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Identification of Heat Shock Protein 60 as the Ligand on Histoplasma capsulatum That Mediates Binding to CD18 Receptors on Human Macrophages

Kristin H. Long,* Francisco J. Gomez,†‡ Randall E. Morris,* and Simon L. Newman2*†

Histoplasma capsulatum (Hc) is a facultative intracellular fungus that binds to CD11/CD18 receptors on macrophages (MΦ). To identify the ligand(s) on Hc yeasts that is recognized by MΦ, purified human complement receptor type 3 (CR3, CD11b/CD18) was used to probe a Far Western blot of a detergent extract of Hc cell wall and cell membrane. CR3 recognized a single 60-kDa protein, which was identified as heat shock protein 60 (hsp60). Biotinylation of viable yeasts, followed by precipitation with streptavidin-coated beads, and Western blotting with anti-hsp60 demonstrated that hsp60 was on the surface of Hc yeasts. Electron and confocal microscopy revealed that hsp60 resided on the yeast cell wall in discrete clusters. Recombinant hsp60 (rhsp60) inhibited attachment of Hc yeasts to MΦ. Recombinant hsp60 and Abs to CD11b and CD18 inhibited binding of yeasts to Chinese hamster ovary cells transfected with CR3 (CHO3). Polystyrene beads coated with hsp60 bound to MΦ, and attachment was inhibited by Abs to CD11 and CD18. Freeze/thaw extract (F/TE), a preparation of Hc yeast surface proteins that contained hsp60, inhibited the attachment of Hc yeasts to MΦ. Depletion of hsp60 from F/TE removed the capacity of F/TE to block binding of Hc to MΦ. Interestingly, rhsp60 did not inhibit binding of Hc yeasts to dendritic cells (DC), which recognize Hc via very late Ag 5. Moreover, F/TE inhibited attachment of Hc to DC even when depleted of hsp60. Thus, Hc hsp60 appears to be a major ligand that mediates attachment of Hc to MΦ CD11/CD18, whereas DC recognize Hc via a different ligand(s).

The protein concentration was quantified by the BCA protein assay. Preparations of the F/TE extract did not contain cystolic proteins as determined by assay for lactate dehydrogenase (8), and <5% of the yeasts were lost during the freeze/thaw procedure.

Preparation of human Mφ and dendritic cells (DC)

Human monocytes were purified from buffy coats obtained from Hoxworth Blood Center (Cincinnati, OH) via sequential centrifugation on Ficoll-Hypaque and Percoll gradients (Amer sham Pharmacia Biotech, Piscataway, NJ). Mφ were obtained by culture of monocytes for 5-7 days in suspension in Teflon beakers as described previously (3). DC were derived from purified monocytes by culture at 6.5 × 10⁷/ml in medium containing human rGM-CSF (115 ng/ml) and human rIL-4 (50 ng/ml; PeproTech-Rocky Hill, NJ) for 6-8 days as described previously (9).

Isolation of human neutrophils and purification of CR3 (CD11b/CD18)

Human neutrophils were purified from buffy coats by centrifugation on Ficoll-Hypaque. Erythrocytes and PMN were separated via dextran sedimentation, and the remaining contaminating RBCs were removed by hypotonic lysis (10). The PMNs were washed in HBSS, and the cell pellet was snap-frozen in liquid nitrogen and stored at −70°C until used.

CR3 was purified from neutrophil lysates by a modification of the procedure of Diamond et al. (11). PMNs were solubilized for 1.5 h at 4°C in cold lysis buffer (100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM MgCl₂, 1% Triton X-100, 5 mM iodoacetamide, 0.025% NaN₃, 1 mM PMSF, 1 mM di-isopropylfluorophosphate, and 0.2 U/ml of aprotinin), with gentle stirring. After removal of insoluble components, CR3 was purified by affinity chromatography over tandem columns of mouse IgG and LMS/2 Ab (anti-CD11b, IgG1) coupled to cyanogen bromide-activated Sepharose (CNBr-Sepharose). CR3 was eluted with 50 mM triethylamine (pH 10.0), 300 mM NaCl, 2 mM MgCl₂, and 1% n-octyl-β-D-glucopyranoside into tubes with neutralizing buffer (10% by volume 1 M Tris-HCl, pH 7.4). Peak protein fractions were pooled, aliquoted, and stored at −70°C. The purified CR3 appeared as a single band on SDS-PAGE (data not shown).

