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In Vivo Selection of Populations of *Plasmodium chabaudi chabaudi* AS Resistant to a Monoclonal Antibody That Reacts with the Precursor to the Major Merozoite Surface Antigen

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Mice bearing a hybridoma secreting a monoclonal antibody (MAb), MAb-3, which significantly delays the onset of a *Plasmodium chabaudi chabaudi* AS, but not *P. chabaudi chabaudi* CB, challenge parasitemia in a passive transfer assay and which is specific for the precursor to the major merozoite surface antigen (PMMSA) of *P. chabaudi chabaudi* AS, were challenged intravenously with 10^3 *P. chabaudi chabaudi* AS-parasitized erythrocytes. The resultant parasitemia was very similar to that in normal mice except that initially the parasitemia was sometimes slightly delayed. Parasites derived from cryopreserved stabilates isolated from MAb-3 hybridoma mice with an unmodified parasitemia, or with a delayed parasitemia, were found to have lost their susceptibility to MAb-3 in the passive transfer assay. A number of anti-PMMSA MAb were used to immunoprecipitate lysates of parasite populations isolated directly from hybridoma-bearing mice. In some instances and with certain of the MAb, immunoprecipitation patterns were modified, but other isolates were not detectably different when compared with unselected *P. chabaudi chabaudi* AS parasites. Using a panel of MAb reacting with the PMMSA of *P. chabaudi chabaudi* AS, immunoprecipitation patterns of parasites derived from cryopreserved stabilates isolated from hybridoma-bearing mice were determined at 2-h intervals through the appropriate part of the parasite maturation cycle. In these derived populations, resistance to MAb-3 was not associated with a change in the immunoprecipitation reaction with the MAb used. These results are discussed in the context of current knowledge of genotypic and phenotypic antigenic diversity of malaria parasites and other protozoa.

A group of high-molecular-weight polypeptides synthesized late in the erythrocytic cycle of *Plasmodium* spp. (24) have been identified as precursors of the major merozoite surface antigens (PMMSA) (14). These antigens, which vary in molecular weight among species (3, 8, 13, 14), have been implicated in the induction of protective immune responses in malaria. Evidence for this protective effect has been derived from in vitro and in vivo studies using anti-PMMSA monoclonal antibodies (MAb) (3, 8) and in vivo immunization experiments using purified PMMSA (1, 11, 13).

Despite this evidence, it is known that animals remain infected long after their sera contain detectable levels of anti-PMMSA antibodies (5). Possible explanations for this persistence of the parasitemia are (i) that merozoites are not accessible to antibodies for sufficient time to allow effective antibody binding (29); (ii) the possible occurrence of capping and shedding of immune complexes at the merozoite surface; (iii) the binding of a plasma component to the merozoite surface as has been suggested for sporozoites (16) which blocks antibody binding in vivo; (iv) the development of anti-idiotype antibodies which inhibit protective antibodies (Moore et al., unpublished results); (v) that the gene coding for the antigen shows an extremely high mutation rate; (vi) that antigen expression undergoes rapid phenotypic variation; (vii) that the protective immunogenicity of PMMSA lies primarily in the stimulation of cellular responses (15); or (viii) that the antigen contains sequences which stimulate suppressor activity (1). The fact that protective anti-PMMSA MAb have been raised by hybridoma techniques using animals immunized by infection as a source of immune cells indicates that protective anti-PMMSA antibodies are generated during infection. This paper describes experiments designed (i) to test the strain specificity of the delaying action of a MAb reacting with the PMMSA of the AS cloned line of *Plasmodium chabaudi chabaudi*, (ii) to test the capacity of this cloned line of *P. chabaudi chabaudi* parasites to become resistant to the anti-PMMSA MAb thus identified (3), and (iii) to examine such resistant lines for possible changes in PMMSA structure which could account for the resistance thus induced. The PMMSA of *P. chabaudi chabaudi* AS has an M_r of 250,000 while the PMMSA from other cloned lines of *P. chabaudi chabaudi* have a slightly different M_r and differ in peptide composition (27). MAb-3 (NIMP M23) was chosen for this study since it had previously been shown to delay *P. chabaudi chabaudi* AS parasitemias by passive transfer assay (3) and to be specific for *P. chabaudi chabaui* AS PMMSA by immunoprecipitation and immunofluorescence.

MATERIALS AND METHODS

Mice. Male CBA/Ca mice and female (CBA/Ca × BALB/c)F_1 hybrid mice (24 to 26 g each) were used. All mice were bred at the National Institute for Medical Research under specific-pathogen-free conditions. They were maintained on 41B cube diet (Grain Harvesters, Wingam, Kent, England) and supplied with water ad libitum.

