Malaria, Malaria and more Malaria

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The proliferation of international meetings focusing on malaria is, in part, a reflection of the increased awareness of the scientific community of the extent of the problem which, despite their significant successes, malaria continues to present. European Commission-supported researchers have been in the vanguard of recent developments in international coordination of attempts to provide new tools to combat malaria. This meeting offered an opportunity to reflect on what had been achieved and what the future might hold.

Sweating it out, with PCR?

Major differences appear to exist in entomological inoculation rate (EIR) within a single village, and even between people in the same household. Such variations do not occur simply as a result of sampling techniques, but also because sympatric *Anopheles* species have greatly different host preferences and, therefore, vectorial roles. For *Anopheles gambiae*, the principal vector in Africa, the emanations of the skin of human feet are the most important cue for attracting mosquitoes. These cues have been located in the fatty acid component of sweat. The emanations are different from person to person and could be related to other host factors, such as genetic background and behaviour. The consequences for generalizations based on EIR in a certain geographic area are clear. In addition, these differences may influence the relative contribution to transmission and the disease history of individuals. Micro- and macro-epidemiology is therefore needed to understand the dynamics of malaria in a given area. Odour-baited traps (OBT) for mosquitoes could soon replace human biting catch for experimental purposes. Their use in combination with electrocution traps is already operational. Further studies will also concentrate on the potential for the use of OBT as a means of control (W. Takken, B. Knols, Wageningen Agricultural University, The Netherlands; L. Mboera, NIMR, Muheza, Tanzania; M. Coluzzi, Universita degli Studi la Sapienza, Rome, Italy; G. Gibson Imperial College, London, UK).

Of course the ability to be able to identify vectors and parasites is crucial. Developments in the PCR (polymerase chain reaction) technique have been further refined in order to study the various parasite life cycle stages in the mosquito gut (oocyst stage) and salivary gland (sporozoite stage). Many difficulties are being experienced with contamination but with some further improvements, the technique will be feasible for study of genetic composition of parasites resulting from feeds on infected people, so that the relative survival rate of clonal types can be studied in more detail (V. Do Rosario, IHTM, University of Lisbon, Portugal; Y. Touré, Ecole Nationale de Médecine et de Pharmacie, Bamako, Mali; M. Coluzzi).

Building on Biology

The sex life of *Plasmodium* in the mosquito midgut, and the success rate of different genotypes in fertilization and transmission influences the composition of the parasite population. Detailed analyses using state-of-the-art molecular techniques has revealed a large number of genotypes in most individuals. The cross-fertilization of parasites in the mosquito does not occur with the expected frequency, confirming that not all clonal types are equally successful in the mosquito. The extent to which clonal or panmictic elements are found in the mosquito and the stability of gene linkage, are related to the intensity of transmission which will in turn influence the development of protection, or disease, as a function of the number of genotypes an individual carries, or has experienced. Drug-resistant phenotypes may be selected as a consequence of the genetic diversity of the parasites spread by mosquitoes. Disease epidemiology and the spread of drug resistance will be better understood when EIR is mapped in conjunction with parasite genotype prevalences (K. Day, University of Oxford, UK; D. Walliker, University of Edinburgh, UK; C. Frontali, Istituto Superiore di Sanità, Rome, Italy; M. Alpers, Institute of Medical Research, Papua New Guinea). The duration of overall patency of infection is inversely correlated with the number of simultaneous clonal infections leading to the speculation that an antigen-dependent stimulation threshold may need to be passed before non-antigen-dependent parasite-killing mechanisms are triggered. Hence, a low level of many different parasites may be maintained for a long period.

Regulation of gene expression has been studied for a variety of genes using the synchronized model of *P. berghei*. Of specific interest are the genes involved in switching from asexual to sexual development. Although the research is fundamental, this work has led to the first stable integration of transfected foreign DNA fragments in malaria parasites. Gametocyte-specific genes in *P. berghei* are clustered on chromosome 5. Work on the analysis of reproducible rearrangements in the sub-telomeric regions of these chromosomes is in progress and may lead to the identification of a molecular basis for the switch to gametocyte production. These findings may be translated to human parasites. In biological terms, the duration of gametocytogenesis is one of the most obvious differences in the life cycles of rodent parasites and *P. falciparum*. Other genes studied include Pb21/Pf28 and ribosomal genes. In the case of Pb21, a gene coding for a protein explosively produced soon after fertilization, accumulation of mRNA in gametocytes was described, the translation of which was triggered by transfer to mosquitoes (C. Janse, University of Leiden, The Netherlands; R.E. Sinden, Imperial College, London, UK; M. Ponzi, Istituto Superiore di Sanitá, Rome, Italy; M. Rodriguez, Instituto Nacional de Salud Publica, Tapechula, Mexico; H. del Portillo, Universidade de São Paulo, Brazil).

