INFECTION AND IMMUNITY, Sept. 1995, p. 3702–3704 Vol. 63, No. 9
0019-9567/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Murine Complement Reduces Infectivity of *Plasmodium yoelii* to Mosquitoes

TAKAFUMI TSUBOI,[1]* YA-MING CAO,[1] MOTOMI TORII,[1]
YASUO HITSUMOTO,[2] AND HIROJI KANBARA[3]

Departments of Parasitology[1] and Clinical Laboratory Medicine,[2] Ehime University School of Medicine, Ehime 791-02, and Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Sakamoto, Nagasaki 852,[3] Japan

Received 27 February 1995/Returned for modification 5 April 1995/Accepted 15 June 1995

The alternative pathway of complement in the mouse serum significantly reduced, but did not eliminate, the infectivity of *Plasmodium yoelii* to *Anopheles stephensi*. The reduction of the infectivity is mainly due to the inability of the zygote to transform into the ookinete in the mosquito midgut.

There are some immune effector mechanisms described concerning the infectivity of malaria parasites to mosquitoes (1). Two known mechanisms are cytokines and antibodies, which mediate transmission-blocking immunity (6). However, little is known about the effect of the alternative pathway of complement (APC) on the infectivity of malaria parasites to mosquitoes. Only one report said that the APC in chicken serum did not reduce the infectivity of *Plasmodium gallinaceum* to *Aedes aegypti* (4). Here we present data on the effect of the APC on the infectivity of *Plasmodium yoelii*. The APC in mouse serum reduced *P. yoelii* oocyst formation on the midgut of *Anopheles stephensi.*

DBA/1 and C5-deficient DBA/2 (8) mice were purchased from the Charles River Co. (Yokohama, Japan). Sera were collected and heat inactivated by incubation for 30 min at 56°C in some experiments. C5-deficient human serum was purchased from Sigma (St. Louis, Mo.), and purified human C5 was purchased from Cordis (Miami, Fla.). The complement activities were measured by the procedure of Hitsumoto et al. (5), by using rabbit erythrocytes (RRBC) sensitized with goat anti-RRBC antiserum (Cappel, West Chester, Pa.). A group of *A. stephensi* mosquitoes were starved and then allowed to feed on the mice 3 days after infection with *P. yoelii* 17X (lethal). The mice were subsequently given each test sample intravenously. Ten minutes after administration, another group of mosquitoes was fed on the mice. The engorged mosquitoes were maintained at 24°C. The midguts from eight mosquitoes in each group were removed at 1, 4, 8, and 15 h after the blood