Parasites. Cloned lines of *P. chabaudi chabaudi* AS and CB obtained from the World Health Organization Registry of Standard Strains of Malaria Parasites (Edinburgh, Scotland) and described previously (19) were used. Mice were maintained on a controlled illumination cycle between 17.30 and

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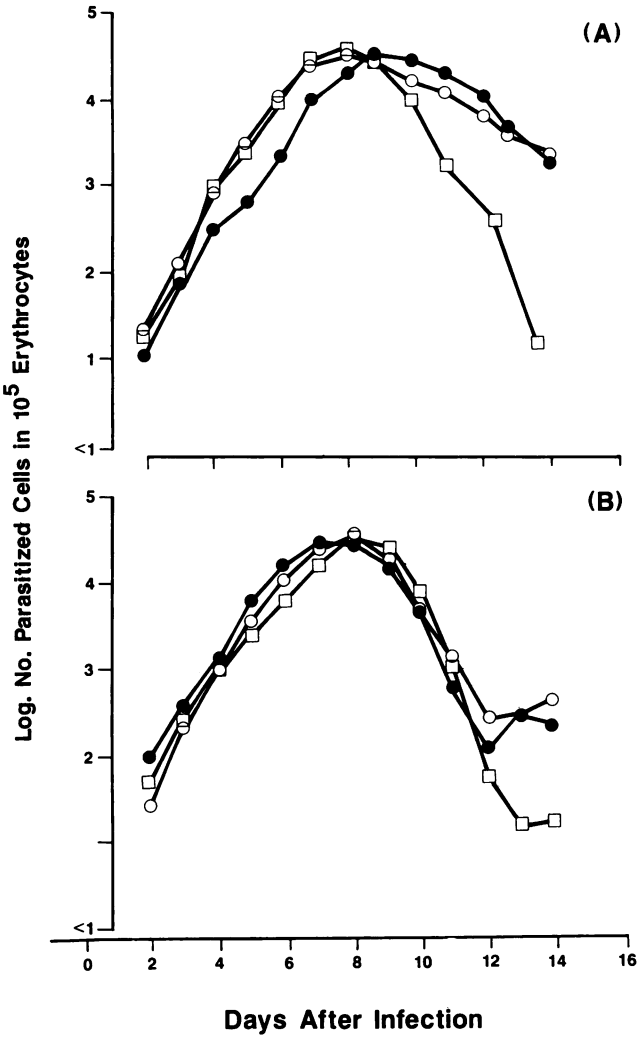

FIG. 1. The effects of administering anti-250-kDa PMMSA ascitic fluid during the course of a *P. chabaudi chabaudi* AS infection in CBA/Ca mice. Log geometric mean parasitemia resulting from infection with 10^5 *P. chabaudi chabaudi* AS (A)- or CB (B)-parasitized erythrocytes per mouse intravenously on day 0 is shown. Mice received intraperitoneally, on days −1, 0, 1, 3, 5, 7, and 9, 0.25 ml of ascitic fluid of MAb-3 (●), MAb-1 (○), or MAb-4 (□). The difference between MAb-3 and MAb-4 with *P. chabaudi chabaudi* AS (panel A) was significant ($P = 0.008$) for days 2 to 8. The difference between MAb-3 and MAb-1 with *P. chabaudi chabaudi* AS (panel A) was significant ($P = 0.008$) for days 2 to 8.

08.30 h to enable mature-stage parasites to be available in the morning.

Selection of hybridoma-resistant *P. chabaudi chabaudi* AS. Hybridoma cells [MAb-3 (NIMP M23) immunoglobulin G1; MAb-8 (NIMP M28) immunoglobulin G2b] or X63-Ag8.653 cells (3) were grown in RPMI 1640 plus 10% fetal calf serum tissue culture medium, and (CBA/Ca × BALB/c)F_1 mice were inoculated with 10^6 cells intraperitoneally. Pristane treatment was omitted in case it influenced parasite multiplication. At 6 days after inoculation, serum from tail blood was tested for MAb activity by the indirect fluorescent-antibody test against *P. chabaudi chabaudi* AS, using methanol-fixed infected erythrocyte antigen smears as previously described (3). Mice positive for MAb at a serum dilution of 1/100 were infected with 5×10^3 *P. chabaudi chabaudi* AS intravenously, and parasitemias were monitored daily. At approximately 20% parasitemia, as determined by Giemsa-stained smears, parasites were either cryoperserved as stabilates or metabolically labeled with [^{35}S]methionine.

Passive transfer assay. Ascitic fluids were clarified by centrifugation at 30,000 × *g* at 10°C for 30 min prior to use. (CBA/Ca × BALB/c)F_1 mice were infected intravenously with 10^4 control *P. chabaudi chabaudi* AS or MAb-3-resistant *P. chabaudi chabaudi* AS on day 0. Each parasite line was used at an equal number of passages from its respective reference stabilate (see Results). Mice were inoculated intraperitoneally with 0.25 ml of ascitic fluid on days −1, 0, 1, 3, 5, 7, and 9. Parasitemias were monitored daily.

