Antagonism of Immunostimulatory CpG-Oligodeoxynucleotides by Quinacrine, Chloroquine, and Structurally Related Compounds

Donald E. Macfarlane and Lori Manzel

*J Immunol* 1998; 160:1122-1131;  
http://www.jimmunol.org/content/160/3/1122

---

**Why The JI?**

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

---

**References**  This article cites 42 articles, 12 of which you can access for free at:  
http://www.jimmunol.org/content/160/3/1122.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at:  
http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at:  
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at:  
http://jimmunol.org/alerts
Antagonism of Immunostimulatory CpG-Oligodeoxynucleotides by Quinacrine, Chloroquine, and Structurally Related Compounds

Donald E. Macfarlane and Lori Manzel

Phosphorothioate oligodeoxynucleotides containing CpG (CpG-ODN) activate immune responses. We report that quinacrine, chloroquine, and structurally related compounds completely inhibit the antipoptotic effect of CpG-ODN on WEHI 231 murine B lymphoma cells and inhibit CpG-ODN-induced secretion of IL-6 by WEHI 231. They also inhibit IL-6 synthesis and thymidine uptake by human unfractionated PBMC induced by CpG-ODN. The compounds did not inhibit LPS-induced responses. Half-maximal inhibition required 10 nM quinacrine or 100 nM chloroquine. Inhibition was noncompetitive with respect to CpG-ODN. Quinine, quinidine, and primaquine were much less powerful. Quinacrine was effective even when added after the CpG-ODN.

Near-toxic concentrations of ammonia plus bafilomycin A1 (used to inhibit vesicular acidification) did not reduce the efficacy of the quinacrine, but the effects of both quinacrine and chloroquine were enhanced by inhibition of the multidrug resistance efflux pump by verapamil. Agents that bind to DNA, including propidium iodide, Hoechst dye 33258, and coralyne chloride did not inhibit CpG-ODN effect, nor did 4-bromophenacyl bromide, an inhibitor of phospholipase A2. Examination of the structure-activity relationship of seventy 4-aminoquinoline and 9-aminoacridine analogues reveals that increased activity was conferred by bulky hydrophobic substituents on positions 2 and 6 of the quinoline nucleus. No correlation was found between published activity and published charges. This article must therefore be hereby marked in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Bacterial DNA is increasingly being recognized as a powerful modulator of immunity (1, 2), stimulating the polyclonal proliferation of B cells and the production of cytokines by monocytes and other cells (3–5). This activity is attributed to unmethylated CpG sequences in bacterial DNA, which are uncommon in vertebrate DNA (6). Selected single-stranded oligodeoxynucleotides (especially those synthesized with a nuclease-resistant phosphorothioate backbone) that include the dinucleotide CpG (CpG-ODN)3 mimic many of the actions of bacterial DNA (7) and powerfully inhibit the induction of apoptosis in mouse WEHI 231 B cells by Ab to cell surface IgM (5, 8). This system is a convenient and reproducible model to study responses to CpG-ODN.

Bacterial DNA immobilized on beads does not stimulate immune responses, suggesting that internalization of the DNA is required for activity (6). DNA and oligodeoxynucleotides are endocytosed into acidic vesicles and are then transported to the cytoplasm and nucleus of cells (9–11).

Chloroquine, hydroxychloroquine, and quinacrine induce remissions of systemic lupus erythematosus and rheumatoid arthritis by an unknown mechanism (12, 13). These drugs bind to DNA by intercalation (14), and they are weak bases and partition into acidic vesicles. At high concentration, chloroquine can collapse the pH gradient and disrupt the action of endosomal hydrolytic enzymes and the trafficking of receptors (15).

Here we report that quinacrine, chloroquine, and similar 4-aminoacridine and 4-aminoquinoline compounds completely block the immunostimulatory action of CpG-ODN at concentrations much below those needed for other reported anti-inflammatory effects of these compounds in vitro. We discuss whether inhibition of immune responses triggered by CpG-ODN could account for the ability of these agents to induce remissions of rheumatoid arthritis and systemic lupus erythematosus, and whether further drug development optimizing this activity might lead to more useful remittive and anti-inflammatory agents.

