Immunocompetence of macrophages in rats exposed to Candida albicans infection and stress

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Rodríguez-Galán, María Cecilia, Claudia Sotomayor, María Eugenia Costamagna, Ana María Cabanillas, Beatriz Saldo Rentería, Ana Maria Masini-Repiso, and Silvia Correa. Immunocompetence of macrophages in rats exposed to Candida albicans infection and stress. Am J Physiol Cell Physiol 284: C111–C118, 2003. First published August 28, 2002; 10.1152/ajpcell.00160.2002.—The integration of innate and adaptive immune responses is required for efficient control of Candida albicans. The present work aimed to assess, at the local site of the infection, the immunocompetence of macrophages in rats infected intraperitoneally with C. albicans and exposed simultaneously to stress during 3 days (CaS group). We studied the 1) ability to remove and kill C. albicans, 2) tumor necrosis factor-α (TNF-α) release, 3) balance of the inducible enzymes NO synthase (iNOS) and arginase, and 4) expression of interleukin (IL)-1 receptor antagonist (ra) mRNA. Compared with only infected animals (Ca group), the number of colony-forming units was significantly higher in CaS rats (P < 0.01), and the macrophage candidicidal activity was ~2.5-fold lower (P < 0.01). Release of TNF-α was diminished in both unstimulated and heat-killed C. albicans restimulated macrophages of the CaS group (Ca vs. CaS, P < 0.03 and P < 0.05, respectively). In Ca- and CaS-group rats, the rates for both the arginase activity and the NO synthesis were significantly enhanced. However, the stress exposure downregulated the activity of both enzymes (CaS vs. Ca, P < 0.05). After in vitro restimulation, the IL-1ra/IL-1β ratio was significantly diminished in CaS-group rats (P < 0.05). Our results indicate that a correlation exists between early impairment of macrophage function and stress exposure. 

CELL-MEDIATED IMMUNITY is essential for host protection against virtually all fungal pathogens, and in individuals with intact immune function, the defense mechanisms are highly efficient at preventing fungal diseases (40). The yeast Candida albicans has evolved as a successful commensal in healthy persons (31) and is also a member of the oral and gastrointestinal flora in immunocompetent humans; however, in immunocompromised hosts, the transition of C. albicans into an opportunistic pathogen is not uncommon, and disseminated candidiasis of endogenous origin may occur (22). A proper integration between the innate and the adaptive immune systems is required for efficient control of C. albicans (40, 41). Resistance to C. albicans infection is determined by phagocytic effector mechanisms enhanced by Th-1 cytokines (40, 41). Regulation of the early fungal burden, the cytokine production, and the expression of costimulatory molecules are possible pathways through which the innate immune system may condition the development of the adaptive response.

Evidence derived from experimental studies demonstrates the importance of polymorphonuclear neutrophils (PMNs) for preventing candidal dissemination. A direct correlation between the level and duration of neutropenia and the host’s susceptibility to candidiasis exists (43). However, the occurrence of systemic candidiasis in hosts with normal PMN function suggests a protective role for other cells including mononuclear phagocytic cells (47). In contrast to several negative reports on the role of macrophages (40, 41, 47), many studies support the involvement of these cells in the resistance to C. albicans infection (21). Accordingly, the selective elimination of macrophages in euthymic (21, 34) or athymic (34) mice is associated with a slow clearance and an enhanced susceptibility to candidiasis.

Thus far, characterized factors that predispose one to C. albicans infection include prolonged use of broad-spectrum antibiotics, disruption of the gastrointestinal barrier by cytotoxic drugs, neutropenia, or T-cell dysfunction (31, 43). Recently we developed an experimental model of candidiasis that is suitable to study the contribution of innate effector cells in host protection against this fungus (38, 39). We found severe alterations in the immune response against C. albicans in rats infected and exposed to chronic varied stress (CVS) during 10 days (38). Interestingly, already after 3 days of stress exposure, we observed a bigger fungal burden in kidneys and livers and a poor inflammatory.