Antibodies

mAb TS-1/22 (12), specific for CD11a (LFA-1); MN-41 (13), specific for CD11b (CR3); Leu-MS (14), specific for CD11c (CR4); and IB4 (15), specific for CD18 (β-chain), were described previously (3). LM2/1 (anti-CD11b) coupled to Sepharose was a gift from Dr. C. Parkos (Emory University, Atlanta, GA).

A polyclonal monospecific Ab to Hc hsp60 was prepared from immune serum obtained from a rabbit immunized with native Hc hsp60 (16). Partially purified IgG was obtained by precipitation with saturated ammonium sulfate to a final concentration of 50%. The IgG then was affinity-purified on a column of recombinant hsp60 (rhsp60) coupled to CNBr-Sepharose. After elution, the affinity-purified Ab was dialyzed against PBS and concentrated, and the protein concentration was determined by the BCA protein assay. The monospecificity of the Ab was verified in two ways. First, both a total cell lysate and a cytosolic preparation of Hc yeasts yielded a single band after separation by SDS-PAGE and analysis by Western blot with the Ab. Second, by flow cytometric analysis, a 10-fold molar excess of rhsp60 completely blocked the binding of the Ab to Hc yeasts (data not shown).

For use as a control in flow cytometry studies, the natural Abs to common fungal cell wall carbohydrates found in naive rabbit IgG were removed by absorption once with β-glucan (5 mg), and twice with zymosan (1 × 10⁹ particles/ml). These absorptions removed ~90% of the cross-reactivity and allowed the naive rabbit IgG to be used as a control for flow cytometry, electron microscopy, and confocal microscopy analysis. The affinity-purified anti-hsp60 IgG was treated in an identical manner without any loss of binding activity to Hc yeasts.

Preparation of Hc hsp60-coated beads

Recombinant Hc hsp60 and native Hc hsp60 (nhs60) were prepared as described previously (16, 17). Polystyrene fluorescent beads (Sigma-Atlas, St. Louis, MO) were washed with 0.1 M borate buffer, pH 4.0, and then resuspended in 1 mg/ml of rhsp60 or BSA and incubated for 24 h at 25°C. The beads were blocked by incubation in BSA (10 mg/ml). Approximately 50% of the protein was adsorbed to the beads, and bound hsp60 was confirmed by flow cytometric analysis (data not shown).

Chinese hamster ovary cells transfected with CR3 (CHO3) culture

CHO3 cells and nontransfected CHO cells (a gift from Dr. G. Ross, University of Louisville, Louisville, KY) were cultured in DMEM (Life Technologies, Rockville, MD) containing 15% heat-inactivated FCS, 16 μM thymidine, 0.1 μM methotrexate, fungizone, and 10 μg/ml of gentamicin (18). Cells were harvested by scraping after reaching 80% confluence and were passaged or used in binding experiments with Hc yeasts.

Binding of Hc yeasts to Mφ, DC, and CHO3 cells

Mφ, DC, and CHO3 cells were suspended to 2.5 × 10⁶/ml in HBSS containing 2% aprotinin. For binding assays with Mφ or CHO3 cells, the cells were adhered in the wells of a Terasaki tissue culture plate (Miles, Naperville, IL) that was coated with 1% human serum albumin. After adherence, 5 μl of test protein or HBSS as a control was added to the monolayers and incubated for 30 min (Mφ) or 1 h (CHO3) at 37°C. Five microliters of FITC-labeled HK Hc yeasts (5 × 10⁶/ml) were added to each well and incubated for 30 min (Mφ) or 2 h (CHO3) at 37°C. Unattached yeasts were removed by washing, and the monolayers were fixed in 1% paraformaldehyde. Binding of the yeasts was quantified via phase and fluorescence microscopy on an inverted microscope (Diaphot; Nikon, Melville, NY). The data are presented as the attachment index (AI), the total number of attached yeasts per 100 cells, or the percent inhibition of binding (1-experimental AI/control AI × 100).

