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AIDS: Caused by development of resistance to drugs in a non-target intracellular parasite

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Summary The origin of acquired immune disorder syndrome (AIDS) has been the subject of substantial controversy both in the scientific community and in the popular press. The debate involves the mode of transmission of a simian virus (SIV) to humans. Both major camps in the argument presume that humans are normally free of such viruses and assume that once the simian virus was transmitted, it immediately infected some T-cells and caused the release of toxic agents that killed off bystander (uninfected) T-cells resulting in AIDS. The evolution of the Simian virus (SIV) into a human virus (HIV) is regarded as an artifact. In contrast, a fundamentally different hypothesis has been proposed [Parris GE. *Med Hypotheses* 2004;62(3):354–7] in which it is presumed that in hyper-endemic areas of malaria (central Africa), all primates (humans and non-human primates) have shared a retrovirus that augments their T-cell response to the malaria parasite. The virus can be called “primate T-cell retrovirus” (PTRV). Over thousands of years the virus has crossed species lines many times (with little effect) and typically adapts to the host quickly. In this model, AIDS is seen to be the result of the development of resistance of the virus (PTRV) to continuous exposure to pro-apoptotic (schizonticidal) aminoquinoline drugs used to prevent malaria. The hypothesis was originally proposed based on biochemical activities of the aminoquinolines (e.g., pamaquine (plasmoquine(TM)), primaquine and chloroquine), but recent publications demonstrated that some of these drugs definitely adversely affect HIV and other viruses and logically would cause them to evolve resistance. Review of the timeline that has been created for the evolution of HIV in humans is also shown to be qualitatively and quantitatively consistent with this hypothesis (and not with either version of the conventional hypothesis). SARS and Ebola also fit this pattern.

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Introduction

The conventional hypothesis concerning the initiation of the acquired immune deficiency syndrome (AIDS) epidemic is that a simian immunodeficiency virus (SIV) was transferred to (previously uninfected) humans within the relatively recent past

(during the 1900s) and that virus proved to be very virulent to humans (as it evolved into HIV). The majority of the scientific community seems to hold the idea that this transfer was an accidental transfer resulting from native hunting/direct contact with infected primates (*circa* 1931, [1]) while a largely non-scientist group has embraced the idea that testing of a vaccine in Congo (*circa* 1957–1960, [2]) caused the transfer of virus from non-human primates to humans. Obviously,

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observations made by these researchers need to be accounted for in any serious scientific hypothesis, but it must be pointed out that this hypothesis leaves many unanswered questions:

- Why are non-human primates in sub-Saharan Africa (and apparently no other place) infected with SIV and why do they seem to not be harmed by the virus?
- Considering that SIV is readily transmitted to humans [3], that HIV has been readily transmitted among humans, that it is apparently impossible to eliminate from humans, and that humans and non-human primates have co-existed in sub-Saharan Africa for many thousands of years, why should we ignore the possibility that humans have carried benign/symbiotic strains of HIV for a very long time [4]?
- Ironically, the HIV virus does not kill the cells it infects (e.g., T-cells and macrophages of the immune system), but rather it causes disease because it kills un-infected bystander cells (T-cells and other cells) with secreted toxins that cause apoptosis [5]. Should not the origin of AIDS explain this unusual type of disease?

The hypothesis that I have published [6–8] addresses these issues as well as the timing issues that are important to identifying specific actions that lead to the AIDS epidemic.

The malaria–aminoquinoline hypothesis

Background concerning malaria and anti-malarial drugs

Beginning in the 1920s, a series of aminoquinoline derivatives were synthesized and screened for anti-malarial activity. In particular 8-aminoquinolines (e.g., pamaquine (plasmoquine(TM)), primaquine) and 4-aminoquinoline (e.g., chloroquine) have been found to prevent or mitigate malarial infections in two ways. One mode of action seems to involve direct toxicity to the parasite in the erythrocytes. The other mode of activity seems to involve induction of apoptosis in liver cells that have become infected with the parasite, thereby preventing the necessary amplification of the parasite to establish and maintain a significant infection in erythrocytes (red blood cells).