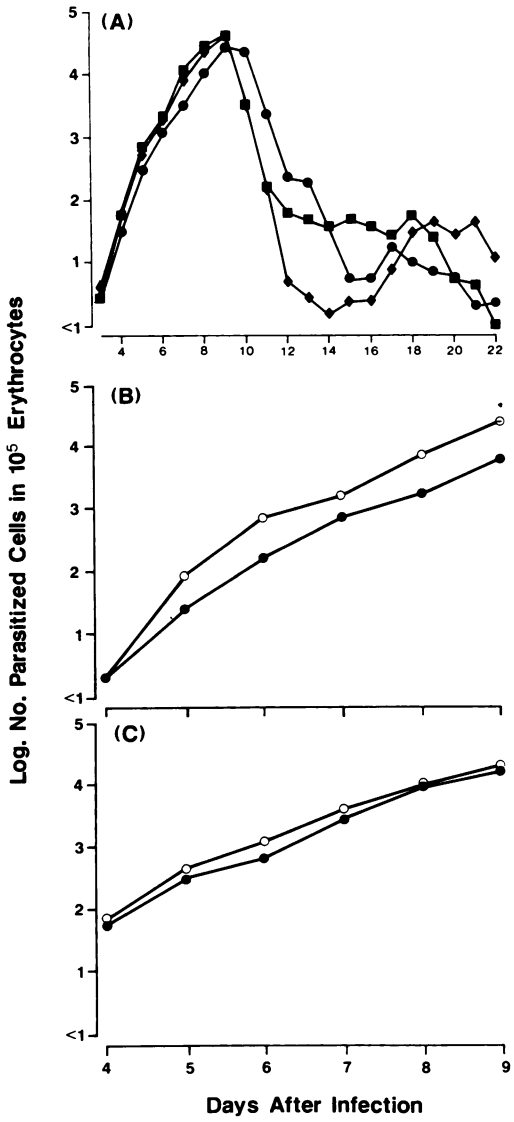

FIG. 2. The pattern of a *P. chabaudi chabaudi* AS infection in anti-250-kDa PMMSA hybridoma-bearing (CBA/Ca × BALB/c)F_1 mice. Log geometric mean parasitemia resulting from infection with *P. chabaudi chabaudi* AS on day 0 is shown. (A) Infection with 5×10^3 *P. chabaudi chabaudi* AS of mice bearing MAb-3 hybridoma (●), MAb-8 hybridoma (■), or Ag8 myeloma (◆). (B) Experiment 1. Infection with *P. chabaudi chabaudi* AS of mice bearing MAb-3 hybridoma (●) or control mice (○). (C) Experiment 2. Infection with *P. chabaudi chabaudi* AS of mice bearing MAb-3 hybridoma (●) or control mice (○).

TABLE 1. History of isolates from MAb-3 hybridoma mice and their experimental investigation

Expt	Delayed parasitemia	Day isolated	% Parasitemia[a]	Test[b]	Result
1	+	10	15	PPT	Difference
	+	10	11	PPT	Difference
	+	9	8,11,13[c]	STAB+2	
				PPT	No difference
				STAB+3	
				PROT	Resistant
2	−	9	18	PPT	No difference
	−	8	28	PPT	No difference
	−	7	5,8[d]	STAB+2	
				PROT	Resistant

[a] Percentage of parasitized erythrocytes in 200 erythrocytes.
[b] PPT, Immunoprecipitation; PROT, passive transfer protection test; STAB+2 and STAB+3, cryopreserved stabilate with two or three subpassages, respectively.
[c] Blood from three mice pooled for stabilate.
[d] Blood from two mice pooled for stabilate.

Results are given as $\log_{10}$ geometric mean parasitemia, and statistically significant differences between parasitemias were evaluated by the sign test for median values (19).

Metabolic labeling of parasitized erythrocytes with [^{35}S]methionine. Erythrocytes infected with mature stage *P. chabaudi chabaudi* AS were harvested from mice with parasitemias of >15%. Metabolic labeling of parasites with [^{35}S]methionine was carried out as described previously (25).

Lysate immunoprecipitation. Lysates were prepared from [^{35}S]methionine-labeled parasites (26) and incubated with 5 μl of serum from *P. chabaudi chabaudi* AS hyperimmune mice (HIS), 20 μl of normal mouse serum, or 10 μl of anti-PMMSA MAb for 30 min at 37°C before solubilization in PBS(A) (0.002 M Na_2HPO_4, 0.002 M KH_2PO_4, 0.17 M NaCl, 0.003 M KCl, pH 7.5) containing 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.), and 20 μg of DNase (type 1; Sigma). Samples were then centrifuged at 150,000 × *g* for 30 min at 4°C to pellet any unsolubilized material. Supernatants were incubated overnight at 4°C with rabbit anti-mouse immunoglobulin, and the resulting precipitates were washed three times in PBS(A)–0.5% Triton X-100.

SDS-PAGE. Immunoprecipitates were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (26).

RESULTS

Activity of MAb for genotypically different cloned lines of *P. chabaudi chabaudi* AS. The activity of MAb-3 against *P. chabaudi chabaudi* AS and CB cloned lines was compared by passive transfer assay. Two other anti-PMMSA MAb, MAb-1 (NIMP M21) and MAb-4 (NIMP M24), both immunoglobulin G2a (3), were used as controls. The delay in parasitemia produced by MAb-3 was specific for *P. chabaudi chabaudi* AS (Fig. 1) and did not occur with CB parasites, a result in keeping with previous immunofluorescence and immunoprecipitation studies (5). MAb-1 and -4 were not active against either strain. The prolongation of the parasitemia occurring after peak parasitemia is difficult to interpret. Similar effects have been seen in passive serum transfer experiments using *P. chabaudi chabaudi* immune serum (20).

Parasitemia in mice bearing a hybridoma secreting MAb-3 and infected with *P. chabaudi chabaudi* AS. The overall parasitemias in mice carrying a hybridoma secreting MAb-3 and with the antibody detectable in their sera were not markedly dissimilar from those in mice carrying the nonprotective MAb-8 hybridoma or mouse myeloma. A typical parasitemia is shown in Fig. 2A. The time to reach 2% parasitemia in one experiment (experiment 1, six mice) was increased by approximately 1 day (Fig. 2B); subsequently the parasitemia progressed normally. In another experiment (experiment 2, seven mice) the pre-2% period and subsequent parasitemia differed very little from those of normal mice (Fig. 2C). Stabilates were cryopreserved from mice in each experiment, as indicated in Table 1.

