Epitopes Recognized by Human T Cells Map Within the Conserved Part of the GP190 of *P. falciparum*

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In a study aimed at developing a vaccine against the asexual blood stages of *Plasmodium falciparum*, two T cell epitopes were identified within a nonpolymorphic region of gp190 of *Plasmodium falciparum* merozoites. The two epitopes, which were revealed by deletion analysis, stimulated human T cell clones. Peptides containing sequences of the epitopes stimulated the cloned T cells and peripheral blood mononuclear cells (PBMC) from malaria-infected individuals. Moreover, the T cell clones responded to 11 different *Plasmodium falciparum* isolates in culture, showing that the epitopes are recognized in native parasites.

IMMUNIZATION OF MONKEYS WITH PURIFIED native gp190 from the blood stage of *P. falciparum* modifies the course of infection by the parasite (*1–3*). Although homologous proteins of gp190 in various species of *Plasmodium* are of known immunological importance (*4, 5*), the polymorphism of large parts of the molecule (*6*) and difficulties in expressing recombinant DNA coding for gp190 have hampered immunological studies of this potentially protective protein. In trials with monkeys, gp190-derived synthetic peptides yielded promising results (*7, 8*), but the peptides required carriers such as tetanus toxoid, indicating the need for T cell stimulation.

We therefore thought it important to identify T cell epitopes within the gp190. An engineered protein containing one or more of these epitopes would elicit humoral immunity through parasite-specific helper T cells and, at the same time, might induce antibody-independent cellular immunity shown to be crucial in the mouse model (*9*). Furthermore, priming with parasite-specific T cell epitopes would permit subsequent boosting of the immune response during a natural infection.

To identify the T cell epitopes we have used a panel of human T cell clones. These have the helper/inducer phenotype $CD3^+CD4^+CD8^-$ and are specific for a highly conserved (95%) portion of gp190 originating from the Thai isolate K1 (*10, 11*). Sequences from this conserved region were subcloned as fragment L (*12*) and fragment F2 (*13*) and expressed in *Escherichia coli*.

To map the epitopes within F2, we produced a set of truncated proteins progressively shortened at the amino terminus (*13*). Each was then tested for its ability to stimulate the panel of T cell clones. The rationale of this approach is based on the notion that T cell epitopes consist of linear sequences of amino acids (*14, 15*). We expected that some proteins would continue to stimulate the T cells but others would not. The latter ones would lack all or part of the T cell epitope. The smallest subfragment still able to stimulate T cells would have the epitope close to its amino terminus.

The partially deleted gp190-specific sequences were expressed as fusion proteins of chloramphenicol acetyltransferase (CAT) in *E. coli* (F2 through 2.12, Fig. 1A). Testing of these proteins against the panel of T cell clones (*16*) revealed two epitopes (Table 1). The epitope recognized by the clones AC68, AC69, AC71, and AC75 lies between the

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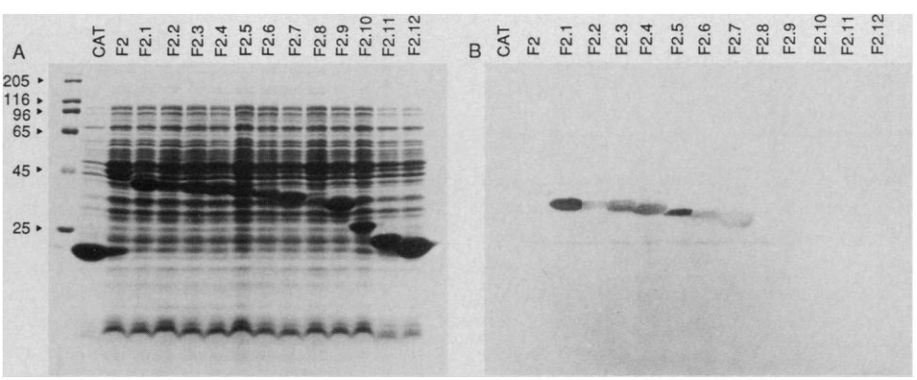

Fig. 1. (A) Expression of fragment F2–derived sequences in *E. coli*. The Coomassie stained electrophoretic pattern (SDS-PAGE 12.5%) show total *E. coli* lysates containing as main products progressively shortened proteins synthesized as CAT-fusions. Molecular weight standards (Sigma, MW SDS-200 Kit) are indicated. **(B)** Binding of the human monoclonal antibody 2D-7 to truncated F2-derived fusion proteins. The protein immunoblot analysis was carried out with total *E. coli* lysates containing the various fusion proteins separated by electrophoresis as shown in (A).

