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Target antigens in malaria transmission blocking immunity

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Malaria transmission blocking immunity has been found to operate against two distinct phases of development of malaria parasites in the mosquito midgut: (i) against the extracellular gametes and newly fertilized zygotes shortly after ingestion by a mosquito of parasitized blood and (ii) against the zygotes during their subsequent development into ookinetes. Immunity is antibody-mediated and stage-specific. A set of three proteins, synthesized in the gametocytes, expressed on the surface of the gametes and newly fertilized zygotes and subsequently shed during later transformation of the zygotes, has been identified as the target antigens of anti-gamete fertilization blocking antibodies. A single protein, synthesized and expressed on the zygote surface during its development to ookinetes, has been identified as the target of antibodies which block the development of the fertilized parasites in the mosquito.

Immunization of human populations against gamete or zygote antigens, while not directly protecting an immunized individual from inflection, would reduce the transfer of malaria within the population. Such immunity, in addition to reducing the overall rate of malaria transmission, would, if combined with a vaccine against the asexual (disease-causing) stages, reduce the chance of selection of parasites that are resistant to the asexual vaccine by preventing their entry into the mosquito population.

1. Introduction

Malaria is transmitted from a vertebrate host to a mosquito vector via the gametocytes, sexual stages of the malaria parasite, which circulate as intraerythrocytic parasites in the blood stream of the host (figure 1). The gametocytes undergo gametogenesis and the extracellular gametes fertilize in the midgut of a mosquito following a blood meal. These events are rapid and fertilization is largely completed within 30 min of ingestion of blood. The resulting zygotes remain within the contents of the blood meal for a period of about 24 h and during this time transform into motile forms known as ookinetes. The mature ookinetes transverse the mosquito midgut wall and continue their development to form oocysts and ultimately sporozoites which may be inoculated into a new host during a subsequent blood meal.

During the 24 h period of development within the lumen of the mosquito midgut the parasites are extracellular and in continuous contact with the blood components of their vertebrate host. Thus, in contrast to their previous condition as intraerythrocytic forms, the midgut stages of the parasite are accessible to antibodies and other immunological agents of the vertebrate host. This situation has been exploited by inducing antibodies in the vertebrate host against the

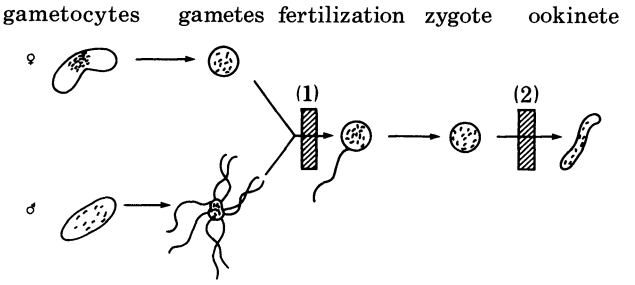

FIGURE 1. Transformations of gametocytes of *Plasmodium falciparum* in the mosquito midgut. The stages at which transmission blocking antibodies have been shown to act are indicated by a hatched bar.

midgut stages of the parasites (gametes, zygotes or ookinetes). These antibodies when ingested as part of a blood meal lead to sterilization of the malarial infection in the mosquito. In view of the potential of these antibodies to suppress transfer of malaria parasites from man to mosquito and hence break the cycle of transmission, we refer to such immunity as transmission blocking immunity.

2. THE PHENOMENA OF TRANSMISSION BLOCKING IMMUNITY

The first evidence for immune suppression of infectivity of malaria parasites to mosquitoes was that of Huff *et al.* (1958) who immunized turkeys intravenously with formalin-killed blood infected with *Plasmodium fallax*, or chickens with *P. gallinaceum*, and showed that subsequently induced blood infections were of greatly reduced infectivity to mosquitoes, although their asexual parasitaemias and gametocytaemias were similar to those in control infections. This phenomenon was re-examined by Gwadz (1976) and by Carter & Chen (1976). As in the previous studies (Huff *et al.* 1958), intravenous immunization of chickens with formalized *P. gallinaceum*-infected blood induced an immunity which reduced infectivity of a subsequent blood infection to *Aedes aegypti* mosquitoes (Gwadz 1976). However, when the birds were immunized with partly purified preparations of extracellular male and female gametes of *P. gallinaceum*, a much more effective immunity was achieved with relatively minute amounts of material (Carter & Chen 1976). Moreover, immunization with parasitized blood in which gametogenesis had been induced *in vitro* gave much more effective immunity than an equivalent amount in which gametogenesis was not induced (Carter *et al.* 1979*a*), indicating that the relevant immunogens are associated primarily with the extracellular gametes of malarial parasites. The efficiency of immunizing with extracellular malarial gametes to induce transmission blocking immunity has been demonstrated with *P. knowlesi* in rhesus monkeys (Gwadz & Green 1978), although in this system Freund's complete adjuvant was required, and with *P. yoelii* in mice (Mendis & Targett 1979) and with irradiated *P. berghei* gametes in hamsters (Kaushik *et al.* 1982), in which immunization was effective without adjuvant.

The mechanisms of transmission blocking immunity induced by gametocyte or gamete immunization have been investigated by feeding mosquitoes through a membrane with *P. gallinaceum*-infected blood cells resuspended in normal or immune chicken sera (Gwadz 1976). The mosquitoes were dissected one week later and the numbers of oocysts on their midguts counted as a measure of infection in the mosquitoes. Gametocytes from normal chickens

were no longer infectious to mosquitoes when resuspended in serum from immunized chickens. Gametocytes from immunized birds were infectious when these were washed free of their own immune serum and fed to mosquitoes after resuspension in normal serum. These results showed that the infectivity of gametocytes in immunized birds was affected by immune serum factors which acted only after ingestion of the gametocytes by a mosquito. In the absence of complement, immune serum was shown to agglutinate male and female gametes *in vitro* (Gwadz 1976; Carter *et al.* 1979*b*); titres of agglutinating antibodies correlated with the efficiency of the transmission blocking immunity. Although the mechanism of action of these anti-gamete antibodies has not been completely defined, it has been demonstrated that they act to prevent fertilization of the parasites in the mosquito midgut but, in the absence of complement, are virtually without effect on the development of the zygote (Carter *et al.* 1979*b*; C. A. Grotendorst, N. Kumar, R. Carter and D. C. Kaushal, unpublished results). In the presence of complement, however, the same anti-gamete antibodies mediate lysis of both gametes and newly formed zygotes and are thus capable of suppressing infectivity to mosquitoes of even the fertilized parasites.

Anti-gamete antibodies capable of mediating significant transmission blocking immunity are not known to occur during natural blood infection (Gwadz & Green 1978). Nevertheless, in an immunized animal a blood infection is a potent boost of waning levels of transmission blocking antibodies as has been found in both *P. gallinaceum* in chickens (Carter *et al.* 1979*b*) and in *P. knowlesi* in rhesus monkeys (Gwadz & Koontz 1984). Indeed, following immunization with gametes of *P. knowlesi* in Freund's complete adjuvant, rhesus monkeys, challenged intermittently with *P. knowlesi*, have maintained effective transmission blocking immunity for at least six years (Gwadz & Koontz 1984). This boost presumably results from antigens in gametocytes and not asexual parasites (see below).

Another form of transmission blocking immunity has been demonstrated which acts against the zygotes during their transformation into ookinetes and not against the gametes. Anti-sera raised in chickens against ookinetes of *P. gallinaceum* suppressed infectivity of zygotes of *P. gallinaceum* to mosquitoes (C. Grotendorst, N. Kumar, R. Carter and D. C. Kaushal, unpublished results). The transmission blocking effect of the anti-ookinete antibodies was completely independent of active complement, in contrast to anti-gamete antibodies which suppressed the infectivity of zygotes to mosquitoes only in the presence of complement. As will be discussed below, the target antigens of the anti-gamete antibodies and the anti-ookinete antibodies are unrelated and stage-specific.

