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Number of Cells from *Plasmodium falciparum*-Immune Donors That Produce Gamma Interferon In Vitro in Response to Pf155/RESA, a Malaria Vaccine Candidate Antigen

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Secretion of gamma interferon (IFN-γ) in response to stimulation of *Plasmodium falciparum*-primed T cells by specific antigens may be a useful indicator of cellular immunity to malaria. An enzyme-linked immunospot (ELISPOT) assay designed to detect IFN-γ at the single-cell level was used to study IFN-γ-producing cells from *P. falciparum*-primed donors from The Gambia after in vitro stimulation with various malarial antigens. IFN-γ secreted into the culture supernatant was measured by conventional enzyme-linked immunosorbent assay (ELISA). There was a good correlation in individual donors between the level of IFN-γ secreted into the culture supernatant and the number of IFN-γ-secreting cells. However, the ELISPOT assay was apparently more sensitive in demonstrating low levels of IFN-γ production than the ELISA was. Thus after stimulation with crude *P. falciparum* antigen from infected erythrocytes, 72% of the primed donors responded positively in the ELISPOT assay but only 55% responded positively in the ELISA. When stimulated with synthetic peptides representing immunodominant epitopes of the malarial antigen Pf155/RESA, a vaccine candidate, 31 to 55% responded in the ELISPOT assay and 21 to 36% responded in the ELISA. Unprimed Europeans did not respond positively to these antigens in either of the assays, and background in antigen-free controls was generally low. These results indicate that measurement of IFN-γ by the ELISPOT assay or ELISA should have wide applications in large-scale epidemiological studies of malaria immunity. In addition, the ELISPOT assay makes it possible to analyze the T cells responding to malarial antigens in terms of both numbers and functional heterogeneity.

The development of effective protective immunity to human malaria is a complex process which is not yet completely understood. Although antibodies contribute to immune protection, priming of T cells is important for the development and maintenance of immunity to malaria (4, 19, 22), and T-cell-derived gamma interferon (IFN-γ) is believed to be an important mediator of cellular effector mechanisms. In rodent malaria models, both $CD8^+$ T cells and IFN-γ are involved in immunity to the exoerythrocytic stages of the malaria parasite (7, 16, 23). IFN-γ can protect rhesus monkeys from *Plasmodium cynomolgi* infection in a dose-dependent manner (10) and is also involved in the activation of macrophages and natural killer cells in nonspecific immune responses to *Plasmodium berghei* infections in mice (11). IFN-γ has also been shown to have adjuvant activity for a malaria vaccine in mice (14).

T cells from humans who are clinically immune to *Plasmodium falciparum* can be induced to proliferate and produce IFN-γ in vitro by using intact Pf155/RESA (12) or peptides corresponding to the 3′ and the central repeat regions of the molecule (6, 20). In individual donors, these different T-cell activities were shown not to be correlated (6, 20). Moreover, both proliferation and IFN-γ release were usually negatively correlated with the concentration of serum antibodies to the peptide used for T-cell activation (M. Troye-Blomberg et al., Proc. Natl. Acad. Sci. USA, in press). Detection by enzyme-linked immunosorbent assay (ELISA) (1) of IFN-γ secretion into culture supernatant requires high cell densities and gives no information about the number of responding cells. We have shown that (in individuals recently vaccinated with tetanus toxoid) small numbers of antigen-specific IFN-γ secreting cells can be detected by specific staining with anti-IFN-γ monoclonal antibody (MAb) but do not produce sufficient IFN-γ to be detected by conventional ELISA of culture supernatants (5a). In this study we have investigated IFN-γ production by Pf155/RESA-specific T cells from *P. falciparum*-immune individuals who were primed to the antigen by natural infection, using the enzyme-linked immunospot (ELISPOT) assay (3), which detects IFN-γ secreted from an individual cell as a precipitate on a solid phase coated with MAb specific to human IFN-γ. We have compared the sensitivity of this method with that of the conventional ELISA, and we have also determined the minimum number of activated, i.e., secreting, cells needed to detect IFN-γ in the ELISPOT assay and in the ELISA. We believe that this new approach will make it possible to determine with some accuracy the precursor frequency of cytokine-producing cells in individuals with various degrees of functional immunity to malaria.