Binding assays with DC (4 × 10⁶/ml) and 50 μl of the test protein, or HBSS as a control, were mixed in polypropylene tubes for 30 min at 37°C. Subsequently, 50 μl of Hc yeasts (2 × 10⁷/ml) were added to each tube and incubated for an additional 30 min. At the end of the incubation period, 10 μl of each sample was mounted on a clean glass slide and coverslipped for immediate quantitation of the attachment index as described above. Binding assays with CHO3 cells coated latex beads were performed in suspension with human Mφ in an identical manner.

Far Western blot analysis with purified CR3

One-dimensional Far Western immunoblotting studies of Hc proteins were performed by electrophoresis of Hc CW/M, F/TE, Hc nhsp60, or rhsp60 in a 12% SDS-PAGE gel. Separated proteins were electrobotted onto nitrocellulose membranes (Bio-Rad, Richmond, CA), blocked with 5% nonfat dried milk in TBS, and incubated for 3 h at 37°C with 25 μg of purified CR3. After washing, the membranes were incubated with anti-human CD11b, followed by HRP-conjugated, goat anti-mouse IgG. The membranes were developed with ECL substrate (Amer sham Pharmacia Biotech) and exposed on Hyperfilm ECL.

For two-dimensional electrophoresis, 100 μg CW/M was electrofocused on Immobiline isoelectric focusing strips, with a linear pH of 5-11 (Amer sham Pharmacia Biotech). After focusing was complete, the isoelectric focusing strips were electrophoresed in a 10% SDS-PAGE gel. Separated proteins on one gel were stained with Coomassie Brilliant Blue R-250, and a duplicate gel was electrotransferred to a nitrocellulose membrane and analyzed by Far Western blot analysis as described above.

Biotinylation and precipitation of surface proteins on Hc yeasts

Viable Hc yeasts (1 × 10⁷/ml) were suspended in PBS or in PBS containing 10 mg/ml of biotin (EZ-Link sulfo-NHS-LC-biotin; Pierce) and incubated overnight at 4°C. After washing, yeast pellets were resuspended in lysis buffer and mixed with an equal volume of glass beads. After sonication, the beads were removed by centrifugation, and insoluble components were removed by centrifugation. Biotinylated proteins were precipitated by incubation with streptavidin (SA) agarose beads (Pierce), and 50 μg of each SA-bead fraction and 0.25 μg of rhsp60 as a positive control were separated on a 10% SDS-PAGE gel. Biotinylated Hc hsp60 was visualized on a Western blot probed with the affinity-purified anti-hsp60 Ab.

Immunolabeling and electron microscopy of hsp60 on Hc yeasts

Hc yeasts were incubated for 1 h at 4°C with either 40 μg of absorbed, affinity-purified anti-hsp60 or absorbed normal rabbit IgG. The yeasts then were incubated with biotinylated goat anti-rabbit IgG, followed by SA-conjugated 5 nm-collodial gold. The samples were fixed in 4% paraformaldehyde/2.5% glutaraldehyde and processed for electron microscopy (19). Samples were viewed on a 100CX electron microscopy (JEOL, Peabody, MA) operating at 80 kV. In identical experiments Hc yeasts were prepared using an anti-hsp60 mAb or an isotype control Ab.

To estimate the number of hsp60 antigenic sites on the surface of Hc yeasts, we analyzed 81 experimental and 30 control electron micrographs using the standard stereological methods of planimetry and point counting (20-22).
Flow cytometric analysis of hsp60 on Hc yeasts

Hc yeasts (1 × 10^6/ml) were incubated for 1 h at 4°C with the affinity-purified anti-hsp60 or absorbed rabbit IgG as a control. After washing, yeasts were incubated with FITC-labeled, goat anti-rabbit IgG and then fixed overnight in 1% paraformaldehyde. Yeasts were analyzed by flow cytometry on a Coulter EPICS XL flow cytometer, and the acquired data were analyzed with Easy 2 software (Beckman Coulter, Brea, CA).