Analysis by immunoprecipitation of polypeptide antigens synthesized by MAb-3-resistant populations isolated directly from hybridoma-bearing mice. Mature trophozoites derived directly from mice as indicated in Table 1 were metabolically

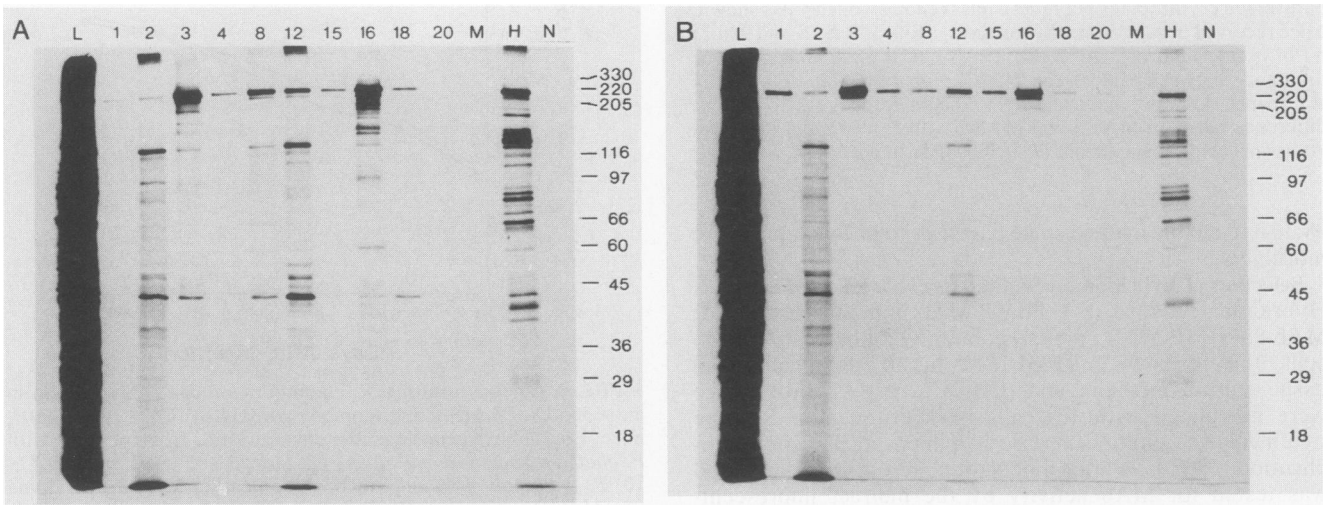

FIG. 3. Immunoprecipitation of metabolically labeled polypeptides of *P. chabaudi chabaudi* AS populations from experiment 1 by anti-250 kDa PMMSA MAb. (A) Control *P. chabaudi chabaudi* AS population. (B) MAb-3-resistant *P. chabaudi chabaudi* AS population. Lanes: L, total lysate; M, myeloma ascitic fluid; H, HIS; N, normal mouse serum; 1 to 20 = anti-250-kDa PMMSA MAb (3). Numbers on the right indicate molecular masses (in kilodaltons).

