Chloroquine Antagonizes the Proinflammatory Cytokine Response to Opportunistic Fungi by Alkalizing the Fungal Phagolysosome

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Recent observations demonstrated that the antimalarial drug chloroquine (CQ) can kill the opportunistic fungus Cryptococcus neoformans. Since CQ blunts lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α release, it was hypothesized that this drug would also interfere with the inflammatory response to C. neoformans and Candida albicans, another fungal opportunist. CQ inhibited TNF-α release from peripheral blood mononuclear cells from healthy and human immunodeficiency virus–positive donors without affecting NF-κB activation. CQ reduced TNF-α mRNA levels by a pH-dependent mechanism in a manner similar to 2 unrelated alkalizing drugs (ammonium chloride and bafilomycin), which also inhibited TNF-α gene expression. Although CQ inhibited release of interleukin (IL)-1β and IL-6, it did not affect IL-10 or macrophage inflammatory protein–1α production. Thus, CQ interferes with fungus-induced TNF-α expression by a mechanism that probably depends on the alkalization of endolysosomes. This contrasts with CQ’s reported pH-independent inhibition of LPS-stimulated TNF-α release and suggests that the mechanism of CQ’s anti-inflammatory effects is stimulus specific.

People with AIDS are highly predisposed to opportunistic infection with fungi, such as Cryptococcus neoformans and Candida albicans. Current therapy for opportunistic fungal disease is limited by the variable efficacy of treatment, which requires patients to undergo lifelong prophylaxis to prevent relapse. Furthermore, because of the prohibitive cost of conventional therapies, these drugs are unavailable to many people with human immunodeficiency virus (HIV) infection or AIDS. This deficit is most pronounced in the developing world, where HIV infection and opportunistic fungal diseases are commonplace. Thus, there is a great need for the development of less expensive and more effective drugs to combat these infections.

Chloroquine (CQ), a diprotic lysosomotropic weak base [1], is an affordable generic drug with >60 years of safe clinical use in the treatment of malaria and inflammatory disorders. CQ’s small, lipophilic nature enables it to readily penetrate the lipid bilayer upon which it diffuses down a pH gradient, accumulating within acidic endosomes and lysosomes. Diprotonation of this drug at low pH results in charge trapping, allowing for CQ to concentrate within acidic compartments >10,000-fold higher than its extracellular concentration [1]. It has long been thought that CQ acts within this compartment to mediate its numerous effects on eukaryotic cells. However, it was recently demonstrated that CQ does not require entry into this subcellular compartment to antagonize the lipopolysaccharide (LPS)-induced proinflammatory cytokine response [2]. Thus, it appears that the alkalization of endolysosomes is not the mechanism by which CQ mediates its anti-inflammatory effects in response to LPS.

Although CQ toxicity to the Plasmodium parasite is well documented, recent observations demonstrated that CQ can enhance survival in a murine model of cryptococcosis [3], an opportunistic fungal disease common in people with AIDS. CQ, at therapeutically relevant concentrations, directly inhibits and kills C. neoformans [4] and augments killing of this fungus by human monocytes [3]. In addition, this drug has been shown to inhibit and kill a variety of other fungal and bacterial pathogens by both direct and indirect mechanisms (for review, see [5]). The potential of this drug to reduce infection by a variety of microbes lends credibility to its potential use as a multiprophylactic agent in immunocompromised people.

Secondary infection with opportunistic fungi, such as C. neoformans and C. albicans, can be further deleterious to the immunocompromised host by activating HIV replication via secretion of proinflammatory cytokines, particularly tumor necrosis factor (TNF)-α [6]. Thus, the inflammatory response mounted against opportunistic organisms could represent a double-edged sword,
pitting eradication of the fungus against aggravation of the underlying immunodeficiency. Of import, after LPS challenge of human peripheral blood mononuclear cells (PBMC), CQ potently inhibits secretion of proinflammatory cytokines, including TNF-α [2]. Thus, in addition to its direct and indirect anticytotoxicity activity, CQ treatment might have the added benefit of blocking infection-induced HIV replication.

