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Parasitic Diseases' Control: Beyond the Adaptive Immunity

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***Abstract* — Global parasite burden may impact population, individual health and livestock production, with socioeconomic consequences. Preventive measures are the first approach for control but peculiarities of parasitic infections when involve sylvatic vectors or reservoirs for instance turn them close to impossible. In this context, an alternative is to induce protective responses in the target hosts. Parasites in general comparing with other microorganisms have long replicating time that apparently would facilitate the immune system to defeat them. However through millions of years these parasites have developed diverse strategies to evade and/or to modulate the host response for their survival. The difficult control of parasites derives in part from characteristics of their life cycle presenting different developmental phases, interactions with different vectors, intermediate and definitive hosts. Thus for effective parasite control, researches focusing different interactions that take place during life cycle are of paramount importance. The articles in this issue highlight some novel facets. Related to these aspects we add some reflections focusing the role of diverse hosts, of vectors and non-specific elements of host defense, many paving route to adaptive immune response, to open fronts that may be tackled aiming parasite control.**

***Keywords* — parasite, vector, mammalian hosts, growth factors, neutrophil.**

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I. Introduction

Global parasite burden may impact population, individual health, livestock production with enormous socioeconomic consequences. The control measures may rely on improvement in sanitary conditions, education, nutrition, preventive actions for sustainable environment and health promotion, medical assistance, etc. However, there are aspects of parasite life cycle that turn almost impossible to apply preventive measures such as control of vectors using insecticide spraying or elimination of intermediate hosts being many of them sylvatic. In this context, an alternative is to induce protective responses in the target hosts and the main focus so far has been to induce specific immune response [1, 2]. Parasites with medical importance comparing with other microorganisms such as bacteria and virus have in general relatively long replicating time. Having molecules that may be easily recognized as foreign by the host, this long replicating time would turn the parasites extremely vulnerable to attack by the host. However, they have co-evolved with human beings through millions of years and developed diverse strategies to evade and/or to modulate the host response for their survival [3]. One of characteristics that turns the control of parasites into a very difficult task is their life cycle that comprises different developmental forms, interactions with different vectors, intermediate and definitive hosts [4]. For effective parasite infection control, we need researches focusing different interactions that take place during life cycle to disclose proper approaches to different facets. Articles published in the present issue on parasitic immunology cover different aspects, show also the complexity of the area and highlight some novel aspects. Related to these aspects we add some reflections on the role of diverse hosts, vectors and non-specific elements of host defense, many paving route to adaptive immune response, to open fronts that may be tackled aiming parasite control.

II. Animal health in the context of parasitic diseases

One health approach focus all species thus integrates human and veterinary medicine and environmental science [5]. It is not by chance that over 70% of human diseases originate from animals, being the majority of emerging infectious diseases zoonoses [6]. Among them vector-borne diseases are mainly zoonotic diseases affecting humans. This issue demands more information on possible interventions to control the

transmission that is frequently directed to the animal hosts. To calculate the cost effectiveness of intervention strategies, consideration on animal health has to be concurrently taken in account [1]. We should further keep in mind that the impact of vector-borne diseases is not merely on human and animal health and welfare but on livestock industries as well [7] bringing great socioeconomic impact [8]. Additional concern is the way the control of the vector and the parasitic diseases is done presently, dependent largely on the use of chemicals that are sometimes extensive and improper bringing several risks for environment and human health.

Over the years, hosts and pathogens became co-adapted, enabling both living beings to survive and reproduce. This phenomenon is commonly referred as co-evolutionary arm race [9] and depend, among other factors, on background mutations plus natural selection of gene variants favoring the propagation of both species [10]. Perhaps the best example is of human population exposed to *Plasmodium spp*. that cause malaria. These populations have higher frequencies of certain polymorphism of haemoglobin and other red blood cell related genes than non-exposed populations [11]. Around the better known haemoglobin mutations, there is gene variants related to the host immune response that are associated with malaria resistance [12].

