The importance of models of infection in the study of disease resistance

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Models currently occupy the crucial first step in the research flow for the development of new drugs and vaccines. Some animal models are better at reflecting the host–pathogen interaction in humans than others; this depends on the pathogen and its host specificity. Data gathered from what are often poorly adapted models provide a mosaic of sometimes contradictory information, yet there is little incentive to better delineate the relevance of models or to exploit recent advances to develop improved ones. This review reports on three particularly intractable human pathogens – *Mycobacterium*, *Plasmodium* and *Schistosoma* – and reflects that the extent to which these model systems mimic infection and protection processes in humans might not be sufficiently well defined.

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Existing animal model systems have paved the way for much of modern biomedical research; without them we would know little. As these models have been instrumental in the acquisition of much of the present knowledge, they might be supposed to be well fitted to current needs. But, by definition, no-one can determine how much has been missed through the use of these models. When models have been relevant, which is fortunately a reasonably frequent event, important developments have been possible. This review addresses three diseases where we believe the relevance of animal models can be questioned because lead concepts defined in the models have so far failed to be applicable to the target host. We aim to draw attention to the limitations of existing models rather than to the more obvious benefits they provide.

Most animal model systems are used because: (1) they have been in use for many years; (2) their features have been documented; (3) work on them has defined the current paradigm; (4) they are easy to work with; and (5) they are readily available from local suppliers. But the price of this pragmatism, still often overlooked, is that findings peculiar to any given model might lead to the selection for further study of potential vaccine or drug candidates that will never be of any use in humans. Equally, the results obtained with models might result in the rejection of candidates that would be of use in humans. Although much of the early development and screening work on drugs and vaccines is done in small mammals, there is often pressure to complete experiments in non-human primates, despite their relative scarcity and cost, and the important ethical concerns over their use. It is not clear whether the data obtained from work in primates are necessarily of any more value in the selection and testing of drug and vaccine candidates than those obtained from smaller mammals. Of course, the morphological and genetic similarities between humans and primates provoke an intuitive feeling that they should provide more reliable data than those obtained from small mammals. But each transfer between species – from rodents to non-human primates to humans – involves assumptions that are rarely questioned. Clinical data would be more relevant to deciding initial research orientations, but are often thought to be unobtainable, whereas in some circumstances they might well be possible using well-defined discriminative clinical differences. Such studies should be encouraged. However, as animal models of infection and disease – particularly those using mice – are likely to continue to occupy a crucial position in the research flow for the forseeable future, there is a need for improved delineation of models and for a review of their merits and limitations.

Models for vaccine development

A mozaic of models

The very large number of available inbred and outbred laboratory mice, and the many species and strains of pathogen, means that the number of combinations used experimentally is extremely large. This heterogeneity has several crucial consequences (the following are based on models for malaria but can also apply to other pathogens). *Infection patterns depend on choice of host.* The pattern of infection is not a fixed characteristic of a given pathogen but depends – particularly for parasites – on the host in which it develops: susceptibility, load of infection, time course and resistance can vary greatly depending on the host. It is the combination of host and microbe that determines the dynamics of their interactions, so it is hardly

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suprising that different mechanisms of defence can be described for the same pathogen when experiments are carried out in different hosts. As is apparent from the contradictory observations reported in many review papers, the difficulty is in trying to identify the key differences between the various host–pathogen combinations used.

High loads, rapidly acquired resistance. The majority of models used for research rely on non-natural host–parasite combinations. In many natural host–pathogen combinations, particularly with parasites, host death is a relatively infrequent event: chronic infection with low pathogen loads are the rule, and continued susceptibility to repeated exposure means that immunity is likely to be partial and take years to establish. By contrast, in non-natural host–pathogen combinations, death can be a frequent outcome, acute infections with high pathogen loads (often determined by experimental design) are common, and protective immunity develops rapidly, even after a single exposure, provided the host survives the acute phase of infection.

Distinct defence mechanisms. The mechanisms of protection in natural host–pathogen combinations have yet to be elucidated for most pathogenic organisms. However, differential patterns of susceptibility/resistance to the same pathogen can at least cast serious doubts on the relevance of such models to the natural host–pathogen combination of the immune interactions reported therein.