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response in these organs. The number of phagocytes (macrophages and neutrophils) recruited at the site of the infection was significantly reduced (39), and the candidicidal activity was diminished (38). The integrin Mac-1, which is involved in migration and macrophage activation, was also significantly diminished after CVS exposure (39). However, PMN oxidative burst was not impaired (38), which is in agreement with previous reports of the resistance of neutrophils to endogenous doses of glucocorticoids (42). The present work aimed to assess at the local site of the infection the immunocompetence of macrophages from rats exposed to C. albicans infection and stress during 3 days. We studied the 1) ability to remove and kill C. albicans, 2) tumor necrosis factor-α (TNF-α) release, 3) balance of the inducible enzymes nitric oxide synthase (iNOS) and arginase, and 4) expression of interleukin (IL)-1β and IL-1 receptor antagonist (ra) mRNA.

METHODS

Animals. Outbred female Wistar rats (body wt, 100–150 g) were collectively housed in cages in the experimental room for at least 3 days before experiments were started. The rats were maintained at 22°C under a 12:12-h light-dark cycle with light onset beginning at 0700. Animals had continuous access to food and water except when food was removed from the stressed groups for 24 h as part of the stress procedure.

Microorganism and infection. The pathogenic C. albicans, strain no. 387, was taken from the stock culture collection of the Mycology Division, Department of Clinical Biochemistry, Faculty of Chemical Science, National University of Córdoba. Yeast cells were grown on Sabouraud glucose agar slant at 28°C, maintained by weekly subculture on the same medium, and periodically checked for assimilation pattern and virulence. For each infection, yeast cells were harvested after 48 h of culture, centrifuged at 1,000 g, washed twice in sterile 0.15 M NaCl supplemented with 0.1% gentamicin, counted, and diluted to the desired concentration. The number of viable cells was checked in triplicate by counting the colony-forming units (CFUs) on the Sabouraud agar after 48 h of incubation at room temperature (RT).

Stress procedure. Animals were exposed to different stressors between 1400 and 1600 except for food deprivation, which lasted for 24 h. In our model of candidiasis and stress (38, 39), rats are infected and exposed to a CVS paradigm that involves a different stressor each day and lasts 10 days (14). In the present work, we focused on the impairment of the immune response observed after 3 days of treatment as reported previously (38, 39). The stress procedure described (see Experimental design) is the fraction of the CVS paradigm that corresponds to the first 3 days, and includes day 0, swim (4°C for 5 min); day 1, restraint (for 2 h); and day 2, food deprivation (for 24 h). Protocols were approved by the Animal Experimentation Ethics Committee, Faculty of Chemical Science, National University of Córdoba.

Experimental design. Rats were assigned to one of four treatment groups: the uninfected and unstressed normal (N), stressed (S), C. albicans-infected (Ca), and infected and stressed (CaS) groups. Rats were infected intraperitoneally with a volume of 1 ml of inoculum (8 × 10⁶ yeasts/ml) on day 0, and stress was imposed immediately after the infection and during the next 2 days. On day 3, animals were killed by decapitation, and peritoneal cells (PCs) were obtained by sterile lavage with 25 ml of RPMI 1640 without phenol red (Sigma) supplemented with 0.1% gentamicin and 50 U/ml heparin.

Peritoneal CFU determination. A 0.5-ml aliquot of peritoneal lavage fluid (from the 20 ml recovered) obtained as described was surface-plated in duplicate on Sabouraud glucose agar. The CFU were counted after 48 h of incubation at RT. Means and SE values were calculated from five rats per group. Experiments were performed three times. Results are expressed as CFU.

Macrophage purification. PCs were adjusted to 2 × 10⁶ cells/ml in RPMI 1640 that contained 10% fetal calf serum and 0.1% gentamicin. Macrophages were purified by adherence during a 2-h incubation (at 37°C with 5% CO₂) and depending on the experiment, 24- or 96-multiwell plates were used. Nonadherent cells were removed by washing the plates twice with cold RPMI 1640 medium. In our experimental condition, adherent cells were ~50% of the PCs plated with a purity of >90% according to morphological analysis or non-specific esterase (Sigma) staining (35). No changes in adherence properties were observed in cells from stressed animals, which is in agreement with previous reports (36).