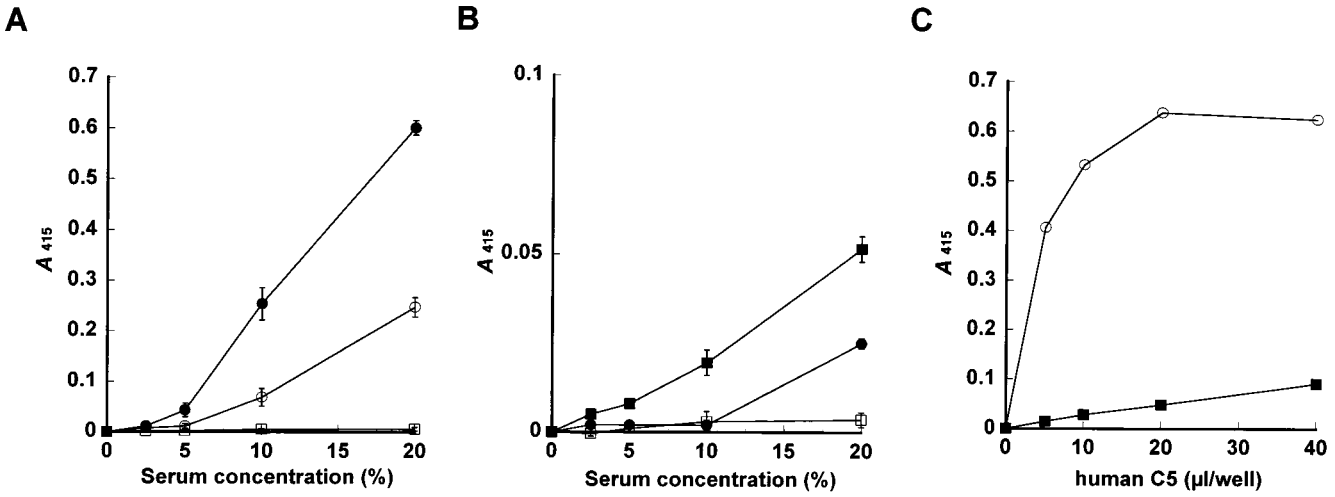

FIG. 1. (A) Hemolysis of sensitized RRBC by complement of mice sera with incubation for 60 min at 37°C. Sensitized RRBC were mixed with the serially diluted sera of female DBA/1 mice (●), female DBA/2 mice injected with 200 μl of fresh DBA/1 sera (○), or female DBA/2 mice injected with 200 μl of heat-inactivated DBA/1 sera (□). (B) Hemolysis of sensitized RRBC by complement of mice sera with incubation for 2 or 6 h at 24°C. Sensitized RRBC were mixed with the serially diluted sera of female DBA/1 mice and incubated for 6 h (■) or for 2 h (●) or with sera of female DBA/2 mice and incubated for 6 h (□). (C) Hemolysis of sensitized RRBC by complement of mouse or human sera with incubation for 60 min at 37°C. Increasing volumes of purified human C5 solution (1,000 50% hemolytic complement U/ml) were added to 20% fresh sera obtained from male DBA/2 mice (■) or to 3% C5-deficient human sera (○).

* Corresponding author. Mailing address: Department of Parasitology, Ehime University School of Medicine, Shigenobu-cho, Ehime 791-02, Japan. Phone: 011-81-899-64-5111, ext. 2064. Fax: 011-81-899-64-5236. Electronic mail address: tsuboi@m.ehime-u.ac.jp.

TABLE 1. *Plasmodium yoelii* oocyst formation in *Anopheles stephensi*

Expt no.[a]	Treatment of mice[b]	Median no. of oocysts (range)[c]	No. of infected mosquitoes/total no. of mosquitoes	*P* value[d]
1	DBA/1	6.0 (0–35)	29/34	<0.001
1	DBA/2	419.5 (30–758)	26/26	
2	Inact pre	397.0 (0–716)	29/31	NS
2	Inact post	381.0 (0–683)	31/33	
2	Fresh pre	364.5 (94–627)	30/30	<0.001
2	Fresh post	33.0 (0–112)	28/30	
3	hC5 pre	505.0 (0–728)	36/37	<0.001
3	hC5 post	24.0 (0–96)	38/40	

[a] Group of experiments done at the same time.
[b] Treatment condition of mice for live feedings. Abbreviations: DBA/1, live feedings on DBA/1 mice; DBA/2, live feedings on DBA/2 mice; inact pre, live feedings on DBA/2 mice before injection with heat-inactivated DBA/1 serum; inact post, live feedings on DBA/2 mice after injection with heat-inactivated DBA/1 serum; fresh pre, live feedings on DBA/2 mice before injection with fresh DBA/1 serum; fresh post, live feedings on DBA/2 mice after injection with fresh DBA/1 serum; hC5 pre, live feedings on DBA/2 mice before injection with human C5; hC5 post, live feedings on DBA/2 mice after injection with human C5.
[c] Number of oocysts per midgut from all mosquitoes.
[d] Probability value of statistical analysis of the number of oocysts between the DBA/1-fed group and DBA/2-fed group or before and after injection. NS, differences between these two groups were not statistically significant.