MATERIALS AND METHODS

Study subjects. Subjects were recruited from the village of Brefet in The Gambia, where *P. falciparum* malaria is holoendemic but seasonal, with maximum transmission between June and November. A 20-ml venous blood sample was obtained from each of 22 adults (aged 22 to 61 years; mean age ± standard deviation [SD], 33 ± 12 years) during

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a 3-week period at the end of the wet season (November). Thick blood smears were stained with Giemsa stain and examined for malaria parasites. Three of the donors (14%) were positive for *P. falciparum* with low levels of parasitemia (10 to 60 parasites per μl). Five European adults with no previous history of malaria exposure were used as control donors.

Isolation and fractionation of human peripheral T cells. Human peripheral blood mononuclear cells were isolated from heparinized venous blood by sodium metrizoate density gradient centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway). T cells were separated by rosette formation with neuraminidase-treated sheep erythrocytes. E-rosette-forming cells (T cells) were separated from the non-rosette-forming cells by sodium metrizoate density centrifugation (5).

Adherent cells. Adherent cells were obtained from peripheral blood mononuclear cells by incubation for 1 to 2 h at 37°C in sterile petri dishes in Iscove medium containing essential amino acids (GIBCO, Paisley, Scotland), gentamicin (100 μg/ml), 5×10^{-5} M mercaptoethanol, sodium bicarbonate (36 mM), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (25 mM), and 10% pooled human AB^+ serum (complete tissue culture medium [TCM]). Nonadherent cells were washed off, and adherent cells were recovered after overnight incubation at 4°C.

Cell cultures. T cells supplemented with 5% autologous adherent cells were cultured at a final cell density of 10^6/ml in TCM and placed in round-bottomed tissue culture tubes (A/S Nunc, Roskilde, Denmark) in the presence of purified protein derivative of *Mycobacterium tuberculosis* (PPD) (National Bacteriological Laboratory, Stockholm, Sweden), crude malarial antigen (E_i), uninfected-erythrocyte antigen (E_o) at a final concentration of 5 μg/ml, or Pf155/RESA peptides (0.1 μM), or in the absence of any stimulant. After incubation for 4 days at 37°C in a humidified atmosphere with 5% CO_2, the cell pellets were washed twice in serum-free medium and used for determination of IFN-γ secretion at the single-cell level (ELISPOT; see below). Parallel cultures were incubated for 5 days, the cells were centrifuged, and the supernatants were collected for IFN-γ determination by ELISA.

Antigens and peptides. A crude preparation of malarial antigen was obtained from infected erythrocytes, and control antigen was prepared from noninfected erythrocytes as described earlier (18). The synthetic peptides (P1, from the 3′ repeat region, and P2, from the 5′ repeat region of Pf155/RESA) were synthesized as described earlier (20). The amino acid sequence of the P1 peptide in single-letter code is EENVEHDAEENVEHDAEENVEENV, and that of P2 is TVAEEHVEEPTVAEE.

Monoclonal anti-IFN-γ antibodies. The production and characterization of the mouse anti-human-IFN-γ MAbs 7-B6-1 and 1-D1K have been described elsewhere (1). Both MAbs recognize distinct epitopes of human IFN-γ. They are of immunoglobulin G1 subclass k and bind recombinant as well as natural IFN-γ.

Reverse ELISPOT assay. IFN-γ-secreting cells were detected by ELISPOT assay as previously described (3, 5a). TCM (200 μl) containing 5×10^4 or 1×10^5 cells was dispensed into duplicate wells of nitrocellulose-bottomed plates coated with the anti-IFN-γ MAb 7-B6-1 as the solid-phase capture. After incubation for 20 h and washing of the wells, the plates were incubated with biotinylated 1-D1K MAb for 2 h, washed, and incubated with horseradish peroxidase conjugated with ExtrAvidin (Sigma Chemical Co., St. Louis, Mo.) for 45 min. The enzyme substrate solution (3-amino-9-ethyl carbazole–H_2O_2–chromogen substrate) was added, and spots were detectable as a red precipitate within 2 to 5 min. The spots were counted under low magnification (×40) with a stereomicroscope.

