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Protection against Malaria in *Aotus* Monkeys Immunized with a Recombinant Blood-Stage Antigen Fused to a Universal T-Cell Epitope: Correlation of Serum Gamma Interferon Levels with Protection

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The major surface antigen p190 of the human malaria parasite *Plasmodium falciparum* contains nonpolymorphic, immunogenic stretches of amino acids which are attractive components for a subunit vaccine against malaria. One such polypeptide, termed 190L, is contained in the 80-kDa processing product of p190, which constitutes the major coat component of mature merozoites. We report here that immunization of *Aotus* monkeys with 190L gives only poor protection against *P. falciparum* challenge. However, addition by genetic engineering of a universal T-cell epitope (CS.T3) to 190L improved immunity, and as a result three of four monkeys were protected following challenge infection with blood-stage parasites. Neither antibody against the immunizing antigens or against blood-stage parasites nor the capacity of the monkeys' sera to inhibit in vitro parasite invasion correlated with protection. However, in contrast to sera from nonprotected monkeys, sera from protected animals contained elevated levels of gamma interferon. These results suggest that gamma interferon is directly or indirectly involved in the process of asexual parasite control in vivo.

The unicellular protozoon *Plasmodium falciparum* is the predominant pathogen causing malaria in humans. The infection starts when sporozoites, present in the salivary glands of *Anopheles* mosquitoes, are inoculated into the blood of susceptible hosts. Sporozoites rapidly penetrate hepatocytes, in which they further develop into liver schizonts. After maturation, infectious merozoites are released into the blood of the host and invade erythrocytes, starting a new schizogonic cycle that is associated with the clinical symptoms of malaria. The complex, mainly intracellular development of *P. falciparum* and the extensive polymorphism of malaria antigens are considered to be major difficulties for the development of malaria vaccines.

An attractive candidate for a blood-stage vaccine is the merozoite protein termed p190, or polymorphic schizont antigen (9, 14). It is a large glycoprotein which is synthesized and extensively processed during merozoite formation, and the 80-kDa processing product of p190 is the major coat protein of mature merozoites (13, 25). Monoclonal antibody probes against p190 and primary sequence analysis revealed that the antigen contains nonpolymorphic as well as polymorphic sequence blocks (16, 27). The function of the protein is unknown, but immunization of monkeys with parasite-derived p190 protein results in partial (1, 9) or complete (21) protection against *P. falciparum* malaria after parasite challenge. In addition, immunization of *Aotus* monkeys with two conserved, p190-based recombinant polypeptides (190L and 190M) contained in a single recombinant fusion protein (termed 190N) (7) induced partial protective immunity to malaria challenge in monkeys (10). 190L is a major target of the humoral immune response against p190 in nature (17) and contains epitopes recognized by human T cells (3, 8, 23, 24). These findings raised the possibility that 190L constitutes the active component of the larger, partially protective 190N antigen.

A T-cell epitope (CS.T3) in the circumsporozoite protein of *P. falciparum* that is recognized by T cells in association with many different major histocompatibility complex class II molecules, both in mice and in humans (22), has been described. For these reasons, it was termed a universal T-cell epitope. It was thus appealing to test whether CS.T3 can improve the immune response to 190L. We thus added the CS.T3 sequence to the 190L antigen, using standard genetic engineering techniques.

Here we report on the capabilities of the 190L and 190L-CS.T3 recombinant antigens to protect *Aotus* monkeys from *P. falciparum* challenge and on our studies of the possible roles of antibodies and cell-mediated immunity in protection against malaria.

MATERIALS AND METHODS

Monkeys and parasites. *Aotus trivirgatus griseimembra* owl monkeys were from the primate center of the Universidad del Valle (Cali, Colombia). Each experimental group contained five animals matched for weight (800 g), size, and age. They had karyotype II or III, and no previous exposure to malaria was detectable before the primary immunization, on the basis of clinical histories and tests for antimalarial antibody. The animals were challenged with 5×10^5 parasites of the FVO isolate of *P. falciparum* (10). The animals were drug cured at a parasitemia of 10%. One animal died before the challenge infection because of heart complications (M78; 190L group), and another died because of a lung infection (F46; 190L-CS.T3 group). C60 (control group) died after parasite challenge, probably as a result of the infection. One anemic animal (M3) required a blood transfusion at day 21 after challenge in order to maintain normal hematocrit levels.