The aim of this study was to determine whether CQ concentrations required for killing C. neoformans would interfere with fungus-induced proinflammatory cytokine secretion. We evaluated whether pretreatment with a micromolar concentration range of CQ would interfere with TNF-α gene expression or release, using PBMC obtained from both healthy volunteers and HIV-positive people. Furthermore, since CQ is a weak base, we determined whether 2 additional drugs known to alkalize endosomes and lysosomes by distinct mechanisms would similarly interfere with TNF-α production.

Materials and Methods

Materials. Reagents were obtained from Sigma Chemical unless otherwise stated. Experiments were designed to minimize endotoxin contamination. All plasticware was obtained prepackaged and endotoxin free. RPMI 1640 and PBS were obtained from BioWhittaker and contained <0.005 U/mL of endotoxin. RPMI 1640 was supplemented with 1-glutamine, penicillin, and streptomycin. CQ and ammonium chloride were dissolved in PBS at stock concentrations of 10 mM and 2 M, respectively, and were filter sterilized. Bafilomycin A1 was solubilized in methanol (100 μM). Pooled human serum samples were obtained by combining serum samples from 10–15 healthy donors under conditions designed to preserve complement activity [7]. CQ, ammonium chloride, bafilomycin A1, and pooled human serum samples were stored in aliquots at −80°C and were thawed immediately before use. After pretreatment with alkalizing drugs and 8 h of fungal stimulation (a time when TNF-α release already has peaked), PBMC viability was measured by use of the Live/Dead Viability/Cytotoxicity Kit (Molecular Probes).

Fungi. Serotype A strain 145 of C. neoformans [8] (gift of Thomas Mitchell, Duke University) was grown at 30°C. CQ kills this strain of C. neoformans at therapeutically relevant concentrations [4]. A well-characterized strain of C. albicans [9] was grown in the yeast phase at 25°C. Fungi were harvested after 3 days of growth on Sabouraud dextrose agar (Remel), were washed 3 times with ice-cold PBS, were counted with a hemocytometer, and were resuspended at the appropriate density. All experiments were done using live fungi.

Isolation of PBMC. Peripheral blood was obtained by venipuncture from healthy volunteers or HIV-positive patients. The latter were recruited from the Immunodeficiency Clinic at Boston Medical Center. Blood samples from each donor were used no more than once per set of experiments. PBMC were purified by use of standard methods [8]. Blood was anticoagulated with 5 U pyrogen-free heparin (Fujisawa USA) per milliliter of blood and was centrifuged at 500 × g for 15 min. Leukocyte-rich buffy coats were then subjected to Ficoll-hypaque density gradient centrifugation, which was followed by collection of PBMC from the density fraction. Cells were washed 3 times with ice-cold PBS before counting by hemocytometer and resuspension in RPMI 1640.

Cytokine release. PBMC (2 × 10⁵ to 1 × 10⁶) were preincubated in the absence or presence of CQ (100 μM), ammonium chloride (100 mM), or bafilomycin A1 (100 nM) for 2 h at 37°C in 96-well polystyrene plates (Dyneatech Laboratories) containing 200 μL of RPMI 1640. Intracellular concentrations of CQ [10] and bafilomycin A1 [11] reach equilibrium within this time period. Cells were then stimulated for 18 h with C. neoformans (10 fungi per mononuclear cell) or C. albicans (1 fungus per mononuclear cell) in the presence of 10% pooled human serum. Preliminary experiments demonstrated that this fungus-to-PBMC ratio induced near maximal TNF-α release [8, 12]. Inhibitors were not washed away before stimulation. Cell-free supernatant was collected and was analyzed by ELISA for human TNF-α, interleukin (IL)-1β, IL-6 [2], macrophage inflammatory protein (MIP)-1α [13], and IL-10. In brief, to assay for TNF-α, MIP-1α, and IL-10, we obtained antibody pairs and recombinant cytokine standards (R&D Systems) and performed the ELISA, according to the manufacturer’s instructions, by using horseradish peroxidase as the detection reagent. IL-1β and IL-6 concentrations were assayed by ELISA (Biosource International), according to the manufacturer’s protocol.

