Raman and Nath for polarized light. I hesitated to publish this at first, since there was no immediate prospect of checking it out experimentally, but soon afterwards I came across a paper by Sackmann *et al.* of Bell Labs (*J Am Chem. Soc.*, 1968, **90**, 3567), who found a cholesteric material with negative molecular diamagnetic anisotropy which enabled them to observe for the first time the appearance of polarized Debye–Sears diffraction patterns. Being a purely experimental paper, they did not consider it necessary to refer to the Raman–Nath theory. At that time there was a lingering doubt in the minds of some, including myself, about the structure of the cholesteric, for a chemist had expressed the view some time earlier that structurally the cholesteric and smectic are similar (a view that he withdrew subsequently). It therefore seemed worthwhile to write a note on the theory of the observations of Sackmann *et al.* firstly to confirm in a direct way that although the cholesteric does have a lameller type of structure, it is actually different from that of the smectic, and secondly to bring out the fact that the RN theory can be used to evaluate the intensities of the polarized diffraction maxima (S C. and J. Shashidhar Prasad in Bhagavantam's 60th birthday volume, Academic Press, 1969). It was an elementary first-order treatment, but it was certainly the *first* paper to point out that RN concept of a phase grating can be applied to a chiral liquid crystal system. The model has since been extended and generalized very nicely to include Smc* (*Liquid Crystals*, 1992, **11**, 73)'.

We publish here a note by K. A. Suresh.

'As it was a Research News item, I presented the news of some current experimental and theoretical work on phase gratings in liquid crystals. It was not intended to be a detailed review, and consequently the pioneering paper of S. Chandrasekhar and J. Shashidhara Prasad (*Physics of Solid State*, edited by S. Balakrishna, M. Krishnamurthi and B. Ramachandra Rao, Academic Press, 1969, p 77) was not directly cited (it was however cited in ref. 7 of the news item) I take this opportunity to clarify the situation'.

Editors

RESEARCH NEWS

Synthetic malaria vaccine, SPf66

V. S. Chauhan

Malaria is perhaps the most important infectious disease of mankind which is responsible for around 100 million clinical cases and more than a million deaths among children in sub-Saharan Africa. With the emergence of insecticide resistance of the vector and now wide-spread resistance of the parasite, the malaria situation is already out of control. In this context, the development of an effective vaccine against the most important malaria parasite, *Plasmodium falciparum*, would be a major breakthrough in combating this killer disease. Efforts in this direction have been hindered mainly because of a lack of proper understanding of the acquired malaria immunity in humans and a very complex life cycle of the parasite.

Malaria infection begins when an infected mosquito inoculates sporozoites into the circulation during a blood meal. Sporozoites invade hepatocytes specifically and develop as exo-erythrocytic forms. After the hepatic stage, merozoites burst from the hepatocytes and invade red blood cells to begin the blood stage in the life cycle. During this stage, the parasite divides asexually within erythrocytes. This is the stage which is responsible for morbidity and mortality of malaria. Finally, gametocytes (sexual forms) develop within the red blood cells and are taken up by the mosquito during a blood meal to begin the life cycle in mosquito's midgut. Each stage in the parasite life cycle is highly complex, and is characterized by the expression of several different proteins specific to these stages The parasite, being intracellular most times, is mostly protected from usual effector immune mechanism. In addition, the parasite has developed several highly complicated mechanisms to evade the immune responses of the host.

Malaria research underwent a dramatic change during the past two decades. Most significant reasons for this were the availability of culture forms of human malaria parasite[1]. *P. falciparum* for the analysis of the parasite structure and function, and the use of molecular biology tools in identifying from the pre-erythrocytic, asexual and sexual stages have been identified as potential vaccine candidates for providing protection against infection, clinical disease and/or transmission of malaria[1–15].

Acquired immunity to malaria in human takes several years to develop and is usually short lived. Many asexual malarial antigens are polymorphic with multiple alternative forms, often characterized by different sequences of tandem amino acid repeats[8]. Despite the fact that some conserved sequences from these antigens have been identified, antigenic polymorphism and the tremendous genetic plasticity of the asexual malaria parasite remains a major concern for malaria vaccine development[15, 16–18].

It is possible that many new antigens will be discovered and may eventually be incorporated into asexual malaria vaccines, but sufficient evidence has accumulated to warrant vaccine testing in human

volunteers of several well-defined asexual malarial protein antigens. Keeping in mind the ability of malaria parasites to alter the structures of potential vaccine target antigens, it is likely that multicomponent vaccines will be developed in order to minimize the possibility of parasite evasion of vaccine-elicited immune responses.

