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Development of the Human Immune Response against the Major Surface Protein (gp190) of *Plasmodium falciparum*

HANS-MICHAEL MÜLLER,[1] KLAUS FRÜH,[1] ALBRECHT VON BRUNN,[1] FULVIO ESPOSITO,[2] STEFANIA LOMBARDI,[3] ANDREA CRISANTI,[1] AND HERMANN BUJARD[1]*

Zentrum für Molekulare Biologie, Im Neuenheimer Feld 282, 6900 Heidelberg, Federal Republic of Germany[1]; Departimento di Biologia Cellulare, Università di Camerino, Camerino, Italy[2]; and Instituto di Parassitologia, Università di Roma "La Sapienza," Rome, Italy[3]

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The 190-kilodalton glycoprotein (gp190) of *Plasmodium falciparum*, the precursor of the major surface proteins of merozoites, is considered a promising candidate for a blood stage malaria vaccine. DNA sequences specific for the gp190 of the two isolates K1 and MAD20 were subcloned and expressed in *Escherichia coli*. The panel of fusion proteins obtained represents about 80% of the polymorphic sequences observed so far within various isolates of *P. falciparum*. Sera from individuals living in a malaria-endemic area of West Africa were tested in immunoblots against the gp190 fusion proteins, and antibody reactivity was mapped to defined regions of the gp190. Depending on the age of the individual and on the presence of parasites in the blood, distinct regions of gp190 were differentially recognized by the respective antibodies. Similarly, the analysis of sera from German patients with acute malaria revealed a distinct pattern. When grouped according to age and to parasitemia, the reactivity of the sera of people living in malaria-endemic areas may indicate a correlation between certain gp190 regions and protective immune response.

The major surface proteins of the merozoites of *Plasmodium falciparum* are processing products of a 190-kilodalton (kDa) precursor glycoprotein, gp190 (8). The function of these proteins is still unknown, though their localization within the coat of the merozoite (6) suggests that they may play a role in the interaction between the parasite and erythrocytes (14).

The gp190 protein is highly immunogenic in humans (15), and when used for the immunization of monkeys it modifies the course of infection by the parasite (5, 16, 18). Moreover, homologous proteins in animal models were shown to be involved in protective immunity (2, 4, 7). These protective effects make gp190 a candidate for a subunit malaria vaccine, although the polymorphism of the molecule (13) may raise questions about the feasibility of gp190-based vaccine development. Comparison of the gp190 sequences of different *P. falciparum* isolates reveals three degrees of variability: isolate-specific runs of amino acids, dimorphic parts which are either MAD20- or K1-like, and clusters of highly conserved sequences (11, 17, 19, 21).

The best protection in gp190-immunized monkeys is achieved when the parasite used for the challenge carries the homologous protein (5, 19). This suggests that the polymorphic sequences (isolate-specific or dimorphic) are, in this animal model, the targets of the protective immunity.

For the development of a gp190-derived vaccine, it is important to identify the targets of the protective cellular and humoral response within gp190. It was therefore of interest to us to map the humoral reactivity against the invariant and polymorphic regions of gp190 in individuals showing different degrees of protection against *P. falciparum* infections.

Thus, we have subcloned and expressed in *Escherichia coli* gp190 sequences of the K1 and the MAD20 types, which together cover about 80% of all gp190 sequences known so far. The fusion proteins obtained were used to analyze via immunoblots sera from individuals living in malaria-endemic areas, from *P. falciparum*-infected German patients, and from individuals living in a malaria-endemic area under chemoprophylaxis since birth.