IFN-γ ELISA. IFN-γ in culture supernatants was assayed by a two-site ELISA using MAbs to recombinant human IFN-γ (7-B6-1 and 1-D1K) in which 1-D1K was labeled with alkaline phosphatase as described earlier (1). Human IFN-γ (Gg 23-901-530; 4,000 U per ampoule; National Institute of Allergy and Infectious Diseases, Bethesda, Md.) was used as a standard. The detection limit of the method was 2 U/ml, and supernatants containing less IFN-γ than this amount were considered negative.

Serology. A small sample of plasma was collected from each blood sample and used to measure antibody titers to *P. falciparum* and Pf155/RESA by conventional immunofluorescence (air-dried monolayers) and by erythrocyte membrane immunofluorescence (glutaraldehyde-fixed monolayers of ring-infected erythrocytes) (12). For antipeptide antibody determinations, an ELISA was used as described previously (13). Briefly, 96-well plastic plates were coated with bovine serum albumin-conjugated (2:1, wt/wt) peptides. Bound antibodies were detected by alkaline phosphatase-conjugated rabbit antibodies specific for human immunoglobulin G with *p*-nitrophenylphosphatase as substrate. The concentration of specific antibodies (in micrograms per milliliter) in the plasma was determined in parallel assays with a known immunoglobulin G standard in wells coated with anti-human $F(ab')_2$ antibodies (13).

Statistics. Differences between the responses induced by malarial antigens and the background responses were analyzed by using paired *t* tests. The results obtained in the two tests (ELISPOT and ELISA) in response to the antigens were corrected for background values and compared by linear regression analysis.

RESULTS

All 22 subjects were permanent residents of the same Gambian village and were thus likely to have been exposed to similar levels of infection and to similar strains of *P. falciparum* over many years. None of them had clinical malaria at the time of sampling, although three had low levels of *P. falciparum* parasitemia (10 to 60 parasites per μl). All sera were positive for *P. falciparum* antibodies by conventional immunofluorescence at a titer of ≥1/1,000 on unfixed air-dried infected erythrocytes, and 65% had antibodies to Pf155/RESA at titers ranging from 1/50 to 1/1,250 as determined by erythrocyte membrane immunofluorescence. Antipeptide antibodies against peptides P1 and P2 ranged from 0 to 59 μg/ml (mean ± SD values were 11.6 ± 16.3 and 5.7 ± 6.7 μg/ml for P1 and P2, respectively). In agreement with our previous findings (Troye-Blomberg et al., in press), the presence of antipeptide antibodies was frequently negatively correlated with T-cell activation parameters in individual donors (data not shown).

Estimation of IFN-γ production at the single-cell level by ELISPOT assay. To determine whether antigen-activated IFN-γ-secreting cells can be visualized by ELISPOT assay, T cells and 5% autologous adherent cells from *P. falciparum*-primed donors were incubated with or without antigen for 4 days, a period which was found to be optimal in preliminary studies (data not shown), and IFN-γ-producing cells were visualized by immunoenzyme staining. The mean number of IFN-γ spots in duplicate wells with 5×10^4 and 1×10^5 cells

TABLE 1. IFN-γ secretion detected at the single-cell level from in vitro-activated T cells from 22 Gambian and 5 European donors

Donor	No. of spots/10^5 cells with stimulant[a]:				
	None	E_i	E_o	P1	P2
G1	3.5	6.5	6.5	12.5	4
G2	4	6	8	8.5	7.5
G3	4	9	8	8	9
G4	4	6	9	10.5	5
G5	4	11	5	8.5	6
G6	4	16	3.0	4	5.5
G7	4.5	5.5	5	7.5	6
G8	5	14	6	5	6.5
G9	5	22.5	7	9.5	5.5
G10	5.5	30	7	14.5	14
G11	5.5	14	3.5	15	11
G12	5.5	10	11.5	8.5	8
G13	6	16	9.5	6	7
G14	6	12	7.5	8	9.5
G15[b]	6	25	16	30	7.5
G16	7	11	7.5	11	7.5
G17	7	15	13	13	9
G18	7	27.5	7.5	16	15.5
G19	7.5	22.5	13.5	20	22.5
G20	8.5	15	9.5	12.5	15
G21[b]	11	36	30.5	44.5	41
G22[b]	12	33.5	27.5	35	38
Mean ± SD	6 ± 2.2	16.5 ± 9	10 ± 6.9	14 ± 10	11.8 ± 9.9
E1	0	4	2	0	0
E2	0	3	0	0	0
E3	0	3	1	0	0
E4	0	1	0	0	0
E5	0	3	2	0	0
Mean ± SD	0	2.8 ± 1	1 ± 1	0	0

