Current status of vaccination against African trypanosomiasis

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SUMMARY

Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigentic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, recent research into the biology of B cells has indicated that the problems might go further than that. This paper reviews past and current attempts to design both anti-trypanosome vaccines, as well as vaccines directed towards the inhibition of infection-associated pathology.

Key words: Trypanosomiasis, vaccination, B cells.

VSG: WHERE IT ALL BEGAN

Antigenic variation of the African trypanosome surface coat has long been considered to be the main defence mechanism against the host immune system (Vickerman, 1978). Although the trypanosome genome already comprises a vast array of variant surface glycoprotein (VSG)-encoding sequences and *vsg* pseudogenes (Berriman *et al*. 2005; Marcello and Barry, 2007; McCulloch and Horn, 2009), recombination-based genome plasticity ensures that African trypanosomes are capable of generating a virtually inexhaustible collection of VSGs (McCulloch and Horn, 2009). This allows these parasites to continuously evade the host antibody response, perpetuating the infection until the host succumbs to either secondary infections or infection-associated complications such as encephalitis (Kennedy, 2009) or inflammatory anaemia (Naessens, 2006). Since the discovery of VSG and the elucidation of the major VSG switching mechanisms, it has been generally accepted that vaccination against the main surface glycoprotein of trypanosomes will never result in the build-up of sterile immunity and protection against trypanosomiasis. Indeed, early studies that were based on the VSG-specific *in vitro* killing of trypanosomes prior to experimental infections with well characterized parasite stocks, showed that many different antigenic parasite types could be generated starting from the same trypanosome source (Van Meirvenne *et al*. 1975*a*, *b*). However, it is important to realize that the vast majority of studies dealing with antigenic variation of trypanosomes are based on VSG switching in bloodstream form parasites. In contrast, when a mammalian host becomes infected with trypanosomes through a tsetse fly bite, the infection is initiated by metacyclic trypanosomes that appear to have a VAT repertoire that is limited to no more than a dozen different VSGs (Le Ray *et al*. 1978; Barry *et al*. 1979; Esser *et al*. 1982; Crowe *et al*. 1983). These discoveries initially lead several authors to propose that vaccination against metacyclic trypanosomes could be feasible. In particular, the use of attenuated irradiated trypanosomes for vaccination resulted in preliminary positive results (Esser *et al*. 1982). Also a combination approach in which mice were infected through tsetse fly bites and subsequently treated with Berenil® resulted in short-term protection against a homologous challenge (Nantulya *et al*. 1980*b*). However, as the mechanisms of VSG switching were being elucidated, it became apparent that while the M-VSG expression sites (M-ES) had particular unique features (Barry *et al*. 1998), the VSGs expressed in these expression sites were not any different from the VSGs present in the B-ES (bloodstream form expression site). Hence, it was suggested in 1985 by Cornelissen and colleagues that due an overlap between the M-*vsg* and B-*vsg* repertoires, and the fact that antigenic variation in both expression sites relies on telomeric exchange, vaccination prospects were 'not good' (Cornelissen *et al*. 1985). It is interesting to note that the initial vaccination experiments that were reported to be promising showed that protective antibodies were of the IgM class (Crowe *et al*. 1984) and that stock-specific immunity against M-VATs was short lived (Nantulya *et al*. 1980*a*). In this context, several other

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reports have indicated the superior anti-trypanosome activity of IgM as compared to IgG antibodies (MacAskill *et al*. 1983; Mitchell and Pearson, 1983). This IgM activity has been proposed to be linked to the capacity of anti-VSG IgM to capture C3 complement fragments which, *in vivo*, would contribute to liver-mediated parasite clearance (Shi *et al*. 2005; Pan *et al*. 2006). It is puzzling that the latter reports show that trypanotolerant mice are characterized by increased IgM anti-trypanosome titres as compared to susceptible animals; even the tolerant animals are not capable of preventing parasites from reaching infection levels of 10^7 parasites/ml of blood in the presence of anti-VSG antibodies. From an alternative point of view than the one of Shi and Pan, one could argue that bloodstream form trypanosomes are perfectly capable of avoiding efficient immune elimination, and that the correlation found between slightly improved parasitaemia control and increased IgM titres is not linked to the actual functional immune control in naturally trypanotolerant hosts that can maintain parasites levels well below 10^3 parasites/ml of blood. In this context, O'Beirne and colleagues suggested that under optimal growth conditions, parasites indeed possess the capacity to circumvent anti-VSG antibody-mediated clearance (O'Beirne *et al*. 1998). This could involve the recently described capacity of trypanosomes to remove VSG-binding antibodies rapidly from their surface (Engstler *et al*. 2007).

