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Babesia with Plasmodium Coinfection : What if the host is benefitting?

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ABSTRACT

Concurrent infections by multiple parasites in a single host have all round implications in shaping their ecological niche. Many aspects of immunobiology of parasites have been shown to have far reaching consequences in such biological existence. Modulation and or suppression of growth, alteration of infectiveness and reduced response to chemotherapy are well documented when parasites exist concurrently. In Host-parasite relationships, any substance, biological or chemical product that can reduce the multiplication of parasites in the blood, without necessarily suppressing it completely, has the potential to allow for host survival while giving a chance for acquired immunity to set in, thus proving right the axiom ***"live and let live."*** *Babesia* with *Plasmodium* co-existence offers a paradigm of rich example and has been cited to suppress the growth of the latter, a stratagem in which the host is a great beneficiary. This cross-reactive and cross-protective algorithm is thought to be due to common antigenic determinants for *Babesia* and *Plasmodium*. Patients who were naturally cured or drug-cured with *Babesia* infection were shown to be protected against *Plasmodium* infection. Such biological paradigm and exposé could be exploited in vaccine design against malaria better than the use of parasite based antigen formulations which have recorded dismal performance in field trials due to extensive clonal diversity of the parasite.

INTRODUCTION

In nature, poly-infection in a single host is a common phenomenon and often, parasites with varying degree of near or distance taxonomy are involved [1]. This may be attributed to their panoramic and ubiquitous abundance in ecosystems. In such scenario, competition for growth, space, nutrient and expression of ecological niche are well documented and can be gleaned from numerous literatures. For example, interacting parasites can directly compete for common host resources during invasion and establishment, and/or affect each other through cross-responsive host immune defenses, thus reducing parasite success [2]. Fundamental to this is the premise that infection by a second parasite species is sufficient to modify host responsesoften the immune responseto the first species, leading to changes in susceptibility and transmissibility, and so introducing nonlinearities into the dynamics and influencing the basic reproductive number of the parasite [3].

A number of examples of interactions between protozoa and viruses, protozoa and bacteria, protozoa and other protozoa, protozoa and helminths, helminths and viruses, helminths and bacteria, and helminths and other helminths are described in literatures. There are studies that report on antagonistic or synergistic effects of co-infecting agents in animal and human models [4]. However, in mixed infections, the burden of one or both of the infectious agents may be increased, one or both may be suppressed or one may be increased and the other suppressed [1]. What is of interest to the immuno-parasitologist is the position of the host with regards to outcomes of interactions; is the host benefiting or affected?

The suppression of growth of a virulent parasite by avirulent co-infecting parasites is a biological expression of ecological niche to the benefit of the host. One key example is the suppression of virulent *Mycobacterium tuberculosis* by a co-infecting avirulent *M. bovis* and this concept was explored as a basis for the production of BCG vaccine against human tuberculosis infection since 1921 by a French physician and bacteriologist, Albert Calmette [5]. Other numerous examples abound in literatures on host ecological benefits with co-infecting agents.

Rhesus Macaques (monkey) infected with *Babesia microti* (avirulent parasite) were shown to be refractive to *Plasmodium cynomolgi* (a virulent parasite in monkey) leading to low parasitaemia of *P. cynomolgi* [6].

*Patients that were drug-cured for *Babesia* infection were shown to be protected with subsequent challenge with *Plasmodium* [7].

*Mice which had recovered from infections with the avirulent piroplasm *Babesia microti* were also resistant to challenge with the virulent malaria parasite *Plasmodium vinckei* [8].

*In mice which had been pre-treated with killed *Corynebacterium parvum*, it was shown that they were completely resistant to infection with *Babesia microti* or *B. rodhaini* and were protected from death caused by *Plasmodium vinckei* or *P. chaubaudi* infection [9].

*Also, infection of mice with *Bacillus* Calmette-Guerin (BCG) provided good protection against *Babesia* species with intensity and duration of protection similar to that established after natural recovery from babesiosis [10].In medical community, exposé on the suppressive mechanism of *Plasmodium*, a type or prototype that was used in the suppression of Mycobacterium will surely evoke interest and concern most especially in this era where effort is geared towards malaria elimination in Sub-Saharan Africa.