[a] Cells from 22 *P. falciparum*-primed Gambian donors (G1 to G22) and 5 European donors (E1 to E5) were tested for IFN-γ production in vitro by ELISPOT assay. Data for individual donors are the mean values of numbers of spots from duplicate wells with 10^5 cells per well. E_i, *P. falciparum*-infected erythrocyte antigen; E_o, uninfected-erythrocyte control antigen; P1, Pf155/RESA peptide EENVEHDAEENVEHDAEENVEENV; P2, PF155/RESA peptide TVAEEHVEEPTVAEE. All positives (>10.4 spots per 10^5 cells) are underlined.

[b] Donors with *P. falciparum* parasitemia at time of assay.

per well were counted. The relative frequencies of IFN-γ-producing cells were similar for both cell concentrations, and therefore 10^5 cells per well were used for counting throughout this work.

All 22 *P. falciparum*-primed donors and all 5 control donors produced spots in response to stimulation with PPD (mean number of spots ± SD, 160 ± 85 and 284 ± 81, respectively). Table 1 shows the results with cells from both the 22 *P. falciparum*-primed donors from The Gambia (G1 to G22) and the 5 controls (E1 to E5) after stimulation with malarial antigens. Cells from all primed donors spontaneously produced some spots in the absence of additional in vitro stimulation (antigen-free wells), but spots were rarely seen in control cultures from unprimed European donors. There was considerable variation in the magnitude of the response to the malarial antigens, and each antigen induced a different pattern of responses in individual donors. The differences between spontaneous (background) response and the antigen-induced responses in the Gambian donors were highly significant (paired *t* test; $P < 0.001$ for E_i, E_o, and P1; $P < 0.002$ for P2). The response to E_i was also significantly greater than the response to E_o (paired *t* test; $P < 0.001$).

Antigen-stimulated cultures were considered to be positive in this assay if the number of spots was greater than the mean + 2 SDs of that in the antigen-free control wells (Table 1). Cells from two primed donors (G21 and G22; Table 1) spontaneously produced rather high numbers of spots in unstimulated cultures; these responses were outside the range of the mean ± 2 SDs for the whole population. In any event, with these criteria, cells from a majority (72%) of primed donors produced IFN-γ spots in response to the crude malaria antigen E_i, whereas cells from a minority (27%) responded to the uninfected erythrocyte ghost antigen (Table 2). For the two Pf155/RESA peptides, the strongest and the most frequent responses were induced by P1 (55% response; mean number of spots ± SD, 14 ± 10); responses to P2 were considerably less frequent (31% response; mean number of spots ± SD, 11.8 ± 9.9). In contrast, cells from unprimed European donors produced a small number of spots (mean ± SD, 2.8 ± 1 and 1 ± 1) in response to the E_i and E_o antigens, respectively, and none at all in response to Pf155/RESA peptides.

TABLE 2. Comparison of ELISA and ELISPOT for detection of IFN-γ production

Assay	% Response to stimulant[a]:			
	E_i	E_o	P1	P2
ELISPOT[b]	72	27	55	31
ELISA[c]	50	18	36	22

[a] Cells from 22 *P. falciparum*-primed donors were tested for IFN-γ production in vitro by conventional ELISA and by ELISPOT assay. IFN-γ values were corrected for background release. Data are percentages of donors whose cells produced IFN-γ in response to each of the stimulants described in Table 1, footnote *a*.

