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Abstract. Malaria is one of the world's most devastating diseases, and *Plasmodium falciparum* (*Pf*) causes significant mortalities particularly in Sub-Saharan Africa. The rise and spread of multi-drug resistant strains of the parasite has coincided with an era of increased travel to malaria endemic regions. In the absence of an effective vaccine against malaria it may be possible to utilize human monoclonal antibodies against the stage transmitted by mosquito bites (sporozoites) as a prophylactic to prevent infection. We report the characterization of an engineered human IgG$_4$ monoclonal antibody against *Pf* sporozoite cloned from a protected individual recognized the sporozoite surface and inhibited sporozoite invasion of human hepatocytes *in vitro*.
The fully human monoclonal antibody PfNPNA-1 IgG$_4$ against (NPNA)$_3$ specifically labels *Plasmodium falciparum* in an IFA. This antibody also inhibits *Plasmodium falciparum* sporozoite invasion of human hepatocytes HepG2-A16 in a dose dependent manner in an *in vitro* assay. PfNPNA-1 IgG$_4$ is a promising candidate for evaluation for the prevention of malaria.

Keywords: Plasmodium falciparum, sporozoite, human monoclonal antibodies

1. Introduction

Malaria is an infectious disease which threatens public health in regions of the world where more than a third of the human population reside (approximately 2.2 billion) [1,2]. A number of vaccine candidates have been evaluated for the prevention of either infection or disease, and while the results have been encouraging, an effective vaccine is still years away [3]. The risk to travellers of malaria infection has increased with the emergence and spread of drug resistant strains of the parasite [4], and the need for new effective prophylactic agents is urgent. Studies carried out more than half a century ago showed that immunization with radiation-attenuated *Plasmodium* sporozoites, the infectious stage of the malaria parasite, could confer protective immunity [5,6]. The role of specific antibody in such protection was demonstrated with passive administration of murine monoclonal antibodies directed against the major repeat epitope of the circumsporozoite protein (CSP), which prevented malaria in naïve

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animals [7]. The corresponding epitope of the human malaria parasite *Plasmodium falciparum CSP* [8] is contained within repeats of the tetramer peptide (NPNA)$_n$ [9,10]. However attempts to generate and maintain protective immunity with repeat peptide based vaccines have been inadequate, whilst immunization with attenuated *Pf* sporozoites induced anti-repeat antibody and protective immunity in many human volunteers [11–14]. Analysis of antibody responses in 5 volunteers immunized with irradiated *Pf* sporozoites implied that induction of anti-repeat antibodies correlated with protection [15]. Moreover the long term persistence of sterile immunity in a volunteer immunized with irradiated *Pf* sporozoites also correlated with the maintenance of high levels of antibodies against the repeat epitope [16]. Due to the T-cell independent activation of B-cells thought to occur with repetitive antigens [17] it is unlikely that that high levels of high affinity specific anti-repeat antibodies will be maintained via conventional immunization with subunit based recombinant vaccines encoding the repeat epitopes. The vaccines however may confer short-term protection, but this cannot be guaranteed for all recipients, since the responses to the vaccines are dependent on each individual. One possible way around this problem is to administer a known anti-parasite inhibitory antibody at a level commensurate with predicted protection. Previously we reported the isolation of PfNPNA-1 a fully human anti-*Pf* sporozoite antibody derived from an immune protected individual [18]. Briefly, peripheral blood lymphocytes donated by a protected *Pf* sporozoite immunized individual were used to recapitulate the antibody repertoire in a phage display library. The library was accessed for antibody fragments binding to the repeat epitope (NPNA)$_3$. The specific anti-repeat antibody response appeared to be restricted to a single V$_H$/V$_L$ combination designated PfNPNA-1. In contrast, many distinct mAbs have been isolated from mice immunized with either sporozoites [19,20] or (NANP)$_n$-based peptide immunogens [21], some of which inhibit invasion of hepatocytes. Here we describe a fully human Pf NPNA-1 IgG$_4$/κ monoclonal antibody candidate for development for the prevention of *P. falciparum* malaria.

