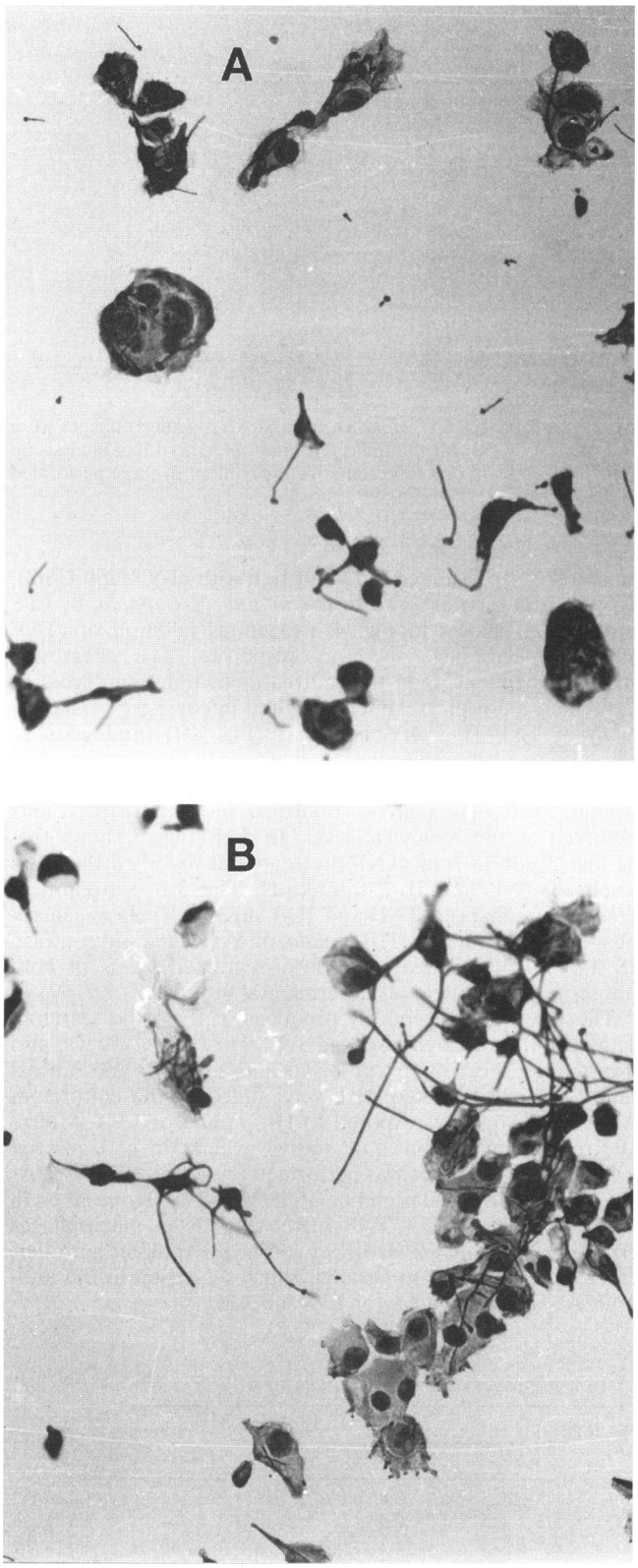

FIG. 2. Microscopic examination of ANA-1 macrophages and Y-*Candida* cocultures. ANA-1 macrophages were incubated for 3 h (A) and 9 h (B) with Y-*Candida*. The cocultures were then stained with periodic acid-Schiff stain and photographed with a Zeiss microscope. Magnification, ×400.