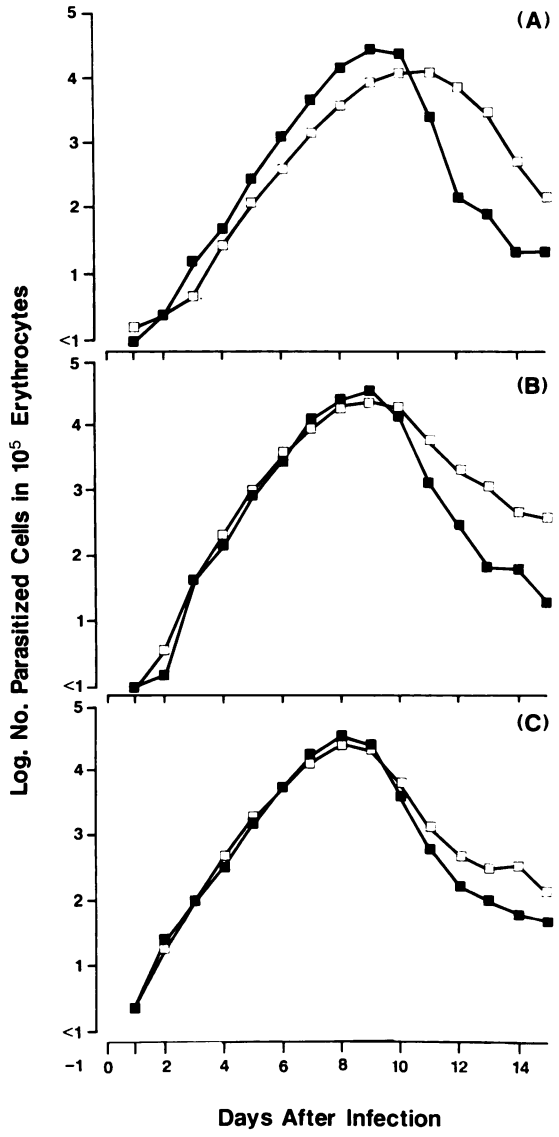

FIG. 4. Passive protection test with MAb-3 on MAb-3-selected *P. chabaudi chabaudi* AS parasites in (CBA/Ca × BALB/c)F_1 mice. Log geometric mean parasitemia resulting from infection with 10^5 *P. chabaudi chabaudi* AS-parasitized erythrocytes per mouse intravenously on day 0 is indicated. Mice received intraperitoneally, on days −1, 0, 1, 3, and 5, 0.25 ml of ascitic fluid of MAb-3 (□) or anti-schistosomular MAb (■). (A) Control *P. chabaudi chabaudi* AS. Parasitemias were significantly different ($P = 0.004$) for days 3 to 10. (B) Experiment 1. MAb-3-selected *P. chabaudi chabaudi* AS. (C) Experiment 2. MAb-3-selected *P. chabaudi chabaudi* AS.

labeled in vitro with [^{35}S]methionine. SDS-PAGE patterns following immunoprecipitation of these MAb-selected parasites were compared in two experiments with original unselected populations. With parasites isolated from a mouse in experiment 1, there were differences between precipitation patterns derived from the MAb-3-selected and an unselected population (Fig. 3), especially in the peptides precipitated by MAb-1, -3, -8, -12, and -16 (3) and HIS. In contrast, with parasites from a mouse in experiment 2, no differences were detected between immunoprecipitates obtained from the selected population and those obtained from unselected parasites. The experiments were repeated with similar results.

Sensitivity to MAb-3 of parasites derived from stabilates isolated from mice carrying a hybridoma secreting MAb-3. Parasites isolated at the second passage of stabilates of pooled blood from experiment 1 (3 mice) and experiment 2 (2 mice) (Table 1) were compared with parasites of the original population for sensitivity to MAb-3 in a passive transfer assay. An anti-*Schistosoma mansoni* MAb (NIMP M46) of the same isotype was used as a control. The result is shown in Fig. 4. Unlike the original AS line passaged an equal number of times from stabilate (Fig. 4A), parasites derived from both MAb-selected stabilates proved to be insensitive to MAb-3, irrespective of (i) whether they originated from mice with a delayed parasitemia (Fig. 4B) or from mice with no such delay (Fig. 4C) and (ii) whether the immunoprecipitation pattern obtained with parasites isolated directly from mice equivalent to those providing the stabilates appeared modified by the MAb selection (Fig. 3).

Polypeptide antigens synthesized by *P. chabaudi chabaudi* AS during a 24-h maturation period. The asexual erythrocytic stage of *P. chabaudi chabaudi* AS in CBA/Ca mice shows a synchronous 24-h cell cycle of development with peak schizogony occurring at 13.00 h under the lighting conditions used. Two experiments were carried out to determine (i) the optimum part of the cell cycle to be used in subsequent experiments to detect antigenic differences between populations by immunoprecipitation with anti-PMMSA MAb and (ii) whether antigens occurred, other than the 250-kilodalton (kDa) PMMSA polypeptides and derived smaller polypeptides, which cross-reacted with the anti-PMMSA MAb used. The pattern of cross-reacting antigens may be modified as a consequence of antibody selection as well as, or instead of, the PMMSA and its products and thus could be inferred as being associated with the observed delay in parasitemia.

Individual mice from a batch infected with *P. chabaudi chabaudi* AS were killed at 2-h intervals over a 26-h period, and the parasites were metabolically labeled for 2.5 h. Lysates were prepared and immunoprecipitated with HIS and MAb-1, -3, and -12, and the results were analyzed by SDS-PAGE and fluorography. The results with HIS and MAb-3 are shown in Fig. 5. With the lighting conditions under which the mice were maintained, the PMMSA antigen was first detected by HIS and the MAb at 8.00 h and became undetectable at 18.00 h. In addition, a number of other polypeptides were precipitated by MAb-3, which were synthesized prior to the PMMSA and presumably were therefore separate gene products. Most prominent were polypeptides with M_r values of 45,000, 50,000, 66,000, and 90,000 as indicated (Fig. 5B). These polypeptides are also precipitated by a number of anti-PMMSA MAb which after passive transfer have no effect on challenge parasitemias (data not shown).

Immunoprecipitation pattern of polypeptide antigens synthesized by parasites derived from a stabilate pool from experiment 1. At 2-h intervals during the last third of the maturation cycle, parasites from one mouse with an infection derived from cryopreserved unselected AS parasites and one mouse with an infection derived from an experiment 1 stabilate (Table 1) were isolated simultaneously and metabolically labeled. Labeled parasite lysates were immunoprecipitated with anti-PMMSA MAb or HIS and analyzed by SDS-PAGE. The result of one of these experiments is shown in Fig. 6. Although MAb-12, MAb-16, and HIS each produced a characteristic pattern of precipitated polypeptides, these patterns were identical for selected and unselected populations (Fig. 6A and B; results for HIS not shown).

