Enhanced Resistance to Cryptococcus neoformans Infection Induced by Chloroquine in a Murine Model of Meningoencephalitis

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Although the pathogenesis of cerebral cryptococcosis is poorly understood, local immune cells, such as microglia and astrocytes, likely play a critical role in containing infection. Chloroquine (CQ) is a weak base that accumulates within acidic vacuoles and increases their pH. Consequently, proteolytic activity of lysosomal enzymes and intracellular iron release/availability are impaired, resulting in decreased availability of nutrients crucial to microorganism survival and growth in the host. We found that CQ enhances BV2 microglial-cell-mediated anticryptococcal activity in vitro. The phenomenon is (i) evident when both unopsonized and opsonized microorganisms are used and (ii) mimicked by NH4Cl, another weak base, and by bafilomycin A1, an inhibitor of vacuolar-type H+-ATPases. In vivo, intracerebral administration of CQ before lethal local challenge with Cryptococcus neoformans results in a significant augmentation of median survival time and a marked reduction of yeast growth in the brain and is associated with the enhancement of local interleukin 1β (IL-1β) and IL-6 mRNA transcripts. Overall, these results provide the first evidence that CQ enhances anticryptococcal host defenses.

Cryptococcus neoformans is a significant cause of opportunistic infections with a marked predilection for the central nervous system (38). Acquired by inhalation, cryptococcosis may occur as an asymptomatic pulmonary infection; however, in immunocompromised hosts, it may result in a fatal disseminated disease generally manifested as meningitis (16).

Numerous in vitro studies indicate that monocytes, macrophages (MΦ), natural killer cells, and polymorphonuclear cells exhibit fungicidal activity against C. neoformans (28, 34, 36). Despite the importance of innate immunity, the capacity of the host to mount an adequate cell-mediated immune response plays an important role in resistance to cryptococcosis. In particular, CD4+ lymphocyte depletion allows rapid dissemination of the fungus from the lung to other organs, including the brain (29, 35).

Chloroquine (CQ) is a drug widely used against malaria and certain kinds of chronic inflammatory diseases (31, 42, 44). Experimentally, it is also used to study the role of vacuole acidification in immune cell functions (30, 39). Indeed, CQ is a weak base that accumulates in acidic vacuoles (endosomes, lysosomes) and increases their pH. Consequently, both the enzymatic activities of such endocellular compartments and the availability of nutrients crucial to microorganism survival and growth are impaired (25, 37, 40).

In previous reports, we demonstrated that brain immune defense mechanisms have a role in the onset and development of cryptococcal meningoencephalitis. In this respect, initial evidence indicates that brain phagocytic effector cells exert local antimicrobial activities (2, 3, 7). In particular, in vitro studies document that microglial MΦ are proficient anticryptococcal effectors, provided they have been exposed to gamma interferon and/or that opsonized microorganisms are used (3, 5). Moreover, it has been shown that in vivo pharmacological impairment of phagocytic functions is detrimental to the host (2, 3), while intracerebral (i.c.) administration of picolinic acid, a potent MΦ costimulator (9), or of heat-killed C. neoformans, which is known to retain immunostimulating properties (8), enhances host resistance to a subsequent lethal challenge with C. neoformans (2, 3, 7).

Using a murine model, we investigated the effects of CQ in vitro on anticryptococcal activity of microglial cells and in vivo on the establishment of cryptococcal meningoencephalitis. We show that in vitro CQ enhances BV2 cell anticryptococcal activity. This phenomenon is evident when unopsonized as well as opsonized microorganisms are used and is mimicked by NH4Cl, another weak base, and by bafilomycin A1 (BAF), an inhibitor of the vacuolar-type H+-ATPases. Moreover, in vivo i.e. administration of CQ before lethal local challenge with C. neoformans results in (i) significant augmentation of median survival time (MST), (ii) marked reduction of fungal brain colonization, and (iii) local induction of IL-6 and tumor necrosis factor alpha (TNF-α) gene expression. Overall, these results provide the first evidence that CQ may enhance host resistance to C. neoformans i.e. infection.

