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The host targeting effect of chloroquine in malaria

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Due to the rapid onset and spread of the COVID-19 pandemic, the treatment of COVID-19 patients by hydroxychloroquine alone or in combination with other drugs has captured a great deal of attention and triggered considerable debate. Historically, the worldwide use of quinoline based-drugs has led to a spectacular reduction in death from malaria. Unfortunately, scientists have been forced to seek alternative drugs to treat malaria due to the emergence of chloroquine-resistant parasites in the 1960s. The repurposing of hydroxychloroquine against viral infections, various types of cancer and autoimmune diseases has been ongoing for more than 70 years, with no clear understanding of its mechanism of action (MOA). Here, we closely examine the MOA of this old but influential drug in and beyond malaria. Better insights into how chloroquine targets the host's cellular and immune responses may help to develop applications against to new pathogens and diseases, and perhaps even restore the clinical utility of chloroquine against malaria.

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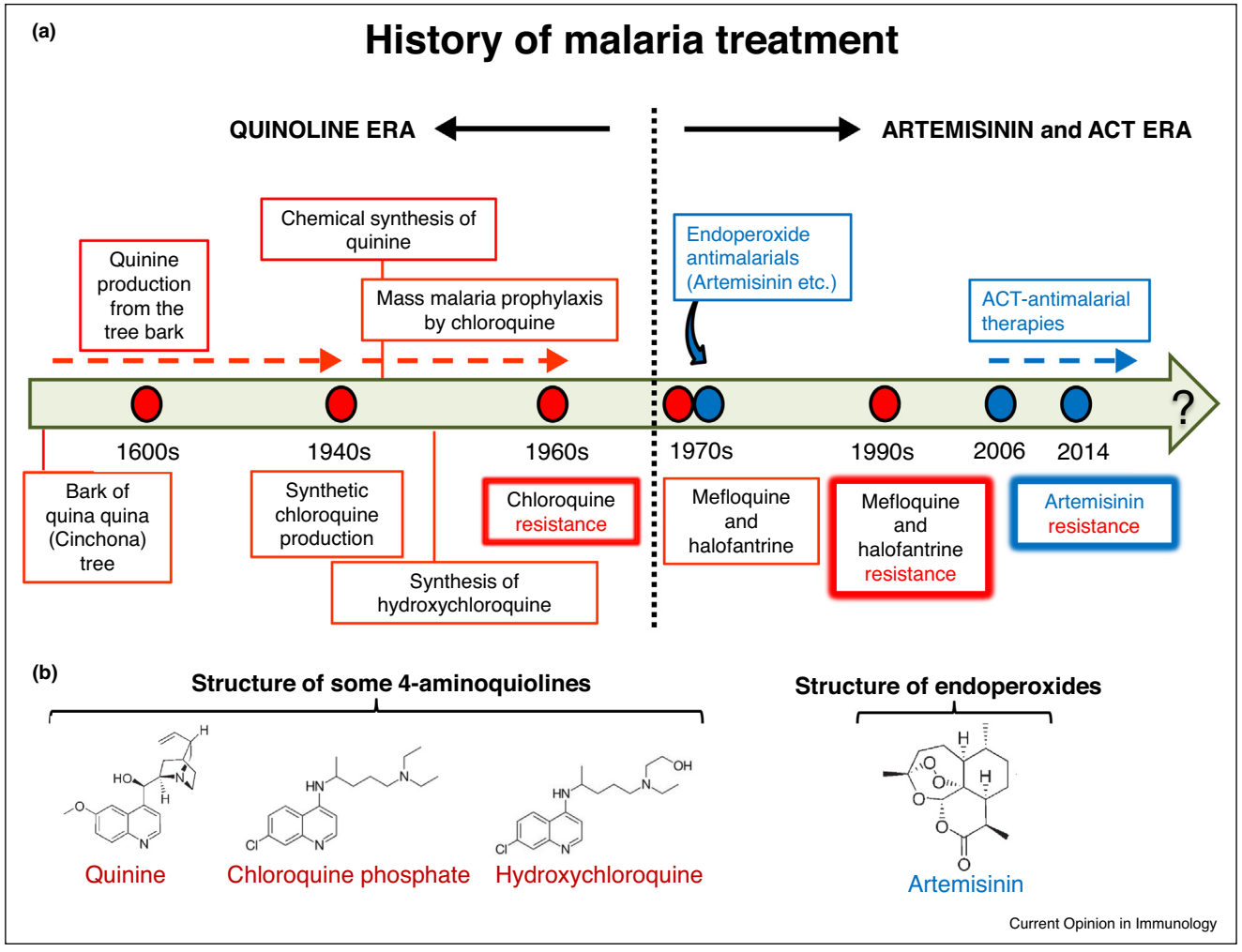
Introduction

Malaria is an ancient disease that co-evolved with human populations and their migratory spread over the globe. Not so long ago, only 100 years, in fact, 77% of the world population was suffering from malaria, this number was reduced to 48% due to a century long elimination effort [1]. Today, almost half a million people still die from malaria every year [2]. The 'success' against malaria is largely due to the quinoline-containing antimalarial drugs such as quinine, chloroquine and mefloquine [3].