The resultant effect of coinfection

Studies have shown that in concomitant parasitic or microbial infections, the science of forces involved in propelling the course of infection could have a resultant effect as follows:

1). Antagonistic Effect (Suppressed infections or growth and development of one or both parasites e.g. direct competition, antigenic cross reaction, immunopotentiation and possibly, harming the host less [11-12].In a study to investigated the influence of coinfection with nonlethal parasites, *P. berghei* XAT (*Pb* XAT) or *P. yoelii* 17X (*Py* 17X), on the outcome of *P. berghei* NK65 (*Pb* NK65) lethal infection, which caused high levels of parasitemia and severe pathogenesis in mice, it was found that the simultaneous infection with nonlethal *Pb* XAT or *Py* 17X suppressed high levels of parasitemia, liver injury, and body weight loss caused by *Pb* NK65 infection, induced high levels of reticulocytemia, and subsequently prolonged survival of mice [13].

2). Synergistic Effect (Enhanced infection of one or both parasites e.g. enhanced pathogenicity or virulence or immunosuppression and possibly harming the host more [11]. In host-parasite interactions, ability to mount an effective immune response to new infections depends largely on Th2 inducible response [14]. A parasite with dominant inducible immune character in concurrent infection is bound to incite cytokine expression that may affect the other while it may use such cytokine expression to its advantage and by extension creates a superimposition of ecological niche. It has been shown that helminth infection can make mice far more susceptible to certain pathogens against which Th1 response are protective (e.g., *Toxoplasma gondii*) and more resistant to pathogens against which Th2 responses are protective (*Trichinella muris*) [15]. In general, helminth coinfection has been reported to be severe with varying detrimental defects to the host [16-17]. Clinically, helminth infections are known for growth impairment, increased micronutrient uptake, eosinophilia and various organ pathologies [17]. In co-occurring synergistic infections, one can only expect to see a resultant pathological consequence and multiple diseases.

3). Inconclusive: No observable effects on either the parasites or the host [11].

Immunology of Babesia with Plasmodium co-infection

Both *Plasmodium* and *Babesia* species are intra-erythrocytic protozoans that infect a wide range of hosts, including humans, and they elicit similar inflammatory responses and clinical manifestations that differ markedly in severity [6]. Biological defects such as sequestration which results in organ damage, cerebral dysfunction and pulmonary oedema are known examples [6]. It has been hypothesized that the severe organ dysfunction and abnormalities that occur during acute *B. bovis* infection are quite similar to that seen in experimental malaria and are mediated in parts by inflammatory cytokines, including gamma interferon (IFN-γ), tumor necrosis factor (TNF) and nitric oxide (NO). Activation of macrophages forms the basis of protection in *Babesia* and in *Plasmodium* infection though in can also result in immunopathological consequences [18].

Shared features in the pathobiology of babesiosis and malaria have been studied and the two have been proven to rank *pari-passu*. Studies of cytokine activation and erythrocyte cytoadherence in babesiosis and malaria have been exploited and their similarities provide new insights into malaria pathobiology [19]. Mice immunized against *B. rodhaini* by means of a drug-controlled infection were subsequently resistant to infection with B. *microti, B. ratti* and *P. vinckei* infection, whereas protection against *P. berghei* did not occur. This may be explained by the occurrence of cross-reacting antibodies found in the plasma [20].

Suppression of growth Plasmodium by Babesia and cross protection of infected host:

Co-infection of rodents with *Babesia* and *Plasmodium* has been reported to induce cross-protection. In a wide range of hosts including humans, both elicit similar inflammatory responses and clinical manifestations that differ in severity. It has been reported that a rhesus macaque monkey that was chronically infected with *Babesia microti* was able to control infection with *P.cynomolgi* better than naïve monkeys [6]. There was a significant decrease in *P. cynomolgi* parasitemia in animals co-infected with *B. microti* compared to the parasitemia in animals infected with *P. cynomolgi* alone. This decrease in *P. cynomolgi* parasitemia correlated with increases in the levels of pro-inflammatory monocytes at the time of *P. cynomolgi* infection and with higher C-reactive protein (CRP) serum levels 1 week after malaria infection. Therefore, it was concluded that ongoing infection with *B. microti* parasites led to suppression of malaria infection. In veterinary applications, *Babesia* has been successfully used as attenuated vaccines and such procedures could be applied on the use of modified *Babesia* as an attractive agent for delivery of live antimalarial vaccines [21].