Materials and Methods

Methods

Cell culture. WEHI 231 cells (ATCC CRL 1702; American Type Culture Collection, Rockville, MD) were grown in log phase in medium consisting of RPMI 1640 medium supplemented with 10% FBS (heat inactivated at 65°C for 1 h), 0.3 g/L L-glutamine, 50 mg/L gentamicin, and 0.05 mM 2-ME in a 37°C humid atmosphere with 5% CO2. Cells were diluted with 0.4% trypan blue in PBS, and live cells were counted in a hemacytometer.

Human unfractionated PBMC were prepared from blood anticoagulated with heparin. The mononuclear cells were isolated using Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) and resuspended at 2 × 106/ml in RPMI 1640 medium supplemented with 10% autologous serum (heat inactivated 56°C, 1 h).

Growth curves. Cells were resuspended in medium at 2 × 105/ml in 24-well tissue culture plates (Costar, Cambridge, MA). Appropriate additions were made, and the cells were incubated and counted daily. Cells were diluted with fresh medium if the concentration exceeded 7 × 105/ml.

Received for publication June 23, 1997. Accepted for publication October 16, 1997.

1 This work was supported by a Merit Review Grant to D.E.M. from the Department of Veterans Affairs.

2 Address correspondence and reprint requests to Dr. Donald Macfarlane, Internal Medicine, University of Iowa, Iowa City, IA 52242. E-mail address: donald-macfarlane@uiowa.edu

3 Abbreviations used in this paper: CpG-ODN, oligodeoxynucleotides containing CpG motif; α-sIgM, goat anti-mouse surface IgM.

Department of Medicine, Veterans Affairs Medical Center and University of Iowa, Iowa City, IA 52242.

Copyright © 1998 by The American Association of Immunologists
\[^{3}H\]Thymidine uptake. Cells (200 \(\mu\)l in duplicate or triplicate) were incubated in a 96-well plate (Costar). Additions were made as indicated. After incubation, \([^{3}H\]|{\text{methyl-}}\[^{3}H\])thymidine (2 Ci/mmol, DuPont/NEN, Boston, MA) was added to each well, and the cells were incubated for 4 h (WEHI 231 cells) or 18 h (human PBMC). Cellular DNA was then captured onto glass fiber filters (no. 934AH, Whatman Ltd., Maidstone, U.K.) using a cell harvester (Brandel, Gaithersburg, MD). The filters were dried, placed into plastic scintillation vials with 10 \(\mu\)l scintillation mixture (EconoSafe, Research Products International, Mount Prospect, IL), and counted in a liquid scintillation counter (no. LS-3133 T, Beckman Instruments, Fullerton, CA). The \(^{3}H\) recovered on the filter was expressed as a fraction of the \(^{3}H\) added.

DNA fragmentation assay. A DNA fragmentation assay was performed as previously described (8). In brief, WEHI 231 cells were incubated with \[^{3}H\]thymidine and resuspended in fresh medium. After incubation for 16–18 h, with additions as indicated, the cells were centrifuged, washed, and resuspended in 20 \(\mu\)l PBS. This suspension was drawn into a pipette tip containing 25 \(\mu\)l 2% SDS, and the mixture was immediately ejected into a well of a 0.8% agarose TBE gel containing 0.5 \(\mu\)g/ml ethidium bromide. The gel was electrophoresed, rinsed 1 h at 65 V, and the lanes of the gel were cut into three approximately equal portions. The first portion included the gel well and the first 2 mm of the running lane. The second and third pieces were the remainder of the lane. The amount of fragmented DNA (\(^{3}H\) in the second and third gel pieces) was expressed as a percentage of the total in the lane.

Assay of IL-6. WEHI 231 cells (2 \(\times\) 10\(^{5}\)/ml) were incubated with or without ODN 1760 (6 \(\mu\)g/ml) and with or without chloroquine or quinacrine. After 24 h, the IL-6 content of the supernatant was assayed by ELISA using two mAbs in a sandwich technique as described by the manufacturer, using the standards supplied (PharMingen, San Diego, CA). IL-6 production by PBMC was assayed similarly, using Abs supplied by R&D Systems (Minneapolis, MN).