The history of the quinoline antimalarials dates back to 400 years ago. The Incas first extracted it from the bark of the quina-quina tree grown in the Andes (Figure 1). The tree later was widely came to be known as the 'Cinchona tree' after the Countess of Chinchon of Spain who was treated with the bark extract in the 17th century, and the tree bark was then brought to Europe [4]. The quinine was chemically extracted from these barks and used for centuries until the discovery of its synthetic analog chloroquine in 1934, which exhibited better tolerability and side effects. Although it took 10 years for chloroquine to come in to use in humans as a cheap, efficacious and affordable drug, it eventually came to be over used for protection from *Plasmodium falciparum* infections in many parts of the world, which resulted in the emergence of drug resistance and its withdrawal from *P. falciparum* treatment in South-East Asia, South America and Africa [3] (Figure 1). In addition, *P. vivax*-chloroquine resistant strains emerged in the 1990s in Southeast Asia, overall making more than 80% of worldwide wild parasite isolates proving resistant to chloroquine [5]. The chloroquine alternatives mefloquine and halofantrine were introduced in the 1970s and used for 30 years until parasite resistance appeared in these drugs towards *P. falciparum* strains.

The studies of the 2015 Nobel laureate Dr. Youyou Tu on Artemisia extracts since the beginning of 1970s led to the discovery of the artemisinin based drugs which do not belong to the quinoline class of drugs [6]. Since 2006 artemisinin combination therapies (ACTs) have been used to treat *P. falciparum* and complicated chloroquine-resistant *P. vivax* infections. The reason why artemisinin is used together with other agents such as quinoline-related drugs is due to the very short half-life of artemisinin, so the additional drugs help to prevent the recrudescence of the parasites [7]. Although recent studies have confirmed the signs of artemisinin resistance in *P. falciparum* [8], artemisinin and its derivatives have nevertheless provided a breakthrough treatment modality for malaria and rendered the quinoline drugs a secondary treatment option in most of the world. In the course of the recent coronavirus pandemic, treatment of COVID-19 patients with hydroxychloroquine has provoked a great deal of a debate. Chloroquine's possible action on viral load and replication, lysosomal function and cellular immune responses has been vigorously discussed [9,10]. Therefore, I here summarize the current knowledge on the mechanisms of action of chloroquine against malaria. I wish to obtain novel insights into the effect of chloroquine on the host, rather than the parasite, which will facilitate its repurposing against various conditions, including viral infections, cancer and autoimmune

Figure 1



(a) History of malaria treatment. **(b)** Chemical structures of some quinoline drugs quinine, chloroquine phosphate and hydroxychloroquine and non-quinoline drug artemisinin.