Hence, despite the capacity of antibodies to cause VSG-specific trypanosome damage under certain conditions, it has been clear for more than two decades that anti-trypanosome vaccination targeting the metacyclic surface coat is being hampered by the intrinsic features of trypanosome antigenic variation and VSG recycling. This is at least the case for *T. congolense* and *T. brucei* infections and also in the cases of related *T. evansi* and *T. equiperdum* infections. Interestingly, at the same time that *T. brucei* and *T. congolense* M-VSGs were discovered, others reported that *T. vivax* metacyclic parasites were devoid of a VSG coat (Tetley *et al*. 1981). Again, this finding resulted in an optimistic view with regards to a soon-to-be-discovered protective vaccine for trypanosomiasis. However, given that more than 25 years after this discovery not a single effective anti-*T. vivax* vaccine has been generated, one can only assume that also this parasite uses an efficient mechanism of antigenic variation of the metacyclic surface to escape the host immune response during the initial stage of infection.

NON-VARIABLE ANTIGEN-BASED VACCINE: CAN IT BE DONE?

Being obligate parasites, African trypanosomes rely on host factors for survival and multiplication. In order to bind and take up exogenous macromolecules, the parasites express a number of non- or less-variable surface antigens such as the transferrin receptor, the product of expression-site associated gene ESAG6/7 (Steverding *et al*. 1994). The organelle specialized in endocytosis and exocytosis and thus containing conserved receptors is the flagellar pocket. In addition, embedded under the VSG coat are multiple copy-number invariant surface glycoproteins, including ISG64 (Jackson *et al*. 1993), ISG65 (Ziegelbauer and Overath, 1992*a*), ISG75 (Ziegelbauer *et al*. 1992*b*), ISG100 (Nolan *et al*. 1997). These externally exposed proteins are development stage-specific, yet their biological functions remain to be elucidated. In principle, these molecules could all be targets for alternative vaccination protocols. Also cytoskeleton proteins constitute an interesting group of non-variable antigens. Indeed, microtubules, composed of tubulin heterodimers and microtubule-associated proteins (MAPs), are distributed beneath the surface membrane, the flagellum, paraflagellar rod and mitotic spindle apparatus of dividing nuclei (Hemphill *et al*. 1991). They play important roles in trypanosome motility, flexibility and mechanical stability (Robinson and Gull, 1991). Actin, another crucial component of the cytoskeleton, is involved in trypanosomal endocytosis and formation of coated vesicles from the flagellar pocket membrane (Kohl and Gull, 1998). In recent years, many of the above candidates have been used for experimental vaccination schemes for trypanosomiasis. However, while most reports usually conclude that results are promising, not a single strategy has brought about the development of a useful effective vaccine. Here we will review the major outcomes of these vaccine trails and the pitfalls that might explain the failure of all strategies.

With respect to the use of non-variable intracellular vaccine targets, both beta-tubulin and actin have produced hopeful results (Lubega *et al*. 2002*a*, *b*; Li *et al*. 2007, 2009), as have different MAP vaccination trials. Indeed, MAP p15 expressed in an adenovirus vaccine delivery system conferred complete protection in mice upon challenge with 500 *T. b. brucei* parasites. Unfortunately, the same level of protection was observed in the negative control mice being challenged with adenovirus particle construct devoid of p15 (Rasooly and Balaban, 2004). Hence, the observed protection was the consequence of non-specific immune modulation and did not involve immune memory. On the other hand, murine immunization with a preparation of MAP p52 together with co-purified glycosomal enzymes (aldolase and GAPHD) from *T. b. brucei* was reported to be protective (Balaban *et al*. 1995). Also trials using renatured recombinant beta-tubulin and recombinant actin expressed in *Escherichia coli* (Li *et al*. 2007, 2009) showed both a partial but similar degrees of protection against *T. evansi*, *T. equiperdum* and *T. b. brucei* infections in mice. This confirmed

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the observations by Lubega and colleagues, who demonstrated that immunization of mice with beta-tubulin was protective against *T. b. rhodesiense* and *T. congolense* (Lubega *et al.* 2002*a*).