Vaccines and Immunology

Vaccine studies remain a key focus of malaria research with the concerted European action for the development of malaria vaccines (VINCOMAL) completing its first year of consultation. VINCOMAL is attempting to develop guidelines for the rational assessment of malaria vaccine candidate antigens; adjuvants and delivery systems. This is not a simple task; the field is highly empirical. In addition, VINCOMAL has

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been charged with responsibility for identifying obstacles and operational bottlenecks in European malaria vaccine development and for providing suggestions as to how these may be overcome (S. Jepsen, Statens Seruminstitut, Copenhagen, Denmark).

Meanwhile, basic studies on vaccines are continuing, sometimes with quite unexpected results. Rather than protecting mice against *P. yoelii*, immunization with heat shock protein HSP70-1 enhanced transmission of the parasite to mosquitoes. This was suggested to be a result of the stimulation of direct gametocytogenesis from liver merozoites. Protection induced by *P. yoelii* MSP-1 (merozoite surface protein 1) C-terminus was strain specific, reflecting considerable sequence diversity between different strains. Other approaches are being followed, including the elution of MHC (major histocompatibility complex)-bound parasite peptides from *P. yoelii* and *P. vivax* infected hepatocytes. Using this approach, several promising new potential vaccine candidate antigens have been identified (D. Mazier, S. Pied, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; A.A. Holder, NIMR, London, UK; V. Chauhan, ICGEB, New Delhi, India; M. Kombila, Université de Libreville, Gabon).

GLURP (glutamine-rich protein), MSP-1 and MSP-2, and variants thereof, have been examined in direct isolates and after short-term cultures. A minimum of seven forms of GLURP (RII region), 13 forms of MSP-1 and 16 forms of MSP-2 intra-allelic variants have been identified. Combining data on the unique occurrences of any particular allele in 19 Beninois children revealed a maximum of 35 different genotypes in this group at a single point in time, ie. 2–5 genotypes per sample. The paucity of suitable markers for other variations suggests that this is a gross underestimate of the potential variability of 'wild' isolates and this may have a significant impact on recombinant vaccine success. This study also highlighted the loss of variability during *in vitro* manipulation (A. Ebrahimzadeh, G.H. Mitchell, Guy's Hospital, London, UK; J. Langhorne, Imperial College, London, UK; A. Sanni, Université Nationale de Benin, Cotonou, Benin).

Studies on AMA-1 (apical membrane antigen 1) have focused on different parasite species and different hosts. In *P. falciparum* and the chimpanzee parasite *P. reichenowi* the AMA-1 possesses a 17 kDa N-terminal (NT) region. This NT region shows 28% variation between *P. falciparum* and *P. reichenowi* while the rest of the molecule shows only 7% difference at the nucleotide level. The PR allelic form was not found in the field. The invasion of human red blood cells by both species is inhibited by a mAb (monoclonal antibody) to the ectodomain of the processed (66 kDa) form of AMA-1 downstream of the NT region. AMA-1 is recognized by antibody specificities in human serum from some endemic regions and these antibodies strongly competed the binding of the mAb which inhibits parasite invasion *in vitro* (A. Thomas, BPRC, Rijswijk, The Netherlands; M. Nwagwu, University of Ibadan, Nigeria; A. Sanni).

Natural and recombinant forms of AMA-1 are often highly immunogenic, even without adjuvant. So far, potentially protective epitopes have been shown to be conformational, so a suitable expression system is essential. The yeast *Pichia pastoris*, which enables relatively easy downstream processing, has been successfully used to express folded, homogeneous *P. vivax* AMA-1 (PV66) ectodomain at very high secretion levels. The product is immunogenic in experimental clinical vaccine trials and induces high antibody levels in rabbits and primates (C. Kocken, BPRC, Rijswijk, The Netherlands; A. Thomas).

One finding which seems to take on more and more importance is that not all endemic areas are equal. For example, in the Amazon region in Brazil, where malaria is hypoendemic, immune resistance does not really develop. The comparison of multiple sites with varied levels of transmission can prove to be most informative. The development of protection (or lack of protection) can be studied against the background of the clinical, parasitological, immunological and genetic characterization of the study population. Immune responses at the humoral and cellular level against *P. falciparum* antigens MSP-1, MSP-2, Pf332 and R23, selected in part for their protective activity in monkey model experiments, have been analysed in longitudinal studies in correlation with the immune status. It has been assessed that immune protection defined by a reduced risk for malaria attacks correlates with the finding of dominant IgG3 responses against total *P. falciparum* antigen extracts. Analysis of isotype specific responses against individual recombinant antigens are in progress (L. Pereira da Silva, Institut Pasteur, Paris, France; C. Roussilhon, Institut Pasteur de Dakar, Senegal; J-F. Trape, ORSTOM, Dakar, Senegal; E. Camargo, Universidade de São Paulo, Brazil).