meal. The blood meal in each midgut was suspended in 50 μl of phosphate-buffered saline (PBS), and 1-μl aliquots were spotted onto Multitest Slides (Flow Laboratories, McLean, Va.), fixed with acetone, and then blocked with PBS containing 5% nonfat dry milk. The remaining mosquitoes were maintained for 9 days, and the number of oocysts was scored. The differences in the number of oocysts formed between the pre- and posttreatment groups were compared by the Mann-Whitney U test. The slides made an hour after the blood meal were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin G (IgG) plus IgM (Tago, Camarillo, Calif.) or anti-mouse C3 (Cappel) sera and then observed. The slides made at 4, 8, and 15 h after the blood meal were incubated with mouse monoclonal antibody which specifically recognizes the 28-kDa zygote and ookinete surface protein of *P. yoelii* for 60 min at 37°C and then stained with FITC-conjugated second antibody. The mature and retort ookinetes and zygotes were distinguished from each other by their morphological features, and then the numbers of parasites were counted. The differences in the number of parasites of each stage between the DBA/2-fed and the DBA/1-fed groups were compared by the Mann-Whitney U test.

Fresh DBA/1 mouse serum showed strong hemolytic activity. DBA/2 mouse serum injected with fresh DBA/1 serum showed half as much hemolytic activity as fresh DBA/1 serum, whereas the DBA/2 mouse serum injected with heat-inactivated DBA/1 serum showed no hemolytic activity (Fig. 1A). Fresh DBA/1 mouse serum retained less, but significant, hemolytic activity with incubation at 24°C (Fig. 1B). Purified human C5 restored the hemolytic activity of serum from male DBA/2 mice in vitro (Fig. 1C).

The effects of complement on the infectivity to *A. stephensi* are shown in Table 1. The infectivity of oocysts to *A. stephensi* mosquitoes fed on DBA/1 mice was significantly less than that to those fed on DBA/2 mice (experiment 1). Heat-inactivated DBA/1 sera injected into DBA/2 mice did not reduce the infectivity of oocysts. In comparison with these controls, fresh DBA/1 sera injected into DBA/2 mice significantly reduced the infectivity (experiment 2). Moreover, male DBA/2 mouse sera restored with human C5 significantly reduced the infectivity (experiment 3). In contrast to the reduction of infectivity, the numbers of infected mosquitoes were not reduced.

The number of mature ookinetes formed in the mosquito midgut fed on DBA/1 mice was significantly decreased compared with that for those fed on DBA/2 mice during 4 to 15 h after the blood meal. On the other hand, the number of zygotes formed in the mosquito midgut fed on DBA/1 mice was not decreased (Table 2). Further proof of the involvement of complement was provided by detection of C3 on zygotes. On examination of the zygotes stained with an FITC-conjugated anti-mouse C3 antibody, the parasites obtained from both strains of mice 1 h after the blood meal gave a fluorescent reaction over the entire cell surface, although those incubated with an FITC-conjugated anti-mouse IgG-IgM antibody were totally negative (data not shown).

The blood meal is digested in the mosquito midgut with digestive enzymes, especially trypsin (3). However, chicken APC remains active in the mosquito midgut (4). We showed that the APC is still active in the mosquito midgut, because C3 was deposited on the surface of zygotes and the defect of C5 caused a marked increase in the oocyst number. Furthermore, the reduction of the oocyst infectivity by APC is mainly due to the inability of the zygote to transform into the ookinete in the mosquito midgut. For consistent and effective natural infection, it does not seem that the mosquito needs to have many oocysts in its midgut because the majority of midguts of naturally infected mosquitoes contain only a few oocysts (2). Penetration of the midgut epithelium by ookinetes (7, 10) results in midgut damage and increases mosquito mortality (9). The present results suggest that the APC in the mouse serum significantly reduced, but did not eliminate, the infectivity of *P. yoelii* to the mosquito, and thereby that the APC likely has a beneficial effect on the vector-parasite association by reducing vector mortality, thus enhancing the probability of parasite transmission.