Despite the apparently promising conclusions of these studies, some considerations could explain the lack of a positive follow-up on these results. Firstly, vaccination with recombinant *E. coli* material carries the risks of being affected by contamination with bacterial compounds, such as LPS that may contribute to non-specific immunity. Hence, the use of PBS control 'vaccination' is irrelevant here; and all results should be compared to the effects obtained with an irrelevant recombinant protein produced in the same manner as that for the target molecule. Secondly, the timing between immunization and challenge could bias the outcomes towards positive protection. A period of six to nine days between the last vaccination boost and the actual infection, as described in the studies of Li and Balaban, might only lead to the detection of non-specific protection. Most likely, a positive outcome of these protocols is due to the immediate effect of immune modulation by the vaccine boost, and not by the presence of any immunological memory. It would be interesting to know what the level of protection would have been if the tubulin- or actin-vaccinated mice were challenged with heterologous parasites three or six months after the last vaccine boost, and actual B cell memory would have been assessed. Thirdly, the infection dose is an important parameter for evaluation of the effectiveness of vaccine candidates. It is well documented that a single bite of an infected tsetse fly can contain up to 10^4 metacyclic parasites. Hence, vaccine/challenge studies in which only 10^3 or fewer bloodstream form parasites are used for infection might be biased towards an unrealistic positive outcome. This argument is supported by vaccine trials with trypanosome flagellar pocket extracts. The flagellar pocket is the principle site of interactions between the parasite and its environment in the host. Hence, purified FP extracts likely contain an array of surface-exposed membrane proteins that are conserved among different trypanosome species (Gull, 2003) and as such present an ideal vaccine target. Unfortunately, so far FP-vaccination has only resulted in partial protection in cattle (Mkunza *et al.* 1995) or mice (Olenick *et al.* 1988; Radwanska *et al.* 2000*a*). In the latter case it was determined that the vaccine efficacy was broken once mice were challenged with 10^3 or more parasites. In addition, Radwanska reported that 'partial' protection in anti-trypanosome vaccination is being used to describe two different effects, *i.e.* (1) the percentage of mice showing sterile immunity towards infection, and (2) the reduction in parasite burden. In case of the latter, it can be questioned whether results are positive or not, as all vaccinated animals acquired the infection and finally suffered from infection-associated pathology and succumbed to the disease.

A major pitfall hampering FP immunization could be that the FP contains vast amounts of the highly immunogenic VSG. Therefore, failure of FP vaccination does not rule out the possibility of obtaining an effective vaccine strategy when VSG could be excluded. In view of this, recent efforts have been focused on vaccination with recombinant trypanosome invariant surface proteins such as ISG75, or highly purified trypanosome proteins such as tomato lectin-binding antigens (TL antigens) (Nolan *et al.* 1999). Interestingly, the induction of antibodies against ISG75 was a prominent result during FP vaccination, indicating that this protein is immunogenic by itself, despite its heavy glycosylation (Radwanska *et al.* 2000*a*). ISG75 is evenly distributed on the surface of the bloodstream-stage parasites and is conserved among all taxa of the *Trypanozoon* subgenus (Tran *et al.* 2006; Ziegelbauer *et al.* 1992*b*). While the structure of the protein remains to be elucidated, it has been proposed that the protein is buried in between the VSG surface molecules and is inaccessible to infection-induced antibodies (Jackson *et al.* 1993; Ziegelbauer and Overath, 1993). An extra-cellular domain of ISG75, heterologously expressed in *E. coli* and purified to high degree of purity (Tran *et al.* 2008), was recently used to immunize mice with the aim of generating antibodies that would possibly differ from the infection-induced antibodies mentioned above and could possibly confer a certain level of protection against trypanosomiasis. This protocol resulted in high titres of ISG75-specific antibodies. At day 21 after the last boost the mice were challenged with 5×10^3 cells of *T. b. brucei*. Upon challenge no protection against infection was observed. It is interesting to note that during infection, vaccine-induced anti-ISG75 antibody titres decreased rapidly to a level similar to that found in non-vaccinated infected mice. Therefore, these results indicate that contact of the vaccine-primed immune system with living parasites failed to trigger an effective B cell memory response, despite the continuous challenge of the immune system with ISG75. This suggests that the active infection of *T. b. brucei* either suppressed or abolished the specific antibody response and possibly destroyed the vaccine memory response. These results fit with data that were previously obtained while recording anti-ISG antibody titres during an active *T. b. brucei* infection (Radwanska *et al.* 2000*b*). While it was shown that the infection onset resulted in the rapid accumulation of mainly IgG2a ISG-specific antibodies, these titres dropped significantly after two weeks of infection despite the continuous challenge of the immune system with the antigen. Hence, we propose that despite the presence of non-variable and immunogenic proteins on the surface of the parasite, immunization against these