2. Materials and methods

2.1. PfNPNA-1 human IgG$_4$ assembly

For expression of whole human IgG$_4$ in eukaryotic cells two plasmids pSG5LCDR3 and pSGHCDR-

G4, which respectively encoded the light and heavy chains of the humanized monoclonal antibody OKT4 (IgG$_4$/k) ligated into pSG5 (Stratagene, La Jolla, CA), were modified by inserting the V$_H$ and V$_L$ genes of PfNPNA-1 [18]. The constructs were evaluated in a transient transfection assay. The two plasmids were mixed in equimolar amounts and transfected into 293 cells (transformed human primary embryonal kidney cells, ATCC # CRL-1573) with lipofectamine (Invitrogen, Carlsbad, CA), according to the manufacturers guidelines. PfNPNA-1 IgG$_4$ was recovered from culture medium using Protein-A sepharose (Pharmacia, Piscataway, NJ) and quantified by ELISA. To establish a stable transfectant, the genes encoding the two chains were transferred into a modified single vector pcDNA3.1/zeo (Invitrogen, Carlsbad, CA). The cell culture, transfection, and cloning were carried out as described by Dreier [22]. The supernatants of isolated colonies were assayed for repeat epitope binding by ELISA and selected IgG$_4$ producers were expanded.

2.2. Indirect immunofluorescence assay

Printed multiwell slides coated with *P. falciparum* NF54 strain sporozoites were used unfixed. Antibodies were applied to the slides and incubated at 37°C in a humid container as follows. Slides were blocked with 4% BSA in PBS for 1 h. Antibodies diluted in PBST were applied for 2 h, the slides were washed 4× with PBS and fluoroscein-conjugated anti-human *k* light chain or anti-mouse IgG (Sigma, St Louis, MO) were applied, diluted 1:25 in PBST. After 2 h slides were washed as before and mounted in SlowFade anti-fade reagent (Molecular Probes, Eugene, OR) and viewed by fluorescence microscopy. Initially a dilution limit point was determined for each antibody preparation and images of staining captured.

2.3. Inhibition of P. falciparum invasion of HepG2-A16 cells

The assays were performed essentially as described by Hollingdale [23]. Human hepatoma (HepG2-A16) cells were cultured at 37°C in 5% CO$_2$ mixed with air in MEM supplemented with 10% heat inactivated fetal bovine serum, 50 U/ml Penicillin and 50 μg/ml streptomycin. The purified monoclonal antibodies were diluted from 20, 10, 5, 2.5 μg/ml in culture medium. Control cultures received mouse mAb 36 to *Pf* CSP [24], or no antibody. Viable *P. falciparum* NF54 sporozoites were isolated from the salivary glands of laboratory

Table 1

Recombinant antibodies bind to *P. falciparum* sporozoites in an IFA: Determination of dilution limit point (DLP). Antibodies were diluted and applied to slides coated with Pf sporozoites, and incubated at 37°C in a humid container as follows Printed multiwell slides coated with PF NF54 strain sporozoites were used unfixed. Slides were blocked with 4% BSA in PBS for 1 h. Antibodies diluted in PBST were applied for 2 h, the slides were washed 4x with PBS and fluoroscein-conjugated anti-human k light chain or anti-mouse IgG (Sigma) were applied, diluted 1:25 in PBST. After 2 h slides were washed as before and mounted in SlowFade anti-fade reagent (Molecular Probes) and viewed by fluorescence microscopy and scored visually for parasite staining. The dilution limit point (DLP) is defined as the concentration of antibody binding sites below which a clear surface immunofluorescence of sporozoites is no longer visible

Antibody	Dilution Limit Point (M)*
2A10	$\sim 1 \times 10^{-10}$
PfNPNA-1 VH/k	$\sim 8 \times 10^{-9}$
PfNPNPA-1 IgG4	$\sim 5 \times 10^{-10}$
PfNPNA-1 IgG1	$\sim 3 \times 10^{-9}$
Vol-IgG	$\sim 3 \times 10^{-7}$

bred *Anopheles stephensi* mosquitoes infected by membrane feeding on blood obtained from *in vitro* culture of the parasite. Salivary glands were pooled in heat inactivated human serum and disrupted in a tissue grinder. Sporozoites were counted, diluted and $\sim$20,000 parasites added to each culture well and incubated for a further 3 h, rinsed twice with Dulbecco's PBS, fixed with methanol and rinsed twice with PBS. Sporozoites that had entered cells were visualized in fixed cultures by immunoperoxidase antibody assay (IPA) [23]. The IPA was carried out by first treating the fixed cultures with mAb 36 followed by incubation with rabbit anti mouse immunoglobulin conjugated with HRP and stained with DAB. The numbers of parasites present in the entire preparation were counted on a Leitz microscope at $40\times$ magnification with a blue filter.