Immunodepletion of hsp60 from Hc F/TE

Hc F/TE was immunodepleted of hsp60 by absorption onto an affinity column prepared with the affinity-purified anti-hsp60 IgG. Two hundred microliters of F/TE was loaded onto the column and incubated overnight at 4°C. The F/TE was run through the column, and the column was washed extensively. All fractions containing protein were pooled, dialyzed against PBS, and concentrated to the original volume.

Results

CR3 recognizes Hc hsp60

During the early stages of infection, unopsonized Hc yeasts attach to Mφ via the CD11/CD18 family of adhesion-promoting glycoproteins (2). As an initial step to identify a putative ligand(s) on Hc yeasts that is recognized by Mφ CD11/CD18, we prepared CW/M from Hc yeasts and found that it inhibited the attachment of yeasts to Mφ by >90% (data not shown). We then used purified CR3 (CD11b/CD18) to probe a Far Western blot of proteins from the CW/M preparation. CR3 recognized a single protein band at ~60 kDa (data not shown). To determine whether the 60-kDa band was comprised of one or more proteins, Hc CW/M was resolved by two-dimensional SDS-PAGE and was re-analyzed by Far Western blotting with CR3. Fig. 1B shows a single protein spot that migrated to an isoelectric point of pH 5.9 and had a Mr of 60 kDa. Fig. 1A shows the Coomassie-stained gel.

Based on studies by Gomez et al. (16, 17), the protein recognized by CR3 had the same isoelectric point and molecular mass as Hc hsp60. To determine whether CR3 could specifically recognize Hc hsp60, rHsp60 and recombinant Hc hsp60 (rhsp60) were examined by Far Western analysis. As shown in Fig. 1C, CR3 avidly bound to both nhsp60 and rhsp60.

Detection and localization of hsp60 on the surface of Hc yeasts

Hsp60 is reported to be predominantly in the cytosolic fraction of cells (23, 24). However, to act as a ligand for host cell CD11/CD18, hsp60 would have to be localized to the surface of Hc yeasts. That hsp60 was indeed on the surface of Hc yeasts was confirmed by four complementary techniques. In one experiment yeast cell surface proteins were biotinylated with a membrane-impermeant reagent, and biotinylated and untreated control yeasts were simultaneously lysed and solubilized. The insoluble fraction was removed by centrifugation, and the supernatants were incubated with SA-agarose-coated beads to precipitate the biotinylated proteins. The SA-coated beads were resolved by SDS-PAGE, electrotransferred to a nitrocellulose membrane, and probed with affinity-purified rabbit anti-hsp60. Hsp60 was detected in the fraction of precipitated biotinylated proteins, but not in untreated controls (data not shown).

The surface expression of hsp60 on Hc yeasts was further confirmed by flow cytometric analysis using the absorbed, affinity-purified anti-hsp60. Both viable and HK-yeasts contained equivalent amounts of hsp60 (Fig. 2, A and B).

To visualize the distribution of hsp60 on the yeast cell wall, Hc yeasts were incubated with the absorbed, affinity-purified anti-hsp60 IgG or absorbed rabbit IgG as a control, followed by incubation with biotinylated goat anti-rabbit IgG, and SA-conjugated 5 nm-collodial gold. The yeasts then were processed for transmission electron microscopy. Immunolabeling of hsp60 on the surface of Hc revealed dense clusters of gold labeling (Fig. 3A), whereas control Ab labeling was virtually undetectable. Both monoclonal (data not shown) and polyclonal Ab demonstrated aggregated surface hsp60 labeling. Fig. 3B shows the quantitation of hsp60 expressed as the number of gold particles per yeast. Analysis by confocal fluorescence microscopy also revealed punctate labeling over the surface of the yeasts (data not shown). Using the standard stereological methods of planimetry and point counting (20–22), and both monoclonal and polyclonal anti-hsp60, we observed that there were approximately three antigenic sites per thin section per yeast. We calculated that each thin section represented 1.2% of the surface area of a yeast cell. Accordingly there are ~250 hsp60 sites/Hc yeast (three sites per section/1.2% of surface area per section).