Killing of C. albicans by macrophages. The candidicidal activity of macrophages was assessed by a colorimetric assay (7). Briefly, C. albicans-macrophage coculture experiments were set up in 96-well, flat-bottom plates (Corning) in a final volume of 200 μl; viable C. albicans were diluted appropriately in RPMI 1640 that contained 0.1% gentamicin, and 10% of normal rat serum (NRS) was added to macrophages at an effector-target (E/T) cell ratio of 100:1. After 72 h (at 37°C with 5% CO₂), plates were treated with 0.1 ml of 1% Triton X-100 in distilled water to lyse the macrophages and were washed twice with 0.1 ml of distilled water. Then 0.1 ml/well of RPMI 1640 without phenol red that contained 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) was added, and the plates were incubated for 2 h at 37°C. After the incubation, the plates were centrifuged, the supernatants were discarded, and the pellets were resuspended in 0.2 ml of isopropanol. When the dark-blue formazan crystals had dissolved, the reaction was read at 540 nm using an automated microplate reader (TiterTek MultiScan). The color developed is proportional to the fungal mass. Wells containing only isopropanol were used as blanks. Control wells containing C. albicans or macrophages alone were included in each experiment. Each sample was assessed in triplicate. The candidicidal activity was expressed as a candidicidal index (CI) that was calculated as follows: CI = [mean optical density (OD) from N group/OD for each rat].

TNF-α production. Purified macrophages from the Ca and CaS groups were cultured with or without opsonized, heat-killed C. albicans (E/T ratio, 100:1), and after 3 or 6 h of incubation, supernatants were sampled to determine TNF-α quantities via an ELISA sandwich protocol. Briefly, 96-well plates were coated with primary anti-TNF-α capture antibody (10 μg/ml; Pharmingen) and blocked with PBS that contained 10% BSA. Supernatants were incubated overnight at 4°C before the diluted biotinylated anti-TNF-α-detecting monoclonal antibody (2 μg/ml) was added. The plates were developed by adding avidin peroxidase and its substrate, and absorbance was measured at 492 nm in a microplate reader. The amount of TNF-α was extrapolated from the standard curve, which was generated in 1:2 dilutions. Results are expressed in picograms.

Assessment of arginine metabolism. Purified macrophages were incubated without stimulation during 48 h (at 37°C with 5% CO₂), and culture medium and cells were sampled for NO measurement and arginase assay, respectively. We determined NO as nitrite in culture supernatants via a
microplate-assay method using Griess reagent (2): 100-μl aliquots were mixed with 200 μl of Griess reagent [1.5% sulfanilamide in 1 N HCl and 0.13% N-(1-naphthyl)ethylenediamine dihydrochloride in H2O]. Absorbance was measured at 540 nm in a microplate reader. Nitrite was measured by using NaNO2 as a standard. Each sample was tested in triplicate, and results are expressed in micromoles (17).

To assess arginase activity, cells were washed with PBS and treated with 0.15 ml of 0.1% Triton X-100 that contained protease inhibitors (13). After 30 min, cell lysate was mixed in a 1:1 ratio with 10 mM MnCl2, and the enzyme was activated by heating for 10 min at 56°C. Arginine hydrolysis was conducted by adding 0.5 M l-arginine at pH 9.7 to the activated lysate. Incubation was performed at 37°C for 60 min, and the reaction was stopped with 400 μl of a solution that contained 96% H2SO4, 85% H3PO4, and H2O (a 1:3:7 ratio, vol/vol/vol). The urea formed was colorimetrically quantified at 540 nm after the addition of 25 μl of 9% α-isonitrosopropiophenone (dissolved in 100% ethanol), which was followed by heating at 100°C for 45 min. After 10 min, the OD of the dark, the OD of the dark was determined in a microplate reader. Each sample was tested in triplicate. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of urea per minute. Results are expressed as milliunits per 10^6 cells.

**Immunoblot analysis.** iNOS expression was assessed in macrophages that were purified in 24-well, flat-bottom plates (Corning) and cultured with or without opsonized, heat-killed C. albicans (E/T ratio, 100:1) for 24 h. Whole cell extracts were prepared as described for arginase activity. Equal amounts of protein (30 μg/lane) were fractionated in a 10% SDS-PAGE, and proteins were electrotransferred onto nitrocellulose membranes. The anti-iNOS polyclonal antibody (Santa Cruz) was diluted to 2 μg/ml and incubated overnight. Immunodetection was performed with the enhanced chemiluminescence detection kit and was followed by exposure for 3–5 min to Amersham Hyperfilm (Uppsala, Sweden).