3. Results

3.1. Indirect immunofluorescence assay

The recombinant human antibodies label sporozoites in an IFA and compare favorably with the *in vitro* protective mAb 2A10 [19,25] as shown in Fig. 1. A dilution limit point determination compared the various antibody preparations in the IFA and again the results were very similar to the murine 2A10 as shown in Table 1. Since the sporozoites were unfixed and therefore impermeable, we infer that the antibodies were only recognizing the sporozoite surface.
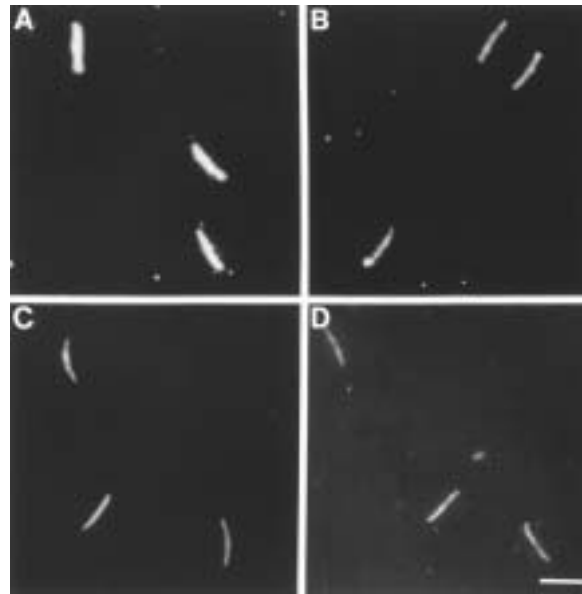

Fig. 1. Recombinant antibodies bind to *P. falciparum* sporozoites in an indirect immunofluorescence assay (IFA) Antibodies were diluted and applied to slides coated with unfixed *Plasmodium falciparum* sporozoite. The following antibody dilutions used; panel A, mAb 2A10 ($\sim$17 μg/ml), panel B, PfNPNA-1 IgG4 (0.5 μg/ml), panel C, PfNPNA-1 IgG1 (0.5 μg/ml), panel D, PfNPNA V$_H$/κ (17 μg/ml). The scale bar in panel D represents 25 μm. The PfNPNA-1 IgG1 was assembled and expressed in baculoviral expression system by J. Donald Capra and Kathy Potter at University of Texas South Western [30].

3.2. Inhibition of P. falciparum invasion of HepG2-A16 cells

The potential of this antibody was evaluated by its efficacy in inhibition of sporozoite invading hepatocytes in an *in vitro* assay [23]. As little as 10 μg/ml whole IgG4 reduced sporozoite invasion of hepatocytes by 74%. This inhibition was dose dependant with 5 and 2.5 μg/ml resulting in 58% and 44% inhibition of sporozoite invasion respectively as shown in Table 2. This degree of inhibition, correlates well with efficacy of murine mAb directed against the repeat in similar assays [23]. At the maximum level tested (20 μg/ml antibody) the protection did not increase. The murine positive control IgM mAb 36 however, conferred 91% inhibition at 10 μg/ml.

4. Discussion

It is well established that polymeric proteins can stimulate B-cells without the help of T-cells [17]. This activation of B-cells is mediated via cross-linking of

Table 2

Human monoclonal antibody PfNPNA-1 IgG4 inhibits *P. falciparum* sporozoite invasion of HepG2-A16 cells. Human hepatoma (HepG2-A16) were cultured at 37°C in 5% CO_2 mixed with air in MEM supplemented with 10% heat inactivated fetal bovine serum, 50 U/ml Penicillin and 50 μg/ml streptomycin. The purified monoclonal antibodies were diluted from 20, 10, 5, 2.5 mg/ml in culture medium. Viable *P. falciparum* NF54 sporozoites were counted and ~20,000 parasites added to each culture well and incubated for a further 3 h, rinsed twice with Dulbecco's PBS, fixed with methanol and rinsed twice with PBS. Sporozoites that had entered cells were visualized in fixed cultures by immunoperoxidase antibody assay (IPA). The numbers of parasites present in the entire well were counted on a Leitz microscope at 40× magnification with a blue filter. The total numbers of parasites from two independent wells were added together prior to determining the % inhibition. % inhibition = 180 – (invasion events) × 100/180