MATERIALS AND METHODS

Cloning and expression of K1- and MAD20-specific sequences of gp190 in *E. coli*. Genomic DNA coding for the gp190 from the K1 and MAD20 isolates of *P. falciparum* (11, 19) was cut with appropriate restriction enzymes to generate a series of sequences coding for 150 to 300 amino acids. The DNA fragments derived from the K1 isolate were fused to *Bam*HI and to *Hin*dIII linkers at their 5′ and 3′ ends, respectively (12), and inserted into the expression vector pUHE31-1, a derivative of the pDS plasmid family (3). The expression unit of this vector is under the control of an inducible promoter and yields fusions between the inserted sequence and the N terminus of the chloramphenicol acetyltransferase (CAT) gene. The DNA fragments derived from the MAD20 isolate were cloned in the same way in an expression vector in which the *cat* gene was replaced by the coding sequence for dihydrofolate reductase (DHFR) of the mouse. The gp190 fusion proteins were obtained in *E. coli* upon induction with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) at an OD_{600} of 0.6 for 3 h. Restriction enzymes, linkers, and T4 DNA ligase were purchased from Boehringer, IPTG was from Biomol, and media were from Difco Laboratories.

Immunoblotting. Total lysates of bacteria expressing the CAT or the DHFR fusion proteins specific for K1 or MAD20 sequences were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10) and electroblotted onto nitrocellulose filters (20). Nonspecific adsorption of antibodies by the nitrocellulose was blocked by saturating the filters with 1% bovine serum albumin solution overnight. The sera were diluted 1:100, and antibodies directed against bacterial proteins were removed by incubation overnight with nitrocellulose-bound lysates of *E. coli* containing CAT or DHFR fusions. Nitrocellulose filters containing the various fusion

* Corresponding author.

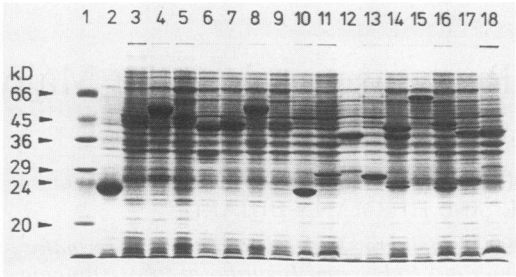

FIG. 1. Electrophoretic separation of total *E. coli* lysates containing various K1- and MAD20-derived fusion proteins. Lanes 3 to 9 contain the K1-derived CAT fusion proteins F2, F4, F5, F7, F8, F9, and F10, respectively, and lanes 11 to 18 contain the MAD20-derived DHFR fusion proteins M2, M3, M4, M6, M7, M8, M9, and M11, respectively. The major proteins in lanes 2 and 10 are CAT and DHFR, respectively; lane 1 shows molecular mass standards (in kilodaltons) (12.5% acrylamide, Coomassie stained).

proteins were incubated with the preadsorbed sera and kept overnight at room temperature. Antibodies bound to the gp190 fusion proteins were detected by a goat anti-human immunoglobulin G antibody conjugated to alkaline phosphatase (Protoblot system; Promega). Positive reactions on the immunoblots were evaluated blindly and independently by three members of the laboratory.

Sera. Samples of sera from 112 inhabitants (63 adults [18 to 67 years], 35 children [1 to 5 years], and 14 infants [3 to 6 months]) of the village of Kuiti in Burkina Faso were collected during a survey conducted in 1985 and 1986. The following information was recorded for each participant: age, sex, and level of parasitemia. The entomological inoculation rate (infective bites per person per night) in 1985 in the village of Kuiti was 10^{-3} in June, 1.96 in August, and 0.15 in November. The proportion of parasitized individuals recorded in June, August, and November was 42, 88, and 90% for the children and 23, 50, and 46% for the adults, respectively. Similar proportions of parasitemia were recorded in 1986. Fourteen serum samples from German patients who were infected with *P. falciparum* for the first time were analyzed as well. Ten serum samples from laboratory volunteers and 10 from African children under malaria chemoprophylaxis since birth served as controls.

All samples from Kuiti villagers, including those of the children under chemoprophylaxis, and 10 samples from German patients were tested against the gp190 fusion proteins of K1. The MAD20 fusions were used to examine 14 serum samples from German patients and, due to the limited amount of serum samples from Kuiti, 14 samples from adults and 10 from infants. The 10 samples from laboratory volunteers were tested against both the K1 and MAD20 fusion proteins.