Materials

CpG-ODN 1760 has the sequence 5'-ATAATCGACGTTCAAGCAAG-3' synthesized with a phosphorothioate or (when indicated) with a phosphodiester backbone. The fluorescent ODN was ODN 1760 with fluorescein linked to the 5' terminus. The oligonucleotides were purchased from Genosys (The Woodlands, TX).

The following were all purchased from Sigma Chemical Co.: goat antimouse surface IgM (\(\alpha\)-sIgM, cat. M8644), LPS (Escherichia coli serotype 012, cat. L3129), amodiaquine, chloroquine, quinacrine, primaquine, quinidine, quinine, coralyne chloride, and thapsigargin. Hydroxychloroquine was purchased from Copley Pharmaceutical, Inc. (Canton, MA). Veparapin was from American Reagents Laboratory (Shirley, NY). C\(_2\)-ceramide was purchased from Matreya, Inc. (Pleasant Gap, PA). Acridine homodimer (bis-(6-chloro-2-methoxy-9-acridinyl)spermine) and 9-amino-6-chloro-2-methoxyacridine were purchased from Molecular Probes (Eugene, OR). The other antimalarials listed in Tables I and II were generous gifts from Dr. Jill Johnson (National Cancer Institute).

Results

Suppression of apoptosis by CpG-ODN

WEHI 231 murine B cells are killed by engagement of \(\alpha\)-sIgM. This phenomenon is believed to simulate the deletion of self-reactive clones during B cell ontology. We and others have shown that single-stranded phosphorothioate CpG-ODNs reverse the inhibition by \(\alpha\)-sIgM of thymidine incorporation by WEHI 231 cells (5, 8).

In Figure 1, we show that several antimalarial drugs added simultaneously with the phosphorothioate CpG-ODN block the effect of the CpG-ODN. WEHI 231 cells were incubated with \(\alpha\)-sIgM, ODN 1760, and the indicated concentration of the antimalarial. After 16 h, \[^{3}H\]thymidine was added. In the absence of other additions, \(\alpha\)-sIgM powerfully inhibits \[^{3}H\]thymidine incorporation. This inhibition is almost completely relieved by ODN 1760. Figure 1 also shows that quinacrine, hydroxychloroquine, chloroquine, and amodiaquine (substituted 4-aminochloroquinolines) at nanomolar concentrations completely inhibited the CpG-ODN effect. Antimalarials added by themselves did not inhibit \[^{3}H\]thymidine incorporation unless added at concentrations in well in excess of 30 \(\mu\)M (data not shown). Quinidine and quinine (substituted 4-methanolquinolines) and primaquine (a substituted 8-aminoquinoline) had little effect on CpG-ODN action at concentrations less than 10 \(\mu\)M (Fig. 1). Quinacrine and chloroquine were selected for further study.

Effect on growth and apoptosis

Quinacrine alone influenced neither cell growth nor cell death induced by \(\alpha\)-sIgM. CpG-ODN protects cells against \(\alpha\)-sIgM-induced apoptosis. Quinacrine reversed this protection by ODN 1760, revealed by measurement of both cell growth (Fig. 2A) and DNA fragmentation (Fig. 2B). Similar results were obtained with chloroquine (data not shown). These data show that the effect of antimalarials on thymidine uptake is a true reflection of their effect on cell growth and protection from apoptosis.

Noncompetitive kinetics

In the experiment shown in Figure 3, we measured the inhibition by \(\alpha\)-sIgM of thymidine incorporation in the presence of a range of concentrations of ODN 1760 and quinacrine. The data show that increasing the concentration of ODN 1760 does not overcome the inhibitory effect of quinacrine, suggesting that the antimalarials do not compete with CpG-ODN for a common site, such as a receptor, an enzyme active site or a transport protein.