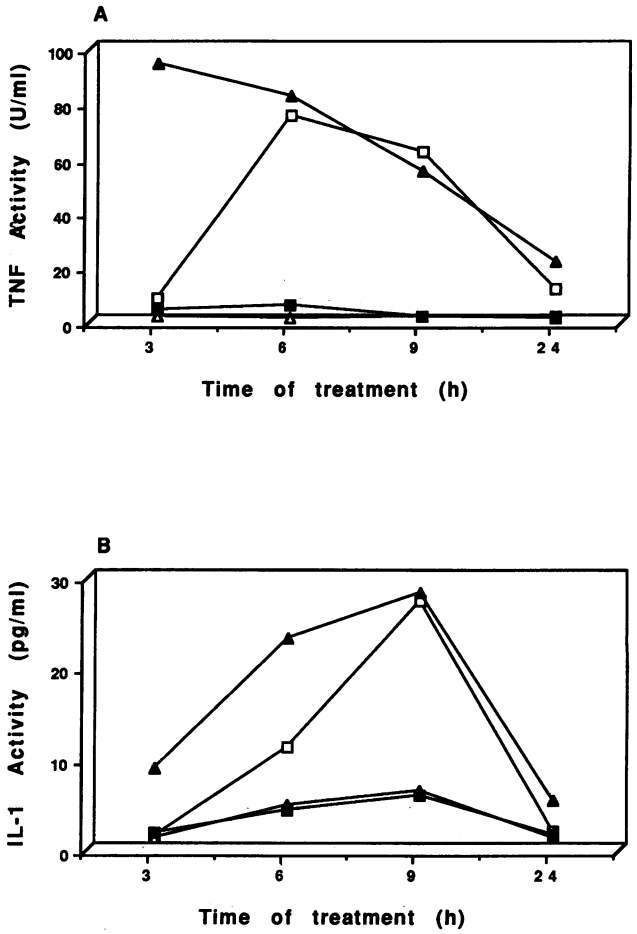

FIG. 3. Kinetics of TNF and IL-1 activities in supernatants from ANA-1 macrophages exposed to killed Y-*Candida* or PCA-2. ANA-1 macrophages were exposed to viable Y-*Candida* (□), killed Y-*Candida* (△), killed H-*Candida* (▲), or PCA-2 (■) for 3, 6, 9, and 24 h. Cell-free supernatants were then harvested and assessed for TNF (A) and IL-1 (B) activities as described in Materials and Methods. The results shown are from one representative experiment of three performed. The values are the means of triplicate determinations. Standard deviations, which were less than 5%, are not shown.

DISCUSSION

In the present paper, we demonstrate the autocrine and paracrine regulatory role of TNF in the macrophage secretory response to the dimorphic fungus *C. albicans*.

Recently, it has been reported that macrophages are able to distinguish between the yeast and hyphal forms of *C. albicans* (7). Although they exert antifungal activity against both fungal forms (4, 35), macrophages identify only the nonphagocytable H-*Candida* as a secretory stimulus. In particular, early interaction (1 to 3 h) between ANA-1 macrophages and H-*Candida*, but not Y-*Candida*, results in a consistent augmentation of TNF activity, as evaluated at both the biological and molecular levels (7).

Using long-term kinetic experiments (3 to 24 h), we show here that macrophages exposed to H-*Candida* produce TNF in a time-dependent manner, with the shape of the curve resembling that observed upon incubation with LPS. Most of the activity is detected within the initial 3 to 6 h, whereas prolonged incubation times result in a gradual decrease in activity,