Vaccine Variants and Extra-erythrocytic Immunity

The transmission-blocking assay is one of the few assays in the field of malaria immunology in which the correlation with 'protective' activity is relatively straightforward to establish. However, 'one has to know one's assays'; and so extensive testing is required with the same assay being applied in different places, such as in Yaounde and Nijmegen, with gametocytes directly from donors or from culture, with and without the serum of the patients. The methods of counting oocysts are important in reducing variation in test results. The TB test is highly specific, but has a relatively low sensitivity, so that utmost care should be taken when analysing negative results. Strain-specific blocking may be found by comparison of the field transmission-blocking data with those in the laboratory with one specific strain. Questions about variability and cell-mediated immunity are emerging in relation to the sexual bloodstages (J.P. Verhave, R. Sauerwein, University of Nijmegen, The Netherlands; C. Boudin, ORSTOM, Paris, France; A. Bilongo Manene, OCEAC, Yaounde, Cameroon).

The *var* genes of *Plasmodium* analysed so far map in subtelomeric positions on the chromosomes. It is now well established that in eukaryotes the telomeres deteriorate as a function of cell division. The enzyme telomerase can elongate the chromosome again to facilitate further divisions. The gene for this enzyme has been found to be expressed in germ-line cells, tumour cells and unicellular organisms. The length of the telomeres in *Plasmodium* is rather constant, which indicates telomerase activity, which has been demonstrated. The gene for *Plasmodium* telomerase is now being cloned and could help substantially in better understanding the chromosomal organization of the malaria parasites, and the possibility of designing attenuated parasites (telomerase knockouts) (A. Scherf, Institut Pasteur, Paris, France).

In the malaria pre-erythrocytic stage (MPES) network, multi-centre, multidisciplinary approaches have been a feature of concerted efforts to understand extra-erythrocytic immunity. Malaria pre-erythrocytic stage vaccine development work has challenged the dogma that irradiated sporozoites

induce strong protection, offering an alternative suggestion that the more artificial the host–parasite combination the easier it was to induce immunity. Immunity is easily achievable against *P. berghei* in some strains of mice, but *P. berghei*-irradiated sporozoites do not induce protection in its natural host *Thamnomys*. Of the many mechanisms that protect rodents against malaria, those which will prove to be effective against human malaria remain to be determined. The focus on crucial events in the liver is welcome. Too often the liver has been viewed as a 'black box', with the single read-out being the assay of parasitaemia. Of the 20 antigens that are the focus of the network LSA-1 (liver-stage antigen 1), LSA-3, SALSA (sporozoite and liver-stage antigen) and STARP (sporozoite threonine- and asparagine-rich protein) are most advanced, with LSA-3 seeming at present to be the strongest candidate for inclusion in a vaccine. LSA-3 is encoded by a highly conserved gene. Based on rodent, primate and human (field) studies LSA-3 appears to play a crucial role in protection. Responses to *Pf* LSA-3 in humans show clear B- and T-cell activity. *Pf* LSA-3 also protects mice against *P. yoelii* challenge, indirect support for the conserved nature of the molecules (P. Druilhe, K. Brahimi, Institut Pasteur, Paris, France; M. Wery, S. Chatterjee, ITM, Antwerp, Belgium; C. Roussilhon, Institut Pasteur de Dakar, Senegal; J. Langermans, BPRC, Rijswijk, The Netherlands).

Re-examination of irradiated sporozoite immunizations in chimpanzees shows that the correct dose of irradiation is essential. Live, attenuated, sporozoites will only protect if they are capable of invading hepatocytes. Hyper-irradiated sporozoites which are incapable of invading the liver do not induce protection. The most obvious hypothesis is that the protection induced by irradiated sporozoites is not sporozoite-based. Responses to LSA-3 NRII, figure prominently in protected volunteers immunized with irradiated sporozoites whereas such responses are absent from non-protected volunteers. Lipopeptides based on some of the antigens under study have demonstrated the capacity to induce strong B- and T-cell responses in various models, including chimpanzees, without the need for adjuvants (L. BenMohammed, Institut Pasteur, Paris, France; A. Tartar, Institut Pasteur, Lille, France; P. Druilhe; A. Thomas).