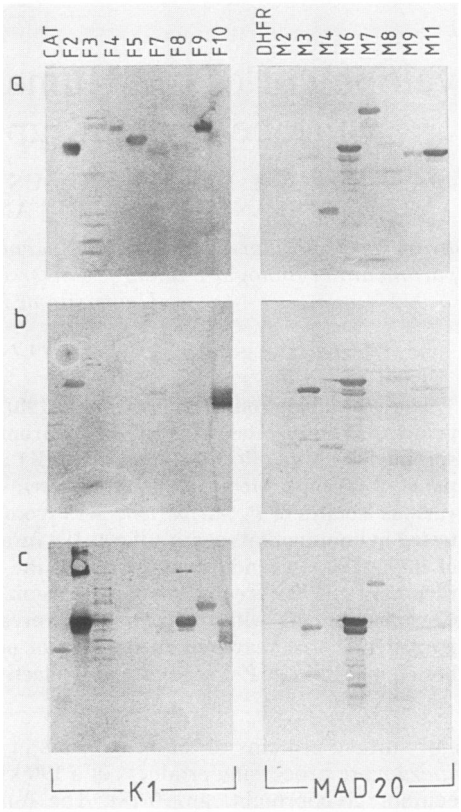

FIG. 3. Immunoblot analysis of human sera with the K1- and MAD20-specific gp190 fusion proteins. Representative serum reactions of adults (a) and infants (b) from a malaria-endemic area in Burkina Faso and of German patients (c) are shown. Authentic CAT and DHFR standards were also run.

Statistical analysis. For each group of individuals, the reactivity of the sera against the various gp190 fusion proteins is given in absolute numbers and as a proportion of positive reactions. Comparison of the frequencies of reac-

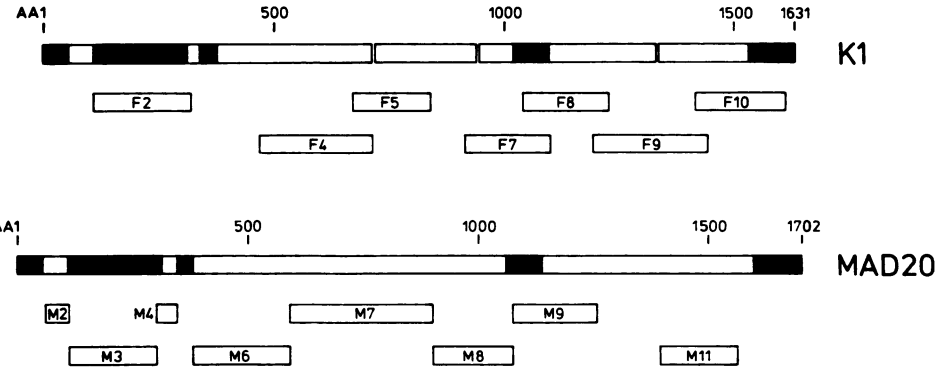

FIG. 2. Schematic representation of the K1 and MAD20 precursor proteins, and the positions of the sequences included in the various fusion proteins. Black boxes represent conserved regions (19). The proposed borders of the 83-, 28- to 30-, 38-, and 42-kDa (9) gp190 cleavage products within K1 are indicated.

TABLE 1. Comparison of reactions against gp190 fusion proteins between adults and children from a malaria-endemic area[a]

Group	K1 gp190 fragments										MAD20 gp190 fragments										
	No. of samples	% Positive on fragment:							No reaction[b] (%)	% Recognized[c]	No. of samples	% Positive on fragment:								No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10				M2	M3	M4	M6	M7	M8	M9	M11		
Adults	63	25	46	86	18	41	38	29	5	40	14	21	50	21	86	43	0	14	57	7	37
Children	35	26	31	29	37	29	17	31	29	28	0										

[a] *P* value for K1 gp190 fragment F5 was significant ($P = 5 \times 10^{-8}$) for adults versus children. Other values were not significant ($P = 0.73, 0.19, 0.05, 0.25, 0.05$, and 0.47 for F2, F4, F7, F8, F9, and F10, respectively). *P* values for percent samples showing no reaction and percent K1 gp190 fragments recognized per individual were significant at $P = 0.003$ and $P < 0.01$, respectively, for adults versus children.