Isolation and quantitation of TNF-α mRNA by Northern blotting and RNase protection assay. PBMC (5 × 10⁶) were incubated in 6-well polystyrene plates in RPMI 1640 for 2 h at 37°C in the absence or presence of CQ (10 or 100 μM), ammonium chloride (100 mM), or bafilomycin A1 (100 nM). Cells then were stimulated with C. neoformans or C. albicans in the presence of 10% pooled human serum for 3 and 2 h, respectively. Total cellular RNA was extracted from PBMC, using reagent (TRIZOL; Life Technologies Gibco BRL), as described elsewhere [8]. Total RNA was separated on 1.2% agarose-formaldehyde gels, was transferred to nylon membranes (Immobilon-Ny+; Millipore), and was analyzed by Northern blotting, using a 32P-labeled human TNF-α cDNA probe exactly as in previous studies [2]. Because of the weaker induction of TNF-α expression by C. neoformans, twice the amount of RNA was loaded after C. neoformans stimulation of PBMC (10 μg/lane), compared with that after C. albicans stimulation (5 μg/lane). RNA integrity and equal loading were evaluated by ethidium-bromide staining of agarose gels before transfer, as well as by stripping and reprobing of membranes with a G3PDH cDNA probe (Clontech). Alternatively, total RNA (5 μg) was analyzed by use of the RNase protection assay (PharMingen). The mRNA content of specific bands was quantified by phosphorimager analysis (Molecular Dynamics). No TNF-α or G3PDH RNA was observed when C. neoformans or C. albicans, without PBMC, were subjected to the same RNA extraction and hybridization procedure (data not shown).

Electrophoretic mobility shift assay. Nuclear translocation of the transcription factor NF-κB was assayed, according to a standard protocol [2, 12]. In brief, nuclear extracts from PBMC were prepared in the presence of protease inhibitors after stimulation for 45 min with C. neoformans or 30 min with C. albicans. Total protein content of the nuclear extracts was determined using a commercial kit (Bio-Rad Laboratories). An oligonucleotide containing the NF-κB consensus binding sequence was obtained (Promega) and end-labeled with [α-32P]dATP and [α-32P]dCTP, using Klenow DNA polymerase (Promega). Unincorporated nucleotides
Chloroquine (CQ) antagonizes fungus-stimulated TNF-α release. We previously showed that CQ interferes with LPS-stimulated release of the proinflammatory cytokine TNF-α [2]. Since the inflammatory response to opportunistic fungi is implicated in the exacerbation of HIV disease [6], we set out to determine whether CQ would similarly interfere with the proinflammatory cytokine response to these organisms. TNF-α release was examined by pretreating PBMC with CQ for 2 h, followed by incubation with C. neoformans or C. albicans for 18 h. CQ inhibited TNF-α release in response to both fungi by a dose-dependent mechanism over a concentration range of 1–100 μM (figure 1A). At the highest concentration, CQ almost completely abrogated this response, reducing TNF-α release to <6.5% of control levels, respectively (P < .001; n = 3). The calculated IC₅₀ value was 18.0 and 7.7 μM of CQ for C. neoformans and C. albicans, respectively. Similar findings were observed after CQ pretreatment of PBMC obtained from HIV-positive donors, with inhibition seen at the lowest CQ concentration studied (10 μM). Nearly complete abrogation was observed at the highest CQ concentration evaluated (100 μM), reducing TNF-α release to 6.4% ± 3.4% and 0.8% ± 0.8% of control levels for C. neoformans and C. albicans, respectively (P < .01; n = 3; figure 1B).