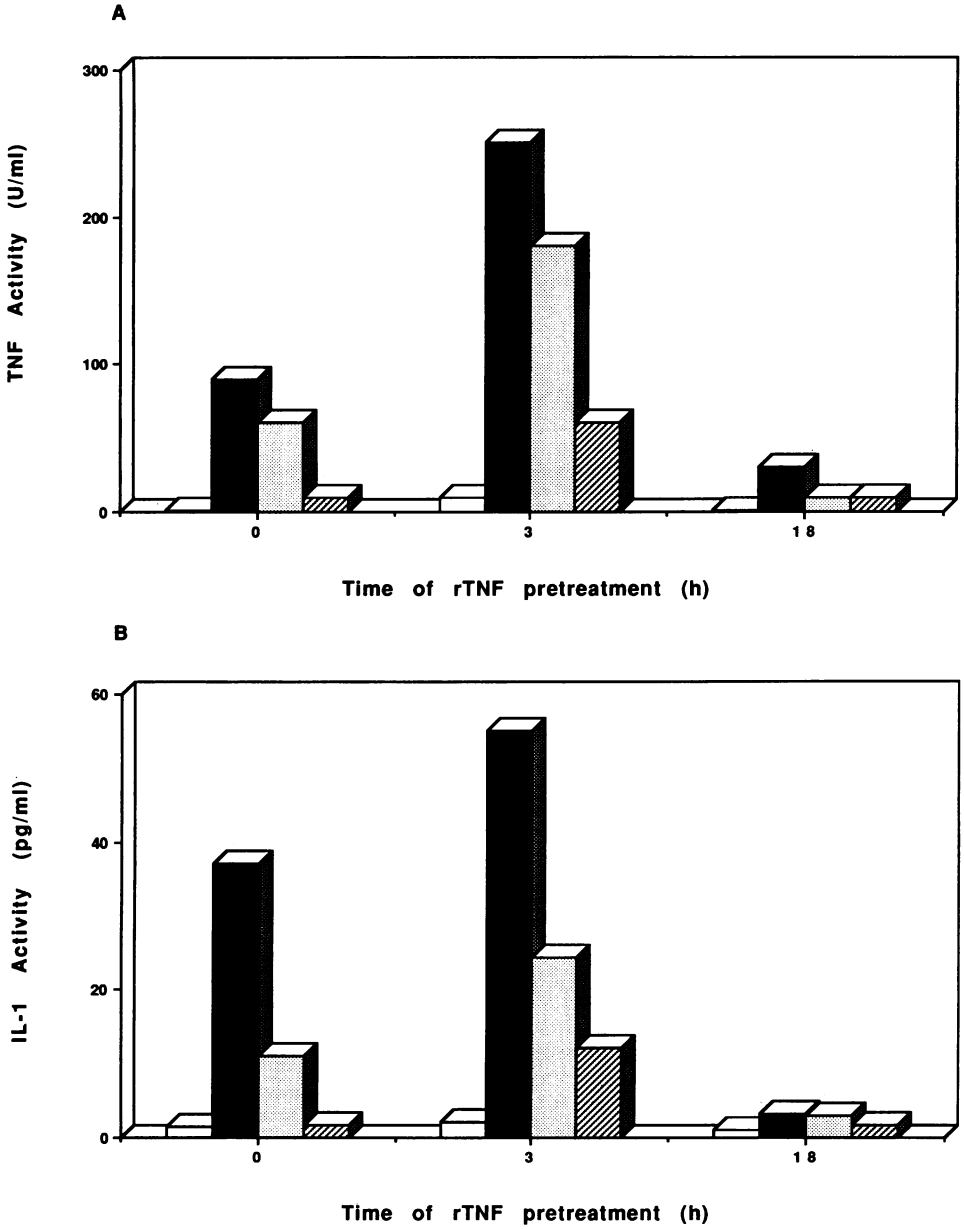

FIG. 4. Effect of rTNF pretreatment on secretory activity of ANA-1 macrophages exposed to *C. albicans* or LPS. ANA-1 macrophages not pretreated or pretreated with rTNF (250 U/ml) for 3 and 18 h were washed and then either untreated (□) or exposed to LPS (■), H-*Candida* (▩), or Y-*Candida* (▨) for 3 h. Cell-free supernatants were then harvested and assessed for TNF (A) and IL-1 (B) activities as described in Materials and Methods. The results are from one representative experiment of five performed. The values are the means of triplicate determinations. Standard deviations, which were less than 5%, are not shown.