**RNA isolation.** Macrophages were purified in 100-mm plastic tissue-culture plates. For stimulation experiments, heat-killed C. albicans diluted in RPMI 1640 with 0.1% gentamicin and 10% NRS was added to macrophages (E/T ratio, 100:1) for 24 h. Whole cell extracts were prepared as described for arginase activity. Equal amounts of protein (30 μg/lane) were fractionated in a 10% SDS-PAGE, and proteins were electrotransferred onto nitrocellulose membranes. The anti-iNOS polyclonal antibody (Santa Cruz) was diluted to 2 μg/ml and incubated overnight. Immunodetection was performed with the enhanced chemiluminescence detection kit and was followed by exposure for 3–5 min to Amersham Hyperfilm (Uppsala, Sweden).

**Northern blot analysis.** Total RNA (12 μg) was subjected to electrophoresis on 1% agarose gel that contained 0.66 M formaldehyde (8). RNA was transferred to nylon membranes by capillary transblotting overnight, baked for 2 h at 80°C, and prehybridized at 42°C in a solution that contained 50% formamide, 5× Denhardt’s solution, 5× sodium chloride-sodium phosphate-EDTA (SSPB), 1% SDS, and 200 μg/ml denatured herring testes DNA. The membranes were then hybridized overnight at 42°C in the same solution, which contained 1-32P-labeled rat IL-1β cDNA, human IL-1α, or an 18S ribosomal RNA (rRNA) probe (20, 25). Blots were washed in 2× sodium chloride-sodium citrate (SSC) that contained 1% SDS at RT, washed twice in 2× SSC with 1% SDS at 55°C, and exposed to Kodak X-Omat film at −70°C. The band intensities were determined via scanning densitometry (Shimadzu Dual-Wavelength Chromato Scanner CS-930) at 500 nm, and the absorbance values of the IL-1β and IL-1ra signals in arbitrary densitometric units were normalized to those of the 18S rRNA in the same lane.

**Serum corticosterone assay.** To determine corticosterone levels, rats were kept undisturbed the night before the experiment. Animals from all experimental groups were killed by decapitation within <3 min of entering the room. Sampling from all rats was carried out within 30 min. Blood was allowed to clot for 45 min, and serum was obtained after centrifugation. Serum corticosterone concentration was determined as previously described (29), and results are expressed as micrograms of corticosterone per deciliter.

**Statistical analysis.** Differences between group means were assessed using ANOVA followed by the Student-Newman-Keuls test for multiple comparisons. A P value <0.05 was considered statistically significant.

**RESULTS**

Increased number of C. albicans and impaired killing activity of macrophages at infection site after stress exposure. Rats infected intraperitoneally with C. albicans and exposed to CVS during 10 days show an increased fungal burden in kidneys and livers together with a poorly developed inflammatory response in these organs (38). The effect of CVS on the immune response against the fungus is significantly pronounced after 3 days of treatment (38, 39). First, we determined the number of viable C. albicans in peritoneal lavage aliquots of infected (Ca) and infected and stressed (CaS) rats. As shown in Fig. 1A, the number of CFU was significantly higher in CaS-group rats compared with the Ca group (P < 0.01), which suggests a reduction of the lytic activity of PCs. Because the aim
of our work was to evaluate the immunocompetence of macrophages exposed to *C. albicans* infection and stress, we assessed the candidicidal activity of purified macrophages after restimulation in vitro with *C. albicans* to define the extent of the impairment of macrophage function (Fig. 1B). The candidicidal index was ~2.5-fold lower in CaS-group rats compared with only infected animals (*P* < 0.01). Clearly, the stress exposure conditioned a poor clearance of *C. albicans* early after the infection.

