Hypothesis links emergence of chloroquine-resistant malaria and other intracellular pathogens and suggests a new strategy for treatment of diseases caused by intracellular parasites

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Summary Chloroquine and related anti-malarial drugs appear to promote apoptosis in T-cells by suppressing NF-kappa-B, which enhances the expression of anti-apoptotic proteins (e.g., Bcl-2). Thus, chloroquine has found applications in autoimmune diseases where it apparently facilitates apoptosis of abnormally persistent T-cell clones. The mode of action of chloroquine in prevention of malaria is not known, but it may be to minimize replication of the parasite in the liver cells, which occurs before invasion of the erythrocytes, by facilitating premature apoptosis of the infected host cells. After introduction of chloroquine in the 1950s world-wide for prophylactic use, chloroquine-resistant malaria emerged. Here it is hypothesized that concurrent with emergence of chloroquine-resistant malaria (presumably with enhanced anti-apoptotic capabilities), other intracellular parasites have evolved to enhance their ability to prevent apoptosis in host cells. Two examples of viral diseases that have emerged from areas of high incidence of chloroquine-resistant malaria are AIDS from HIV and SARS from *coronavirus*. The hypothesis holds that prophylactic exposure to pro-apoptotic chloroquine drugs caused natural selection for strains of viruses and other parasites that have enhanced anti-apoptotic abilities. When transmitted to host organisms that are not under the influence of the pro-apoptotic drug, the new "anti-apoptotic" strains may cause unexpected diseases. In the case of SARS, the *coronavirus* appears to have accessed a new niche where it proves to be lethal to its host. In the case of AIDS, the HIV (which has had a long-term symbiotic relationship with primates) has run amuck because the infected cells are now substantially more tolerant to the toxins (i.e., resistant to apoptosis) that they secrete than the uninfected bystander cells, which are not unusually resistant to apoptosis. A corollary to the hypothesis is that if the level of resistance to apoptosis in the infected cells were no higher than the level of resistance in the bystander cells, then the infected cells would preferentially kill themselves through apoptosis. It appears that in the case of HIV, the increased resistance to apoptosis is provided by expression of Bcl-2 and suppression of p53. Hence, drugs that suppresses Bcl-2 or restore p53 function might be effective in restoring the parity of resistance to apoptosis between infected and uninfected cells. Currently, an antisense drug targeting Bcl-2 (G3139/Genasense™, Genta, Inc.) is in late-stage cancer trials and may be on the market for those indications in months. It would be interesting to try these drugs against various intracellular parasites including HIV. This approach to prevent or eliminate active infections might be particularly attractive against a range of parasites (virus, bacteria, protozoa, fungus) when safe and effective vaccines are not available.
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Introduction

Quinine has been used since the 1800s as prophylaxis for malaria, primarily by non-indigenous visitors. Quinine was largely replaced by chloroquine, hydroxychloroquine and related compounds in the 1950s, and these drugs have been used widely and intensely by natives of endemic areas as well as visitors. The mode of action of "chloroquine anti-malarial drugs" (Cq) is not clear. However, Cq may suppress the transcription-factor NF-kappa-B [1], which normally up-regulates Bcl-2 and other anti-apoptotic proteins, leading to apoptosis [2,3]. Recently, it has been shown that caspase-independent disruption of the mitochondrial membrane regulated by the Bcl-2 family proteins is the rate-limiting step for apoptosis induced by hydroxychloroquine [4]. It is also noted that Cq drugs are finding applications in autoimmune diseases where they seem to facilitate apoptosis of abnormally persistent clones of T-cells [5]. Thus, it is reasonable think that Cq drugs may prevent malaria by minimizing reproduction of the parasite in the first stage of its life cycle in the liver by premature apoptosis of schizonts. The malaria parasite invades erythrocytes only after extensive replication (up to 10,000 times) in the liver.