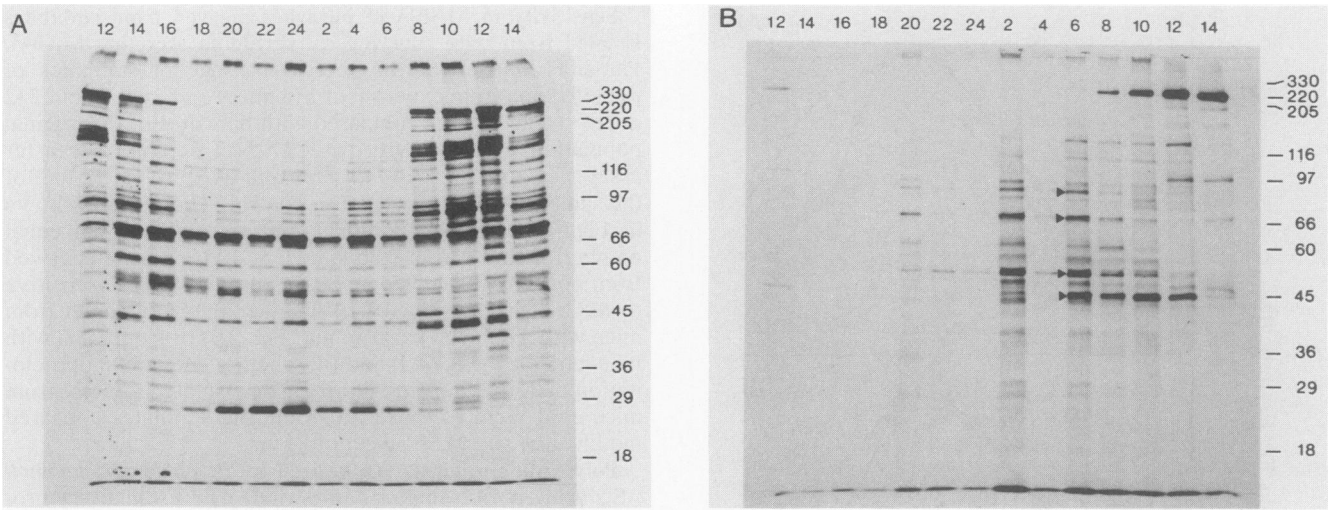

FIG. 5. Immunoprecipitation of metabolically labeled polypeptides of *P. chabaudi chabaudi* AS during a 26-h maturation period. (A) Immunoprecipitation by hyperimmune serum. (B) Immunoprecipitation by anti-250-kDa PMMSA MAb-3. Numbers at tops of lanes indicate times (in hours) of isolation for metabolic labeling. ▶, Predominant polypeptides synthesized prior to the PMMSA. Numbers on the right indicate molecular masses (in kilodaltons).

There were some minor differences between the precipitation patterns with MAb-1 and -3, but these could not be reproduced in a repeat experiment (Fig. 6C and D).

DISCUSSION

The PMMSA and the merozoite surface antigens derived therefrom represent an apparent paradox in terms of immunity to malaria. If, as seems to be the case, the merozoite surface antigens are exposed to antibody action during escape from the infected erythrocyte and prior to invasion of a fresh host cell, why is erythrocytic malaria chronic and subject to recrudescence? This paradox is heightened by the fact that a strong antibody response to the PMMSA occurs very early in an infection (5). In addition to the possible explanations listed in the introduction, other factors also need to be considered. (i) In *P. chabaudi chabaudi* infections initiated by cloned lines of parasites, populations partially or totally resistant to protective antibodies including presumably anti-PMMSA antibodies, soon emerge (20, 22). (ii) In the present investigation, not only was the MAb under study specific in its delaying action for the cloned AS line and without activity against the genotypically different CB parasites, but subpopulations of *P. chabaudi chabaudi* AS resistant to this MAb readily emerged in vivo. This occurred in some instances without any detectable reduction in parasite multiplication as judged by the progress of the parasitemia. These resistant populations were not clearly differentiated by immunoprecipitation analysis of their PMMSA and associated polypeptides from the original population. (iii) In *Plasmodium falciparum* at least, the gene for the PMMSA analog is apparently present in a single copy (31), although subject to considerable antigenic diversity among *P. falciparum* isolates, with conserved and variable regions distributed along the molecule. The variable areas, with the exception of a section of tripeptide repeats, apparently show allelic dimorphism; the tripeptide repeats, however, are polymorphic (28, 31) and possibly represent an area of frequent mutation. It has not been possible to detect in the genome silent copies of variable regions as possible sources of phenotypic diversity of the *P. falciparum* PMMSA within cloned populations of a single genotype. Thus, present evidence obtained by gene cloning techniques has been interpreted as indicating that sexual recombination in the mosquito stage of the life cycle and mutation are the most likely cause of epitope diversity in this group of molecules and its products (28).

The region of the *P. falciparum* tripeptide repeats differs in five out of six isolates examined so far (28, 31) or may be absent (6). It is conceivable that these tripeptide repeats could have a very high mutation frequency when under continuous immune pressure in vivo but remain relatively stable during repeated subculture in a uniform in vitro environment and in the absence of immune pressure. Such instability would imply that no specific cellular function is located in this part of the molecule, as suggested by Peterson et al. (28). Thus, genetic heterogeneity and rapid mutation of the tripeptides within parasite populations during an ongoing infection could be the normal state but could remain undetected in in vitro-maintained parasites. Mutation in a merozoite surface antigen of *Plasmodium knowlesi*, apparently not derived from the PMMSA, has been reported (7), but in this instance the mutant forms were detected after some delay in a later recrudescence. Their polypeptides appeared different by MAb immunoprecipitation.