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proteins might never result in a significant B cell memory-based protection in experimental mouse models. Indeed, it appears that in order for an anti-trypanosome vaccine to be effective it should have the ability to eliminate all circulating parasites prior to B cell memory suppression or destruction. A successful vaccine strategy should therefore result in the permanent presence of high effective/protective anti-trypanosome titres that can prevent the onset of infection. These antibody titres must be maintained in the absence of continuously circulating trypanosome antigens. This apparent contradiction seems to suggest that the race between (1) the parasite that aims to modulate the B cell memory response and (2) the B cell immune response that aims to eliminate the parasite has been decided in favour of the parasite. Hence, based on experimental mouse infections, it appears that anti-trypanosome vaccination might never be feasible in model systems that are characterized by an excessively high parasite burden early on in infection. This conclusion questions the usefulness of the mouse model as a tool in vaccine development against African trypanosomiasis. In addition, as discussed below, this conclusion suggests that mechanisms of B cell memory destruction in trypanosusceptible hosts (including humans) could have major implications for other vaccination programmes in trypanosome-exposed regions.

TRANSMISSION BLOCKING VACCINES: WHERE DO WE FLY FROM HERE?

Conventional vaccine strategies, such as those discussed above, concentrate on targeting the infectious agent, the trypanosome. However, in contrast to most bacterial and viral agents, trypanosomes pass their life cycle through a second host, the tsetse fly (*Glossina* sp.) that serves as insect vector. Hence, problems encountered with direct anti-trypanosome vaccination could be circumvented by alternative approaches such as transmission blocking vaccines (TBVs) where the obligate blood feeding biology of tsetse flies could offer unique possibilities. The rationale of a TBV approach is to reduce transmission through immunization against insect parasite stages or exposed or concealed arthropod antigens in order to (1) interfere with the parasite life cycle in the vector by targeting specific interactions that are required for parasite development, (2) reduce the vector fitness or (3) block the parasitaemia onset in the host. In several vector-parasite-host models, TBV has been demonstrated to target at least one of each of these three mechanisms (as will be discussed below). An important factor in all these strategies is that the targeted antigens have not been under evolutionary pressure of the mammalian immune system and are therefore expected to display much less antigenic variability.

TBVs that interfere with the parasite life cycle rely on targeting antibodies to the parasite surface or the vector midgut to inhibit parasite colonization in the arthropod. This type of approach does not protect the vaccinated individual against infection but would result in reduced numbers of infectious vectors and reduced parasite transmission. With respect to trypanosomiasis, the presence of anti-procylic *T. brucei* or *T. congolense* antibodies in the tsetse blood meals suppresses the development of each parasite respectively in *Glossina morsitans* (Maudlin *et al*. 1984; Nantulya *et al*. 1980*b*). Similar results were obtained for *T. brucei*, *T. congolense* and *T. vivax*, when tsetse flies were fed on goats that were immunized against *in vitro*-propagated parasites (Murray *et al*. 1985). Unfortunately, it seems that the lack of information on the antigens responsible as well as the publication of contradictory results (Honigberg *et al*. 1991) halted the progress in this field. An interesting feature of trypanosomes in the tsetse fly is the presence of GPI-anchored glycoproteins other than VSG, which is replaced in procyclic trypanosomes by GPEET or EP procyclins (reviewed in Roditi and Lehane, 2008) and in epimastigotes by alanine-rich protein isoforms (BARP) (Urwyler *et al*. 2007) that could represent putative targets for TBVs. Alternatively, tsetse midgut antigens could be selected as vaccine candidates using expression analysis of tsetse lines with differential susceptibility to trypanosome infection (Haddow *et al*. 2005). Support for this approach comes from immunization of rabbits against *Glossina pallidipes* midgut extracts, resulting in reduced *T. b. rhodesiense* infection rates in tsetse flies that were fed on the immunized animals (Kinyua *et al*. 2005). It is important to mention that the feasibility of TBVs is supported by a number of results obtained in other infection models. For example, vaccination against the sexual stages of *Plasmodium falciparum* and *P. vivax* was able to abrogate parasite development in the mosquito and subsequent transmission to a new host (Outchkourov *et al*. 2008). Similarly, the Leishmune vaccine (FML, fucose-mannose ligand) against visceral leishmaniasis exhibits a TBV activity by interfering with the adherence of procyclic promastigotes to the *Lutzomyia* sand fly midgut (Saraiva *et al*. 2006).