In the present issue, the review by Ferrolho et al [13] shows most recent progress in the cattle immune response against *Theileria annulata*, agent of theileriosis that ensues profound anemia. They demonstrate intriguing particularities among ruminant species and breeds, and how the immune response can be a major contributor to pathology as well as protection. Interestingly, infection with *Theileria annulata* in the susceptible *Bos taurus taurus* induces a chronic severe inflammatory response leading to high levels of fatality. In contrast in the *Bos taurus indicus* (Zebu cattle), i.e. the Sahiwal breed, and water buffaloes that lives in endemic areas, the survival rate is higher occuring control of infection with *T. annulata*. It is seemingly due to the control of over-stimulation of pathways involved in the pro-inflammatory cytokine-dependent acute phase response [10]. The members of the signal regulatory protein family, MHC Class II (BoLA-DQ), musculoaponeurotic fibrosarcoma, prion protein, toll-like receptor-10 and transforming growth factor (TGF)-β2, at least in part, are differently regulated among these ruminant species and breeds. Discovery of such gene variants may also be crucial to identify novel pathways and mechanisms that control protection and/or pathology of relevance for vaccine and therapeutic development [10, 14, 15]. Thus, advances in the knowledge and science in an era of the study of whole parasite genome open up opportunities to select genes controlling disease resistance to achieve an ideal proposal to control vector-borne diseases, resulting in environmentally and economically sustainable approach.

There is another interesting set of evidences showing the fine specificity of parasites. Some parasites from one host are genotypically and antigenically closely related to another mammal host and significant proportion of specific host-derived parasite population cannot be transmitted to other mammal host suggesting a relative parasite strain specificity what was observed with *Theileria parva* in ruminants (buffalo versus cattle). These findings suggest that during parasite evolution there may been immune-imposed selection for sequence diversity in the target antigens of the protective immune response [16]. Another parameter related to the animal species that should be considered is MHC diversity and recognition of antigenic epitopes by lymphocytes that may differ among animals, leading to strain-specific epitope recognition [10].

III. Vector - parasite interactions

Blood-feeding arthropods are vectors for a wide spectrum of diseases including malaria and leishmaniases with cutaneous or visceral lethal forms, responsible for more than 17 percent of global infectious diseases causing more than one million human deaths annually [17]. Interactions between vector arthropods, pathogens and their hosts are complex since vectors are not just a device to inject the parasite but possess immune system and produce saliva that exerts important effect due to its complex mixture of active molecules either inducing host immune response or helping pathogens to evade host defenses. Studies in this area on control of parasite infection explore the possibility of control of parasite growth or spread at the level of vector.

In the present issue, the study of Couto et al [18] brings in the area of malaria control an innovative approach to explore vector (*Anopheles spp*) – parasite (*Plasmodium spp*) interaction focusing vector's immune system. They show differences in expression of immune related genes between resistant and susceptible species of *Anopheles spp* upon pathogen control at vector level. It constitutes new approach on pathogen invasion, providing basis to seek for potential candidate genes for malaria control. It is very important since it targets directly the anophelines that are the definitive host for *Plasmodium spp* .

In another angle of study it has been searched antigens to induce host immune response using vectors' saliva of genus *Lutzomiya* sand fly, vector of *Leishmania*, of genus *Anopheles* mosquito, vector of malaria and genus *Ixodes* ticks. Saliva of *Leishmania* vector was seen influencing the neutrophilic influx and further affecting parasite virulence [19, 20] and some particular salivary proteins inducing different types of immune response either of TH2- or TH1-type or both concomitantly [21, 22]. These findings are important since TH2-type response is related to susceptibility and TH1-type to resistance to leishmaniases development in mouse model. In another type of approach recombinant proteins were generated using cDNA libraries from salivary glands to trigger the host immune response such as salivary proteins LJM19 and LJM11 from *Lutzomiya* that elicit a desirable strong protective delayed-type hypersensitivity reaction in mice and hamsters but unfortunately not in dogs that is important intermediate host that could be targeted aiming control of the propagation of *Leishmania infantum*, the causative agent of visceral leishmaniasis. [23].

In malaria vector the results from experiments using similar approaches are controversial whether repeated mosquito bites confer protection against *Plasmodium* infection [24]. However

there is another interesting approach with host antibodies being used to block the development of *Plasmodium* within its vector, *Anopheles*. Different studies disclosed some promising proteins present in stages of malaria parasite cycle that can be chosen for transmission-blocking immunity, e.g. surface protein present on male and female gametes (P230 and P48/45), proteins of oocyst (P25 and P28), and vector's protein (APN1). In this transmission-blocking immunity, antibodies produced by the vaccinee act outside the host, specifically inside the vectors. Foreseen the possibility to induce effective transmission-blocking immunity in humans either by natural infection or by immunization with recombinant vaccines, recent efforts resulted in three functional effective recombinant vaccines in phase 1 trial: Pfs230 Pfs25-EPA/Alhydrogel and Pfs230-EPA/Alhydrogel [25].