MATERIALS AND METHODS

Mice. Female C57Bl/6 (H-2b) mice, 6 to 8 weeks old, were obtained from Charles River Breeding Laboratories, Calco, Milan, Italy. C. neoformans. An encapsulated strain of C. neoformans, ATCC 11240, was used. Stock cultures were maintained by biweekly passages on Sabouraud dextrose agar. The plates were kept at room temperature. Yeast cells were harvested from agar plates, washed twice in saline by low-speed centrifugation (1,000 × g).
and diluted to the appropriate concentration in RPMI 1640 medium or saline prior to use in the in vitro or in vivo assays.

*C. neoformans* was opsonized as follows. Microorganisms (10^10) were incubated for 20 min at 37°C with 50 μg of monoclonal antibody 2H1 (immunoglobulin G1, IgG1) in Rinds glass ammonium and was kindly supplied by A. Casadevall (Departments of Medicine and of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, N.Y.), in a total volume of 200 μl of RPMI 1640. Then the yeast cells were washed, and appropriate dilutions for the in vitro assays were prepared in RPMI 1640 complete medium.

**Cell lines.** The BV2 microglial cell line used in this study was obtained as previously described (1). Briefly, primary microglial cultures were infected with a vβ1 and vα11 oncoencephalitis virus and maintained by biweekly passages in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (HyClone), gentamicin (50 μg/ml), and L-glutamine (2 mM).

**Drugs.** CQ, NH₄Cl, and BA were purchased from Sigma Chemical Co., St. Louis, Mo. Stock solutions, prepared with sterile phosphate-buffered saline, were stored at −80°C and used in the experiments described. Only preparations showing lipopolysaccharide contamination of <0.5 ng/ml (as detected by Limulus amyles assay) were used.

**Measurement of anticytotoxic activity.** BV2 microglial cells were plated (5 x 10⁵ cells/ml) in triplicate on 12 mm diameter plates (Corning Glass Works, Corning, N.Y.) and incubated with the drugs for 1 h. Then *C. neoformans* (5 x 10⁵/ml) was added. After different periods of incubation at 37°C in 5% CO₂, Triton X-100 (0.1% final concentration) was added to the wells, and the plates were vigorously shaken. Seven microliters of each well was mated with distilled water and plated on Sabouraud dextrose agar. The colonies were counted after 48 to 72 h of incubation at room temperature. Control cultures consisted of *C. neoformans* incubated without effecter cells. Results were expressed as percentage of anticytotoxic activity, according to the following formula:

\[
\text{% anticytotoxic activity} = \frac{\text{CFU for experimental groups} - \text{CFU for control cultures}}{\text{CFU for control cultures}} \times 100
\]

**Inoculation.** Inoculations (i.e.) were performed on anesthetized mice as detailed below. Mice received inocula (30 μl per mouse) in the brain 1 mm laterally and posteriorly to the bregma at a depth of 2 mm with a 0.1 ml glass microsyringe and a 27-gauge disposable needle. CQ-treated mice received CQ, and untreated mice received phosphate-buffered saline according to schedules described below prior to challenge with *C. neoformans*. Mice recovered from trauma within 30 to 60 min. Surgical mortality was less than 3% and always occurred within 1 to 5 min after inoculation.

**Quantitation of *C. neoformans* in the brain.** At different times after challenge, brains from individual mice (three per group) were removed aseptically and placed in a tissue homogenizer with 3 ml of sterile distilled water. The number of CFU was determined by a plate dilution method on Sabouraud dextrose agar. Colonies of *C. neoformans* were counted after 48 or 72 h of incubation at room temperature, and the results were expressed as the number of CFU per organ.