Eukaryote cells under stress (genotoxic, metabolic or physical stress) tend to undergo p53-dependent apoptosis. Invasion by an intracellular parasite is expected to cause stress in the host cell. This stress would normally be adequate to provoke p53-dependent apoptosis and this would typically limit such infections. However, it is common for intracellular parasites (viruses, bacteria, protozoa, etc.) to manipulate the apoptosis pathway of their host cells to allow maximum replication of the parasite before the host cell is killed [6–9]. Sometimes intracellular parasites provide their own anti-apoptotic proteins [10] and sometimes they induce the host cells to over-express the host's anti-apoptotic proteins (e.g., Bcl-2, IAP, caspase inhibitors, FLIP). Thus, exposure of infected host cells to drugs that work against the parasite-driven up-regulation of anti-apoptotic proteins is an ecological pressure on the parasite that will result in evolution of strains of parasites that are more effective (quantitatively and/or qualitatively) at preventing pre-mature apoptosis of host cells. While these new "anti-apoptotic strains" may be subdued under the continuous influence of the pro-apoptotic drug, they may prove quite virulent without it.

The hypothesis

The crux of the hypothesis posed here is that Cq-drugs (more potent than quinine and used in unprecedented quantities in native populations starting in the 1950s), have brought forth not only chloroquine-resistant malaria (which is well recognized), but also a host of "anti-apoptotic strains" of other common parasites (which are generally not recognized). Some of the new "anti-apoptotic strains" of common parasites have become important pathogens because they can replicate in greater numbers in tissues where they previously may have had no niche (e.g., new strains of *coronavirus* that produce SARS) or because they can produce levels of secreted toxins that are lethal to uninfected bystander cells while the infected host cells (that express high levels of anti-apoptotic proteins) can survive (e.g., new strains of HIV that produce AIDS). Resistance to apoptosis also facilitates cell fusion and cell-to-cell spread of pathogens [11].

Finally, it is noted that pre-existing cell clones that resist apoptosis by over-expressing anti-apoptotic proteins (e.g., memory T-cells or (pre-) cancerous clones) may be easy targets for initiation of infection in an organism [12–14].

A corollary

It follows from this hypothesis that suppression of the over-expressed anti-apoptotic proteins in the host cells (or potential host cells) would be an attractive way to indirectly control the parasite. This is particularly true in cases where it has proved impossible to develop safe and effective vaccines against the parasite itself. But, care must be taken because some parasites (e.g., HIV) have become lethal because their enhanced resistance to apoptosis allows them to survive while spreading extracellular toxins especially some that kill (normal, unprotected) bystander cells by inducing apoptosis. General suppression of the anti-apoptotic proteins in all cells might cause slaughter of the bystander cells before the cells hosting the virus could be killed, unless intracellular toxins can be neutralized or nullified first.

Interestingly, a drug (G3139, Genta, Inc.) that is intended to suppress a key anti-apoptotic protein (Bcl-2) is in the final stages of clinical trials (December 2003) for several cancer indications. G3139 is an "antisense" drug that has been shown to reduce Bcl-2 protein levels in vivo with minimal side effects [15]. Moreover, G3139 is an antisense ODN

with unmethylated CpG units, which induce normal T-cells to resist apoptosis by up-regulating c-FLIP and Bcl-x(L), while having little effect on Bcl-2 [16,17]. It is relevant that Bcl-2 only protects cells against apoptosis from internal stress, but Bcl-x(L) has a distinct function in protecting cells against external death signals such as TRAIL [18]. Verthelyi et al. [19] have advocated using unmethylated-CpG ODNs in HIV-1 infected individuals to protect against secondary infections. Thus, the tools to test the hypothesis posed here are at hand.

An example: The emergence of AIDS from HIV

One of the puzzles of acquired immune deficiency syndrome (AIDS) is why this disease has emerged now and only in humans, when the basic virus (HIV) has been around for a very long time and similar viruses (SIV) are carried by other primates without disease. It is understood that HIV inhabits certain host T-cells and the infected cells release pro-apoptotic toxins that induce extensive apoptosis in bystander cells (other T-cells and cells of other tissues). This phenomenon is not unlike the release of toxic NO (nitric oxide) by immune cells to fight infection and there may actually have been a symbiotic arrangement between the humans (primates) and the virus (HIV) to fight off malaria [20—22] and other tropical diseases [23—25]. (Although this may seem like an extreme adaptation, remember that sickle cell mutation, which produces a sever disease, apparently has been retained in the gene pool because of its ability to defeat malaria.) Originally, the population of HIV infected T-cells was kept in check by the fact that they were also susceptible to the pro-apoptotic proteins they secreted. However, the infected host cells carried just enough anti-apoptotic protein to protect themselves while attacking cells (especially liver cells) hosting other parasites.