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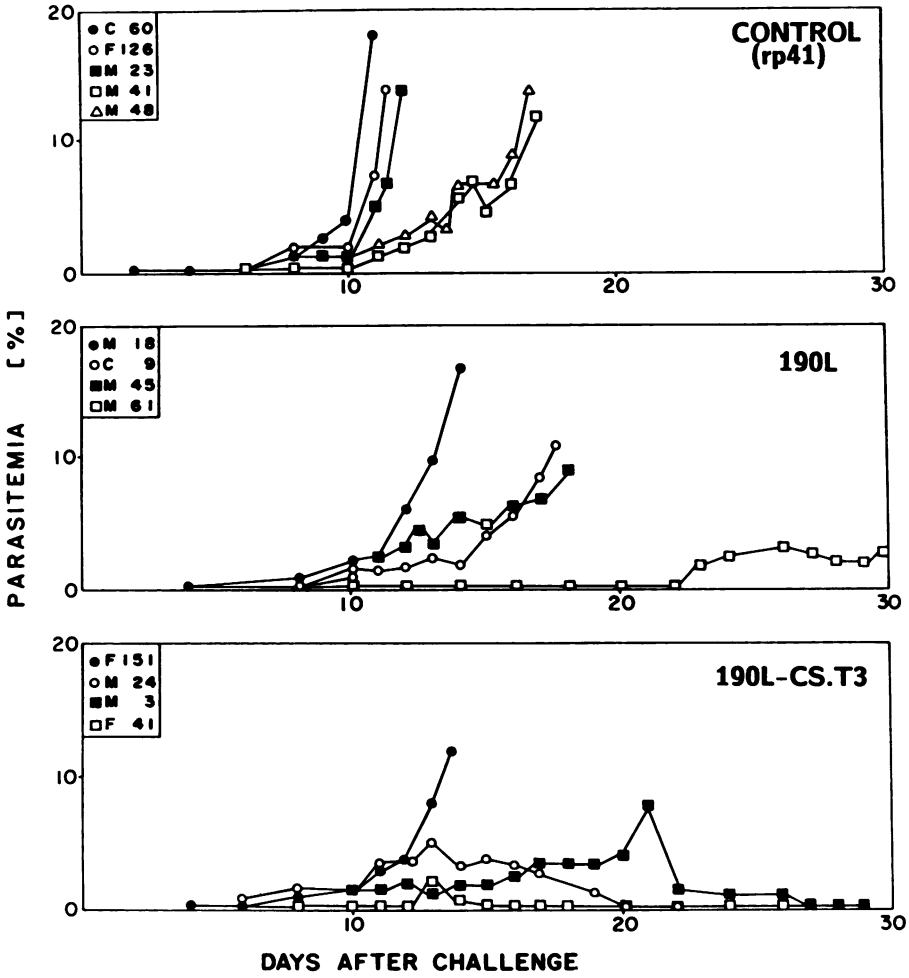

FIG. 1. Course of parasitemia in three groups of monkeys immunized with *P. falciparum* recombinant p41 aldolase (control group), 190L, or 190L-CS.T3 as indicated. At day 0, the monkeys were challenged with the virulent FVO isolate, and terminated curves indicate the point of chemotherapy in nonprotected animals.

Construction of the plasmid p190L-CS.T3. The insert of p190L (7) was released with *Bam*HI and cloned in a derivative of the vector pDS6/RBSII,6xHIS (26), and the plasmid expressing 190L was termed p190L-6H. This recloning step added four additional histidine residues, required for affinity purification, to 190L (7). The universal T-cell epitope CS.T3, corresponding to circumsporozoite (CS) protein residues 378 to 398 (but with cysteines 384 and 389 replaced by alanine), was fused to the carboxy terminus of 190L. Briefly, a unique *Bgl*II restriction site was introduced by in vitro mutagenesis downstream of the 190L-6H coding sequence, which was then used to insert synthetic DNA oligomers encoding CS.T3 (5'-GATCCGAAAAAAAAATCGCTAAAATG GAAAAAGCTAGCAGCTTTTCAACGTTGTAA-3', coding strand; 5'-GATCTTACAACGTTGAAAACGCTGC TAGCTTTTTCCATTTTAGCGATTTTTTTTTCGG ATC-3', noncoding strand). The recombinant protein expressed by the plasmid p190L-6H-CS.T3 was termed 190L-CS.T3.

