Impairment of Granulomatous Inflammatory Response to
Histoplasma capsulatum by Inhibitors of Angiotensin-Converting
Enzyme

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Systemic infection with Histoplasma capsulatum induced a granulomatous inflammatory response in the lymphoreticular organs of C57BL/6 mice that was associated with elevated levels of angiotensin-converting enzyme (ACE) in the spleens. To determine the influence of ACE on the granulomatous response, either captopril or MK 421, two inhibitors of ACE, were administered intraperitoneally to mice 6 h after intravenous injection of H. capsulatum and then daily for 1 week. Each ACE inhibitor sharply reduced ACE activity in the spleens of infected mice. Both drugs worsened the clinical severity of infection and significantly increased the growth of H. capsulatum in livers and spleens of mice infected for 1 week. The histopathological changes in mice given captopril were more severe, with massive infiltrates of macrophages in proximity to large aggregates of yeasts. Conversely, the administration of captopril for 2 weeks during the resolving phases of infection did not slow the healing of the granulomatous lesions, nor did it provoke a relapse of infection. Captopril did not promote the growth of H. capsulatum in artificial medium. This drug was not cytotoxic to peripheral blood leukocytes or to splenic leukocytes from normal and infected mice. Administration of captopril to normal mice for 1 week did not depress the response of splenocytes of concanavalin A or to phytohemagglutinin, nor did it diminish delayed-type hypersensitivity responses in vivo. Finally, captopril did not augment the growth of H. capsulatum within macrophages. Our results suggest that ACE may participate in the regulation of the granulomatous inflammatory response to H. capsulatum and that ACE inhibition impairs the protective effects of granulomatous inflammation during acute H. capsulatum infection.

Angiotensin-converting enzyme (ACE) is an exopeptidase that converts angiotensin I to angiotensin II and inactivates bradykinin (17, 26). ACE activity is elevated in the sera of some individuals with granulomatous diseases, including sarcoidosis (6), berylliosis (12), leprosy (11), and histoplasmosis (18). Furthermore, increased ACE activity has been detected within granuloma-containing lymph nodes of patients with sarcoidosis (24) and in granulomas from livers, colons, ilea, and lungs of mice experimentally infected with Schistosoma mansoni (29).

At present, the biological significance of increased ACE activity in these granulomatous diseases is unknown. However, recent reports have suggested that ACE may be involved in the regulation of granulomatous inflammation. Thus, the administration of captopril, a specific inhibitor of ACE, to mice with schistosomiasis reduces ACE activity and the volumes of the egg-induced granulomas in the livers, gastrointestinal tracts, and lungs (29). Likewise, captopril substantially diminishes the granulomatous inflammatory response in the lungs and spleens of mice given an intravenous (i.v.) inoculum of heat-killed Mycobacterium bovis BCG (21).

The studies described above were performed in model systems that employed nonreplicating antigens. However, the effect of ACE inhibition has not been assessed in a model of granulomatous disease caused by replicating pathogens in which even subtle modification of the granulomatous tissue response may alter survival. We did this in C57BL/6 mice systemically infected with Histoplasma capsulatum. After 1 week of infection, ACE activity in the granulomatous mouse spleens was elevated markedly. Administration of captopril or MK 421, both specific inhibitors of ACE, to mice for 1 week reduced splenic ACE activity. Moreover, these drugs worsened the clinical severity of disease and resulted in a significant increase in the number of H. capsulatum colony-forming units recovered from livers and spleens of mice compared with infected control mice. In infected recipients of either ACE inhibitor there was massive, but ineffectual, proliferation of the granulomatous inflammatory response compared with infected control mice.

MATERIALS AND METHODS

Mice. Male C57BL/6 mice were purchased from Jackson Laboratories, Bar Harbor, Maine. All animals were housed under controlled conditions of humidity, temperature, and light. Food and acidified, chlorinated water were provided ad libitum.

