and only ~7% of RU 486-GR) (Fig. 4*b*). Two series of complementary experiments confirmed this observation. First, when transformed (4S) TA- and RU 486-GR were isolated on glycerol gradients, their binding to DNA-cellulose was identical (data not shown). Second, when DNA-cellulose bound TA- and RU 486-GR were eluted with increasing concentrations of KCl in the washing buffer (20–200 mM)[20], the same patterns of elution were obtained (binding was decreased by 50% at 133.2 ± 10.4 and 128.2 ± 9.5 mM for TA- and RU 486-GR, respectively). Our results agree with recent findings indicating that agonist-, antagonist- and ligand-free GR and progesterone R, bind to HREs in mouse mammary tumour virus long terminal repeat (MMTV-LTR) DNA and the uteroglobin gene promoter, respectively (refs 1 and 2 and A.G., G.S.G., F.C., unpublished experiments). Moreover, our study explains DNA-binding data showing that RU 486-GR is less transformed than TA-GR and behaves in some aspects like an nt⁻ agonist-GR complex[16,21].

Even if alternative explanations may be presented[22], our data may reconcile different results obtained for DNA interaction of RU 486-GR *in vivo*[3] and *in vitro*[1,2]. Indeed, the limited ability of the 8S, hetero-oligomeric, RU 486-GR to undergo subunit dissociation supports the proposal that, *in vivo*, the antagonistic activity of RU 486 is mainly attributable to the stabilization of the non-transformed form of GR, and that, once transformed to 4S *in vitro*, both agonist-GR and antagonist-GR are able to bind to sequence-specific GRE. Whether other differences between agonist- and antagonist-GR are involved in the expression of the anti-hormonal activity of RU 486 remains to be elucidated.

Note added in proof: Recent work (J. M. Renoir, J. Devin and E.E.B., in preparation) suggests a similar result when RU 486 is used as an antiprogesterone in the rabbit progesterone system.

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Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA-DR antigen

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Mature T cells segregate phenotypically into one of two classes: those that express the surface glycoprotein CD4, and those that express the glycoprotein CD8. The CD4 molecule is expressed primarily on helper T cells whereas CD8 is found on cytotoxic and suppressor cells[1–3]. A more stringent association exists, however, between these T-cell subsets and the major histocompatibility complex (MHC) gene products recognized by their T-cell receptors (TCRs)[2–4]. CD8⁺ lymphocytes interact with targets expressing class I MHC gene products, whereas CD4⁺ cells interact with class II MHC-bearing targets. To explain this association, it has been proposed that these 'accessory' molecules bind to monomorphic regions of the MHC proteins on the target cell, CD4 to class II and CD8 to class I products[4–9]. This binding could hold the T cell and its target together, thus improving the probability of the formation of the trimolecular antigen:MHC:TCR complex. Because the TCR on CD4⁺ cells binds antigen in association with class II MHC, it has been difficult to design experiments to detect the association of CD4 with a class II molecule. To address this issue, we devised a xenogeneic system in which human CD4 complementary DNA was transfected into the murine CD4⁻, CD8⁻ T-cell hybridoma 3DT-52.5.8, the TCR of which recognizes the murine class I molecule H-2D^d. The murine H-2D^d-bearing target cell line, P815, was cotransfected with human class II HLA-DR α, β and invariant chain cDNAs. Co-culture of the parental T-cell and P815 lines, or of one parental and one transfected line resulted in a low baseline response. In contrast, a substantial increase in response was observed when CD4⁺ 3DT-52.5.8 cells were co-cultured with HLA-DR⁺ P815 cells. This result strongly indicates that CD4:HLA-DR binding occurs in this system and that this interaction augments T-cell activation.