Table 1. Response of T cell clones to gp190 fusion proteins. Fusion proteins progressively shortened at their amino terminus (F2.1 to F2.10) stimulated the T cells until the epitope was partially or totally deleted so that there was no response. Two T cell epitopes were mapped: One (A) is localized within the amino termini of F2.3 and F2.4 and the other (B) within the amino termini of F2.7 and F2.8. Results with CAT and the intact F2 fusion protein are included as controls. The mean incorporation of [^{3}H]thymidine into triplicate cultures is given. The standard errors (SEM) are below 20.0%. Positive responses are underlined.

T cell clones	Radioactivity (cpm × 10^3)											
	CAT	F2	F2.1	F2.2	F2.3	F2.4	F2.5	F2.6	F2.7	F2.8	F2.9	F2.10
AC68	0.2	45.0	35.4	29.6	30.4	0.2	0.2	0.2	0.3	0.2	0.2	0.2
AC69	0.1	55.6	39.5	53.4	62.0	0.7	0.3	0.4	0.2	0.4	0.2	0.2
AC71	0.1	68.0	36.2	48.5	39.6	0.3	0.3	0.2	0.3	0.3	0.2	0.1
AC75	0.3	110.0	96.6	85.7	98.4	0.5	0.4	0.5	0.3	0.4	0.5	0.4
AC63	0.1	30.3	26.6	28.0	9.0	35.0	29.0	16.0	38.0	0.2	0.2	0.2
AC66	0.1	36.8	28.6	25.6	8.5	34.4	32.3	23.3	21.6	0.4	0.2	0.2
AC74	0.1	19.6	17.8	10.9	5.0	14.9	18.7	17.6	12.0	0.2	0.4	0.2

termini of F2.3 and F2.4 (epitope A). Similarly, the epitope recognized by AC63, AC66, and AC74 lies between the ends of F2.7 and F2.8 (epitope B).

Peptides designed to contain the T cell recognition sites were synthesized (*17*). Their sequences contained amino acids spanning from the amino terminus of the shortest still active protein into the amino terminal region of the first inactive fusion protein. The reactivity of the T cell clones against peptides A1 (YKLNFYFDLLRAKL) and B1 (LDNIKDNVGKMEDY) is shown in Table 2. As little as 25 to 250 ng/ml of the synthetic peptides A1 and B1, respectively, were sufficient to activate the clones, showing that the T cell recognition sites lie within the amino acid sequences of these two peptides.

We then synthesized analogs of peptide A (A2 through A4) that contained nonconservative amino acid substitutions. The comparison of the dose response effect of these analogs on T cell recognition shows that the substitution in position 5 (F → A) has profound effects: clone AC68 is not stimulated by A4 whereas AC75 is still stimulated but to a much lesser extent. Two substitutions at positions 2 and 7 had no effect.

The two identified T cell epitopes are not predicted by the structural analysis of their amino acid sequence (*18*). However, by using an algorithm based on the pattern of amino acid sequences (*19*), 12 T cell epitopes were suggested within the 216 amino acid long F2 fragment. The T cell epitopes A and B show extensive homology to 2 of the 12 predicted structures.

Within the amino termini of F2.7 and F2.8 we have localized the binding site of a human monoclonal antibody (Fig. 1B). This antibody is produced from a fusion cell line established with B cells from an immune West African adult (*12*). Fine mapping of this B cell epitope (by means of additional deletion constructs) showed that it lies between amino acids 225 and 237 of gp190. Thus the antibody binding site is located in close proximity to the T cell epitope B1 and might even overlap the B1 sequence. However, the peptide B seems not to contain the entire B cell epitope since it does not react with the human antibody.

The location of the two T cell epitopes A and B within a highly conserved region of gp190 suggests that these epitopes might also be invariant. However, the number of gp190 sequences analyzed so far is small and the data derived from these analyses might not be representative for all *P. falciparum* isolates in culture (and even less for those causing natural infection). We therefore examined our T cell clones for their ability to respond to 11 different *P. falciparum* isolates in culture (*20*). The identified T cell epitopes or cross-reacting sequences were present in all strains tested (Table 3). Within epitope A, the sequences of the K1 and the MAD-20 isolates differ in one amino acid (F → Y) (*21*). As shown with peptide A3, an amino acid exchange in this position appears not to affect T cell recognition.

The identification of invariant T cell epitopes of the gp190 protein may have important implications for the development of a peptide vaccine against the merozoite stage of malaria. Such a merozoite vaccine must induce an antibody-independent cellular (T cell) response, as well as humoral immunity (*9, 22, 23*). Peptide vaccines consisting of the gp190 invariant T cell epitopes in combination with the B cell epitopes would induce both humoral and cellular parasite specific immunity.