Unfortunately, this speculative partnership between humans and the HIV virus apparently turned sour when humans introduced Cq, which not only produced apoptosis in the liver cells as they became infected with malaria, but also killed off many of the cells hosting the virus [26—30]. This ecological pressure selected for strains of the HIV virus that produced higher levels of anti-apoptotic proteins in the host cells and consequently allowed the cells hosting the virus to survive while releasing higher levels of toxic proteins into the blood stream (where the bystander T-cells were their first new victims). As overall T-cell counts fall,

humans become prey to a series of opportunistic infections (known as AIDS).

In the hypothesis developed here, the basic strain(s) of HIV (per-1950) were kept in check by self-induced apoptosis while providing some protection against malaria. The apoptosis-resistant strain of HIV that emerged [10,31—33] facilitates survival of its host and cell fusions of its host with cell-to-cell transmission of the virus [11] under conditions where the (uninfected) bystander cells are killed in large numbers; ultimately resulting in a deficiency of T-cells and the symptoms of AIDS. It is proposed here that use of a drug that effectively suppresses the resistance to apoptosis in all the cells will essentially level the playing field and make the infected cells kill themselves (before they can kill disproportionate numbers of bystander cells).

References

[1] Bierhaus A, Hemmer CJ, Mackman N, et al. Antiparasitic treatment of patients with *P. falciparum* malaria reduces the ability of patient serum to induce tissue factor by decreasing NF-kappa B activation. Thromb Haemost 1995;73:39—48.

[2] Picot S, Burnod J, Bracchi V, Chumpitazi BF, Ambroise-Thomas P. Apoptosis related to chloroquine sensitivity of the human malaria parasite *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 1997;91:590—1.

[3] Meng XW, Feller JM, Ziegler JB, Pittman SM, Ireland CM. Induction of apoptosis in peripheral blood lymphocytes following treatment in vitro with hydroxychloroquine. Arthritis Rheum 1997;40:927—35.

[4] Boya P, Gonzalez-Polo RA, Poncet D, et al. Mitochondrial membrane permeabilization is a critical step of lysosome-initiated apoptosis induced by hydroxychloroquine. Oncogene 2003;22:3927—36.

[5] Potvin F, Petitclerc E, Marceau F, Poubelle PE. Mechanisms of action of antimalarials in inflammation: induction of apoptosis in human endothelial cells. J Immunol 1997;158:1872—9.

[6] Barber GN. Host defense, viruses and apoptosis. Cell Death Differ 2001;8:113—26.

[7] Gangappa S, van Dyk LF, Jewett TJ, Speck SH, Virgin HW. 4th.Identification of the in vivo role of a viral bcl-2. J Exp Med 2002;195:931—40.

[8] Hardwick JM, Bellows DS. Viral versus cellular BCL-2 proteins. Cell Death Differ 2003;10:S68—76.

[9] Heussler VT, Kuenzi P, Rottenberg S. Inhibition of apoptosis by intracellular protozoan parasites. Int J Parasitol 2001;31:1166—76.

[10] Greenway AL, McPhee DA, Allen K, et al. Human immunodeficiency virus type 1 Nef binds to tumor suppressor p53 and protects cells against p53-mediated apoptosis. J Virol 2002;76:2692—702.

[11] Sandstrom PA, Pardi D, Goldsmith CS, Chengying D, Diamond AM, Folks TM. bc1-2 expression facilitates human immunodeficiency virus type-1 mediated cytopathic effects during acute spreading infections. J Virol 1996;70:4617—22.

[12] Stebbing J, Patterson S, Gotch F. New insights into the immunology and evolution of HIV. Cell Res 2003;13:1—7.