3. Target antigens on gametes and newly fertilized zygotes

(*a*) *Anti-gamete immunity mediated by monoclonal antibodies*

Identification of the target antigens of anti-gamete immunity has been pursued using mouse monoclonal antibodies (Mabs) specific for surface components on extracellular gametes of malaria parasites. The initial studies were conducted using *P. gallinaceum* (Rener *et al.* 1980; Kaushal *et al.* 1983*b*). Mabs were screened for their ability to react by indirect immunofluorescence (i.i.f.) with the surface of extracellular female gametes and newly fertilized zygotes and to agglutinate male gametes. Those Mabs that were positive in these reactions were tested for their ability to suppress infectivity of *P. gallinaceum* gametocytes to *Ae. aegypti* mosquitoes. These studies led to the identification of a variety of Mabs that suppressed gametocyte

infectivity, some individually, others only when administered in appropriate paired combinations (Rener *et al.* 1980; Kaushal *et al.* 1983*b*). These results are summarized in table 1.

Effective suppression by an individual antibody in the absence of complement was found in only one instance, that of μ isotype Mab, IA1-D5, which suppressed infectivity to between 1 and 2% of control levels (Kaushal *et al.* 1983*b*). In other instances, effective suppression was the result of synergism between two different Mabs. Synergistic suppression was first noted with

TABLE 1. EFFECTS OF ANTI-GAMETE MONOCLONAL ANTIBODIES ON INFECTIVITY OF *PLASMODIUM GALLINACEUM* GAMETOCYTES TO *AEDES AEGYPTI* MOSQUITOES

Mabs	isotypes	infectivity† (% of control)
IA1-D5‡	μ	2%
10G3§	μ	70%
11C7§	γ_1	70%
10G3+11C7§	$\mu+\gamma_1$	10–20%
IA1-B3‡	γ_1	20%
IID3-B3‡	γ_{2a}	40%
IA1-B3+IID3-B3‡	$\gamma_1+\gamma_{2a}$	2–10%

† Gametocyte-infected blood was resuspended with the appropriate Mab in the absence of complement and fed to mosquitoes through a membrane. Infectivity is expressed as the mean number of oocysts which developed in the mosquitoes fed with gametocytes in the presence of the Mabs relative to the number of oocysts in mosquitoes in which normal mouse immunoglobulins were substituted for the Mabs.

‡ Data from Kaushal *et al.* (1983*b*).

§ Data from Rener *et al.* (1980).

a combination of a μ isotype Mab, 10G3, and a γ_1 isotype Mab, 11C7, which reduced infectivity to mosquitoes to between 10 and 20% of control levels (Rener *et al.* 1980). More effective synergistic suppression was subsequently achieved with a combination of a γ_1 isotype Mab, IA1-B3, and a γ_{2a} isotype Mab, IID3-B3, with which infectivity was reduced to less than 10% of control levels (Kaushal *et al.* 1983*b*). For all Mabs or combinations of Mabs, suppression was shown to be mainly due to prevention of fertilization as none were effective in suppressing infectivity of the parasites to mosquitoes when fertilization was induced before exposing them to the antibodies (Kaushal *et al.* 1983*b*) (table 2).

An enhanced suppression of infectivity has been noted in the presence of complement with certain Mabs as had also been found with anti-gamete immune chicken serum. Thus, in the presence of native human serum as a source of complement, the combination of 11C7 and 10G3 reduced infectivity to between 2 and 5% of control (cf. table 1). Examination of extracellular gametes and newly fertilized zygotes of *P. gallinaceum* in the presence of this combination showed

TABLE 2. EFFECTS OF ANTI-GAMETE MABS ON INFECTIVITY OF *PLASMODIUM GALLINACEUM* GAMETOCYTES TO *AEDES AEGYPTI* MOSQUITOES WHEN THE PARASITES WERE EXPOSED TO ANTIBODY EITHER BEFORE OR AFTER FERTILIZATION

	infectivity (% of control)	
Mabs	exposed to Mabs before fertilization	exposed to Mabs after fertilization
IA1-D5	2%	40%
IA1-B3+IID3-B3	8%	100%

Data from Kaushal *et al.* (1983*b*).

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that the parasites were rapidly lysed by complement in an antibody-dependent fashion (Kaushal *et al.* 1983*b*).

Mouse monoclonal antibodies have also been derived against surface antigens of gametes of *P. falciparum* (Rener *et al.* 1983) obtained from gametocytes grown in continuous *in vitro* culture (Ifediba & Vanderberg 1981). These Mabs, selected for reactivity by i.i.f. with female gametes of *P. falciparum*, were tested for their effect on infectivity to *Anopheles freeborni* mosquitoes of *P. falciparum* gametocytes from *in vitro* culture. In analogous circumstances to those previously found with *P. gallinaceum*, a combination of two Mabs, both of the γ_{2a} isotype and designated IA3-B8 and IIC5-B10, synergized to suppress infectivity of gametocytes of a Brazilian isolate of *P. falciparum*, 7G8, to less than 1% of control levels in the absence of complement (Rener *et al.* 1983). Individually neither Mab mediated suppression of infectivity of the parasites to mosquitoes. In the presence of complement (from fresh native human serum), one of the Mabs, IA3-B8, mediated a significant suppression of infectivity of 7G8, generally to less than 5% of control, while IIC5-B10 was without effect.

(*b*) *Characterization of target antigens of anti-gamete immunity*

Transmission blocking anti-gamete antibodies have been used to immunoprecipitate their target antigens from gametes or zygotes of the malaria parasites. Following lactoperoxidase-catalysed radioiodination of intact gametes and newly fertilized zygotes of *P. gallinaceum*, the surface-labelled cells were extracted in Triton X-100, the extracts immunoprecipitated with the Mabs and analysed by SDS-polyacrylamide gel electrophoresis and radioautography. With the exception of the μ isotype Mabs, IA1-D5 and 10G3, for which target antigens have not been identified by either this or various attempted biosynthetic labelling techniques, each of the other anti-gamete Mabs involved in suppression of gametocyte infectivity precipitated the same set of three gamete surface-labelled proteins of M_r 240000, 56000 and 54000 (figure 2) (Kaushal *et al.* 1983*b*). These three proteins appear to occur in similar or identical form on both male and female gametes of *P. gallinaceum* as well as on the newly fertilized zygotes (Kaushal & Carter 1984). An apparently analogous set of three surface proteins of M_r 260000, 59000 and 53000 were immunoprecipitated from gametes of the *P. falciparum* isolate, 7G8, by each of the transmission blocking Mabs IIC5-B10 and IA3-B8 (as well as another Mab IID2-A10 of properties similar to IA3-B8) (figure 3, lanes A to D).

Thus proteins in these two molecular mass regions appear to represent a generic set of surface proteins on the gametes of malaria parasites. They have been identified collectively as target antigens of antibodies mediating fertilization blocking and complement-dependent anti-zygote transmission blocking immunity. The biochemical and immunological relations within this class of three gamete surface proteins are unclear. The high and low molecular mass proteins do not appear to exist in a precursor–product relationship. In both *P. gallinaceum* and *P. falciparum* during pulse chase experiments following biosynthetic labelling of gametocytes with [^{35}S]methionine, no change in the relative intensity of label was found in the three proteins with increasing chase time (figure 4; N. Kumar and R. Carter, unpublished results). Moreover, in *P. gallinaceum*, an immunological reagent is available which immunoprecipitates only the M_r 56000 and 54000 gamete surface proteins but not the M_r 240000 protein. In *P. falciparum*, it has been shown that the M_r 59000 and 53000 proteins are glycosylated but not the M_r 260000 protein (N. Kumar and R. Carter, unpublished results). Neither of these situations would be expected if the lower molecular mass proteins were breakdown products of the high molecular mass protein.