Antibody	Concentration μg/ml	Number of Invaded Sporozoites per well $n = 2$	% Inhibition
mAb 36	10	11, 5	91
PfNPNA-1 IgG4	20	37, 19	68
PfNPNA-1 IgG4	10	22, 25	74
PfNPNA-1 IgG4	5	41, 35	58
PfNPNA-1 IgG4	2.5	54, 46	44
No Antibody	0	105, 75	0

the B-cell surface Ig through specific interactions of the binding sites. The consequence of this route of B-cell stimulation is the lack of formation of germinal centers, lack of somatic mutations and the failure to establish real memory. These antibodies tend to be predominantly IgM with some class switching to IgG with low affinity, yet retain a physiologically relevant avidity. This may be occurring in the natural human immune response against the *P. falciparum* CS protein repeat $(NPNA)_n$. Antibody phage display technology is based on affinity selection [26]. Invariably, it selects for the antibody fragment with the highest affinity for the selecting ligand. Despite exhaustive searching of a human antibody fragment phage display library derived from a *Pf* sporozoite immunized (protected) donor PBLs only a single antibody was recovered designated Pf NPNA-1 V_H/κ [18]. The binding affinity of the monovalent Pf NPNA-1 V_H/κ for the repeat peptide was in the μM range.

The various recombinant human antibodies based on the PfNPNA-1 V_H/V_L sequences all label sporozoites in an IFA and compared favorably with the *in vitro* protective mAb 2A10. Moreover the dilution limit point determinations compared the various antibody preparations in the IFA and were very similar to the murine 2A10 as shown in Table 1. The IgG$_1$ and IgG$_4$ had comparable binding characteristics in the IFA and similar dilution limit points.

The antibody titers against sporozoite in the sera of individuals living in endemic regions tends to increase with age [27], and this also correlates with naturally acquired immunity [28]. Natural infection follows seasonal patterns in which the antibody response may rise after exposure, but is usually too late to be preventative and then wanes until the next season, and then

the pattern repeats. We speculate that natural protection against the sporozoite may be correlated to levels of specific antibody in the circulation at the time of exposure and the subsequent development of cell mediated immunity. However we further speculate that the continuous presentation of CS proteins may be required to maintain a high level of anti-CS antibody response. Hepatocytes infected with developmentally arrested parasites, as a result of irradiation, may continue to produce and release CS protein in such cases. This is an obstacle for conventional vaccination. Indeed an immunization protocol establishing a chronic release of CS proteins may be an ideal vaccine [13]. However until such a concept is developed further, the administration of human monoclonal IgG antibody may provide an alternative passive immunization route to protection. The passive immunization approach is independent of factors that impose limits on conventional active vaccination strategies, such as the nature of the antigen and adjuvant effects. Also, the natural restrictions imposed by the requirements to be effective in a broad range of human leukocyte class I and II antigens (HLA) for effective antigen presentation leading to induction and maintenance of immunity is negated [29]. This candidate prophylactic antibody is directed against the repeat epitope $(NPNA)_3$ fortunately which is conserved within the CS protein of *P. falciparum* isolates from different regions [10,19], and thus should be broadly effective against all *P. falciparum*. Moreover the tandem arrays of repeats on the CS protein molecule reduce the likelihood of variants arising, which completely disrupt the epitope and thus evade antibody recognition. Finally, with the administration of a human monoclonal antibody of desired affinity, specificity and at a level required, effective protection can be assured for the

period of risk to parasite exposure. Polyclonal antibodies induced against the repeat epitope by various procedures have been correlated to protection [15,16]. It has been estimated that as little as 15–19 μg/ml of specific IgG may be sufficient to provide complete protection against malaria infection. The use of pooled immunoglobulin preparation to confer passive protection is no longer a therapeutic option due to the risk of transmission of HIV, HBV, HCV and other blood borne infectious diseases. Hence, in the absence of an effective vaccine for the prevention of malaria and the appearance of multi-drug resistant parasites, reducing the effectiveness of conventional chemoprevention, human monoclonal antibodies may prove to be an effective safe prophylactic agent for the prevention of *P. falciparum* malaria.