**TNF-α production.** In response to pathogen constituents, macrophages release a battery of cytokines and other inflammatory mediators that have important local and systemic effects (7, 40, 41). In particular, TNF-α is an inducer of a local inflammatory response that helps to contain infections (24, 30, 33, 41). In this context, we evaluated the production of TNF-α after 3 days of treatment in purified macrophages from the Ca and CaS groups with or without in vitro stimulation (Fig. 2). In 3-h cultures, the release of TNF-α was significantly diminished in unstimulated macrophages of the CaS group compared with the Ca group (*P* < 0.03), and the same result was obtained after restimulation with opsonized, heat-killed *C. albicans* (Ca vs. CaS group, *P* < 0.05). A similar trend was observed after 6 h of incubation (data not shown).

**Downregulation of iNOS-arginase activity in peritoneal macrophages of infected rats.** In response to infection by several pathogens and inflammatory cytokines, noticeable changes occur in macrophage arginine metabolism (1, 28). These include, for instance, increases in NO synthesis via iNOS and catabolism of arginine to ornithine and urea via arginase. We assessed the release of NO and the arginase activity in unstimulated purified macrophages cultured in vitro for 48 h. As shown in Fig. 3, A and B, in infected rats (Ca and CaS groups), both the arginase activity and the NO synthesis were significantly enhanced compared with N and S groups (*P* < 0.01). However, whereas the Ca group exhibited a 10-fold increase in arginase activity (Fig. 3A), the stress exposure downregulated significantly the activity of this enzyme in the CaS animals (*P* < 0.05). A similar pattern of response was observed for the release of NO (Fig. 3B), which suggests that although the infection with the fungus induced the two metabolic pathways, the stress products downregulated the activity of both enzymes.

Furthermore, the expression of the enzyme iNOS was evaluated in lysates of unstimulated macrophages or macrophages restimulated with opsonized, heat-killed *C. albicans* for 24 h. As shown in Fig. 3C, in unstimulated macrophages of infected groups, increased expression of iNOS was observed; however, in the in vitro restimulation with the fungus was associated with diminished expression of the enzyme in the CaS group.

**IL-1β and IL-1ra mRNA expression.** Upon exposure to inflammatory stimuli, transcriptional activation of genes that encode proinflammatory cytokines occurs. Therefore, the expression of mRNA for IL-1β and IL-
1ra was evaluated in macrophages after 3 days of treatment. As shown in Fig. 4, A and B, in unstimulated macrophages, an approximately twofold expression of IL-1β mRNA was observed in infected groups (Ca and CaS vs. N group, *P < 0.01), whereas the amount of IL-1ra mRNA relative to noninfected groups showed a clear trend without reaching significant differences (Ca and CaS vs. N group, #P < 0.03, Ca vs. CaS).

Additionally, in a separate experiment, RNA was obtained from macrophages that were restimulated in vitro with opsonized, heat-killed C. albicans for 16 h. As shown in Fig. 4, C and D, in infected groups, the expression of IL-1β was significantly diminished compared with uninfected groups (P < 0.01), whereas the expression of IL-1ra mRNA was reduced only in the CaS group (*P < 0.05).

The IL-1ra-to-IL-1 production ratio may change with certain viral infections, and some authors associate the severity of inflammatory illnesses with a decrease in this ratio (9). We calculated the ratio for infected groups in each experimental condition asayed: for unstimulated cells (Fig. 4B), ratios of 1.17 and 1.22 were obtained for the Ca and CaS groups, respectively, which suggests a sustained expression of mRNA for IL-1ra. However, after in vitro restimulation (Fig. 4D), the ratio was significantly diminished in CaS rats (1.22 and 0.93 for Ca and CaS groups, respectively; *P < 0.05) consequently with a lower expression of IL-1ra mRNA.

Corticosterone levels. In an attempt to correlate impaired killer activity and cytokine imbalance with the continuous release of products of the hypothalamus-pituitary-adrenal (HPA) axis, we determined corticosterone levels after 3 days of treatment. As shown in Fig. 5, corticosterone levels increased in stressed groups, but although a clear trend was observed in S-group rats (*P < 0.07), a significant increment was evident only in rats exposed to stress and infection (P < 0.05). Interestingly, when the S and CaS groups were exposed to an additional acute stress on day 3, a consistent increment of both ACTH and corticosterone levels was shown (data not shown). On the other hand, an additive effect of stressors was observed after 10 days of treatment, and the S and CaS groups exhibited increments in both ACTH and corticosterone levels (Ref. 38; data not shown).

