Aspects of Immunity for the AMA-1 Family of Molecules in Humans and Non-Human Primates Malarias

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The apical membrane antigen (AMA-1) family of malaria merozoite proteins is characterised by a high degree of inter-species conservation. Evidence that the protein (PK66/AMA-1) from the simian parasite Plasmodium knowlesi *was protective in rhesus monkeys suggested that the 83kDa* P. falciparum *equivalent (PF83/AMA-1) should be investigated for protective effects in humans. Here we briefly review pertinent comparative data, and describe the use of an eukaryotic full length recombinant PF83/AMA-1 molecule to develop a sensitive ELISA for the determination of serological responses in endemic populations. The assay has revealed surprisingly high levels of humoral response to this quantitatively minor antigen. We also show that PK66/AMA-1 inhibitory mAb's are active against merozoites subsequent to release from schizont-infected red cells, further implicating AMA-1 molecules in red cell invasion.*

Key words: *Plasmodium falciparum* - *Plasmodium knowlesii* - AMA-1 - PK66 - PF83 - merozoite - seroepidemiology

To date the AMA-1 family of molecules can be partitioned into those with an apparent MW of approximately 66kDa, for example rodent (*P. chabaudi*, Marshall et al. 1989), simian (*P. knowlesi*, Deans et al. 1982) and human (*P. vivax*, unpublished observations) species, and those of approximately 83kDa so far characterised by human (*P. falciparum*, Peterson et al. 1989) and ape (*P. reichenowi*, unpublished observations) species. In the light of the promise shown by PK66/AMA-1 as a vaccine in rhesus monkeys (Deans et al. 1988) it is helpful to compare some of its properties with those of the *P. falciparum* equivalent, PF83/AMA-1 (Table I). AMA-1 synthesis is restricted in both species to very late schizogony, and both molecules are N-terminally post-translationally cleaved, although the timing of this event appears to differ between the species. In cultures of *P. knowlesi* mature schizonts to which chymostatin and/or leupeptin has been added, the appearance of the 42kDa processed fragment is specifically inhibited (unpublished observation). No effect on the appearance of the 66kDa *P. falciparum* form was noted under similar conditions. In both species, two differentially expressed hybridising mRNA populations have been identified. Intriguingly, for PF83/AMA-1 the appearance of the larger of the two mRNA transcripts would seem to correspond to protein synthesis (Jaikaria et al. 1993, Narum & Thomas 1994. Even at the time of peak synthesis, the molecules in both species comprise only quantitatively minor fractions of the overall protein synthetic activity, such that on SDS-PAGE fluorographs of total radiolabelled metabolic incorporation during schizogony, PK66/AMA-1 and PF83/AMA-1 synthesis cannot readily be distinguished (Deans et al. 1984 and unpublished observations). Nevertheless we show that, in humans exposed to blood stage infection, this quantitatively minor antigen is strongly recognised.

It has been suggested that the AMA-1 molecule may function in red cell invasion (Thomas et al. 1984) and/or in the release of merozoites from mature schizont infected red cells (Peterson et al. 1989). Here we show evidence that a mAb that inhibits *P. knowlesi* multiplication in vitro retains

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this activity against free merozoites, suggesting that PK66/AMA-1 has an important functional role subsequent to schizont rupture.

TABLE I

PK66/PF83 AMA-1 comparison

	PK66	PF83
Synthesis	7 nuclei	9 nuclei
Processing	Sz rupture -> 44 and 42 kDa	Rapid -> 66 kDa
Processing site	N-Terminal	N-terminal
Chymostatin / Leupeptin inhibit processing	Yes	No
mRNA	2 transcripts	2 transcripts
Localization	Mz: Apical /circunfrencial	Mz: Apical /circunfrencial
Ring incorporation	Yes	Yes
Immune recognition	Strong in rhesus	Strong in human