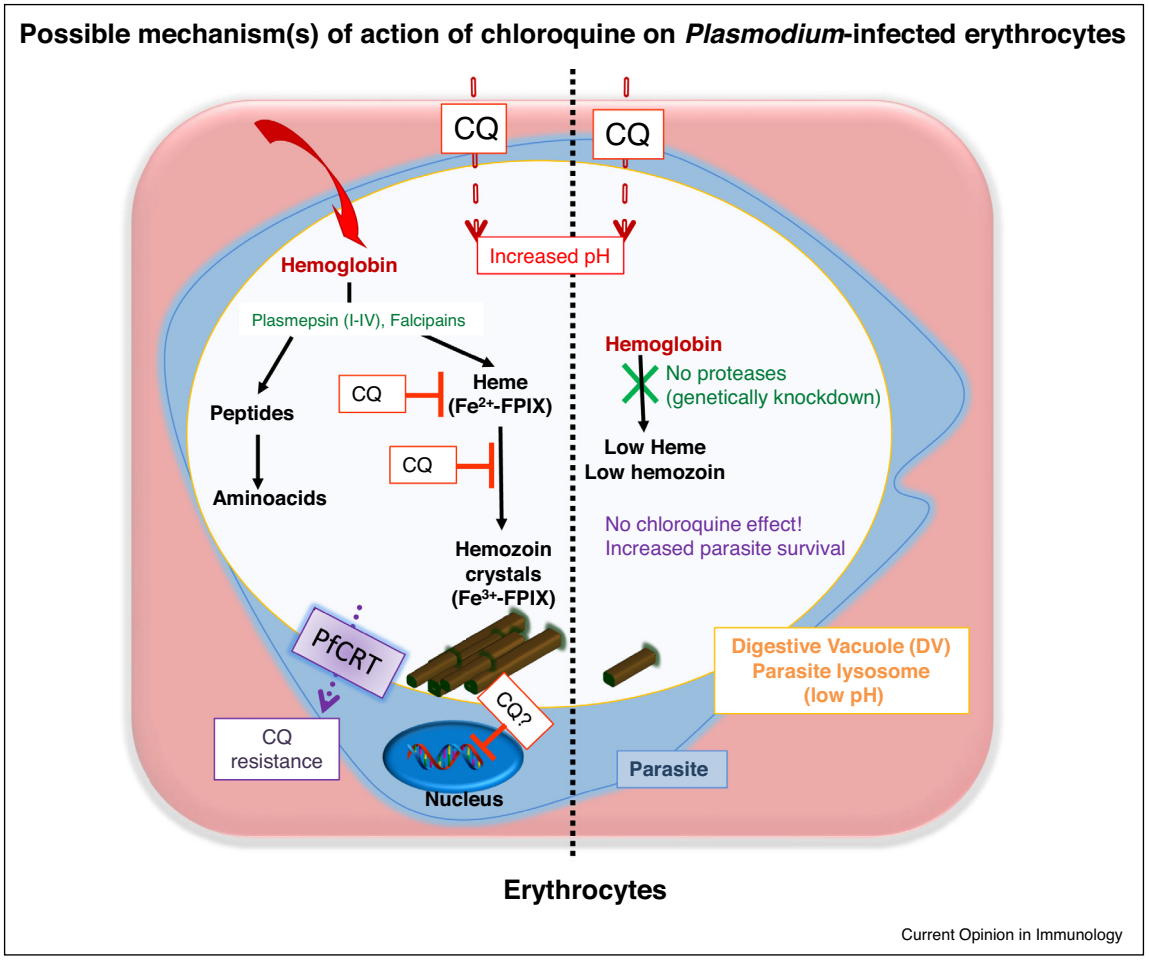
diseases, and perhaps may even help to restore its clinical utility against malaria.

The mechanism of action of chloroquine on *Plasmodium* infected erythrocytes

Chloroquine generally refers to chloroquine phosphate (C_{18} H_{26} ClN_3), a weak base drug that belongs to the first group of quinolone derivatives, the 4-aminoquinolines. Chloroquine's hydroxyl derivative hydroxychloroquine (C_{18} H_{26} ClN_3 O) that was developed in the 1950s presumably has a similar mechanism of action along with a higher safety profile. How chloroquine acts against malaria is still not well understood, although it is known that chloroquine affects only erythrocytic-stage parasites after diffusing across the erythrocyte and parasite membranes due to its small size and lipophilic characteristics. Two possibilities are suggested (Figure 2).

First, chloroquine has long been known to bind to DNA and RNA. Early studies suggested it could inhibit DNA and RNA synthesis by binding to nucleic acids via electrostatic forces, hydrogen bonds, and van der Waals forces [11,12]. This may explain why chloroquine as well as hydroxychloroquine can inhibit replication of certain viruses *in vitro*, such as HIV, Zika virus, influenza A virus, herpes simplex virus, SARS-CoV, and chikungunya virus [13]. Hence, it is reasonable that chloroquine may interact in such a manner with the *Plasmodium* DNA/RNA machinery within erythrocytes. However, chloroquine-*Plasmodium* DNA interactions inside the parasite nucleus were found to require rather high concentrations of the drug (at a toxic level that exerts an inhibitory effect even on the growth of host cells), which would be far more than the concentration required for the clearance of parasites *in vivo* [4]. Therefore, this idea was found to be unfavorable.

Figure 2



Possible mechanism (s) of action of chloroquine during blood stage malaria infection. After invasion of erythrocytes, *Plasmodium* parasites form their own DV, a lysosome-like acidic compartment important for parasite metabolism and survival. In acidic DVs, the host-hemoglobin is degraded by parasite proteases for the vital needs, such as amino acids and the free-heme (Fe^{2+}–protophorphyrin IX) is detoxified by converting it into insoluble crystals hemozoin (Fe^{3+}–protophorphyrin IX). A weak base chloroquine accumulates in DVs, increases DV pH and binds heme and crystal surfaces, thereby blocks every steps of hemozoin formation which eventually leads heme toxicity and parasite death. In the absence of hemoglobin degrading proteases hemoglobin remains undigested and free heme is significantly diminished and the effect of chloroquine on parasites does not occur. Ineffective presence of chloroquine, on the other hand, may create the chloroquine-resistant parasites via a mutation in *P. falciparum* chloroquine resistance transporter (PfCRT) and possibly other genes.