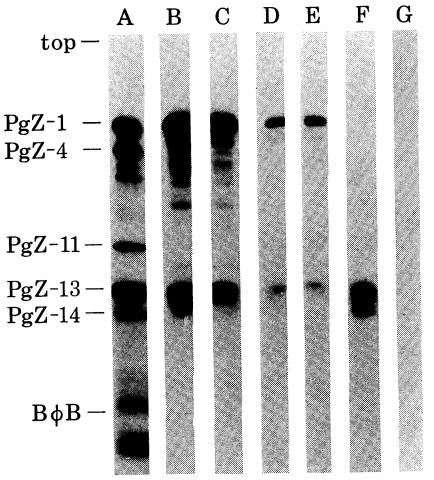

FIGURE 2. Surface proteins on female gametes and zygotes of *P. gallinaceum* labelled with ^{125}I and immunoprecipitated with Mab against surface antigens on these stages. The precipitated proteins were separated on 5–15% SDS-polyacrylamide gradient gel electrophoresis under reducing conditions and made visible by radioautography. The designation PgZ-1 indicates the position of the M_r 240000 gamete surface protein; PgZ-13 indicates the M_r 56000 and 54000 surface proteins. Lane A, total antigen extract; lanes B and C, proteins precipitated by Mabs IID3-B3 and IID3-E8, respectively; lanes D and E, proteins precipitated by Mabs IA1-B5 and IA1-F2, respectively; lane F, proteins precipitated by uncloned hybridoma IID4; lane G, proteins precipitated with normal mouse serum. The mobility of immunoprecipitated, labelled proteins in the M_r 50000–60000 region (PgZ-13) is distorted by the presence of the immunoglobulin heavy chain. BϕB marks the position of the bromophenol blue dye marker. Data from Kaushal *et al.* (1983*b*) with permission.

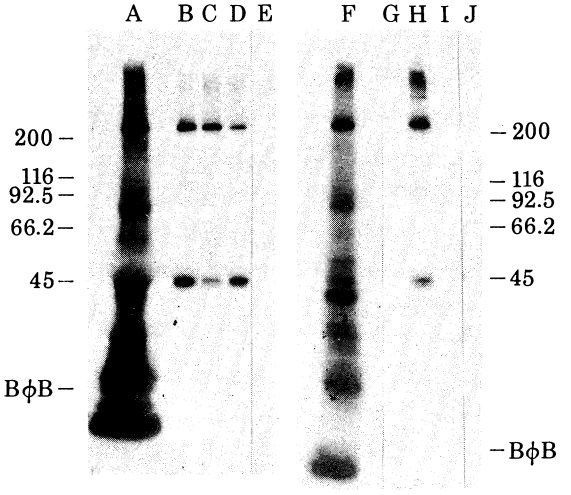

FIGURE 3. Immunoprecipitation of ^{125}I-surface labelled female gametes of *P. falciparum* and separation on SDS-polyacrylamide gel electrophoresis under non-reducing conditions and radioautography. *P. falciparum*, 7G8: lane A, total gamete antigen and lanes B, C, D and E, immunoprecipitated with Mab IA3-B8, Mab IIC5-B10, Mab IID2-A10 and normal mouse serum, respectively. *P. falciparum*, L.E5: lane F, total gamete antigen and lanes G, H, I, and J, immunoprecipitated with Mab IA3-B8, Mab IIC5-B10, Mab IID2-A10 and normal mouse serum, respectively. The positions of the standard molecular mass markers are shown by numbers ($\times 10^{-3}$). (P. M. Graves & R. Carter, unpublished data.)

(*c*) *Biosynthesis and cellular processing of targets of anti-gamete transmission blocking antibodies*

In both *P. gallinaceum* and *P. falciparum* the synthesis and expression of the three gamete surface proteins precipitated by fertilization blocking Mabs have been studied during the growth and transformation of gametocytes to gametes and zygotes. In this regard a significant point of difference should be noted between the two species of malaria parasite, namely the

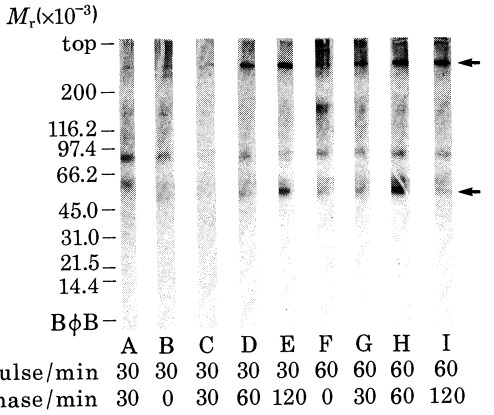

FIGURE 4. Pulse-chase labelling of *P. falciparum* gametocytes with [^{35}S]methionine. Gametocytes of 7G8 were labelled with [^{35}S]methionine for either 30 min (lanes A–E) or 60 min (lanes F–I) at 37 °C, washed with medium RPMI 1640 containing 10% normal human serum and 2 mM L-methionine and chased for various times periods. Immediately following the pulse and after each chase period, samples of the cells were washed and extracted with Triton X-100 and used for immunoprecipitation with Mab IIC5-B10 (lanes B–I) or normal mouse serum (lane A). Immunoprecipitates were analysed using SDS-5–15% polyacrylamide gradient gels. Positions of standard marker proteins are shown. Pulse and chase periods are under each lane. (N. Kumar & R. Carter, unpublished data.)

longevity of the gametocytes before maturation. Whereas gametocytes of *P. gallinaceum* develop from invasion of erythrocytes by merozoites to infectious gametocytes within 36–48 h, those of *P. falciparum* reach maturity only 8–10 days after erythrocyte invasion. In spite of this difference the two species of parasites are basically similar in their patterns of synthesis and expression of the three gamete surface proteins under discussion.

These proteins are neither synthesized nor otherwise detectable in asexual blood stage parasites as had been shown in i.i.f. studies on air-dried preparations in both *P. gallinaceum* (J. Rener and R. Carter, unpublished observations) and *P. falciparum* (P. Graves and R. Carter, unpublished observations). Incorporation of [^{35}S]methionine into the three proteins in *in vitro* blood stage cultures of *P. falciparum* was dependent on the presence of significant numbers of gametocytes at least two to three days after invasion of erythrocytes (N. Kumar and R. Carter, unpublished results). From this stage through to maturity, gametocytes of *P. falciparum* readily incorporated exogenously administered [^{35}S]methionine into the three gamete proteins as immunoprecipitated by the anti-gamete Mabs (figure 5). Moreover when the biosynthetically labelled gametocytes were stimulated at maturity to undergo gametogenesis, the three proteins could be detected as labelled products on the surface of the extracellular gametes, thus demonstrating that these gamete surface proteins are synthesized in the gametocytes before gametogenesis. Similar results have been obtained with *P. gallinaceum* (N. Kumar and R. Carter, unpublished results). Once formed, the female gametes and zygotes of *P. falciparum* no longer incorporated label into these proteins. In *P. gallinaceum* their synthesis continued in the female gametes and newly fertilized zygotes for several hours after gametogenesis, but they failed to appear on the cell surface (N. Kumar and R. Carter, unpublished results).

Shortly after gametogenesis and fertilization the parasites begin to shed the three gamete associated surface proteins (figure 6) apparently intact, into the surrounding medium (Carter & Kaushal 1984). By about 12 h after fertilization, the surface of transforming zygotes cultured *in vitro* were virtually devoid of the three transmission blocking Mab target proteins (figure 6). Shedding was largely restricted to these three proteins and some other relatively minor surface