Fig. 4. Northern blot analysis of interleukin (IL)-1β and IL-1 receptor antagonist (ra) mRNA expression in macrophages of different experimental groups. Total RNA was obtained from purified macrophages (~2 × 10⁶ cells) of different experimental groups after 3 days of treatment (A and B). In another set of experiments, macrophages were restimulated for 16 h with opsonized, heat-killed C. albicans (E/T ratio of 100:1; C and D). Representative autoradiograms are shown (A and C). Relative IL-1β and IL-1ra abundance was normalized with 18S rRNA and expressed as the mean of 2 or 3 separate experiments (B and D). *P < 0.01, N vs. Ca and CaS; **P < 0.05, N vs. Ca and CaS; #P < 0.03, Ca vs. CaS.

Fig. 5. Corticosterone levels. After 3 days of treatment, rats from the different groups were killed by decapitation, and sampling was carried out within 30 min. Serum corticosterone concentration was determined as described in METHODS. Results are means ± SE for at least 5 rats/group; data from 3 experiments were pooled. *P < 0.05.
DISCUSSION

Working with a model of candidiasis and stress, we demonstrated the impairment of the immune response against the fungus after exposure to CVS (38). Phenotypic and functional studies showed that macrophages were more affected by stress exposure than were PMNs (39). In this regard, our model is suitable to assess the biology of the macrophage under an integrated interplay of both neuroendocrine and immune systems as occurs in vivo. The present work aimed to determine how the immunocompetence of this cell is affected after stress exposure at the site of the infection.

Our first interesting observation was that in CaS-group rats, the number of CFU recovered from the peritoneal lavage fluid was notably larger than in animals that were only infected. Purified peritoneal macrophages showed a significantly reduced killer capacity, which suggests either a direct effect of the CVS treatment on the candidicidal activity, or a modified ability to release cytokines to respond to, or both. Phagocytosis of C. albicans by macrophages must be accompanied by killing; otherwise, macrophages could promote the dissemination of the pathogen and help the fungus to avoid the immune attack. Killing of C. albicans can be upregulated in macrophages by cytokines such as interferon (INF)-γ or TNF-α (47), chemokines, or neuroendocrine products (18, 19, 44, 51). Early during C. albicans infection, proinflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-18 participate both in the control of the fungus and in the induction of protective Th-1 immunity (40, 41). TNF-α is one of the major secretory products of macrophages (47) with an important role in host defense against disseminated candidiasis (6, 37, 49). TNF-α production is dose dependent, and blood levels are directly related to the C. albicans-inoculum in both neutropenic and nonneutropenic animals (46, 48). As expected in infected rats, the infection triggered TNF-α production. However, in infected and stressed animals, which had a higher fungal burden at the site of the infection, the spontaneous and stimulated release was diminished. Differences observed between Ca- and CaS-group animals correlated with impaired autocrine and paracrine effects of this cytokine: candidicidal activity or cell recruitment, respectively (39).

L-Arginine can be metabolized by alternative pathways that involve the enzymes iNOS and arginase (11, 26). The competition between arginase and iNOS is more pronounced when substrate availability is compromised as occurs during sepsis and in other inflammatory sites (1). Under determined experimental conditions, the induction of one enzyme is accompanied by the suppression of another, which indicates two competitive states in murine macrophages (28). Here we demonstrated that early in the infection, during the innate immune response against C. albicans, both metabolic pathways were activated. In rats only infected (Ca group), iNOS expression and NO production were significantly increased. However, in macrophages from CaS-group animals, NO production decreased after stress exposure (38) as did the enzyme that limits the NO production. In contrast to the well-documented role of iNOS in C. albicans infection (47), no data exist about the alternative l-arginine pathway. This is the first report that demonstrates the induction of arginase activity during the course of this mycosis. After fungal infection, both enzymes exhibited increased activity. However, in macrophages from CaS-group animals that were exposed simultaneously to fungus, inflammatory cytokines, and increased glucocorticoid levels, the arginase function was clearly downmodulated. In agreement with this, induction of arginase activity by lipopolysaccharide (LPS) is largely abolished by dexamethasone with levels only twofold above control values (27).