The second is the effect of the weak base chloroquine on heme-like structures. During the life cycle of parasites in erythrocytes, the host hemoglobin is degraded by parasite proteases called Plasmepsins (I–IV) and Falcipains [14] for their own amino acid needs. The liberated free heme is subsequently polymerized into hemozoin, a black crystalloid metabolite and the hallmark of *Plasmodium* parasites, in a process that develops in the acidic lysosome-like parasite digestive vacuole (DV) residing inside the erythrocytes (Ref. [15] and Figure 2). The structural similarity between heme (monomeric) and hemozoin (dimeric) is well known and it is preserved at a low pH, but readily disassociates in alkaline solutions [16]. Thus, the accumulation of the weak base chloroquine in the DV perhaps naturally prevents crystallization dynamics due to the pH increase. Supporting this, recent studies have clearly shown that chloroquine binds heme (Ferriprotoporphyrin IX, Fe^{+2}) as well as crystal surfaces with a strong affinity and thus ends up blocking hemozoin (Ferriprotoporphyrin IX, Fe^{+3} dimers) formation at every step of crystallization [17•,18•], thus allowing free-heme toxicity to parasites. On the other hand, there are other important facets of the mechanism of action of chloroquine against erythrocytic stage parasites. Recent studies showed that chemically labeled chloroquine molecules could be detected in DVs and on parasitic membranes,

but not on red blood cell membranes [19,18•]. Chloroquine probably compromises the DV membrane and leads to an extrusion of DV proteases such as plasmepsin-IV which hydrolyze hemoglobin, in both *in vitro* and *in vivo* conditions [20]. A recent *in vivo* study using genetically modified mutant-parasite model confirmed this. In the absence of *P. berghei* hemoglobin degrading proteases (plasmepsin-IV and berghepain-2), hemoglobin was shown to be undigested and free heme release was significantly diminished. Therefore, no effect of chloroquine on parasites occurred, so no parasite death resulted [21]. This study also implies that the *Plasmodium* DNA/RNA machinery remains intact even in the presence of chloroquine.

However, ineffective presence of chloroquine has been linked to genetic pressure on the parasites resulting in chloroquine-resistant parasites. The main mechanism of resistance to chloroquine by *P. falciparum* parasites has been shown to involve mutation of the *P. falciparum* chloroquine resistance transporter (PfCRT), a transmembrane protein located in the DV membrane, which effluxes chloroquine into the cytosol [3] (Figure 2). However, other studies have indicated that this chloroquine-resistance phenotype does not involve all *P. falciparum* parasite strains, rather more multigenic involving other genetic loci [22,23]. Furthermore, chloroquine's effect on the *P. falciparum* and *P. vivax* parasites seems to involve different mechanisms. While *P. falciparum* trophozoites with a single large food vacuole are fully susceptible to chloroquine, *P. vivax* trophozoites with many small vacuoles are not [24••,25]. Further studies are clearly needed to elucidate chloroquine's pleiotropic effects on infected-erythrocytes and how *Plasmodium* parasites develop resistance to this drug. Because chloroquine resistance in *P. falciparum* seems to be decreasing in Africa after years of withdrawal [26], the opportunity for bringing back this cheap and safe drug to the field is growing.

Interestingly, the blood fluke *Schistosoma mansoni* similarly digests host hemoglobin and release free heme which is detoxified through hemozoin formation [27]. Based on this finding, *in vivo* experimental studies showed that chloroquine treatment decreased hemozoin formation, the viability of the worms and the severity of infection in *S. mansoni*-infected mice [27], confirming chloroquine's pleiotropic effects on various pathogens.

