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PONENCIA

THE ROLE OF IMMUNOLOGY IN COMBATING *TRYPANOSOMA CRUZI* INFECTION AND CHAGAS DISEASE

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ABSTRACT

Chagas disease is a solvable problem and if it is to be solved, applying what we already know about immunity to *T. cruzi* will be crucial. But currently too many people get infected, few of those who are infected get diagnosed, especially before irrevocable damage is done, and an insufficient number of those diagnosed get effective treatment. To alter this state of affairs, setting appropriate priorities and demanding more of the research that is ongoing and of the funders of that research is an absolute necessity. We are fortunate to be in a period of significant interest in Chagas disease by the pharmaceutical industry. This interest is unlikely to last for long, so it is especially critical now to translate what we know and can learn into more effective interventions.

Keywords: Chagas disease. Immunity. Biological markers. Vaccine.

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RESUMEN

El papel de la inmunología en combatir la infección por *Tripanosoma cruzi* y la Enfermedad de Chagas

La enfermedad de Chagas es un problema resoluble y la aplicación de los conocimientos actuales de la inmunidad contra *T.cruzi* será crucial. Pero en la actualidad muchas personas se infectan, sólo algunas se diagnostican antes que el daño cardíaco se haga irreversible y pocas de las diagnosticadas reciben un tratamiento efectivo. Para cambiar este estado de cosas es necesario el establecimiento de prioridades adecuadas y más exigentes en la investigación, así como convencer a los financiadores de que esta es una necesidad absoluta. Tenemos la suerte de que actualmente la industria farmacéutica tiene interés en la enfermedad de Chagas, aunque es poco probable que dure mucho tiempo, por lo que es especialmente importante aplicar ya lo que sabemos hacia intervenciones más eficaces.

Palabras clave: Enfermedad de Chagas. Inmunidad.Biomarcadores- Vacuna.

IMMUNE CONTROL OF *T. CRUZI* INFECTION

The immune effector mechanisms that are crucial to controlling, containing or, under the best of circumstances, eliminating an infectious agent are largely predictable from the lifestyle of the pathogen. This is no less true for *Trypanosoma cruzi* which spends the vast majority of its time in mammals replicating in the cytoplasm of a range of host cell types. The hundreds of parasites produced over the approximately 4 day intracellular phase eventually overwhelm and destroy the host cell. The released trypomastigotes may infect other host cells, or, if parasite release is coincident with the taking of a blood meal by triatomine insects, be transmitted to these vectors and thus capable of spreading to other mammalian hosts.

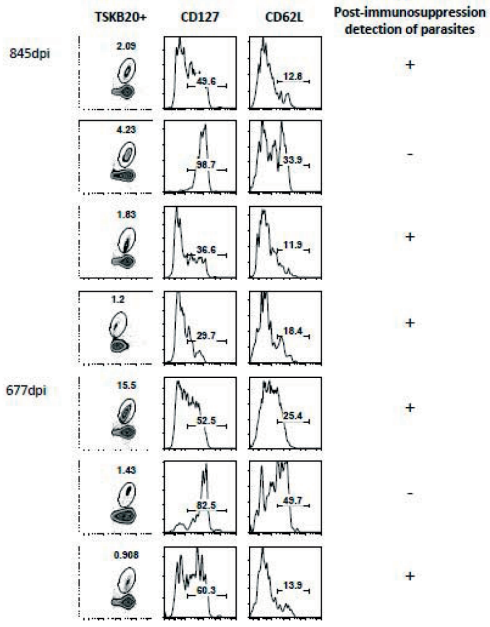
As expected from this lifestyle, immune control of *T. cruzi* infection in mammals is most heavily dependent on cytolytic T cells capable of recognizing and destroying parasite-infected host cells and antibodies that recognize extracellular parasite stages[1-3]. Th1 cells that provide the appropriate helper function for B and CD8+ T cells as well as cytokines that enhance macrophage killer functions are also crucial to control of the infection. While a number of other effector mechanisms have been noted to influence *T. cruzi* infection, including NK cells, gamma/delta T cells, Th2, Th17, etc., the relative impact of these appears to be rather modest.

An often ignored aspect of the *T. cruzi*: host interface is that immune control of the infection is in general incredibly efficient. With the exception of very high dose infections or infection in immunocompromised hosts, *T. cruzi* infection is nearly always controlled acutely and even occasionally eliminated. Parasite burden in chronically infected hosts is maintained at low levels, often below the level of detection by even the most sensitive methods. Some of the most convincing evidence supporting this fact is the proficiency with which parasites within a chronically infected host are restricted from spreading to "new" tissues (e.g. implanted muscle tissues[4]), and the inefficiency by which the infection is transmitted to vectors. In one remarkable study utilizing serial xenodiagnostic testing of infected subjects over several years, the majority of subjects had detectable parasites in less than 25% of tests and one patient had as few as 2 positive bugs among the 1440 bugs that fed[5].

