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Human *Plasmodium* Liver Stages in SCID Mice: A Feasible Model?

E. Badell, V. Pasquetto, W. Eling, A. Thomas and P. Druilhe

In a recent issue of Parasitology Today, *Stanley and Virgin*[1] *have stressed the potential of B- and T-cell deficient mice, among which severe combined immunodeficiency (SCID) mice are most frequently used, as models for the study of parasites. One of the most tantalizing prospects has been in the development of liver stages (LS) of human* Plasmodium.

The report by Sacci *et al.*[2] on the establishment of *Plasmodium falciparum* LS in SCID mice generated considerable interest and excitement, as it appeared to offer a means to overcome the most critical limitation faced in human LS research. The technique promised a means to improve our understanding of the biology and development of human LS, because it increased the quantity of LS available for study, and offered the potential to store human hepatocytes, *in vivo*, thus facilitating the development and evaluation of pre-erythrocytic-stage vaccines and drugs.

Because of the enormous potential of the technique, we have (over the past two years) made extensive efforts to achieve similar results, using the published procedures and various alternative procedures; so far, we have had no success. Although this has led us to develop some reservations about the potential of the method described, particularly because *P. falciparum* LS could be produced in parallel using the *in vitro* techniques that we designed previously[3,4], we considered our results to be preliminary. Furthermore, human LS research is notorious for problems associated with the provision of infectious sporozoites and suitable hepatocytes, such that variation between laboratories may be attributed to a wide range of variables.

In recent communications, others[5] have expressed doubts about the suitability of SCID mice for LS development. These reservations were based, in large part, upon observations that: (1) hepatocytes from a number of sources (human fetal, cryopreserved human adult, hamster and mouse) and human red blood cells (RBC) did not survive transplantation into the SCID mouse; and (2) that transplanted HepG2 (hepatocyte tumor) cells did not support sporozoite invasion by *P. berghei* or *P. vivax*, *in vivo*. However, HepG2 cells, infected with LS *in vitro* and subsequently transplanted to SCID mice would support LS development of *P. berghei*. Although our own experiments have led us to the same reservations regarding the method of Sacci *et al.*[2], some arguments put forth by Butcher *et al.*[5] are not in agreement with our observations and, in our opinion, are not sufficient to rule out the potential of this model.

Of crucial concern is the mid- to long-term viability of xeno-transplanted material in the SCID mouse. Over the past two years, we have performed parallel *in vitro* cultures and *in vivo* experiments in SCID mice, aimed at producing *P. falciparum* LS. A total of 28 SCID mice, checked as being 'non-leaky' (not reverting to immunocompetence), have received implants of human hepatocytes, either as collagenase-dissociated cells (in ten mice) or as minced slices (in 18 mice): three in the kidney capsule, three in intrasplenic locations, six in hepatic locations and 19 in other organs. The survival of these hepatocytes was investigated (at intervals that varied from one week to four months) using histological studies and by labeling with anti-human albumin monoclonal antibody (Sigma). Functionally active hepatocytes were demonstrated in 15 out of 28 mice after delays as long as four months (three out of three animals). On the basis of criteria that included morphology and the proportion of albumin-secreting cells, spleen and liver-transplant locations were found to be superior to the kidney capsule. Our study thus suggests that, despite the persistence of polymorphs, monocytes and natural killer (NK) cells, a human liver graft can survive for a useful period in immunocompromised animals. This convinced us that the model was not as impractical as suggested[5]. However, attempts were unsuccessful to infect these hepatocytes, *in vivo*, using *P. falciparum* sporozoites (NF54 strain) inoculated intravenously [in numbers between 20 and 50 times higher (10^6 per mouse) than those used by Sacci *et al.*[2]]. There was no observation of any LS developing in sections taken from these implants (250 to 500 sections per biopsy, one in five being screened). An immunohistological screen was used, using mouse antibodies to peptides derived from the LS-expressed molecules LSA1, LSA3, SALSA[6] and CS, and African adult serum, all of which stained LS in control slides that had been produced *in vivo* (in *Cebus apella*[7] and in chimpanzee[8]), or *in vitro*. They were further checked following Giemsa staining. In parallel experiments, however, the same sporozoites invaded and developed into mature LS in cultured human hepatocytes. In one case, hepatocytes