TABLE 2. *Plasmodium yoelii* zygote development to mature ookinete in *Anopheles stephensi* midgut[a]

No. of h after blood meal	Median no. of mature ookinetes (2×10^{-2})/midgut (range)		Median no. of retort ookinetes (2×10^{-2})/midgut (range)		Median no. of zygotes (2×10^{-2})/midgut (range)	
	DBA/2	DBA/1	DBA/2	DBA/1	DBA/2	DBA/1
4	27.5 (10–55)	0[b] (0–0)	31.0 (11–38)	0.5[b] (0–4)	69.5 (45–85)	71.0 (47–104)
8	46.0 (10–80)	3.0[b] (0–6)	22.5 (21–28)	20.5 (14–28)	57.5 (42–72)	99.0[c] (53–120)
15	17.0 (8–45)	1.0[b] (0–2)	31.0 (20–42)	14.0[b] (4–20)	46.0 (30–89)	64.0 (32–87)

[a] Number of mature ookinetes, retort ookinetes, or zygotes obtained from eight mosquitoes.
[b] Statistically significant compared with the corresponding group fed on DBA/2 ($P < 0.01$).
[c] Statistically significant compared with the corresponding group fed on DBA/2 ($P < 0.05$).

We wish to express our thanks to K. Ohno and K. Okugawa, Laboratory Animal Center, Ehime University School of Medicine, for their assistance in caring for the experimental animals.

This work has been supported in part by Grants-in-Aid for Scientific Research 05807020 and 06807024 from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. **Carter, R., N. Kumar, I. Quakyi, M. Good, K. Mendis, P. Graves, and L. Miller.** 1988. Immunity to sexual stages of malaria parasites. Prog. Allergy **41:**193–214.
2. **Collins, F. H., F. Zavala, P. M. Graves, A. H. Cochrane, R. W. Gwadz, J. Akoh, and R. S. Nussenzweig.** 1984. First field trial of an immunoradiometric assay for the detection of malaria sporozoites in mosquitoes. Am. J. Trop. Med. Hyg. **33:**538–543.
3. **Graf, R., and H. Briegel.** 1989. The synthetic pathway of trypsin in the mosquito *Aedes aegypti* L. (Diptera: Culicidae) and in vitro stimulation in isolated midgut. Insect Biochem. **19:**129–137.
4. **Grotendorst, C. A., R. Carter, R. Rosenberg, and L. C. Koontz.** 1986. Complement effects on the infectivity of *Plasmodium gallinaceum* to *Aedes aegypti* mosquitoes. I. Resistance of zygotes to the alternative pathway of complement. J. Immunol. **136:**4270–4274.
5. **Hitsumoto, Y., H. Ohnishi, A. Nakano, F. Hamada, S. Saheki, and N. Takeuchi.** 1993. Inhibition of homologous complement activation by the heat-stable antigen. Int. Immunol. **5:**805–808.
6. **Kaslow, D. C.** 1993. Transmission-blocking immunity against malaria and other vector-borne diseases. Curr. Opin. Immunol. **5:**557–565.
7. **Meis, J. F., G. Pool, G. J. van Gemert, A. H. Lensen, T. Ponnudurai, and J. H. Meuwissen.** 1989. *Plasmodium falciparum* ookinetes migrate intercellularly through *Anopheles stephensi* midgut epithelium. Parasitol. Res. **76:** 13–19.
8. **Nilsson, U. R., and H. J. Muller-Eberhard.** 1966. Deficiency of the fifth component of complement in mice with an inherited complement defect. J. Exp. Med. **125:**1–16.
9. **Ramasamy, M. S., and R. Ramasamy.** 1990. Effect of anti-mosquito antibodies on the infectivity of the rodent malaria parasite *Plasmodium berghei* to *Anopheles farauti*. Med. Vet. Entomol. **4:**161–166.
10. **Torii, M., K. Nakamura, K. P. Sieber, L. H. Miller, and M. Aikawa.** 1992. Penetration of the mosquito (*Aedes aegypti*) midgut wall by the ookinetes of *Plasmodium gallinaceum*. J. Protozool. **39:**449–454.