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Ebola Virus and Malaria Coinfection: How Infection with One Pathogen May Protect Against Another

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SUMMARY Both malaria and Ebola virus have been the centre of global health care efforts in recent years, due to the massive ongoing disease burden of malaria and the largest Ebola outbreak in history in West Africa. However, little is known about the interplay of these two diseases within the same host, and it has been suggested that coinfection with Ebola virus and *Plasmodium falciparum*, the causative agent of malaria, may impact survival outcomes in Ebola patients. While the existing literature is conflicting, it is obvious that there is some level of interaction that needs to be further explored. I propose a potential mechanism by which *P. falciparum* infection prior to infection with Ebola virus could have a protective role, namely in the ability of *P. falciparum* to induce an antiviral-like immune response to the protozoan, which in turn protects against severe Ebola virus infection. Secreted glycoprotein of Ebola virus normally functions to inhibit proinflammatory cytokine production and inhibits macrophage activation in order to provide a pool of susceptible host cells, however following the priming of the immune system by *P. falciparum* infection, Ebola's immune modulating actions are inhibited which leaves the immune system more able to effectively clear the infection. In order to elucidate the mechanism at play, *in vivo* studies will be crucial in determining direction of correlation as well as factors involving severity of viremia or parasitemia. The immune modulation at play could be elucidated by utilizing CyTOF technology (a mass spectrometry based technique) in order to get a better understanding of the cytokine milieu and activation states of the immune cells of the innate immune system.

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INTRODUCTION

Malaria and Ebola virus have both made headlines in recent years due to their massive global disease burden and potential for large outbreaks, respectively. Malaria caused 438 000 deaths in 2015 alone, and while Ebola virus is not a particularly common disease, it can be one of the most deadly, especially during massive disease outbreaks such as that seen West Africa in 2014, which killed 11 310 people (1,2). When comparing data from outbreaks