CQ interferes with TNF-α release when added concurrently with the fungal stimulus or when added after stimulation. To determine the kinetics of CQ’s effects, we added the drug at the time of fungal stimulation or 2 h after the addition of C. neoformans or C. albicans. Consistent with the effects observed after CQ pretreatment, the addition of the drug at later time points reduced TNF-α release but to a lesser degree. For example, treatment with CQ (100 μM) concurrent with the addition of C. neoformans or C. albicans reduced TNF-α release to 26.1% ± 7.6% and 4.8% ± 1.5%, re-
respectively, of control levels (P < .005; n = 3). However, CQ (100 μM) treatment 2 h after stimulation reduced Cryptococcus- and Candida-induced TNF-α release to 39.4% ± 5.2% and 21.5% ± 7.6%, respectively, of control levels (P < .004; n = 3). Similar trends were observed using CQ concentrations of 10 and 30 μM (data not shown).

CQ interferes with fungus-induced TNF-α mRNA accumulation. To identify the step in TNF-α production at which CQ is active, Northern blotting was performed to determine the effect of CQ treatment on TNF-α mRNA accumulation. PBMC were preincubated with concentrations of CQ, which were shown to inhibit TNF-α release (10 and 100 μM), for 2 h before stimulation with C. neoformans and C. albicans for 3 and 2 h, respectively. Previous studies suggested that each fungus induced peak TNF-α mRNA levels at the respective time points [8, 12]. Total RNA was extracted, and Northern blotting was performed to quantitate cellular levels of mRNA for TNF-α and the constitutively expressed gene, G3PDH. Compared with unstimulated PBMC, C. neoformans and C. albicans induced TNF-α mRNA levels by 4.1 ± 2.9-fold and 6.4 ± 3.4-fold, respectively (mean ± SE; n = 5). Preincubation with the highest level of CQ tested resulted in the reduction of TNF-α mRNA levels to 29.4% ± 18.0% and 20.5% ± 12.7% of control levels induced by C. neoformans and C. albicans, respectively, as measured by phosphorimager analysis of multiple independent experiments (P < .04; n = 5; figure 2). However, mRNA levels of the constitutively expressed gene, G3PDH, were not significantly affected by any concentration of CQ examined.

Endolysosomal alkalization by CQ and other alkalizing drugs reduces fungus-stimulated TNF-α release. Since CQ is a weak base, the next set of experiments was done to determine whether this drug acted by a pH-dependent mechanism. To evaluate whether alkalization of endosomes and lysosomes was sufficient to inhibit TNF-α release, we used the chemically unrelated weak base, ammonium chloride, and a specific inhibitor of lysosomal v-ATPase, baflomycin A₁, to evaluate the effect of endolysosomal alkalization on TNF-α release. Preincubation with ammonium chloride (100 mM) and baflomycin A₁ (100 nM) reduced Cryptococcus-stimulated TNF-α release to 7.9% ± 2.3% and 14.6% ± 4.8%, respectively, of control levels (P < .002; n = 3; figure 3A). Candida induction was reduced to 25.7% ± 10.7% and 10.4% ± 2.8% of control TNF-α levels by ammonium chloride and baflomycin A₁, respectively (P < .002; n = 3; figure 3A). These results argue that endolysosomal alkalization is sufficient to reduce fungus-stimulated release of TNF-α and suggest that CQ could mediate its effects by a similar mechanism of action.

Drugs that alkalize endosomes and lysosomes reduce TNF-α mRNA levels. To determine whether endolysosomal alkalization similarly interfered with TNF-α release at the pretranslational level, we evaluated TNF-α mRNA levels after pretreatment with the same alkalizing drugs. PBMC were pretreated with CQ (100 μM), ammonium chloride (100 mM), or baflomycin A₁ (100 nM) for 2 h before stimulation with C. neoformans (3 h) or C. albicans (2 h). Although these drugs raise endolysosomal pH by distinct mechanisms, each reduced levels of TNF-α mRNA to a similar degree, as measured by the RNase protection assay (figure 3B). These effects appeared to be specific for TNF-α since neither drug interfered with expression of the constitutively expressed gene, G3PDH.