There are many routes to identifying and evaluating molecules or constructs for their potential to become vaccine components. Data generated *in vivo* (using natural antibodies or cells) can be double-checked *in vitro* (ADCI) and subsequently tested by transfer studies, either in animal models or in humans ('curative' Ig fractions), followed by retrospective analysis of the active fractions in these purified Ig preparations. For GLURP, antibodies can be detected in Liberian adults to at least two specific regions of the molecule, which were preliminarily associated with protection. In fact there are at least three reactive types: R0 specific, RII specific and crossreactive (R0 + RII) antibodies. Neither the antibodies nor the monocytes alone have a direct inhibitory effect *in vitro*. But, if antibodies and monocytes are combined, an inhibitory effect is observed, with R0 or RII antibodies. The relevance of the novel merozoite antigen, MSP-3, was confirmed by further ADCI assays and epidemiological studies, showing a correlation between the acquired protection and the titres of cytophilic (IgG1 and IgG3) classes of anti-MSP-3 antibodies. Development of the SCID-Hu (severe combined immunodeficiency mice–human) system has enabled the group to establish parasitaemias between 0.1% and 3% for periods of up to two months. ADCI tests can be performed in such animals after addition of selected human (or primate) components. In preliminary experiments only a combination of specific antibodies and monocytes caused a drop in parasitaemia (S. Jepsen; P. Druilhe; M. Theissen, Statens Serum-institut, Copenhagen, Denmark; C. Oeuvray, E. Badell, Institut Pasteur, Paris, France).

A pilot project has assessed the feasibility of immunizing vertebrate hosts with mosquito-derived antigens in order to shorten the life span of mosquitoes after feeding on such immunized individuals, thereby reducing transmission. Some mosquito midgut fractions induce responses that have a significant effect on longevity and fecundity of mosquitoes in the laboratory situation, as has been established for certain monoclonal antibodies (mAbs). In these studies, subcellular fractions of midguts from sugar-fed *A. stephensi* were used as antigens and varying antibody titres were observed after immunizations. Any significant effects on mosquito fecundity or longevity were confounded by the high variability in the assay system (P.F. Billingsley, University of Aberdeen, UK; A. Almeida, IHTM, University of Lisbon, Portugal; E. Lyimo, The Ifakara Centre, Tanzania).

Getting High on Drugs

Drug resistance will remain a major problem, but there have been some developments that provide hope that new drugs may be developed. The rationale for development of antimalarial compounds should normally be based on a fundamental knowledge of crucial pathways in parasite metabolism. Such has been the case for inhibitors of phospholipid synthesis, where work has been driven by the need to bridge the gap to industrial development by performing initial preclinical and clinical trials. CTP:phosphocholine cytilyltransferase, a crucial enzyme in the pathway is now cloned, and can regulate the phospholipid synthesis. The interference of the synthetic compounds is based on their interaction with the choline carrier in the red blood cell membrane, where they mimic choline and become an inhibitor of the phospholipid metabolism. In the preclinical phase the therapeutic index of the lead compounds is very promising. Of the 25 monkeys with parasitaemias close to lethal levels that were treated with the compounds, 24 cleared their parasitaemia rapidly (within four days) and showed no sign of recrudescence in a longitudinal follow-up monitored by PCR. Only one monkey showed rapidly increasing parasitaemia, followed by death after the start of the treatment, which might be explained by the possible release of large numbers of sequestered parasites. Comparison of G25, one of the lead compounds, against five other drugs (chloroquine, quinine, mefloquine, halofantrine, arthemeter) revealed that the G25 showed no crossresistance with any of these drugs. Furthermore, prolonged *in vitro* studies on stability of sensitivity of cloned parasites did not reveal any development of resistance (H. Vial, M. Calas, Université Montpellier II, France; J. Bickii, OCEAC, Yaounde, Cameroon; S. Herrera, Universidade Del Valle, Cali, Colombia; D. Warhurst LSHTM, London, UK; A. Thomas).

Protease inhibitors are a more conventional target for antimalarial development. Inhibitors of these enzymes could conceivably work at all stages of invasion of the parasite (merozoites, sporozoites, salivary glands and liver) and ookinetes (midgut). The production of pseudopeptides could be one effective way to establish a blockade. A putative gene for the merozoite

proteinase for erythrocyte invasion (MPEI) has been isolated and cloned, revealing a 72% AT-rich gene, related to zinc metallopeptidases. Expression of this gene was demonstrated in erythrocytic stages, and correlates with the observed peak of co-presence of MPEI and aminopeptidases. Additional fluorescence studies show that MPEI expression is compartmentalized within the parasite (J. Schrevel, I. Florent, Museum National D'Histoire Naturelle, Paris, France).