Mechanisms of action of chloroquine: beyond infected erythrocytes

It is well known, mainly from empirical non-malarial usage of chloroquine in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), that chloroquine has a wide range of different effects on immune cells and inflammation. Therefore, chloroquine may exert an effect on the immune system during malaria treatment, a possibility which has been largely overlooked. Early studies in animals and a few post-mortem studies in humans have shown that chloroquine accumulates at high concentrations in the eye (due to potent binding to melanin), lung, liver and spleen (possibly due to preferred accumulation in lysosomes), with low levels in muscle, brain tissue and bone, albeit only after several months of medication [12]. In fact, one of the reasons for the widespread investigation into the side effects of chloroquine and its derivatives is due to the prolonged treatment needs of SLE and RA patients (i.e. several months or even years). These studies have concluded that hydroxychloroquine is effective with fewer side effects than many other drugs for SLE treatment, even during pregnancy [10]. It is of note that the overall cumulative doses of chloroquine used for autoimmune disease treatment is at least 100 times greater (200−400 mg/day over weeks and years depending on the patient) than for prophylaxis (100−250 mg/week during and after stay in endemic area) or treatment of malaria (400 mg/day over 3 days) [12]. Chloroquine has been used safely for the treatment of pregnant women (chloroquine can easily cross the placenta with no apparent harmful effects to the fetus) as well as in lactating women and newborn/infants during cases of malaria [28]. In contrast, certain other quinolines, such as primaquine, may exert hemolytic effects in people with glucose-6-phosphate dehydrogenase deficiency (G6PD) [29]. The rare acute side effects of chloroquine during malaria treatment include gastrointestinal symptoms and itching, but it comparatively rarely induces neurological or cardiovascular symptoms, such as cardiac arrhythmia due to prolongation of cardiac repolarization (the QTc interval), and when such effects do occur, they are usually associated with high doses due to rapid intravenous infusion of the drug and/or high peak concentrations [10]. Importantly, children 4–8 years-old who are infected with chloroquine-resistant *P. falciparum* and treated with double or nearly triple-doses of the standard chloroquine protocol appear to tolerate the drug and are completely cured of malaria, albeit with prolonged QT intervals, but with no cardiac arrhythmias [30••]. Of note, hydroxychloroquine alone seems to be well tolerated if not given at a bolus concentration, and additional consideration is needed when it is given along with additional drugs such as digitoxin, tamoxifen, methotrexate and cyclosporine, as well as primaquine and azithromycin (the drug–chloroquine interactions are extensively summarized in Ref. [10]).

Similar to the case of the DV of *Plasmodium* parasites, chloroquine accumulates in any low-pH (i.e. a pH of less than 4–5) organelle such as lysosomes due to its weak base property and reduces the organelle's acidification. Weak base chloroquine is usually uncharged and readily diffused into the lysosome, where it easily becomes protonated, allowing chloroquine to concentrate within compartments higher than its extracellular concentration and

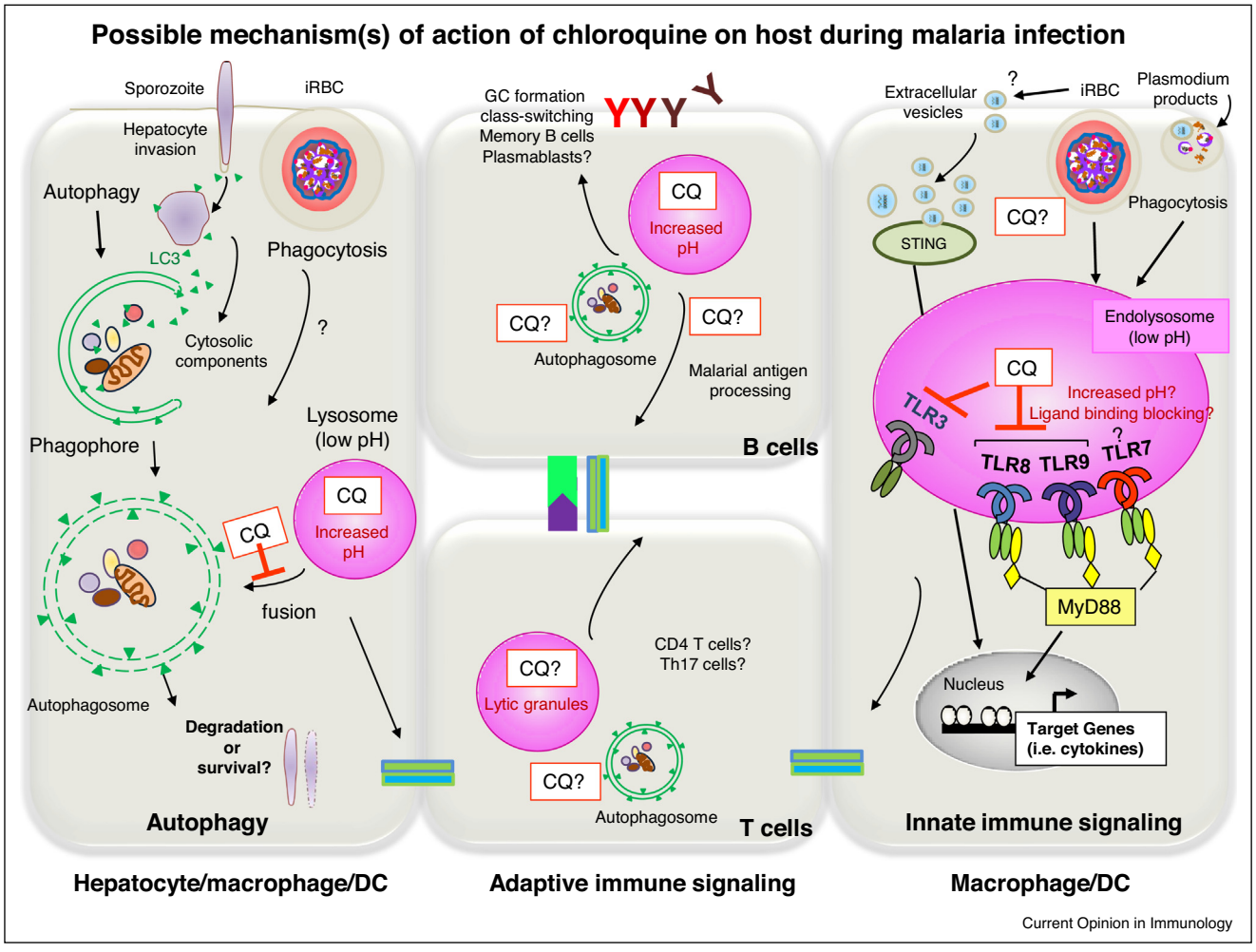
increasing the pH up to 6. The increase in the lysosomal pH in immune cells by chloroquine has three possible consequences during malaria.