A CD4⁻ variant of the murine CD4⁺ T cell hybridoma 3DT-52.5, named 3DT-52.5.8, was obtained by single-cell cloning. This cell line bears high levels of cell-surface receptor as evidenced by staining with KJ12-98 (Fig. 1*a*), a murine anti-TCR idiotype antibody which identifies the hybrid H-2D^d-restricted receptor unique to 3DT-52.5 and its subclones[10]. Recent work by Blackman *et al.*[11] has shown that this TCR is a hybrid molecule formed by the fusion partner BW5147 α chain and the parental T cell $V_\beta 8$-containing β chain. The TCR derived from the incoming T-cell blast can also potentially be expressed by these cells. Therefore, KJ16-133 (ref. 12), an allotypic antibody which recognizes the $V_\beta 8$ family, was used to detect any surface receptor using the $V_\beta 8$-containing β chain with a different α chain. Equivalent staining was obtained with both antibodies indicating that the hybrid TCR was the predominant if not the exclusive receptor species present on the 3DT-52.5.8 cell surface (data not shown). The line 3DT-52.5.8/S4, a subclone of 3DT-52.5.8, expressed considerably lower levels of TCR as evidenced by staining with KJ12 and KJ16 (Fig. 1*c*, data not shown). Neither 3DT-52.5.8 nor 3DT-52.5.8/S4 cells stained with antibodies to either human CD4 (Fig. 1*b*, *d*) or murine CD4 (data not shown). It should be noted that numerous murine T-cell hybridomas generated in our laboratory lack surface CD4

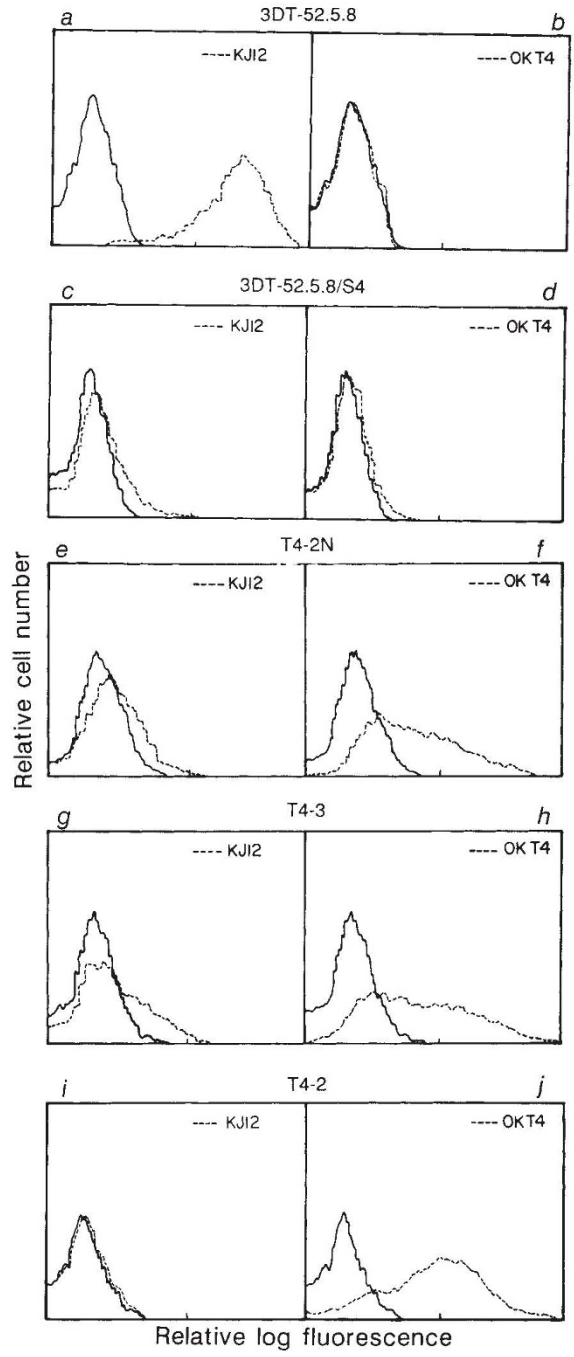

Fig. 1 Cytofluorographic analysis of parental and CD4$^+$ T-cell hybridomas. T-cell hybridomas were stained with either KJ12-98, a mouse antibody directed against an idiotypic determinant of the 3DT-52.5 T-cell receptor (TCR, ref. 10), (1:60), or with OKT4A (ref. 4), a mouse anti-human CD4 monoclonal reagent (1:100). After a 30-min incubation at 20 °C, the cells were washed twice in phosphate buffered saline (PBS) containing 2% fetal bovine serum (FBS), 0.2 M sodium azide and resuspended in fluoroscein isothiocyanate (FITC)-conjugated rat anti-mouse immunoglobulin κ light chain antibody (187.1) for another 30 min at 20 °C. After a second set of washes in PBS/FBS/azide, the cells were suspended in 2% paraformaldehyde/0.09 M sodium phosphate/0.2% sodium chloride (*p*H 7.4). Cells were analysed for fluorescence using a three decade log scale on a Coulter EPICS V cytofluorograph. Control cells stained only with the secondary antibody, FITC-187.1, are represented in all panels as solid lines (——).