DNA replication enzymes are another new target. Earlier EC-funded studies had indicated structural differences between plasmodial DNA polymerases and topoisomerases and those of the host. A series of 31 DNA minor groove binders with preference for AT-rich sequences were tested for antiplasmodial activity in culture and against partially purified plasmodial topoisomerase I and II; the most potent derivative had an IC_{50} of 20 nM in *P. falciparum* cultures with an *in vitro* therapeutic index of 1000. A large number of acyclic nucleoside phosphonates, potential DNA polymerase inhibitors, some of which [9-(2-phosphonylmethoxyethyl)adenine (PMEA) and 9-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC)] are potent inhibitors of viral DNA polymerases and currently in progress of being registered as antiviral drugs – were tested for antiplasmodial activity, revealing that three conditions are essential for such activity: a purine base, a hydroxyl group in the acyclic side chain and a phosphonate group terminating this chain. HPMPA [9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] was the most potent antiplasmodial of all. Its activity is parasite-stage dependent: it does not kill erythrocytic parasites instantly but abruptly inhibits DNA replication in mid-schizogony. UV-induced resistance to it was re-lated to specific point mutations in the 3'–5' exonuclease domain of the DNA polymerase-delta gene, identifying this polymerase as the main target enzyme. Most importantly, HPMPA (in contrast to almost all regular antiplasmodial drugs) is even more active against liver stages of the parasite, without abolishing liver-stage-induced protective immunity. This opens the way to specific host cell targeting of the drug in order to enhance its effectivity and reduce its toxicity (J.P. Overdulve, University of Utrecht, The Netherlands; P. Wilairat, Mahidol University, Bangkok, Thailand; E. De Clercq, J. Balzarini, Rega Institute for Medical Research, Leuven, Belgium).

The fact that compounds occur 'naturally' does not guarantee that they will be more effective and less toxic. Nevertheless, some herbs used in traditional medicine may contain active compounds with potential for development as antimalarials. Oxygenated chalcones have been identified as lead compounds from Chinese herbal remedies. When one of these, LICA, was tested on mice infected with lethal strains of rodent malaria parasites, the mice were cured and blood transfusion to naive mice did not result in infection. The therapeutic index of LICA in mice is around 100, and parasite-specific enzyme inhibition is indicated (A. Kharazmi, Statens Seruminstitut, Copenhagen, Denmark; S. Christensen, S. Frokjaer, Danmarks Farmacuetisk Højskole, Copenhagen, Denmark; S. Herrera).

Drug Resistance: Back to Basics

The mechanism of the action of chloroquine and the development of resistance to chloroquine has exercised the minds of many scientists. Recent developments have thrown some 'new' light on an old topic. The Na^+–H^+ exchanger is a candidate chloroquine transporter, and chloroquine uptake is carrier mediated. The process is temperature sensitive, and the uptake of labelled chloroquine can be inhibited by addition of 'cold' chloroquine. The kinetics of this uptake differ in sensitive and resistant strains of *P. falciparum*. The maximum uptake is the same, but the affinity of the carrier system for chloroquine in the resistant parasites is much lower. If the parasites from a genetic cross are analysed based on the differences in this system, the expression of resistant and sensitive phenotypes fits the genotypic patterns. The same holds true for all field isolates from different regions tested so far (M. Lanzer, University of Würzburg, Germany).

The drug EISA specifically inhibits uptake in both sensitive and resistant parasites. This drug is very specific for the sodium proton exchanger. These findings represent a major step forward in our basic understanding of the development of chloroquine resistance in malaria parasites. The monitoring of the development of chloroquine resistance in the field, has to be revisited with a view to standardizing the methodologies and making some quality control assessment of the drugs which are in use. Another important point raised in discussion was that naturally occurring chloroquine-sensitive strains may eventually cease to exist. We may need to consider them endangered species and preserve them, or face having to engineer them in the future.

More work on the genetic aspects of chloroquine resistance was reported from a study carried out in Gambian children. It showed that infections that resisted treatment with chloroquine or amodiaquine had a significantly raised prevalence of the Tyr86 allele of Pfmdr1. Not only were infections showing this allele more likely to resist treatment, but the recrudescent parasites showed a significant rise in Tyr86 prevalence. This is the first time that selection of a chloroquine-resistance-related mutation has been demonstrated in the human host during drug treatment (M.T. Duraisingh, C. Drakeley, R. Bailey, D. Warhurst, G. Targett, B. Greenwood, LSHTM, London, UK; O. Muller, MRC Laboratories, Fajara, The Gambia; G. Snounou, Northwick Park Hospital, London, UK).

