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Immunology

T-cell epitopes in Pf155/RESA, a major candidate for a *Plasmodium falciparum* malaria vaccine

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ABSTRACT **Immunogens included in a subunit vaccine should contain both B- and T-cell-activating sites to ensure anamnestic responses following reinfection after vaccination as well as antibody-independent cellular immunity. The *Plasmodium falciparum* antigen Pf155/RESA, a major candidate for a vaccine against the asexual blood stages of this malaria parasite, was investigated for T-cell epitopes in its C-terminal amino acid repeat region, a region known to be conserved in different *P. falciparum* strains. It was found to contain several related sequences that activated T cells from humans primed to *P. falciparum* by natural exposure, to proliferation, and/or interferon-γ release *in vitro*. T cells from approximately half of the donor group investigated responded to the intact protein, and 65% of these responders also responded to short synthetic peptides, probably representing a small number of partly overlapping T-cell epitopes. Thus, sequences from the C terminus of Pf155 may be suitable constituents of a *P. falciparum* subunit vaccine and also provide a basis for epitope-specific epidemiological studies relating cellular immune responses *in vitro* to clinical immunity and *P. falciparum* endemicity.**

Antibody-binding structures (B-cell epitopes) of several antigens from the asexual blood stages of the malaria parasite *Plasmodium falciparum* have recently been identified as potential vaccine candidates for the control of morbidity and mortality of this disease (1). However, in spite of the fact that both antibody-dependent and antibody-independent immune protection to the malaria parasite are controlled by T cells (2), nothing is known of T-cell-activating structures (T-cell epitopes) in these blood stage antigens, a knowledge crucial for the design of efficient subunit vaccines (3).

An antigen generally considered a major candidate for a merozoite vaccine is Pf155/RESA (4–6). This antigen contains two extensive blocks of tandemly repeated amino acid sequences. The major units in its C-terminal block of repeats are the sequence Glu-Glu-Asn-Val-Glu-His-Asp-Ala, tandemly repeated five times, and the first half of this sequence (Glu-Glu-Asn-Val), repeated >30 times, including a few variants and deletions (7). A major proportion of the human anti-Pf155 antibodies, which also efficiently inhibit erythrocyte invasion by merozoites *in vitro*, is directed against linear epitopes formed by these sequences (8, 9), which also appear to be conserved between different *P. falciparum* strains (7, 10).

We have earlier shown that intact Pf155 induces *in vitro* proliferation, interleukin 2 release, and interferon-γ (IFN-γ) production in T cells primed to this antigen by natural infection (11, 12). In Pf155-seropositive donors, the antigen has also been found to induce production of T-cell-dependent anti-Pf155 antibodies *in vitro*, suggesting that it possesses T-helper cell stimulating epitopes (13). To define T-cell-activating regions for possible inclusion in a subunit vaccine, we have now investigated *in vitro* responses of T cells from donors with previous malaria experience to short synthetic peptides representing C-terminal repeats of Pf155/RESA. Structures inducing proliferation and/or IFN-γ release have been identified in large proportions of donors whose T cells also responded to intact Pf155.

MATERIALS AND METHODS

Study Subjects. Samples of venous blood were obtained by informed consent from 150 donors (adult males), most of them living in an endemic *P. falciparum* area in Liberia. Blood samples from Swedish donors not previously exposed to the parasite served as controls.

Antibodies. A small aliquot of plasma was prepared from each sample and used for determination of *P. falciparum* antibodies by conventional immunofluorescence and for determination of anti-Pf155 antibodies by indirect immunofluorescence on air-dried monolayers of infected erythrocytes (EMIF, erythrocyte membrane immunofluorescence) (4).

Preparation and Fractionation of Peripheral Blood Lymphocytes. Blood samples of both malarious donors and controls were processed in Stockholm within 30 hr after blood had been taken. Human peripheral blood lymphocytes were isolated by gelatin sedimentation, carbonyl iron treatment, and Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) centrifugation (14). T cells were separated by rosette formation with neuraminidase-treated sheep erythrocytes. E-rosette-forming cells (T cells) were separated from non-E-rosetting cells by Ficoll-Isopaque density centrifugation. The T cells were ≥98% pure and free of surface immunoglobulin-positive B cells. Sheep erythrocytes attached to T lymphocytes were lysed by osmotic lysis (15). In some experiments, B-cell-enriched fractions containing ≈45% cells with surface immunoglobulin were collected from the interphase.