Infection with H. capsulatum. Preparation and inoculation of H. capsulatum G217-B yeast cells have been described previously (1). Briefly, yeast cells were harvested after culture for 36 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at a gyroratory speed of 200 rpm and were washed three times with Hanks balanced salt solution (HBSS). A final centrifugation at 3 × g was performed for 2 min to remove larger yeast cell aggregates. The yeast forms were enumerated with a hemacytometer. Mice 6 to 8 weeks old were inoculated via the tail vein with 6 × 10^3 yeast cells in 0.2 ml of HBSS. This number of organisms is a sublethal inoculum that induces less than 5% mortality in mice at 30
days postinoculation. Uninoculated mice housed under identical conditions were used as age-matched controls.

**Drug treatment.** Captopril was a gift from Z. P. Horovitz, E. R. Squibb & Sons, Princeton, N.J., and MK 421 was a gift from Charles Sweet, Merck Sharp & Dohme, West Point, Pa. Both drugs were dissolved in physiological saline and sterilized by filtration before use. Captopril and MK 421 were given in 0.2 ml volumes intraperitoneally (i.p.) at the dosages and intervals indicated below. In all experiments, control mice were given 0.2 ml of saline at concomitant time intervals. A cortisone acetate suspension purchased from Merck Sharp & Dohme was diluted in sterile saline prior to use and given to mice in 0.2 ml volumes i.p.

**Organ culture for *H. capsulatum* and histopathology.** Spleens and livers from groups of four to eight mice were removed aseptically and homogenized in 10 ml of sterile HBSS by using a Teflon tissue grinder. Homogenates were diluted serially, and 0.1 ml of each dilution was plated in triplicate onto brain heart infusion agar (2% [wt/vol] agar containing 5% defibrinated sheep erythrocytes [SRBC], 0.01% [wt/vol] cysteine hydrochloride, 10 μg of gentamicin per ml, and 1% dextrose [BHIA/B medium]). Cultures were incubated at 30°C for 7 to 10 days, and the mycelial colonies were enumerated with a colony counter. Addition of hydroxamic acid growth factors did not increase recovery of *H. capsulatum*. Tissue specimens from spleens were fixed in 10% buffered Formalin, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin, methylene-silver (Grocott), periodic acid–Schiff stain, and methyl green-pyronin.

**Induction and measurement of delayed-type hypersensitivity to SRBC.** Groups of normal mice that received saline or captopril were sensitized by i.v. injection with 0.2 ml of a 0.01% SRBC suspension. After 5 days, the mice were challenged intradermally with 0.05 ml of a 20% SRBC suspension injected into a hind footpad. Footpad swelling was measured 24 h later with a digital micrometer (Brown and Sharpe Co., North Kingstown, R.I.) and the delayed-type hypersensitivity response was expressed as the percent increase in footpad size over the size measured immediately before the SRBC challenge. Mean values were determined from groups of six mice. As an additional control, the degrees of banal inflammatory response to the injection of the SRBC challenge dose only were measured in footpads of nonimmunized mice given either saline or captopril.

**Mitogenic stimulation assay.** Cell suspensions were prepared from a pool of at least two spleens by teasing the spleens between two glass slides in HBSS. The cells were allowed to settle for 5 min, washed twice, and suspended at a density of 2 × 10⁶ cells per ml in RPMI 1640 medium containing 5% fetal calf serum and 0.1% gentamicin. To 4 × 10⁶ cells in 0.2 ml, we added 0.2 μg (0.05 ml) of concanavalin A or 1.0 μg of phytohemagglutinin in microtiter plates (BD Labware, Oxnard, Calif.). Cells were cultured in triplicate for 3 days at 37°C in a 5% CO₂ atmosphere. These concentrations of mitogens had been established as optimal in preliminary testing. At 2 h before cell harvest, the cultures were pulsed with 0.5 μCi of [3H]thymidine (specific activity, 6.7 Ci/ml per mol; New England Nuclear Corp., Boston, Mass.). Cells were collected with an automatic sample harvester (MASH II; MA Bioproducts, Walkersville, Md.), and uptake of radioactivity was measured with a liquid scintillation counter.