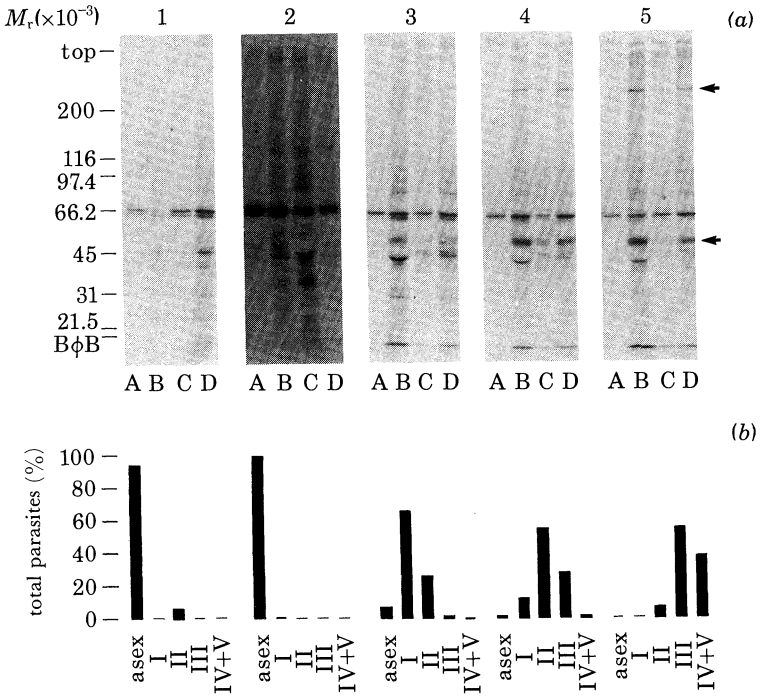

FIGURE 5. (*a*) SDS-polyacrylamide gel electrophoresis analysis of [^{35}S]methionine-labelled polypeptides synthesized by different stages of *P. falciparum* in culture. After expansion in fresh erythrocytes for two days (panel 1), six days (panel 2), nine days (panel 3), 12 days (panel 4) and 15 days (panel 5) the parasites were labelled with [^{35}S]methionine for 2 h and extracted in Triton X-100. Extracts equivalent to 500000 TCA insoluble counts per minute were immunoprecipitated with normal mouse serum (lane A), Mabs IA3-B8 (lane B), IIC5-B10 (lane C) and IID-2A10 (lane D). Reduced samples were analysed on a 5–15% SDS-polyacrylamide gel electrophoresis. Molecular mass marks are indicated and positions of target antigens are marked by arrows. The gel in panel 2 was overexposed to detect presence of metabolically labelled target antigens. None were detected.

(*b*) Stages of *P. falciparum* gametocytes in culture at the time of metabolic labelling in (*a*). Thin blood films were made on the day of labelling (panels 1–5), fixed with methanol and stained with Giemsa. Bars represent each stage (asexual or gametocyte stages I, II, III or IV and V as described in Carter & Miller (1979)) as percentage of parasites. (N. Kumar & R. Carter, unpublished data.)

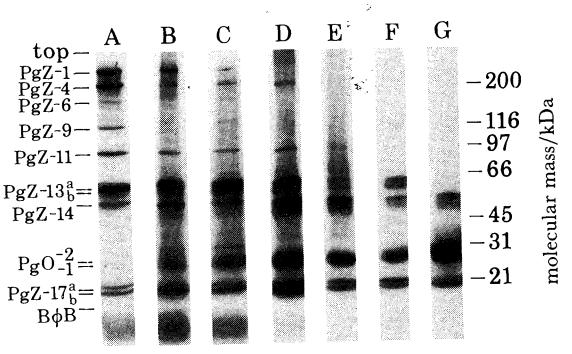

FIGURE 6. Zygotes of *P. gallinaceum* surface-labelled with ^{125}I at different times during transformation *in vitro* into ookinetes. At each time point the labelled cells were immediately extracted with Triton X-100 and the extracts subsequently separated on 5–15% SDS-polyacrylamide gel electrophoresis under reducing conditions. Lane A, zygotes labelled 2–3 h after gametogenesis; lane B, 4–5 h; lane C, 6–7 h; lane D, 8–9 h; lane E, 10–11 h, lane F, 12–13 h; and lane G, 24 h. The designation PgZ-1 indicates the M_r 240000 gamete protein; PgZ-13a and b the M_r 56000 and 54000 gamete proteins; PgO-1 and PgO-2 indicate the M_r 26000 and 28000 proteins synthesized during the transformation of zygotes to ookinetes. Data from Carter & Kaushal (1984) with permission.

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components above about M_r 50000. Other, lower molecular mass proteins found only on female gametes and zygotes, remained bound to the zygote surface throughout their transformation into ookinetes (figure 6).

(*d*) *Antigenic diversity in the targets of anti-gamete transmission blocking antibodies*

Following a survey of the reactions of the anti-gamete Mabs with gametes of different isolates of *P. falciparum* one line, L.E5 derived from a Liberian (West Africa) isolate, was found to respond differently from the other isolates tested (including 7G8, a second Brazilian isolate, ItD12, and an isolate from Thailand, T4) (P. Graves, R. Carter, J. Burkot, J. Rener, D. Kaushal and J. Williams, unpublished results) (table 3). Mab IA3-B8, which suppressed infectivity of other isolates to mosquitoes in the presence of complement and reacted with gamete surface antigens both by i.i.f. and by immunoprecipitation, reacted in none of these tests with the isolate L.E5 (figure 3, table 3). Mab IIC5-B10, on the other hand, reacted by i.i.f. and immunoprecipitation with gametes of all isolates including L.E5 but in contrast to its effects on other isolates, IIC5-B10 suppressed infectivity of L.E5 to mosquitoes in the presence of complement (table 3). In the absence of complement the combination of IA3-B8 and IIC5-B10, which suppressed infectivity of other isolates, was without effect against L.E5.

TABLE 3. REACTIONS OF TWO DIFFERENT ISOLATES (7G8 AND L.E5) OF *P. FALCIPARUM* WITH ANTI-GAMETE MABS IA3-B8 AND IIC5-B10

	suppression of infectivity to mosquitoes				indirect immuno-fluorescence with gamete surface	
	without complement		with complement			
Mab isolate...	7G8†	L.E5†	7G8	L.E5	7G8	L.E5
IA3-B8	no	no	yes	no	yes	no
IIC5-B10	no	no	no	yes	yes	yes

† Studies performed with a Brazilian isolate 7G8 and a Liberian isolate L.E5. Unpublished data from P. Graves, R. Carter, T. Burkot, J. Rener, D. C. Kaushal and J. Williams.

These results indicate diversity among the isolates of *P. falciparum* in the epitope recognized by IA3-B8. Although IIC5-B10 reacted with the gamete surface antigens on all isolates tested its effect on their infectivity varied. This could be due to isolate differences other than diversity of the epitope recognized by IIC5-B10.

4. TARGET ANTIGENS OF POST-FERTILIZATION TRANSMISSION BLOCKING IMMUNITY

While the target antigens of anti-gamete immunity are being shed from the surface of transforming zygotes of *P. gallinaceum*, a new set of proteins is synthesized and expressed on the surface of mature ookinetes (Carter & Kaushal 1984) (figure 6). As already indicated, these proteins include immunogens which give rise to antibodies capable of blocking the post-fertilization development of zyotes of *P. gallinaceum* in *Ae. aegypti* mosquitoes.

To define these target antigens, Mabs were derived that reacted by i.i.f. with surface antigens on mature ookinetes of *P. gallinaceum* (C. A. Grotendorst, N. Kumar, R. Carter and D. C. Kaushal, unpublished results). These Mabs were tested by feeding pre-fertilized parasitized blood to *Ae. aegypti* mosquitoes. A single γ_1 isotype Mab, IID2-C5 and its subclones, was

identified which suppressed infectivity of the fertilized parasites to less than 5 % of control levels. Numerous other Mabs that reacted strongly by i.i.f. with the surface of intact ookinetes of *P. gallinaceum* were relatively ineffective in reducing infectivity of the parasitized blood to mosquitoes.

The Mabs were used to immunoprecipitate proteins from extracts of surface radioiodinated ookinetes of *P. gallinaceum* or from zygotes that had been biosynthetically labelled with [^{35}S]methionine during transformation into ookinetes. Following SDS-polyacrylamide gel electrophoresis two distinct surface proteins were identified by the Mabs. Mab IID2-C5, which mediated post-fertilization suppression of parasite infectivity to mosquitoes, immunoprecipitated a protein of M_r 26000, thus identifying this ookinete surface protein as the target antigen of post-fertilization transmission blocking immunity (figure 7). The other Mabs, none of which blocked transmission, immunoprecipitated a protein of M_r 28000.