**RNA extraction.** Total RNA was isolated from the brains of six mice per experimental group by solubilization with guanidine isothiocyanate as previously described (15). Following digestion in DNase, a 15 μg aliquot of RNA was electrophoresed on a 1% agarose formaldehyde denaturing gel containing ethidium bromide to detect the intact 18S and 28S rRNAs and to confirm the integrity of the isolated mRNA. The amount of RNA was calculated by measuring the optical density at 260 nm in a spectrophotometer (Beckman). Total RNA was then processed for CDNA synthesis and PCR amplification assay.

**RT.** Following heating at 65°C for 3 min and subsequent chilling on ice, a 5-μg aliquot of total RNA in 13.6 μl of diethylpyrocarbonate-treated water was used in each reverse transcription (RT) reaction. RT buffer for each sample contained the following: 1.1 μl of 1 M Tris-HCl (pH 8.3) (Bethesda Research Laboratories [BRL]; Gaithersburg, Md.), 0.13 μl of 1 M MgCl₂ (BRL), 0.22 μl of 150 mM dithiothreitol (Promega, Madison, Wis.), 1.33 μl of deoxyxynucleoside triphosphates (25 mM each; Promega), 1.4 μl of 100 mM Tris-HCl (pH 8.3) (Bethesda Research Laboratories [BRL]), 0.22 μl of 100 mM KCl (BRL), 0.22 μl of 100 mM dithiothreitol (Promega, Madison, Wis.), 1.33 μl of deoxyxynucleoside triphosphates (25 mM each; Promega), 1.4 μl of oligo(dT)₅, primer (0.8 μg/μl; Promega), 0.33 μl of RNasin (40 U/μl; Promega), and 1.33 μl of avian myeloblastosis virus reverse transcriptase (7.5 U/μl; Promega). The total reaction volume was 20 μl, and the reaction was allowed to continue at 42°C for 90 min. The reaction was stopped by the addition of 980 μl of TE buffer (Tris-EDTA, pH 8.2) (final volume of each sample of cDNA, 1 ml).

**PCR.** The PCR mixture for each sample contained 6.5 μl of double-distilled sterile water, 3.2 μl of 10× PCR buffer (Pharmacia, Uppsala, Sweden), 3.2 μl of 1.25 mM deoxyxynucleoside triphosphates (Promega), 1 μl each of 3′ and 5′ primers (25 PM final concentration; Promega), and 0.1 μl of Taq polymerase (5 U/μl; Pharmacia). Each cycle consisted of denaturation at 94°C for 1 min; annealing at 55°C for 2 min (for IL-12p40), 60°C for 1 min (for β-actin, TNFa, and IL-6), or 65°C (for IL-1β and inducible nitric oxide synthase); and extension at 72°C for 1 min. Before each cycle, the samples were heated to 100°C for 2 min and then cycled to 80°C before being added to the reaction mixture. Amplification was performed using 30 cycles in a Perkin-Elmer Cetus DNA thermal cycler. Ten microliters of the PCR amplification products were separated on an ethidium bromide-stained 1.5% agarose gel, visualized by UV transillumination, and photographed. Aliquots of 0.05 μg of 4X174 replicative-form DNA HaeIII fragments (New England Biolabs, Beverly, Mass.) were run in parallel as molecular size markers (providing bands at 1,353, 1,078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 bp). The amplified bands showed their predicted sizes. Cytokine-specific primers were DNA specific and nonreactive with RNA. The following oligonucleotide 5′ and 3′ primer sequences (synthesized in our laboratory) were used: TNFa, AGCCCCAGCTGGAGCAGCCAAACA and ACA CCCATCTTCTACAGACATGC and IL-6, ATGAAGTTCTCTTCTGCAGAAC and GACTACATGGTGGAGAGATCTC; IL-1β, TGAGGGTCTGC ITCCAAACCTTGGACC and TGCTCATGTAGGTGAGACTGTTCAGGC; INOS, CCCTTCGGAAATGTTATTCGACCG and GGGCTGTGAAAGCCTC GTGGCTTGG; IL-12p40, CAGAAAGTAAAACCTCCTCCTGTG and TCCGGATTTACATTTGCGTTCACAC; and β-actin, CTGAGATCCCAT GTAACATGGC and CAGAGGCTAATTCTCTTCTGAT. Positive-control DNAs for each cytokine were obtained from Clontech Laboratories, Palo Alto, Calif., while negative controls consisted of samples in which (i) RNA was replaced by diethylpyrocarbonate-purified water, (ii) RT was omitted to detect any contamination by previously amplified cDNA, and (iii) the primers were not added.