Attempts to immunise humans against sporozoites date back to 1936, but by seventies it was clearly established that immunization with irradiated sporozoites could provide complete protection against malaria infection. Since then the pre-erythrocytic stage has been the focus of intense research for malaria vaccine development. Most early studies were concentrated on the circumsporozoite protein (CS protein) which was shown to be a target for neutralizing antibodies[3, 15]. However, human trials using recombinant antigens or chemically synthesized peptides developed to be antisporozoite vaccine have shown no evidence of protection against malaria. In comparison, a much larger number of blood stage antigens have been identified and although several of these are considered to be promising vaccine candidates, relatively few human trials have been reported.

The outlook for a blood stage vaccine

There are reasons to believe that in the next decade or two, effective, at least partially, asexual malaria vaccines will be developed. A synthetic peptide vaccine, SPf66, which contains peptide sequences from three malaria proteins, has shown promising results in initial human trials. Secondly, recombinant proteins representing fragments of asexual antigens have shown significant protection against blood stage infection. Further, so far only a few antigens have been actually tested. It is very likely that valuable vaccine antigens will be discovered and will contribute in modifying the existing vaccines at that time. Lastly, it must be stressed that vaccination results obtained in one host species may not be extendable to another species. In fact, monkey trial results in case of SPf66 were quite different from trials in human volunteers. There is a need for increased emphasis on vaccine studies in human volunteers.

Synthetic malaria vaccine: Rationale and possibilities

The hope for a peptide-based vaccine against malaria, and indeed other diseases, is largely based on the following observations:

1. It is possible to obtain antigen-neutralizing antibodies using a small, but suitable, portion of the antigen, i.e. a synthetic peptide fragment.
2. T-cell determinants (both Th and CTL) can be identified in an antigen and specific T-cell activation and proliferation can be induced using short synthetic peptides.
3. Covalent linking of the B and T-cell determinants can enhance the immunogenicity to provide B-epitope specific antibodies. The use of carrier proteins to enhance immunogenicity of short peptides may, therefore, be avoided.
4. Synthetic peptides allow to focus immune response to regions which in the native antigen may not be immunodominant. It has been found, particularly in malaria antigens, that more effective immune responses were obtained with synthetic peptides representing the desired epitopes than when the whole recombinant antigens were used.
5. Easy availability, high purity and stability of synthetic peptide make them attractive targets for vaccine development.

However, several problems exist in using synthetic peptides as immunogens, such as the question of genetic restriction of the immune response, presentation of the synthetic immunogen, possible generation of new dominant but nonsense epitopes, epitope dominance, etc. Some of these questions are under active investigation in different laboratories including this author's. The overall view of current vaccine development research is that the synthetic peptides do offer an attractive alternative to recombinant or native antigens. In fact, in some cases synthetic peptides have proved to be more effective.

Malaria peptide vaccine, SPf66 (the Colombian vaccine)[19–33]

Design and field trials of SPf66

Sera from apparently immune individuals were used for immunoblotting of *P. falciparum* schizonts and merozoite lysates; the molecules identified in a range of 20–200 kDa were isolated and tested as immunogens in lotuc monkeys. None provided consistently high degree of protection but four, including MSA-1 (195 kDa) and RESA (155 kDa) were chosen as the possible vaccine target antigens. The other two antigens still remain uncharacterized and unlocated on the parasite. Several dozens of peptides were synthesized and checked for their protective potential[27]. None provided complete protection, and only delayed parasitaemia was observed in some cases. A combination of three peptides linked to BSA, worked somewhat better[30]. These peptide sequences were then joined together along with two NANP sequences and a cysteine residue each was placed at the amino and carboxyl terminals. Aerial oxidation of this 44 residue peptide provided the polymeric vaccine candidate, Spf 66. With this polymer several immunization/protection experiments were carried out in monkeys; none provided complete protection even when a strong adjuvant (FCA, IFA) was used[25, 27, 31].

Immunizations in humans were done with $Al(OH)_3$ as the adjuvant[19, 20, 32], The IgG response was variable allowing a division into high, medium and low responders, which was ascribed to genetic control of the immune response to Spf66 (ref. 23, 32). Several thousands of individuals in Latin American countries have been vaccinated and protection levels of 30–50% were reported[19, 20]. A surprising but consistent feature of these trials in animals and in humans was that there was no correlation between the antibody titres against SPf66 and the incidence of malaria episodes. However, it has been concluded that the chemically synthesized SPf66 vaccine is safe, immunogenic and partially protective against *P. falciparum* in a semi-immune population living in a malaria-endemic area.