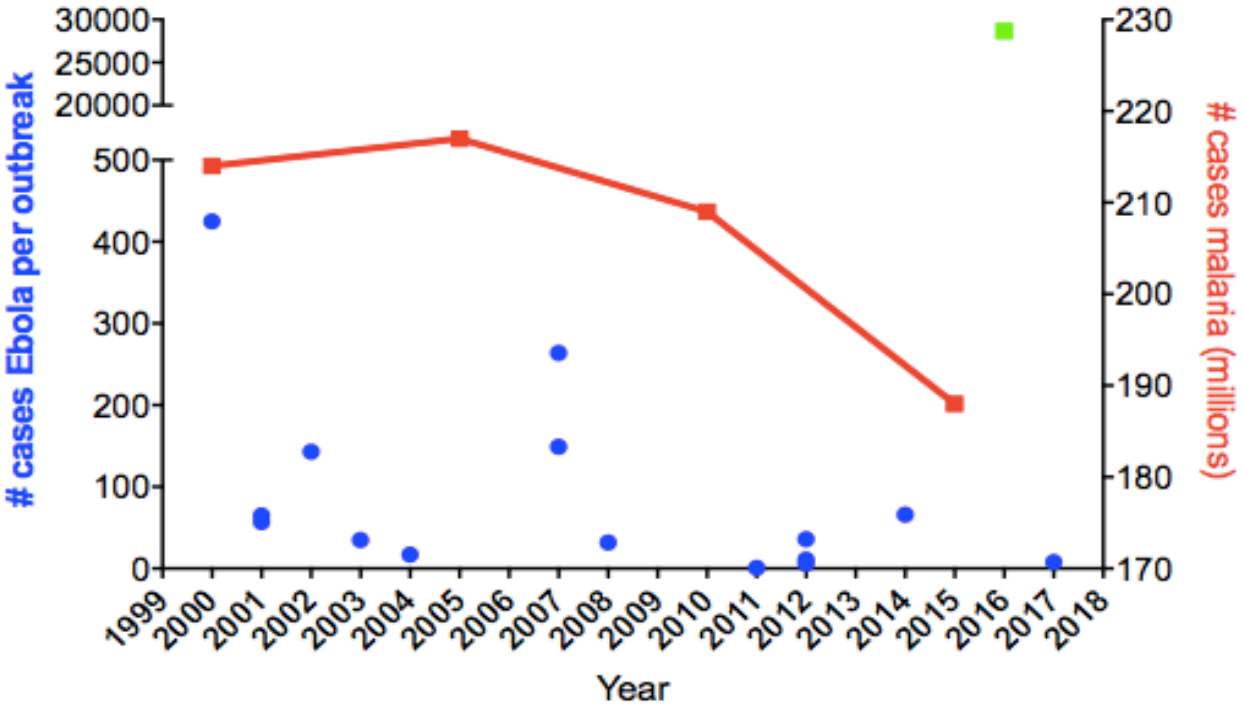

FIG. 1 Ebola and Malaria in Africa. The number of cases per Ebola outbreak in Africa, where each blue point is an outbreak (left axis), compared to the number of cases of malaria infection in Africa (red trend line, right axis). The green square data point depicts the West Africa outbreak.

of both diseases in Africa, the region to which both diseases are endemic and have the highest burden, there appears to be a correlation between the Ebola virus outbreak size and the number of malaria cases. While both disease incidences appeared to steadily decrease, this correlation fails in 2014 when the lowest incidence of malaria allows a spike in Ebola virus cases, culminating in the West Africa outbreak (Fig. 1) (3,4). Therefore it may be possible that malaria may be protective against large-scale Ebola virus outbreaks. It is important to consider that coinfection may have unforeseen consequences, and may cause different effects on disease course and mortality than would otherwise be observed with either infection alone. While their interactions within a host pose a major knowledge gap, independently, substantial research has elucidated their pathogenesis and interactions with the host immune system.

The negative-sense RNA Ebola virus genome is comprised of 7 genes, and of particular importance is the glycoprotein gene, which has multiple transcription products due to polymerase stuttering and subsequent frameshift mutations at an adenosine-rich site within the gene (5,6). These products are a full-length surface glycoprotein ($GP_{1,2}$), a shorter secreted glycoprotein (sGP), and an even further truncated small secreted glycoprotein (ssGP) (Fig. 2) (6,7). The sGP product is the dominant transcriptional product, and while its function is not completely understood, it has been shown to play important roles in modulation of the host antiviral response by inhibiting macrophage chemotaxis and production of pro-inflammatory cytokines (8). Ebola pathogenesis proceeds by the virus infecting a host through mucosal surfaces where it can then infect most cell types to induce symptoms of fever, diarrhea, vomiting, and haemorrhage (9). The virus modifies the host immune system by interfering with type 1 interferon (IFN) production and perturbs cytokine production in dendritic cells (DCs) and macrophages to induce massive release of pro-inflammatory cytokines (9).

The infectious agent of malaria is a protozoan, of which four species cause disease in humans, but the most prevalent species in Africa is *Plasmodium falciparum* (10). The parasite is transmitted via a mosquito bite and then travels to the liver to infect hepatocytes (10). Following liver-stage infection, the parasites enter a blood-stage infection where they infect