Regarding the pro-apoptotic effects of chloroquine [6,7], the aminoquinolines seem to act by suppressing the activity of a very important transcription factor NF-kappa-B, which activates

anti-apoptotic proteins (including Bcl-2 and Bcl-xL). NF-kappa-B also plays a key role in reactivation of latent HIV [9]. Indeed, chloroquine has been shown to interact with NF-kappa-B and apparently enhances its binding to specific segments of DNA [10] in such a way that it prevents NF-kappa-B from acting as a transcription factor. This effect of chloroquine on the binding of NF-kappa-B to DNA may be the basis for chloroquine's suppression of HIV replication [11]. Chloroquine also affects expression of TNF [12,13].

The 8-aminoquinolines seem to be more potent in this role than the 4-aminoquinolines (e.g., chloroquine) because chloroquine does not eliminate all malaria infected cells in the liver (although it does appear to prevent them from becoming mature schizonts through premature apoptosis). However, the 8-aminoquinolines are more toxic to the host (humans) and it is more difficult to achieve a safe and effective dose. Chloroquine actually builds up in the blood stream and it proved easier to achieve continuous protection over a long period of time with chloroquine. The history of these developments will be discussed in detail below [8,14].

First proposition: malaria leads to a symbiotic relationship between T-cells and primate T-cell retroviruses (PTRV)

If you consider the important ecological and evolutionary context of sub-Saharan Africa, the dominant feature has been the plague of malaria on primates there. Malaria has been a continuous plague on primates and other animals for at least 10,000 years. In the hyper-endemic area of central Africa, the malaria parasite substantially reduces the number of children who make it to reproductive age. This attrition produces an ecological stress on humans and humans are known to have incurred some significant evolutionary changes as adaptation to the ecological stress caused by malaria. For example, the sickle-cell trait in humans from sub-Saharan Africa produces a life-threatening and debilitating disease of its own and yet has been retained in the human population there because it confers some protection against malaria. In other areas of the globe, malaria has been a problem, but it has not noticeably affected human evolution. Extreme problems call for extreme measures.

The life-cycle of the malaria parasite is quite complex in humans. The form of the parasite injected by mosquitoes does not go directly to the

erythrocytes (red blood cells). There are not enough of them to successfully evade the immune system and survive the normal attrition of erythrocytes. Thus, the initial malaria infection invades the liver where hepatocytes are converted into factories for making thousands of copies of the malaria organism. The infected liver cells are called schizonts as they fill up with parasites. The bursting of these schizonts begins the major cycle of fever associated with the disease. The parasites released from the liver infect the erythrocytes and also use them to multiply. Periodically, the erythrocyte schizonts burst with new attacks of chills and fever. In adults, the immune system and the attrition of erythrocytes gradually get the upper hand over the parasite and symptoms are usually eliminated over a period of years.

Two points need to be made: First, cells that are placed under metabolic, genotoxic or mechanical stress usually undergo spontaneous apoptosis (programmed cell disassembly and recycling, *i.e.*, suicide). In the case of intracellular parasites, apoptosis of the newly infected host cells is a desirable defense against the establishment of infections that burden the whole animal. Obviously, for the malaria parasite to successfully reproduce so many copies of itself that it distorts and expands the hepatocytes means that it has mastered the art of blocking apoptosis of the host cell. Suppression of apoptosis by intracellular parasites is usually accomplished by either producing parasite mimics of the host's anti-apoptotic proteins or inducing the host cell to over-express its own anti-apoptotic proteins (or suppress the host's pro-apoptotic proteins). Second, in the arms race between the intracellular parasites and the immune system (T-cells), T-cells have evolved an array of weapons including the release of several proteins that target "death receptors" on the surface of cells (*e.g.*, TNF [12,13]) and even the ability to spew toxic nitrous oxide (NO produced from the nitrous oxide synthetase (NOS) system) at invaders.