Selection by the host. The host specificity of a given natural pathogen arises from progressive selection over thousands of years for parasite molecules that ensure chronicity. When the same parasite is introduced into an abnormal host, the same molecular fine-tuning is absent. As a consequence, there could be acute pathogenicity to the host and greater acquired resistance to infection, possibly because a greater number of parasite molecules are antigenic to the host and stimulate a more efficient immune response. It might then be expected that a larger number of 'vaccine candidates' will be identified in such models, although these might not be extended to the natural host. Moreover, the successes in extending any promising findings from animals to humans are more likely to be reported than the failures.

A new fit. Serial passages are usually needed to establish a pathogen in a novel or abnormal host. Not much is known of what lies behind this progressive 'adaptation' process but, as the pattern of infection improves over time, molecular modifications are likely to be taking place. One can speculate that the 'fittest' clones from the original pathogen isolates, or favourable mutations arising from them, are positively selected. A new host–pathogen combination will have been created, but only at the expense of substantial and potentially significant changes in the pathogen from that originally isolated.

Which model for which goal? Some of these models might still be relevant to the goals of some studies but the difficulty is in knowing which. It is not necessary for a model system to mimic every aspect of the real system; conversely, it is necessary to spend time identifying which features are mimicked faithfully. In the field of vaccines, establishing the relevance of a model would require one to be in the position of knowing how protection is induced in the target host – humans. In practice, the limited knowledge of protective responses in both the target host and the model results in a vicious circle of debate.

Harder but better: clinical data. Examination of the first key events in the clinic is an interesting option often ignored or dismissed because accessing clinical data is considered difficult. However, clinical testing could help determine which of the various models can best fit the needs of any given research and development programme. This requires additional effort but would undoubtedly be rewarding in the longer term.

Biological differences between models

A most striking example of biological differences between models is supplied in the field of malaria by the irradiated sporozoite model. Immunization by the whole irradiated sporozoite stage of the parasite (the stage that is injected by the bite of an infected *Anopheles* mosquito) can achieve full, sterile protection in both mice and humans, although with substantial differences in the protocols [1]. Large numbers of *Plasmodium berghei* parasites are needed to establish a patent (blood stage) infection in Balb/c mice, whereas protection can be induced in the same mouse strain by small numbers of parasites (a single inoculation of 1000 irradiated sporozoites). In effect, there appears to be an inverse relationship between the number of parasites needed to demonstrate susceptibility, and the number of parasites needed to induce protective immunity. With *P berghei* in the tree-rat (*Grammomys*) – its natural host – exactly the opposite is true. *Grammomys* is highly susceptible and a patent infection can be achieved by inoculation of fewer than five sporozoites. It is, however, hardly possible to induce protection against *P berghei* in *Grammomys*. It is worth noting that in humans 12–14 immunizations by a few hundred irradiated mosquitoes carrying sporozoites is needed to induce solid protection against *P. falciparum*. There is neither proof nor consensus that the same mechanisms are responsible for protection in these three host–pathogen combinations. In mice, irradiated sporozoites invade hepatocytes but remain blocked at the one-nucleus stage, whereas in the chimpanzee – the closest relative of humans – they develop into fully mature schizonts, containing thousands of nuclei.

Despite the abundance of publishable data generated by studies of artificial host–pathogen combinations,

antigens and delivery systems that have proved efficient in protecting these 'easy' (and possibly misleading) rodent models often fail to immunize humans [2]. *Grammomys* could be considered a valuable alternative, but they are difficult to maintain and, because of the limited availability of reagents, their immune system is barely accessible to investigation. At the other end of the scale are chimpanzees. As well as their genetic similarities to humans, they are fully susceptible to major pathogens with strong host specificity, such as malaria, tuberculosis (TB), hepatitis B virus (HBV) and hepatitis C virus (HCV), and are amenable to immunological investigations as precise and detailed as those possible in humans or mice [1]. However, their scarcity, cost and the related ethical constraints are strong counter-arguments against their use and go some way towards explaining why, despite their remarkable significance, they have been used so little.