The most important outcome of these studies in the field of vaccine development research has been that it is shown for the first time that a peptide-based construct containing antigenic determinants from different proteins, synthesized chemically, can be used in humans. Further, this represented the first successful synthetic vaccine against malaria and indeed the first one against a parasite disease in humans, and clearly indicated the potential of chemically synthesized peptide vaccines, in general.

At the same time, other research groups,

one from Colombia[27] itself and another from USA[22, 32], found SPf66 to be a poor immunogen which provided no protection against *P. falciparum* infection. This, and the manner in which the early human trials were conducted led to a lot of controversy. In June 1990, the WHO committee concluded that while the vaccine was safe and immunogenic, the evidence for its efficacy was not conclusive.

In January 1992, the Swiss Tropical Institute, the Ifakara Centre, Tanzania, The London School of Hygiene and Tropical Medicine and the Spanish Science Council jointly developed a protocol to carry out trials of SPf66 in the Kilombero District of Tanzania, an area under malaria investigation and transmission studies since 1960. This phase III efficacy trial became focus of attention for malaria vaccine development and also of the media, given the desperate situation due to the increasing drug resistance of *P. falciparum*, and the controversy that had come to surround SPf66 about its immunological efficacy. Of equal importance was the fact that this was to be the first human trial outside the Latin America, and which was to be conducted in much more severe malaria transmission conditions so often seen in Africa.

Recently, a Spanish group[23] has fully characterized SPf66 (batch 9, used in Tanzanian trials), for the first time, and found it to be highly water soluble and its molecular weight ranging from 10 to 25 kDa. They also found it to be pure, free of metallic contaminants, atoxic and stable at 4°C. It was reported some time back, again through popular media (the BBC taking the lead here) that the synthetic vaccine was safe and produced an antibody response in children aged between 1 and 5 years.

The secrecy code to determine the level of protection provided by SPf66 immunization in Tanzania was to be opened in October 1994. It was reported (BBC again!) that the vaccine has provided a protection cover of up to 30% among the vaccinated children. These results have been published recently[33]. Five hundred and eighty six children aged 1–5 years formed the study group. No severe side effects were observed among those who received three doses of the vaccine. The vaccine was highly immunogenic with detectable anti-SPf66 antibodies in all the recipients. The vaccine efficacy estimate was 31%, lower than the 50% level that the child was designed to detect. However, whether anti-SPf66 antibodies have any relationship with clinical protection is not yet determined; the mechanism of protection remains unclear, and needs to be established for any further improvements in the design of the vaccine. In summary, SPf66 appears to be effective in both Africa and Latin America and even though the exact mechanisms involved in protection are not clear it is suggested that SPf66 may mimic naturally acquired strain-transcending immunity. Whether SPf66 will be used as a vaccine for malaria will be a matter of debate, even though its reported efficacy is much lower than most other vaccines currently in use in humans.

The partial success of SPf66 as a synthetic vaccine, even though limited in terms of the level of protection it provides, is remarkable in that it is shown for the first time that a chemically synthesized molecule based on antigenic determinants can be safe and immunogenic in humans This is a landmark achievement in the field of synthetic vaccine development in general, and particularly in malaria vaccine against the blood stage parasite. It is quite obvious that this strategy of vaccine development will soon be attempted in other infectious diseases. This may lead to the development of a new generation of synthetic molecules capable of producing specific immune responses in addition to being cheaper, easy to obtain and more stable than the existing vaccines. It is further significant that this work has come out of a third world country where malaria is a major health problem. Given the modern tools of molecular biology, synthetic chemistry and access to knowledge of the field situations (disease-endemic areas), it would be most rewarding and at the same time a tremendous intellectual challenge to work towards understanding and controlling pathogens which cause these life-threatening diseases in humans.

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Cell-type and stage-specific gene knockouts in mice: An achievement of a long-sought goal

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The importance of gene action in regulating the biology of higher eukaryotes is hardly a matter of dispute. But until recently we have been unable to say whether a given gene was essential for the functioning of a particular developmental pathway or not. The reason was that there was no way to ensure that a gene was inactivated in just one cell type, and not in others. This situation has now changed.

Technical advances in handling and manipulating mouse embryos have made a great difference to the study of mammalian biology. It was the ability to create uniparental embryos by nuclear transplantation that pinpointed the essential features behind the phenomenon of genomic imprinting[1,2]. Studies with transgenic mice led to an improved understanding of the various aspects of tissue-specific gene expression and gene imprinting. Adding yet another dimension to the problem of tissue-specific gene expression is the control of developmental time sequences in higher eukaryotes. Developmental biologists long cherished the hope that they would one day be able to 'knock out' endogenous genes at will and so learn more about *when* a gene is essential (in addition to where it is essential).