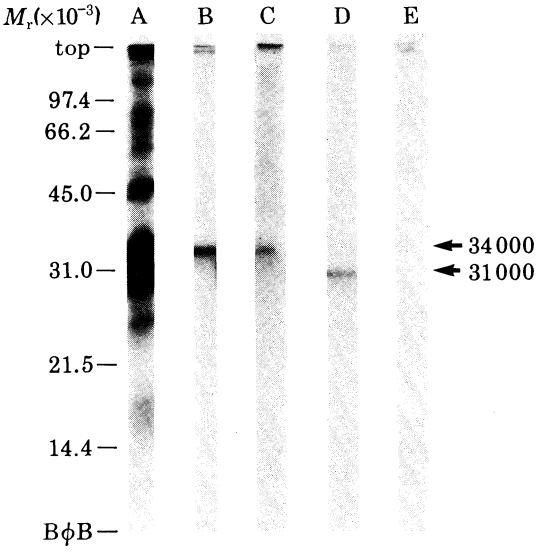

FIGURE 7. Immunoprecipitation of proteins biosynthetically labelled with [^{35}S]methionine during the transformation of zygotes of *P. gallinaceum* to ookinetes. The cells were extracted with Triton X-100 and immunoprecipitated with rabbit anti-ookinete serum (lane A), with Mab IID2-B3B3 (lane B), with Mab IID2-E6 (lane C), with Mab IID2-C5I (lane D), and with normal rabbit serum (lane E). The immunoprecipitated proteins were separated on 12.5 % SDS-polyacrylamide gel electrophoresis under non-reducing conditions (C. A. Grotendorst, N. Kumar, R. Carter and D. C. Kaushal, unpublished data.)

Studies were carried out to define the kinetics of synthesis and expression of the M_r 26000 and 28000 ookinetic surface proteins (N. Kumar and R. Carter, unpublished results). Neither of these proteins could be identified by immunoprecipitation from mature gametocytes of *P. gallinaceum* biosynthetically labelled with [^{35}S]methionine. However, within minutes of initiation of gametogenesis and fertilization, exogenously added [^{35}S]methionine was readily incorporated into the M_r 26000 protein but not the M_r 28000 protein; the M_r 26000 protein could not be detected on the zygote surface until 2–3 h after gametogenesis and fertilization. Biosynthesis of the M_r 26000 protein peaked between 6 and 12 h after fertilization and thereafter declined. Active synthesis of this protein could not be detected in the mature ookinetes

of *P. gallinaceum*, even though it represented a major component of the ookinete surface. Synthesis of the M_r 28000 protein was first demonstrated 5–6 h after fertilization; shortly thereafter this protein could be detected on the zygote surface. Synthesis of the M_r 28000 protein increased throughout the subsequent maturation of the zygote and continued at a maximum rate in the mature ookinete after 24 h of development.

These proteins have been further studied by isolation and *in vitro* translation of parasite (*P. gallinaceum*) mRNA in rabbit reticulocyte extracts (C. French, D. F. Wirth, R. Carter and L. H. Miller, unpublished results). Message coding for these proteins was isolated from newly fertilized zygotes of *P. gallinaceum* and was found to be entirely polyadenylated. The products of *in vitro* translation of zygote mRNA were identified by immunoprecipitation with monoclonal and polyspecific antibodies specific for the *in vivo* synthesized M_r 26000 and 28000 proteins. Interestingly, in contrast to the differential rates of synthesis of the two proteins *in vivo* (N. Kumar and R. Carter, unpublished results), both the M_r 26000 and 28000 products were synthesized in the *in vitro* translation system in similar amounts. Thus the mRNAs coding for these proteins in newly formed zygotes of *P. gallinaceum* appear to be present in similar amounts. The differential rates of synthesis of the M_r 26000 and 28000 proteins during the transformation of the zygote therefore appear to be due to post-transcriptional control of protein synthesis.

Interestingly, certain Mabs that immunoprecipitated the products of *in vivo* synthesis, including IID2-C5 (specific for the M_r 26000 *in vivo* product) and IID2-B3B3 (specific for the M_r 28000 product of *in vivo* synthesis), failed to precipitate the products of *in vitro* translation. This suggests that formation of the epitopes recognized by these Mabs is probably dependent upon post-translational modification. Indeed, the M_r 26000 and the 28000 proteins have been found to be glycosylated *in vivo* (N. Kumar and R. Carter, unpublished results; C. French, D. F. Wirth, R. Carter and L. H. Miller, unpublished results) and to be acylated during synthesis *in vivo* (N. Kumar, unpublished results).

5. Conclusions

Our studies have identified target antigens of malaria transmission blocking immunity at two distinct phases of parasite development in the mosquito midgut: (i) on the surface of the gametes and newly formed zygotes and (ii) on the surface of the zygotes during their transformation into ookinetes. Antigens on the surface of the extracellular gametes are the targets of antibodies that block fertilization and, in the presence of complement, mediate destruction by lysis of both gametes and early zygotes. The target antigens on the gametes are a set of three surface proteins of approximately M_r 250000, 60000 and 55000 which are present on both male and female gametes as well as newly formed zygotes. These antigens, which are specific to the sexual stages of the parasites, are synthesized in the gametocytes during their development in the blood stream. They are, therefore, preformed at the time of gametogenesis and, indeed, their synthesis terminates at, or shortly after, the formation of the gametes.

Shortly after gametogenesis and fertilization, the target antigens of anti-gamete transmission blocking antibodies begin to be shed from the zygote surface. At this time, a new protein of M_r 26000 is synthesized and expressed on the zygote surface. This protein is the target of antibodies that block the development of zygotes during their transformation into ookinetes in the mosquito. The M_r 26000 protein is not synthesized by the gametocytes in the blood stream

but appears on the zygote surface 2–3 h after gametogenesis and fertilization in the mosquito midgut.

While neither anti-gamete nor anti-zygote immunity can be expected to protect directly the immunized individual against malaria either could prevent the spread of the infection to other individuals via the prevailing mosquito vectors. Either or both forms of immunity administered throughout a region of transmission would, therefore, serve to suppress the transfer of infections within the human community. Anti-gamete immunity has the attractive feature, presumably due to the presence of the target antigens in circulating gametocytes, of being boosted by the blood infection. This ensures that immunity is effective when it is needed, that is during an active parasitaemia. Immunity against the transforming zygote probably would not share this feature since the target antigen is not present in the blood.

The evidence for antigenic diversity in the targets of anti-gamete transmission blocking antibodies in *P. falciparum* may not be relevant in practice to vaccine development; Mabs failed to suppress infectivity of a variant isolate only when complement had been inactivated. Nevertheless, antigenic diversity must be faced as a potential or actual problem for all stages of the parasite against which a vaccine may be contemplated, since immunization will tend to select for parasites expressing antigens not present in the vaccine. The only way to prevent the spread of such resistant forms would be to prevent their entry into the mosquito vector population. In this context the incorporation of a transmission blocking component into any vaccine would serve the important function of impeding the selection and spread of vaccine-resistant parasites.

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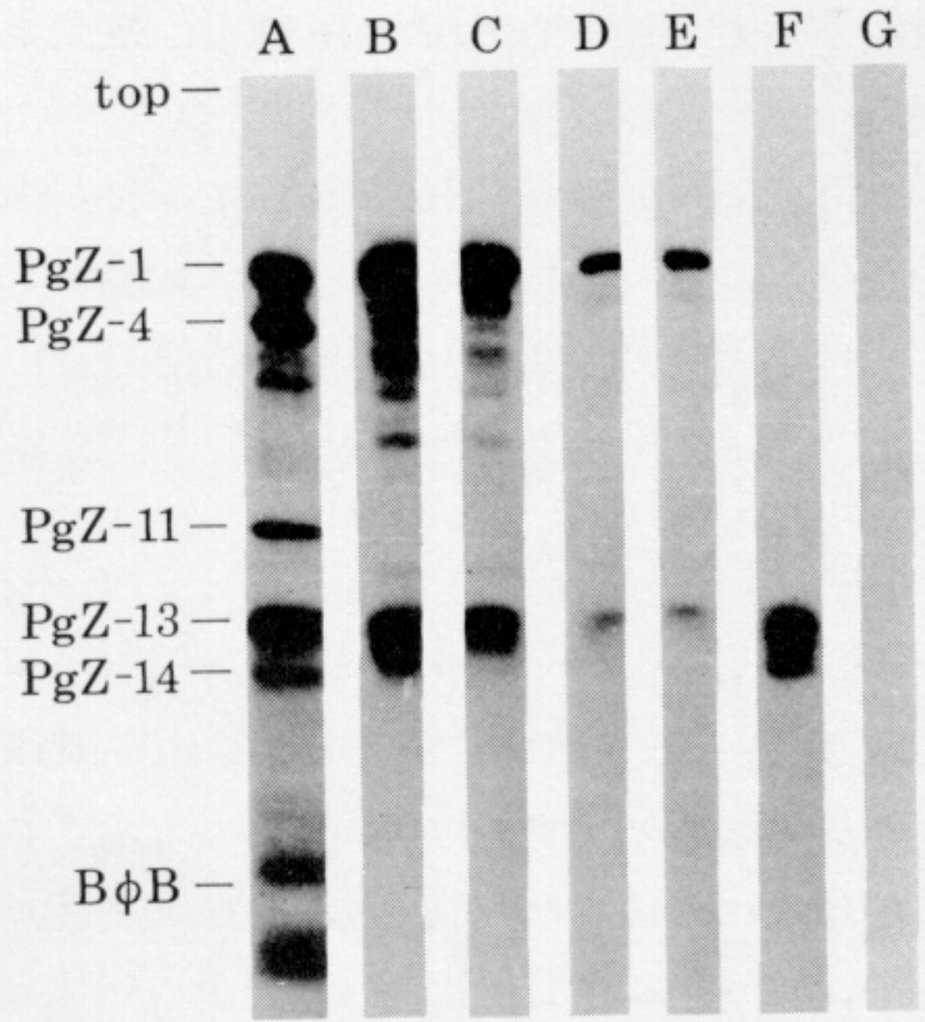