Another instructive example of major bias that can result from the use of models is given by experimental investigations on the role of the spleen in resistance. In a recent review of immunity to the blood stages of malaria, the crucial role of the spleen in protection in various models was documented by no fewer than 27 citations. However, the review failed to mention a single publication where humans resident in endemic areas had undergone splenectomy (usually following a road accident or other trauma). In fact, the parasite densities and frequency of malarial attacks is no greater in these individuals than in those with an intact spleen [3]. The main lesson from this is that although the spleen is absolutely essential in non-adaptive defences at an early stage of infection, it is no longer needed when antigen-specific immunity has developed.

Besides precise molecular interactions of pathogens with their normal hosts, each different host brings with it differences in the immune system that can lead to further complications when attempting the rational development of vaccines that will give improved levels of protection in humans. One notable example is a novel adjuvant that was selected from a large series in mice, partly because of its capacity to induce high levels of CD8 T cell reactivity. When assessed in humans, it proved instead to induce extremely high antibody titres and very low, if any, cytotoxic T-lymphocyte (CTL) activity. Even so, it induced a substantial degree of protection, blurring the rationale for further improvements [4].

In vitro assays offer limited help

In vitro studies are favoured when animal models are deemed to have practical limitations, but they present a different set of problems. For instance, adaptation of *Plasmodium* to *in vitro* conditions of growth selects a limited subset of the original clonal diversity [5]. At the liver stage level, in view of the difficulty of obtaining access to metabolically active human hepatocytes, many studies of defence mechanisms in the liver have relied on hepatoma cell lines, particularly the HepG2-A16 line, which shares most of its proteins with normal human hepatocytes. It was thought that invasion of HepG2 by *P. falciparum* would mimic invasion in normal hepatocytes but, despite the similarities between the cell types, *P. falciparum* cannot develop in HepG2. Furthermore, multiple comparative studies have revealed major discrepancies in the inhibitory effect of antibody on sporozoite invasion molecules. Whereas antibodies can totally inhibit sporozoite invasion of HepG2 cells, their influence on the invasion of hepatocytes seems to be much weaker [6,7]. In one dramatic example, antibodies found to have a significant inhibitory effect on the invasion of HepG2 cells by *P. falciparum* sporozoites enhanced the invasive capacity of sporozoites for human hepatocytes [8]. Data from three malaria vaccine trials in humans show a lack of correlation between levels of invasion and/or inhibition in HepG2 and protection status. Similar important differences between hepatoma cell lines and hepatocytes have also been described for hepatotropic viruses.

Dose dilemma

Similar limitations are apparent in the mouse models used for the study of the immunology of pulmonary tuberculosis. The consequences of infecting the lungs of mice by aerosol or – preferably – by direct intratracheal injection, depend on: the dose of bacteria; the way in which the bacterial suspension was grown, stored and prepared; and the strain of mouse used. Very-low doses lead to latent infection, in which bacteria are detectable by PCR, immunohistochemistry or culture, but bacterial numbers remain low and stable for the life of the mouse, without causing disease. In the C57Bl × DBA2 F1 hybrid, this state of stable latent infection can be achieved by direct intratracheal injection of 10^4 *Mycobacterium tuberculosis* H37Rv [9]. By contrast, it is claimed that as few as 200 bacteria delivered by aerosol will cause progressive disease in C57Bl/6 mice. This figure, usually determined by culture of lung tissue some hours after exposure to the aerosol, is likely to be an underestimate because it ignores the large number of organisms that lodge in the nose or on the fur that are subsequently swallowed; however, it also ignores the organisms that are killed within hours of entry into the lungs.

Using a dose that will cause progressive disease in all animals (about 10^6 in Balb/c mice, less in C57Bl/6 mice), this phase of initial killing of bacteria, which lasts a few hours, is followed by a phase of rapid bacterial

growth, characteristically with a doubling time close to 24 h. This rapid growth phase lasts 3–4 weeks. There is then a plateau in bacterial counts, correlating closely with the development of a Th1 response. The Th1 nature of this plateau, and the requirement for major histocompatibility complex (MHC), CD8+ T-cells, and other cytokines such as interleukin-2 (IL-12) and tumour necrosis factor α (TNF-α), have been studied extensively using neutralizing antibodies and gene knockout mice [10–12].