Antigens. The recombinant proteins 190L and 190L-CS.T3 were produced in *Escherichia coli* cells harboring the plasmids p190L-6H and p190L-6H-CS.T3 as described previously (7). Purification was performed by metal chelate affinity chromatography and an additional preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel purification step, as reported earlier (10). Enzymatically active recombinant *P. falciparum* aldolase (rp41) was prepared by a published procedure (4). Prior to each immunization, the antigens displayed a single band on SDS-polyacrylamide gels (data not shown).

Immunization and challenge. Animals were immunized in complete (first immunization) or incomplete (booster injections) Freund's adjuvant at days 0, 21, and 42 with 350 μg of *P. falciparum* aldolase, 190L, or 190L-CS.T3. The challenge was on day 57. Recombinant aldolase served as a parasite-related but nonprotective control antigen (10). Serum was collected before each immunization and 16 days after parasite challenge.

Analysis of the immune response. Antibody levels were determined by enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay, and Western blot (immunoblot) as described previously (10) with crude parasitized erythrocytes of the FVO strain as the antigen.

IFN-γ assay. Serum gamma interferon (IFN-γ) levels were determined with a solid-phase sandwich immunoenzymatic assay for human use described by Gallati et al. (6). Results are given in arbitrary units (see the legend to Fig. 2).

Inhibition assay for intraerythrocytic growth. Parasites of

TABLE 1. Pre- and postchallenge antibody responses in immunized animals

Antigen and monkey	Protection status[a]	IFA titer[b,c]		ELISA titer[b,d]		Immunoblot titer[b,e]	
		Pre	Post	Pre	Post	Pre	Post
rp41							
C60	−	80	NT	8,000	NT	+	NT
F126	−	640	6,120	500	500	+	+
M23	−	20	1,280	100	500	+	++
M41	−	320	320	400	16,000	+	+
M48	−	320	10,240	500	500	+	++
190L							
C9	−	640	5,120	128,000	128,000	+	+++
M18	−	1,280	2,560	16,000	32,000	+	++
M45	−	320	640	16,000	32,000	+	++
M61	+	320	640	16,000	64,000	+	++
190L-CS.T3							
F41	+	5,120	5,120	32,000	64,000	++	+++
F151	−	80	1,280	64,000	64,000	++	++
M3	+	320	640	8,000	16,000	++	++
M24	+	640	1,280	64,000	128,000	++	++

[a] + and −, protection and nonprotection, respectively, against parasite challenge.

[b] Pre, prechallenge serum titers at day 57 after immunization; Post, antibody titers 14 days after challenge; NT, not tested.

[c] Data are expressed as reciprocal endpoint dilutions. IFA, indirect immunofluorescence assay.

[d] Data are expressed as reciprocals of the dilutions that yield an absorption of 1.0 U at 405 nm.

[e] Total proteins (10 μg per lane) of the FVO isolate of *P. falciparum* were electroblotted onto a nitrocellulose membrane and incubated with a 1/200 dilution of each monkey serum. Iodinated protein G was used for detection of the antigen-antibody complexes. +, positive detection after overnight exposure, ++, strong reactivity under the same conditions; +++, overexposed.

TABLE 2. In vitro inhibition of *P. falciparum* development by prechallenge sera of immunized monkeys

Monkey or serum[a]	Antigen	Protection status[b]	% Inhibition in assay of:	
			Invasion[c]	Growth[d]
Monkeys				
F126	rp41	−	NI	90
M23	rp41	−	NI	NI
M48	rp41	−	NI	97
C9	190L	−	92	97
M18	190L	−	NI	NI
F151	190L-CS.T3	−	82	95
F41	190L-CS.T3	+	NI	NI
M3	190L-CS.T3	+	NI	NI
M24	190L-CS.T3	+	NI	24
Sera				
NMS			NI	NI
HMS			100	100

[a] NMS, normal monkey serum; HMS, hyperimmune monkey serum (which served as a positive control in both assays).

[b] + and −, protection and nonprotection, respectively, after parasite challenge.

[c] Inhibition of parasite invasion relative to a negative control serum. NI, no inhibition.