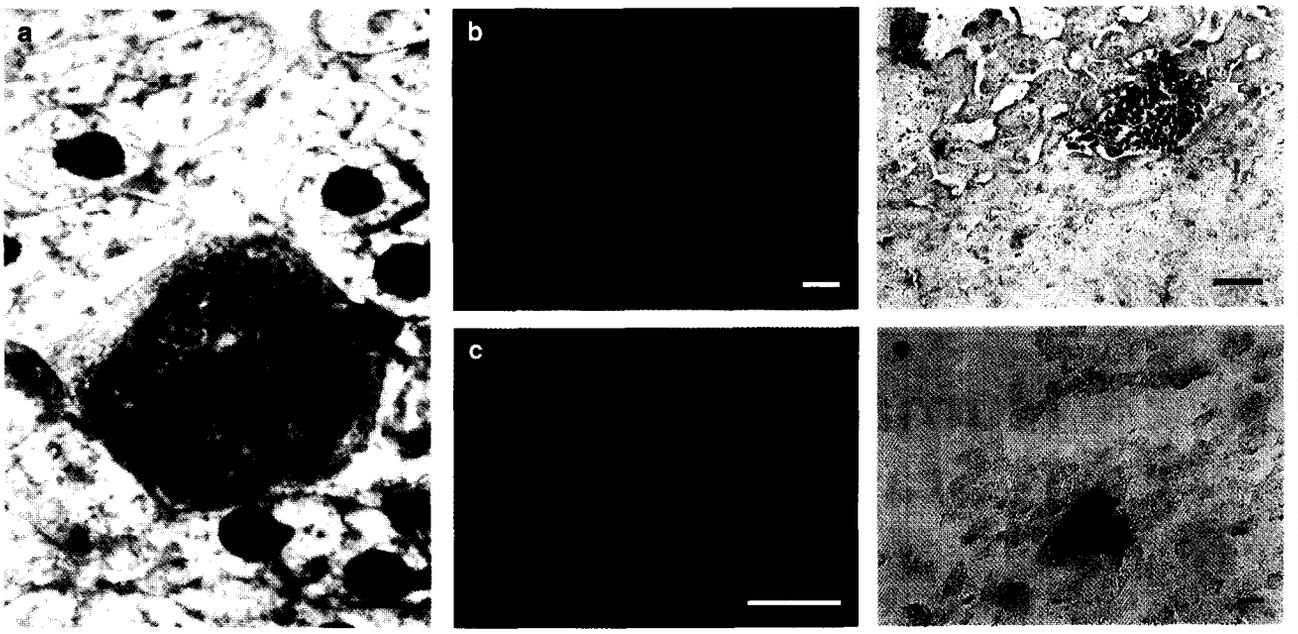

Fig. 1. Giemsa-stained mature liver stage (LS) produced in Saimiri monkey seven days post-injection of *Plasmodium falciparum* sporozoites (P. Druilhe, unpublished) (a). IFA labeling of mature LS obtained in Saimiri using a human hyperimmune serum (b) and of LS produced in chimpanzee (Ref. 8) using mouse antibodies directed against the liver-stage specific molecule LSA1 (c). Results from two *in situ* hybridization assays shown in (d) and (e) of LS produced in chimpanzee with *P. falciparum* labeled DNA, according to Ref. 9. Scale bars = 20 μm.

of the same origin were tested *in vitro* and *in vivo*, and were infected with the same batch of sporozoites. Again, LS were readily detectable *in vitro*, but not *in vivo*. These results were disappointing in view of the reproducibility of *P. falciparum* sporozoite inoculation reported for the SCID/human hepatocyte model (34 successful out of 34 tested)[2].