Eenie, meenie, minie … mouse

The duration and sequellae of this Th1-mediated plateau depend on the mouse strain. In C57Bl/6 mice, bacterial numbers stay more-or-less constant, and the Th1 response persists without an increase in IL-4 secretion, but the Th1-mediated granulomata increase steadily in size and coalesce until the animals die from lack of lung tissue. By sharp contrast, in Balb/c mice (or C57Bl × DBA2 F1 hybrids) the bacteria start to grow again (although more slowly than in the early phase of the disease) and there is a progressive increase in IL-4 mRNA and in IL-4 protein by immunohistochemistry or by enzyme-linked immunosorbent assay (ELISA) of lung extracts. As IL-4 expression increases, so TNF-α becomes toxic [13,14], as is also seen in models of schistosomiasis and *Trichinella spiralis*. As anticipated, knocking-out the IL-4 gene has no effect on the progress of tuberculosis in mice that respond in the same way as C57Bl/6 [15], but it profoundly ameliorates the progression of the disease in Balb/c mice (R. Hernandez-Pando *et al.*, unpublished results). Similarly, there is accelerated disease in mice with pre-existing Th2 responses to the 'common' antigens shared with environmental saprophytic mycobacteria [13], to protein antigens of *M. tuberculosis* [16] or even to a single 16-amino-acid epitope from ovalbumin expressed within the strain of recombinant *M. tuberculosis* used for challenge [17].

An increased understanding of the immunology of tuberculosis in humans [18–22] suggests that it is appropriate to select a mouse model in which Th2 cytokine expression increases, such as pulmonary infection in Balb/c or C57Bl × DBA2 F1 hybrids. How then should these models be used to answer the crucial questions? The current gold-standard protective vaccine is Bacille Calmette Guérin (BCG), an attenuated strain of bovine tuberculosis. When human populations are exposed to *M. tuberculosis*, about 10% develop progressive disease. In some populations, BCG has no protective effect, but in others it is able to reduce this figure by 80% [23]. In other words, it reduces the incidence of disease in those exposed from 10% to 2%. Unfortunately, nobody has ever done the same experiment in animals. Ideally, mice would be infected with a low dose of organisms so that only 10% develop progressive disease. Then vaccines could be sought that can reduce this incidence not to 2% but rather to 0%. Such experiments would require very large numbers of animals. In practice, BCG is used as a standard in mouse models where a large infecting dose is used so that all animals develop disease whether BCG-vaccinated or not. One crucial endpoint is prolongation of survival time, but all the animals will eventually die, so the gold standard is not effective in such assays. Another endpoint is bacterial kill during the first 10 days, but this does not correlate with the presence, absence or severity of subsequent disease progression after the plateau phase, and so is not related to the result sought in humans. It is not clear whether such assays are relevant to the selection of vaccines for human use.

Real-world hosts are not axenic

A second problem is that mice in modern laboratories have few activated T cells and little experience of mycobacteria. Humans, however, have inevitably encountered huge numbers of antigenically cross-reactive saprophytic environmental mycobacteria, of which there are >80 species, common in mud and untreated water. Moreover, in developing countries where the need for a vaccine is most acute, a large percentage, perhaps 80%, of adults will have encountered *M. tuberculosis* itself [24]. It is clear that this prior exposure to mycobacteria is crucial. In mice, if exposure has evoked a Th2 response the animals are more susceptible than unimmunized animals, whereas if it has evoked a Th1 response the animal is partially protected [14]. A further implication is that 'partial protection' might be sufficient to kill subsequently administered BCG vaccine before it can boost protection, but insufficient to protect the individual from *M. tuberculosis* itself [25].

Effective immunotherapy for tuberculosis could shorten chemotherapy (which currently takes a minimum of 6 months), and allow treatment of multidrug-resistant disease. In the 1890s, Robert Koch observed that attempts to treat tuberculosis by injecting killed organisms or culture supernatants resulted in necrosis both at the site of injection and in the tuberculous lesions [26]. Such 'immunotherapy' was therefore effective for skin tuberculosis, which underwent necrosis and sloughed off, but fatal in patients with pulmonary or spinal lesions. This exacerbation of disease by antigens of *M. tuberculosis* was also documented extensively in the 1940s, in the work of G. Wilson and of A. Rich, and the importance of a change in the role of TNF-α from protective to toxic was noted subsequently [13,14]. These phenomena have recently been rediscovered [27]. Similarly, various protective vaccine candidates have been tried as therapeutic vaccines in mice; all have failed to exert beneficial effects on established disease [28]. The only