**Generation time of *H. capsulatum* within peritoneal macrophages.** Intracellular growth of *H. capsulatum* in macrophages was assayed by the method of Wu-Hsieh and Howard (30); peritoneal macrophages were harvested with RPMI 1640 medium containing 10 U of heparin per ml from mice inoculated i.p. 3 days previously with 2 ml of 10% Proteose Peptone (Difco). The macrophages were washed and then suspended in RPMI 1640 medium containing 10% fetal calf serum and 0.1% gentamicin to a final concentration of 10⁶ cells per ml; 1-ml portions were placed on 25-mm round glass cover slips in 35-mm plastic dishes and incubated for 2 h at 37°C. The dishes were then washed extensively, and the peritoneal macrophages were incubated with captopril or medium for 3 h. Subsequently, the macrophage monolayers were inoculated with 2 × 10⁴ yeast cells. The extracellular yeast cells were removed after 1 h, and cover slips from each group were removed and stained with Diff-Quik (Dade Diagnostic Inc., Aquada, P.R.). The remaining monolayers were incubated for an additional 15 h before staining. The number of yeast cells within 100 infected macrophages was counted, and the mean number of yeast cells per macrophage was calculated. Generation times, which represent the growth of intracellular *H. capsulatum*, were derived from the increase in the number of yeast cells within macrophages over a 15-h interval. Thus, generation time was calculated as follows: generation time = incubation interval/number of divisions. The number of divisions was determined from the following formula: number of divisions = (log Nf − log No)/log 2, where Nf is the mean number of yeast cells per infected macrophage at the end of the incubation period (16 h), and No is the mean number of yeast cells per infected macrophage at zero time (after 1 h of incubation).

**Assay for ACE activity and protein concentration.** A 200-μg sample of tissue from the spleens of infected or normal mice (pool of three animals was homogenized in 3 ml of HBSS with a Teflon grinder for 1 to 2 min on ice and washed. The homogenate was then sonicated for 30 s at 4°C. ACE activity was determined by a spectrophotometric method, using hippuryl-L-histidyl-L-leucine as the substrate (10). Preliminary experiments demonstrated that addition of up to 10⁷ viable *H. capsulatum* yeast cells did not alter measurement of ACE in specimens. Protein concentrations of homogenates were determined by the Coomassie blue technique (2).

**Statistics.** The Wilcoxon rank sum test was used to determine levels of significance between groups.

### RESULTS

**Effect of captopril on ACE activity in spleens from *H. capsulatum*-infected mice.** Captopril or saline was adminis-

### TABLE 1. ACE activity in spleens of *Histoplasma*-infected mice treated with captopril or MK 421

<table>
<thead>
<tr>
<th>Spleen source</th>
<th>Dose (mg/kg per day)</th>
<th>Splenic ACE activity (nmol/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Captopril</td>
<td>MK 421</td>
</tr>
<tr>
<td>Normal mice</td>
<td>None</td>
<td>0.27 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.09 ± 0.16</td>
</tr>
<tr>
<td>Infected mice</td>
<td>None</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
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<tr>
<td></td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

* a Data are means ± standard errors of the mean for at least five mice. Values for normal mice were from pools of three spleens and represent five separate determinations.
tered i.p. 6 h after i.v. inoculation of \(6 \times 10^5\) yeast-phase *H. capsulatum* cells, and treatment was continued daily for 1 week. As shown in Table 1, the ACE activity in extracts of spleens of mice that received placebo treatment with saline. Signs of infection (i.e., inactivity, weight loss, and ruffled fur) appeared earlier (day 4) in mice given captopril than in infected mice given saline (day 7). By day 7 of infection, 70% of the infected captopril recipients were severely ill. Indeed, by day 10 90% of mice given captopril were dead, compared with none of the control, infected mice. Administration of the same captopril regimen to age-matched, normal mice produced no clinical illness. Furthermore, both gross and histological examinations of tissues were normal. In other studies, infected mice were given captopril in dosages of 75 or 25 mg/kg per day. These animals also manifested greater severity of illness than infected controls given saline; however, there were no deaths in the infected mice given captopril or in the control infected mice after 10 days.