Chloroquine and host autophagy during malaria

Autophagy (canonical or macroautophagy) is a cellular process that help cells to degrade and recycle their own components through an intracellular engulfment process which involves the formation of double membrane vesicles known as autophagosomes, which fuse with lysosomes for the final enzymatic digestion of components for their nutrient content [31,32] (Figure 3). This final acidic lysosomal activity is a key step in autophagy and its neutralization by weak alkaline chloroquine, or inhibition with bafilomycin A1 (that inhibits the lysosomal proton pump) disturbs lysosomal function, and either event leads to the failure of autophagy.

Several types of autophagy against pathogens have been described over the last two decades. In the course of selective autophagy (xenophagy), the autophagosome forms around the engulfed pathogens. Xenophagy helps

Figure 3



Possible mechanism (s) of action of chloroquine on immune cells during malaria infection. Chloroquine has a clear anti-inflammation and anti-cytokine effect during malaria. It may accumulate in any low-pH organelle such as lysosomes in immune cells due to its weak base property and increases pH. This may affect autophagy of cells which require final acidic lysosomal activity that chloroquine can easily neutralize this process. Chloroquine may be affecting host autophagy machinery targeting sporozoites inside the hepatocytes or alternatively might have an effect on the accumulated $CD8^+$ T cells, liver-resident T cells or activated T cells and B cells during infection. Chloroquine may have direct effect on pattern recognition receptors (PRRs), particularly TLR-mediated cytokine induction, because the acidic pH of endosome is a requirement for endosomal TLR activation. Therefore, chloroquine may interfere with the interaction of *Plasmodium* DNA, hemozoin and RNA with TLR9, TLR7 and TLR8 or inhibit the maturation of *Plasmodium*-containing phagosome. Chloroquine might modulate the extracellular vesicles (EVs) secreted from infected RBCs containing parasite small RNA and genomic DNA which activate cytosolic STING pathway. Overall, all these possible effects may modulate antigen presenting cells (APCs, mainly monocytes/macrophages and dendritic cells), lytic granule processing and modulate adaptive immune responses to malaria.

eliminate pathogens, but in turn, may be hijacked by pathogens for their own survival. The recently described microtubule-associated protein 1 light chain 3 (LC3)-associated phagocytosis (LAP) involves only a few of the autophagy initiation complex proteins and does not result in autophagosome formation, but helps to eliminate pathogens via direct fusion with lysosomes [33]. During the pre-erythrocytic stages of malaria infection sporozoites invade hepatocytes and make a shield out of a membrane-bound parasitophorous vacuole (PV) and then replicate in it. The host autophagy machinery targets these PVs by decorating PV membranes with autophagy markers, including LC3. As a result, *Plasmodium* sporozoites either hijack the hepatocyte autophagy pathway and gain nutrients for their growth (elimination is avoided by PV transmembrane protein UIS3 [34]), or are degraded by a xenophagy-like mechanism. If the immune system is successfully activated (such as by the secretion of IFN-γ), approximately 50% of the intracellular sporozoites are cleared by a LAP-like process in hepatocytes [35].

Chloroquine has a direct effect on erythrocytic-stage but not sporozoite stage parasites. However, chloroquine has been used as an autophagy inhibitor in experimental studies investigating host autophagy in hepatocytes during sporozoite development [36]. On the other hand, the protection against *Plasmodium* parasites has been shown in animal and human experimental studies when individuals are immunized with infectious sporozoites under the cover of chloroquine chemoprophylaxis [37••,38,39•]. Although the role of chloroquine in these studies was to suppress following blood stage parasites, whether chloroquine induces autophagy inhibition or exerts an immunomodulatory effect has not been elucidated. For instance, chloroquine's prophylactic effect during live sporozoite immunizations was compared with radiation-attenuated sporozoite (RAS) immunizations in humans and found to be 20 times more efficient, requiring only 45 mosquitoes bites versus 1000 bites, although the induction of the efficient parasite-specific $CD8^+$ T cells response and IFN-γ production was comparable [40]. As it is believed that the presence of the liver stage parasite is a prerequisite for the induction of protective responses after sporozoite immunization (confirmed by concurrent primaquine treatment, which abrogated protection), chloroquine prophylaxis may need a different interpretation. The difference between live sporozoites and RAS sporozoites might be due to the sporozoites' interaction with the hepatocyte autophagy machinery and chloroquine's direct effect on it. Alternatively, chloroquine might have an effect on the accumulated $CD8^+$ T cells or liver-resident T cells as autophagy helps to maintain liver-resident $CD8^+$ T cells and their mitochondrial fitness [41]. These are open questions to be answered in the future.