RESULTS AND DISCUSSION

HUMAN SERUM RESPONSES TO PF83 / AMA-1

For all malaria vaccine components, except those directed towards some post-fertilisation stages, there will be a pre-existing immune response in the target population in endemic areas. The intriguing manner in which immunisation with PK66/AMA-1, in combination with exposure to infection, engendered a strong protective immunity in rhesus macaques (Deans et al. 1988) has prompted our interest in the naturally occuring response to this antigen family. A full length baculovirus recombinant PF83 / AMA-1 molecule has been expressed and purified by hydrophobic anion exchange chromatography (Narum et al. 1993). This product has been used in an ELISA in which a mAb specific for the C-terminal cytoplasmic AMA-1 region is used to capture recPF83/AMA-1 (CT-capture ELISA). Human serum responses have been determined in populations from Guinea Bissau (50 children aged 2 - 9 years, moderate endemicity, 22% thick film *P. falciparum* positive at time of serum sample) and Senegal (199 individuals, 2 - 86 years, holo-endemic, 65% of individuals thick film *P. falciparum* positive at time of serum sample). CT-capture ELISA end point titrations corresponded closely with the strength of signal from single dilutions, so the simpler single dilution readout was used throughout. Responses were also determined by ELISA against a sonicated homogenate of whole schizont-infected red cells.

Fig. 1: results of CT-capture ELISA against recPF83/AMA-1 and total parasite antigen ELISA for Senegalese (A) and Guinea Bissau (B) serum samples. Sera (1:200) were assayed in duplicate and the ELISA index as an arithmetic mean and the SEM are shown. Association between recPF83/AMA-1 ELISA index and age for the Guinea Bissau population is statistically significant (Wilcoxon test P).

Full details of the study population and the ELISA techniques are in press.

All individuals within the Guinea Bissau study, and 94% of the Senegalese population showed naturally acquired serum IgG responses to PF83/AMA-1. The average CT-capture ELISA index (calculated by division of the observed arithmetic mean by the geometric mean (+ 2 S.D.) of a panel of normal sera) was grouped by age within the two populations. While a statistically significant increase in specific serum IgG with age was observed for responses to total parasite antigen in the Senegalese population, this was not the case for responses to PF83/AMA-1 (Fig. 1A). In contrast a statistically significant age related response to PF83/AMA-1 was evident in the Guinea Bissau study (Fig 1B). This may reflect the relatively higher intensity of exposure in the Senegalese population and suggests that responses to PF83/AMA-1 may be more readily induced than responses to some other parasite antigens. Taken together with the overall prevalence of PF83/AMA-1 specific responses these data clearly indicate that, after exposure to malarial infection, this antigen is very well recognised. A similar response has been observed in rhesus monkeys that had been immunised with *P. knowlesi* merozoites, or by repeated infection and drug cure, where PK66/AMA-1 was also one of the more strongly recognised antigens (manuscript in preparation). The implications of such strong responses to AMA-1 are not yet clear. Further studies to more closely define antibody (isotype and specificity) and cellular responses to PF83/AMA-1 in these, and other endemic populations are now underway.

PK66 / AMA-1 MAB FUNCTION AGAINST MEROZOITES

The "cell-sieve" method of Dennis et al. (1975) was used to isolate free viable merozoites from *P. knowlesi* W1 strain schizonts that had been isolated from the blood of an infected rhesus monkey as previously described (Deans et al. 1983). Merozoites were eluted in RPMI 1640 supplemented with 1 mg ml^{-1} glucose, 40mM TES and 1 unit ml^{-1} heparin, maintained at 23°C during centrifugation (2800 x g, 10 mins), and added to rhesus erythrocytes in the presence of various concentrations of mAb prepared as previously described (Thomas et al. 1984). The results from one assay of parasite growth under these conditions are shown in Fig. 2. Data from the same experiment when analysed by microscopic evaluation of Giemsa stained thin films gave comparable results (not shown). It is evident that antibody R3/1C2, previously shown to be inhibitory when incorporated into cultures initiated with *P. knowlesi* schizonts (Thomas et al. 1984), retains this activity against isolated merozoites. While this does not preclude a funtional role for PK66/AMA-1 in the process of schizont rupture and merozoite release, it does implicate the molecule in events subsequent to merozoite release. The joint apical and circumferential surface localisation of PK66/AMA-1 (Thomas et al. 1990) in combination with the activity against free merozoites strongly suggests that AMA-1 has a vital role in the process of erythrocyte invasion.