**Statistical analysis.** Differences in survival times were determined by the Mann-Whitney test. Differences in the number of CFU were determined by Student’s t test.

**RESULTS**

To investigate the effects of CQ on BV2 cell anticytotoxic activity, we performed in vitro experiments in which BV2 microglial cells were incubated for 1 h alone or in the presence of CQ at concentrations ranging from 1 to 20 μM. Then BV2 cells were infected with *C. neoformans* at an effector-to-target ratio of 10:1, and after 8 h of coincubation, a CFU assay was performed. As reported in Fig. 1, CQ increased the percentage of CFU inhibition by BV2 cells in a concentration-dependent manner. In particular, maximal levels of such activity were evident at a CQ concentration of 10 μM. As assessed by trypan blue exclusion and lactate dehydrogenase release, CQ was not toxic for BV2 cells. Similarly, the presence of CQ did not alter the viability and growth of *C. neoformans* as evaluated by CFU assay (data not shown).

To better characterize this phenomenon, kinetic studies on BV2 cell anticytotoxic activity were performed. BV2 cells, incubated alone or with CQ (10 μM) for 1 h, were infected with *C. neoformans*. CFU assays were performed at different times. The results obtained by comparing untreated versus CQ-treated BV2 cells are depicted in Fig. 2. While at 2 and 4 h

**FIG. 1.** Effects of different CQ concentrations on anticytotoxic activity by BV2 microglial cells. BV2 microglial cells (5 x 10⁵ cells/ml) were cultured for 1 h in medium alone (c) or in medium containing increasing concentrations of CQ. Then *C. neoformans* (5 x 10⁵ yeast cells/ml) was added, and after 8 h of coincubation, a CFU assay was performed. The CFU data are expressed as the percentage of CFU inhibition by BV2 cells in a concentration-dependent manner. Data shown are the means ± standard deviations of three independent experiments. *, P < 0.01 (CQ-treated versus untreated cells).
the percentage of CFU inhibition was significantly augmented in CQ-treated BV2 cells, and the maximal inhibition was observed at later time points (Table 1). We showed previously that BV2 cells exhibit enhanced anticytotoxic activity when opsonized yeast cells are used (3). In order to establish whether CQ effects might be further enhanced, BV2 cells were treated with CQ (10 μM) for 1 h and then exposed to opsonized C. neoformans. After 8 and 24 h, a CFU assay was performed. Unopsonized yeast cells were included as baseline controls. When opsonized yeast cells were used, anticytotoxic activity of CQ-treated BV2 cells was consistently higher than that of untreated BV2 cells: 75% versus 42% and 96% versus 62% at 8 and 24 h, respectively (Table 1). As expected, CQ pretreatment enhanced BV2 cell anticytotoxic activity against unopsonized microorganisms at both time points (Table 1).

FIG. 2. Kinetics of anticytotoxic activity by BV2 microglial cells treated or not with CQ. BV2 microglial cells (5 x 10^5/ml) were incubated for 1 h with (C) or without (O) CQ (10 μM). Then C. neoformans (5 x 10^5/ml) was added, and CFU assays were performed. Percentage of anticytotoxic activity was calculated as described in Materials and Methods. Data shown are the means ± standard deviations of three independent experiments. *, P < 0.01 (CQ-treated versus untreated cells).