Possible Factors of suppression of Plasmodium by Babesia

1. The role of Pro-inflammatory cytokines: The biological processes taking place in *Babesia* and *Plasmodium* co-infection that result in growth suppression and or modulation may vary. Some evidence suggests that, excessive production of pro-inflammatory cytokines has a role to play. Markedly elevated serum concentrations of tumor necrosis factor (TNF), IFN-γ, interleukin-2 (IL-2), IL-6, E-selectin (expressed in the endothelium), vascular cell adhesion molecule 1 (VCAM-1), and

intracellular cell adhesion molecule 1 (ICAM-1) have been reported to occur during an acute phase of human *B. microti* infection and the concentrations returned to the baseline level one month after the resolution of infection [6]. Such elevated pro-inflammatory cytokines may be hostile to the development of another parasite in concurrent infection.

2. Effect of IFN-γ and TNF-α: Studies in mice with *B. microti*, *P. yoelii, P. vinckei* and *P. chaubaudi* demonstrated that IFN-γ, TNF-α and TNF-β are important components of immunity to these parasites. IFN-γ facilitates the phagocytosis of *P. falciparum* -infected erythrocytes by human macrophages. In addition, IFN-γ producing human $CD4^+$ Th-cell clones inhibited the growth of *P. falciparum* in the presence of adherent peripheral blood mononuclear cells (PBMC) *in vitro* [18]. This paradigm is thought to explain that pre-infection of host with *Babesia* will scale up IFN-γ level and subsequent infection with *Plasmodium* suppresses growth due to high level of TNF-α [22].

Studies has shown that early in malaria infection, it may play a protective role in that administration of recombinant TNF to infected mice inhibits the multiplication of both liver and blood stage parasites [22]. In particular, it has been demonstrated that erythrocytes infected with malaria parasites (whether live or dead) induce activated macrophage to release TNF, or some of their soluble products, and have been found to be heat stable [23]. By hypothetical extrapolation, it could be inferred that, pre-inoculation or immunization with live or attenuated *Babesia* with subsequent rise in TNF level could mar the expression of *P. falciparum*.

3. The role of increase in CD cells: *Babesia microti* produces a self-limiting infection in infected host. Mice which recovered from infection were resistant to re-infection with CD^{4+} T cells playing a prominent role in resolution of infection [24]. Marked exponential increases of CD monocytes were observed in the peripheral blood 3weeks after *B. microti* infection [6]. By biological extrapolation, it seems likely that the higher levels of CD cells monocyte populations and the effect of IFN-γ cytokine at the time of *Babesia* infection will scale up pro-inflammatory cytokines to the disadvantage of the in-coming *Plasmodium*. Such biological paradigm could be exploited in vaccine design against malaria.

D. The role of C-reactive protein (CRP): Acute phase or C-reactive proteins (APP or CRP) are produced in response to existing infections mainly by pro-inflammatory stimuli [25]. CRP is able to recognize damaged cells of the host to help with their elimination. It can activate the complement pathway as an opsonic protein or by binding C1q, and by binding Fcγ receptors it can also lead to complement-independent phagocytosis [26]. Infection of host by *Babesia* results in accumulation and activation of granulocytes and mononuclear cells, which in turn release acute phase cytokines [27]. Subsequent exposure of *Plasmodium* to an already harsh environment filled with inflammatory cytokines could mar its growth and development.

E. The role of Nitric oxide (NO) production: Macrophages are believed to be important for immunity to *Babesia* via removal of parasitized RBCs by phagocytosis and as APCs for T-helper (Th) lymphocytes [18]. Th1 cytokine responses are thought to dominate in *B. microti* infections especially during the acute phase. IFN-γ has been reported to play some role in the resolution of acute infection of mice infected with *B. microti* and to be involved in protection against other intracellular parasites [28]. Nitric oxide is proposed to be a mediator of a Multiple Organ Dysfunction Syndrome (MODS), which is seen in complicated infections [29].

Activation of macrophages by IFN-γ and other cytokine induces the production of NO, which serves as the effector molecule of parasite killing [30]. The possible explanation to this is that the activation of macrophages and NK cells produces NO which interferes with the development of the incoming *Plasmodium* [28].

CONCLUSION

Babesia and *Plasmodium* are intra-erythrocytic protozoan parasites that infect a wide range of hosts, including man and they elicit similar inflammatory responses during infection based on their shared antigenic characters. Co-infection between the two has been shown to induce cross-protection. This concept, if well extrapolated, can be used in vaccine production in the control of malaria infection.

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