[b] Proportion of individuals showing no reaction against the gp190 fusion proteins.

[c] Proportion of fragments recognized per individual in the group.

tions against each individual gp190 fragment between groups was evaluated by the *P* values derived from Fisher's twice one-sided exact test (1). The *P* values for the comparison of the total frequencies between groups were calculated with Yates's continuity-corrected χ^2 test (1). Only *P* values of ≤ 0.01 were considered significant.

RESULTS

Expression of gp190 fusion proteins in *E. coli*. Seven DNA fragments were generated from genomic clones containing the gp190 gene of the K1 isolate (11): F2 (amino acids [AA] 106 to 321), F4 (AA 469 to 714), F5 (AA 671 to 833), F7 (AA 915 to 1100), F8 (AA 1038 to 1224), F9 (AA 1194 to 1439), and F10 (AA 1412 to 1608). They were expressed as CAT fusion proteins in *E. coli* (Fig. 1) and covered 78% of the gp190 of K1. Similarly, eight DNA fragments were produced from genomic clones containing the gp190 gene of the MAD20 isolate (19): M2 (AA 61 to 121), M3 (AA 123 to 302), M4 (AA 304 to 343), M6 (AA 384 to 595), M7 (AA 595 to 897), M8 (AA 898 to 1079), M9 (AA 1078 to 1251), and M11 (AA 1397 to 1563). These sequences were expressed in *E. coli* as DHFR fusion proteins (Fig. 1). They represent 77% of the MAD20-specific gp190. The positions of the K1- and MAD20-derived fragments along the parental proteins are shown in Fig. 2.

Reactivity of sera against the gp190-derived fragments. The serum samples from 63 adults and 35 children from Kuiti were subjected to immunoblot analysis against the various gp190-derived fusion proteins. A set of typical immunoblots as used for evaluation is shown in Fig. 3. The proportion of sera which reacted with each region of the gp190 of the K1 isolate is shown in Table 1.

The gp190 fusion proteins F5 (K1) and M6 (MAD20), both part of the dimorphic regions of gp190, were recognized with the highest frequency by sera from adults, whereas the sera of children did not show any preference for either of the K1-specific fragments tested. Statistical analysis showed a highly significant difference in the frequency of positive reactions against F5 when the serum samples from adults and children were compared (Table 1). A corresponding analysis with the MAD20-specific fusion proteins could not be carried out due to the lack of serum samples from the children.

There was also a quantitative difference between the sera of adults and children. Comparison of the sum of all reactions as well as comparison of the frequency in both groups of individuals that did not react against any gp190 fragment revealed a statistically significant difference (Table 1).

Reactivity of sera against gp190 upon primary infection. The gp190-specific humoral immune response induced upon a putative primary infection with *P. falciparum* was analyzed by examining sera from African infants (3 to 6 months) as well as from German patients infected with *P. falciparum* (Fig. 3). The results (Table 2) showed that all fragments corresponding to the invariable and dimorphic regions of the MAD20 sequence were recognized with almost identical frequencies by samples from African infants and German patients. Both M2 and M4, which seemed to be recognized differentially within these two groups, belong to the highly polymorphic region of gp190. Differences, although statistically significant only for F8, were observed between German patients and African infants for the K1 fragments. A prevalence of *P. falciparum* infection of the MAD20 type in our collection of patient sera and mixed (K1 and MAD20) infections in the African infants could account for such a difference.