Methods. The generation and characterization of the T-cell hybridoma 3DT-52.5 have been described elsewhere[10]; 3DT-52.5.8, a CD4$^-$ variant of 3DT-52.5, was obtained by single-cell cloning of 3DT-52.5; 3DT-52.5.8/S4 was obtained by single-cell cloning of 3DT-52.5.8 cells presorted for low TCR density. CD4 cDNA isolation, plasmid transfection and infection procedures have been described by Maddon *et al.*[13].

and CD8 but retain the specificity of their CD4$^+$ parents for antigen/MHC. Therefore, CD4 does not appear to contribute to the specificity of the TCR.

The three human CD4$^+$ populations (T4-2, T4-2N and T4-3) were obtained by infection of 3DT-52.5.8 cells with murine Maloney leukaemia (Mo-MLV) recombinant virus contaning a full-length human CD4 cDNA driven by a murine LTR. This vector also contains the bacterial gene conferring resistance to the antibiotic neomycin[13]. Populations of cells infected with retrovirus were identified by resistance to neomycin and were then analysed for expression of surface CD4 and TCR. As can be seen, these cells expressed reasonable levels of CD4 but considerably lower levels of TCR than the parent 3DT-52.5.8 (Fig. 1*e–j*). After observing a number of CD4 infectants, some correlation was noted between high CD4 expression and low TCR expression (data not shown) but it is unclear whether this was due to the conditions of Mo-MSV infection or down-regulation of receptor expression by CD4. These infected cells were also phenotypically unstable, losing 80–100% of surface TCR in 5–7 days in culture. As we were unable to stabilize these populations by single-cell cloning, all cell lines were carefully monitored for surface expression of a variety of antigens just before each test of function.

The antigen-presenting cell populations P815/HTR and HLA-DR-transfected P815/HTR (B2-P815) were analysed by cyto-fluorograph for surface expression of H-2D^d and HLA-DR. As Fig. 2 illustrates, the parental P815 cells and the HLA-DR transfectants exhibited identical staining patterns with respect to cell surface H-2D^d antigen. In contrast, only the HLA-DR transfectants were positive for staining with D1.12, a pan anti-human HLA-DR antibody.

The relative quantity of IL-2 produced by co-culture of the T-cell hybrids with H-2D^{d+} stimulatory cells was used as an indication of relative T-cell activation. As seen in Fig. 3, the mouse CD4$^-$ T-cell hybridoma 3DT-52.5.8 produced considerable amounts of IL-2 upon incubation with either the parental P815 or with the HLA-DR$^+$ transfectant, B2-P815. Expression of HLA-DR by the P815 transfectants appeared to have no effect upon stimulation of this hybrid. 3DT-52.5.8/S4, the sub-clone of 3DT-52.5.8, produced much lower concentrations of IL-2 when cultured with either P815 population, presumably because this T cell bears much lower levels of cell-surface receptor than its parent. In other respects, however, 3DT-52.5.8/S4 responded similarly to 3DT-52.5.8, responding equally well to both P815 populations.

The human CD4$^+$ T-cell hybridomas T4-2N and T4-3, both bearing levels of TCR similar to that on 3DT-52.5.8/S4, responded as poorly as that line to P815 target cells (T4-3 cells with higher TCR density cells did respond slightly better to this stimulus than did either S4 or T4-2N). The T hybrid T4-2, with almost undetectable amounts of TCR, did not produce any IL-2 in response to P815. Expression of CD4 by the CD4-infectants appeared to have no effect upon either TCR recognition of H-2D^d on the P815 cells or subsequent stimulation to produce IL-2.