IGURE 2. Surface proteins on female gametes and zygotes of *P. gallinaceum* labelled with ^{125}I and immunoprecipitated with Mab against surface antigens on these stages. The precipitated proteins were separated on 5–15% SDS-polyacrylamide gradient gel electrophoresis under reducing conditions and made visible by radioautography. The designation PgZ-1 indicates the position of the M_r 240000 gamete surface protein; PgZ-13 indicates the M_r 56000 and 54000 surface proteins. Lane A, total antigen extract; lanes B and C, proteins precipitated by Mabs IID3-B3 and IID3-E8, respectively; lanes D and E, proteins precipitated by Mabs IA1-B5 and IA1-F2, respectively; lane F, proteins precipitated by uncloned hybridoma IID4; lane G, proteins precipitated with normal mouse serum. The mobility of immunoprecipitated, labelled proteins in the M_r 50000–60000 region (PgZ-13) is distorted by the presence of the immunoglobulin heavy chain. BϕB marks the position of the bromophenol blue dye marker. Data from Kaushal *et al.* (1983*b*) with permission.

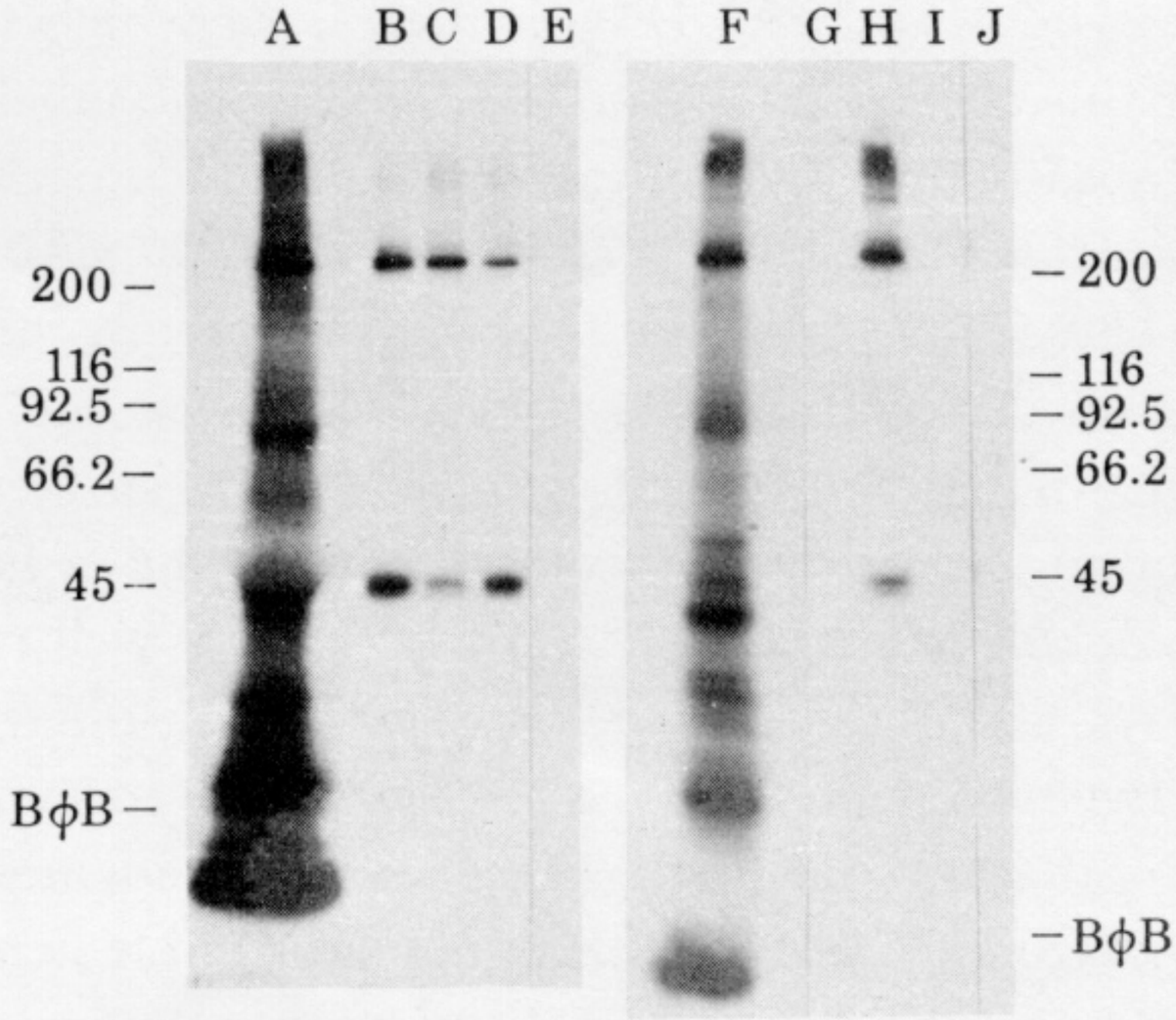

FIGURE 3. Immunoprecipitation of ^{125}I-surface labelled female gametes of *P. falciparum* and separation on SDS-polyacrylamide gel electrophoresis under non-reducing conditions and radioautography. *P. falciparum*, 7G8: lane A, total gamete antigen and lanes B, C, D and E, immunoprecipitated with Mab IA3-B8, Mab IIC5-B10, Mab IID2-A10 and normal mouse serum, respectively. *P. falciparum*, L.E5: lane F, total gamete antigen and lanes G, H, I, and J, immunoprecipitated with Mab IA3-B8, Mab IIC5-B10, Mab IID2-A10 and normal mouse serum, respectively. The positions of the standard molecular mass markers are shown by numbers ($\times 10^{-3}$). (P. M. Graves & R. Carter, unpublished data.)