We have no knowledge of the PMMSA gene of *P. chabaudi chabaudi*, but if one assumes that, as appears to be the case with *P. falciparum*, it exists as a single gene copy, then protective action by anti-PMMSA MAb and the rapid emergence of MAb-resistant cloned lines might also indicate that the gene is subject to extremely frequent mutation. On the other hand, the rapidity with which resistance emerges without, under certain conditions, detectable parasite destruction is a characteristic shared with phenotypic variation in the *P. knowlesi* SICA antigen, which is expressed at the surface of schizont-infected erythrocytes (4). In addition, McLean et al. (22), working with *P. chabaudi chabaudi* AS, demonstrated that development of antibody resistance by a cloned line of this parasite is a regular feature during chronic infection, followed by a return to susceptibility after mosquito transmission (23). Furthermore, immunization studies with MAb affinity-purified *P. chabaudi chabaudi* AS

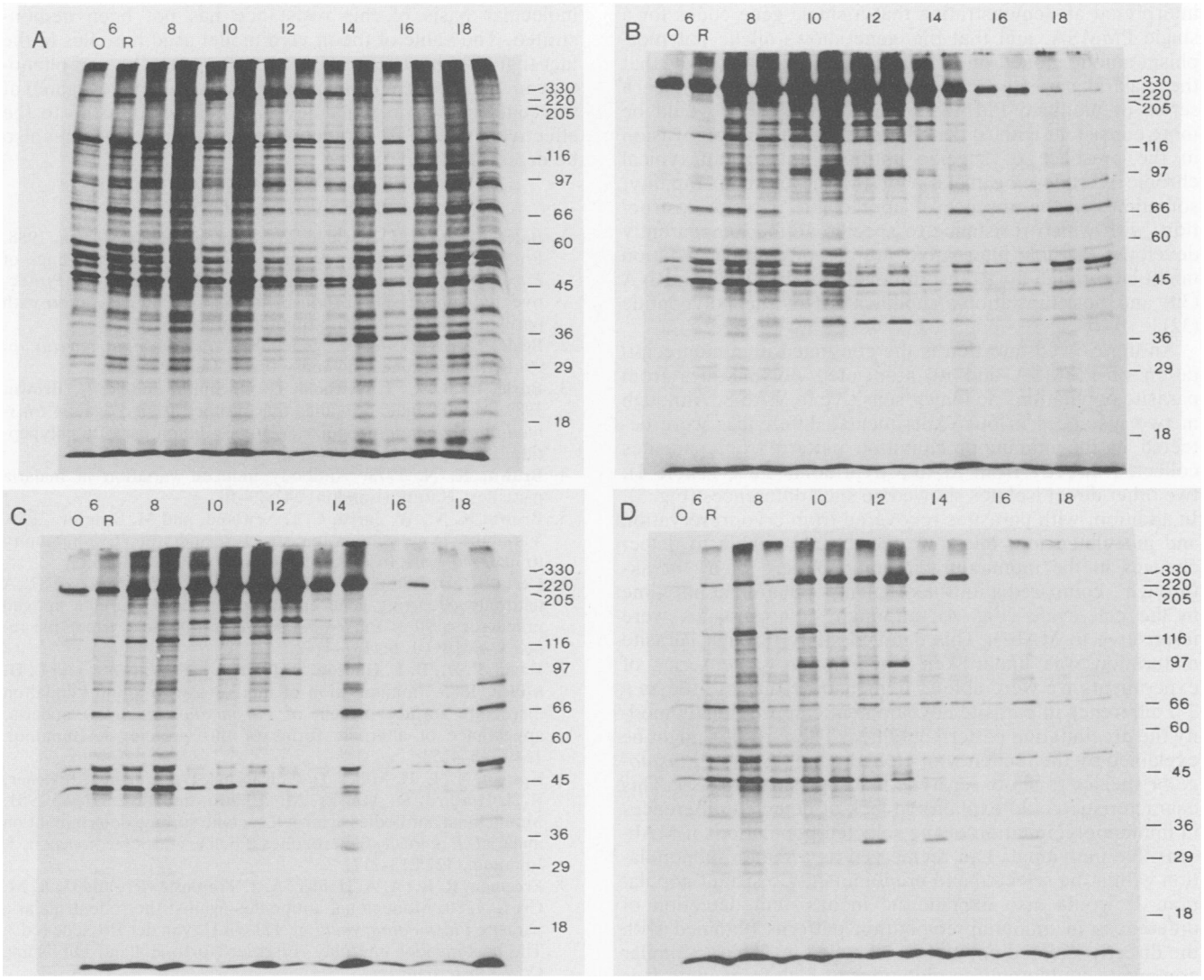

FIG. 6. Immunoprecipitation of metabolically labeled polypeptides of control and MAb-3-resistant *P. chabaudi chabaudi* AS during a 12-h period. (A) Immunoprecipitation by anti-250-kDa PMMSA MAb-12. (B) Immunoprecipitation by anti-250-kDa PMMSA MAb-16. (C) Immunoprecipitation by anti-250-kDa PMMSA MAb-1. (D) Immunoprecipitation by anti-250-kDa PMMSA MAb-3. Lanes O (alternate lanes), Control *P. chabaudi chabaudi* AS; lanes R (alternate lanes), MAb-3-resistant *P. chabaudi chabaudi* AS. Numbers at tops of lanes (in pairs) indicate times (in hours) of isolation for metabolic labeling. Numbers on the right indicate molecular masses (in kilodaltons).