I have proposed [6] that in a desperate situation, improved survival in the face of malaria might be achieved in individuals carrying T-cells armed with unusually potent pro-apoptotic proteins. Hence, in hyper-endemic malaria areas, fast evolving retroviruses providing toxic agents (especially pro-apoptotic proteins) may have found a home with T-cells and developed a symbiotic relationship. However, the delicate balance between benefit and overdose has apparently been maintained by a very simple mechanism: When the viral-infected T-cells become too toxic, they kill themselves. The population of primate T-cell retroviruses (PTRV) is normally limited by the

lethality of the toxins to the infected cells. However, if the infected host cells acquired too much protection from their internal viral toxins relative to the bystander cells, then the bystander T-cells will be preferentially killed off (potentially leading to immune deficiency) and the toxin may also directly kill non-immune cells.

The first proposition (above) is plausible but would be difficult to prove. It presumes that HIV existed in a symbiotic relationship in humans and other primates in malaria endemic areas for a long time (thousands of years). In all likelihood, the viruses have crossed and re-crossed the species barriers many times [3], generally with no noticeable adverse effects [4]. These ideas are consistent with the ideas discussed by Levin [15] on the evolution of microparasites. It is relevant that there is, indeed, a "primate T-cell lymphotropic retrovirus" (PTLV) that has repeatedly crossed between humans and other primates over thousands of years in central Africa [16,17]. The evidence that chloroquine interacts with NF-kappa-B [10] gives credence to the hypothesis that chloroquine's actions on HIV and other viruses are to facilitate apoptosis of infected cells [18,19]. Thus, development of chloroquine-resistance in PTRV(HIV) would require development of enhanced resistance of infected cells to apoptosis.

Second proposition: pro-apoptotic chloroquine cause evolution of HIV and other viruses towards resistance

The mode of action of chloroquine is generally agreed to be on the malaria protozoa once it enters the erythrocytes. However, considering its pro-apoptotic effects [6,7] and the fact that chloroquine is used to prevent malarial infection (which starts in the liver) it would seem odd if chloroquine did not have any effects on the parasite in the liver. Chloroquine certainly has a biochemical effect on expression of NF-kappa-B [10] and down-stream (anti-apoptotic) proteins. The agents (*e.g.*, primaquine) that do cure established liver infections are more toxic. Chloroquine has very moderate toxicity, but it bioconcentrates and it is excreted slowly. Thus, the dosing schedule for prophylaxis can be flexible.

It is well known that the malaria parasites developed resistance to chloroquine in only a few years of intense use [14]. It is also well known that HIV develops resistance to anti-AIDS drugs quickly [18,19]. It has been found that chloroquine has effects on HIV infections [11,20–22]. Thus, it seems

self-evident that chloroquine would cause evolution of HIV strains toward resistance to the drug. Of course, this would also be true if humans were infected with a benign strain of PTRV (as defined above).

Please note that the affect of chloroquine on HIV [11,20–22] and coronavirus [23] are experimental observations (not speculation), and although the mode of action discussed in the first proposition may be wrong, the rest of the hypothesis would be unaffected.

Third proposition: the history of evolution of primate T-cell retrovirus (PTRV) into an AIDS-causing strain (HIV-1) is consistent with the history of 8-aminoquinoline and 4-aminoquinoline usage in central Africa

Korber et al. [1] have analyzed the mutations in numerous samples of retrovirus (HIV, here called PTRV) from people who have developed AIDS. Information about the presence of retroviruses in persons not displaying AIDS symptoms is not readily available (as noted above humans are generally presumed to be free of PTRVs, except in the case of HIV/AIDS). There has been much discussion of the date assigned to the last common ancestor of the known AIDS-causing strains of HIV(PTRV). But, unfortunately, much of the important information has been overlooked. Briefly, assuming mutations (caused by erroneous transcription of RNA from the DNA template) are the principal reason for the divergence of HIV(PTRV) strains, a phylogenetic tree was developed and an absolute timeline was created [1]. Almost all the samples were collected after 1980 and extrapolation back in time is accompanied with progressively greater uncertainty. The consensus last common ancestor of these AIDS-causing strains was estimated to be 1931 with 95% confidence interval of 1915–1940. Similar results were obtained by Salemi et al. [24], who showed that the transmission of the predecessor of HIV (*i.e.*, PTRV) to humans could have come as early as the late 1600s (late XVII century) and that the strains of HIV-1 began to diverge in the 1930s. The absolute uncertainty in these dates is thus quite large, but the relative errors are fairly small. Thus, special attention should be applied to the qualitative features of the phylogenetic tree.