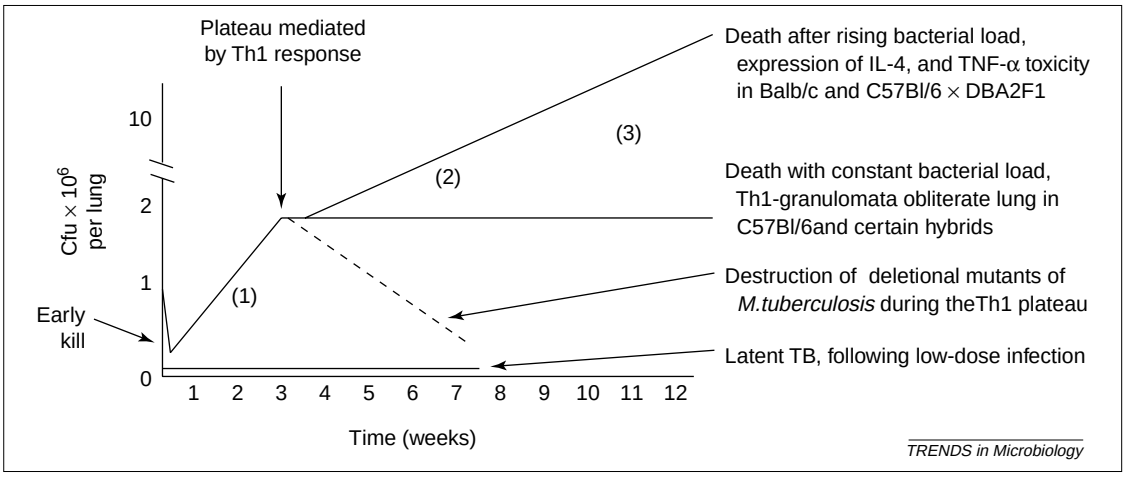

Figure 1. Patterns of replication of *M. tuberculosis* in mice

After intrapulmonary infection of mice with a sufficient number of virulent *Mycobacterium tuberculosis,* there is initially some rapid kill, after which the organisms double in number approximately every 24 h for about 3 weeks, when a plateau develops. This plateau is attributable to a Th1 response, plus CD8+ cells and tumour necrosis factor α (TNF-α). In some mouse strains, the organisms then start to proliferate again; interleukin 4 (IL-4) is expressed and TNF-α becomes toxic, as in human disease. In other mouse strains, however, the bacterial count stays constant but the lungs are obliterated by granulomata, without increased expression of IL-4. (1) Some researchers assay vaccines by looking for decreased growth at 10 days during the phase of development of the Th1 response. (2) Others look at bacterial counts later, during progression after the plateau, and (3) some look at time to death. None of these endpoints corresponds to the role of a vaccine in humans, which is to decrease the percentage of individuals developing disease. Abbreviations: TB, tuberculosis.

vaccines shown to exert a therapeutic effect are a DNA vaccine encoding a mycobacterial heat shock protein [29] and an environmental saprophyte [30] with the unusual property of powerfully downregulating Th2 responses by evoking regulatory T cells [31]. This raises the intriguing possibility that immunotherapy of tuberculosis should aim not only to boost the Th1 response, but rather to downregulate the 'subversive' IL-4-secreting response. So, once again, the appropriate models might be those, such as the Balb/c mouse, that show activation of IL-4 production during progressive pulmonary disease. This last point leads to a further important concept. If the failure of the protective response is related to IL-4 secretion, and if most humans have encountered mycobacterial antigens before vaccination, it is possible that an effective vaccine needs to eliminate any pre-existing IL-4 response, rather than merely boost the Th1 response, which develops rapidly after infection anyway. In support of this notion, it has been observed that antigens of *M. tuberculosis* activate expression of IL-4 in cultures of human peripheral blood mononuclear cells, even when these are derived from healthy BCG-vaccinated donors [32].