[d] Inhibition of the intracellular development of the parasite relative to inhibition in normal monkey serum. The percentage of inhibition was calculated from the counts per minute of [^{3}H]hypoxanthine incorporation (average of triplicate microcultures) for each monkey serum.

the FVO isolate were synchronized essentially as described previously (12). A 100-μl volume of RPMI 1640 containing 5% erythrocytes with 3% parasitemia was supplemented with 10 μl each of test serum, normal monkey serum (negative control), or hyperimmune monkey serum (positive control). Inhibition of intraerythrocytic growth in triplicate microcultures was determined microscopically on Giemsa-stained smears and by [^{3}H]hypoxanthine incorporation after 36 h in culture.

Inhibition assay for parasite invasion. Parasites highly synchronized to mature schizonts were incubated for 8 h in triplicate microcultures as described above after test and control sera were added. Inhibition of invasion on Giemsa-stained smears was assayed microscopically and quantified by [^{3}H]hypoxanthine incorporation. Inhibition in both assays is expressed as a percent as follows: % inhibition = [1 − (test serum cpm/negative-control serum cpm)] × 100. Counts per minute are the average of triplicate measurements. The negative-control serum gave 25,000-cpm incorporation (0% inhibition of growth), and the positive control gave 320 cpm (100% inhibition of growth). Because of a serum shortage, only a random subset of monkey sera was available for both inhibition assays.

RESULTS AND DISCUSSION

Immunity to conserved regions of the p190 protein conferred partial protection in monkeys immunized with recombinant antigens (10). In the present study, we questioned whether the addition of the broadly recognized T-cell epitope (CS.T3) to one of these conserved p190-derived polypeptides can improve antimalaria protection in *Aotus* monkeys.

Figure 1 shows the development of parasitemia in each of three groups of monkeys immunized with *P. falciparum* recombinant aldolase (rp41; control group) (10), 190L, or 190L-CS.T3. In the rp41-immunized group, as expected, parasites were detectable in all animals 8 days after challenge. Three of the animals were drug treated at or before day 12, and two (M41 and M48) were drug treated at day 16. Following immunization with 190L, all animals but one (M61) had to be drug treated between days 14 and 18. One animal, M61, was well protected until day 22. From then on, its parasitemia increased slowly, necessitating drug treatment at day 34 after parasite challenge. In contrast, in the group immunized with 190L-CS.T3, only one animal (F151) needed drug treatment 13 days after parasite challenge. The remaining three animals in this group cleared the parasites without chemotherapy. These experiments suggest that the protection afforded by 190L may be improved by the addition of the CS.T3 epitope.

We have previously reported that the CS.T3 sequence is able to induce T-helper cell function in vivo (22). To investigate whether increased T-helper cell function might contribute to the different patterns of protection obtained with the two immunogens, we examined the antibody responses in the different groups of monkeys.

Antibody titers measured by three independent assays are shown in Table 1. The sera from the monkeys immunized with the different recombinant proteins recognize the corresponding parasite proteins in the native state (indirect immunofluorescence assay and ELISA) or denatured in Western blots. In addition, no increased T-helper function due to CS.T3 could be demonstrated in this experiment, as the antibody titers were high and of the same order of magnitude in the groups immunized with 190L and 190L-CS.T3. These