Reactivity of sera against gp190 sequences upon secondary infections. The parasitemia observed in African individuals who live for more than 1 year in a malaria-endemic area with a high rate of transmission, such as the village of Kuiti in Burkina Faso, indicates secondary *P. falciparum* infections. We analyzed the effect of such infections on the antibody response by comparing the proportion of gp190-specific fragments recognized by antibodies from parasitized and

TABLE 2. Comparison of reactions against gp190 fusion proteins between infants from a malaria-endemic area and German malaria patients[a]

Group	K1 gp190 fragments										MAD20 gp190 fragments										
	No. of samples	% Positive on fragment:							No reaction (%)	% Recognized	No. of samples	% Positive on fragment:								No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10				M2	M3	M4	M6	M7	M8	M9	M11		
Infants	10	90	50	20	90	100	20	80	0	64	10	0	90	50	90	10	50	60	30	0	54
Patients	10	60	40	40	30	30	40	30	0	39	14	29	64	14	100	21	21	50	29	0	47
P[b]		0.30	0.93	0.62	0.02	<u>0.01</u>	0.62	0.07		<u>≤0.01</u>		0.23	0.34	0.15	1.00	0.87	0.30	0.87	1.00		<0.50

[a] See Table 1, footnotes *b* and *c*.

[b] Significance, infants versus patients. Significant values are underlined.

TABLE 3. Comparison of reactions of parasitized and nonparasitized individuals against gp190 fusion proteins[a]

Group[b]	No. of samples	% Positive on K1 gp190 fragment:							No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10		
Adults										
Parasitized	20	40	60	95	45	55	65	30	0	56
Nonparasitized	43	19	40	81	5	35	26	28	7	33
P		0.13	0.19	0.29	0.0005	0.20	0.006	0.90		<0.001
Children										
Parasitized	25	28	36	36	52	40	24	40	20	37
Nonparasitized	10	20	20	10	0	0	0	0	50	7
P		0.92	0.60	0.26	0.01	0.06	0.36	0.03		<0.001

[a] See Table 1, footnotes *b* and *c*.
[b] *P* values for parasitized versus nonparasitized individuals. Significant values are underlined.

nonparasitized individuals. The proportion of the fragments reacting with the sera from African adults and children, grouped according to the presence and absence of parasitemia, is shown in Table 3.

The reactivity of the sera from parasitized individuals, both adults and children, compared with that from the nonparasitized individuals showed a high proportion of positive reactions against all the gp190 K1 fragments tested (Table 3). This difference in reactivity was statistically significant for some fragments. Furthermore, comparison of the proportion of fragments recognized per individual showed a statistical significance for the two groups (Table 3).

Similar differences in the proportions of reactivity against the gp190 K1 fragments could be detected by comparing adults and children at two time points during the year, August 1985 and June 1986, during transmission and at the beginning of the next transmission season (Tables 4 and 5).

The reactivity against the K1 gp190 fragments of serum samples from the two control groups, laboratory volunteers and children living in a malaria-endemic area under chemoprophylaxis since birth, is shown in Table 6. Among the samples from laboratory volunteers, only one reacted with one MAD20 gp190 fragment.

DISCUSSION

We have analyzed the antibody reactivity in immunoblots against the gp190 of *P. falciparum* by using a panel of 15 protein fragments which represent about 80% of the known gp190 sequences. In *P. falciparum*, a major part of the gp190 is found in two allelic forms of the K1- or the MAD20-type (19). Since our panel of fragments is composed of K1 as well as MAD20 sequences, the potential bias of the dimorphism on the results is largely eliminated. Moreover, the use of this panel of fragments permits mapping of the antibody response to defined regions of the gp190. Analysis of the reactivity of sera by this experimental system reveals qualitative as well as quantitative differences in the humoral response among different groups of individuals exposed to *P. falciparum* infection.

From our analysis, it emerges that adults living in a malaria-endemic area react more frequently against some dimorphic regions of the gp190 (F5) than children.

When the serum reactivity of the adults and children was analyzed with respect to a detectable infection, parasitized individuals showed a higher proportion of reactivity against all the gp190 fragments. Especially for F7, the difference was highly significant. Thus, the apparent bias between children and adults found for F7 when parasitemia is not taken into account reflects the high proportion of infections among children. It therefore appears that the increased reactivity against the gp190 fragments among parasitized individuals reflects an antigenic boost due to the parasite infection.