After infection, humans who do not develop overt disease nevertheless harbour live organisms (i.e. latent infection), and these can reactivate later in life [33]. These organisms constitute an important reservoir of infection. Recent work using an *in situ* PCR has shown that these latent bacteria are found in numerous cell types, including fibroblasts, endothelial cells and type II pneumocytes, in addition to macrophages, and that these infected cells are found in areas of lung devoid of any inflammatory infiltrate [34]. Thus, their presence in non-professional antigen-presenting cells lacking many costimulatory molecules leads to the suspicion that they might play a role in the distortion of the immune response. This phenomenon can be studied by using very-low-dose infection in Balb/c or C57Bl × DBA2 F1 hybrids, in which a very similar pattern of infection of non-macrophage cell types, in areas of tissue devoid of inflammatory infiltrate, can be seen [9]. It is important not to confuse this state of latency with the plateau in high bacterial counts that occurs from 3 weeks when the Th1 response is maximal (Fig. 1). Studies that identify genes required for *M. tuberculosis* to maintain its numbers during the plateau phase in the presence of a powerful Th1 response have yielded some important results [34,35]. However, such studies are unlikely to illuminate the latent state, which, in both humans and mice, can be achieved by exposure to a subinfectious dose.

Size matters

Tuberculosis and malaria present major problems for the immune system; schistosomes add to the challenge owing to their size and therefore antigenic complexity of (adult worms live in blood vessels and are some 2 cm long) [36]. However, the relative lack of host specificity of the three main species of schistosome that infect humans – *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum* – means that there is a wide-range of hosts available for studies of resistance to infection [37]. Rats, mice, hamsters, jirds (gerbils) and guinea pigs have all been used extensively. Transfer to other species is simpler; goats, sheep, pigs, cows, buffaloes, monkeys, baboons

and chimpanzees are also susceptible to infection [37] and, in endemic areas, many of them are naturally infected with a variety of schistosome species. Not surprisingly, these larger animals have other difficulties. In mice, assessing the survival of a challenge infection by perfusion of worms from the mesenteric and portal blood vessels is a relatively simple procedure but one that takes on a whole different dimension when buffalo are involved [38]. For practical reasons, most preliminary work on the testing of vaccine candidates has used *S. mansoni* in mice [39]. In addition to their ease of use, mice were selected for studies of schistosome infection primarily because they develop the full spectrum of egg-induced immunopathology [40]. Only later was its value in studying immunological resistance to infection fully exploited.

Divergent data

The ease with which laboratory studies could be conducted, and the difficulties in measuring infection levels and exposure to schistosomes in humans, meant that the paradigm for resistance to schistosome infection was determined largely by the laboratory work. However when new single-dose schistosomicides became available, which enabled reinfection studies in humans, it became clear that there were unresolved issues. Much of the animal data, including those generated from cytokine knockouts and the use of anticytokine antibodies, indicated that Th1 responses and interferon γ (IFN-γ) were playing a dominant role in protection, whereas Th2 responses appeared to be associated with egg-induced pathology [41–43]. The reinfection studies and subsequent genetic analyses, indicated that protection in humans was associated with high levels of eosinophilia and immunoglubulin E (IgE) antibody responses, which are classed as Th2 responses [44–47]. Additional experiments indicated that protection in mice need not be associated only with Th1-type responses [48,49].

Baboons might be better

Although the complex ethical issues surrounding the use of non-human primates in biomedical research are unresolved, it is clear from the relatively little work that has been done that they offer significant advantages over mice [50]. The most exciting developments have come from work on baboons, in which the pathology and disease course of schistosomiasis, including periportal fibrosis, appear to be similar to that observed in humans [51]. In addition, protection against infection in repeatedly exposed baboons was associated with antiworm IgE, a situation similar to that observed in human populations exposed to schistosome infection [44–47]. Additional work is needed to build on the promising results that have already been obtained from the use of baboons as a model for human schistosome infection. However, ethical constraints and limited availability will always serve to limit their use.