That hope was realized by two groups, those of Capecchi in USA and Gruss in Germany, when they succeeded in using homologous recombination to create knockouts[3,4]. Capecchi and co-workers used embryonic stem cells (ES cells) and a phenotypic selection method to screen for homologous recombination involving the gene of interest, whereas Zimmer and Gruss designed a PCR approach to fish out ES cells that had undergone homologous recombination (Figure 1). In both these approaches the selected ES cells were used to create chimaeric mice. Subsequently, a transgenic line was established by selection of second-generation mice that carried the transgene. The next challenge was of achieving selected gene knockouts in a specific tissue type at a specific stage in development.

What looked improbable until the other day has now been achieved by Gu *et al.*[5]. A bacteriophage system provided the key. Phage P_1, a bacteriophage of *E. coli*, has a recombinase of the integrase family which helps in circularizing phage DNA during both the lytic and the lysogenic cycles. The system, called Cre-lox, comprises a 34 kDa protein (Cre, Cyclization

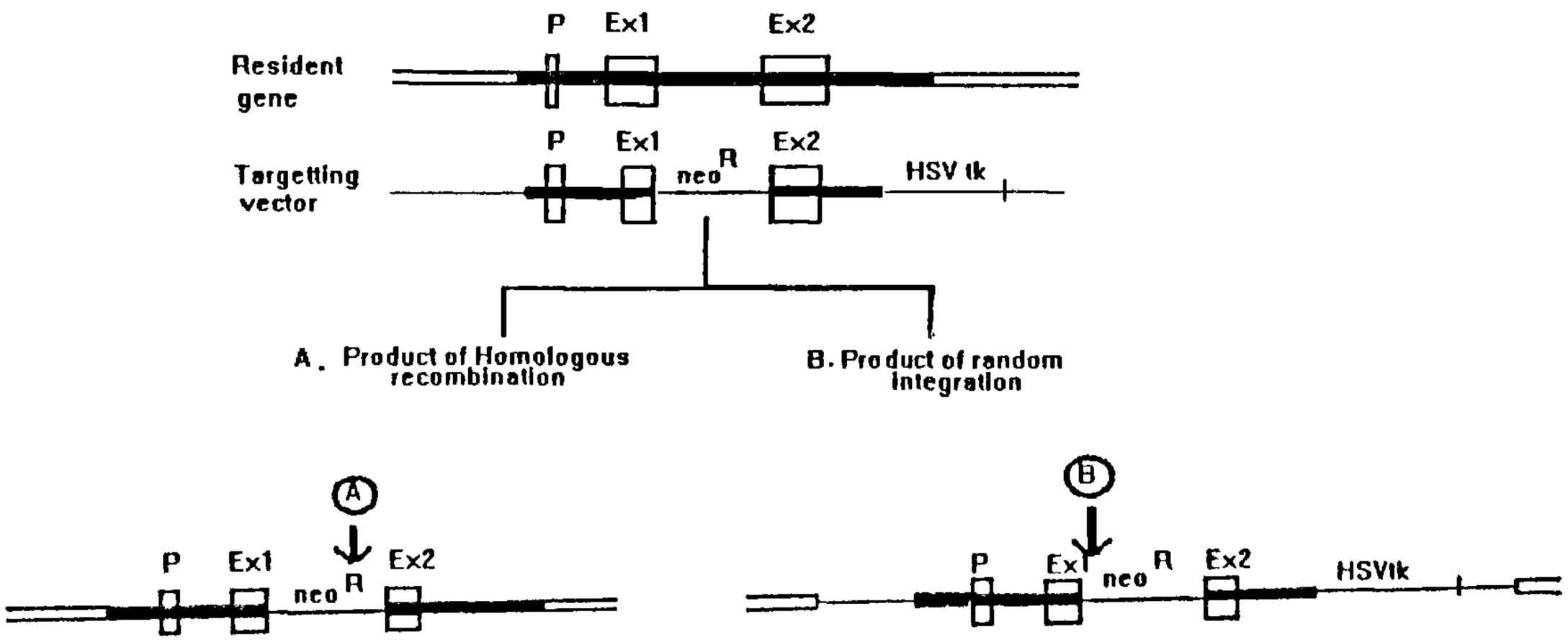

Figure 1. Strategy for creating knockout mice by homologous recombination. P is a promoter sequence, Ex1 and Ex2 are two exons of the gene to be disrupted, neo^R is the bacterial neomycin gene. HSVtk is the thymidine kinase gene from herpes simplex virus. After transformation, embryonic stem cells are selected for homologous recombinants on a medium containing G418 and gancyclovir; only type-A transformants survive. Selection can also be carried out by PCR with appropriate oligonucleotides.