**Captopril worsens the clinical severity of infection.** Captopril, given as described above, greatly intensified the severity of clinical disease in mice compared with infected mice that received placebo treatment with saline. Signs of infection (i.e., inactivity, weight loss, and ruffled fur) appeared earlier (day 4) in mice given captopril than in infected mice given saline (day 7). By day 7 of infection, 70% of the infected captopril recipients were severely ill. Indeed, by day 10 90% of mice given captopril were dead, compared with none of the control, infected mice. Administration of the same captopril regimen to age-matched, normal mice produced no clinical illness. Furthermore, both gross and histological examinations of tissues were normal. In other studies, infected mice were given captopril in dosages of 75 or 25 mg/kg per day. These animals also manifested greater severity of illness than infected controls given saline; however, there were no deaths in the infected mice given captopril or in the control infected mice after 10 days.

**Captopril increases growth of *H. capsulatum* yeast cells in**

\[\text{groups} \]

\[\text{i.v.} \]

\[\text{and then} \]

\[\text{per} \]

\[48, \text{week}. \]

\[\text{As} \]

\[\text{capsulatum cells,} \]

\[\text{infection} \]

\[\text{compared} \]

\[\text{ed,} \]

\[\text{in} \]

\[\text{activity} \]

\[\text{captopril} (75 \text{ and } 25 \text{ mg/kg per day}) \]

\[\text{also significantly diminished ACE} \]

\[\text{activity in the spleens of infected mice} (P < 0.05). \]

**Captopril worsens the clinical severity of infection.** Captopril, given as described above, greatly intensified the severity of clinical disease in mice compared with infected mice that received placebo treatment with saline. Signs of infection (i.e., inactivity, weight loss, and ruffled fur) appeared earlier (day 4) in mice given captopril than in infected mice given saline (day 7). By day 7 of infection, 70% of the infected captopril recipients were severely ill. Indeed, by day 10 90% of mice given captopril were dead, compared with none of the control, infected mice. Administration of the same captopril regimen to age-matched, normal mice produced no clinical illness. Furthermore, both gross and histological examinations of tissues were normal. In other studies, infected mice were given captopril in dosages of 75 or 25 mg/kg per day. These animals also manifested greater severity of illness than infected controls given saline; however, there were no deaths in the infected mice given captopril or in the control infected mice after 10 days.

**Captopril increases growth of *H. capsulatum* yeast cells in**

\[\begin{align*}
\text{A. Liver} \\
\text{B. Spleen}
\end{align*}\]

\[\text{CFU/Organ} (x 10^6) \]

\[\text{0} \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \]

\[\text{0} \quad 10 \quad 20 \quad 30 \quad 40 \]

\[\text{Captopril} (mg/kg/day) \]

\[\begin{align*}
0 & \quad 25 & \quad 75 & \quad 150 \\
0 & \quad 2 & \quad 4 & \quad 6
\end{align*}\]

**FIG. 2.** Effect of MK 421 on the number of colony-forming units recovered from livers and spleens of *H. capsulatum*-infected mice. Groups of 10 mice were inoculated with \(6 \times 10^5\) *H. capsulatum* cells i.v. and then given i.p. either saline or MK 421 (25 mg/kg) daily for 1 week.

**livers and spleens.** Counts of *H. capsulatum* colony-forming units per liver and per spleen were made after 1 week of infection. As shown in Fig. 1A, the number of colony-forming units cultured from livers of mice given 150 mg of captopril per kg per day \((43.2 \times 10^6 \pm 9.5 \times 10^6 \text{ CFU [mean ± standard error of the mean]})\) was significantly greater \((P < 0.005)\) than the number recovered from livers of infected controls given saline \((6.6 \times 10^5 \pm 1.8 \times 10^5 \text{ CFU})\). Similarly, the number of colony-forming units cultured from spleens of captopril-treated mice \((10.7 \times 10^5 \pm 2.9 \times 10^5 \text{ CFU})\) was significantly greater \((P < 0.01)\) than the number recovered from spleens of infected controls \((1.7 \times 10^6 \pm 0.5 \times 10^6 \text{ CFU})\) (Fig. 1B). Captopril \((75 \text{ mg/kg per day})\) also significantly increased the number of *H. capsulatum* yeast cells cultured from the livers and spleens of infected mice \((P < 0.05)\) for both; even at a dosage of 25 mg/kg per day, it significantly enhanced the growth of *H. capsulatum* in spleens \((P < 0.05)\) (Fig. 1B).