[b] ELISPOT data are percentages of donors with IFN-γ-secreting cells that exceed the limits of the mean ± 2 SDs of IFN-γ spots (mean, >10 spots per 100,000 cells) in control cultures without antigen stimulation.

[c] ELISA data are percentages of donors in whose cells the secreted IFN-γ level was above 2 U/ml after subtraction of spontaneous IFN-γ release in control wells.

Secreted IFN-γ measured by ELISA. Supernatants from 5-day lymphocyte cultures were used for determination of secreted IFN-γ. In accordance with previous results, a response of ≥2 U/ml was considered positive (20). Cells from malaria-primed Gambian and control European donors produced significant amounts of IFN-γ (17 to 22 U/ml) after activation with PPD, but IFN-γ was not detectable in cultures from unprimed European donors stimulated with malaria antigens (E_i, E_o, or Pf155/RESA peptides), confirming our previous findings (6, 20).

Table 3 shows the IFN-γ ELISA responses for the malaria-primed donors in response to malaria antigens. Cells from the two primed donors who spontaneously produced high numbers of spots in unstimulated cultures (Table 1) also spontaneously released ≥2 U of IFN-γ per ml in the unstimulated cultures, (Table 3). The magnitude of the response varied among donors. The percentage of positive responders to each antigen in the ELISA is shown in Table 2.

Comparison of ELISPOT assay and ELISA for detection of antigen-induced IFN-γ production. We compared the two methods of measuring IFN-γ responses of cells from individual donors to crude malarial antigen (E_i), ghost antigen (E_o), or Pf155/RESA peptides (P1 and P2) (Fig. 1). There was a statistically significant correlation between the results obtained in the two tests for all the antigens used for stimulation ($r = 0.9$; $P < 0.001$). However, the ELISPOT method appeared to be more sensitive than the conventional ELISA method, as it detected IFN-γ-producing cells even in the absence of detectable amounts of secreted IFN-γ in

TABLE 3. ELISA responses of cells of primed donors to malarial antigens in vitro[a]

Donor	Amt of IFN-γ (U/ml) in supernatant of T cells stimulated with:				
	No antigen	E_i	E_o	P1	P2
G1	0	0	0	2.5	0
G2	0	0	0	0	0
G3	0	0	<2	0	0
G4	0	0	0	<2	0
G5	0	0	0	0	0
G6	0	5	0	0	0
G7	0	0	0	0	0
G8	0	3	0	0	0
G9	0	3	0	0	0
G10	0	5	0	2.5	2
G11	0	3	0	2.5	0
G12	0	0	0	0	0
G13	0	3	0	0	0
G14	0	0	0	0	0
G15	0	3	2	4	0
G16	0	0	0	0	0
G17	0	0	3	0	0
G18	0	3	0	4	2.5
G19	0	2.5	0	2.5	3
G20	0	0	0	0	0
G21	2	12.5	9	8	5
G22	2.5	14	13.5	11.5	10
Mean ± SD	0.2 ± 0.7	2.6 ± 3.9	1.3 ± 3.4	1.8 ± 3.0	1 ± 2.4

[a] Cells from 22 *P. falciparum*-primed donors were tested for IFN-γ release by ELISA after 5 days of incubation. IFN-γ values for donors G21 and G22 were corrected for IFN-γ release in antigen-free controls. The stimulants used were those described in Table 1, footnote *a*. All supernatants from control donors (E1 to E5) were negative in this assay.

culture supernatants. In general, IFN-γ could be detected by ELISA if ≥14 spots per 100,000 cells were detected by the ELISPOT method. In 2 of 22 instances, IFN-γ could not be detected by ELISA even when ≥14 spots per 100,000 cells were detected by the ELISPOT method.

DISCUSSION

IFN-γ is believed to be an important mediator of antibody-independent immunity to malaria (10, 16, 17), and potential malaria vaccine antigens should therefore be assessed for the ability to induce secretion of IFN-γ from malaria-specific T cells (6, 21). Synthetic peptides derived from the 3′ and 5′ repeat regions of the malarial antigen Pf155/RESA induce T cells of *P. falciparum*-primed donors to release IFN-γ into culture supernatant in vitro. IFN-γ secretion appears to be independent of lymphocyte proliferation, suggesting that the two assays may measure stimulation of T cells belonging to two different subsets, and thus IFN-γ represents an additional indicator of T-cell activation (6, 20).

