How reliable are models for malaria vaccine development? Lessons from irradiated sporozoite immunizations

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ABSTRACT
Models occupy a key position in the development of anti-parasitic vaccines, yet their relevance has been seldom addressed. It is customary to admit that malaria vaccine development requires easy-to-handle, laboratory models. Animal models involving predominantly inbred rodents and primates as parasite hosts are currently the basic tools for the study of host-parasite interactions. Literature however indicates that the induction of host protection is more difficult in natural host-parasite pairs than in experimental models of parasite infection. Moreover different models delineate a wide range of host-pathogen relationship profiles providing a mosaic of contradictory informations, yet there is little incentive to delineate their relevance or to exploit recent advances to develop improved model systems. In this context the analysis of natural host-parasite interactions between Plasmodium berghei and its mammalian host and reservoir, the tree rat Grammomys surdaster could be of relevance in the study of host-parasite interactions.

KEY WORDS: Grammomys, host-parasite interactions, irradiated sporozoites, laboratory models, Plasmodium, vaccine

Immunization against the pre-erythrocytic stages of malaria has attracted considerable efforts, since it induces a strong, sterile protection in humans upon vaccination with live radiation-attenuated, sporozoite stage parasites. Irradiated sporozoites readily transform but become blocked at the liver stage of development in vivo. The same is observed with human parasites in rodents. Resistance to laboratory rodents in which high parasite loads and death is followed by a blood infection. C57BL/6 mice are highly susceptible to the development of P. berghei (but difficult to protect from malaria), whereas the BALB/c mice are poorly susceptible to P. berghei infection (but readily protected). Therefore, protection appears to be inversely correlated to the susceptibility of the host to sporozoite infection, the natural host being both highly susceptible to infection and difficult to protect.

In terms of susceptibility to infection, there are also large differences from one host to the other: Humans are extremely susceptible, 1-2 mosquito bites delivering a dozen sporozoites are followed by a blood infection. C57BL/6 mice are highly susceptible to the development of P. berghei (but difficult to protect from malaria), whereas the BALB/c mice are poorly susceptible to P. berghei infection (but readily protected). Therefore, protection appears to be inversely correlated to the susceptibility of the host to sporozoite infection, the natural host being both highly susceptible to infection and difficult to protect.

Consideration of this led us to launch studies of P. berghei in Grammomys surdaster, a natural host-parasite combination in which natural infection evolves in a chronic manner (in contrast to laboratory rodents in which high parasite loads and death are the most frequent outcome). Surveys of wild rodents revealed chronic Plasmodium carriage at low density. The same is observed with human parasites in repeatedly exposed individuals. G. surdaster are exquisitely susceptible to infection since < 12 sporozoites could induce a blood infection,
but they are extremely difficult to protect. Immunization schedules effective in laboratory rodents such as BALB/c mice and C57BL/6 mice failed to protect G. surdaster. Five immunizations with 50,000 irradiated sporozoites provided protection in only a fraction of immunized animals and did not prevent death (Van Ham and Coosemans, personal communication). A higher dose of 100,000 irradiated sporozoites was also ineffective. Similar results were obtained when using Thammomys gazelle, the natural host of P. yoelii (S. Chatterjee, unpublished). The similarity of behaviour of P. berghei in G. surdaster and of P. falciparum in humans appears as a valuable advantage, as compared to models currently used.

One drawback however to the present use of the Grammomys is that they are difficult to breed, are not commercially available and their immune system is poorly characterized. In the absence of any other alternative natural rodent host system, the Grammomys model needs to be better characterised, especially for immune responses. At present a small colony of Grammomys surdaster is maintained in the Netherlands and could be offered to researchers developing malaria vaccines, to test their efficacy. Thus rather than focusing on a single mouse strain or a single host species to test vaccine efficacy, researchers could select such natural host-parasite systems of Grammomys - P. berghei or man - P. falciparum. In the latter case, malaria challenges and vaccinations may need to be attempted on a background of previous or current infection with malaria or other parasites and pre-existing immunity to malaria; which is the case for many humans in need of vaccination.