**FIG. 1.** Effect of captopril on the number of colony-forming units recovered from livers and spleens of *H. capsulatum*-infected mice. Groups of 12 mice were inoculated with \(6 \times 10^5\) *H. capsulatum* cells i.v. and then given i.p. either saline or 25, 75, or 150 mg of captopril per kg daily for 1 week.

The weights of livers \((1.3 \pm 0.07 \text{ g})\) and spleens \((0.195 \pm 0.13 \text{ g})\) from infected mice given the highest dosage of captopril did not differ \((P > 0.05)\) from the weights of livers \((1.25 \pm 0.07 \text{ g})\) and spleens \((0.228 \pm 0.35 \text{ g})\) from saline recipients. Thus, the increases in number of *H. capsulatum* colony-forming units recovered from the livers and spleens of captopril recipients cannot be explained by organomegaly.

To test the possibility that captopril may have enhanced the recovery of *H. capsulatum* by acting directly upon the organism to promote growth, 100 budding yeast cells were dispensed onto plates containing either BHIA/B medium (see above) or BHIA/B medium supplemented with captopril \((200 \mu \text{g/ml})\) and incubated at 30°C for 7 to 10 days. In three separate experiments, the recovery from the plates containing BHIA/B medium alone was 34 ± 2 CFU (mean ± standard error of the mean) whereas 28 ± 2 CFU was recovered from the plates containing BHIA/B medium plus captopril. Clearly, captopril did not enhance the growth of *H. capsulatum*.

**Effect of MK 421 on ACE activity in spleens and on recovery of *H. capsulatum*.** MK 421 is a specific ACE inhibitor that is chemically distinct from captopril and lacks a sulfhydryl moiety (16). To determine whether this ACE inhibitor could exert iminical effects similar to those caused by captopril administration, 25 mg of MK 421 per kg per day was given to mice 6 h after injection of *H. capsulatum* yeast cells and then daily for 1 week. The mean ACE activity in the spleens of mice given this drug was significantly lower \((P < 0.05)\) and the activity in the spleens of control infected mice (Table 1).
MK 421 also worsened the clinical status of infected mice and significantly increased the number of yeast cells recovered from the livers ($P = 0.01$) and spleens ($P < 0.05$) compared with infected controls (Fig. 2). Thus, two chemically different inhibitors of ACE impaired host defense mechanisms against *H. capsulatum* during acute phases of infection.

**Histopathology of spleens.** Histopathological examination of blind-coded specimens of spleens from infected mice given saline or captopril (150 mg/kg per day) provided clear evidence of more severe disease in the latter group. In mice given saline, the red pulp was heavily infiltrated by macrophages; as described previously (1), marginal zones and periarteriolar lymphocyte sheaths of the white pulp were invaded by well-demarcated infiltrates of these cells (Fig. 3A). Scattered yeast cells (15 to 20 cells per high-power field) were present in both the red and white pulp. In striking contrast, the granulomatous lesions within spleens of mice given captopril were far more extensive and less well circumscribed (Fig. 3C). There was extensive disruption of malpighian corpuscles by poorly organized, granulomatous infiltrates in which foci of karyorrhexis were numerous. These areas of karyorrhexis appeared not to be caused by the death of rapidly dividing lymphocytes since we detected no differences in the number of pyroninophilic lymphocytes (5 to 10 lymphocytes per high-power field) in the spleens of mice given captopril or saline.

In all sections of spleens from captopril-treated animals, periodic acid-Schiff stain revealed macrophages (two to four per high-power field) engorged with yeasts, a phenomenon that did not occur in infected controls. In addition, large aggregates of yeast cells (75 to 125 cells per high-power field) were present throughout the red and white pulp areas (Fig. 3D). Spleens from infected mice given MK 421 also demonstrated more extensive granulomatous inflammation than did controls, with disruption of the white pulp areas and increased numbers of yeast cells.