Reliably inducing protection

As with other pathogens, irradiated schistosomes have been used to stimulate a high degree of protective immunity. In the late 1970s, the efficacy of an irradiated vaccine was demonstrated in classical field studies in cattle [52]. Although not a practicable solution to the problem of schistosomiasis in humans, the irradiated vaccine remains an important tool and is the gold standard for the induction of protection. Recently, the radiation-attentuated *S. mansoni* vaccine was tested successfully for the first time in chimpanzees [53]. Group sizes were small and care is needed in data interpretation, but it was found that morbidity was lower in the vaccinated group, with faecal egg-outputs reduced by 38%, compared with challenge controls. Unfortunately, the successful induction of protection with irradiated schistosomes has not often been matched, even by some of the leading schistosome vaccine-candidate antigens [54].

Models for drug development

At first glance, the situation for drug development is not as problematic as for vaccine development or for studies of pathology. The subtle host–pathogen immune interactions that are unique to the final host and might bias the antigenicity of any vaccine candidate are no longer a concern. However, differences can still influence the selection or rejection of a given compound. The most obvious ones are the metabolism, bioavailability and pharmacokinetics of the drug in the animal model. There are numerous examples of huge differences between mammals in this respect [55] and it is of particular concern for rodents, which are by far the most widely used first *in vivo* screens; rodent hepatocytes and liver functions differ markedly from those of humans.

Less obvious, or more difficult to monitor, are the differences in the protein expression of the parasite in its abnormal model environment. This probably concerns only a limited subset of the transcriptome. However, if by chance this new host environment produces a qualitative or quantative modification in the drug ligand or in a protein involved in the cascade of events triggered by the drug or its metabolites, it can either reduce or abrogate the efficacy of the tested compound.

Limitations for drug development

Unlike vaccine development, an indication of responses in humans is normally possible only when a new drug has reached the end of the development process, so

go–no-go decisions rely entirely on available models. For instance, the two most effective compounds available today against malaria – cinchona bark alkaloids and artemisin derivatives – have been selected as antimalarials by traditional healers (effectively from phase III trials) but would not be selected by current screens in rodents because of their relatively short half-lives. In the case of pre-erythrocytic stages of malaria in the liver, technical limitations of both *in vitro* and *in vivo* screening protocols have not enabled the screening of drug libraries against this important stage, and so have prevented the identification of agents that are active before the emergence of the pathogenic blood stages.

The number of malaria parasite species, their complexity and the still as-yet limited genome data, have consequences for data mining and further drug screening. Antigenic make-up and metabolic pathways are best known for the human species of *P. falciparum* but homologues of characterized proteins in rodent species are frequently difficult to identify because of distinct codon usage. This situation can bias screening in rodents of those compounds identified in *P. falciparum* as potential targets of a given metabolic pathway, because it is impossible to distinguish between a true lack of efficacy or molecular differences between the parasite species.

For malaria, there is a severe lack of coherence in the various screens used sequentially: *in vitro* techniques are available to screen novel compounds against the asexual blood stages of the major human pathogen *P. falciparum*, whereas *in vitro* screening is not available for rodent species, nor for the three remaining human malaria species. By contrast, *in vivo* models, which are essential in the study of drug bioavailability, rely almost entirely on rodent malaria species, whereas for *P. falciparum* they are limited by the small numbers of primates that can be enrolled, and by their cost. An alternative is *ex vivo* experiments, in which the serum from treated rodents is assessed for biological activity *in vitro* using *P. falciparum* cultures, thereby ensuring greater coherence in the system. However, this assay is limited by the serum concentrations of the test compound and toxicity of large amounts of mouse serum in *P. falciparum* cultures.

In one series, screening of several thousands of compounds from a novel drug family – a compound active in the nanomolar range against multidrug-resistant *P. falciparum* and against three rodent malaria species *in vivo* – was abandoned after its failure to clear the parasitaemia of five *Aotus panamanensis* monkeys infected with *P. falciparum*. In this particular case, it was neither possible to complete an *in vitro* study of the serum metabolites from these primates nor to study the efficacy of the drug on the parasite line used to infect the primates. Hence, uncertainties over the relevance of the different systems and lack of coherence among the three models clearly had severe consequences for the development of this family of compounds.

Models for the screening of antimalarial compounds are thus not well adapted to current needs. It is possible that they have led to the identification of a number of new compounds, although no-one knows how many have been rejected as a result of the limitations of the primary screens.

Towards novel models

Genomics and proteomics can be expected to make important contributions to a better evaluation of the research areas where data from models can be trusted. The principle of synteny will enable identification of those animal pathogen genes that are similar to the genes of human pathogens, and the same could apply – to some extent – to a selection of the best-fit between host molecules, perhaps enabling the selection of an improved model for a particular research target.

