Transmission Blocking Immunity as Observed in a Feeder System and Serological Reactivity to Pfs 48/45 and Pfs230 in Field Sera

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Monoclonal antibodies (mAbs) and human sera from gametocyte carriers were applied in the bio-assay to test for their transmission-blocking capacity.

Competition ELISA's have been developed for the detection of natural transmission blocking antibodies. Approximately 55% of the sera blocking in the bio-assay gave positive results in these competition ELISA's.

Key words: *Plasmodium falciparum* - transmission-blockade - natural antibodies - competition ELISA's

Gametocytes and macrogametes/zygotes of *Plasmodium falciparum* synthesize 230 kDa and 48/45 kDa molecules (Vermeulen et al. 1986). Several transmission-blocking mAbs have been described which react specifically with these proteins of the sexual stages of *Plasmodium falciparum* (Rener et al. 1983, Vermeulen et al. 1985a, 1985b, Quakyi et al. 1987). Both surface proteins (Pfs230, Pfs48/45) are also target antigens of natural transmission-blocking immunity (Graves et al. 1988). As their epitopes seem to express a conformational configuration, peptide-constructs cannot be used as immunogens for transmission-blocking antibodies.

The main objective of the present study was to develop a reliable method for the assessment of transmission reducing antibodies in sera of people exposed to malaria infections. Reduced transmission as demonstrated using the laborious bio-assay with *Anopheles gambiae* was considered the standard.

RESULTS AND DISCUSSION

SOLUBILIZING THE ANTIGENS FROM GAMETOCYTES TO MAKE IT SUITABLE FOR APPLICATIONS IN ELISA

Gametocytes were extracted with Triton X-114 (TX-114) as described by Bordier (1981). Phase separation of Triton X-114 extracts results in the separation of Pfs230 in the aqueous phase (AP) and Pfs48/45 in the detergent phase (DP). Immune reactivity of Pfs230 was preserved for at least 8 weeks after freeze-drying and storage under vacuum or under N_2, and Pfs48/45 over one year if stored at -80^0C.

No Pfs230 was detected with the -Pfs230 mAbs after directly coating of AP onto the surface of the ELISA-plate. After precoating with poly-L-lysine before coating with AP an one-site ELISA using HRPO-coupled rabbit-anti-mouse conjugate to detect binding of the mAbs could be established. With the DP only a two-site ELISA could be done.

EPITOPES OF THE SEXUAL ANTIGENS

In studies using several mAbs with specificity for each of both proteins, it has been established that each protein has more than one epitope, but per molecule of protein each epitope is expressed only once. Some of these epitopes interact with blocking others with non-blocking mAbs as observed in the bio-assay. Also it became evident that the simultaneous presence of more than one of these protein specific antibodies, interacting with the same protein, might cause potentiation of the blocking effect.

MAbs against the 230 kDa protein of the IgG2a isotype, made by Dr R Carter, appeared to give a complement dependent blockage by lysis of the macrogametes. The relevance of this phenomenon in sera from endemic areas is presently under study and point to a role for isotypes.

This study was supported by the EEC STJ 004/2, 633.

TABLE I

Comparison of activity in the Pfs230 (C230-ELISA) and Pfs48/45 (C45-ELISA; combined results with 32F3 and 32F1 competition) competition ELISA and transmission reduction activity (TBA) in 46 sera of gametocyte carriers from Cameroon

		TBA		
		≥ 85% (n=19)	<85% (n=27)	Total
C230-ELISA	positive	5	1	6
	negative	14	26	40
C45-ELISA	positive	11	2	13
	negative	8	25	33

In one-site competition ELISA's we have checked all available Pfs230 mAbs against each other. At least 5 singly expressed epitopes have been defined on Pfs230. Three are targets of mAbs with no transmission-reducing activity. The other two are defined through its reactivity with transmission-reducing and nonreducing mAbs.

The two-site ELISA now appeared utilizable for testing material in epidemiological settings for the testing of specificity to Pfs230 next to that of Pfs48/45.