Subsequent addition

We suspected that antimalarials might interfere with the transport of ODNs to an intracellular site of action, in which case antimalarials would prevent, but not to reverse, the effect of (nuclelease-resistant) CpG-ODNs. This prediction does not appear to be correct. Figure 4 shows that quinacrine blocks the action of the phosphorothioate CpG-ODN even when added 8 h after the CpG-ODN, suggesting that quinacrine interferes with the mechanism the cells use to recognize CpG-ODN (or with the subsequent signal-response coupling) rather than transport.

Action on phosphodiester ODN

The CpG-ODN used in typical experiments was synthesized on a nuclease-resistant phosphorothioate backbone. The phosphodiester ODN with the same base sequence as ODN 1760 is considerably less potent than ODN 1760. Figure 4 shows that quinacrine is effective in blocking the action of phosphodiester ODN, although the inhibitory effect is less complete than with phosphorothioate ODNs.

Effect on CpG-ODN uptake

We evaluated the effect of chloroquine on the cellular incorporation of fluorescein-labeled ODN 1760 by fluorescence microscopy of WEHI-231 cells after they were fixed with paraformaldehyde. Preliminary experiments showed that this fixation had little effect on the distribution or the intensity of the fluorescence compared with live cells. Fixation was used to eliminate the potential of chloroquine to alter fluorescence intensity by altering vesicular pH. The fluorescein-labeled CpG-ODN is taken up into numerous small perinuclear structures, and this uptake is not inhibited by chloroquine; indeed, uptake appeared to be enhanced (Fig. 5).

Inhibition of acidification

4-Aminoquinoline antimalarials partition into acidic vesicles (15). We wondered whether preventing this concentrating effect might reduce the potency of the antimalarials. We used a combination of ammonium acetate (a permeable cation that collapses proton gradients, at concentrations ranging up to 10 mM) and bafilomycin A1 (an inhibitor of the vacuolar type of proton pumping ATPase (16,
17), up to 5 nM) to try to block vesicular acidification. This combination of agents at the higher concentrations is toxic to the cells, reducing thymidine uptake. It marginally reduced the efficacy of the CpG-ODN to protect from apoptosis (without affecting the ability of \( \alpha \)-sIgM to induce growth arrest), but the combination did not block the action of quinacrine (data not shown).

DNA binding agents
Quinacrine and chloroquine bind to dsDNA by intercalating between adjacent bases. We wondered whether other agents known to bind to dsDNA could influence the action of CpG-ODN. We found that neither the intercalating agent propidium iodide nor the minor groove binding Hoechst dye 33258 (both added at 5 \( \mu \)g/ml) influenced the action of CpG-ODN on WEHI 231 cells. Quinacrine also stabilizes triple-helix DNA, but coralyne chloride at 1 \( \mu \)M (which stabilizes triple-helix as effectively as quinacrine) (18) did not inhibit CpG-ODN responses (data not shown).

No effect of quinacrine on LPS response
The B cell repertoire is regulated by a number of factors that modulate clonal deletion, including LPSs (endotoxin, LPS, derived...
from Gram-negative bacteria), which promote B cell growth and inhibit B cell apoptosis. Chloroquine and other antimalarials have been reported to block LPS-induced responses in mononuclear cells (19, 20). In the experiment shown in Figure 6, we examined the influence of LPS on $\alpha$-sIgM-induced suppression of thymidine incorporation. Even at high concentrations, LPS has much less ability than ODN 1760 to reverse $\alpha$-sIgM-induced growth inhibition in WEHI 231 cells. Quinacrine at a concentration that completely reverses the effect of ODN 1760 had no effect on the action of LPS. Thus (at the concentration we used), quinacrine does not block the detection of LPS and subsequent signal-response coupling in the same way as it does with CpG-ODN.