In Fig. 4 of Korber et al. [1], there is a lot of very important information: Recall that the mutations of the RNA virus is continuous (though not con-

stant), however, the actual evolution of the AIDS-causing strains of HIV(PTRV) follows a distinct pattern. Either version of the prevailing hypothesis would presumably produce a continuous random branching of the tree. Contrary to this expectation, the evolution of the AIDS-causing strains of HIV(PTRV) was initially very fast. The first four major branches of the tree (HIV-1A; C/J/H; B/D; and F) all split off soon after the last common ancestor (*i.e.*, 1930–1932). Subsequently, strains H and J/C split from H/J/C and B and C split for B/C between 1936 and 1939. Then, very little happened until 1955 when multiple splittings of all the strains occurred, but this intense period of splitting mysteriously stopped around 1980.

Before explaining the qualitative pattern [1,24], it should be mentioned that the main sources of uncertainty in the absolute dates are sampling time uncertainty (which should be random), recombination (which would suggest that the earlier dates are too early) and latency of the virus (that would suggest that the earlier dates are too late). Regardless, it should be noted that the first 8-aminoquinoline anti-malarial drug pamaquine (plasmoquine(TM)) was synthesized in 1926 [25] by I.G. Farben (Bayer) in Germany. It was almost immediately (1927) tested in Kinshasa (Leopoldville), Congo [26]. Belgium had an active School of Tropical Medicine dating from 1906, which specifically addressed diseases in the Free State of Congo. Plasmoquine(TM) continued to be investigated under the auspices of the League of Nations throughout the 1930s [27]. Thus, the date targeted by Korber et al. [1,24] agrees very well with the arrival of 8-aminoquinolines in Kinshasa, Congo.

The search for anti-malarial drugs was just beginning. Chloroquine and other 4-aminoquinoline derivatives were synthesized in 1934–1935 in the laboratories of Bayer (I.G. Farben) in Germany as candidate anti-malarial drugs. Chloroquine was originally called “Resochin” by the Germans. Dr. J.B. Rice, who was active in anti-malarial drug research from 1937–1960, gave some rare details of its early history as testimony in a court case in 1969. According to Rice [28], chloroquine and other 4-aminoquinoline derivatives were superficially screened in canaries (the Germans did a lot of work on avian malaria) and found to be active against malaria by Dr. Kikuth. Dr. Kikuth then sent samples of the drug candidates to Dr. Seolli (Rome, Italy) for more detailed toxicological testing. Dr. Seolli deemed chloroquine (Resochin) to be too toxic for human use and instead pursued a related compound (designated “Sontochin”). Sterling Drug, Inc. (for whom Dr. Rice worked) had a cross-licensing agreement with I.G. Farben and received this

information in the late 1930s, but did not pursue chloroquine. Once the Japanese invaded Java, the US was deprived of a source of quinine and launched a high priority program to find new anti-malarial drugs. However, Rice did not become interested in chloroquine until he was involved in a post-war review of German technology in mid-1945. Looking for an anti-malarial for use in a planned continuation of the war with Japan, the US reviewed and tested (in the US) numerous compounds and selected chloroquine, which won approval from the US Food and Drug Administration in 1946. No reference to use of the 4-aminoquinolines in Congo prior to 1946 has been found. However, it is possible that some may have been tested there between 1935 and 1939 (when WWII started).