Juggling with the Genome

The stable integration and expression of foreign DNA in *Plasmodium*, and the knockout of specific genes have now been achieved. The introduction of entirely heterologous sequences into the *P. knowlesi* genome has now been realized with plasmids derived from *P. berghei* and *P. falciparum*, which indicates fairly common regulatory sequences in different species. Transfection of this species offers the opportunity to analyse further the biology of antigens, not only in a natural host, but also in hosts that are closely related to humans. Vectors have been constructed that allow the expression of any open reading frame at varying levels and timing of transcription that function in both the rodent and primate species. For example AMA-1 from *P. falciparum* has been introduced into *P. berghei*; resulting patterns of expression and subcellular distribution are highly dependent on promoter control. Heterologous expression and artificially induced over-expression in transformed parasites may prove to be highly effective in inducing high antibody titres against this antigen. The possibility of gene over-expression, heterologous expression or gene knockout through genetic modification of malaria parasites brings improved possibilities for functional studies, adding a new dimension to our ability to design attenuated vaccines (C. Janse; A. Thomas; H. del Portillo; A. Waters, University of Leiden, The Netherlands).

Concertation

The INCO-DC (Scientific and Technological Cooperation with Third Countries and International Organizations) programme now has the possibility of supporting 'concerted actions' (CA) These are peer-reviewed projects where the costs of concertation, rather than the research costs themselves, are covered. These contracts offer an excellent mechanism to identify and optimally exploit existing facilities. In the field of malaria (mainly concentrated on vaccine R&D) a number of such CAs have been established.

VINCOMAL As described above, VINCOMAL regularly brings together a group of interested scientists from nine countries (six in Europe and three developing countries; DCs) to discuss the ins and outs of malaria vaccine development. The main issues are the rationalization of the vaccine development process by addressing the conceptual and practical hurdles in vaccine development for malaria. VINCOMAL also tries to identify ways to alleviate some of these barriers for the European situation. For more downstream issues VINCOMAL has close collaboration with other concerted actions described below, such as PVEN for pre-clinical evaluation in primates and AMVTN for the evaluation of vaccines in the field and a structural link to field studies for human immunology (S. Jepsen).

PVEN The Primate Vaccine Evaluation Network aims at co-ordinating and developing primate centres around the globe, with emphasis on centres in primate source countries (which are mainly DCs). It is an association of primate centers with research interest in parasitic diseases and other diseases of particular significance to developing countries. The core group has developed, with the help of internationally recognized experts, a book with guidelines covering a large number of important topics related to primate research. In addition, PVEN is busy developing initiatives to enable more regular exchange of reagents, techniques and knowledge via a primate reference programme, aimed at improved efficiency and reproducibility. This should not only improve the overall efficiency and quality of primate related research, but also improve animal welfare and contribute to gradual reduction of the use of primates (A. Thomas; S. Hererra; G.H. Mitchell; C. Bambra, IPR, Nairobi, Kenya; Q.S. Chen, Guangdong Shunde Institute of Laboratory Animals, China; N. Herrenschmidt, Centre de Primatologie, Fort Foch, France).

AMVTN (African Malaria Vaccine Testing Network) This CA has the principle objective of facilitating multicentre malaria vaccine trials. Already it has made excellent progress in obtaining and publishing an inventory containing information on potential malaria vaccine testing sites throughout Africa. Courses relevant to the design and conduct of malaria vaccine trials have been held and others are planned. Training, particularly for African scientists who may be involved in future malaria vaccine trials, has already started and more courses are being devised. The AMVTN provides an important forum for discussions on malaria vaccine trials. The first issue of the *AMVTN Newsletter* is now available. One of the most encouraging aspects for the future of the AMVTN and a clear indication of the achievements to date, has been the coordinated investment in its activities of a number of supporters of malaria vaccine research (W. Kilama, NIMR, Dar es Salaam, Tanzania; P. Jakobsen, State University Hospital, Copenhagen, Denmark).

SHARED The concerted action SHARED aims at an inventory of all projects in collaboration between Europe and DCs in the field of Health. Not only the research projects, but in a second phase also the projects directed at disease control or general health projects. In several European countries, focal points are presently under development (M. Traore, INRSP, Bamako, Mali; B. Mons, NWO, The Netherlands; C. Oepen, R. Korte, GTZ, Eschborn, Germany).

Final Discussions

In general, there was consensus among the participants that The European Union (EU) Programme for Research and Technological Development (RTD) and in particular the Science and Technology for Development (STD) and INCO-DC programmes have been instrumental in changing bilateral to multilateral research. In many cases the liaison among laboratories had created an essential critical mass of researchers in a given area. While networking had been highly successful, care was taken to ensure that there remained the capacity to absorb new topics and new researchers in the programme. The present malaria research portfolio of INCO-DC is geared towards meeting the health needs and demands of developing countries by providing the tools to tackle the continuing problem of malaria. The activities are 'investigator-initiated' and therefore also science-driven, and there was strong support for the view that it should remain this way. The cooperation of DC and EU scientists in the review of proposals and the consultation with experts on regional priorities were considered to be extremely valuable aspects of proposal assessment. Nevertheless, the need for an improved dialogue between scientists and policy makers was recognized.