PILOT STUDY ON THE RELEVANCE OF THE TEST FOR FIELD SAMPLES

In view of the epitope results with the mAbs further studies were made with a positive serum, negative sera and a panel of 46 sera taken from gametocyte carriers in an endemic area.

The positive serum (St1) collected from a missionary who worked for thirty years in Tanzania, was positive in all assays, blocked transmission in the bio-assay and was used as a reference serum. A pool of negative sera (N56) was obtained from Dutch bloodbank donors. The gametocyte carriers were recruited at a dispensary in Yaounde, Cameroon.

Six (13%) out of 46 gametocyte carriers were able to compete HRPO-mAbs in the Pfs230 competition ELISA at dilution varying from 1/40 to 1/80.

Eleven (24%) out of 46 gametocyte carriers were able to compete HRPO-32F3 and 8 (17%) sera compete the HRPO-32F1 in the Pfs48/45 competition ELISA at dilution varying from 1/40 to 1/320.

Nineteen (41.3%) out of 46 gametocyte sera consistently reduced infectivity in the Nijmegen bio-assay significantly to less than 15% of the control.

In Table I the results are presented of the bioassay (TBA) for transmission reduction (positive ≥ 85%, negative < 85%) and competition ELISA for Pfs230 and Pfs48/45. Of the 19 transmission blocking sera 5 (26%) were positive in the C230-ELISA and 11 (58%) in the C45-ELISA (32F3 and/or 32F1 epitope). The Pfs230 test was positive in 6 of the 46 sera; one of these had a TBA score of 73%. Two of the 13 positive sera in the Pfs48/45 test had oocyst counts which did not meet the 85% reduction standard. There is a positive correlation (for C45-ELISA $X^2=14.02$, $P<0.001$; for C230-ELISA $X^2=5.03$, $P<0.04$) between TBA and C45/C230-ELISA, which means if TBA ≥ 85% more positives in the C45/C230-ELISA were found. In Table II, the results of C230-ELISA and C45-ELISA are compared of the sera with transmission reduction ≥ 85%. All 5 sera, positive in the C230-ELISA was also

TABLE II

Results of Pfs48/45 (C45-ELISA) and Pfs230 (C230-ELISA) competition ELISA's from 19 sera with high transmission reduction

		C230-ELISA		
		Positive	Negative	Total
C45-ELISA	positive	5	6	11
	negative	0	8	8
	Total	5	14	19

positive in the C45-ELISA. Of the 11 sera, positive in the C45-ELISA, 6 were negative in the C230-ELISA.

In this selection of sera from gametocyte carriers from Cameroon, the Pfs230 specific ELISA's seems to add little or no effect to the transmission-reduction predictive value of the competition ELISA with the Pfs48/45 blocking epitope as target antigen.

The use of a capturing antibody for the Pfs230 assay is optional as both mAbs react also with the antigen directly coated to the microtiter plate. In the one-site ELISA, the 5 Pfs230 specific mAbs, labelled with HRPO, were added together with serial dilutions of sera in competition for the specific epitope. No differentiation in antibody responses to the Pfs230 epitopes was found; sera positive in one test reacted also with the other epitopes of Pfs230. This is in contrast with the results of our epitope specific Pfs48/45 competition test (C45-ELISA with 32F3 or 32F1 mAb).

The combined competition ELISA's predict about 50% of the transmission reductions higher than 85%. Five sera with 100% blockade were absolutely negative in the ELISA's, although the blockade was IgG related.

These results might indicate that other Pfs230 epitopes or other *P. falciparum* antigens are involved in inducing the production of transmission reducing antibodies. Moreover, transmission-reduction is probably a relatively short-lived phenomenon that mainly depends on the temporary presence of gametocytes. The incidence of reduction that is prominently present in gametocyte carriers is now being studied in a longitudinal way.

ACKNOWLEDGEMENTS

To T Derks and M Sieben for production and purification of monoclonal antibodies, to M Bolmer, A Huisman and GJ van Gemert for parasite cultures and transmission experiments.

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