Since ACE inhibitors modified the inflammatory response to *H. capsulatum* during acute stages of infection, we examined the evolution of this response after completion of captopril administration. Thus, we performed studies on mice inoculated with *H. capsulatum* and then given either
EFFECT OF ACE INHIBITORS ON GRANULOMATOUS INFLAMMATION

TABLE 2. Cell counts in peripheral blood and spleens of normal and infected mice given captopril

<table>
<thead>
<tr>
<th>Mice</th>
<th>Treatment</th>
<th>No. of cells per mm³ in peripheral blood</th>
<th>No. of cells (×10⁶) in spleens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leukocytes</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Normal</td>
<td>Controla</td>
<td>5,650 ± 437b</td>
<td>4,778 ± 354</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>6,308 ± 269</td>
<td>5,340 ± 285</td>
</tr>
<tr>
<td>Infected</td>
<td>Control</td>
<td>4,067 ± 495</td>
<td>2,352 ± 454</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>3,792 ± 334</td>
<td>2,145 ± 237</td>
</tr>
</tbody>
</table>

* Mice were given saline i.p. daily for 7 days.

Values are means ± standard errors of the mean for five or more mice.

* Mice were given 150 mg of captopril per kg per day i.p. for 7 days.

saline or captopril in a lower dosage (75 mg/kg) daily for 1 week to permit survival from infection. Again, the granulomatous lesions in the spleens of captopril recipients were more extensive on day 7 than the lesions in the spleens of saline recipients. These differences persisted until day 10 of infection. However, by day 14 and thereafter, the histologies of the spleens from the two groups were indistinguishable; in both, the granulomatous inflammation had begun to resolve, as indicated by a reduction in the areas of infiltrate and in the numbers of yeast forms observed. At day 21, there was further resolution of the granulomatous infiltration, and yeast forms were detected infrequently in both groups. Thus, the adverse effect of captopril on cell-mediated defenses against H. capsulatum appears to be lost rapidly after cessation of drug administration, and subsequent maturation of an "effective" inflammatory response is not delayed substantially.

Is captopril cytotoxic or immunosuppressive? Drugs that are cytotoxic for lymphocytes and macrophages or otherwise suppress cell-mediated immunity may enhance susceptibility to infection with H. capsulatum (5, 27). The possibility that captopril might have such properties was explored by giving 150 mg/kg per day to normal and infected mice for 1 week and counting the leukocytes in the peripheral blood and in the spleens. The drug did not alter the absolute counts of leukocytes and lymphocytes in the peripheral blood of normal mice compared with saline-injected controls (Table 2). Likewise, it did not change the total number of splenocytes or the total number of splenic lymphocytes and macrophages. In mice infected for 1 week, there is a lymphopenia in the peripheral blood and increased splenic cellularity (1). Captopril did not worsen the lymphopenia of the blood, nor did it alter the splenic cellularity in infected mice (Table 2).

To determine whether captopril could modify the delayed-type hypersensitivity response, normal mice were sensitized to SRBC and then challenged in the footpads with SRBC 5 days later. The increase in footpad swelling of animals given the drug (37 ± 2.8%) was similar to that of mice given saline (35 ± 1.7%). Furthermore, the banal inflammatory responses of unimmunized mice that were footpad challenged with SRBC alone did not differ between saline recipients (9.0 ± 1.8%) and captopril recipients (8.0 ± 1.7%). Thus, captopril does not appear to be cytotoxic, nor does it act as a nonspecific immunosuppressant in the assay systems which we used.

Captopril does not alter intracellular growth of H. capsulatum. Macrophages are the principal effector cells that mediate resistance to H. capsulatum (8). In preliminary experiments, we determined that captopril did not impair phagocytosis of H. capsulatum yeast cells by normal murine peritoneal macrophages (data not shown). Subsequently, two experiments were performed to determine whether captopril augmented intracellular growth of H. capsulatum (see above). At 15 h after macrophages were infected with H. capsulatum, the number of intracellular yeast cells had increased from 2.6 ± 0.1 cells at zero time to 5.6 ± 0.2 cells, with a generation time of 13.6 ± 1 h. The number of yeast cells in macrophages that were exposed continuously to 200 µg of captopril per ml increased from 2.8 ± 0.1 to 6.4 ± 0.2 cells, with a generation time of 15.2 ± 0.8 h. Thus, in vitro captopril did not promote intracellular growth of H. capsulatum.