A dual-activation paradigm has been proposed for macrophages: although classically activated macrophages secrete proinflammatory cytokines such as IL-1 and TNF-α, alternatively activated macrophages preferentially express anti-inflammatory cytokines such as IL-1ra and IL-10 (28). The dichotomy also involves L-arginine metabolism and the expression of receptors implicated in attachment of microorganisms and phagocytosis: IgG receptor expression is associated with classic activation; and mannose receptor (MR), β-glucan receptor, and scavenger receptor type I are associated with the alternative activation (28, 45). β-Glucans, chitin, and mannose polymers (10) of the C. albicans wall could interact with β-glucan receptor and the MRs of macrophages, thereby activating the alternative pathway and upregulating the expression of associated products. Also, the binding of yeast to the MRs results in the elevation of mRNA of IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor (50).

In our model, the singular interaction of this opportunistic pathogen with macrophages triggers these two metabolic pathways, and stress products downmodulated both. The relevance of these findings deserves further study.

IL-1α, IL1-β, and IL-1ra belong to the IL-1 system and share the IL-1 receptors; by competitively binding to the receptor, IL-1ra neutralizes the biological actions of IL-1α and IL1-β without showing any IL-1-like activity. The main source of cytokines in this system are macrophages, and the balance between IL-1ra and IL-1 can vary on the course of some infections (9). In candidiasis, the role of IL-1 is quite controversial (40, 41, 47) and little is known about the ratio of IL-1ra to IL-1. In our model, the infection upregulated the IL-1β and IL-1ra transcripts in macrophages from both Ca- and CaS-group animals with no differences associated to stress exposure. Interestingly, after restimulation with the fungus, lower levels of IL-1β were observed in Ca- and CaS-group animals, whereas only CaS-group macrophages showed reduced levels of IL-1ra transcripts. The expression of IL-1 and IL-1ra genes may be differently regulated (3, 32). Arzt et al. (4) reported that glucocorticoids have an inhibitory effect on both genes and demonstrated that the ability of glucocorticoids to suppress the synthesis of IL-1ra after stimulation with LPS is endotoxin-dose dependent: the final
effect depends on the balance between the inflammatory stimulus and the dose of glucocorticoids employed (4). Steroids inhibit the synthesis of IL-1β by blocking the transcription or diminishing the stability of the mRNA (19). The in vivo effects of other HPA-axis hormones such as corticotropin-releasing hormone and ACTH on IL-1 system expression are less known.

The neutralization of IL-1 biological action by IL-1ra has an important physiological role during a sharp inflammatory process (9). The balance between endogenous IL-1 and IL-1ra in vivo is an important determinant of the host response to infection. In a murine myocarditis model, the increased levels of IL-1β correlated with myocardial fibrosis, whereas the increased serum levels of IL-1ra improved survival rates and decreased myocardial inflammation and fibrosis (23). The clinical severity of certain inflammatory illnesses is usually associated with a decrease in the relationship of IL-1ra to IL-1 (9). After a new contact with the fungus, CaS-group macrophages showed a diminished IL-1ra-to-IL-1 relationship; this suggests a compromised plasticity of the cell to a new challenge with the pathogen, which could have physiological relevance.

Stress can affect various facets of the immune response (5, 15). On the other hand, proinflammatory cytokines produced by activated immune cells are able to activate the HPA axis at each level: the hypothalamus, the pituitary, and the adrenal gland (16, 44). Several studies have supported a close link between the HPA axis and the adrenomedullary response during stress and have shown that this link is bidirectional in that it receives input from the nervous and immune systems (16). Serum corticosterone levels were significantly increased only in animals exposed to two associated stimuli: infection and stress. It is conceivable to speculate that both ACTH and non-ACTH-mediated input on the adrenal cortex contributed to the higher corticosterone release that was achieved in this experimental group.

Our work provides clear evidence of the in vivo relevance of macrophages during the initial control of C. albicans infection. Our findings also contribute to the understanding of how the immunocompetence of the host can be conditioned by neuroendocrine factors, thereby increasing the susceptibility for this opportunistic fungal infection.

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