When the PBMC of individuals who had *P. falciparum* malaria were examined, four

Table 2. Stimulation of T cell clones by different concentrations of the synthetic peptides A1 and B1. The incorporation of [^{3}H]thymidine into triplicate cultures is given as the mean (cpm) ± SEM. Positive responses are underlined. ND, not determined.

Peptides (μg/ml)	Radioactivity (cpm × 10^3)		
	AC68	AC75	AC63
YKLNFYFDLLRAKL (A1)			
10.0	8.5 ± 0.8	36.3 ± 1.2	0.25 ± 0.02
1.0	31.1 ± 1.0	108.4 ± 1.9	ND
0.1	27.6 ± 1.3	95.0 ± 2.6	ND
0.01	6.9 ± 0.7	41.6 ± 1.2	ND
LDNIKDNVGKMEDY (B1)			
10.0	0.20 ± 0.01	0.30 ± 0.05	49.3 ± 1.7
1.0	0.27 ± 0.03	0.28 ± 0.01	30.5 ± 1.1
0.1	ND	ND	9.7 ± 0.8
0.01	ND	ND	0.9 ± 0.07

Table 3. T cell stimulation by red blood cells infected with various *P. falciparum* isolates. T cells specific for the epitope A respond to 11 *P. falciparum* isolates but not to uninfected red blood cells (RBC). T cells specific for the epitope B reacted correspondingly. The incorporation of [^{3}H]thymidine into triplicate cultures is given as the mean (cpm) ± SEM. Fr-2 is included as positive control.

Antigen	Radioactivity (cpm × 10^3)		
	T cell clones		
	AC68	AC69	AC75
Noninfected RBC	1.3 ± 0.1	1.0 ± 0.1	1.7 ± 0.2
Fr 2	67.8 ± 3.4	90.0 ± 2.8	77.7 ± 3.0
Infected RBC isolate			
Ro-33	17.6 ± 0.9	14.2 ± 1.1	17.0 ± 0.6
Ro-56	16.3 ± 1.3	16.4 ± 0.4	16.2 ± 1.0
K1	28.6 ± 1.9	21.8 ± 0.9	22.3 ± 1.7
NF-54	27.5 ± 2.0	27.4 ± 1.3	30.6 ± 2.1
Palo Alto	17.1 ± 1.0	18.8 ± 2.1	22.2 ± 0.6
542	7.8 ± 1.0	5.3 ± 0.4	7.4 ± 0.1
CP6	9.2 ± 1.1	5.6 ± 0.4	5.8 ± 0.3
RFCN-3	12.8 ± 0.9	7.5 ± 1.2	15.1 ± 1.3
FCH-5-c_2	21.6 ± 2.4	30.5 ± 2.9	20.8 ± 0.8
Geneva 13	25.4 ± 1.7	21.2 ± 1.3	27.3 ± 2.6
MAD-20	18.2 ± 1.0	10.1 ± 0.9	16.1 ± 1.2

Table 4. Response of T cell clones to peptides A and B, respectively, presented by autologous APC in presence of antibodies to DQ (mAb Tü22) (*26*), DP (mAb B7/21) (*27*), and DR (mAb E-31) (*28*). The stimulation of epitope A–specific T cells is blocked by antibodies to DQ and of epitope B–specific T cells by antibodies to DR. AC is homozygous with DR-6. The incorporation of [^{3}H]thymidine into triplicate cultures is given as the mean (cpm) ± SEM.

Clones	Peptides	Antibody to HLA class II		
		Anti-DR	Anti-DP	Anti-DQ
AC68	A1	23.9 ± 1.3	18.2 ± 0.2	0.4 ± 0.0
AC75	A1	67.8 ± 0.8	73.7 ± 0.6	0.6 ± 0.0
AC66	B1	0.61 ± 0.01	35.7 ± 1.0	29.2 ± 1.7
AC74	B1	0.79 ± 0.02	14.8 ± 3.7	13.3 ± 0.2

out of six responded to peptide A1 and one out of three to peptide B1. Since T cell response is under HLA control it is not expected that all individuals respond to any given T cell epitope (*24*). The epitopes identified here interact with HLA class II molecules DQ (peptide A1) and HLA DR-6 (peptide B1, Table 4), as shown by the effect of monoclonal antibodies to DQ, DR, and DP in a T cell stimulation assay.

It is evident that for the design of a vaccine information is required not only on B and T cell epitopes but also on relevant HLA class II molecules within a population.