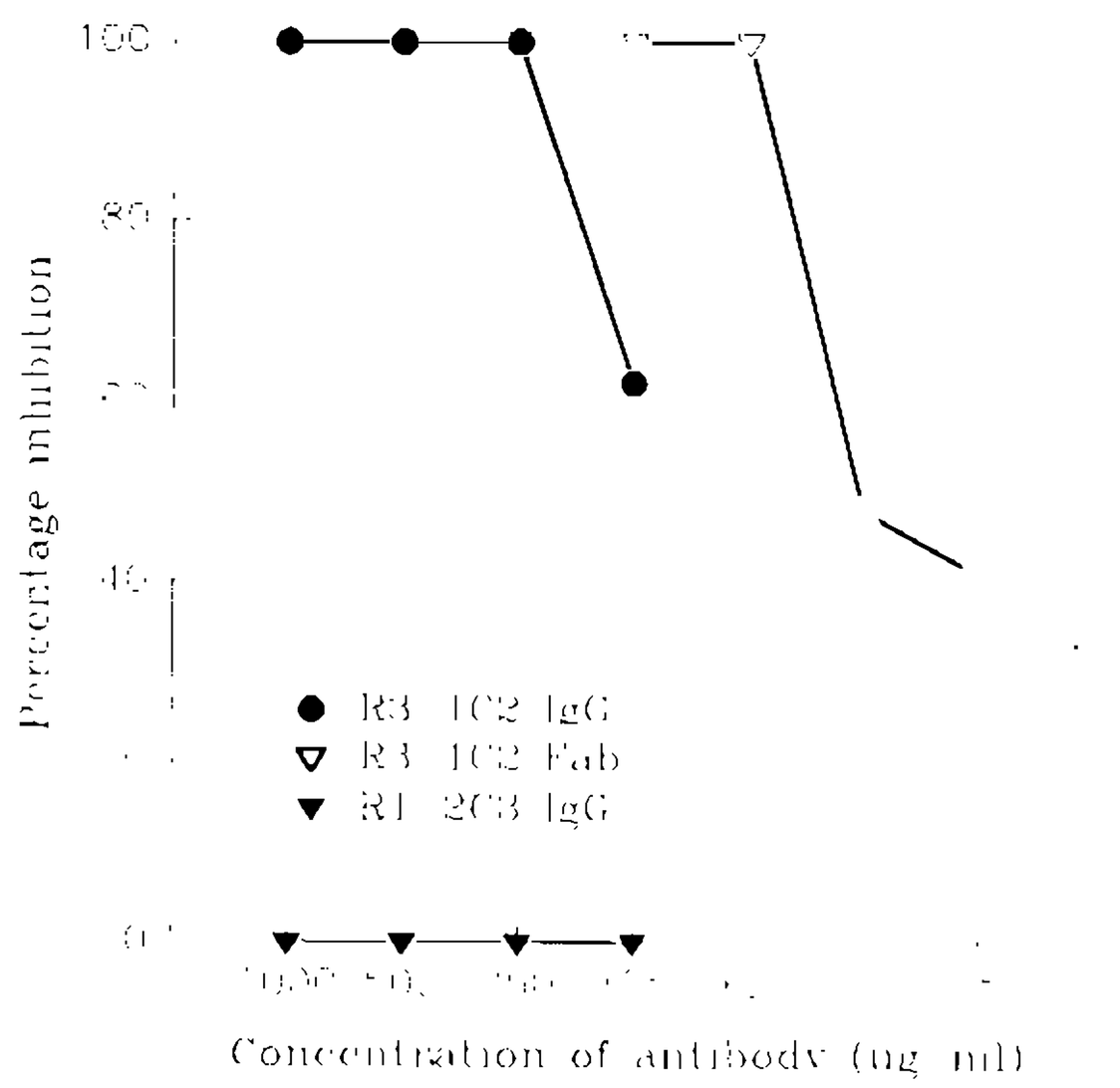

Fig. 2: inhibition of invasion by isolated *P. knowlesi* W1 strain merozoites (mz). Viable mz were isolated, in the absence of antibody, from mature schizonts by nucleopore filtration. Mz were added to wells containing rhesus RBC at a 1% haematocrit (1:1 ratio mz:RBC) in the presence of the indicated concentrations of monoclonal antibody, and cultured under standard conditions in the presence of [3H] amino acids. Inhibition was calculated from the incorporation of TCA precipitable material 6 hours later.

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REFERENCES

Deans JD, Alderson T, Thomas AW, Mitchell GH, Lennox ES, Cohen S 1982. Rat monoclonal antibodies

which inhibit the *in vitro* multiplication of *Plasmodium knowlesi*. *Clin Exp Immunol 49:* 297-309.

Deans JA, Thomas AW, Cohen S 1983. Stage-specific protein synthesis by asexual blood stage parasites of *Plasmodium knowlesi*. *Mol Biochem Parasitol 8:* 31-44.

Deans JA, Thomas AW, Alderson T, Cohen S 1984. Biosynthesis of a putative protective *Plasmodium knowlesi* merozoite antigen. *Mol Biochem Parasitol 11:* 189-204.

Deans JA, Knight AM, Jean WC, Waters AP, Cohen S, Mitchell GH 1988. Vaccination trials in rhesus monkeys with a minor, invariant, *Plasmodium knowlesi* 66 kDa merozoite antigen. *Parasitol Immunol 10:* 535-552.