Recent progress in gene replacement methods has also opened a very wide array of novel screening models, which are still in their infancy but can predictably provide artificial, yet very convenient (and possibly more relevant) models.

Knockout mice lacking adaptative B-cell- and T-cell-immune functions, such as severe combined immune deficiency (SCID), beige-xid-nude (BXN) and Rag-2, can be reconstituted by heterologous bovine red blood cells (RBCs) and sustain the replication of the cattle parasite *Babesia bovis*. (When the same is attempted with human RBCs and *P. falciparum*, the parasite becomes pyknotic in less than 30 minutes.) Further studies have shown that the partial depletion of tissue and circulating phagocytes from these animals can lead to the successful grafting of human RBCs that can be infected by *P. falciparum* and can sustain a parasitaemia for several months. This, incidentally, showed the importance of non-adaptive immune defences in the host-specificity of *P. falciparum*. The main difficulty in the development of this model was preventing the parasite-triggered recruitment and increase in number of cells of the macrophage lineage. This model has been shown to be of value in drug screening [56] and in the assessment of immune effectors – in particular, selected antibody subsets – against the recently identified merozoite surface-protein-3 antigen [57] and other vaccine-candidate antigens.

In this system, *P. falciparum* interacts with its normal host cell (human RBCs), and selected human cell types (such as mononuclear cells) can be grafted into the same mice. The ability of the same mice to be grafted with human lymphocytes creates opportunities for the production of human monoclonal antibodies and, perhaps more desirable, preclinical assessment of the immunogenicity of

novel vaccine formulations. Although promising results have been already obtained [58], these 'human' immunogenicity models are yet to be developed fully.

The receptivity of immunodeficient mice to xenogenic grafts of human origin is now being used in an attempt to solve a longstanding bottleneck: access to functionally intact human hepatocytes. This has obvious applications to the study of hepatotropic pathogens, which include some of the major killers: HBV, HCV, human malaria liver stages and *Listeria*. Two recent reports, combining immunodeficiency with genetic defect that permit the depletion of mouse hepatocytes (UPA-SCID and FAH-Rag2), have been extremely promising; one showed the susceptibility of grafted hepatocytes to HBV [59], and the other to HCV [60]. In theory, these approaches offer an almost unlimited range of possibilities for the development of new models in the short-to-medium term but, like other models, they might prove to be technically demanding. The introduction of human IgG genes and even the entire human chromosome 3 into mice is another novel development that enables the production of human antibodies by immunization of the mice [61,62]; however, any antigen presentation in this situation is still made in the context of mouse MHC genes.

Modern transgenesis tools have also been applied to the modification of pathogens. *P. falciparum* vaccine-candidate molecules have been expressed in the rodent species *P. berghei* and in the primate species *P. knowlesi*. In these cases, the immunization process is still biased by the rodent or primate immune systems and their immune responses to the vaccine-candidate antigens could differ from those of humans. However, these systems might prove to be useful for testing effectors against a given vaccine candidate. Similar models can be envisaged for drugs that target a well-identified metabolic pathway of a given pathogen. Transgenic parasites expressing the green fluorescent protein are now available for *P. berghei* (and soon for *P. falciparum*), and will assist the development of high-throughput techniques for screening novel antimalarial compounds. Almost unlimited possibilities are opened up by these new technologies. Nevertheless, each will require time to be developed and, above all, to be assessed and validated.

Concluding remarks

Although humans remain the best 'model' for studies of human diseases, much of the currently available knowledge relies on model data, which will be a better fit for some host–pathogen interactions than for others. Existing animal models of infection and disease need to be improved and new model systems need to be developed. The ethical issues raised by rapid transfer to clinical trials have to be balanced against those associated with the use of animals in experimentation, particularly when non-human primates are involved. The difficulties of interpreting data from animal models stem, at least in part, from a failure to invest time and effort in defining the parameters for any given host–pathogen combination and from the failure to make a systematic evaluation of the advantages and disadvantages of the existing models. New models will undoubtedly bring advances in our understanding but, like the current models, their relevance should be precisely defined.

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