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13. The coding sequence of gp190 between the unique Pvu II and Hae III sites (bp 316–964) henceforth called F2, was fused to Bam HI (5′ end) and Hind III (3′ end) linkers and cloned into the polylinker of the expression vector pUH130,RBSII a derivative of pDS5 (*25*). Upon induction by IPTG fusion proteins containing the gp190 portion at the NH_2-terminus of CAT were obtained. After cleavage of the plasmid DNA at the Bam HI site the F2-specific sequence was shortened from the 5′ end by exonuclease III (40, 60, 80, 100, and 120 seconds) and then treated with nuclease S1 and Klenow enzyme. Bam HI-linkers were ligated to the blunted ends and after digestion with Bam HI and Hind III fragments of the desired length were isolated from polyacrylamide gels and recloned into pUH130,RBSII. The progressively shortened fusion proteins (2.1–2.12) differing from each other by four to ten amino acids were expressed in *E. coli*.
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Lactate-Supported Synaptic Function in the Rat Hippocampal Slice Preparation

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The present study was undertaken to examine the possibility that cerebral energy metabolism can be fueled by lactate. As a sole energy substrate, lactate supported normal synaptic function in rat hippocampal slices for hours without any sign of deterioration. Slices that were synaptically silent as a result of glucose depletion could be reactivated with lactate to show normal synaptic function. When slices were exposed to the glycolytic inhibitor iodoacetic acid, lactate-supported synaptic function was unaffected, whereas that supported by glucose was completely abolished. This indicated that lactate was metabolized directly via pyruvate to enter the tricarboxylic acid cycle. Thus, under conditions that lead to lactate accumulation (cerebral ischemia) this "end product" may be a useful alternative as a substrate for energy metabolism.

GLUCOSE IS GENERALLY ACCEPTED to be the primary substrate in both aerobic and anaerobic brain energy metabolism (*1*), whereas lactate is the end product of such metabolism in the absence of oxygen (*1*). The oxidation of other substrates by different brain tissue preparations has been reported (*2*). However, the physiological significance of such capability in vivo has not been determined. Alternative substrates to glucose could be important in hypoglycemia or hypoxia, and especially during cerebral ischemia, which appears to cause increased damage under hyperglycemic conditions (*3, 4*). Lactic acidosis has been suggested as a major detrimental factor under these conditions (*4*). Our studies in vitro have indicated a beneficial effect of hyperglycemic levels of glucose on synaptic function recovery from hypoxia (*5*). Moreover, lactic acidosis had no adverse effect on hypoxic brain tissue in vitro (*5, 6*). We report here that synaptic function in vitro can be supported by lactate as the sole substrate for energy metabolism.

One possible explanation for the lack of adverse effects from lactic acidosis in brain tissue is rapid lactate metabolism. Is this metabolism simply a way to reduce lactic acid to safe levels or is it an important alternative as an energy substrate under certain circumstances? We used the rat hippocampal slice preparation to answer these questions.

We performed all experiments in a dual, linear-flow incubation chamber (*7*) using the rat hippocampal slice preparation and electrophysiological methods (*5–9*). Extracellular recording of evoked population responses (population spikes) from the stra-

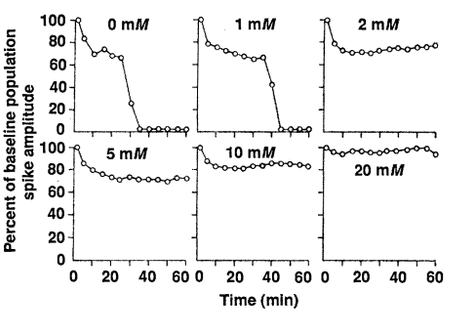

Fig. 1. The effect of a 60-min perfusion of rat hippocampal slices with the concentrations shown of lactate in ACSF on the evoked population spike amplitude at 34° ± 0.5°C. Slices were prepared, maintained, and stimulated orthodromically (Schaffer collaterals) as described (*5–9*); recordings (once per minute) were from the CA1 stratum pyramidale layer. Data show percent of baseline population spike amplitude at 5-min intervals. Lactate at 2 m*M* or higher could maintain synaptic function in hippocampal tissue in vitro as well as, or better than, 2 m*M* glucose (*9*). A dual chamber was used and control and experimental slices originated from one rat brain for any given experiment. Records were stored and analyzed later (*5–9*). Lactate (Sigma; 30% L-lactic acid solution) was neutralized with NaOH before use. Osmolarity was kept constant in the lactate ACSF solution by adjusting the concentration of NaCl.

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