Hsp60 blocks attachment of Hc yeasts to Mφ and CHO3 cells and binds to CD11/CD18

As hsp60 bound to CR3 and clearly was expressed on the surface of Hc yeasts, it was an excellent candidate as a ligand for Mφ CD11/CD18. Therefore, we next tested the ability of Hc rhsp60 to inhibit the binding of Hc yeasts to Mφ or to CHO cells transfected with CR3 (CHO3 cells). Mφ or CHO3 cells were incubated with varying concentrations of rhsp60 or H-Ag as a control and then incubated with FITC-labeled Hc yeasts. Recombinant hsp60, but not H-Ag, inhibited the attachment of Hc yeasts to both Mφ (Fig.
A) and CHO3 cells (Fig. 5A) in a concentration-dependent manner. The rH-Ag was chosen as a control because, like hsp60, immunization with rH confers protection in a murine model of pulmonary histoplasmosis (25), but, unlike hsp60, H-Ag resides in the cytosol (26). Fig. 4B shows that M/H9278 bound viable and HK Hc yeasts equally well, and that binding was inhibited by rhsp60, but not by rH-Ag. The attachment of Hc yeasts to CHO3 cells also was inhibited by mAbs to CD11b and CD18, but not by mAbs to CD11a or CD11c (Fig. 5B). Untransfected CHO cells did not bind Hc yeasts at all (data not shown). Finally, rhsp60 also inhibited the binding of Hc microconidia to M/H9278 (Table I), but not quite to the extent that it inhibited the binding of Hc yeasts. Microconidia contained levels of hsp60 on their surface equivalent to viable and HK yeasts (Fig. 2C).

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To confirm that hsp60 could bind M/H CD11/CD18, 490 M/H9278 CD18 RECOGNIZES Hc hsp60 by guest on July 25, 2017 http://www.jimmunol.org/ Downloaded from

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We next sought to determine whether hsp60 was the only ligand on Hc yeasts that mediated binding to M/H. An F/T prepared from viable Hc yeasts was highly enriched for surface proteins, as we
detected no lactate dehydrogenase activity in the F/TE, and <5% of the yeasts were lost during the freeze/thaw procedure (data not shown). Further, hsp60 was detected in a Western blot of F/TE probed with the affinity-purified rabbit anti-hsp60 (Fig. 7A). Yeasts frozen and thawed to prepare the F/TE bound minimally to Mφ, even though they contained about half the amount of hsp60 as viable and HK yeasts (Fig. 2D). We depleted hsp60 from F/TE using a column of anti-hsp60 Ab immobilized on CNBr-activated Sepharose. An affinity column prepared with rabbit IgG was used for mock depletion. The F/TE flow-through was concentrated to its original volume and tested for its ability to inhibit the binding of Hc yeasts to Mφ. Western blotting of the immunodepleted F/TE demonstrated that ∼90% of the hsp60 protein was removed by affinity chromatography (Fig. 7A). Untreated F/TE inhibited the binding of Hc yeasts to Mφ by 70%, whereas F/TE immunodepleted of hsp60 had no inhibitory activity (Fig. 7B).

**Hsp60 does not promote binding of Hc yeasts to DC**

Recently, we demonstrated that immature human DC recognized Hc yeasts via the fibronectin receptor very late Ag 5 rather than CD11/CD18 (9). These data suggest that DC also might recognize Hc yeasts via a different ligand than does Mφ CD11/CD18. To test this hypothesis, DC were preincubated with F/TE, hsp60-depleted F/TE, mock-depleted F/TE, rhsp60, or rH-Ag and then incubated with FITC-labeled Hc yeasts. Although F/TE was found to inhibit the binding of Hc to DC, rhsp60 and rH-Ag were without effect. Moreover, F/TE depleted of hsp60 was just as inhibitory as undepleted F/TE, indicating that a different component of the F/TE inhibited the attachment of Hc yeasts to DC (Fig. 8).

**Discussion**

Mφ serve as a critical interface in the pathogenesis of histoplasmosis. Initially these cells provide a permissive intracellular environment that allows Hc yeasts to gain a foothold in the host and to disseminate from the lung to other organs. After the induction of Th1-type immunity, Mφ become activated and kill the invading fungus. The ability of Hc and other intracellular pathogens to subvert the normally hostile confines of the Mφ phagosome to a “friendly” environment may in part depend on which receptors the pathogen uses to gain entrance into Mφ.