### TABLE 1. Effect of lysosomotropic compounds on BV2 microglial cell anticytotoxic activity

<table>
<thead>
<tr>
<th>Lysosomotropic compound</th>
<th>Concentration (μM)</th>
<th>Unopsonized</th>
<th>Opsonized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 h</td>
<td>24 h</td>
<td>8 h</td>
</tr>
<tr>
<td>None</td>
<td>14</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>CQ</td>
<td>10</td>
<td>25a</td>
<td>66a</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>20</td>
<td>28a</td>
<td>73a</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>10</td>
<td>26a</td>
<td>62a</td>
</tr>
<tr>
<td>BAF</td>
<td>5</td>
<td>10</td>
<td>54a</td>
</tr>
<tr>
<td>BAF</td>
<td>300</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>BAF</td>
<td>200</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>BAF</td>
<td>150</td>
<td>10</td>
<td>31</td>
</tr>
</tbody>
</table>

a BV2 cells were treated with CQ (10 μM) or different concentrations of NH₄Cl or BAF for 1 h. Then C. neoformans was added to the cultures.
b Anticytotoxic activity was assessed at 8 and 24 h against unopsonized or opsonized C. neoformans as detailed in Materials and Methods. Data shown are the means of three independent experiments. Standard deviations, which were less than 5%, have been omitted.
c P < 0.01 (treated BV2 cells versus untreated).

d P < 0.05 (treated versus untreated mice).

e P < 0.01 (treated versus untreated mice).

### TABLE 2. Effect of CQ on survival of mice challenged i.c. with C. neoformans

<table>
<thead>
<tr>
<th>Treatment with CQ (μM)</th>
<th>C. neoformans i.c. challenge</th>
<th>MST (days)</th>
<th>Survival range (days)</th>
<th>D/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10^4</td>
<td>20</td>
<td>10–30</td>
<td>30/30</td>
</tr>
<tr>
<td>10</td>
<td>10^4</td>
<td>30</td>
<td>15–45</td>
<td>30/30</td>
</tr>
<tr>
<td>1</td>
<td>10^4</td>
<td>48</td>
<td>23–60</td>
<td>30/30</td>
</tr>
<tr>
<td>0.1</td>
<td>10^4</td>
<td>22</td>
<td>11–28</td>
<td>30/30</td>
</tr>
</tbody>
</table>

a Mice received CQ or saline i.c. 24 and 3 h before challenge.
b C. neoformans (10^4 cells per mouse) was given i.c. on day 0.
c Number of dead mice at 60 days/total number of animals tested.
d P < 0.05 (treated versus untreated mice).
e P < 0.01 (treated versus untreated mice).

### TABLE 3. C. neoformans recovered from brains of CQ-treated and untreated mice

<table>
<thead>
<tr>
<th>Treatment with CQ (μM)</th>
<th>CFU (10^4) of C. neoformans recovered from brains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>None</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>0.7e</td>
</tr>
<tr>
<td>1</td>
<td>0.2e</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a Mice were treated i.c. with CQ or saline 24 and 3 h before i.c. challenge with C. neoformans (10^4 cells per mouse).
b CFU recovery from brains was assessed at the indicated days as detailed in Materials and Methods. Data shown are the means of three independent experiments. Standard deviations, which were less than 5%, have been omitted.
c P < 0.01 (treated versus untreated mice).
Micewere treated i.c. with saline or CQ (1 μM) before a assessment of TNF-
neoformans (24 and this purpose, untreated naive mice and saline-or CQ-treated
treated and processed, and RNA was employed for RT-PCR analysis as detailed
in Materials and Methods. Results from six brains per experimental group are
shown.