**CpG-ODN blockade of apoptosis induced by other agents**

We have previously shown that CpG-ODNs block apoptosis in WEHI 231 cells induced by stimuli other than $\alpha$-sIgM (8). To determine whether the action of antimalarials is restricted to $\alpha$-sIgM-induced apoptosis, we examined the effect of quinacrine on the protection by ODN 1760 from cell death induced by other agents. C2-ceramide mediates receptor-induced cell death (21) and inhibits thymidine incorporation. Figure 7A shows that this effect of C2-ceramide is reduced by ODN 1760, and that quinacrine ...
blocks this protective effect. Thapsigargin induces an increase in intracellular calcium, resulting in massive DNA fragmentation. Figure 7B shows that quinacrine also blocks the protective effect of ODN 1760 against thapsigargin-induced DNA fragmentation. Thus, quinacrine seems to block the protective effect of phosphorothioate CpG-ODN generally, not just the protection against at α-sIgM-induced apoptosis.

Inhibition of multidrug-resistance transport

Multidrug resistance is caused by the active transport of drugs out of the cell. This transport mechanism is inhibitable by verapamil. Chloroquine-resistant variants of malaria accelerate efflux of the drug, an effect reversed by verapamil (22). In Figure 8 we demonstrate that verapamil increases the potency of chloroquine (Fig. 8, top) or quinacrine (Fig. 8, bottom). This results suggests that these compounds act intracellularly, in a compartment of the cell from which they are pumped by the multidrug-resistance mechanism. Verapamil has little effect on the action of CpG-ODNs at 10 μM.

Structure-activity relationship

A large number of analogues of antimalarial compounds have been synthesized, many with structures similar to chloroquine or quinacrine (23, 24). We determined the activity of several of these compounds using the thymidine incorporation technique. Each compound yielded dose-response curves similar to those in Figure 1. From these curves, we estimated the concentration of the test compound required for half maximal blockade of the CpG-ODN effect. The results are shown in Tables I and II, revealing that bulky substituents on positions 2 and 6 of the quinoline ring tend to enhance activity. Figure 9 illustrates how some of these substituents on the quinoline nucleus impact on this activity.

Several attempts have been made to determine the structural requirements for antimalarial action (25–27). We tested seven compounds in common with these studies. We found no correlation between the reported antimalarial activity of these seven compounds and their ability to inhibit CpG-ODN-driven responses.

Effect on IL-6 synthesis

Our strain of WEHI 231 cells does not produce substantial amounts of IL-6 in response to LPS. We were able to select a clone of WEHI 231 (designated 231M) by limiting dilution that was more responsive to LPS. Figure 10 shows that IL-6 production by
this clone induced by ODN 1760 is inhibited by chloroquine (Fig. 10, top) and quinacrine (Fig. 10, bottom) at a much lower concentration than IL-6 production induced by LPS. These data show that the effect of the antimalarials is specific for CpG-ODN.

Effect on human PBMC

The effects of the test compounds was not restricted to immortal mouse cells. Unfractionated human peripheral blood cells incorporate thymidine (Fig. 11A) and produce IL-6 (Fig. 11B) when exposed to ODN 1760. Both effects are inhibited by quinacrine and chloroquine.

Discussion

Our results show that the antimalarials, quinacrine, chloroquine, and many structurally related 4-aminoquinolines and 9-aminoacridines are extremely potent inhibitors of the action of CpG-ODNs. Quinacrine, added in low nanomolar concentration, completely

Table I. Activities of quinacrine analogs

<table>
<thead>
<tr>
<th>Quinacrine</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>9-Sidechain</th>
<th>EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>-</td>
<td>OMe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCHMe(CH2)3NEt2</td>
<td>14.1</td>
</tr>
<tr>
<td>26</td>
<td>-</td>
<td>-</td>
<td>OMe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.2</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(3-pyridyl)(CH2)3NEt2</td>
<td>19.1</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(C6H4Cl)(CH2)3NEt2</td>
<td>30.2</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(C6H4F)(CH2)3NEt2</td>
<td>42.7</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(C6H4OMe)(CH2)3NEt2</td>
<td>52.4</td>
</tr>
<tr>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(1-naphthyl)(CH2)3NEt2</td>
<td>57.5</td>
</tr>
<tr>
<td>27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(2-thienyl)(CH2)3NEt2</td>
<td>60.3</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(C6H10Cl)(CH2)3NEt2</td>
<td>60.3</td>
</tr>
<tr>
<td>59</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NH2</td>
<td>110</td>
</tr>
<tr>
<td>58</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Acridine homodimer</td>
<td>417</td>
</tr>
</tbody>
</table>

The structures are listed as changes from quinacrine or chloroquine. The symbol - indicates no change. Me and Et indicate -CH3 and -CH2CH3, respectively. Aromatic substituents are in the para position except as indicated. The EC50 is the concentration (nM) required for half-maximal inhibition of CpG-ODN effect on thymidine uptake in the presence of α-sIgM (see Fig. 1). The left most column indicates our reference number.