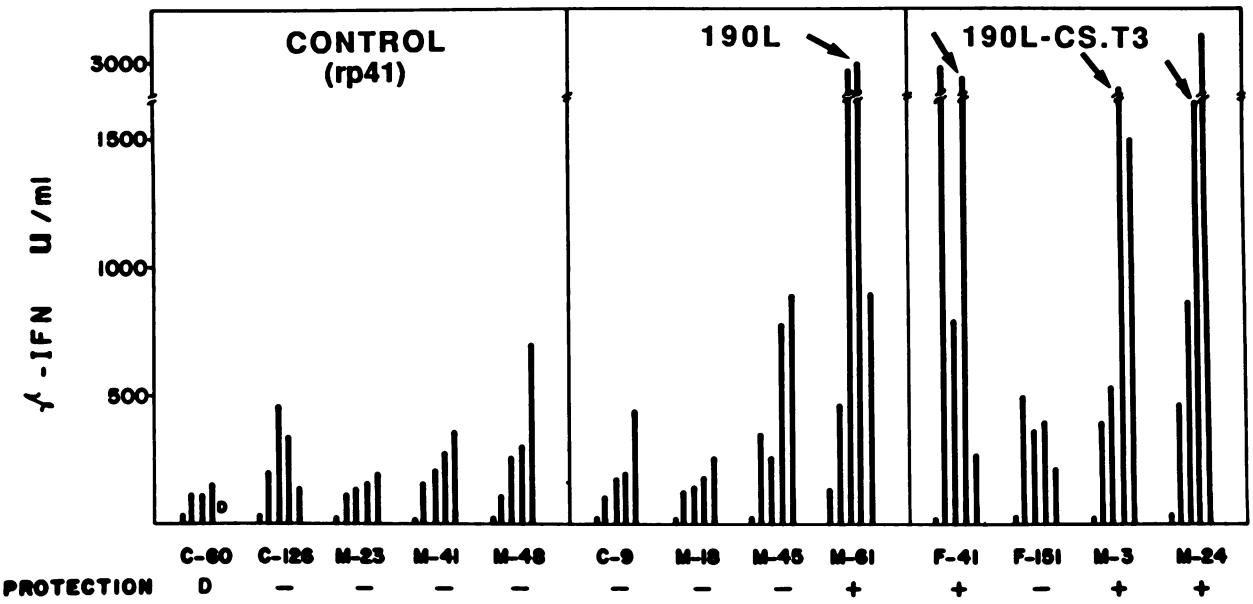

FIG. 2. IFN-γ concentrations in sera of immunized monkeys at five time points of the experiment. First line, preimmune serum; second, third, and fourth lines, 21, 42, and 57 days after the immunization, respectively; fifth line, 71 days after immunization and 14 days after challenge. The code for each monkey appears below each set of data, and the antigen used for immunization is indicated at the top of each panel. D, death of the animal before challenge; −, not protected; +, protected. Note the significant correlation ($P < 0.05$ by Mann-Whitney U test) between protection (measured at day 20 after challenge) and the prechallenge concentrations of IFN-γ (fourth line). Arrows indicate the serum IFN-γ levels at the point of parasite entry in protected animals. Values are given in units per milliliter, where one arbitrary unit is defined as the amount of *Aotus* IFN-γ which is measured as 1 pg in the assay standardized for human IFN-γ.

titers were not correlated with protection. For example, the nonprotected monkey C9 in the 190L-immunized group had pre- and postchallenge antibody titers virtually identical to those of the protected animal M24 in the 190L-CS.T3 group.

Since antibody titers did not correlate with protection against *P. falciparum* infection, we next questioned whether the sera from the immunized monkeys differed in their ability to inhibit the multiplication of parasite growth in vitro. We monitored the development of either synchronized ring stages (intraerythrocytic growth assay) or schizonts (invasion assay) in the presence of prechallenge monkey sera from protected and nonprotected animals. Sera inhibiting the intracellular development of parasites were found in each group of monkeys (Table 2). The sera from monkeys C9 (190L group; nonprotected) and F151 (190L-CS.T3 group; nonprotected), for example, were positive in both assays, although these animals were not protected against *P. falciparum* infection. On the other hand, sera from the protected animals F41 and M3 did not affect parasite growth in vitro. Thus, neither antibody titers nor antibody specificities seem to account for the protection observed in the 190L- or 190L-CS.T3-immunized monkeys, suggesting that non-antibody-mediated T-cell mechanisms might be responsible for this protection.

IFN-γ, produced by antigen- or mitogen-activated T cells, has previously been shown to play a crucial role in the protective immunity to malaria (2, 5, 15). We thus measured the levels of IFN-γ present in the sera of the monkeys immunized with the different recombinant proteins before and after the first immunization, after the booster injections, and before and after parasite challenge. Strikingly, the highest prechallenge concentrations of this cytokine in serum were found in sera of animals which later controlled the malaria infection (Fig. 2). These results suggest that IFN-γ is directly or indirectly involved in the biological process of asexual parasite control in vivo.

It has previously been shown that in IFN-γ-treated chimpanzees, the infectivity of *Plasmodium vivax* is virtually neutralized, and in mice injected with IFN-γ, the development of *Plasmodium berghei* is strongly inhibited (5). These observations have been described as the effect of IFN-γ against the exoerythrocytic forms of *Plasmodium*. However, a protective effect of IFN-γ against the intraerythrocytic stage of the parasite has also been suggested (11, 18, 19). IFN-γ activates macrophages and neutrophils, which are important effector cells in plasmodial infections. Activated macrophages and neutrophils have been shown to inhibit the growth of *P. falciparum* in vitro, presumably by enhancing the release of reactive oxygen intermediates (19). Although the precise roles of IFN-γ in the host's defense mechanisms still remain to be defined, it is likely that IFN-γ release by antigen-activated T cells stimulates macrophages or neutrophils to exert an antiparasitic effect.