Ated with the modulation of local cytokine gene expression,
molecular studies were performed by RT-PCR analysis. For
this purpose, untreated naive mice and saline- or CQ-treated
(−24 and −3 h) mice were sacrificed at time 0 before C.
neoformans challenge. Brains were isolated and processed for
assessment of TNF-α, IL-6, IL-1β, IL-12, and iNOS-specific
mRNA levels. As depicted in Fig. 3, naive and saline-treated mice showed undetectable levels of all gene transcripts inves-
tigated. In contrast, CQ treatment resulted in the expression of
TNF-α and IL-6 genes in six of six mice assessed, while IL-1,
IL-12, and iNOS gene transcripts remained undetectable.
β-Actin transcripts were detected in all the samples tested.

DISCUSSION

During inflammatory processes and/or infections in the
brain, local defense elements, such as microglia and astrocytes,
have several immunological functions, including phagocytosis
(1, 43), antigen presentation (21, 22, 24), cytokine production
(20, 23, 26, 27, 33), respiratory burst, and antimicrobial activ-
ities (14, 32, 45). In particular, microglial cells are proficient
anticytotoxic and/or elements in vitro. Our previous studies dem-
onstrated that BV2 microglial cells are capable of ingesting C.
neoformans and inhibiting its growth. This is accomplished in a
time- and effector-to-target-ratio-dependent fashion and is
markedly potentiated upon opsonization of the target (3). In
the present report, we demonstrate that, following in vitro
exposure to CQ, BV2 microglial cells exhibit increased anti-
cytotoxic activity, which is particularly evident at a CQ
concentration of 10 μM and after 8 and 24 h of infection with C.
neoformans. This finding is in line with several reports demon-
strating that murine as well as human M6 are susceptible to
CQ and respond with enhanced antimicrobial properties (12,
18, 37, 41). The molecular mechanisms involved in this phe-
nomenon have been partially identified. Indeed, CQ interferes
with the endosomal/lysosomal functions. By raising intravacu-
olar pH, CQ inhibits (i) the proteolytic activities of lysosomal
enzymes (40) and (ii) the release of iron from its transferrin
binding sites within the endosome (12, 42). In our experimen-
tal system, we observed enhancement of anticytotoxic ac-
vity in BV2 cells exposed to CQ as well as to other com-
ounds, such as NH₄Cl or BAF, which are known to impair
intracellular acidification processes through similar (NH₄Cl)
or different (BAF) mechanisms (11). Altogether, our results
strongly suggest that within certain subcellular compartments,
pH is important for the accomplishment of BV2 cell anticyt-
otoxic activity. It remains to be elucidated whether CQ acts
on BV2 cells via alteration of proteases and/or of iron metab-
olism. We tend to exclude the second hypothesis. In a recent
report, we established that anticytotoxic activity of BV2 cells
is potentiated by iron loading (via iron nitrotriaceacetate
[FeNTA]). Thus, it appears that two compounds, CQ and FeNTA, which are known to mediate opposite effects in terms
of intracellular iron availability, both enhance anticytotoxic
activity. The mechanisms through which CQ acts on BV2 cells are
therefore probably related to alteration of lysosomal enzymatic
activities rather than to interference with iron metabolism.
This conclusion is supported by preliminary data showing that
BV2 cell exposure to FeNTA does not abrogate the CQ anti-
cytotoxic effect (data not shown).

According to the literature, enhancement of intravacuolar
pH can be detrimental to some intracellular pathogens, prob-
cably causing a reduction in the availability of nutrients critical
for their survival and growth via inactivation of the acidic
proteases (11, 25). On these bases, we can speculate that, once
ingested by CQ-treated cells, C. neoformans encounters an
intracellular environment that is less favorable, as it is deprived
of crucial nutrients. This hypothesis is supported by the fact
that promotion of phagocytosis via C. neoformans opsonization
is associated with the expression of maximal levels of anticyt-
otoxic activity by CQ-treated BV-2 cells. Finally, the induc-
tion of autocrine activation loops by CQ is unlikely, since we
failed to detect TNF-α, IL-6, IL-12, and nitric oxide in super-
natants of BV2 cells exposed to CQ and then infected with C.
neoformans (data not shown).