likely attributable to a reuptake of the cytokine by the macrophages. Moreover, unlike what was previously shown in short-term studies (7), by prolonging the coincubation time to 6 h or more, we observed TNF production even in macrophages exposed to Y-*Candida*. According to these results, two hypotheses can be formulated. First, both H-*Candida* and Y-*Candida* are recognized as secretory stimuli by macrophages, whose different response kinetics likely reflect different mechanisms of TNF induction by each of the two morphogenetic forms of *Candida*. Alternatively, the dimorphic transition, occurring during macrophage–Y-*Candida* cocultures of >3 h, may be responsible for the TNF production observed. In order to determine which hypothesis is correct, two experimental approaches were employed. Microscopic evaluation of 3-h cocultures stained with periodic acid-Schiff stain demonstrates that most of the microorganisms have undergone the transition to the hyphal form, regardless of their intra- or extracellular localization. At later times, the hyphal size gradually increases in such a way that macrophage morphology is modified according to whether the fungi are growing inside the cell. Furthermore, experiments with killed Y-*Candida* and the agerminative strain PCA-2 demonstrate that macrophages exposed to the yeast form are unable to produce TNF, regardless of the coincubation time. Taken together, these results support the

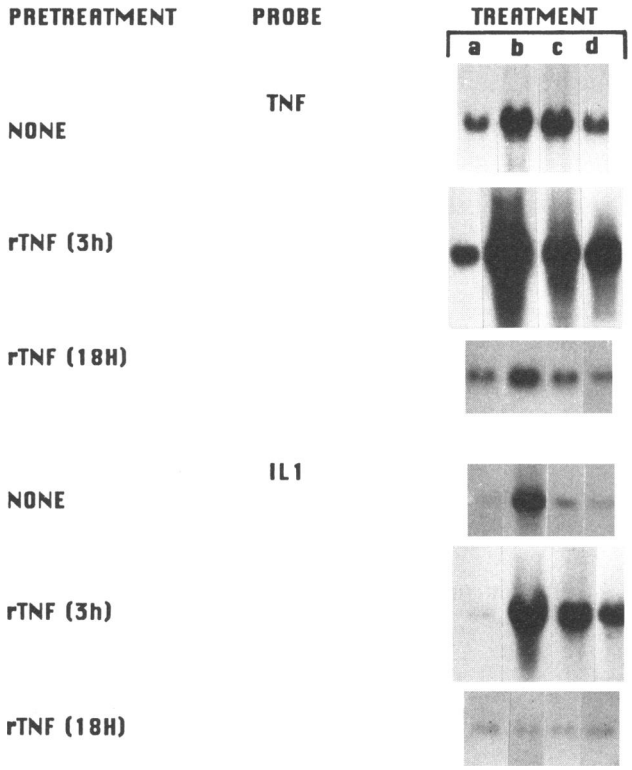

FIG. 5. Effect of rTNF pretreatment on the ANA-1 macrophage secretory response to *C. albicans* or LPS, analyzed at the molecular level. ANA-1 macrophages pretreated with rTNF (250 U/ml) for the indicated times were untreated (lane a) or were exposed to LPS (lane b), H-*Candida* (lane c), or Y-*Candida* (lane d) for an additional 3 h. Northern blot analysis was then performed as described in Materials and Methods. The results shown are from one representative experiment of three performed.

conclusion that the fungus acquires macrophage-activating properties by the dimorphic transition to hyphal form, which occurs despite the presence of macrophages. While we provide initial evidence on the heat-stable properties of the fungal structures mediating the phenomenon (i.e., killed H-*Candida* is as effective as viable H-*Candida* in inducing the macrophage response), their biochemical nature remains to be elucidated.

TABLE 2. Effect of anti-TNF Ab on TNF mRNA levels produced by ANA-1 macrophages exposed to H-*Candida* or LPS

Treatment[a]	Incubation time (h)	TNF mRNA level[b] with:	
		Unrelated Ab	Anti-TNF Ab
H-*Candida*	3	5.7	5.5
	6	4.3	1.5 (65.1)
LPS	3	6.6	6.7
	6	5.6	2.5 (55.4)

[a] ANA-1 macrophages were exposed to H-*Candida* or LPS in the presence of an unrelated Ab (1:100) or an anti-TNF Ab (1:100) for 3 and 6 h.