The extreme difficulties over interpretation are highlighted by collaborative experiments in which SCID mice that had been implanted in Baltimore with human hepatocytes were inoculated intravenously with *P. falciparum* sporozoites in two European laboratories. Although immunofluorescent bodies that resembled those reported previously could be seen, Giemsa staining of material under the kidney capsule five to six days after infection did not reveal any clear LS, and *in situ* DNA hybridization was also negative, while the same DNA probe was positive when using LS produced *in vivo* in monkeys (Fig. 1). From an arithmetical point of view, the efficiency with which *P. falciparum* sporozoites transformed into developing LS according to Sacci *et al.*[2] appears to be surprisingly high. Results obtained in the liver of chimpanzee[8] and that of the Cebus monkeys[7] suggest that, for high sporozoite loads from dissected salivary glands, a transformation rate of approximately 30% may be attainable, *in vivo*. Even assuming all *P. falciparum* sporozoites inoculated migrate to the transplant under the SCID kidney capsule, and assuming an optimal transformation rate, the resulting LS density reported following inoculation of 5×10^4 sporozoites[2] remains unexpectedly high. There is thus some concern as to the nature of the fluorescent bodies observed by Sacci *et al.* If these were indeed LS, it is crucial that the inter-laboratory reproducibility of the method is rapidly established.

On the basis of the poor survival of human hepatocytes, LS development of *P. falciparum* was not attempted by Butcher *et al.*[5] However, successful LS development of the rodent species *P. berghei* was reported by transplant of infected HepG2 cells *subsequent* to *in vitro* infection with sporozoites, based on emergence of blood infection in the receivers. The possibility that this transplant to the SCID mouse kidney capsule 5 h after infection also contained viable sporozoites that would then be able to invade the recipient's *own* liver was discounted by referring to prior observation rather than by parallel control. We have found that, although sporozoites maintained at 37°C and recovered from the supernatant of culture wells after 3, 4 and 7 h showed decreasing infectivity with time, even after 7 h there remained enough infectious sporozoites to initiate infection in mice. Such residual infectivity at ambient temperature and at 37°C has been demonstrated in five separate experiments: four with *P. yoelii* (265BY strain) and one with *P. berghei* (ANKA strain). With a readout (namely the occurrence of a subsequent blood infection) that does not allow discrimination of the LS developmental site, the question of sporozoite longevity *in vitro* becomes crucial. As it would appear that sporozoite infectivity can be retained, under some circumstances, beyond a 5 h window, it may be a possibility that the bloodstage infections observed are consequent to LS development in SCID mouse liver, rather than in transplanted liver. This concern may also be supported by the apparent contradiction that transplanted HepG2 cells are not available for sporozoite invasion and yet, according to the ratio of onset of infection (ROI) quoted, merozoites released from HepG2 transplants are essentially as numerous and as effective in RBC invasion as those that derive from normal liver infections[5].

The potential of the SCID mouse model for human malaria parasites was also diminished according to Butcher *et al.* due to the rapid clearance of human RBC after injection into SCID mice. This is a crucial issue because the last phase of LS schizont maturation and the subsequent invasion of RBC by liver merozoites are extremely difficult to achieve *in vitro*, at least with *P. falciparum*. Our own experiments confirm that *P. falciparum* asexual bloodstages fail to survive in human RBC for more than 48 h in the blood of SCID mice. However, we have found that, by intraperitoneal

inoculation, we can reproducibly substitute between 50 and 90% of mouse RBC with human RBC, and that these can be maintained for up to four weeks (ascertained with both anti-Glycophorin and anti-Rh monoclonal antibody labeling, and by agglutination assay).