As mentioned above, besides targeting the parasite/vector interaction, TBVs could also be designed to affect the fitness of the disease vector that feeds on the immunized host. By reducing the fecundity and survival of the arthropod, this type of vaccine would affect the size of the vector population and thereby reduce parasite transmission. In the case of tsetse flies, it is important to mention that upon feeding, the blood meal is stored in a protease-free crop, where a fast dehydration occurs. Strikingly, anti-albumin antibodies that are absorbed into the haemolymph (Nogge and Giannetti, 1979) can have devastating effects on osmoregulation and survival of

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the tsetse fly if they are provided in a single albumin-free meal (Nogge and Giannetti, 1980). This results in the sequestration of albumin, perturbed sodium and potassium concentrations in the haemolymph and problems with the primary excretion and crop emptying. However, it remains unclear whether antibodies can be raised in mammals to exert a similar effect *in vivo*. Interestingly, in an alternative fitness-reducing approach, immunization of rabbits against *Glossina pallidipes* midgut extracts also resulted in a reduced survival and fecundity (Kinyua *et al*. 2005), suggesting that concealed tsetse antigens could represent TBV targets, although protective antigens still remain to be identified. The feasibility of a fitness-reducing TBV approach is supported by reports in other models, in particular in tick transmitted diseases. Here, a major achievement is the development and marketing of a vaccine against the Bm86 midgut antigen of *Rhipicephalus* (*Boophilus*) *microplus* that induces mortality in the post-blood meal tick population and thereby reduces the incidence of babesiosis (Willadsen *et al*. 1989, 1995; de la Fuente *et al*. 1998). The concealed nature of this type of TBV antigen has the disadvantage that natural boosting of vaccine-induced immunity does not occur.

A third TBV strategy could rely upon reducing the parasite transmission efficiency by immunizing against exposed salivary antigens which was proven successful for the sandfly/*Leishmania* model (Kamhawi *et al*. 2000; Thiakaki *et al*. 2005). This is the only approach that would benefit from natural boosting by exposure to the vector. However, immunizations in mice and rabbits with total *Glossina morsitans* saliva did not yield promising outcomes at the level of trypanosome transmission and tsetse blood feeding efficiency and survival (Caljon *et al*. 2006*a*, *b*). Nevertheless, the recent finding that tsetse fly saliva enhances trypanosomiasis onset (Caljon *et al*. 2006*b*) might suggest that individual recombinant salivary proteins still could represent TBV candidates.

Collectively, several antigens have now been proposed as candidates for experimental TBV vaccination schemes. Given the example of the anti-tick vaccine, TBVs are no longer a utopia and might actually contribute to disease transmission control. In the case of African trypanosomiasis, protective antigens remain to be identified while it is still unclear whether vaccination can be realistically adapted to field conditions, where the concealed nature of the antigens would exclude natural boosting.

ANTI-DISEASE VACCINATION: TO BE SICK OR NOT TO BE SICK, IS THAT THE QUESTION?

Besides TBVs, anti-disease vaccine strategies can be considered as an alternative approach to control trypanosomiasis. At least in the case of animal trypanosomiasis it is considered useful to protect the host from disease-associated complications (Authié *et al*. 2001). Here it is important to note that many mammals can harbour natural trypanosome infections without developing severe disease symptoms. This suggests that the negative outcome of trypanosomiasis in both HAT (Human African Trypanosomiasis) and livestock infections is due to the nature of the host immune reaction, rather than to the parasite itself. This hypothesis is based on (1) the comparative study of trypanosusceptible and tolerant cattle, where the latter develop a strong IgG antibody response against the cathepsin-L like cysteine protease congopain (CP) of *T. congolense* (Authié *et al*. 1992, 1993; Mbawa *et al*. 1992) and (2) by experimental mouse trypanosome infections, where the induction of immunopathology and disease development are not correlated to the actual parasite load (Magez *et al*. 2004). Hence, based on the knowledge available on disease-inducing factors, two main anti-disease vaccination strategies have been proposed.