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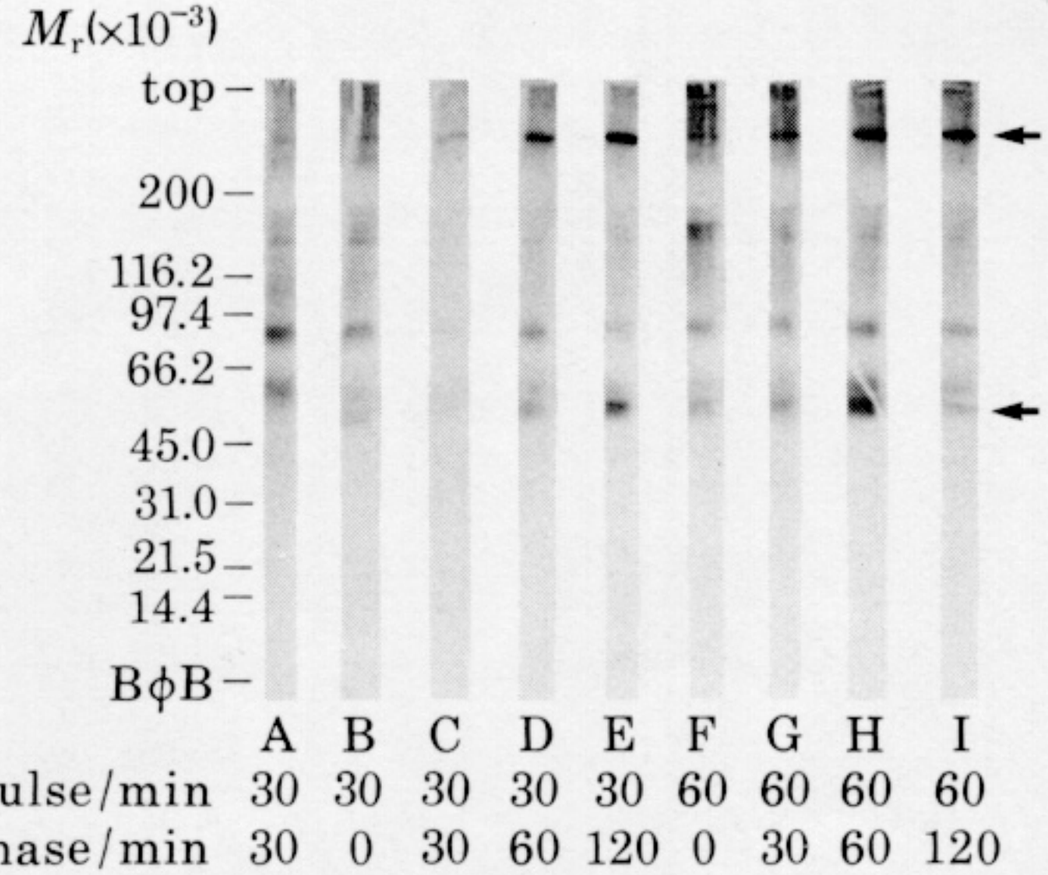

FIGURE 4. Pulse-chase labelling of *P. falciparum* gametocytes with [^{35}S]methionine. Gametocytes of 7G8 were labelled with [^{35}S]methionine for either 30 min (lanes A–E) or 60 min (lanes F–I) at 37 °C, washed with medium RPMI 1640 containing 10% normal human serum and 2 mM L-methionine and chased for various times periods. Immediately following the pulse and after each chase period, samples of the cells were washed and extracted with Triton X-100 and used for immunoprecipitation with Mab IIC5-B10 (lanes B–I) or normal mouse serum (lane A). Immunoprecipitates were analysed using SDS-5–15% polyacrylamide gradient gels. Positions of standard marker proteins are shown. Pulse and chase periods are under each lane. (N. Kumar & R. Carter, unpublished data.)

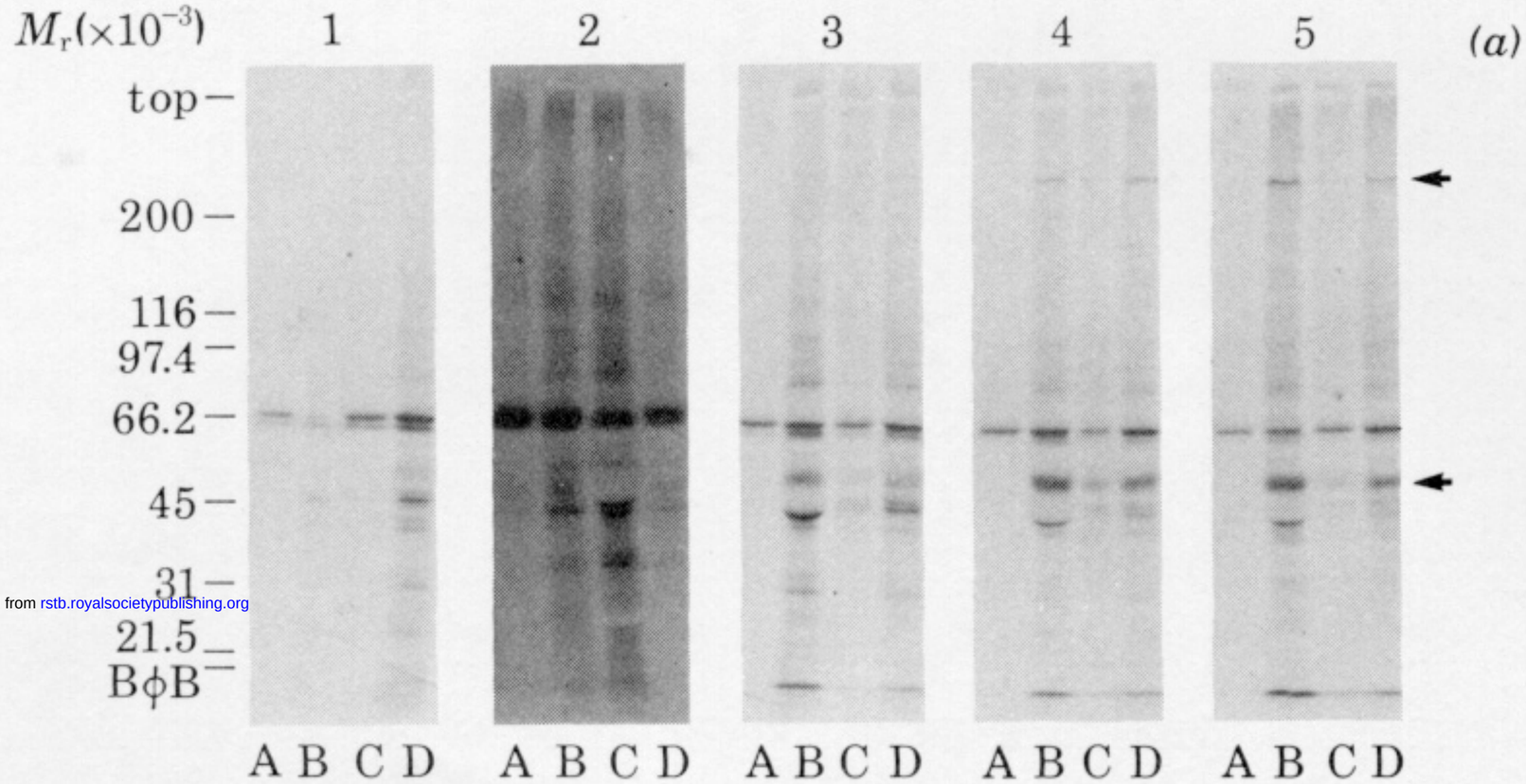

FIGURE 5. (*a*) SDS-polyacrylamide gel electrophoresis analysis of [^{35}S]methionine-labelled polypeptides synthesized by different stages of *P. falciparum* in culture. After expansion in fresh erythrocytes for two days (panel 1), six days (panel 2), nine days (panel 3), 12 days (panel 4) and 15 days (panel 5) the parasites were labelled with [^{35}S]methionine for 2 h and extracted in Triton X-100. Extracts equivalent to 500000 TCA insoluble counts per minute were immunoprecipitated with normal mouse serum (lane A), Mabs IA3-B8 (lane B), IIC5-B10 (lane C) and IID-2A10 (lane D). Reduced samples were analysed on a 5–15% SDS-polyacrylamide gel electrophoresis. Molecular mass marks are indicated and positions of target antigens are marked by arrows. The gel in panel 2 was overexposed to detect presence of metabolically labelled target antigens. None were detected.

(*b*) Stages of *P. falciparum* gametocytes in culture at the time of metabolic labelling in (*a*). Thin blood films were made on the day of labelling (panels 1–5), fixed with methanol and stained with Giemsa. Bars represent each stage (asexual or gametocyte stages I, II, III or IV and V as described in Carter & Miller (1979)) as percentage of parasites. (N. Kumar & R. Carter, unpublished data.)