The fully human monoclonal antibody PfNPNA-1 IgG4 against (NPNA)₃ specifically labels *Plasmodium falciparum* in an IFA. This antibody also inhibits *Plasmodium falciparum* sporozoite invasion of human hepatocytes in a dose dependent manner in an *in vitro* assay. The *in vitro* data suggest that this antibody may play a role in the prevention of malaria infection.

Acknowledgements

References

[1] J.G. Breman, A. Egan and G.T. Keusch, The intolerable burden of malaria: a new look at the numbers, *Am J Trop Med Hyg* **64**(1–2) (2001), iv–vii.

[2] J. Sachs and P. Malaney, The economic and social burden of malaria, *Nature* **415** (2002), 680–685.

[3] U. D'Alessandro, A. Leach, C.J. Drakeley, S. Bennett, B.O. Olaleye, G.W. Fegan, M. Jawara, P. Langerock, M.O. George and G.A. Targett et al., Efficacy trial of malaria vaccine SPf66 in Gambian infants, *Lancet* **346**(8973) (1995), 462–467.

[4] D. Baudon, Malaria and travelers–practical aspects, *Trop Med Parasitol* **44**(3) (1993), 246–249.

[5] H. Mulligan, P. Russell and B. Mohan, Active immunization of fowls against Plasmodium gallinaceum by injection of homologous sporozoite, *J Malaria Institue India* **4** (1941), 25–34.

[6] R. Nussenzweig, J. Vanderberg and H. Most, Protective immunity produced by the injection of x-irradiated sporozoites of Plasmodium berghei. IV. Dose response, specificity and humoral immunity, *Mil Med* **134**(10) (1969), 1176–1182.

[7] P. Potocnjak, N. Yoshida, R.S. Nussenzweig and V. Nussenzweig, Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection, *J Exp Med* **151**(6) (1980), 1504–1513.

[8] J.B. Dame, J.L. Williams, T.F. McCutchan, J.L. Weber, R.A. Wirtz, W.T. Hockmeyer, W.L. Maloy, J.D. Haynes, I. Schneider and D. Roberts et al., Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite Plasmodium falciparum, *Science* **225**(4662) (1984), 593–599.

[9] H.J. Dyson, A.C. Satterthwait, R.A. Lerner and P.E. Wright, Conformational preferences of synthetic peptides derived from the immunodominant site of the circumsporozoite protein of Plasmodium falciparum by 1H NMR, *Biochemistry* **29**(34) (1990), 7828–7837.

[10] F. Zavala, A. Masuda, P.M. Graves, V. Nussenzweig and R.S. Nussenzweig, Ubiquity of the repetitive epitope of the CS protein in different isolates of human malaria parasites, *J Immunol* **135**(4) (1985), 2790–2793.

[11] D.F. Clyde, V.C. McCarthy, R.M. Miller and R.B. Hornick, Specificity of protection of man immunized against sporozoite-induced falciparum malaria, *Am J Med Sci* **266**(6) (1973), 398–403.

[12] W. Hockmeyer and W. Ballou, in: *Progress in Allergy*, P. Perlmann and H. Wigzell, eds, Karger: Basel, 1988, pp. 1–14.

[13] S.L. Hoffman, L.M. Goh, T.C. Luke, I. Schneider, T.P. Le, D.L. Doolan, J. Sacci, P. de la Vega, M. Dowler, C. Paul, D.M. Gordon, J.A. Stoute, L.W. Church, M. Sedegah, D.G. Heppner, W.R. Ballou and T.L. Richie, Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites, *J Infect Dis* **185**(8) (2002), 1155–1164.

[14] T.C. Luke and S.L. Hoffman, Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated Plasmodium falciparum sporozoite vaccine, *J Exp Biol* **206**(21) (2003), 3803–3808.

[15] J.E. Egan, S.L. Hoffman, J.D. Haynes, J.C. Sadoff, I. Schneider, G.E. Grau, M.R. Hollingdale, W.R. Ballou and D.M. Gordon, Humoral immune responses in volunteers immunized with irradiated Plasmodium falciparum sporozoites, *Am J Trop Med Hyg* **49**(2) (1993), 166–173.