PBMC viability after pretreatment with alkalizing drugs and fungal stimulation. To determine whether these observations involved cellular cytotoxicity, we performed viability staining 8 h after stimulation, a time beyond the point at which fungus-induced TNF-α release has reached a plateau [12, 14]. Live/dead staining did not reveal any significant difference in the viability of mononuclear cells under any of the pretreatment conditions (table 1). However, following incubation with live fungi, there was a trend toward reduced mononuclear cell viability, consistent with previous studies showing killing of mononuclear phagocytes following incubation with live C. albicans [15]. These data strongly suggest that CQ cytotoxicity is not responsible for the anti-inflammatory effects observed in this study.

CQ does not interfere with NF-κB nuclear translocation. The transcription factor NF-κB is known to induce transcription of numerous inflammatory genes, including TNF-α. On the basis of the finding that CQ pretreatment decreased TNF-α mRNA levels, we determined the effect of CQ on NF-κB entry into the nucleus following fungal stimulation. PBMC were preincubated in the absence or presence of CQ (100 μM) for 2 h before stimulation with C. neoformans (45 min) or C. albicans (30 min). Adherent cells were harvested, and nuclei were extracted. As described elsewhere, unstimulated PBMC contained quantifiable amounts of NF-κB in the nucleus [16]. Stimulation with C. neoformans and C. albicans led to 2.0 ± 0.7-fold and 3.0 ± 1.1-fold induction of NF-κB translocation, respectively (P < .03; n = 3; figure 4). The highest level of CQ evaluated...
(100 μM) did not reduce NF-κB translocation to the nucleus, nor did CQ appear to alter the balance of stimulatory p50/p65 heterodimers (figure 4, arrow I) and inhibitory p50/p50 homodimers (figure 4, arrow II), as measured by antibody supershift analysis. In fact, there was a trend toward elevated nuclear levels of NF-κB after CQ treatment.

CQ antagonizes release of other proinflammatory cytokines but not IL-10 or the β-chemokine MIP-1α. Consistent with the anti-inflammatory efficacy of CQ, pretreatment reduced release of both IL-1β and IL-6 after stimulation with either Cryptococcus or Candida (figure 5). Pretreatment of PBMC with CQ concentrations as low as 10 μM significantly reduced release of both cytokines. Consistent with the effects of CQ on TNF-α release, addition of this drug concurrently or 2 h after stimulation inhibited release of both IL-1β and IL-6, although in a less robust fashion than exhibited after drug pretreatment (data not shown). However, release of IL-10 and the proinflammatory β-chemokine MIP-1α was not affected significantly by pretreatment with the highest concentration studied. Thus, CQ treatment appeared to interfere with release of a specific subset of inflammatory cytokines while not affecting the production of others.

Discussion

In this study, we demonstrate that CQ mediates dose-dependent inhibition of TNF-α release from human PBMC after stimulation with the opportunistic fungi Cryptococcus neoformans and Candida albicans. CQ appears to act at the pretranslational level, reducing levels of TNF-α mRNA without interfering with NF-κB activation. Previous findings from this laboratory suggest that CQ does not interfere with posttranslational processing or secretion of TNF-α [2]. The observation that endolysosomal alkalization with ammonium chloride or bafilomycin A1 is sufficient to reproduce these effects suggests that CQ acts by a pH-dependent, lysosomotropic mechanism. These findings expand on the mechanism by which this enigmatic drug blunts the proinflammatory cytokine response and begin to elucidate the diverse ways that CQ interferes with cellular function.