Three of four monkeys immunized with 190L-CS.T3 produced high levels of IFN-γ, whereas only one of the 190L-immunized monkeys did so. It is reasonable to propose that the CS.T3 epitope, strongly binding to several different HLA-DR molecules, is involved in the activation of T lymphocytes to produce IFN-γ. In this context, it is worth mentioning previous experiments demonstrating that the affinity of a peptide for major histocompatibility complex class II molecules has a profound influence on its T-cell-stimulatory capacity (20). Although we have defined the CS.T3 epitope as universal, we have recently found that HLA-DR3 molecules do not bind CS.T3 (21a). No information on the major histocompatibility complex antigens in *Aotus* monkeys is currently available, but the observation that three of four monkeys in the 190L-CS.T3 group are

protected suggests that a similar situation might have occurred in the experimental animals. In any event, our findings further support the concept that for effective immunity against *P. falciparum* there is a requirement for cellular immunity and, in particular, an important role for IFN-γ.

We are currently further exploiting the 190L-CS.T3 molecule as a human vaccine candidate by repeating the experiments described above with clinical-grade proteins in conjunction with different adjuvants.

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REFERENCES

1. **Cheung, A., J. Leban, A. R. Shaw, B. Merkli, J. Stocker, C. Chizzolini, C. Sander, and L. H. Perrin.** 1986. Immunization with synthetic peptides of a Plasmodium falciparum surface antigen induces antimerozoite antibodies. Proc. Natl. Acad. Sci. USA **83**:8328–8332.
2. **Clark, I. A., N. H. Hunt, G. A. Butcher, and W. B. Cowden.** 1987. Inhibition of murine malaria Plasmodium chabaudi in vivo by recombinant interferon-g or tumor necrosis factor, and its enhancement by butylated hydroxyanisole. J. Immunol. **139**: 3493–3496.
3. **Crisanti, A., H. M. Muller, C. Hilbich, F. Sinigaglia, H. Matile, M. Mackay, J. G. Scaife, K. Beyreuther, and H. Bujard.** 1988. Epitopes recognized by human T cells map within the conserved part of the gp190 of *P. falciparum*. Science (Washington, D.C.) **240**:1324–1327.
4. **Doebeli, H., A. Trzeciak, D. Gillessen, H. Matile, I. K. Srivastava, L. H. Perrin, P. Jakob, and U. Certa.** 1990. Expression, purification, biochemical characterization and inhibition of recombinant *Plasmodium falciparum* aldolase. Mol. Biochem. Parasitol. **41**:259–268.
5. **Ferreira, A., L. Schofield, V. Enea, H. Schellekens, P. van der Meide, W. E. Collins, R. Nussenzweig, and V. Nussenzweig.** 1986. Inhibition of development of exoerythrocytic forms of malaria parasites by g-interferon. Science (Washington, D.C.) **232**:881–884.
6. **Gallati, H., I. Pracht, J. Schmidt, P. Haring, and G. Garotta.** 1987. A simple, rapid and large capacity ELISA for biologically active and recombinant human IFN-γ. J. Biol. Regul. Homeostatic Agents **1**:109–118.
7. **Gentz, R., U. Certa, B. Takacs, H. Matile, H. Doebeli, J. R. L. Pink, M. Mackay, N. Bone, and J. G. Scaife.** 1988. Major surface antigen p190 of *Plasmodium falciparum*: detection of nonvariable epitopes present in a variety of plasmodia isolates. EMBO J. **7**:225–230.
8. **Guttinger, M., P. Romagnoli, L. Vandel, R. Meloen, B. Takacs, J. R. L. Pink, and F. Sinigaglia.** 1991. HLA polymorphism and T cell recognition of a conserved region of p190, a malaria vaccine candidate. Intern. Immunol. **3**:899–906.
9. **Hall, R., J. E. Hyde, M. Goman, D. L. Simmons, I. A. Hope, M. Mackay, J. Scaife, B. Merkli, 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.
10. **Herrera, S., M. A. Herrera, B. L. Perlaza, Y. Burki, P. Caspers, H. Doebeli, D. Rotmann, and U. Certa.** 1990. Immunization of Aotus monkeys with *Plasmodium falciparum* blood-stage recombinant proteins. Proc. Natl. Acad. Sci. USA **87**:4017–4021.
11. **Kumaratilake, L. M., A. Ferrante, and C. Rzepczyk.** 1991. The role of T lymphocytes in immunity to *Plasmodium falciparum*. Enhancement of neutrophil-mediated parasite killing by lymphotoxin and IFN-γ: comparisons with tumor necrosis factor effects. J. Immunol. **146**:762–767.
12. **Lambros, C., and J. P. Vanderberg.** 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J. Parasitol. **65**:418–420.
13. **Lyon, J. A., R. H. Geller, J. D. Haynes, J. D. Chulay, and J. L. Weber.** 1986. Epitope map and processing scheme for the 195,000-dalton surface glycoprotein of *Plasmodium falciparum* merozoites deduced from cloned overlapping segments of the gene. Proc. Natl. Acad. Sci. USA **83**:2989–2993.
14. **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 merozoite surface antigens of *Plasmodium falciparum* merozoites. EMBO J. **4**:3823–3829.
15. **Maheshwari, R. K., C. W. Czarniecki, G. P. Dutta, S. K. Puri, B. N. Dhawan, and R. M. Friedman.** 1986. Recombinant human gamma interferon inhibits simian malaria. Infect. Immun. **53**: 628–630.
16. **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.
17. **Müller, H.-M., K. Früh, A. van Brunn, F. Esposito, S. Lombardi, A. Crisanti, and H. Bujard.** 1989. Development of the human immune response against the major surface protein (gp190) of *Plasmodium falciparum*. Infect. Immun. **57**:3765–3769.
18. **Okenhouse, C. F., S. Schulman, and H. L. Shear.** 1984. Induction of crisis forms in the human malaria parasite *Plasmodium falciparum* by γ-interferon-activated, monocyte-derived macrophages. J. Immunol. **133**:1601–1608.
19. **Playfair, J. H. L., H. Dockrell, and J. Taverne.** 1985. Macrophages as effector cells in immunity to malaria. Immunol. Lett. **11**:233–237.
20. **Schaeffer, E. B., A. Sette, D. L. Johnson, M. C. Bekoff, J. A. Smith, H. M. Grey, and S. Buus.** 1989. Relative contribution of "determinant selection" and "holes in the T-cell repertoire" to T-cell responses. Proc. Natl. Acad. Sci. USA **86**:4649–4653.
21. **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. Kan.** 1987. Merozoite surface coat precursor protein completely protects *Aotus* monkeys against *Plasmodium falciparum* malaria. Proc. Natl. Acad. Sci. USA **84**:3014–3018.