The effect of parasite infection on the antibody reactivity against the gp190 was also shown with serum samples taken before and during the transmission season. It appears that the antibody response against many of the gp190 regions is short-lived, especially in children and infants. This is indicated by the differences recorded for parasitized and nonparasitized individuals at two different time points during the year. For many children, a positive reaction was scored in August that was no longer detectable the following June, 6 months after the end of the transmission season. At this time point, nonparasitized children showed almost no positive reactions against the gp190 fragments (Table 5).

The corresponding analysis suggests that in adults the reactivity against some regions (e.g., F7) of the gp190 is also short-lived. However, the dimorphic region represented by F5, which was specifically recognized mainly by sera from

TABLE 4. Comparison of reactions against gp190 during transmission (August) and at the beginning of the next transmission season (June)[a]

Group[b]	No. of samples	% Positive on K1 gp190 fragment:							No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10		
Adults										
August	36	25	58	89	31	53	53	25	6	48
June	27	26	30	82	0	26	19	33	4	31
P		0.87	0.04	0.58	0.002	0.06	0.01	0.54		<0.001
Children										
August	16	19	38	38	63	50	13	50	9	38
June	19	32	26	21	16	11	21	16	37	20
P		0.60	0.44	0.46	0.01	0.03	0.76	0.07		<0.01

[a] See Table 1, footnotes *b* and *c*.
[b] *P* values, August versus June. Significant values are underlined.

TABLE 5. Proportion of parasitized and nonparasitized children reacting against the gp190 fusions at the beginning of transmission (June)[a]

Group	No. of samples	% Positive on K1 gp190 fragment:							No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10		
Parasitized	9	44	33	33	33	22	44	33	22	35
Nonparasitized	10	20	20	10	0	0	0	0	50	7

[a] See Table 1, footnotes *b* and *c*.

TABLE 6. Proportion of reactions against the gp190 fusion proteins from nonexposed donors (lab volunteers) and children under prophylaxis since birth[a]

Control group	No. of samples	% Positive on K1 gp190 fragment:							No reaction (%)	% Recognized
		F2	F4	F5	F7	F8	F9	F10		
Children	10	60	10	20	0	10	0	10	20	16
Lab volunteers	10	0	0	0	0	0	0	0	100	0

[a] See Table 1, footnotes *b* and *c*.

adults, seems to be recognized equally well at the time of transmission and several months later.

Furthermore, our data seem to suggest that the MAD20-specific serum reactivity of samples from German patients, i.e., individuals with primary infections, resembles more closely that of infants for the constant and dimorphic regions, though the small number of samples does not allow us to conclude this firmly.

In conclusion, our data indicate that the dimorphic regions of the gp190 are preferentially recognized by adults and that antibody reactivity, especially against F5, can be detected several months after recovery from patent or subclinical infection. In this context it is interesting that the best protection in monkeys immunized with gp190 is achieved when the parasite used for challenge carries the homologous protein (6, 18), suggesting that polymorphic and dimorphic sequences may play a major role in inducing protective immunity.