Hc yeasts and conidia are recognized and ingested by Mφ via β2 integrin receptors (CD11/CD18) (2, 3). The CD18 family consists of three distinct α-chains noncovalently linked to a common
CR also are involved in the recognition and phagocytosis of other intracellular pathogens, including *Leishmania major* (30, 31), and *Klebsiella pneumonia* (32). Thus, under certain circumstances CR may act as a safe portal of entry into Mφ.

As the initial contact of Hc yeasts with Mφ may be crucial to its ability to survive intracellularly, we sought to identify the ligand(s) on the surface of yeasts that is recognized by Mφ CD11/CD18. The results presented herein demonstrate that Hc hsp60 is a major surface molecule recognized by human Mφ. As hsp60 generally is considered a cytoplasmic molecule, we used four separate techniques to demonstrate that hsp60 was indeed expressed on the surface of Hc yeasts. First, surface proteins on viable Hc yeasts were biotinylated, precipitated with SA agarose beads, and analyzed by SDS-PAGE and Western blotting. Anti-hsp60 Ab detected hsp60 on biotinylated yeasts, but not on untreated control yeasts. Surface expression of hsp60 on Hc yeasts also was demonstrated by flow cytometry with the absorbed, affinity-purified anti-hsp60 Ab. Finally, immunoelectron microscopy with both the affinity-purified anti-hsp60, and an mAb to hsp60 showed that hsp60 was present on the outer surface of the electron translucent cell wall in distinct clusters; this topology was confirmed by confocal microscopy.

Using standard stereological methods, we calculated that there are ~250 hsp60 antigenic sites. This low number of sites aligns with our previous observation that disruption of Mφ actin microfilaments with cytochalasin D inhibits binding of Hc yeasts to Mφ (3). Thus, Mφ CD11/CD18 receptors must be mobile within the plane of the membrane to efficiently make contact with the relatively few hsp60 antigenic sites that are on the surface of the yeasts. Despite the relative paucity of hsp60 on Hc yeasts, once the yeasts bind to Mφ, they are rapidly ingested, thus achieving their goal of gaining entrance into a safe environment in which to replicate.

Hsp60 is a 60-kDa hsp homologous to GroEL of *Escherichia coli* (33). A general role ascribed to hspss is to chaperone nascent or aberrantly folded proteins. Hsps also have been shown prevent protein aggregation, promote proper folding of nonnative proteins, assist in the assembly of multisubunit protein complexes, as well as target proteins for membrane translocation (23, 24). From an immunological standpoint, hsps are interesting in that they are immunogenic for a remarkable number of microbial pathogens, including *Borrelia burgdorferi* (34, 35), *L. pneumophila* (36), *Chlamydia trachomatis* (37, 38), *Coxiella burnetti* (39), *Neisseria
meningitidis (40), Rickettsia tsutsugamushi (41), M. tuberculosis (42), Helicobacter pylori (43), Yersinia enterocolitica (44), Plasmodium yoelii (45), Pseudomonas aeruginosa (46), and Coccidoides immitis (47). In addition, adoptive transfer of hsp60-reactive T cell clones protects mice from a normally lethal infection of Y. enterocolitica (44), and vaccination of mice with hsp60 DNA from P. yoelii is partially protective (45). Finally, vaccination of mice with Hc rhes60 is protective against pulmonary histoplasmosis (16). Thus, hsp60 serves a dual function in the immunopathology of histoplasmosis.

In addition to being a potent Ag, hsp60 has been implicated in the attachment and invasion of nonphagocytic host cells by pathogenic microorganisms. Thus, H. pylori uses cell surface hsp60 to promote attachment to gastric epithelia (48), and hsp60 on the surface of Clostridium difficile is involved in the attachment to various epithelial cell lines (49). L. pneumophila hsp60 is important for invasion of HeLa cells (50, 51). Of particular interest is that latex beads coated with L. pneumophila hsp60 were more efficiently taken up by HeLa cells than BSA-coated beads, and phagosomes that contained hsp60-beads did not exhibit phagolysosomal fusion, while phagolysosomal fusion occurred in HeLa cells that had ingested BSA-coated beads (51). Whether Hc hsp60 is involved in the capacity of Hc to inhibit phagolysosomal fusion (52) is unknown.