In contrast, when HLA-DR$^+$ P815 cells (B2-P815) were used as the target cells, IL-2 production by T4-2N and T4-3 increased dramatically over that seen with P815 (Fig. 3). More surprisingly, the unresponsive T hybrid, T4-2, now responded significantly. The increase in response could not be explained by an increase in available antigen because (1) both parental and HLA-DR$^+$ lines had identical amounts of H-2D^d on their surfaces (Fig. 2) and (2) an increased response was not observed for either CD4$^-$ hybrid. The boost in activation required the presence of human

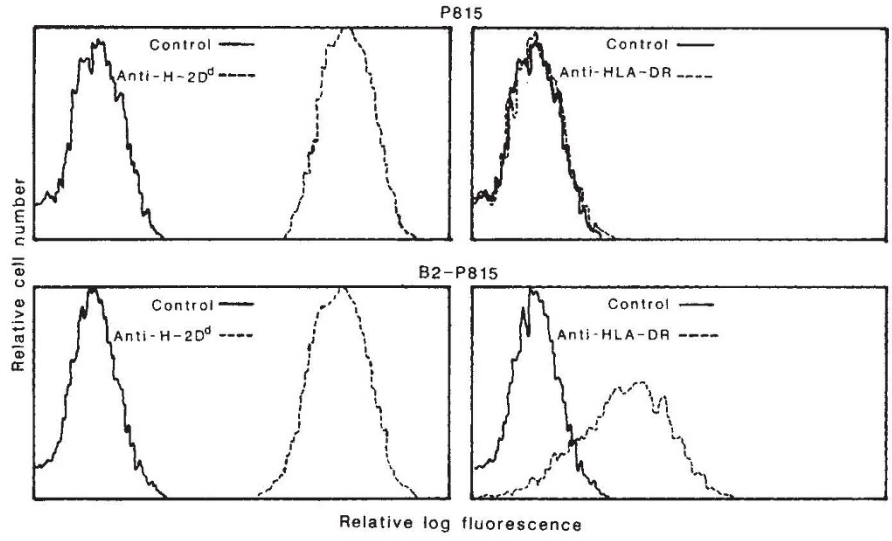

Fig. 2 Cytofluorographic analysis of H-2D^{d+} P815 populations. The murine DBA/2 mastocytoma line P815/HTR and a human DR1-transfected P815/HTR subpopulation (B2-P815) were stained with either 34-5.8, a mouse anti-murine class I H-2D^d antibody (1:300, ref. 22) or with D1.12, a mouse anti-human HLA-DR antibody (1:100, ref. 21) by the procedure described in Fig. 1. Control cells incubated only with FITC-187.1 are represented as solid lines (——). Both parental P815 cells and HLA-DR$^+$ transfectants were 100% positive for 34-5.8 staining and exhibited identical shifts in peak and mean channels.

Methods. Plasmids used for transfection were isolated from a cDNA library constructed with messenger RNA of a human B-cell line as described by Tonnelle *et al.*[23]. The library was made in an expression vector carrying the SV40 early promoter and termination sequences. The HLA-DR β chain cDNA corresponds to the HLA-DR1 allelic specificity and has been completely sequenced (Tonnell). The cDNAs for HLA-DR α and for the invariant chain are full-length and expressible as described by Sekaly *et al.*[24]. The P815 cell transfections were carried out using calcium phosphate coprecipitation. In a typical experiment, 5×10^6 P815 cells were transfected with 10 μg supercoiled DNA of each α,β, invariant plasmids and 1 μg HSVTK plasmid[24]. Cells were incubated with calcium phosphate reprecipitated DNA in 1 ml for 60 min at 37 °C, then washed and incubated at 37 °C in fresh DMEM/10% FBS. HAT selection was applied 48 h after initiation of the transfection.

CD4 on the T cell as well as human HLA-DR on the stimulating cell.

Antibody inhibition studies illustrated in Table 1 showed that addition of anti- H-2D^d antibody abrogated the response by all the T hybrids to either target population. In contrast, anti-CD4 and anti-HLA-DR antibodies only affected stimulation when the appropriate antigens were on the cell surface and then only when augmented stimulation was noted. Under these conditions, the antibodies usually inhibited down to base-line response. The specificity of the TCR on the CD4$^+$ transfectants was not altered to recognize a crossreactive determinant common to murine H-2D^d and human HLA-DR because (1) anti-H-2D^d antibody completely abrogated the response of these cells to HLA-DR$^+$ P815 cells and (2) human HLA-DR-1$^+$ T-cell blasts could not stimulate the CD4$^+$ hybridomas to produce IL-2 (data not shown). Also, the T-cell blast-derived TCR did not contribute to this interaction because 3DT-52.5 negative variants containing only this receptor (KJ16$^+$, KJ12$^-$) responded to neither P815 nor HLA-DR$^+$ P815 cells (data not shown). The above data strongly indicate that a primary function of the CD4 molecule is to bind to the stimulatory cell HLA-DR and physically boost TCR-MHC interaction. Furthermore, the above data substantiates earlier work that MHC restriction of the TCR does not dictate function of CD4[9]. It should be noted, however, that dual recognition of one MHC molecule by both accessory molecule and TCR may be more efficient for T-cell stimulation.