21a. **Sinigaglia, F.** Unpublished results.

22. **Sinigaglia, F., M. Guttinger, J. Kilgus, D. M. Doran, H. Matile, H. Etlinger, A. Trzeciak, D. Gillessen, and J. R. L. Pink.** 1988. A malaria T-cell epitope recognized in association with most mouse and human MHC class II molecules. Nature (London) **336**:778–780.
23. **Sinigaglia, F., M. Guttinger, P. Romagnoli, and B. Takacs.** 1990. Malaria antigens and MHC restriction. Immunol. Lett. **25**:265–268.
24. **Sinigaglia, F., B. Takacs, H. Jacot, H. Matile, J. R. L. Pink, A. Crisanti, and H. Bujard.** 1988. Nonpolymorphic regions of p190, a protein of the *Plasmodium falciparum* erythrocytic stage, contain both T and B cell epitopes. J. Immunol. **140**:3568–3572.
25. **Strych, W., A. Miettinen-Baumann, F. Lottspeich, and H. G. Heidrich.** 1987. Isolation and characterization of the 80,000 dalton *Plasmodium falciparum* merozoite surface antigen. Parasitol. Res. **73**:435–441.
26. **Stueber, D., I. Ibrahimi, D. Cutler, B. Dobberstein, and H. Bujard.** 1984. A novel in vitro transcription-translation system: accurate and efficient synthesis of single proteins from cloned DNA. EMBO J. **3**:3143–3148.
27. **Tanabe, K., M. Mackay, M. Goman, and J. S. Scaife.** 1987. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J. Mol. Biol. **195**:273–287.
28. **Guttinger, M., P. Romagnoli, L. Vandel, R. Meloen, B. Takacs, J. R. L. Pink, and F. Sinigaglia.** 1991. HLA polymorphism and T cell recognition of a conserved region of p190, a malaria vaccine candidate. Intern. Immunol. **3**:899–906.