There is very little information on the role of host autophagy during blood stage malaria infection, although *Plasmodium* parasites' own autophagy in the blood stage has been studied [42,32]. Rapid acidification of phagosomes occurs when macrophages are stimulated with infected-erythrocytes *in vitro*, which may block the efficient signaling required for cytokines, as shown by the blocking of this this pathway by the acidification blocker Baf-A1 [43]. However, whether this actually occurs as a result of a suppression of acidification by chloroquine has not been reported.

Autophagy is known to be enhanced during T cell activation and proliferation [44], therefore whether chloroquine treatment inhibits the autophagic flux of activated T and/or B cells during malaria infection needs to be investigated.

Chloroquine and the innate immune system during malaria

In addition to its anti-parasitic effects, when chloroquine is given to *P. falciparum*-infected children it clearly exerts anti-inflammatory and anti-cytokine effect [30••]. Chloroquine's suppression of cytokinesis during malaria is suspected to be due to its direct effect on pattern recognition receptors (PRRs), particularly TLR-mediated cytokine induction [45]. Although further studies are needed, there are a few possible explanations of the target molecule(s) for chloroquine on immune cells. During the blood stage, infected erythrocytes, ruptured merozoites and parasite products such as hemozoin are continuously phagocytized by monocytes/macrophages or DCs. TLR3, TLR7, TLR8 and TLR9 are the only TLRs among the 13 in the TLR family that are located on acidic organelle endosomes, and mainly recognize different classes of nucleic acids of either endogenous or exogenous origin. The acidic pH of endosomes is a requirement for endosomal TLR activation. However, chloroquine was found to have more activities than just this, for example directly interacting with cognate ligands (nucleic acids), changing the chemical environment and masking TLR ligand-binding epitopes [46]. CpG ODN-induced immune activation was inhibited by chloroquine via directly competing with CpG ODN for binding to the TLR9-ectodomain and changing its conformation. Moreover, poly (I:C) (dsRNA) interaction with its receptor TLR3 was affected similarly by chloroquine. In contrast, chloroquine's inhibition of TLR8 was mostly due to the manipulation of endosomal pH by chloroquine [47]. *Plasmodium* DNA and RNA either alone or complexed with malarial products are ligands recognized by either endosomal TLR9 or TLR7 as well as cytosolic DNA sensing pathways such as STING (recently reviewed in Ref. [48]). Although malarial hemozoin's recognition by TLR9 in DCs [49] has been extensively debated [48], this is the only study that has showed direct evidence that *Plasmodium* hemozoin-mediated cytokine activation was blocked by chloroquine treatment, and that hemozoin directly interacted with the TLR9-ectodomain protein via its heme molecule and thereby changed its

confirmation [50]. Just recently, TLR8 was found to be involved in *P. falciparum* RNA recognition in human TLR8-expressing cells and this interaction was similarly blocked by chloroquine [51•]. Therefore, it is possible that chloroquine, in addition to its ability to inhibit hemozoin formation in erythrocytes, may also interfere with the interaction of *Plasmodium* hemozoin, DNA and RNA with TLR9, TLR7 and TLR8. Alternatively, it may inhibit the maturation of *Plasmodium* product-containing phagosomes, thereby inhibiting subsequent innate immune activation (Figure 3) and not have a direct effect on endocytic cell entry or the replication machinery, as in the case of viral infections. There is also the possibility that macrophage/monocyte and DC acidification mechanisms and levels may interfere with endosomal TLR recognition in different cells [43]. Nevertheless, the contribution of these various ligands from the *Plasmodium* parasite in the control of the immune system via the TLRs and chloroquine's direct effect on innate immunity needs *in vivo* investigation.