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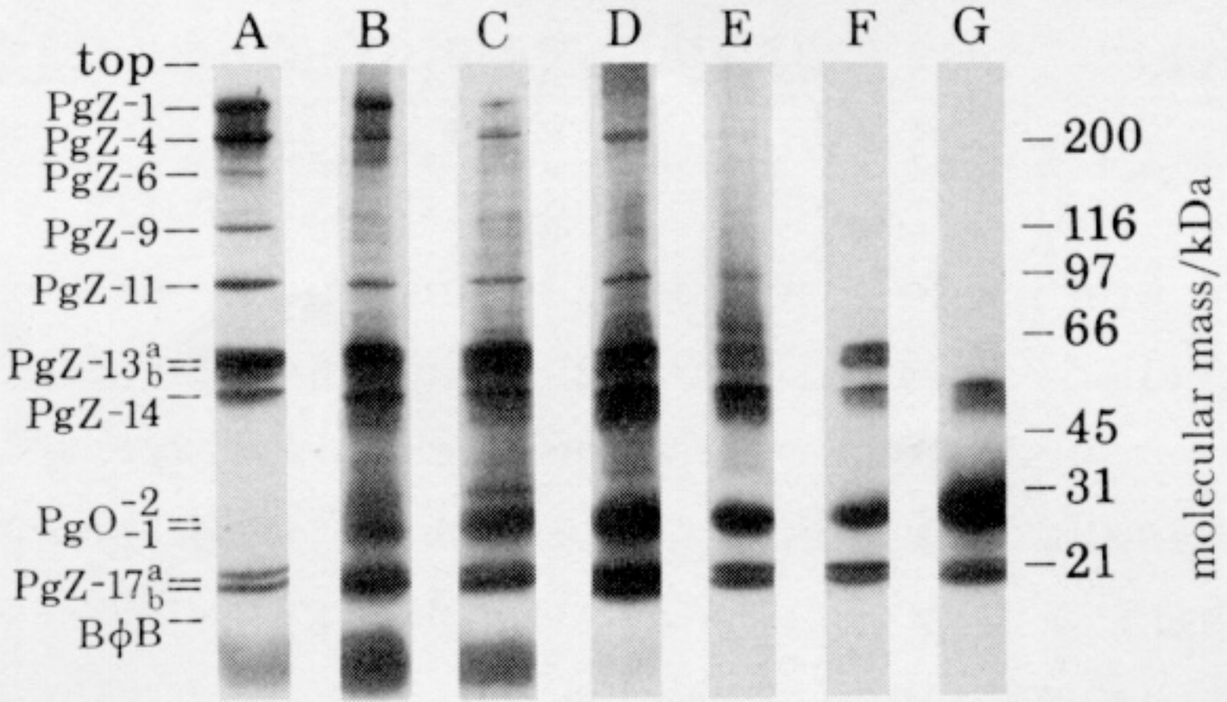

FIGURE 6. Zygotes of *P. gallinaceum* surface-labelled with ^{125}I at different times during transformation *in vitro* into ookinetes. At each time point the labelled cells were immediately extracted with Triton X-100 and the extracts subsequently separated on 5–15% SDS-polyacrylamide gel electrophoresis under reducing conditions. Lane A, zygotes labelled 2–3 h after gametogenesis; lane B, 4–5 h; lane C, 6–7 h; lane D, 8–9 h; lane E, 10–11 h, lane F, 12–13 h; and lane G, 24 h. The designation PgZ-1 indicates the M_r 240000 gamete protein; PgZ-13a and b the M_r 56000 and 54000 gamete proteins; PgO-1 and PgO-2 indicate the M_r 26000 and 28000 proteins synthesized during the transformation of zygotes to ookinetes. Data from Carter & Kaushal (1984) with permission.

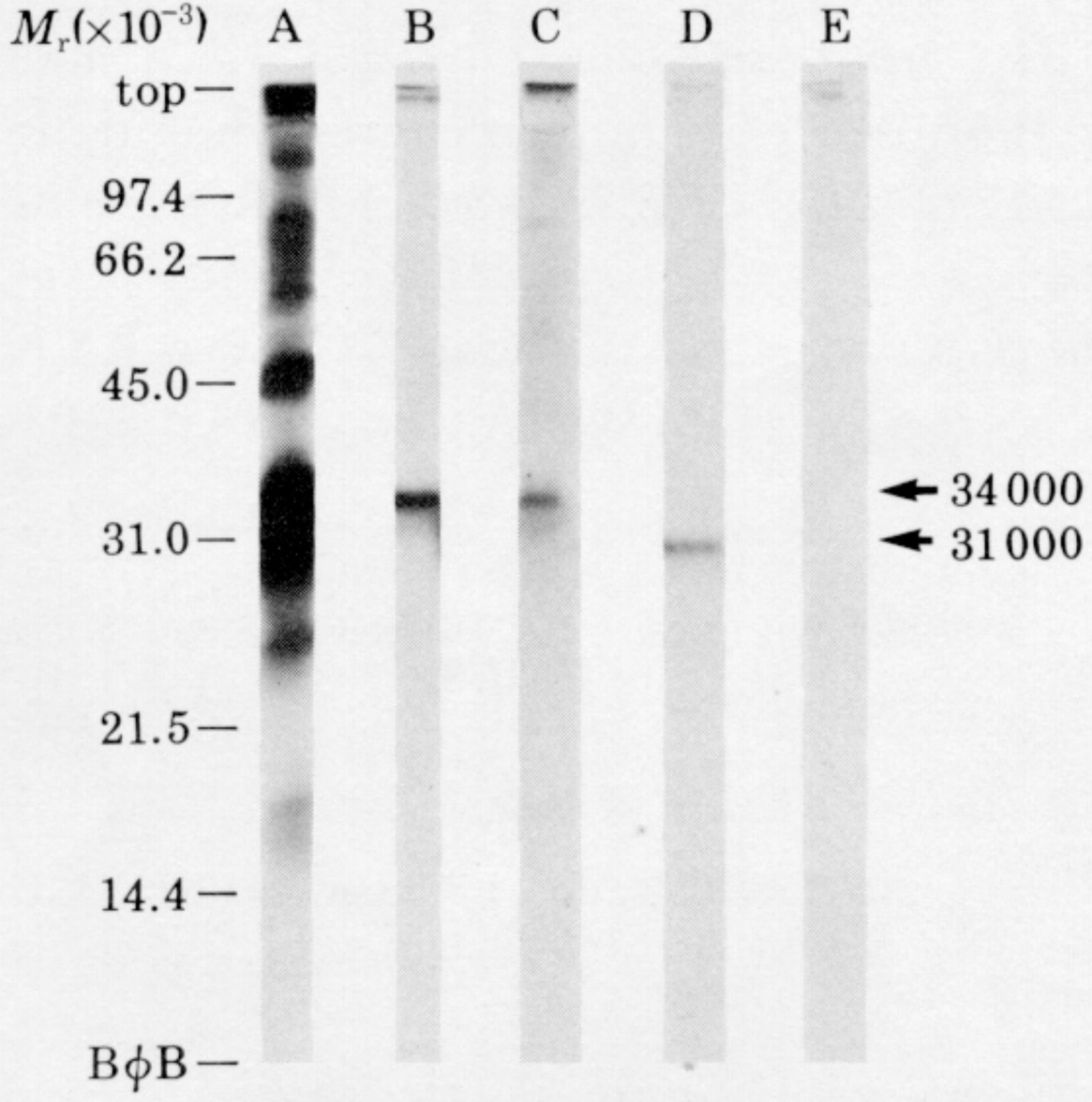

FIGURE 7. Immunoprecipitation of proteins biosynthetically labelled with [^{35}S]methionine during the transformation of zygotes of *P. gallinaceum* to ookinetes. The cells were extracted with Triton X-100 and immunoprecipitated with rabbit anti-ookinete serum (lane A), with Mab IID2-B3B3 (lane B), with Mab IID2-E6 (lane C), with Mab IID2-C5I (lane D), and with normal rabbit serum (lane E). The immunoprecipitated proteins were separated on 12.5% SDS-polyacrylamide gel electrophoresis under non-reducing conditions (C. A. Grotendorst, N. Kumar, R. Carter and D. C. Kaushal, unpublished data.)