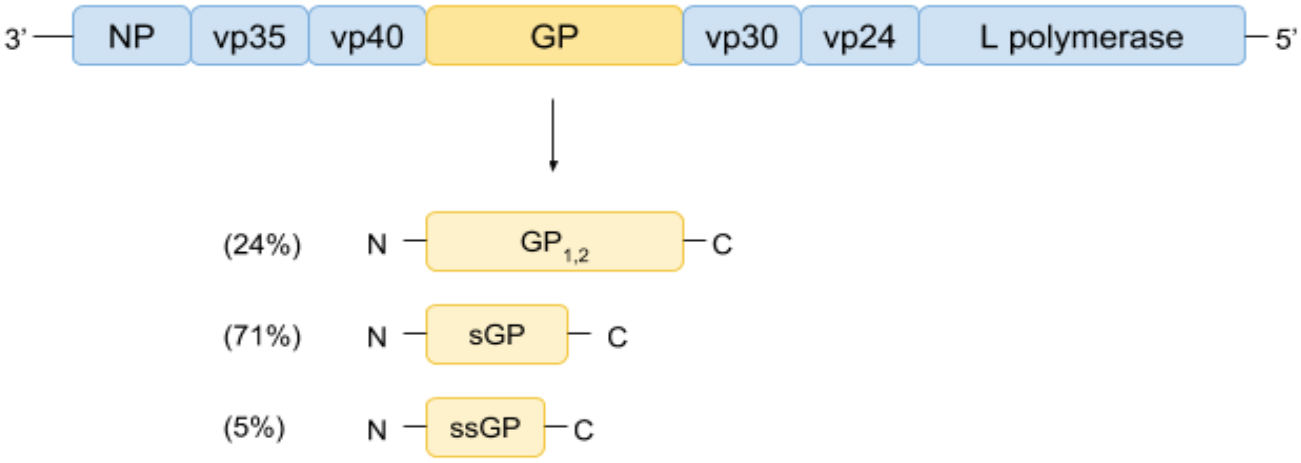

FIG. 2 Ebola virus genome and the protein products of the *GP* gene. The sGP product is produced in the highest abundance, followed by full-length $GP_{1,2}$, then ssGP (71%, 24%, and 5%, respectively).

erythrocytes (10). While most infections are asymptomatic, severe cases of malaria can cause death due to single- or multi-organ failure or cytokine storms (10). While *P. falciparum* can express and secrete a number of factors that may play a role in its pathogenesis and host immune modulation, of particular importance is its expression of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on the erythrocyte surface. This molecule has been suggested to reduce NF-κB activity and inhibit release of IFNγ from peripheral blood mononuclear cells (PBMCs) (11). PfEMP1 may play a role in skewing the immune response away from a protective Th1 response towards the induction of an antiviral response, allowing the parasite to evade the host immune system (6,11).

RESEARCH QUESTIONS

Some data regarding Ebola virus and malaria coinfection has emerged following the Ebola outbreak in West Africa since both Ebola virus and malaria were evaluated as standard practice upon admission to Ebola treatment units (ETUs) (12). The literature poses conflicting results where some studies suggest that the presence of both malaria and Ebola virus infections lead to increased mortality, while others suggest the polar opposite (13,14,15). Regardless of the direction of the association, the evidence suggests that the pathogens are interacting to affect disease progression, and with this finding it is evident that this mechanism needs to be elucidated. In order to explore this interaction, first the existing literature needs to be critically evaluated to establish what aspects of the studies may be influencing their results to give conflicting findings. Next, potential mechanisms of the interaction between *Plasmodium* and Ebola virus will need to be considered and experiments will need to be carried out in both *in vivo* models and on collected human samples to help clarify the direction of the association and to study the mechanism by which the interaction occurs.

PROJECT NARRATIVE

What experimental or statistical analysis differences between existing studies could account for the conflicting results?

The literature describing the coinfection of *P. falciparum* and Ebola virus are largely dependent on retrospective cohort studies from data collected from ETUs (13,14,15). This in itself is a limitation since the researchers have no control over what samples and information were collected or how the samples were handled and analyzed. The variation of sample assay methods and statistical analysis of the data may also have contributed to the differences in findings. Table 1 summarizes the findings and methods used by several studies that discuss malaria and Ebola virus coinfection.

Waxman *et al.* and Kerber *et al.* have published studies that suggest coinfection results in decreased survival rates (13,14). The findings by Waxman *et al.* showed a significant increase

Study	% Mortality malaria positive	% Mortality malaria negative	Effect on survival	Malaria detection method	Data analysis categories	Notes
Rosenke *et al.* (15)	42	54	20% increase in survival	RT-qPCR	Malaria Ct value, Ebola Ct value, age	Small group sizes
Gignoux *et al.* (23)	54.5*	63.5	Increased survival	RDT	Malaria status	Not the study focus * of patients given artemether-lumefantrine (standard treatment)
Kerber *et al.* (14)	60.2**	56.6**	20% decrease in survival (5-14 years), otherwise no effect	RDT	Malaria status, age, Ebola Ct value	** Not significant
Waxman *et al.* (13)	66	52	~30% decrease in survival	RDT	Malaria status	% Mortality does not consider age or viral load