Adherent Cells. These were obtained from peripheral blood mononuclear leukocytes by incubation for 1–2 hr at 37°C in tissue culture flasks (Falcon) in 50% heat-inactivated human AB^+ serum. Nonadherent cells were washed off and adherent cells were recovered after overnight incubation at 4°C (16).

Cell Cultures. Ten thousand adherent cells were seeded into round-bottomed microtiter plates (Linbro) and prepulsed overnight with 0.2 ml of antigen at various concentrations in complete tissue culture medium [Hepes-buffered RPMI 1640 medium (Biocult Laboratories, Paisley, Scotland) supple-

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Abbreviations: IFN-γ, interferon-γ; EMIF, erythrocyte membrane immunofluorescence; SI, stimulation index; La, leukoagglutinin; E_o, noninfected erythrocytes.

mented with 2 mM L-glutamine/25 μg of gentamicin per ml/10% human AB^+ serum]. Supernatants were removed and replaced with fresh tissue culture medium without antigen and 2×10^5 autologous T cells were then added to each well. Proliferation was assayed 5 days later by determining incorporation of [^{3}H]thymidine (Radiochemical Centre; specific activity, 7.0–7.8 Ci/mmol; 1 μCi per well; 1 Ci = 37 GBq) for 16 hr in triplicate and expressed as stimulation index (SI). Mean incorporation in the absence of antigen in T cells from 50 donors was 3582 cpm (±518 SEM; 95% confidence limits, 2541–4623. On this basis, tests having SI ≥ 2.5 were considered positive.

Culture Conditions for Antigen-Induced T-Cell-Dependent B-Cell Activation. T cells supplemented with 5% autologous adherent cells were mixed with autologous B cells at a T-cell/B-cell ratio of 2:1. The cells, at a final concentration of 5×10^5 lymphocytes per ml, were suspended in tissue culture medium, supplemented with 10% heat-inactivated fetal bovine serum instead of human AB^+ serum. Cultures containing adherent cells and either B or T cells were set up in parallel. The cell mixtures were put into round-bottomed tissue culture tubes (A/S, Nunc) in the presence or absence of antigen (10 ng/ml). After incubation for 4 days at 37°C in humidified 95% air/5% CO_2, the medium was removed and fresh tissue culture medium without antigen was added. After further incubation for 12 days, the supernatants were harvested and stored at −20°C until analyzed for total immunoglobulin and anti-malaria antibodies.

Antigen Preparations. The *P. falciparum* antigen Pf155 was prepared by adsorbing spent *P. falciparum* culture medium to polyacrylamide beads conjugated with human glycophorine and subsequent elution with 3 M KSCN (17). These preparations were highly enriched in Pf155 but also contained some faster migrating ($NaDodSO_4$/PAGE) but serologically cross-reacting parasite components (ref. 17; K. Berzins, personal communication). Sonicates of schizont-enriched preparations were used as crude *P. falciparum* antigen and supernatants from sonicates of noninfected erythrocytes as control antigen (16). Peptides were synthesized by solid-phase techniques, purified by HPLC, analyzed, and desalted as described (9). The peptides used were >80% pure and nontoxic.

IFN-γ Determination. For IFN-γ estimation, supernatants were collected after 4 days of incubation when release was optimal. They were assayed undiluted in ELISA, with two distinct IFN-γ-specific monoclonal antibodies used as catcher and indicator antibodies, respectively (18). Human IFN-γ (Gg 23-901-530, 4000 units per ampoule; National Institute of Allergy and Infectious Diseases, National Institutes of Health) was used as standard. The limits of sensitivity of the method were 2.5 units/ml and supernatants containing less than this were considered negative.