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LITERATURE CITED

1. **Armitage, P., and G. Berry.** 1987. Statistical methods in medical research, 2nd ed., p. 125–140. Blackwell Scientific Publications, London.
2. **Boyle, D. B., C. I. Newbold, C. C. Smith, and K. N. Brown.** 1982. Monoclonal antibodies that protect in vivo against *Plasmodium chabaudi* recognize a 250,000-dalton parasite polypeptide. Infect. Immun. **8**:94–102.
3. **Bujard, H., R. Gentz, M. Lanzer, D. Stüber, H.-M. Müller, I. Ibrahimi, M.-T. Häuptle, and B. Dobberstein.** 1987. A T5 promoter-based transcription-translation system for the analysis of proteins *in vitro* and *in vivo*. Methods Enzymol. **155**:416–433.
4. **Epstein, N., L. H. Miller, D. C. Kaushel, I. J. Udeinya, J. Rener, R. J. Howard, R. Asofsky, M. Aikawa, and R. L. Hess.** 1981. Monoclonal antibodies against a specific surface determinant on malarial (*Plasmodium knowlesi*) merozoites block erythrocyte invasion. J. Immunol. **127**:212–217.
5. **Hall, R., J. E. Hyde, M. Goman, D. L. Simmons, I. A. Hope, M. Mackay, and J. Scaife; Merkli, B., R. Richle, and J. Stocker.** 1984. Major surface antigen gene of a human malaria parasite cloned and expressed in bacteria. Nature (London) **311**:379–382.
6. **Heidrich, H.-G., M. Matzner, A. Miettinen-Baumann, and W. Strych.** 1986. Immunoelectron microscopy shows that the 80,000-dalton antigen of *Plasmodium falciparum* is localized in the surface coat. Z. Parasitenkd. **72**:681–683.
7. **Holder, A. A., and R. R. Freeman.** 1981. Immunization against blood-stage rodent malaria using purified parasite antigens. Nature (London) **294**:361–364.
8. **Holder, A. A., and R. R. Freeman.** 1984. The three major antigens on the surface of *Plasmodium falciparum* merozoites are derived from a single high molecular weight precursor. J. Exp. Med. **160**:624–629.
9. **Holder, A. A., J. S. Sandhu, Y. Hillman, L. S. Davey, S. C. Nichols, H. Cooper, and M. J. Lockyer.** 1987. Processing of the precursor to the major merozoite surface antigens of *Plasmodium falciparum*. Parasitology **94**:199–208.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Mackay, M., M. Goman, N. Bone, J. E. Hyde, J. Scaife, U. Certa, H. Stunnenberg, and H. Bujard.** 1985. Polymorphism of the precursor for the major surface antigens of *Plasmodium falciparum* merozoites: studies at the genetic level. EMBO J. **4**:3823–3829.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **McBride, J. S., C. I. Newbold, and R. Anand.** 1985. Polymorphism of a high molecular weight schizont antigen of the human malaria parasite *Plasmodium falciparum*. J. Exp. Med. **161**: 160–180.
14. **Perkins, M. E., and L. J. Rocco.** 1988. Sialic acid-dependent binding of *Plasmodium falciparum* merozoite surface antigen, Pf200, to human erythrocytes. J. Immunol. **9**:3190–3196.
15. **Perrin, L. H., and R. Dayal.** 1982. Immunity to asexual erythrocytic stages of *Plasmodium falciparum*: role of defined antigens in the humoral response. Immunol. Rev. **61**:245–269.
16. **Perrin, L. H., M. Loche, J.-P. Dedet, C. Roussilhon, and T. Fandeur.** 1984. Immunization against *Plasmodium falciparum* asexual blood stages using soluble antigens. Clin. Exp. Immunol. **56**:67–72.
17. **Peterson, M. G., R. L. Copppel, P. McIntyre, C. J. Langford, G. Woodrow, G. V. Brown, R. F. Anders, and D. J. Kemp.** 1988. Variation in the precursor to the major merozoite surface antigens of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **27**:291–302.
18. **Siddiqui, W. A., L. Q. Tam, K. J. Kramer, G. S. N. Hui, S. E. Case, K. M. Yamaga, S. P. Chang, E. B. T. Chan, and S.-C. Kan.** 1987. Merozoite surface coat precursor protein completely protects *Aotus* monkeys against *Plasmodium falciparum* malaria. Proc. Natl. Acad. Sci. USA **84**:3014–3018.
19. **Tanabe, K., M. Mackay, M. Goman, and J. Scaife.** 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. **195**:273–287.
20. **Towbin, K., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
21. **Weber, J. L., W. M. Leininger, and J. A. Lyon.** 1986. Variation in the gene encoding a major merozoite surface antigen of the human malaria parasite *Plasmodium falciparum*. Nucleic Acids Res. **14**:3311–3323.