TABLE 1 Summary of findings and methods by various studies. Organized from lowest coinfection mortality rate to highest

in mortality in patients that were coinfected with malaria compared to those that were only infected with Ebola virus, whereas Kerber *et al.* showed an increase in mortality among 5-14 year olds, but overall they showed no significant change in mortality (13,14). Both studies used rapid diagnostic tests (RDTs) to assay for malaria coinfection, which potentially excludes patients that are effectively controlling *P. falciparum* infection due to the relatively high limit of detection for RDTs compared to RT-qPCR, as well as excludes the possibility that the extent of parasitemia may be a factor in coinfection mechanisms (16). Regarding the statistical analysis of the samples, the exclusion of stratification by age in the study by Waxman *et al*. dismisses the possibility that age may be a confounding factor as has been suggested by other studies (13,14,15).

The opposing finding by Rosenke *et al.* is that coinfection with the malaria parasite is associated with an increase in survival by 20% overall, with this number being even higher in certain age groups (5). Rosenke *et al.* utilized RT-qPCR to assay for malaria, which provided the researchers with quantitative data regarding the extent of parasitemia as well as the level of Ebola viremia (5). During their statistical analysis, this study stratified the data by age to show that age may be an important factor in the outcome of coinfection (5). Of particular interest, their results showed that when Ebola viremia was low or moderate, the patients that had the highest level of parasitemia had the greatest increase in survival, with some groups showing a 100% survival rate (5). However, it should be noted that many of these groups had very small sample sizes, so no claims regarding significance can be made. The thorough data analysis attempts to eliminate as many confounding variables as possible, which gives their claims credibility, even if the small sample size may result in overstatement of the effect of coinfection.

By what mechanisms might concurrent malaria infection affect Ebola virus disease progression?

Interactions between Ebola and other infections, as well as malaria and other infections, have been documented; therefore it is conceivable that these two infections could also be interacting with each other (17,18). Some theories regarding this mechanism include malaria-induced immunosuppression, and malaria-induced NK cell priming (5). However, I believe that neither theory tells the full story, and that a mechanism that includes aspects of both hypotheses may be plausible. Since both *P. falciparum* and Ebola virus produce molecules that are immunomodulatory, these molecules could be inhibiting each other, specifically malaria-induced molecules could be inhibiting the actions of Ebola-secreted molecules.

Ebola pathogenesis involves the impairment of macrophage and DC function and ability to disseminate. Macrophages show reduced production of proinflammatory cytokines in the presence of sGP, which suggests that sGP may suppress the inflammatory state, specifically by inhibiting IL-6 and TNFα by promoting IRF4 activity (8). Ebola virus has also been shown to impair the antiviral state of host cells, and this role has also been attributed to sGP (8).

It has been shown that an suboptimal immune response to *P. falciparum* may occur if there is a shift away from a protective Th1 immune response. Microarray analyses at various stages of malaria disease course have shown that PBMCs increase expression of IFNγ and induce IFNα/β to trigger an increase in NK cell cytotoxicity (19). NK related genes were shown to be upregulated, including IL-15, which induces NK differentiation and proliferation (19). Additionally, *P. falciparum* that express PfEMP1 on the erythrocyte surface have been shown to induce lower levels of NF-κB than their PfEMP1-negative counterparts (11). While this protein was shown to decrease NF-κB signalling, it was shown to not affect the production of type 1 IFNs (11). These results suggest that *P. falciparum* infection can cause downregulation of the proinflammatory Th1 response and could cause a subsequent upregulation of the type 1 IFN-mediated antiviral response. When the immunomodulatory activities of *P. falciparum* and Ebola virus are considered together, it becomes apparent that they induce opposing effects on the host immune response.