It is increasingly evident that CD4 and CD8 serve a more complicated function in T-cell activation than that of simple adhesion molecules. T-cell stimulation which does not involve the putative target ligands of these molecules can be inhibited and sometimes enhanced with anti-CD4 and anti-CD8 antibodies (refs 14–17 and C. Janeway, in preparation). CD4 cross-linking can induce Ca^{2+} mobilization and antigen-modulation and under certain conditions T cell proliferation[17–19]. Also, under certain circumstances, association between the TCR and CD4 can be demonstrated[19,20]. Additional functions of CD4 and CD8 might therefore be to transduce signals across the T-cell membrane.

Alternatively, if the molecules are in close association with the TCR, concordant recognition of class II or class I MHC molecules by these accessory proteins and the TCR might facilitate formation of the TCR/antigen/MHC tertiary complex. In either case, then, the additional function of the CD4 molecule requires engagement of a ligand. The data presented in this paper supports the view that this ligand is a class II molecule. It is of interest that low TCR expression can be compensated for by presence of CD4 on the T cell and HLA-DR on the stimulatory cell. This indicates that effective interaction between T cells and targets is not exclusively controlled by the affinity and density of TCRs but rather is the result of multiple contributions made by a combination of surface receptors and ligands. This example of the involvement of several different receptor-

Table 1 Units of IL-2 produced by T hybrid cell lines in responses to P815 or HLA-DR$^+$ B2-P815 cells

T hybrids:	3DT-52.5.8		3DT-52.5.8/S4		T4-2		T4-2N		T4-3	
Stimulators:	**P815**	**B2-P815**	**P815**	**B2-P815**	**P815**	**B2-P815**	**P815**	**B2-P815**	**P815**	**B2-P815**
Antibodies to:										
None	640	640	40	40	<10	40	10	80	40	640
H-2D^d	<10	<10	<10	<10	<10	<10	10	10	10	10
CD4	640	640	40	40	ND	10	10	20	80	320
HLA-DR	640	640	40	40	ND	20	10	10	40	80

Before assaying, T-hybridoma cells were incubated with OKT4B antibody (1:50, ref. 4) and target cells were incubated with either 34-5.8 antibody (1:400) or with cocktail of the following murine anti-human HLA-DR antibodies; BT-2.9 (1:500); D4.22 (1:50); D1.12 (1:200); LG2/72 (1:200); AA3.84 (1:1000) for 1 h at 37 °C[21]. Untreated cells were resuspended in a comparable volume of cell culture medium. IL-2 assay conditions were as described in Fig. 3. Coculture of T hybrid cells and target cells was carried out in the continued presence of the antibodies. These experiments were performed independently from those represented in Fig. 3. Magnitude of IL-2 production by the different T-cell hybrids appears to vary with TCR concentration. ND, not determined.