PMMSA (1) demonstrated that breakthrough parasites from cloned lines could multiply at the same rate in specifically sensitized hosts as unselected populations could in normal controls. These results suggest that phenotypic antigenic variation may occur with merozoite surface antigens as well as parasite-encoded antigens expressed at the surface of parasitized erythrocytes (12, 17, 18, 21), although the mechanism for this variation has not as yet been detected at the genetic level. Expression of a range of alternative surface polypeptides by individual protozoa according to environmental circumstances is not necessarily inconsistent with the occurrence of single gene copies coding for the variant polypeptides or with sequence homologies between equivalent polypeptides, both characteristics of the PMMSA. For example, extensive phenotypic variation of surface polypeptides is known to occur in these circumstances in the well-studied free-living ciliate *Paramecium* species (10). It is difficult to see the advantage to the malaria parasite in constantly varying molecules exposed at the infected erythrocyte surface without a similar capacity to vary expression of antigens exposed at the merozoite surface.

If there is phenotypic antigenic variation in PMMSA-derived surface polypeptides, then by analogy with *Paramecium* species, the uniform environment of in vitro culture used to maintain *P. falciparum* could result in the expression of a single variant gene out of the many alternatives available to the organism. With different stocks of *Paramecium* species, the variant polypeptides induced by particular environmental conditions all tend to be alleles of the same gene, although the genome codes for many other variants expressed under different conditions. In fact, the presence of dilute antiserum specific for a particular expressed polypeptide is one means that can be used to modify surface polypeptide expression of individual ciliates (2). The alleles expressed in a certain environment show polymorphism among the various stocks. By analogy, analysis of *P. falciparum* of different isolates maintained under standard in vitro conditions and based on cDNA libraries might well be

interpreted as demonstrating that a single gene codes for a single PMMSA, and that this gene shows allelic polymorphism among cloned lines. It is conceivable, however, that the malaria parasite does have the capacity to produce a series of modified PMMSA. Such a possibility would be more consistent with (i) the continuous erythrocyte invasion in the presence of specific antibody throughout typical chronic recrudescing malaria or (ii) the extreme rapidity, sometimes in the absence of appreciable parasite destruction, with which resistance to specific antibody apparently develops. Possible alternative sources of epitope variation might be through posttranscriptional modification of mRNA (30) and posttranslational modification of the polypeptide (32).

An unresolved question is the continued immunoprecipitation of PMMSA and its associated polypeptides from parasite populations no longer sensitive to MAb-3. Although in two instances in our experiments, differences were detected in the immunoprecipitation pattern from parasites collected directly from MAb-3 hybridoma mice (Table 1), two other direct isolates showed no such differences (Fig. 3). In addition, with parasites recovered from cryopreservation and inoculated into mice, we were again unable to detect changes in the immunoprecipitation pattern of a "breakthrough" compared with the original population at any time in the cell cycle (Fig. 4), although such parasites were insensitive to MAb-3. This detailed study through parasite maturation was undertaken since in the same series of experiments we were able to demonstrate that as little as a 2-h difference in parasite development can profoundly modify the precipitation pattern obtained. These results might be explained by the tendency of cloned populations of *Plasmodium* species to show serotype heterogeneity (18, 22). This characteristic could explain the lack of detected differences in immunoprecipitation of the selected populations if MAb-sensitive individuals had reemerged as a minor subpopulation within the selected and predominantly resistant population. It would also explain the inconsistent detection of differences in immunoprecipitation patterns obtained with the directly isolated populations detailed in Table 1. Similar arguments could apply to mutations at high frequency. It is interesting that the emergence of parasite populations resistant to a merozoite rhoptry protein of *Plasmodium yoelii* has been reported without loss of precipitating or fluorescent-antibody test activity (9). In this instance, resistance was associated with a change in erythrocyte age preference.

It is unlikely that the delaying action of MAb-3 is due to binding with a cross-reacting polypeptide which is a quite distinct gene product, rather than with the PMMSA. Polypeptides with M_r values of 45,000, 50,000, 66,000, and 90,000 which reacted with MAb-3 but were synthesized prior to the PMMSA and therefore presumably coded by distinct genes were not detectably different by immunoprecipitation in the breakthrough population from those of the original population, a result which appears to argue against the explanation of an alternative gene product being involved. In any event, the immunogenic status of these polypeptides is somewhat doubtful since they are sometimes seen as precipitates with normal mouse serum (J. C. Wood and K. N. Brown, unpublished data).

In conclusion, we have shown that parasite populations resistant to an anti-PMMSA MAb which delays parasitemia are readily generated in *P. chabaudi chabaudi* infections. The ease with which this resistance developed was remarkable and consistent with previous immunization studies (1) and the chronic, recrudescent nature of malaria. As yet, the molecular basis of this resistance has not been demonstrated. The value of the in vivo model used here lies in the fact that changes occurring in parasite genotype and phenotype in vivo can readily be identified and then investigated at the cellular and molecular levels. Their relationship to the effectiveness of host protective immune responses can also be determined.

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