**Mechanisms of Protection in Natural Versus Artificial Models**

The distinct pattern of susceptibility to infection/ease of induction of protection suggests distinct protective mechanisms and reduces confidence that initial vaccine successes in animal models can be scaled up in humans. This is well illustrated by the recent history of pre-erythrocytic malaria vaccines. These were initially aimed at inducing only sporozoite neutralizing antibodies (based on mAb passive transfer experiments in mice); then at inducing CD8-CTLs destroying infected hepatocytes Later reports however have identified CD4-IFN-gamma secreting cells as a critical component of defence.

Each of these quite different approaches are derived from results gathered in models. They have absorbed decades of research and considerable amounts of money, yet cannot predict with surety the efficacy of similar vaccines in humans. The most successful Plasmodium vaccine used to date, RTS/S, relies on an adjuvant inducing optimal CTL activity in models. In humans it induces very high antibody levels, no CTL activity, yet generates a degree of protection. These recent experiences clearly show that mechanisms of protection in artificial models differ from those in natural hosts and draw attention to four aspects: (a) innate immunity, (b) targets of adaptive immunity, (c) molecular mimicry and (d) efficacy of defence mechanisms depending on the host.

a. The innate immune response prevalent in unnatural hosts (rats, mice) is characterized by an infiltrate of mononuclear cells, neutrophils and eosinophils around late stage schizonts and emerging merozoites. In contrast it is absent in the natural host, Grammomys. The lower susceptibility of artificial hosts could be explained by the stronger local intra-hepatic innate response.

b. Recent experiments established that liver forms resulting from irradiated sporozoites are both persistent and essential for protection. However, in laboratory mice, both immunizing and challenge sporozoites were arrested at the uninucleate, trophozoite stage. This is in contrast to observations of P. berghei in Grammomys and P. falciparum in chimpanzees (Thomas A. and Druilhe, unpublished), where development to submature schizonts were noted. This suggests distinct targets, as well as distinct immune mechanisms.

c. The host specificity of a given parasite relies in great part on molecular mimicry, i.e., on the antigen “compatibility” between a given parasite and its usual hosts. Understandably, when the same parasite is introduced in an abnormal host, even one that is phylogenetically closely related, the number of shared epitopes will be less. This dramatically increases the number of targets for effector mechanisms and may explain, at least in part, the greater effectiveness of vaccines against a given parasite in an abnormal host.

d. Finally, the efficacy of defence mechanisms depends on the host, a fact often overlooked. A telling example comes from sporozoite-hepatocyte interactions. When examining the effect of inhibitory antibodies, diametrically opposite results were obtained when using the same antibodies with either human hepatocytes or the human Hep-G2 hepatoma that is the closest known to normal hepatocytes.

To summarize, the “adaptation” of a parasite to an artificial host actually translates in immunological terms into defence mechanism that in most circumstances are more effective than those seen in the natural host. Since we do not understand well enough human immunity to Plasmodia, it is not possible to determine which model if any may best reflect the desired pattern of immune responses. Similar situations prevail for other parasites, e.g., Schistosomes. Models can be at best considered marginally valuable until results have been ascertained using the same parasite in its natural host.

**Perspectives**

In today’s post-genomic era with Plasmodia, Anopheles and human genomes at hand, optimism abounds that new therapies and vaccines will emerge. However, researchers have become even more dependent upon models for screening molecules involved in protection. The existing models seem to be employed based more on availability in laboratory settings than on demonstrated relevance. Consequently vaccine candidates that show promise in a given model might fail in humans or worse, experimental results may lead to the rejection of vaccine candidates that would be effective in humans. Each transfer between species - from rodents to non-human primates...
to humans - involves assumptions that are rarely questioned. Since animal models will play an even more crucial position there is an urgent need for an improved evaluation of their merits and limitations.