[13] Bermudes D, Zheng LM, King IC. Live bacteria as anticancer agents and tumor-selective protein delivery vectors. Curr Opin Drug Discov Devel 2002;5:194—9.

[14] Edwards SJ, Dix BR, Myers CJ, et al. Evidence that replication of the antitumor adenovirus ONYX-015 is not controlled by the p53 and p14 (ARF) tumor suppressor genes. J Virol 2002;76:12483—90.

[15] Wacheck V, Krepler C, Strommer S, et al. Antitumor effect of G3139 Bcl-2 antisense oligonucleotide is independent of its immune stimulation by CpG motifs in SCID mice. Antisense Nucleic Acid Drug Dev 2002;12:359—67.

[16] Davila E, Velez MG, Heppelmann CJ, Celis E. Creating space: an antigen-independent, CpG-induced peripheral expansion of naive and memory T lymphocytes in a full T-cell compartment. Blood 2002;100:2537—45.

[17] Kojima Y, Xin KQ, et al. Adjuvant effect of multi-CpG motifs on an HIV-1 DNA vaccine. Vaccine 2002;20:2857—65.

[18] Kim IK, Jung YK, Noh DY, et al. Functional screening of genes suppressing TRAIL-induced apoptosis: distinct inhibitory activities of Bcl-XL and Bcl-2. Br J Cancer 2003;24:910—7.

[19] Verthelyi D, Gursel M, Kenney RT, et al. CpG oligodeoxy-nucleotides protect normal and SIV-infected macaques from Leishmania infection. J Immunol 2003;170:4717—23.

[20] Nahrevanian H, Dascombe MJ. Expression of inducible nitric oxide synthase (iNOS) mRNA in target organs of lethal and non-lethal strains of murine malaria. Parasite Immunol 2002;24:471—8.

[21] Brunet LR. Nitric oxide in parasitic infections. Int Immunopharmacol 2001;1:1457—67.

[22] Boutlis CS, Tjitra E, Maniboey H, et al. Nitric oxide production and mononuclear cell nitric oxide synthase activity in malaria-tolerant Papuan adults. Infect Immun 2003;71:3682—9.

[23] Alam MS, Akaike T, Okamoto S, et al. Role of nitric oxide in host defense in murine salmonellosis as a function of its antibacterial and antiapoptotic activities. Infect Immun 2002;70:3130—42.

[24] Brunet LR, Beall M, Dunne DW, Pearce EJ. Nitric oxide and the Th2 response combine to prevent severe hepatic damage during Schistosoma mansoni infection. J Immunol 1999;163:4976—84.

[25] Richardson JO, Forsyth LM, Brown CG, et al. Nitric oxide causes the macroschizonts of Theileria annulata to disappear and host cells to become apoptotic. Vet Res Commun 1998;22:31—45.

[26] Boelaert JR, Sperber K, Piette J. The additive in vitro anti-HIV-1 effect of chloroquine, when combined with zidovudine and hydroxyurea. Biochem Pharmacol 2001;61:1531—5.

[27] Boelaert JR, Sperber K, Piette J. Chloroquine exerts an additive in vitro anti-HIV type 1 effect when associated with didanosine and hydroxyurea. AIDS Res Hum Retroviruses 1999;15:1241—7.

[28] Savarino A, Gennero L, Sperber K, Boelaert JR. The anti-HIV-1 activity of chloroquine. J Clin Virol 2001;20:131—5.

[29] Savarino A, Gennero L, et al. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. AIDS 2001;15:2221—9.

[30] Neely M, Kalyesubula I, Bagenda D, et al. Effect of chloroquine on human immunodeficiency virus (HIV) vertical transmission. Afr Health Sci 2003:61—7.

[31] Zhang M, Li X, Pang X, et al. Bcl-2 upregulation by HIV-1 Tat during infection of primary human macrophages in culture. J Biomed Sci 2002;9:133—9.

[32] Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4(+) T lymphocyte cell death in human immunodeficiency virus infection and AIDS. J Gen Virol 2003;84:1649—61.

[33] Aillet F, Masutani H, Elbim C, et al. Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistant infection of CD4$^+$ T- or monocytic cell lines. J Virol 1998;72:9698—705.

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