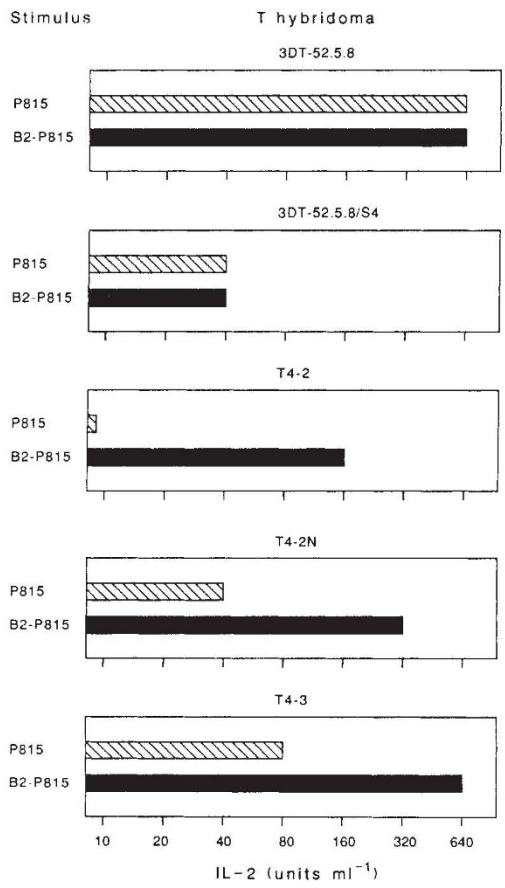

Fig. 3 Response of T-cell hybridomas to HLA-DR$^+$ and untransfected P815 cells. T hybrid cells (10^5) were cocultured with 10^5 target cells at 37 °C, 5% CO_2 in 96-well culture plates (Flow); final volume of 300 μl in culture medium (modified Mishell-Dutton medium/10% FBS/5×10^{-5} M 2-mercaptoethanol/50 μg ml^{-1} gentamycin (Schering)). After 24 h, the culture supernatants were assayed for the presence of IL-2 by their ability to support the growth of the IL-2-dependent T-cell line HT-2 (ref. 25). The highest of 2-fold serial dilutions capable of maintaining 90% HT-2 cell viability defined the IL-2 concentration of the supernatant. Ten units ml^{-1} IL-2 was the minimum concentration noted. T-cell hybrids cultured in the absence of the appropriate antigen/MHC stimulus failed to produce any detectable IL-2 (data not shown).

ligand pairs may be a general phenomenon of cell recognition.

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Induction of protective immunity against experimental infection with malaria using synthetic peptides

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Synthetic peptides are potential vaccine candidates because they may be able to induce high antibody titres and specific cellular immune responses against native proteins and thus the whole invading organism[1]. In a previous study we showed that immunization with molecules of relative molecular mass (M_r) 155,000 (155K) 83K, 55K and 35K, specific for the late schizont and merozoite stages of *Plasmodium falciparum*, could elicit either partial or total protection in *Aotus trivirgatus* monkeys experimentally infected with *P. falciparum*[2]. Here we have chemically synthesized 18 peptides corresponding to different fragments of these proteins to immunize *Aotus trivirgatus* monkeys. Some peptides gave partial protection from challenge with *P. falciparum* parasites, but none provided complete protection individually. A combination of three partially protective peptides gave complete or almost complete protection, however, suggesting that this particular combination of peptides is a good candidate for a malaria vaccine.

The 155K, 83K, 55K and 35K proteins were isolated from schizont and merozoite lysates, in quantities ranging from 200–400 μg each, using preparative SDS-PAGE. These proteins showed a high degree of purity as seen by analytical SDS-PAGE and Western blots with hyperimmune sera from malaria patients. The 55K protein provided incomplete protection (defined as a significant delay in the onset of parasitaemia) and the 35K protein partial sterilizing protection (spontaneous control of the experimental infection without drug therapy) on challenge of immunized monkeys with *P. falciparum*[2].

We determined the sequences of the first 21 N-terminal amino acid residues of the 55K and 35K proteins. Based on these data and the amino acid sequences described for the 155K[3] or RESA molecule by Coppel *et al.*[4] together with the complete amino acid sequence of the 195K precursor of the 83K molecule[5], 18 peptides corresponding to different segments of the 35K, 55K, 83K and 155K proteins were synthesized using Multiple Solid Phase Synthesis[6]. These peptides were chosen randomly to represent different predicted conformations as determined by the Chou and Fasman method[7] (Table 2), taking into account the suggestion that certain structures can preferentially induce humoral and cellular immune responses[8,9].

Groups of four to six Colombian *Aotus trivirgatus* monkeys were immunized with purified peptide (250 μg) coupled to BSA (250 μg) on days 0, 30, 45, 60 and 75. Blood samples for antibody studies were taken on days 50, 70 and 80. On day 90, 15 days after the last immunization, the monkeys were challenged with live *P. falciparum*.

Of the 18 different synthetic peptides used for immunization, 15 elicited antibodies against *P. falciparum* schizonts, as detected by immunofluorescence and were found to be immunogenic by