Can captopril administration reactivate a latent H. capsulatum infection? After i.v. inoculation of 6 × 10⁵ yeast cells the number of H. capsulatum colony-forming units recoverable from livers and spleens decreases progressively after day 7; by week 8 less than 3,000 CFU can be recovered from these organs in 18% of mice (1). Therefore, to determine whether captopril could reactivate disease during recovery from systemic infection, 150 mg of captopril per kg per day was given i.p. for 2 weeks to mice that had been inoculated with H. capsulatum 6 weeks previously. A second group of 6-week-infected mice was given cortisone acetate (50 mg/kg per day), and a third group received saline for 2 weeks. The animals were then sacrificed, and the livers and spleens from each group were cultured for H. capsulatum. Yeast cells were not recovered from the spleens of eight captopril-treated mice. However, low numbers of yeasts (<200 CFU) were cultured from the spleens of two of eight cortisone-treated mice and from the spleens of two of four saline recipients. Low numbers of yeasts were recovered from the livers of two of six captopril-treated mice, five of eight cortisone-treated mice, and two of six saline controls. There were no histological differences between the inflammatory responses in spleens from mice given the drug and spleens from saline recipients.
from mice given saline. Thus, captopril given over a 2-week period did not reactivate the infection.

**DISCUSSION**

This study demonstrated that systemic infection of C57BL/6 mice with the dimorphic fungus *H. capsulatum* induces a granulomatous inflammatory response in the lymphoreticular system which is associated with elevated levels of ACE activity in the spleens. Our results complement and extend previous reports that ACE levels are elevated in the sera, pulmonary lavage fluids, and tissues of experimental animals and some humans with various granulomatous diseases (3, 21, 24, 29).

In infected mice treated for 1 week with captopril or MK 421, there appeared to be poor inhibition of *H. capsulatum* yeast cell multiplication, as indicated both by the large numbers of mononuclear phagocytic cells that were filled to capacity with intact yeast cells and by the significantly increased number of *H. capsulatum* colony-forming units cultured from the spleens of these animals. Concomitantly, there was massive infiltration by macrophages in the spleens of mice given either ACE inhibitor compared with spleens from control infected mice. Therefore, this response may represent an ongoing cellular response that is ineffective and is secondary to impairment of effector mechanisms by ACE inhibitors.

When captopril was administered to mice during the resolving phase of infection (6 to 8 weeks), it did not impede healing of the granulomatous lesions in the spleens, nor did it increase the recovery of yeast cells from the livers and spleens. Therefore, it appears that the immunomodulatory effects of ACE inhibitors are dependent upon the phase of the cell-mediated immune response during which they are given. More specifically, granulomatous inflammation may be susceptible to alteration by ACE inhibitors only when the response is developing acutely rather than during later stages of resolution.

There are several possible mechanisms by which ACE inhibitors might favor the growth of *H. capsulatum* within macrophages. First, ACE inhibitors may impair directly the capacity of macrophages to inhibit replication of *H. capsulatum*. However, the results of our studies on the growth of *H. capsulatum* within normal macrophages that were continuously exposed to captopril suggest that this hypothesis is unlikely.

Since captopril did not augment the number of *H. capsulatum* colony-forming units per organ in a dose-dependent fashion a second possibility is that the heightened severity of infection induced by captopril could be explained by an effect of the drug unrelated to its ACE inhibitory activity. For example, this drug contains a sulhydryl group (16), a moiety that is required for growth of *H. capsulatum* yeast cells in culture media and for transition from the mycelial phase to the yeast phase (19, 20). However, when examined for its potential to promote growth of yeast phase organisms, captopril failed to enhance growth of yeasts in vitro. Other experiments demonstrated that captopril was not cytotoxic to lymphocytes or to macrophages in vivo. It did not depress the responses of splenic T cells to mitogens in vitro, nor did it suppress the delayed-type hypersensitivity response to SRBC in SRBC-immunized mice. Moreover, captopril did not impair phagocytosis of yeast phase organisms by murine peritoneal macrophages.

Alternatively, it has been reported that angiotensin II, the principal biological product of ACE, can depress intracellular killing of *Candida albicans* by rat macrophages at concentrations ranging from $10^{-6}$ to $10^{-4}$ M (7). However, these concentrations of angiotensin II are $10^4$ to $10^5$ fold greater than the levels in the sera of humans (23) or rats (13) given captopril. Thus, it is unlikely that angiotensin II impairs the growth-inhibitory activity of macrophages against *Histoplasma*.