CQ was shown to have a calculated IC50 of 18.0 μM for C. neoformans–induced TNF-α release, compared with 7.7 μM for C. albicans. The discrepancy between these 2 values could result from differences in the stimulation conditions used in these studies. Specifically, it was determined that the ratio of live fungi to PBMC yielding near maximal TNF-α release was 10:1 for C. neoformans, compared with 1:1 for C. albicans. This laboratory has previously shown that CQ accumulates to high levels within a complex vacuolar system of C. neoformans [4]. Thus, addition

Table 1. Effect of alkalizing drug treatment on peripheral blood mononuclear cell (PBMC) viability.

<table>
<thead>
<tr>
<th>Alkalizing drug</th>
<th>Stimulant</th>
<th>None</th>
<th>Chloroquine (100 μM)</th>
<th>Ammonium chloride (100 μM)</th>
<th>Bafilomycin A1 (100 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>94.5 ± 4.6</td>
<td>90.3 ± 5.2</td>
<td>95 ± 2.0</td>
<td>95.2 ± 2.2</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>85.5 ± 9.8</td>
<td>86.2 ± 5.5</td>
<td>83.2 ± 5.2</td>
<td>86.0 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>89.8 ± 3.8</td>
<td>87.8 ± 1.2</td>
<td>88.0 ± 4.0</td>
<td>88.8 ± 3.8</td>
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NOTE: Data are mean percentage ± SE. PBMC were pretreated with the indicated alkalizing drug for 2 h. Cells were either stimulated with the indicated fungus or were left unstimulated for 8 h. Data are representative of 3 experiments performed in triplicate (P = not significant for all alkalizing drug treatment conditions).
Transcriptional machinery required for TNF-α transcription factors or the interaction of these factors with the promoter requires additional transcription factors, including cAMP response element binding protein and Egr-1 [22].

NF-κB previously demonstrated a similar effect of CQ on LPS-stimulated macrophage proinflammatory cytokine release possibly because of induction of the transcriptional activator NF-AT [23]. The finding in this study that weak bases, such as CQ and ammonium chloride, both of which would be expected to alkalize the cytoplasm, antagonize TNF-α gene expression is consistent with this hypothesis. However, our results using bafilomycin A₁, a drug that alkalizes endolysosomes at the expense of acidifying the cytoplasm, would argue against this thesis. Furthermore, considering that similar effects were observed with CQ, ammonium chloride, and bafilomycin A₁ treatment, it would appear that the most likely unifying mechanism would involve alkalization of the endolysosome.

This study is in agreement with previous publications suggesting that CQ antagonizes TNF-α release from human mononuclear cells [2, 24, 25]. However, the current observations using fungal stimulation contrast with previous work from this laboratory and others that used LPS as a stimulus. In those studies, CQ interfered with TNF-α release by a pH-independent mechanism [2, 25, 26]. It remains to be determined how CQ interferes with fungus- and LPS-stimulated TNF-α expression by distinct mechanisms. One possibility is that these mechanistic discrepancies result from functional differences in the signaling pathways triggered by different stimuli. Since C. neoformans and C. albicans, both particulate stimuli, are ingested into the phagolysosome, CQ could potentially interfere with signaling events emanating from this acidic subcellular compartment [27–30] by alkalizing its contents. In contrast, the soluble stimulus LPS is thought to transduce signals across the cell surface via the Toll-like family of receptors, without traversing the endocytic pathway or acidic compartments [31]. These differences argue that CQ acts by multiple distinct mechanisms, both pH-dependent and -independent, to achieve its anti-inflammatory effects.