RESULTS AND DISCUSSION

T-Cell-Dependent Antibody Production *in Vitro*. Using the previously described T-cell/B-cell cooperation system (13), we further investigated the T-cell-dependent production *in vitro* of anti-malaria antibodies in 30 donors, all primed to *P. falciparum* by natural infection. While T-cell stimulation with a crude *P. falciparum* antigen induced anti-parasite antibodies in 48% of the cultures, regardless of the donors' anti-Pf155 serum titers, antibodies to Pf155 were only induced in cultures from donors with anti-Pf155 titers ≥1:625 (6/19). Very similar results were obtained when cultures were stimulated with nanogram amounts of an antigen preparation highly enriched in Pf155. Induction of antibody production *in vitro* was strictly T-cell dependent and no anti-malaria antibodies were induced with control antigen. These results confirm and extend previous findings (13), indicating that intact Pf155 possesses T-helper cell stimulating epitopes.

Induction of DNA Synthesis (Proliferation Assay). To map Pf155 for T-cell-activating epitopes, synthetic peptides representing repeated sequences from its C terminus (7) were prepared and studied for their capacity to induce *in vitro* proliferation of T cells from *P. falciparum* primed donors. Fig. 1 shows the results of a typical experiment with T cells from four Liberian donors. Three donors (Fig. 1 *a–c*) responded well to an optimal dose of intact Pf155, while one donor (Fig. 1*d*) did not. Two of the donors (Fig. 1 *a* and *b*) also responded positively (SI ≥ 2.5) in a dose-dependent manner but to different degrees to two slightly different peptides, each including several copies of the C-terminal repeat unit Glu-Glu-Asn-Val. These peptides were chosen because they were predicted to include dominant T-cell epitopes on the basis of high α-amphipathic scores when analyzed by the algorithm recently developed by Margalit *et al.* (19). However, one of the donors also responded to a dimer of Glu-Glu-Asn-Val-Glu-His-Asp-Ala (Fig. 1*c*), which had a low α-amphipathic score. This donor did not respond to the Glu-Glu-Asn-Val repeat peptide p2a. The donor who did not