[16] R. Edelman, S.L. Hoffman, J.R. Davis, M. Beier, M.B. Sztein, G. Losonsky, D.A. Herrington, H.A. Eddy, M.R. Hollingdale

and D.M. Gordon et al., Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated Plasmodium falciparum sporozoites, *J Infect Dis* **168**(4) (1993), 1066–1070.

[17] M. Brunswick, C.H. June, F.D. Finkelman, H.M. Dintzis, J.K. Inman and J.J. Mond, Surface immunoglobulin-mediated B-cell activation in the absence of detectable elevations in intracellular ionized calcium: a model for T-cell-independent B-cell activation, *Proc Natl Acad Sci USA* **86**(17) (1989), 6724–6728.

[18] J.A. Chappel, W.O. Rogers, S.L. Hoffman and A.S. Kang, Molecular dissection of the human antibody response to the structural repeat epitope of Plasmodium falciparum sporozoite from a protected donor, *Malar J* **3**(1) (2004), 28.

[19] F. Zavala, J.P. Tam, M.R. Hollingdale, A.H. Cochrane, I. Quakyi, R.S. Nussenzweig and V. Nussenzweig, Rationale for development of a synthetic vaccine against Plasmodium falciparum malaria, *Science* **228**(4706) (1985), 1436–1440.

[20] R. Anker, F. Zavala and B.A. Pollok, V_H and V_L region structure of antibodies that recognize the (NANP)3 dodecapeptide sequence in the circumsporozoite protein of Plasmodium falciparum, *Eur J Immunol* **20**(12) (1990), 2757–2761.

[21] H.M. Etlinger and A. Trzeciak, Towards a synthetic malaria vaccine: cyclization of a peptide eliminates the production of parasite-unreactive antibody, *Philos Trans R Soc Lond B Biol Sci* **340**(1291) (1993), 69–72.

[22] T. Dreier, H.N. Lode, R. Xiang, C.S. Dolman, R.A. Reisfeld and A.S. Kang, Recombinant immunocytokines targeting the mouse transferrin receptor: construction and biological activities, *Bioconjug Chem* **9**(4) (1998), 482–489.

[23] M.R. Hollingdale, E.H. Nardin, S. Tharavanij, A.L. Schwartz and R.S. Nussenzweig, Inhibition of entry of Plasmodium falciparum and P. vivax sporozoites into cultured cells; an *in vitro* assay of protective antibodies, *J Immunol* **132**(2) (1984), 909–913.

[24] B.J. Sina, C. Wright, R. Ballou and M. Hollingdale, A protective monoclonal antibody with dual specificity for Plasmodium falciparum and Plasmodium berghei circumsporozoite proteins, *Exp Parasitol* **74**(4) (1992), 431–440.

[25] E.H. Nardin, V. Nussenzweig, R.S. Nussenzweig, W.E. Collins, K.T. Harinasuta, P. Tapchaisri and Y. Chomcharn, Circumsporozoite proteins of human malaria parasites Plasmodium falciparum and Plasmodium vivax, *J Exp Med* **156**(1) (1982), 20–30.

[26] J. McCafferty, A.D. Griffiths, G. Winter and D.J. Chiswell, Phage antibodies: filamentous phage displaying antibody variable domains, *Nature* **348**(6301) (1990), 552–554.

[27] E.H. Nardin, R.S. Nussenzweig, I.A. McGregor and J.H. Bryan, Antibodies to sporozoites: their frequent occurrence in individuals living in an area of hyperendemic malaria, *Science* **206**(4418) (1979), 597–599.

[28] I.A. McGregor, Studies in the Acquisition of Immunity of Plasmodium Falciparum Infections in Africa, *Trans R Soc Trop Med Hyg* **58** (1964), 80–92.

[29] A.V. Hill, C.E. Allsopp, D. Kwiatkowski, N.M. Anstey, P. Twumasi, P.A. Rowe, S. Bennett, D. Brewster, A.J. McMichael and B.M. Greenwood, Common west African HLA antigens are associated with protection from severe malaria, *Nature* **352**(6336) (1991), 595–600.

[30] K.N. Potter, Y. Li and J.D. Capra, Antibody production in the baculovirus expression system, *Int Rev Immunol* **10**(2–3) (1993), 103–112.