FIGURE 9. Structure-activity relationship. The data are from Tables I and II. Each box contains our identification number, the structural change, and the activity of the compound (EC50). The EC50 of each compound is the concentration (nM) at which the ODN effect on α-sIgM in WEHI 231 cells is inhibited 50%. Substitutions in position 7 replace chlorine.
blocks the ability of CpG-ODN to protect WEHI 231 cells from growth arrest, DNA fragmentation, and inhibition of thymidine incorporation induced by the engagement of its surface IgM and by other pro-apoptotic agents. The antimalarials inhibit CpG-ODN-induced IL-6 production by WEHI 213 cells and by unfractionated human PBMCs, and they inhibit the mitogenic effect of CpG-ODN.

See Table I legend for details.

### Table II. Activities of chloroquine analogs

<table>
<thead>
<tr>
<th>C2</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>Sidechain</th>
<th>EC50 (nM)</th>
<th>Chloroquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(CH2)3N(CH2)2NMe2</td>
<td>21.9</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>C3H5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NHCH(CH2)3N(CH2)2NMe2</td>
<td>37.2</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>C(Me)=C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38.0</td>
<td></td>
</tr>
</tbody>
</table>

See Table I legend for details.
on human PBMC. This inhibitory effect is noncompetitive with respect to CpG-ODN, and it occurs even when quinacrine is added after the CpG-ODN. The potency of chloroquine and quinacrine is magnified by inhibition of the multidrug-resistance pump, suggesting that the site of their action is intracellular. The specificity of the effect toward CpG-ODN is illustrated by the fact that quinacrine does not influence the ability of LPS to inhibit apoptosis in, or induce IL-6 production by, WEHI 231 cells. These results can be reviewed in the light of the known biologic effects in vitro of quinacrine and chloroquine.

**Inhibition of lysosomal function**

Being a diprotic weak base, chloroquine (and probably many of the other compounds we use) partitions into the acidified vesicles such as lysosomes (28). At high concentration, chloroquine can collapse the pH gradient of lysosomes (29, 30) and induce their swelling (30). Chloroquine delays the recycling of proteins to the cell surface from lysosomes, resulting in altered trafficking of lysosomal enzymes and receptors (15). These effects on lysosomal function are seen only when cells are exposed to higher concentrations of chloroquine than we use here, so it is improbable that the suppression by the antimalarials of CpG-ODN responses can be attributed solely to these relatively gross actions, even though our experiments with ammonia plus bafilomycin A1 do suggest that the CpG-ODN response requires vesicular acidification.

**Uptake of ODNs**

DNA and oligonucleotides bind to cells and are internalized via acidified vesicles (9–11), and this internalization is thought to be required for their effect (6). Two lines of evidence make it unlikely that the antimalarials acts by inhibiting this transport. Phosphorothioate ODNs persist in cells in undegraded form for many hours (11), but we find that quinacrine blocks the action of CpG-ODN after internalization, suggesting that quinacrine blocks a later step. More directly, chloroquine did not decrease (rather, it appeared to increase) the cellular uptake of fluorescein-labeled ODN.

**DNA binding**

Chloroquine and its congeners bind to dsDNA. Modeling suggests that the planar nucleus of the drugs intercalate between adjacent...
base residues, and the side chain amines interact with the phosphatase residues of opposing strands (14). The binding affinity of chloroquine for DNA is not high (of the order of $10^4 \text{ M}^{-1}$), and it has little affinity for ssDNA. We found that the more powerful DNA intercalator, propidium iodide, is not an effective anti-CpG-ODN agent, and that acridine homodimer, which has extraordinarily high affinity for DNA especially at acid pH (31), is less effective than chloroquine. Quinacrine stabilizes triplex DNA, but another triplex stabilizer, coralyne chloride (18, 32, 33) does not inhibit CpG-ODN effects. These experiments do not lend support to the supposition that the antimalarials operate by binding to CpG-ODN, although an action to block an intracellular CpG-ODN recognition site (at which duplex DNA could be formed) is not improbable.

