Short Report

Anti-Pfs25 monoclonal antibody 32F81 blocks transmission from *Plasmodium falciparum* gametocyte carriers in Cameroon

Bert Mulder[1,2], Will Roeffen[2], Robert Sauerwein[2], Timoléon Tchuinkam[1], Christian Boudin[1] and Jan Peter Verhave[2] *[1]Malaria Department, OCEAC, P. B. 288, Yaoundé, Cameroon; [2]Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands*

Keywords: malaria, *Plasmodium falciparum*, transmission-blocking antibody

Pfs25 is a target protein for the induction of antibodies that block transmission of *Plasmodium falciparum*. Anti-Pfs25 monoclonal antibody (mAb) 32F81 has been generated against gametes of strain NF54 and shown to block the oocyst development in mosquitoes fed on red blood cell suspensions containing NF54 gametocytes (VERMEULEN *et al.*, 1985). Comparison of the genes encoding Pfs25 in 8 isolates of different geographical origin showed almost complete sequence homology (KASLOW *et al.*, 1989). Since transmission blocking activity of 32F81 has been reported for NF54 only, we wanted to test the capacity of this mAb to block transmission of field isolates by experimentally feeding local *Anopheles gambiae* on blood from naturally infected carriers of *P. falciparum* gametocytes. mAb 32F81 was added to the blood meal at a concentration of 50 μg/mL. A blood meal from the same gametocyte carrier without the mAb was used as a control. Gametocyte density was estimated from the parasite/leucocyte ratio by counting gametocytes against 1000 white blood cells and assuming an average leucocyte count of 8000/μL. In 5 mosquitoes, fed on persons with >200 gametocytes per μL, round forms (activated macrogametocytes and zygotes) were searched for 4 h after the blood meal, using mAb 32F81 labelled with fluorescein isothiocyanate. Ookinetes were also looked for in 5 mosquitoes 24 h after the blood meal. After 7 d, surviving mosquitoes were examined for the presence and number of oocysts. Thirty-three infection experiments were performed and 2292 mosquitoes were dissected. The mean age of the gametocyte carriers was 20·3 years (range 4–44 years) and the mean gametocyte density was 252/mm³. Mean numbers (per mm³) of round forms (10·4±9·6) and ookinetes (0·6±1·3) were lower in the 32F81 group, but not significantly so, than in the control group (18·7±26·7 and 9·0±20·1, respectively). Blood meals from 13 gametocyte carriers gave rise to oocysts (Table). Their mean age was 18·5 years and their mean gametocyte density was 400/mm³. In the experimental group 430 mosquitoes were dissected, and in the control group 498. The mean percentage of oocyst-infected mosquitoes in the control group was 35, while in those fed on blood containing mAb 32F81 it was 3·75 ($P<0·002$, Mann–Whitney U test). While the mean oocyst number in the control group was over 7, the 32F81 experimental group yielded a mean of less than one oocyst per mosquito ($P<0·003$, Mann–Whitney U test). The 3 subjects with the highest gametocyte numbers showed significantly higher infection percentages even in the 32F81 group ($P<0·05$ Mann–Whitney U test).

Table. Infections of *Anopheles gambiae* fed on whole blood samples from 13 *Plasmodium falciparum* gametocyte carriers in the presence and absence of monoclonal antibody 32F81

	Control			32F81		
Gametocyte number/mm³	No. dissected	Percentage infected	No. of oocysts (mean)	No. dissected	Percentage infected	No. oocysts (mean)
8	30	23·3	1·3	30	0	–
48	50	22·0	1·3	36	0	–
96	26	7·7	1·0	22	0	–
96	30	16·7	5·2	30	3·3	1·0
136	50	74·0	2·6	50	0	–
168	30	56·7	2·6	30	16·7	1·6
248	30	10·0	1·0	30	3·3	2·0
296	50	10·0	1·8	30	0	–
360	28	35·7	7·4	19	0	–
544	50	14·0	3·1	50	0	–
652	50	90·0	32·9	50	8·0	4·3
1000	25	36·0	10·2	30	3·3	2·0
1240	47	59·6	16·7	21	14·3	1·3

We conclude that mAb 32F81 can block transmission from naturally infected *P. falciparum* gametocyte carriers to a local strain of *A. gambiae*, as has been shown by VERMEULEN *et al.* (1985) and MULDER *et al.* (1994) for a laboratory-adapted strain of *P. falciparum*. These findings are in agreement with the almost complete genetic homology of Pfs25 in different isolates, which has been considered to support the development of a transmission-blocking vaccine based on this protein. Transmission blocking is more effective at low gametocyte densities, which are prevalent under natural conditions, and this confirms previous findings in experimental infections (PONNUDURAI *et al.*, 1987). In addition to naturally occurring transmission blockage of *P. falciparum* in endemic countries (MULDER *et al.*, 1994), immunization using a Pfs25 vaccine might further reduce malaria transmission (KASLOW *et al.*, 1991; KASLOW, 1993).

Acknowledgements

We thank the staff and patients of the Messa dispensary, Yaoundé, for their co-operation, Jullienne Essong and Marianne Sieben for technical assistance, and Isabella Quakyi for critically reading the manuscript. Written permission for blood taking was given to OCEAC by the Cameroonian Ministry of Public Health.

References

Kaslow, D. (1993). Transmission-blocking immunity against malaria and other vector-borne diseases. *Current Opinion in Immunology*, 5, 557–565.

Kaslow, D., Quakyi, I. A. & Keister, D. B. (1989). Minimal variation in a vaccine candidate from the sexual stage of *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, **32**, 101–104.

Kaslow, D., Isaacs, S. N., Quakyi, I. A., Gwadz, R. W., Moss, B. & Keister, D. B. (1991). Induction of *Plasmodium falciparum* transmission-blocking antibodies by recombinant vaccinia virus. *Science*, **252**, 1310–1312.

Mulder, B., Tchuinkam, T., Dechering, K., Verhave, J. P., Carnevale, P., Meuwissen, J. H. E. Th. & Robert, V. (1994). Malaria transmission-blocking activity in experimental infections of *Anopheles gambiae* from naturally infected *Plasmodium falciparum* gametocyte carriers. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 121–125.

Ponnudurai, T., van Gemert, G. J., Bensink, T., Lensen, A. H. W. & Meuwissen, J. H. E. Th. (1987). Transmission blockage of *Plasmodium falciparum*: its variability with gametocyte numbers and concentration of antibody. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 491–493.

Vermeulen, A. N., Ponnudurai, T., Beckers, P. J. A., Verhave, J. P., Smits, M. A. & Meuwissen, J. H. E. Th. (1985). Sequential expression of antigens on sexual stages of *P. falciparum* accessible to transmission blocking antibodies in the mosquito. *Journal of Experimental Medicine*, **162**, 1460–1475.

Received 12 June 1995; revised 25 September 1995; accepted for publication 27 September 1995