Finally, captopril may alter the ability of macrophages to inhibit the growth of *H. capsulatum* by increasing the production of prostaglandins (22) and bradykinin (23). These inflammatory mediators are agonists of adenylate cyclase and can depress the macrophage function by activating intracellular cyclic 3',5'-adenosine monophosphate (15). Furthermore, the action of prostaglandins may be potentiated by the increased level of bradykinin since this mediator promotes prostaglandin release (9, 14). Whether the elevated levels of prostaglandins or bradykinin induced by captopril are sufficient to perturb the capacity of macrophages to inhibit intracellular growth of *H. capsulatum* remains to be determined.

Intracellular replication of *H. capsulatum* yeast cells can be inhibited only by activated macrophages, whereas the organisms multiply within resting cells (30). Therefore, we favor the hypothesis that ACE inhibitors impair the effector function of macrophages during acute *Histoplasma* infection by interfering with the process of macrophage activation. In this regard, recent evidence has suggested that the T-lymphokine gamma interferon can activate macrophages to inhibit growth of *H. capsulatum* (30). In preliminary experiments, we examined the generation of another T-lymphocyte product, interleukin-2, by splenocytes from normal mice treated with captopril. Our results indicate that captopril depresses production of interleukin-2, whereas it does not inhibit generation of interleukin-1 (Deepe, unpublished data). Thus, it is conceivable that captopril may suppress production of other critical lymphokines involved in host defenses, including gamma interferon. Studies are in progress to address this issue.

Two other immunopharmacological drugs, cyclophosphamide and cimetidine, have been shown to increase the size of granulomas in livers of mice infected with *S. mansoni*, whereas ACE activity in these granulomas is reduced (28). This finding is similar to our own finding with captopril. Taken together, the results of these pharmacological studies of drugs with disparate mechanisms of action suggest that although ACE may act as a modulator of granulomatous inflammation, it undoubtedly is only one of several regulators. For example, exogenous prostaglandins also have been shown to alter the size of granulomas in the lungs of mice with schistosomiasis (4).

In contrast to the exuberant but functionally impaired granulomatous inflammatory response to *Histoplasma* infection associated with captopril administration reported here, other workers have observed that treatment with this drug reduces the volume of liver granulomas induced by *Schistosoma* eggs (29). In addition, it reduces the intensity of granulomatous inflammation evoked in the lungs of mice by i.v. inoculation of heat-killed *M. bovis* BCG (20). However, a very fundamental difference between these studies and our own lies in the fact that we used viable, multiplying *H. capsulatum* yeast cells, whereas in the previous studies heat-killed or otherwise nonreplicating antigens were used. The granulomatous inflammation that develops around *Schistosoma* eggs in livers may function to sequester the eggs but also may lead to fibrosis and eventually to portal hypertension in 10 to 15% of heavily infected humans (25). Therefore, a reduction of the granulomatous response to egg
antigens might serve to ameliorate the serious, long-term consequences of persisting antigen. Although reduction of the granulomatous response to nonreplicating antigenic moiety may be desirable under conditions of chronic persistence in tissues, this clearly does not appear to be the case with viable, replicating agents such as *H. capsulatum*. Indeed, the granulomatous inflammatory response to *H. capsulatum* yeast cells and probably to other replicating intracellular pathogens constitutes a critical mechanism whereby the host may limit successfully the extent of infection and destroy an infectious disease agent. Therefore, impairment of the effector mechanism(s) of granulomatous inflammation by such compounds as ACE inhibitors in an acute phase of the response may permit proliferation of microorganisms and result in overwhelming infection.

In summary, our results demonstrate that administration of ACE inhibitors during acute *Histoplasma* infection effects a marked alteration of the granulomatous response. More important, these compounds clearly subvert the protective effects of the granulomatous inflammatory response. Further investigation is warranted to determine whether the iminol effects of ACE inhibitors, as observed in this study, are applicable to other replicating intracellular pathogens.

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LITERATURE CITED