[b] Slot blots were prepared with total cellular RNA (5, 2.5, 1.25, and 0.16 μg) and hybridized with the probe for TNF. Results are expressed as arbitrary optical density units, which were calculated from scanning densitometric analysis of X-ray films by comparing the optical density of H-*Candida*- or LPS-treated macrophages with that of untreated controls. In parentheses is the percent inhibition (anti-TNF Ab-treated versus unrelated Ab-treated macrophages). Results are from one representative experiment of three performed.

Also, the potential involvement of contaminating minimal amounts of endotoxin has been ruled out by the addition of polymyxin B, which does not alter the expected pattern of macrophage response (data not shown).

In vitro and in vivo studies show the beneficial effects of TNF in fungal infections (4, 15, 17, 31, 33, 37). The pleiotropic role of this cytokine also involves regulation of soluble factor production by a variety of cell types (21). The present report provides direct evidence that TNF produced upon H-*Candida* stimulation has autocrine and paracrine regulatory effects on macrophages. Addition of an anti-TNF Ab consistently impairs endogenous TNF production, while pretreatment with exogenous rTNF enhances TNF transcription and secretion in macrophages exposed to either H-*Candida* or LPS. Furthermore, macrophages pretreated with rTNF become responsive to Y-*Candida*, with a significant enhancement of TNF production. This observation suggests that not only the morphogenetic status of the fungus but also the stage of macrophage activation influences the pattern of the secretory response. Such a conclusion is supported by recent data indicating that macrophages from different anatomic sites differ in their secretory responses to the dimorphic fungus *C. albicans* (8). The rTNF potentiating effects are dose dependent (data not shown) and strictly related to the time of pretreatment, since prolonged exposure (18 h) to rTNF down-regulates any transcriptional and secretory activities of macrophages, through mechanisms still unknown.

The protective activity of IL-1 in systemic and localized *Candida* infections has been reported (25, 26, 39), as has the activating role exerted in vitro by this cytokine on macrophage candidacidal activity (4, 42). IL-1 production occurs in human mononuclear cells and murine peritoneal macrophages following exposure to TNF (1, 14). In our model, IL-1 production is observed in ANA-1 macrophages exposed to H-*Candida*. Interestingly, the kinetics of production is delayed with respect to that of TNF (6 versus 3 h), while LPS induces both transcription and production of the two cytokines in the same time-dependent fashion. Taken together, the earlier data (1, 14) and the present data suggest that IL-1 production in response to H-*Candida* is mediated by endogenous TNF. This hypothesis is supported by preliminary experiments showing that an anti-TNF Ab significantly impairs IL-1 production by H-*Candida*-treated macrophages.

As previously reported (4, 35, 42), macrophages exert anti-*Candida* activity against both morphogenetic forms of *C. albicans* in a time- and effector-to-target cell ratio-dependent fashion. The effector-to-target cell ratio of 1:1 employed in our experimental protocol, although below the optimal level for observing antifungal activity (4, 35, 41), allows detection of the macrophage secretory response to *C. albicans*. Thus, we suggest that macrophages are proficient secretory cells during fungal infections, even when their potential role as killer cells is unlikely to occur.

Morphological studies allow us to conclude that, once ingested, the yeast may survive and eventually undergo transition to the hyphal form. Nevertheless, macrophages retain the capacity to act as immunocompetent cells by secreting cytokines. It remains to be clarified whether the macrophage-activating properties should be ascribed to the intracellular hyphae or rather to the few uningested *Candida* cells, which are indistinguishable from each other in their abilities to undergo transition.

Overall, by demonstrating the involvement of endogenous TNF on the macrophage secretory response to *C. albicans*, our findings add to the understanding of the complex cytokine

circuits controlling amplification of immune responses during fungal infection.

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