There is anecdotal evidence of spontaneous infection cures in humans and unambiguous proof of cure in mice (figure 1). The only way that these extremely low parasite loads can be maintained for decades and that spontaneous cures can occur is if immune control is in fact highly effective. If this is so, then why is the infection not always cured? Why is cure a rare event? An obvious answer is that like all infections, there is a range of efficiencies of control of *T. cruzi* in host populations. HLA and other polymorphisms provide a genetic basis for this variability in control and past and current exposures and conditions (nutritional, hormonal, etc) further assure that there will be a spectrum of responses to any infection within a population. So it is reasonable to believe that individuals on the high efficiency end of the spectrum could cure *T. cruzi* infection while those on the other extreme will be relatively high parasitemic "transmitters" and/or the most likely to develop severe disease. And in between these two poles is the majority of individuals who remain persistently infected but with mild or unapparent disease.

An alternative view of *T. cruzi* infection has been presented asserting that there are potent immunoregulatory mechanisms that prevent immune clearance of *T. cruzi* infection. However there is comparatively little concrete support for this view. As already

Figure 1
T cell phenotype and failure of immunosuppression to reveal parasitic infection documents spontaneous parasitological cure in selected chronically infected mice



Peripheral blood collected from 2 sets of mice at 845 and 677 days post infection with the CL strain of *T. cruzi* have *T. cruzi*-specific CD8+ T cells, as detected by staining with the MHC-peptide tetramer H2Kd loaded with the trans-sialidase peptide TSKb20 (first column)[26]. In 2 of these mice (rows 2 and 6), a high proportion of the TSKb20+ cells express CD127 and CD62L, markers of central memory T cells and indicative of parasitologic cure[8]. Cure is confirmed by the failure to detect parasites in the blood or tissues in these 2 mice using direct microscopic examination of blood, hemaculture and PCR following immunosuppression with cyclophosphamide[8].

noted, the infection is well controlled by strong anti-*T. cruzi* immune responses that are readily measured in nearly all hosts. And if these responses are abrogated, as in the case of HIV infection[6,7] or chemical immunosuppression[8], overwhelming parasite burden results. Furthermore, immunoregulatory mechanisms known to play important roles in other infections, including IL-10, TGF-beta, Treg cells, PD-1, etc., show modest, at best, expression during or impact on *T. cruzi* infection immunity[9, 10].

While the persistence of *T. cruzi* infection does not appear to be dependent on overt immunosuppression, infection persistence itself may result in the gradual deterioration of immune responses over time as lymphocytes become exhausted and the resources of the immune system are depleted. Although more apparent in infections characterized by high antigen burden[11,12], there is also good evidence of immune deterioration in patients with decades-long *T. cruzi* infections[13-15]. Such induced inefficiency is associated with increased incidence of clinical disease[16], however the cause and effect relationship between these two is not established (i.e. does the decline in immune control of the infection contribute

to clinical disease or is exhaustion and disease both a consequence of persistent and perhaps relatively high parasite load?).

T. cruzi appears to be very adept at persistence and it is clearly the chronic nature of the infection that drives disease development. However this ability to persist is not perfect; *T. cruzi* seems to frequently teeter on edge of elimination in hosts, occasionally falling off that edge. Thus neither chronic infection nor disease is an inevitable outcome of infection.

APPLYING IMMUNOLOGICAL KNOWLEDGE

Given that immune responses to *T. cruzi* are normally potent and effective, the protective immune mechanisms are known (as are many of the parasite targets of these responses), and at least on occasion, these responses result in cure, it is reasonable to ask how one could use this knowledge in the treatment or prevention of Chagas disease. Prophylactic vaccination is the holy grail of any infectious disease but has been achieved only selectively and very inconsistently with parasitic infections. Vaccine research got a slow start in Chagas disease, in large part due to the fear that vaccination might intensify the anti-self immune responses that were thought to be responsible for pathology in the infection[17]. However with increasing appreciation for the fact that Chagas disease is the result of parasite persistence rather than anti-self (autoimmune) responses, significant interest in vaccine discovery for *T. cruzi* infection has emerged. These efforts are continuing and are significantly enhanced by the ability to use species that are natural hosts of *T. cruzi* (principally rodents) in vaccine trials. As a result, there are a significant number of protocols and vaccine target antigens that provide a degree of protection from death after a normally lethal challenge infection, again demonstrating that *T. cruzi* is susceptible to immune control. However none of these vaccines have provided sterile protection - e.g. complete clearance of the infection.