Firstly, the correlation between the capacity to mount an antibody response against congopain and the relative tolerance of bovines towards the infection-associated pathology suggests that CP is a putative anti-disease vaccine candidate. While the mechanisms underlying the pathogenic action of this protease are not yet fully understood, artificial induction of antibodies against the protease could render a disease-susceptible host more tolerant (Authié *et al*. 1992; Lalmanach *et al*. 2002). Interestingly, when trypanosusceptible cattle were vaccinated with CP, the induced IgG titres were much lower compared to those in trypanotolerant cattle, suggesting that susceptibility correlated with the intrinsic incapacity to mount an immune response against the trypanosome protease (Lalmanach *et al*. 2002). Even the susceptible breed showed reduced levels of infection-associated anaemia and leucopenia during the chronic stage of infection, when vaccinated with baculovirus expressed central domain of CP (Authié *et al*. 2001). Together, these results suggest that anti-disease vaccination is a realistic option, although it remains to be shown if vaccine-induced memory retains its protective capacity for prolonged periods of time during infection.

Secondly, based on experimental mouse infections and data obtained in cattle, it was suggested that the inflammatory cytokine TNF plays a major role in the development of trypanosomiasis-associated disease complications (Sileghem *et al*. 1994; Magez *et al*. 1999). With the identification of the VSG-GPI anchor as the main TNF-inducing trypanosome moiety (Magez *et al*. 1998), a liposome-based GPI-vaccination strategy was developed in order to prevent excessive immune activation upon infection (Magez *et al*. 2002; Stijlemans *et al*. 2007). The proposed strategy resulted in a positive outcome for the host in

terms of (1) parasitaemia control; (2) prolongation of survival; and (3) limitation of infection-associated complications such as anaemia, weight-loss and impairment of locomotor activity. A detailed analysis of the underlying vaccine mechanisms elucidated the lack of B cell and memory involvement. Indeed, GPI-vaccination was shown to modulate host macrophages to become biased to anti-inflammatory alternative activation rather than pro-inflammatory classical activation (Stijlemans *et al*. 2007). This response was found to be short lived, and could even be evoked in B cell-deficient μMT mice. It is interesting to note that also in case of *Plasmodium* infections, a GPI-based anti-disease vaccination has been proposed (Schofield *et al*. 2002). Here, a synthetic carbohydrate malaria GIP, differing in only one mannose residue from the host-GPI carbohydrate core, was used in a conventional Complete Freund-based vaccination. While this vaccination prevented infection-induced excessive inflammation, it failed to protect mice from rapid parasite growth and parasitaemia-induced death. With regard to the relevance of this anti-disease vaccination it is important that clinical immunity to human malaria infections has been linked to increased circulating serum levels of anti-GPI antibodies (Naik *et al*. 2000). Again, this response is short lived, and does not appear to involve the induction of a B cell memory response but relies upon continuous challenge of the host with parasite antigen (Boutlis *et al*. 2002).

IMMUNE SUPPRESSION OR IMMUNE DESTRUCTION: WHY DOES B CELL MEMORY FAIL?