Currently, some areas of malaria research are not well represented in the INCO-DC programme, but this reflects, in part, the types of applications that have been received. The programme remained open for topics like severe malaria. Opportunities for health systems research, with malaria as the focus, were also under-exploited. Aspects such as the organization of home- and self-treatment, health care at the peripheral level, the relation of non-medical control measures to the health sector, routine monitoring and evaluation and quality control of drugs were topics being supported by others. Where appropriate, these could be considered for support within the INCO-DC programme but it was accepted that the programme did not have the capacity to do everything. This led to further discussion on ways of harnessing other resources to support malaria research and, in particular, malaria vaccine development. The possibility was raised of a 'multi-country programme', where a number of interested EU Member States together with the EC take a special initiative to support activity on a given topic (drawing on articles 130 k, l and n of the Maastricht Treaty). This idea received strong support.

Recommendations

The previous Malaria Contract Holders meeting, in Copenhagen, produced a set of recommendations, many of which have since been implemented, while some remain relevant today. This meeting added several others:

(1) The widespread and science-driven approach of the INCO-DC programme which is responding to the needs and demands of developing countries should be continued, and proposals driven by southern researchers should be strongly encouraged. Steps should be taken to empower southern scientists in terms of accessibility of information.

(2) Support for provisional visits to develop common research proposals should be provided on a competitive basis.

(3) There is a major challenge presented by resistance to antimalarial drugs. The paucity of candidate drugs in the pipeline of pharmaceutical companies makes this situation even more serious. Based on the successful start with the better organization in the field of vaccines, the drug field has to unite for discussions with industry.

(4) Recent studies on the variations of mosquito genetics and behaviour, variation in parasite genotypes and human populations have indicated the crucial importance of micro- and macro-epidemiological differences in transmission intensity. Optimal use of existing tools and development of novel approaches should be encouraged and use of these tools in other research areas and control programmes should be facilitated.

(5) Remarkable progress has been made in our fundamental knowledge of the parasite genome, especially stable transfection, cross-species expression of genes and specific knockouts. These results should be exploited with full consideration of the likely availability in the near future of the sequence of the *P. falciparum* genome, which will open enormous possibilities in all areas of activity of the programme.

(6) Standardization of assays is important and should be facilitated.

(7) Based on all the above recommendations the continued support of the Concerted Actions SHARED, VINCOMAL, PVEN, AMVTN and relevant novel ones (such as drug development and vectors) should be considered.

(8) AMVTN and PVEN: The possibility of opening these networks for drug evaluation studies should be considered.

(9) Leading scientists who are not contract holders, and working in fields which are under-represented in the INCO-DC malaria portfolio, should be invited to future meetings.

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Comment

Why does *Plasmodium* have a Pre-erythrocytic Cycle?

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There are two extraordinary features of the biology of members of the genus *Plasmodium*, etiological agents of malaria: (1) the counter-intuitive homing of the parasites to the liver of the mammalian host and (2) the unusual features of immunity engendered in the host, particularly its short-lived nature. I propose that these two features are related.

The extra-erythrocytic cycle. The mode of entry of plasmodial parasites into the mammalian host through the bite of infected mosquitoes introduces them directly into systemic capillaries during the vector's bloodmeal. Despite the fact that these parasites have evolved to live primarily within the erythrocytes of the mammalian host, the very first round of replication does not occur in the erythrocytes, which are immediately available in the capillary. Instead, the parasites home to the liver, where they undergo either a single round (*Plasmodium falciparum*) or multiple rounds (*P. vivax*) of extra-erythrocytic schizogony in hepatocytes. This would seem to be counter-intuitive and unlike the usually parsimonious strategies used by biological systems. Distribution over the systemic circulation would seem to reduce the chances of encounter with the target hepatocyte and to increase the probability of clearance by the spleen and other components of the reticulo-endothelial system (RES).

One possible reason for this seemingly bizarre behavior might be a specific nutritional requirement that can be provided only by the hepatocyte. It can be argued that during its sojourn in the mosquito vector, the parasite loses the ability to generate one or more metabolites that are subsequently required for intra-erythrocytic life, and that the cycle within the hepatocyte helps the parasite acquire this metabolite. Other protozoan hemoparasites, such as *Babesia*, however, do not seem to require such an intermediate step and enter erythrocytes immediately after introduction into the mammalian host.