The US was not alone in pursuing chloroquine. By 1946, chloroquine (designated as SN 7618) was being tested as a prophylactic agent for malaria in Kinshasa, Congo [29,30]. This work was apparently facilitated by the Prince Leopold Institute of Tropical Medicine (established in 1931 on the "Congo Docks" in Antwerp, Belgium). It appears that after a couple of years of testing (1946–1948) in Kinshasa that worked stopped and the attention of Europe turned to the World Health Organization [31–33], which planned field test in Algeria, Italy, Portugal and/or Yugoslavia (no mention is made of central Africa or Congo). The Europeans were too busy trying to recover from the war to go far from home.

Once the initial field trials were over, chloroquine was used by visitors to Congo (1948–1955), but it is unlikely the native population had access to chloroquine. However, in 1955, the World Health Organization launched a world-wide eradication campaign using DDT to kill mosquitoes and chloroquine to reduce the number of infected carriers [14]. In sub-Saharan Africa, eradication of mosquitoes with DDT was considered to be impractical, but chloroquine was made widely available. The scale of this program was many orders of magnitude larger than anything that had come before. Although the WHO eradication program was accepted as a failure by 1967, extensive use of chloroquine continued in central Africa until chloroquine-resistant malaria reached Congo in 1978–1988 [34].

The correlation of the history of 8-aminoquinoline and 4-aminoquinoline anti-malarial drugs in central Africa with the evolution of AIDS-causing HIV(PTRV) is remarkable, but it would not be unexpected given what is now known about the effect of chloroquine on HIV [11,20–22]. Note that I recently attributed the entire history of HIV(PTRV)

evolution to chloroquine [8], but that is unnecessary and unlikely in view of the history of pamaquine (plasmoquine(TM)). Overall, the evolution of a human strain of PTRV, which existed before 1931 (probably as early as 1700 [24]) without causing disease, probably got started during field test with pamaquine *circa* 1931–1935. The modified virus strains were presumably initiated in only a handful of native people who had the virus and participated in the tests (in and near Kinshasa, Congo). This situation readily explains why all the known strains of HIV-1 are found in Kinshasa, and nowhere else. From the mid-1930s until 1955–1956, these people probably transferred the modified virus to other natives *via* sexual contacts. When the WHO anti-malaria program was initiated in 1955–1956, some people carrying the modified virus (which still did not cause AIDS) were exposed to further selective pressure from intense use of chloroquine. AIDS-causing strains soon emerged. The AIDS-causing strains stopped evolving after 1980, because they were no longer exposed to chloroquine. Indeed, the withdrawal of chloroquine in many cases likely allowed clinical AIDS to develop among people infected with the virus. In most cases, the viruses being sampled were now (post-1980) carried by people who were not in malaria endemic areas and/or the level of chloroquine use dropped (*i.e.*, chloroquine was used to treat individual malaria victims rather than provide prophylaxis) because of lack of funding and lack of utility. A similar pattern of evolution has been observed for HIV-2 [35].

Other parasites

As discussed elsewhere [6], other intracellular parasites (viruses, bacteria, protozoa, and fungi) are likely to evolve from areas of endemic malaria where chloroquine-resistant malaria has had time to develop. In particular, the history of SARS [23,36] can be mapped similar to HIV/AIDS. The deadly strains of Ebola virus also emerged in the right timeframe (post-1976) and in the areas covered by the WHO anti-malarial program (Congo, Sudan, Philippines) [37] and have the same sort of bystander effect [38] found in HIV that leads to the idea that a similar event may have started it.

The cure

If pro-apoptotic stresses are removed from AIDS-causing strains of HIV(PTRV), Muller's ratchet

suggests that the disease-causing strains will soon disappear [39,40]. Individuals might be treated with an agent that suppresses the anti-apoptotic proteins in the host cells so that they will kill themselves before killing the bystander cells [6]. To eliminate latent viruses, an agent is needed to activate the host cells [6]. Of course, chloroquine has anti-HIV activity [11,20–22] and pamaquine might work better.

Test of the malaria–chloroquine hypothesis

Although it might prove risky, an AIDS like syndrome can likely be induced in a SIV-infected, non-human primates by intensive dosing with chloroquine over a period of 5–10 years. A pattern of selected mutations might be observable within a year. The disease would probably not be manifest until after chloroquine treatment is stopped.

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