The mechanism by which Hc hsp60 reaches the surface of the cell wall is unclear, as the gene does not contain a classical secretory leader sequence (GenBank accession no. P50142). Indeed, the Hc hsp60 gene possesses a unique leader sequence upstream from the amino terminus of the mature polypeptide that has high homology with the leader sequence of two other pathogenic fungi, Coccidioides immitis (GenBank accession no. O660008) and Paracoccidioides brasiliensis (GenBank accession no. U81786). This leader sequence has not been found in the hsp60 sequence of higher organisms such as mammals, in nonpathogenic yeasts such as Saccharomyces cerevisiae (GenBank accession no. M33301), or in commensal opportunists such as Candida albicans (GenBank accession no. AF085694). However, we have found that the hsp60 associated with the CW/M of Hc is N-glycosylated, while the cytosolic form is not, suggesting that the surface form of hsp60 traffics through the Golgi apparatus. RACE analysis of the hsp60 message suggests an alternative exon with a canonical leader sequence that may direct the export of an hsp60 subpopulation to the surface of the yeasts. Additional experiments are aimed toward confirming these preliminary findings.

Several lines of evidence demonstrated that Hc hsp60 served as a ligand for MΦ CD11/CD18. First, rhes60 inhibited the attachment of Hc yeasts to MΦ and CHO cells transfected with CR3 (CHO3). Second, mAbs to CD11b and CD18 inhibited the binding of Hc yeasts to CHO3 cells. Third, polystyrene beads coated with Hc hsp60 bound to MΦ, and binding was partially inhibited by mAbs to CD11b and CD11c. Further, a mixture of anti-CD11-specific mAbs and an mAb to CD18 inhibited the binding of hsp60 beads to the background level of control BSA-coated beads. Fourth, immunodepletion of hsp60 from Hc F/TE completely removed the capacity of the F/TE to inhibit binding of Hc yeasts to MΦ. Hsp60 also blocked the attachment of Hc microconidia to MΦ. Thus, both Hc conidia and yeasts appear to enter MΦ using the same ligand-receptor combination (3).

It is intriguing that Hc hsp60 failed to block the attachment of Hc yeasts to DC, and that removal of hsp60 from F/TE did not abrogate its ability to inhibit the binding of yeasts to DC. Thus, DC not only recognize Hc yeasts via very late Ag 5 rather than CD11/CD18 (9), but also recognize a different ligand on Hc yeasts than MΦ. This utilization of different receptor-ligand combinations by MΦ and DC may in part account for the ability of DC to kill Hc yeasts (9), while MΦ provide a permissive intracellular environment (4).

Besides the present study, to our knowledge there is only one other report of a microbial hsp mediating attachment to MΦ. Thus, the 68-kDa protein of M. avium, which exhibits strong homology with the 65-kDa hsp of M. tuberculosis, M. leprae, and M. bovis, binds to the vitronectin receptor, αVβ3, on human monocyte-derived MΦ (53). Further, binding through the αVβ3 receptor enhances the expression of CR3 (CD11b/CD18). Since CR3 also plays a role in the phagocytosis of M. avium (54), the data suggest a complex interaction between these receptors.

It also has been reported that human hsp60 activates human MΦ via CD14 in a manner similar to LPS (55), and others have reported that human hsp60 stimulation of mouse MΦ to produce NO and secrete TNF-α is dependent on Toll-like receptor 4 (56). Finally, human and chlamidial hsp60 have been shown to activate the stress protein kinases and mitogen-activated protein kinases via Toll-like receptors 2 and 4 in transfected human fibroblasts (57). Overall, it is clear that both self and non-self hsp may have a profound effect on the host immune response. In addition, the current data suggest that the mode of entry of Hc into host cells, as determined by its microbial ligand-to-host cell receptor interaction, determines its intracellular fate. Further studies of receptor-ligand interactions on MΦ and DC with Hc, and the different signal transduction pathways that are activated, should provide additional knowledge of the mechanism(s) employed by Hc to promote pathogenesis.

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References