**Antimalarial activity**

The mechanism of the antimalarial action chloroquine and other 4-aminoquinolines and 9-aminacridines has been intensively studied (34, 35). These agents are effective in the erythrocytic stage of malaria. Chloroquine binds with high affinity to hematin (ferriprotoporphrin IX, a toxic metabolite generated during the digestion of hemoglobin by the parasite) and interferes with its polymerization and detoxification (35). The structure-activity relationship we report does not correlate with published antimalarial activity (25, 26), suggesting that the inhibition of CpG-ODN-driven responses involves a different mechanism than inhibition of the malarial parasite.

**Remittive action**

The antimalarial drugs chloroquine, hydroxychloroquine, and quinacrine induce remissions of rheumatoid arthritis and systemic lupus erythematosus (12, 13). The beneficial effect of these antimalarials becomes apparent after several weeks of treatment. To account for this therapeutic action, a number of investigators have explored the effects of antimalarials on immune and inflammatory responses in vitro, revealing a wide range of inhibitory actions.

Quinacrine inhibits phospholipase A$_2$ (36–38), blocks ion channels (39, 40), binds to receptors (41, 42), and inhibits FMLP-induced superoxide production and enzyme release by granulocytes (43). Chloroquine and quinacrine have little influence on other functional assays of neutrophils (44). Chloroquine inhibits cytokine release from mononuclear cells induced by endo- and exotoxins (19, 20, 45) and inhibits binding of inositol 1,4,5-trisphosphate (IP$_3$) to its intracellular receptor (46). Each of these described effects requires concentrations of chloroquine or quinacrine markedly higher than the concentration we use to inhibit CpG-ODN driven responses in WEHI 231 cells and none of them satisfactorily explain the remittive effect of these drugs on autoimmune disorders.

CpG-ODNs are powerful activators of immunity in vitro. The role of DNA from pathogens in the regulation of immunity is undefined (2, 47), but it has been speculated that autoimmunity may be influenced by circulating pathogen DNA (48). Indeed, bacterial DNA provokes the synthesis of anti-mammalian dsDNA Abs resembling spontaneous autoantibodies (49). The exquisite sensitivity of CpG-ODN-driven responses to quinacrine, chloroquine, and their analogues prompts us to speculate that the remittive effects of this class of drugs may be due to the blockade of the effect of pathogen DNA. If this is correct, our in vitro assays provide the basis for a search for more useful drugs for treating rheumatoid arthritis and systemic lupus erythematosus. In addition, drugs that inhibit B cell production (and hence Ab production) could be useful in conditions caused by humoral immunity including autoimmune hemolytic anemia and acquired hemophilia. If the resulting drugs inhibit the release of cytokines, they could be useful in treating septic shock, inflammatory bowel disease, respiratory and other infections, and graft-vs-host disease amplified by virus or other infection.

**Acknowledgments**

We thank Dr. Arthur Krieg for his encouragement and many valuable discussions. We thank KWM for donating blood. We also thank Dr. Jill Johnson (Drug Synthesis and Chemistry Branch, National Cancer Institute) for providing many of the antimalarial compounds used in this study and Ms. Jennifer Shultz for her technical assistance.

**Note added in proof:** Chloroquine and other inhibitors of vesicular acidification are recently shown to inhibit the generation of reactive oxygen species and NFkB activation in leukocytes treated with CpG-ODN. A-K. Yi, R. Tuuetken, T. Redford, M. Waldschmidt, J. Kirsch, and A. M. Krieg. CpG motifs in bacterial DNA activate leukocytes through the pH-dependent generation of reactive oxygen species. Journal of Immunology, in press.

**References**


30. T-A-T