IV. Innate host mechanisms in parasitic infection.

Most studies on immunity are directed to specific immune mechanisms, however another stage that we consider important to search is the early phase that starts when the parasite is introduced into the host. The study of Santos-Mateus et al [26] on immune response in leishmaniases published in the present issue discuss this aspect. Leishmaniases suit to address the role of initial players in the interaction of parasite and host as some relevant studies have being developed on neutrophils as mentioned in this study but also complement, growth factor and lipoproteins.

In addition to information on neutrophils shown in that study, with these cells supposed to kill the parasites initially more complex role defining the fate of infection was shown recently. Neutrophils harboring *Leishmania* undergo apoptosis enabling recognition by macrophages to trigger the phagocytic process. Depending on the mouse genetic background, this phagocytosis may lead to destruction with production of tumour necrosis factor and neutrophil elastase or growth of *Leishmania* with production of TGF-beta and Prostaglandin E within macrophages, in the latter case contributing for the transfer of captured *Leishmania* to the macrophage [27]. Besides these differences dependent on host genetic background, diverse susceptibility of *Leishmania* was observed to the effect of neutrophil extracellular traps (NET), a fibrous structure produced by neutrophils that can trap and kill microbes [28]. Further, remarkably recent findings link the *L. mexicana*-induced neutrophil recruitment in the lesion to the retarded influx of dendritic cells in the lesion, delaying the development of adaptive immune response [29].

Another initial player the complement system that supposedly contributes for the destruction of *Leishmania* soon after injection of the parasite rather works for the establishment and development of the parasite infection. A specific review article was published in the International Trends in Immunity [30] analyzing this aspect.

Another still poorly recognized factor that we highlight here is insulin-like growth factor-I (IGF-I) studied in leishmaniases. IGF-I is a polypeptide of 7.5 kD, effector element of growth hormone with pleiotropic effect on different cells and tissues [31]. Recombinant IGF-I but not IGF-II induces *in vitro* proliferation of different species of *Leishmania* and pre-incubation of *Leishmania (L.) amazonensis* with IGF-I induces bigger lesion in infected BALB/c mice containing greater number of intracellular parasites [32]. In *L. (L.) amazonensis*-infected peritoneal macrophage *in vitro* culture, IGF-I affect L-arginine metabolism, promoting decrease of nitric oxide (NO) production and of expression of inducible NO synthase but increase of arginase expression and activity in both the parasite and macrophage with/without the modulation of cytokine production [33]. Macrophages express IGF-I intrinsically and it affects intracellular *Leishmania major* growth [34]. Preliminary data in experiments using small interfering IGF-I RNA (siRNA) in *L. (L.) major*-infected RAW 264.7 macrophage cell line show that when IGF-I mRNA expression is decreased, the effect of IL-4 is nullified (personal communication) showing the link of expression of non-specific growth factor IGF-I to the TH2 cytokine effect.

Again in the article by Santos-Matheus et al [26] in the present issue, a spectrum of *Leishmania infantum* infection is presented relating to delayed-type leishmanin test and serology results trying to disclose the final outcome [35]. In this matter we would like to show another complete different finding, a strong correlation for the development of symptomatic visceral leishmaniasis with trygliceride levels and lipoprotein lipase polymorphism that reached odds ratio of 21.3 comparing symptomatic visceral leishmaniasis with non infected control from the same endemic area [36]. This study indicates that contribution of non-specific factor, lipoprotein, may have extraordinary contribution for parasite development and suggests requirement for further studies in this field.

V.Conclusions

For the parasitic diseases' control we should think beyond adaptive immunity. Here we presented some initial interactions and non-specific elements and, importantly, influencing the development of ongoing adaptive response. Different interfaces of parasites with vectors and hosts with their diversity have to be carefully searched to find vulnerable points to tackle aiming parasite infection control.

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