It is my hypothesis that interactions between malaria-derived factors may cause a skewed immune reaction towards an antiviral response, thus inhibiting Ebola virus activity. To start the chain of events, blood-stage PfEMP1-producing *P. falciparum* infection induces IFNα/β production in circulating monocytes and activates NK cell activity. This monocyte may then differentiate and become activated, but fail to produce molecules of the NF-κB pathway. Since macrophages are a key replication site for Ebola virus, this altered macrophage may come into contact with circulating virus particles and the sGP produced by Ebola virus would not be able to effectively prevent the antiviral state in the macrophage since it had already been induced. Nearby, activated NK cells are able to cytolytically kill Ebola virus infected macrophages and prevent its spread. In summary, an antiviral immune response against PfEMP1-expressing *P. falciparum* is able to protect against subsequent Ebola virus infection by preventing Ebola virus' immunomodulatory effects (Fig. 3).

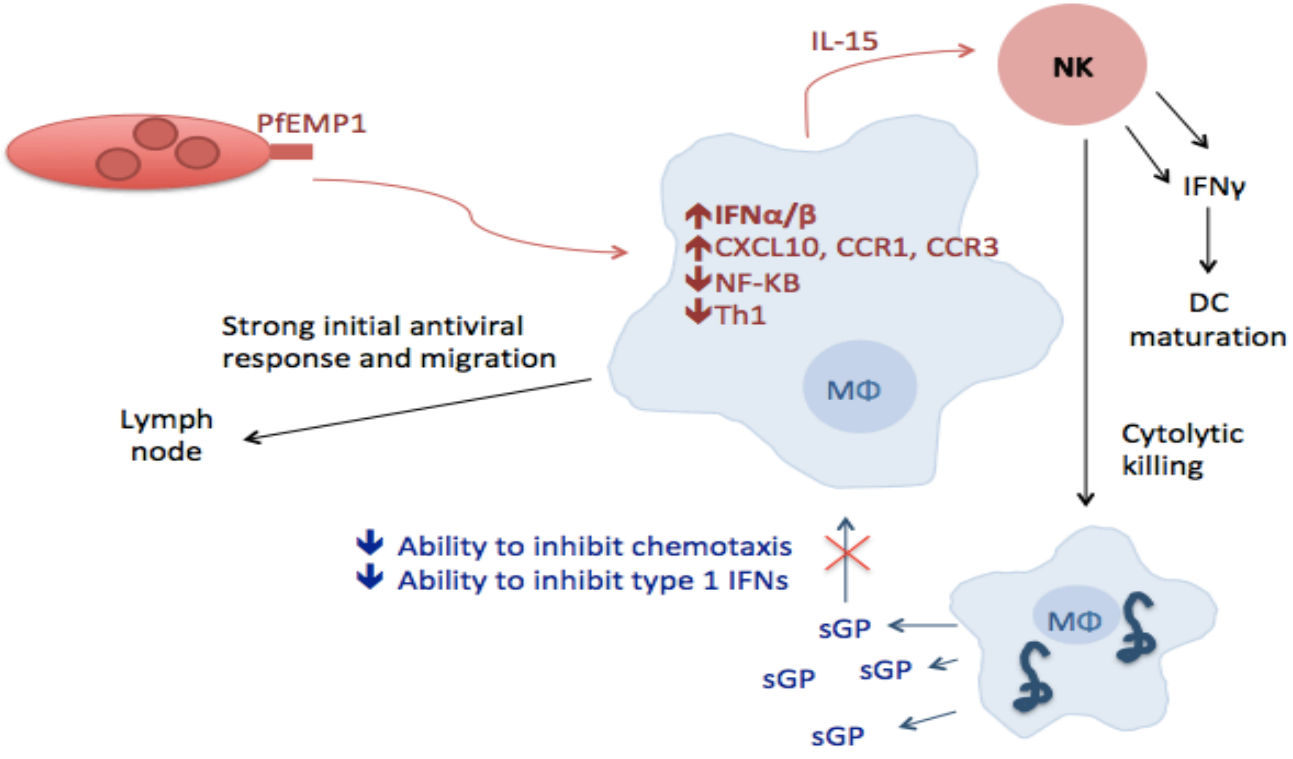

FIG. 3 Pathway of the potential mechanism of coinfection with Ebola virus and *P. falciparum*.

What research needs to be done to further elucidate the mechanism of coinfection?