The dilemma can be summarized as follows: either we rely on substantially more difficult models for vaccine development (e.g. humans, Grammomys) and accept that progress will be slow, though results should be more relevant; or, we continue to employ easier models, generating data which may not be extrapolated to the target host!

In fact, there might be a third way - though unlikely to be implemented. Indeed, it would require a strong commitment to gather an improved understanding of essential defence mechanisms prevailing in this humans, leading to improved models that reflect them best. In the field of malaria pre-erythrocytic vaccine development, the strategy would require (a) the induction of protection by irradiated sporozites in a sufficient number of volunteers and (b) better definition of naturally occurring pre-erythrocytic stage immunity under field conditions, together with an analysis of the immune responses associated with protection in those two situations.[11] Based on this knowledge, the rational choice of an improved model, as well as the development of novel ones, could be envisaged.

Modern genomics may provide valuable alternatives for the selection of both the parasite and the host. For instance, availability of the full genomes of most rodent plasmodia may lead to the selection of a preferred rodent plasmodium species based on knowledge of molecular homologies with the corresponding human plasmodium gene. The same can apply to the selection of the best fit between host molecules. Another approach promoted by some groups is gene replacement to substitute P. falciparum genes for rodent plasmodium genes and similarly, to substitute human genes for mouse ones. Finally, the successful grafting of human hepatocytes into immunodeficient mice opens novel areas of research where the interaction of human plasmodium sporozites with their normal host cells can be dissected in an experimental model.[12]

The delays and investments needed to develop improved models have to be balanced by the ethical and financial concerns, associated with the rising number of clinical trials performed in human volunteers with vaccine formulations designed in insufficiently documented models.

Acknowledgements

We would like to acknowledge the initiation, support and encouragement of Professor Marc Wéry in the Grammomys work mentioned in this paper. This work was supported by the Inter-University Poles of Attraction Program (Grant P5/20) Services of the Prime Minister Federal Agency for Scientific, Technical and Cultural Affairs and by the European Commission programmes STD 2, 3 and INCO DC (grants TS 2-M-0122-F, ERB 350 4PL, 910123, EC‘18 CT 95016).

References


Spot the Diagnosis

**Answer: Spina Ventosa**

The present case had spina ventosa, the tubercular osteomyelitis of small bones of the hands and feet. Her physical examination revealed the presence of generalized lymphadenopathy including matted cervical lymph nodes, bronchopneumonia and hepatosplenomegaly.

The radiograph of the hands and feet showed multiple osteolytic lesions (middle phalanges of middle and little fingers in the left hand, first metatarsals of both the feet and first and fourth proximal phalanges of the left foot) with minimal periosteal reaction [Figure 1]. Chest radiograph showed patchy consolidation in the right upper zone and left lower zone with hilar lymphadenopathy. Mantoux test was positive. These findings confirmed the diagnosis of disseminated tuberculosis with spina ventosa, the tubercular osteomyelitis of small bones. The swellings subsided within six weeks of initiation of anti-tubercular therapy. The treatment is being continued for a year. Short and bent fingers were the sequels of the lesion.

**Discussion**

Spina ventosa refers to a form of tuberculous osteomyelitis where underlying bone destruction, overlying periosteal reaction and fusiform expansion of the bone results in cystic-like cavities with diaphyseal expansion.[1] The word is derived from Latin (Spina-'a thorn'; ventosa-‘full of wind, distended’).[2] It typically involves phalanges, metacarpal and metatarsal bones in children. Though tuberculosis is a common disease in India, spina ventosa is a rare manifestation.

The characteristic findings include the slow progression of the disease, minimal pain despite the fusiform swelling, minimal new bone formation. Similar lesions can be encountered with acute osteomyelitis, dactylitis of sickle cell disease, syphilis, hereditary acro-osteolytic conditions, histiocytosis X and bone tumors. However, the classical lesion, associated lymphadenopathy, primary lung lesion, positive mantoux test and response to anti-tubercular therapy confirmed the diagnosis in this case.

**References**