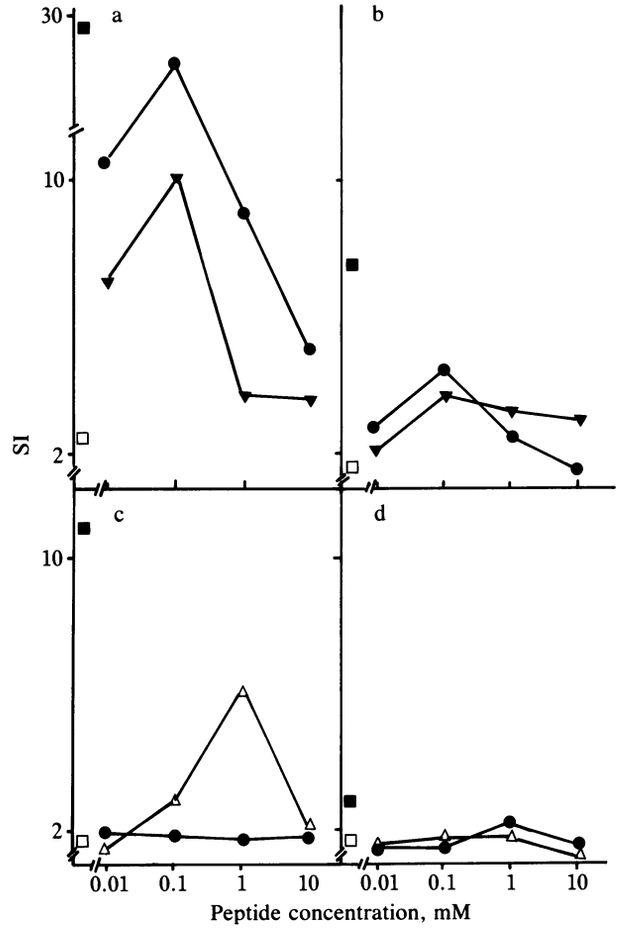

FIG. 1. Stimulation of T cells from four donors primed to *P. falciparum* malaria by natural infection with either intact Pf155, erythrocyte control antigen, or different synthetic peptides corresponding to repeats in the C-terminal region of Pf155. Background incorporation of [^{3}H]thymidine in the absence of antigen was 611 ± 29 (*a*, donor L135), 738 ± 185 (*b*, L136), 414 ± 126 (*c*, L141), and 220 ± 104 (*d*, L140). The Pf155 serum titers (endpoint titers) of the four donors in *a–d* were 1/625, 1/5000, 1/25,000, and 1/125, respectively. They were determined by EMIF according to ref. 4. Ordinate: ■ and □, T cells stimulated with Pf155 or erythrocyte control at optimal concentrations of 0.5 or 5 μg/ml, respectively; ●, with peptide p1a, Ala-Glu-Glu-Asn-Asp-Glu-Glu-Asn-Val-Glu-Glu-Val-$(Glu\text{-}Glu\text{-}Asn\text{-}Val)_2$; ▼, with p2a, Tyr-$(Asn\text{-}Val\text{-}Glu\text{-}Glu)_4$-Cys; △, with p3, Lys-$(Glu\text{-}Glu\text{-}Asn\text{-}Val\text{-}Glu\text{-}His\text{-}Asp\text{-}Ala)_2$.

respond to intact Pf155 also did not respond to any of the peptides (Fig. 1*d*).

Thus far, 96 donors (adult males) have been studied in detail. All were from an endemic *P. falciparum* area in Liberia (20) and were seropositive when tested for *P. falciparum* antibodies by conventional immunofluorescence. Moreover, ≈75% had anti-Pf155 titers (4) ranging from 1:50 to 1:25,000. In the T-cell-proliferation assay, there was no correlation between these serum titers and the magnitude of the lymphocyte response. In total, ≈50% of the donors responded positively to intact Pf155. Table 1 summarizes the responses of the T cells from 50 Pf155 responders and 46 Pf155 nonresponders. When tested with the T-cell mitogen leukoagglutinin (La) (66 donors so tested), there was no difference between the two donor groups, indicating that nonresponsiveness to Pf155 was not due to a generalized T-cell suppression in the latter group. Moreover, although some donors (17/95) also responded to lysates of noninfected erythrocytes (E_o)—obviously a reflection of the erythrocyte response seen frequently in malaria infected donors (11, 12)—the difference in this response between the two donor groups was not statistically significant. Nevertheless, the present data do not prove that Pf155 nonresponsiveness was truly antigen specific as it may also reflect factors such as the donors' immune status as well as partial loss of antigenic sites from the test antigen during isolation. Although the number of Pf155 seropositives and their antibody titers were higher among the Pf155 responders than the nonresponders (40/50 or 29/46; geometric mean titers, 1/774 and 1/472, respectively), these differences were not statistically significant. In addition, several donors who did not respond to Pf155 in the proliferation assay did so in the IFN-γ release assay (see below).

Table 1 also summarizes the responses to two synthetic peptides, representing the two major C-terminal repeat units of Pf155. With some exceptions, donors who did not respond to intact Pf155 also did not respond to these peptides. The few Pf155 nonresponders whose T cells were stimulated by peptides (6/43) may have been primed by cross-reacting plasmodial antigens (21). On the other hand, only ≈65% of the Pf155 responders were stimulated above background by one or several of the peptides, suggesting the existence of additional T-cell-activating sites in the protein, sites not represented by any of the synthetic peptides used. Ongoing studies with a larger number of peptides suggest that such sites may exist preferentially in the second more N-terminally located repeat region of the molecule (M.T.-B., unpublished data).