A recent study showed that chronic accumulation of *Plasmodium* products in the bone marrow induces bone loss, but this pathology does not occur when either MyD88 (the adaptor protein for most TLR signaling) is lacking or mutant parasites lacking hemozoin (i.e. lacking Plasmepsin IV and berghepain 2) were used [52]. This study suggests that intact parasite DNA or RNA alone, although sensed via MyD88, have a limited capacity to induce pathology in the absence of hemozoin. Instead, hemozoin and the accumulation of other as yet unknown *Plasmodium* products activate cytokines via MyD88 are responsible for malaria-induced bone loss. Furthermore, repeated chloroquine treatment was shown to play a minimal role for the accumulation of hemozoin in the bone marrow which caused bone loss, suggesting the hemozoin crystals that form under chloroquine treatment remain intact and continuously induce immune activation and pathology even after parasite clearance. Clearly, further investigation of this bone pathology *in vivo* using animal models and in humans is needed to understand chloroquine's long term effects during malaria infection.

Hydroxychloroquine was also shown to inhibit the activity of another nucleic acid sensor, cyclic GMP-AMP synthase (cGAS), by interfering with its binding to cytosolic DNA upstream of STING as well as by blocking the sorting of STING vesicles to lysosomes for STING degradation [53,54•]. During malaria, it is likely that extracellular vesicles (EVs) secreted from infected RBCs containing small RNA and genomic DNA of the parasite activate the cytosolic STING pathway [55•,56], although whether chloroquine directly targets these EVs has not been investigated. It is of note that recent studies have suggested another possibility that chloroquine can enhance the production of EVs [57,58]. Thus, how chloroquine interferes with *Plasmodium*-secreted EVs needs further investigation.

There is also a very likely possibility that chloroquine may block the interaction of cytosolic DNA as well as RNA with other sensing pathways such as the AIM2 or IPS1 (MAVS) [53]. This raises the possibility that recognition of *Plasmodium* RNA in hepatocytes [59] might be blocked by chloroquine, which has also not been investigated during sporozoite infection and vaccination.

Chloroquine and the adaptive immune system during malaria

The lysosomotropic properties of chloroquine may have an effect on antigen presenting cells (APCs, mainly monocytes/macrophages and dendritic cells) and modulate several innate signaling pathways, such as TLRs (mentioned above), proinflammatory cytokine production, and co-stimulatory molecule signaling, thus helping to elicit a proper adaptive immune responses. Furthermore, chloroquine's long half-life (~50 days) may also result in the modulation of several adaptive immune cell functions such as that results in impaired antigen processing, or suppression of antigen presentation to $CD4^+$ T-cells, or inhibition of the differentiation into and secretion of cytokines from Th1 and Th17 cells [10,60,61], or inhibiting the perforin processing [62]. In contrast, several other studies have shown that chloroquine enhances DCs' cross-presentation of soluble antigens, but not particulate antigens, to $CD8^+$ T cells. This has been shown under both *in vitro* and *in vivo* conditions, for example during vaccination or in the course of viral infections, and is most probably due to the reduced degradation of antigens in the presence of weak base agents, resulting in a higher accumulation in endosomes and subsequent efficient export into the cytosol leading to efficient cross-presentation [63].

During malaria, in general, while antibodies are important in controlling blood-stage parasitemia, it is the $CD8^+$ T-cell responses are critically involved in pre-erythrocytic immunity. Unexpectedly, RAS immunization under prophylaxis with or without chloroquine induced equally strong $CD8^+$ T cells responses [40], suggesting that chloroquine's effect was on other, as yet unknown mechanism(s) or on other cells. Interestingly, a recent study showed that humans immunized with live sporozoites under chloroquine chemoprophylaxis reacted to a broad repertoire of novel antigens in both breadth and magnitude, as assessed by a comprehensive *P. falciparum* protein microarray of blood [39•]. Furthermore, the direct effect of chloroquine/primaquine prophylaxis/treatment doses on vaccination using model antigens in mice was investigated and it was found that chloroquine clearly modulates antigen-specific B cell responses [64]. In fact, chloroquine together with an adjuvant (i.e. alum) increases the protective efficacy of whole-killed blood-stage vaccines via humoral immune responses due to an

expansion of GC B cells and class-switch recombination [65]. A synthetic analog of malarial hemozoin has been proposed to be a universal adjuvant [66]. It will be interesting to see in further studies investigating the effect of chloroquine on this particular malaria-derived adjuvant and whether it has the ability to bind directly to the hemozoin surface [18•].

Conclusions

Chloroquine has been one of the most affordable, relatively safe and widely used medications in the history of humankind, with pleiotropic functions under protozoan, viral, bacterial and inflammatory conditions. We owe a debt to chloroquine for its effectiveness in ameliorating the impact of malaria cases all over the world, but we still do not understand its mechanism of action, and how *Plasmodium* parasites develop resistance to it. Based on the information gained from non-malarial studies, we emphasize that more research is required to understand the host-mediated activity of chloroquine during malaria. We may repurpose chloroquine and its derivatives and facilitate its return to the shrinking list of antimalarials.

Authors' contributions

Review was written and figures were prepared by CC.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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