Dennis ED, Mitchell GH, Butcher GA, Cohen S 1975. *In vitro* isolation of *Plasmodium knowlesi* merozoites using polycarbonate sieves. *Parasitology 71:* 475-481

Jaikaria NS, Rozario C, Ridley RG, Perkins ME 1993. Biogenesis of rhoptry organelles in *Plasmodium falciparum*. *Mol Biochem Parasitol 57*: 269-280.

Marshall VM, Peterson MG, Lew AM, Kemp JD 1989. Structure of the apical membrane antigen I (AMA-1) of *Plasmodium chabaudi*. *Mol Biochem Parasitol 37:* 281-284

Narum DL, Welling GW, Thomas AW 1993. Ion-exchange/immuno-affinity purification of a recombinant baculovirus *Plasmodium falciparum* apical membrane antigen, PF83/AMA-1. *J Chromatogr 657:* 357-363

Narum DL, Thomas AW 1994. Differential localization of full-lengh and processed forms of PF83/AMA-1 an apical membrane antigen of *Plasmodium falciparum* merozoites. Mol Biochem Parasitol 67: 59-68

Peterson MG, Marshall VM, Smythe JA, Crewther PE, Lew A, Silva A, Anders RF, Kemp DJ 1989. Integral membrane protein located in the apical complex of *Plasmodium falciparum*. *Mol Cell Biol 9:* 3151-3154.

Thomas AW, Jeans JA, Mitchell GH, Alderson T, Cohen S 1984. The Fab fragments of monoclonal IgG to a merozoite surface antigen inhibit *Plasmodium knowlesi* invasion of erythrocytes. *Mol Biochem Parasitol 13:* 187-199.

Thomas AW, Bannister LH, Waters AP 1990. Sixty-six kilodalton-related antigens of *Plasmodium knowlesi* are merozoite surface antigens associated with the apical prominence. *Parasite Immunol 12:* 105-113