Table 2 shows some representative results of the stimulation assay in greater detail. It includes 10 malaria-primed Liberian donors and 7 Swedish controls not previously exposed to *P. falciparum*. In line with previous findings, none of the controls responded to intact Pf155 (11, 12) or to any of the peptides representing three related sequences from its C-terminal repeat region. All Liberian donors included in this table responded above background (SI > 2.5) to intact Pf155. The responses seen were of a magnitude also found by others in similar investigations (22). With the peptides, the strongest and most frequent responses were obtained with a 20-mer consisting essentially of 4 Glu-Glu-Asn-Val repeats surrounding the tripeptide Glu-Glu-Val (p1a, Tables 1 and 2). Equivalent results were obtained with a similar peptide differing from p1a in only one position (p1b, Table 1). Most but not all donors responding to these two peptides also responded to the peptide Tyr-(Asn-Val-Glu-Glu)$_4$-Cys (p2a) or the related peptide Lys-(Glu-Glu-Asn-Val)$_4$ (p2b). However, all donors responding to p2a (or p2b) always responded to p1a (or p1b). The minimal epitope involved in these reactions appears to comprise 3 Glu-Glu-Asn-Val units (data not shown). Many of the Pf155 responders also responded to p3, a dimer of the C-terminal 8-amino acid repeat unit Glu-Glu-Asn-Val-Glu-His-Asp-Ala of Pf155. However, in this case and in contrast to what was seen with p2a or p2b,

Table 2. SI of T cells from 10 Liberians and 7 Swedish donors

Donor	EMIF	E_o	Pf155	p1a	p2a	p3	None,* cpm
L133	1:25000	1.8	3.6	4.0	6.5	8.0	424 ± 14
L161	1:5000	1.8	8.8	9.2	6.7		2773 ± 569
L191	1:1250	3.9	9.4	8.8		5.4	2700 ± 241
L200	1:1250	0.4	6.0	2.1	0.1		2640 ± 902
L217	1:1250	0.8	3.3	6.8		6.9	605 ± 30
L192	1:250	1.5	2.8	3.7†		5.3	3360 ± 898
L199	1:250	0.8	5.0	1.5	0.8		3052 ± 1694
L231	1:50	7.6	11.4	7.9		6.0	2031 ± 420
L260	1:50	1.1	6.5	2.5		7.1	2639 ± 182
L149	<1:50	5.9	12.6	6.7†	6.6‡		3183 ± 709
S1			0.3	0.3	0.3	0.3	3830 ± 136
S2			1.2	1.3	0.7	1.3	802 ± 88
S3			1.0	1.4	0.9	0.8	1477 ± 18
S4			0.6	0.6	1.4	0.4	910 ± 128
S5			0.3	0.9	0.5	0.3	1425 ± 154
S6			0.7	1.8	1.5	0.4	761 ± 28
S7			0.7	1.1	1.3	1.5	540 ± 17

All Liberian donors were Pf155 responders. Anti-Pf155 serum titers were determined by EMIF (4). Peptides used as stimulants were as follows: p1a, Ala-Glu-Glu-Asn-Asp-Glu-Glu-Asn-Val-Glu-Glu-Val-(Glu-Glu-Asn-Val)$_2$; p2a, Tyr-(Asn-Val-Glu-Glu)$_4$-Cys; p3, Lys-(Glu-Glu-Asn-Val-Glu-His-Asp-Ala)$_2$.
*[^{3}H]Thymidine incorporation in absence of stimulant (background).
†Stimulated with p1b, Lys-Ala-(Glu-Glu-Asn-Val)$_2$-Glu-Glu-Val-(Glu-Glu-Asn-Val)$_2$.
‡Stimulated with p2b, Lys-(Glu-Glu-Asn-Val)$_4$. For other explanations see Table 1.

Table 1. Summary of T-cell responses in the proliferation assay

Stimulant	Pf155 responders			Pf155 nonresponders		
	%	No. responding/ no. tested	SI	%	No. responding/ no. tested	SI
Pf155	100	50/50	6.1 ± 0.6	0	0/46	1.3 ± 0.1
p1*	67	29/43	5.3 ± 1.0	14	6/43	1.5 ± 0.2
p3†	42	11/26	3.1 ± 0.5	17	4/24	1.6 ± 0.2
E_o	26	13/50	2.2 ± 0.3	9	4/45	1.5 ± 0.2
La	100	36/36	13.8 ± 2.1	100	30/30	18.8 ± 5.8