We have previously described an experimental model of i.c.
infection with C. neoformans in which mice develop a lethal
disease associated with a massive colonization of the brain (3).
Here, we show that CQ significantly reduces host susceptibility
to lethal i.c. infection with C. neoformans. Our data demon-
strate that injection of this compound directly into the brain
results in a twofold increase in the MST of lethally infected
mice. This phenomenon, which is accompanied by a marked
delay in fungal colonization of the brain, is most evident at the
1 μM concentration, while at 10 μM, the benefit of CQ is only
partial. Whether toxic effects or rather down-regulating feed-
back signals may be involved is currently being investigated.
Since the efficacy of CQ against malaria and histoplasmosis has
been documented (31, 37), these results include cryptococcosis
as another infectious disease against which CQ may be bene-

Evidence indicates that immunocompetent brain elements,
such as microglia and astrocytes, have many functions during
inflammatory and/or infectious diseases at the cerebral level.
In particular, they exhibit phagocytic activity, act as potent
antigen-presenting cells, and produce and are stimulated by
numerous biological products, including cytokines, whose au-
tocrine and paracrine roles as enhancers of antimicrobial func-
tions have been described elsewhere (13). Using RT-PCR
analysis, we demonstrate here that, unlike control brains from
naive or saline-treated mice, brains from CQ-treated mice
show induction of TNF-α and IL-6 gene expression. The phe-
nomenon is highly consistent (six of six mice) and does not
involve all cytokine genes, since IL-1β, IL-12, and iNOS gene
transcripts remain undetectable. To our knowledge, this is the
first evidence ascribing an immunostimulating role to CQ. In
this respect, it is of interest that a closely related compound,
hydroxychloroquine, has recently been reported to improve

<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>NAIVE</th>
<th>SALINE</th>
<th>CQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IL-1β</td>
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<td></td>
</tr>
<tr>
<td>iNOS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β ACTIN</td>
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several immunological parameters in human immunodeficiency virus-infected patients (41). In contrast, numerous reports document the wide spectrum of anti-inflammatory properties of CQ in vivo. Because of them, CQ is given as a useful therapeutic agent in several forms of arthritis and in experimental models of hemorrhagic shock, where monocyte/macrophage secretory functions appear to be CQ inhibited (17, 19). While further studies are required to better elucidate the phenomenon, our results imply that the brain compartment behaves differently from other tissues/organs, thus elaborating a peculiar biomolecular response(s) to a chemical compound such as CQ. In this respect, it is worth noting that once stimulated, Mδ of diverse anatomical origins express different patterns of effector and secretory functions (10). Also, the existence of a cell type other than the Mδ that is specific to the brain and is capable of elaborating a positive response to CQ (IL-6 and TNF gene expressions) is conceivable. Experiments will be performed to establish the susceptibility of astrocytes and endothelial or neuronal cells to CQ in vitro. Regardless of the responder cell type, CQ treatment is beneficial to the host in our experimental model. Interestingly, the phenomenon occurs only if the compound is given i.c. Administration of CQ intraperitoneally fails to affect mouse survival after cryptococcal i.c. challenge (data not shown), further strengthening the possibility of involvement of cerebral defense elements in the above-described phenomenon. As previously shown (4), the periphery is little or not at all related to the establishment of brain resistance. We consistently found induction of brain cytokine-specific gene expression in mice rendered resistant to lethal i.c. cryptococcal challenge by appropriate i.c. manipulation (4, 6).

In conclusion, while the exact mechanism(s) by which CQ confers brain anticytotoxic resistance remains to be clarified, we can speculate that there is at least a dual effect on brain immune cells: (i) enhancement of pH within the endosomal/lysosomal compartment and (ii) induction of cytokine gene expression. By acting separately and/or synergistically, such mechanisms may contribute to the observed containment of cerebral cryptococcosis in vivo.

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REFERENCES