Sterile protection is a high bar for any vaccine, but it is an appropriate one for *T. cruzi* infection. As noted above, *T. cruzi* is normally very well controlled with low parasite burden in most cases. However despite this apparently successful outcome, clinical disease frequently develops. So an effective vaccine is going to have to do better than just limit the parasite burden as already happens in unvaccinated hosts; it is likely going to have to clear the infection completely if disease is to be avoided. Life-threatening acute infection is very rare in *T. cruzi* and thus should not be the target of prevention for vaccine development for *T. cruzi*. Rather, preventing persistent infection by achieving sterile cure has to be the goal.

So can a vaccine be developed that will sterilely protect humans from *T. cruzi* infection? A vaccine of such efficiency has not been achieved even in highly controlled animal studies employing a variety of formats that induce potent immune responses. Additionally, multiple rounds of infection followed by drug-induced cure, provides vigorous boosting of protective immune responses but fails to protect mice from reinfection (Bustamante and Tarleton, unpublished). This ability of *T. cruzi* to establish infections in hosts even in the face of apparently "protective" and certainly very potent anti-parasite immune responses, presents an enormous challenge for vaccine development. It may be that parasites like *T. cruzi*, that invade host cells relatively silently[18,19] and establish infection without alerting host danger receptors[20] will elude even the best efforts of vaccinologists.

It is possible that a prophylactic vaccine (by reducing parasite load lower through more efficient immune responses) could prevent the development of Chagas disease despite not completely clearing the infec-

tion. However, there is no evidence that further reducing parasite levels below their already low points would reduce pathology. Furthermore, testing such a vaccine would require decades of monitoring of infected subjects, presenting both a logistical and ethical nightmare.

Therapeutic vaccines have also been explored as possible treatments for Chagas disease, with the goal of pushing this controlled infection over the edge toward eradication[21]. Therapeutic vaccines in general have a tough duty to perform and they have not found great success with any infection. Essentially, they must substantially enhance a response that has been developed over time – decades in the case of many subjects of Chagas disease – and that has effectively controlled the infection but not eliminated it. While this may be possible, it is not likely to be easy in the case of Chagas disease. And it does not seem likely that a therapeutic vaccine that may or may not do the job will gain wide acceptance when there are available drugs that can eliminate the infection.

ALTERNATIVE APPLICATIONS OF IMMUNOLOGY FOR CHAGAS DISEASE

If the prospects for conventional vaccines are really as dismal as presented here, are there alternative ways that immunological knowledge of *T. cruzi* infection can be applied to more effectively deal with Chagas disease? An obvious application is through the development of better diagnostics. The current practice of using 2 or 3 "conventional" diagnostic tests that predominantly depend on crude antigen preparations is inefficient and insensitive[22]. Antigen discovery efforts have unveiled better diagnostic targets and revealed that antibody responses in *T. cruzi*-infected subjects are highly variable not only in terms of the target antigens but also in potency[23-25]. The inability of PCR, hemaculture or xenodiagnosis to consistently detect *T. cruzi* in all infected subjects makes it very likely that serology will remain the primary way of screening for *T. cruzi* infection. So modernization of *T. cruzi* diagnostics, and in particular developing a multiplex platform that allows for detection of responses to many individual antigens, is long overdue.

While it may end up being impossible to develop a prophylactic vaccine that can prevent *T. cruzi* infection in humans, we already have vaccines that can reduce parasite load in animals. Applying such vaccines in the field, particularly in companion animals in endemic areas – the chief source of infection for reduviid bugs in many infested houses - could reduce transmission of infections to humans. Such vaccines would not need to prevent infection in animals, but simply reduce parasite levels to a point that transmission is much less efficient. An easy to distribute vaccine of this type could be used in concert with other transmission-reducing techniques to prevent new infections.

Lastly, it is also often ignored that there are already effective drug treatments for Chagas disease. Unfortunately these drugs, nifurtimox and benznidazole, are not as widely used as they should be because they have side effects in a significant (although a minority) of subjects, and are perceived to be ineffective in treating established (chronic) *T. cruzi* infection. Contrary to this perception, both drugs have well-documented benefit in chronically infected subjects. The problem is that it is difficult to know when they work and when they fail because of the lack of a test of cure in these patients. Just as the low parasite burden makes direct detection of *T. cruzi* an undependable diagnostic of infection in chronically infected subjects, it is likewise unsuitable also as a test of cure. The absence of a dependable test of cure is not only an impediment to the wider use of these acknowledged suboptimal drugs that are now available but also makes evaluation of new drugs nearly impossible. This is another place where immunological assays may

provide an answer. Drug cure in rodents results in unambiguous alterations in T cell phenotypes[8] and treatment (and presumed cure) in humans is also accompanied by alterations in both T cell and B cell responses[24]. Using this knowledge as the basis for developing a test of cure should be the highest priority – without such we will have to continue to settle for inadequate and underutilized therapies.

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