Ninety-six Liberian donors were divided into Pf155 responders (SI ≥ 2.5) and Pf155 nonresponders (SI < 2.5). SI values are means ± SEM from the total number of individuals tested with optimal concentrations of Pf155 (0.5 μg/ml), peptide p1 or peptide p3 (0.1 or 1 mM), erythrocyte control antigen (E_o, 5 μg/ml) or T-cell mitogen (La, 10 μg/ml). Significance of differences between SI (unpaired *t* test): Pf155, $P < 0.0002$; p1, $P < 0.0005$; p3, $P < 0.004$; E_o and La, not significant.
*Ala-Glu-Glu-Asn-Asp-Glu-Glu-Asn-Val-Glu-Glu-Val-(Glu-Glu-Asn-Val)$_2$ or Lys-Ala-(Glu-Glu-Asn-Val)$_2$-Glu-Glu-Val-(Glu-Glu-Asn-Val)$_2$.
†Lys-(Glu-Glu-Asn-Val-Glu-His-Asp-Ala)$_2$.

some donors who responded significantly to p3 did not respond to either p1a or p1b (e.g., Fig. 1*c*; donor L260, Table 2).

From the results it cannot be concluded to what extent the lymphocyte reactions obtained with the different peptides represent responses of different cells seeing different epitopes or to what extent they are cross-reactions. As the peptides have similar sequences, it is likely that they represent a few overlapping and cross-reacting epitopes. Current experiments with T-cell clones support this conclusion. However, the fact that some donors who responded poorly to the Glu-Glu-Asn-Val repeat peptides were significantly stimulated by the Glu-Glu-Asn-Val-Glu-His-Asp-Ala dimer suggest that the latter formed distinct non-cross-reacting epitopes seen by the T cells of only some Pf155 responders. The important question is whether or not the different response patterns reflect differences in major histocompatibility complex restriction. Further experiments, utilizing a modified T-cell/B-cell cooperation system *in vitro*, are also needed to establish whether or not the epitopes defined in the proliferation assay are indeed T-helper cell activating sites, which induce B cells to produce Pf155-specific antibodies. It is noteworthy that some of the prevalent anti-Pf155 antibodies in serum react with epitopes similar to those seen by the T cells discussed above (8, 9). As IgG antibody formation to these epitopes depends on T-cell/B-cell cooperation (13) it may well require help by other T-cell-activating sites present on Pf155 or on other parasite molecules (23–25).

Induction of IFN-γ Release. We have previously reported that stimulation *in vitro* of T cells from primed individuals with crude *P. falciparum* schizont antigens or Pf155 induces release of IFN-γ and that this may be indicative of the existence of an antibody-independent cellular immunity (11, 26). No IFN-γ release was induced in control donors' lymphocytes exposed to the same antigens. Similar results have recently been reported by others studying lymphocytes from Gambian adults (22). In the present investigation, 31 of the Liberian donors were studied in detail for IFN-γ release after *in vitro* stimulation with intact Pf155 or synthetic peptides. The 31 donors were chosen because their T cells released significant amounts of IFN-γ (31.7 ± 14.6 units/ml; mean ± SD) when stimulated with the T-cell mitogen La (18). Without antigen stimulation, no or only small amounts (5.4 ± 8.4 units/ml) of IFN-γ were detected in the supernatants. Although some erythrocyte-sensitized donors responded well to erythrocyte lysates, for the test group as a whole this response was not significantly increased over that seen without antigen (7.7 ± 12.7 units/ml; $P < 0.4$, unpaired *t* test). In contrast, 15 of the 31 donors released IFN-γ significantly above background when incubated with Pf155, suggesting the presence of Pf155-responsive T cells in half of this group. For the responding donors, the mean IFN-γ release was 20.8 ± 12.4 units/ml. Although lower than the release obtained with mitogen, it was significantly increased above background release in the absence of antigen ($P < 0.007$, unpaired *t* test) and in line with findings recently made with other *P. falciparum* antigens and lymphocytes from immune Gambians (22).

Table 3 shows representative results obtained with the cells of 15 donors, all tested with intact antigen and two of the peptides. IFN-γ release was not correlated to the anti-Pf155 serum titers. Only donors responding positively to intact Pf155 released IFN-γ with the synthetic peptides. On the average, the response to peptide p1a was slightly lower than that to Pf155 (18.2 ± 17.4 units/ml) but this difference was not statistically significant ($P < 0.8$, unpaired *t* test). Only few donors responded above background to p3 (Table 3). However, available results are too few to permit conclusions regarding possible differences in the response of individual donors to different peptides.