The use of the SCID/human hepatocyte system for the propagation of malaria LS is a technically demanding operation in which a number of variables are involved. Given the enormous potential of such a system, which may be able to support the full liver stage and subsequent erythrocytic cycle, it is critical that analysis is thorough. The use of immunodeficient mice with other defects, such as SCID/Beige, or derived on backgrounds other than the traditional BALB/c strain, such as NIH III and/or transgenic animals (Rag 1 and 2) might be worth investigation. Despite the data produced by Butcher *et al.*, we believe that the demonstrated survival of human hepatocytes in SCID mice by ourselves (particularly in splenic and hepatic locations) and others[10], and the observation that, under some circumstances, SCID mice can be repopulated with human RBC and support the erythrocytic cycle, should promote rather than discourage further efforts to define reproducible conditions under which *P. falciparum* LS may be studied in immunodeficient mice. A combined effort between several research groups at the international level would be welcomed for this purpose.

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Outlook

Use of a Simplified Polymerase Chain Reaction Procedure to Detect *Trypanosoma cruzi* in Blood Samples From Chagasic Patients in a Rural Endemic Area

P. Wincker and colleagues *Am. J. Trop. Med. Hyg.* 51, 771–777

In the initial acute phase of *T. cruzi* infection, diagnosis of Chagas disease is relatively straightforward because of the high levels of parasites in the human host circulation. However, the individuals then enter a chronic phase which lacks clinical symptoms, and sometimes develop clinical forms of the disease, leading to morbidity and death. During this long and indeterminate phase, parasites cannot be detected, and people have questioned the involvement of *T. cruzi* in disease pathology. Wincker *et al.* here look at the applicability of PCR technology for detecting *T. cruzi* DNA in blood samples of Chagas patients. Previous large-scale attempts to do this involved a complex chemical treatment for splitting the kinetoplast DNA, but were unsatisfactory because reagents were expensive and there was a risk of blood sample contamination. The present study circumvents these problems by boiling the DNA.

The study group was from a rural endemic area in which Chagas disease has been researched for 18 years, and many individuals have frequently been serologically tested for *T. cruzi*. A larger study group than this will be needed before definite conclusions can be made, but results show PCR to be a powerful complement to serology for the diagnosis of Chagas disease, particularly: (1) when serology cannot serve as a good indicator because the patient is undergoing specific treatment; and (2) in chronic Chagas disease, when serology cannot detect parasites in the circulation. Parasite DNA can be detected in most Chagas patients (by PCR), which corroborates those studies that have shown the presence of *T. cruzi* in heart biopsies with chronic disease (using the immunoperoxidase method of parasite antigen detection).

The whole nature of the role of *T. cruzi* in the pathology of the chronic stages of Chagas disease has to be revisited. ■

A New Method for Active Surveillance of Adverse Events From Diphtheria/Tetanus/Pertussis and Measles/Mumps/Rubella Vaccines

P. Farrington and colleagues *Lancet* 345, 567–569

This paper reports a record-linkage method for effective identification of vaccine-attributable adverse events. In situations where vaccine safety relies on passive reporting of adverse vaccine reactions by clinicians, under-reporting is a common inevitability. In 1993, for instance, such passive surveillance failed to detect an unacceptably high risk of aseptic meningitis with measles/mumps/rubella (MMR) vaccines that contained the Urabe strain. The *Haemophilus influenzae* β vaccines have been recently introduced, and acellular pertussis vaccines are probably to be introduced soon, and there is clearly an urgent need to find a more reliable method for the surveillance of adverse reactions.

By correlating vaccination records with computerized hospital admission records, Farrington *et al.* report a study of the association between diphtheria/tetanus/pertussis (DTP) vaccination and febrile convulsions, and between MMR vaccination and febrile convulsions and idiopathic thrombocytopenic purpura.

They found an increased relative incidence for convulsions 0–3 days after DTP vaccination, which was limited to the third dose of vaccine (risk, one in 12 500 doses). For MMR vaccinations, 67% of admissions for convulsions 6–11 days later were attributable to the measles component (risk, one in 3000 doses) for one in 2600 Urabe doses.

The need for active surveillance of adverse effects is justified by the fact that the level of risks ascertained by this method was up to five times that calculated by more passive methods. ■