It is interesting to note that CQ interferes with Cryptococcus- and Candida-induced release of TNF-α, IL-1β, and IL-6 while not affecting either IL-10 or MIP-1α production. It appears that CQ interferes with signaling pathways required for proinflammatory cytokine gene expression. As the above-mentioned cytokines are coregulated and simultaneously induced by inflammatory stimuli, it is possible that certain cellular signaling pathways are required for the expression of this group of inflammatory genes. Thus, interference with a single signaling pathway could potentially interfere with the expression of multiple target cytokine genes. Alternatively, as each of these cytokines is known to potently up-regulate their own expression as well as the expression of other cytokine genes, it is possible that interference with TNF-α release could secondarily lead to reduced expression of IL-1β and IL-6 by blocking autocrine signaling loops required for potent IL-1β and IL-6 production.

Recent studies suggest that CQ antagonizes HIV replication by interfering with posttranslational processing and production of mature viral membrane glycoproteins, resulting in the assembly of noninfectious virions [32–35]. CQ also appears to potentiate the activity of conventional antiretroviral drugs when given in combination [36]. Consistent with these in vitro findings, 2...
Figure 5. Chloroquine (CQ) reduces Cryptococcus- and Candida-stimulated release of interleukin (IL-1β and IL-6) but not of IL-10 or macrophage inflammatory protein (MIP)-1α. Peripheral blood mononuclear cells were pretreated for 2 h with the indicated concentration of CQ and were stimulated for 18 h. ELISA was used to evaluate cell-free supernatants for human IL-1β, IL-6, IL-10, and MIP-1α after stimulation with Cryptococcus neoformans (A) and Candida albicans (B). Data are expressed as percentage of Cryptococcus- and Candida-induced TNF-α release in the absence of CQ (percentage of control). Without CQ pretreatment, Cryptococcus neoformans induced the release of 0.4 ± 0.2 ng/mL, 1.1 ± 0.5 ng/mL, 35.9 ± 14.1 pg/mL, and 38.9 ± 21.4 ng/mL of IL-1β, IL-6, IL-10, and MIP-1α, respectively, whereas C. albicans stimulation resulted in the release of 4.2 ± 1.3 ng/mL, 25.2 ± 19.3 ng/mL, 140.7 ± 109.5 pg/mL, and 22.8 ± 2.0 ng/mL of IL-1β, IL-6, IL-10, and MIP-1α, respectively. Data represent mean ± SE for 3–6 experiments done in duplicate. * P < .01; ** P < .005. ND, not done.

small clinical studies demonstrated some benefit of CQ in the treatment of HIV infection [37, 38]. The data presented here also suggest that CQ treatment might further reduce viral burden in patients by interfering with infection-induced release of proinflammatory cytokines, such as TNF-α, which are known to potentially up-regulate virus replication [6]. Thus, it is clear that CQ could potentially interfere with production of infectious virus at multiple points in the virus replication pathway.

It is important to note that the effects observed in this study are achieved at therapeutically relevant concentrations of CQ [10]. It is unlikely that nonspecific cytotoxicity could explain these findings because several cellular functions were shown to be unaffected by CQ pretreatment. Consistent with previous studies, CQ did not reduce cellular viability [2, 24, 26], nor did CQ inhibit NF-κB translocation, protein phosphorylation (unpublished data), expression of the constitutively expressed gene, G3PDH, or release of IL-10 and MIP-1α.

Considering CQ’s potential utility as adjuvant therapy for cryptococcosis [3], as well as for a variety of other serious fungal and bacterial infections (for review, see [5]), our data suggest caution must be exercised to ensure that CQ therapy is not deleterious to the host inflammatory response. However, in animal models of cryptococcosis, TNF-α is critical within 2 weeks following infection [39, 40]. Once an adaptive T cell response has been established within this period, blockade of TNF-α is no longer deleterious in this model. Extrapolating these findings to patients with cryptococcosis, it is likely that most will have either already mounted an adaptive response to the organism or be incapable of doing so because of preexisting severe immunosuppression. Thus, there is reason to believe that CQ therapy of an established cryptococcal infection will not be deleterious to the host response to this organism.

Acknowledgments

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References

4. Harrison TS, Griffin GE, Levitz SM. Conditional lethality of the diprotic...