Although the overall number of donors responding to Pf155 with IFN-γ release was similar to that responding in the proliferation assay (15/31 as compared to 13/31, respectively), inspection of Table 3 shows that these responses were not correlated in individual donors (e.g., the IFN-γ^+/SI$^-$ donors L190, L194, L237 and the IFN-γ^-/SI$^+$ donors L227, L232, L262). This is in accordance with previous results (11) and suggests that the two assays may measure stimulation of primed T cells belonging to different subsets (27, 28). Whatever the reasons, the results indicate that the IFN-γ assay is an important complement to the proliferation assay and should be included in the test panel whenever one wants to determine the degree of T-cell sensitization in different donor populations.

Concluding Remarks. Taken together the present results show that the C-terminal repeat region of Pf155/RESA, known to contain some of the molecule's immunodominant B-cell epitopes, also includes some of its important T-cell

Table 3. SI and IFN-γ release of T cells from 15 Liberians

Donor	EMIF	La, IFN-γ	E_o		Pf155		p1a		p3	
			IFN-γ	SI	IFN-γ	SI	IFN-γ	SI	IFN-γ	SI
L190	1:25000	56	49	1.2	35	1.8	64	ND	ND	1.6
L224	1:25000	50	4	2.3	9	3.1	4	1.1	4	1.5
L189	1:1250	14	13	1.2	19	4.8	4	6.4	ND	2.0
L216	1:1250	45	5	2.9	18	2.7	5	4.8	4	1.4
L226	1:1250	50	0	3.8	12	11.3	5	7.0	0	4.2
L227	1:1250	23	0	0.6	0	3.1	0	1.6	0	0.7
L252	1:1250	50	0	1.5	0	1.5	0	2.1	0	1.0
L194	1:250	27	0	1.5	31	1.2	22	ND	ND	1.7
L225	1:250	48	0	1.8	0	2.0	0	0.7	0	1.3
L237	1:250	38	13	1.5	14	0.9	15	1.1	24	1.5
L232	<1:50	12	0	0.6	0	3.2	0	1.2	0	2.4
L235	<1:50	39	12	2.1	25	2.7	33	0.9	7	0.4
L251	<1:50	35	0	0.3	0	0.9	0	1.1	0	1.6
L262	<1:50	49	0	0.9	0	2.8	0	1.5	0	1.1
L265	<1:50	22	0	0.9	0	2.0	0	0.3	0	0.3

All IFN-γ values (units/ml) are corrected by subtracting background release obtained in some donors in the absence of added stimulants. For IFN-γ determination, supernatants were collected after 4 days of incubation and for SI determination, cells were harvested on day 5. Peptides used as stimulants were as follows: p1a, Ala-Glu-Glu-Asn-Asp-Glu-Glu-Asn-Val-Glu-Glu-Val-(Glu-Glu-Asn-Val)$_2$ and p3, Lys-(Glu-Glu-Asn-Val-Glu-His-Asp-Ala)$_2$. Anti-Pf155 serum titers were determined by EMIF (4). ND, not done. For other explanations see Table 1.

epitopes, capable of stimulating T cells to proliferate and/or to release IFN-γ. In this respect, Pf155 appears to be different from the circumsporozoite protein of *P. falciparum*, the immunodominant T-cell domains of which have recently been shown to map to polymorphic regions outside its B-cell immunodominant repeat region (29). Since the C-terminal repeats of Pf155 appear to be conserved among different *P. falciparum* strains (7, 10), the T-cell-activating sequences described in this report appear to be suitable components to be included in a subunit malaria vaccine. They also provide a basis for epidemiological studies relating epitope-specific T-cell responses *in vitro* to clinical immunity and malaria endemicity. Further work is needed, however, to establish the basis of the apparent Pf155 nonresponsiveness of T cells seen in about half of the donor population tested herein.

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