Since the very early days of the analysis of host-trypanosome interactions and immune modulations, infection-induced immune suppression has been recognized as a hallmark of trypanosomiasis (Murray *et al*. 1974*a*, *b*; Askonas *et al*. 1979; Clayton *et al*. 1979; Boutlis *et al*. 2002). This suppression could in part explain the trypanosomiasis-associated reduction of the vaccine efficacy against louping-ill virus (Whitelaw *et al*. 1979), foot and mouth disease (Sharpe *et al*. 1982), *Brucella abortus* (Rurangirwa *et al*. 1983), anthrax (Mwangi *et al*. 1990), and swine fever (Holland *et al*. 2003). Recently it has been reported by Radwanska and colleagues (2008) that, in addition to this suppression, several host B cell compartments are rapidly and permanently destroyed during the onset of a trypanosome infection (Radwanska *et al*. 2008). This is the case for the IgM-producing Marginal Zone B cell compartment, as well as the Follicular and Memory B cell compartments. In order to show the general implication of the latter, a vaccination experiment was performed in which mice were exposed the commercially available DTPa vaccine Boostrix®. This vaccine protects mice from challenge with *B. pertussis*, but is rendered inactive in the presence of trypanosomes. While this could be explained by active parasite-driven immune suppression, treatment of mice with Berenil® did not restore vaccine efficacy, despite the curative effect of the treatment on trypanosomiasis. Based on these results it is concluded that the presence of living and dividing trypanosomes can result in the destruction of the host B cell memory compartment which is not restricted to anti-parasite responses alone. In the near future, it will be crucial to validate these results in more natural infection settings. Indeed, if the same infection-associated immune complications were to occur in field-trypanosomiasis, they could (1) provide an explanation to general failure of vaccine trials for trypanosomiasis, and (2) suggest that a number of additional immune problems will occur in trypanosomiasis endemic regions. Firstly, infection-induced B cell memory destruction could explain the failure of vaccine efficacy when antigens such as ISGs are used. Indeed, while these antigens have been shown to be good immunogens, the active memory-recall response during infection appeared to be absent. Secondly, B cell memory destruction could also explain the failure of the cysteine protease-based anti-disease vaccine strategies. Again, while this antigen is immunogenic by itself, a protective memory-recall response appeared to be absent in trypanosusceptible hosts. Interesting is the notion that trypanotolerant cattle do manage to mount a protective anti-cysteine protease response. This finding suggests that the difference between a susceptible and a tolerant host could be linked to the relative capacity to maintain intact B cell compartments during infection, a hypothesis that is supported by recent transcriptional profiling data of both trypanosusceptible and trypanotolerant cattle (O'Gorman *et al*. 2009). Lastly, if memory immune destruction were to appear during HAT, this could have a detrimental impact on non-trypanosomiasis vaccination programmes that are currently ongoing in sub-saharan Africa such as the WHO Meningitis Vaccine Project (MVP) and the Pediatric Dengue Vaccine Initiative (PDVI), as well as on future anti-HIV/AIDS and anti-malaria vaccine programmes. Indeed, in this case it would be necessary to not only treat HAT victims for trypanosomiasis, but subsequently also re-vaccinate these people with all previously administered vaccines, in order to restore the B cell memory compartment.

CONCLUSION

Over the last three decades, many different approaches have been proposed for the development of protective vaccine strategies for trypanosomiasis. Despite all efforts, to date not a single strategy can be considered successful. It has become obvious that trypanosomes have developed multiple mechanisms to (1) protect themselves from the efficient immune attack by antibodies (O'Beirne *et al*. 1998), and (2) actively eliminate the B cell memory

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compartment (Radwanska *et al*. 2008). Hence, there seem to be various problems with the available vaccine development technologies and with the intrinsic mechanisms of immune memory development. First, while most experimental vaccine studies are performed in mice, this model might not represent an optimal host-parasite context to allow for the generation of relevant results, due to parasitaemia characteristics that are not representative for natural infections. Second, while *T. b. brucei* (*Trypanozoon*) and *T. congolense* (*Nanomonas*) are both used in model systems for experimental 'African Trypanosomiasis' these species of parasites have unique and different interaction with the immune system, and an incomparable anatomical distribution in the host, and hence cannot be considered similar from an immunological point of view. This is, for example, illustrated by the fact that IgM antibodies and the inflammatory mediators TNF and iNOS have different functions in *T. brucei* and *T. congolense* mouse models (Magez *et al*. 2006, 2007). Even in cattle, the role of antibodies in parasitaemia control might depend on the trypanosome species involved. Indeed, while various reports have shown a correlation between tolerance in cattle to *T. congolense* infection and increased anti-parasite IgG titres and antibody effector responses (Kamanga-Sollo *et al*. 1991; Taylor *et al*. 1996; Williams *et al*. 1996; Taylor, 1998), this link is absent in case of *T. b. brucei* cattle infections (Pinder *et al*. 1984). Hence, while infections of cattle with *T. congolense* would probably benefit from an IgG vaccine memory response, the data available to date from mice infected with *T. brucei* suggests that an efficient anti-trypanosome vaccine should most likely be based on the induction of a high affinity IgM memory response. In both models however, the maintenance of high circulating anti-trypanosome antibody titres in the absence of parasite antigen might allow the immediate elimination of metacyclic parasite upon entry in the body, thereby avoiding the potential initiation of active B cell memory destruction by living and dividing parasites. In particular, this last requirement appears extremely hard to achieve and hence the 25 year-old conclusion of Cornellissen and colleagues (1985) *i.e.* that 'if the interpretation of the data is correct, then vaccination prospects are not good', remains up-to-date for now.

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