Immunity to malaria. Another intriguing feature about malaria is the extremely short-lived immunity that is engendered in endemic populations. People raised in an endemic area often modulate their parasite burdens to low levels, compatible with an almost normal life. Indeed, many individuals become parasite free. However, if they leave the endemic area and live for longer than three months in a non-endemic area, subsequent exposure to the parasite results in the development of clinical malaria. This short-lived immunity appears to be unique to this infectious agent.

The liver as an immune organ. I propose that these phenomena are linked by the unusual immunological properties of the liver. The first documentation of these features was by Cantor and Dumont in 1967 (Ref. 1). It had been shown previously by Chase[2] that oral feeding of haptens induces systemic tolerance, the so called Chase–Sulzberger phenomenon. Cantor and Dumont demonstrated the importance of the liver in mediating this phenomenon. They showed that oral feeding of 1-chloro 2,4-dinitrobenzene induces systemic tolerance, but that this tolerance could be prevented if the portal

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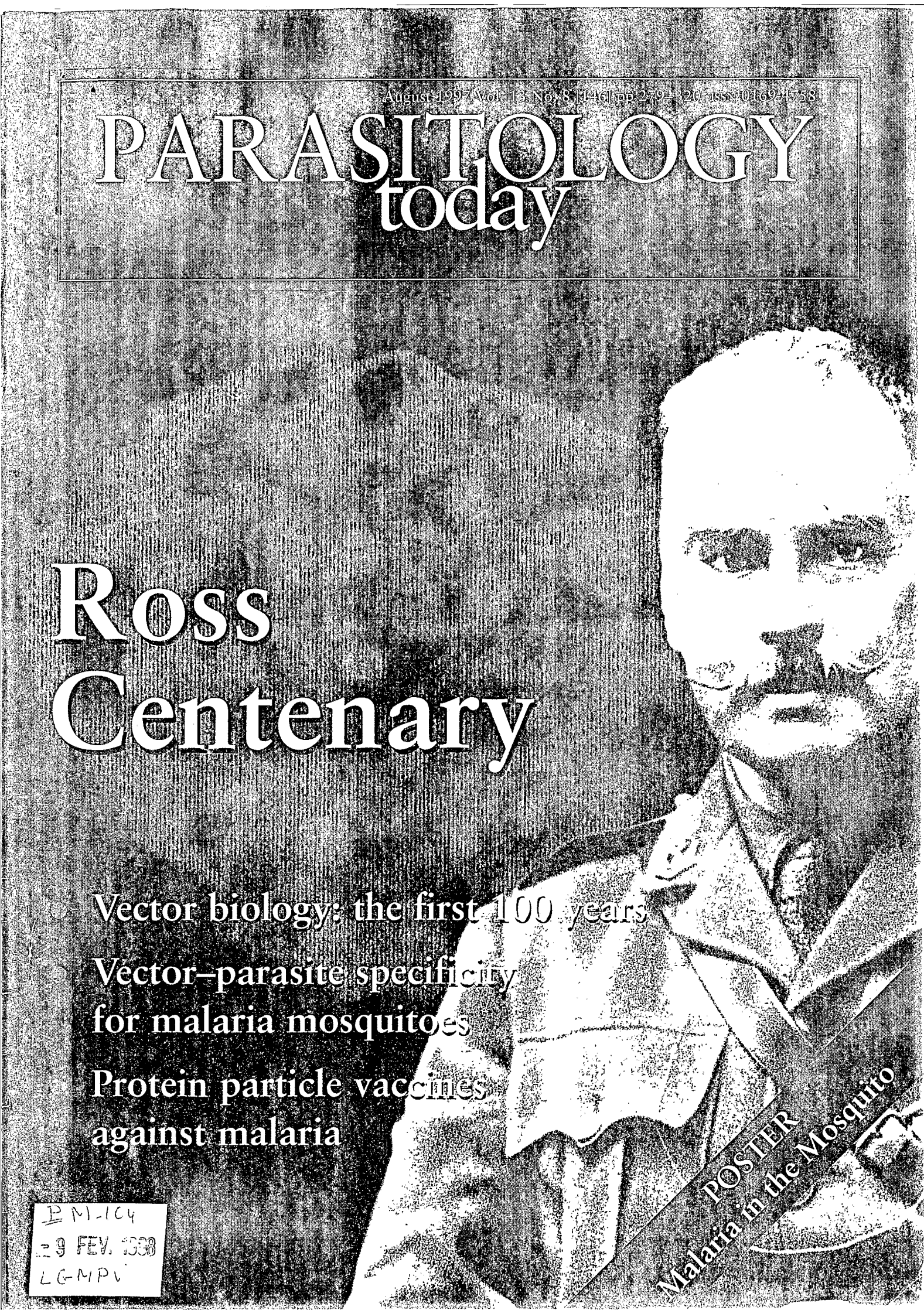

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