There is a desperate need for *in vivo* studies that look into the mechanism of coinfection between malaria and Ebola virus. Both pathogens already have established animal models, including mouse-adapted virus models and guinea pig models that can be transiently infected with wildtype Ebola virus or with a guinea pig-adapted virus (20). However, the most useful animal model to study Ebola virus infection is the nonhuman primate (NHP) since it does not require adapted virus and exhibits symptoms most similar to human infection (20). Since the blood-stage and liver stage of *P. falciparum* infection remain unable to be studied simultaneously in small rodent models, NHPs remain the most relevant disease model (22).

Using an NHP model, the question regarding the direction of the correlation between concurrent *P. falciparum* infection and Ebola survival rate can be answered. A group of NHPs would be infected with *P. falciparum* and allowed to establish blood-stage infection prior to infecting the same group with Ebola virus. The survival rate would be compared to the survival rate in Ebola virus monoinfection conditions to determine if *P. falciparum* infection prior to Ebola virus infection increases survival rates. This opens the door for a second research question: does the Ebola virus inoculum size play a role in the ability of malaria to protect against Ebola virus? This is an important question because Rosenke *et al.* showed that there were differences in survival rates in patients that had mild Ebola viremia compared to patients with high viremia (15). These research questions could aid in determining any confounding factors in the retrospective cohort studies as well as provide insight into a potential mechanism.

In order to discover a potential mechanism of this interaction, cytometry by time of flight (CyTOF) could be utilized in order to gather a large amount of data from samples simultaneously. CyTOF works by measuring the signal of heavy metal isotope-tagged antibodies that target various antigens on labeled cells by mass spectrometry, generating a complete summary of every antibody that had been bound to each cell (22). This is particularly useful in this instance since there are limited patient samples that had been collected from the ETUs during the West Africa outbreak, and since there are still many questions regarding the mechanism, it is a virtue to be able to extract as much information from these samples as possible. By using antibodies that target a variety of cytokines and cell type markers, the use of CyTOF will allow for simultaneous characterization of cells including NK cells, DCs, and macrophages; characterization of the cytokine milieu, including whether there is a skew towards a Th1 or antiviral response; and characterization of parasite-specific molecules including sGP and PfEMP1.

SUMMARY AND CONCLUSION

The concept of *P. falciparum* infection playing a protective role against Ebola virus is thought provoking, and it may call into question whether the global efforts to eradicate malaria are warranted since eradicating malaria may have the potential to lead to an increase in devastating outbreaks of Ebola virus. However, the disease burden of malaria is so vast that malaria reduction efforts are of great importance to the world as a whole. Instead of focusing on the potential benefits of natural *P. falciparum* infection, the culmination of the research proposed here should spark further research regarding potential treatment for Ebola virus. By understanding the mechanism through which Ebola virus survival increases during coinfection, treatment could be geared towards providing molecules that will function to stimulate the immune system similarly to *P. falciparum* infection. Understanding this mechanism may also contribute to vaccine development against Ebola virus. While vaccine candidates are already in clinical trials, more efficacious vaccines could potentially come of the knowledge of how best to stimulate the immune system to fight Ebola virus. Additionally, prophylactic medicine during an outbreak could be a potential therapeutic avenue to explore since the proposed mechanism involves a prior and ongoing infection with *P. falciparum*. If at the onset of a disease outbreak members of the community could take a drug that supplied molecules that mimicked those produced by *P. falciparum*, they could potentially be protected against developing severe Ebola virus.

A research void exists at the intersection of Ebola virus pathogenesis and malaria pathogenesis, where both infections are known to occur simultaneously in a patient, yet their

interactions within a host have not been explored. The West Africa outbreak provided a unique opportunity to evaluate the dynamic of these two pathogens since both were screened for upon admission to an ETU. While the existing literature is not conclusive, it raises a lot of questions and potential areas for molecular researchers to pursue. One such area is the mechanism by which malaria may interfere with the ability of Ebola virus to disseminate throughout the body. I proposed a mechanism by which this interaction may occur, but substantial research needs to be conducted to make any concrete claims about the actual interactions and mechanism at play during coinfection.

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