Previously, we measured IFN-γ secretion into culture supernatants by conventional ELISA (1). However, detection of IFN-γ by this method usually requires high cell numbers and gives no indication of the actual number of IFN-γ-secreting cells (5a, 21). Estimation of the precursor frequency of cells producing different lymphokines in an antigen-specific system is important for selecting vaccine immunogens capable of inducing appropriate protective effector responses. Limiting-dilution assays have been used to localize the murine T-cell response to erythrocytic stages of *Plasmodium chabaudi* and to estimate the precursor frequencies of *P. chabaudi*-reactive T cells (8, 9). In this study, we have used an alternative approach to estimate the number of IFN-γ-producing cells induced by a crude malarial antigen (E_i) and by synthetic peptides corresponding to two immunodominant regions of the *P. falciparum* merozoite antigen Pf155/RESA. For example, the precursor frequency of IFN-γ-secreting cells induced by the crude E_i ranged from 5.5 to 36 spots per 100,000 cells. The ELISPOT system has several advantages over limiting-dilution analysis, since it is a direct, sensitive assay and does not require long-term culturing of cells. Furthermore, the ELISPOT assay has been shown to detect low numbers of IFN-γ-secreting cells even in the absence of secreted IFN-γ detectable by the conventional ELISA in culture supernatants (5a). A few IFN-γ spots (≤8 spots per 100,000 cells) were generally seen in unstimulated cultures from *P. falciparum*-primed Gambian individuals. In contrast, spots were rarely seen in control cultures from unprimed European donors. The elevated number of spontaneously produced IFN-γ spots seen in the blood from two donors with confirmed *P. falciparum* parasitemia (donors G21 and G22; Table 1) probably reflected the presence of IFN-γ-producing cells activated in vivo by malarial antigens. Recent infection by malarial or other common pathogens may explain the presence of low numbers of spontaneously IFN-γ-producing cells in other Gambian donors but not in the European donors. Whether the spontaneous IFN-γ secretion seen in *P. falciparum*-primed individuals has any relevance in vivo for clearance of parasitemia, as has been suggested for the murine *P. chabaudi chabaudi* system (8), is presently not clear. It is of interest to note that spontaneous IFN-γ production has been detected by the ELISPOT assay in people with active autoimmune disease (T. Olsson, W. W. Zhi, B. Höjeberg, V. Kostulas, J. Yu-Ping, G. Andersson, H.-P. Ekre, and H. Link, submitted for publication).

Despite the presence of spontaneously IFN-γ-producing cells, a significant induction of IFN-γ-producing cells was detected after in vitro activation of cells from malaria-primed donors with *P. falciparum* antigens. Presumably these spots represent specific secondary activation of in vivo-primed T cells. Seventy-two percent of the *P. falciparum*-primed individuals responded to the crude malarial antigen (E_i), while 55% and 31% responded to two Pf155/RESA-derived peptides, P1 and P2, respectively. The higher response to the E_i antigen was to be expected, since this preparation was relatively crude and certainly contained multiple T-cell epitopes. Crude malarial antigens have been reported to be mitogenic (2, 18). However, since E_i induced only a few spots in only one or two of the unprimed European donors, it is unlikely that the antigen preparations used in our experiments were mitogenic. A response to the uninfected erythrocyte E_o antigen was seen in 27% of the malaria-exposed donors but not in any of the European donors. This confirms our previous finding (17) that individuals exposed to malaria become sensitized to erythrocyte components.

Of the two Pf155/RESA peptides tested, the strongest and most frequent responses were induced with peptide P1, which corresponds to the 3′ repeat region. T cells from approximately half of the donors (12 of 22) could be activated with either one or the other of the peptides, but only about one-third (7 of 22) responded to both peptides. These results confirm earlier findings for defined epitopes of the Pf155/RESA molecule (15, 20) and may indicate the fact that epitope-specific T-cell responses are in some way genetically regulated.

When the results obtained by the ELISPOT assay were compared with those of the conventional ELISA, there was

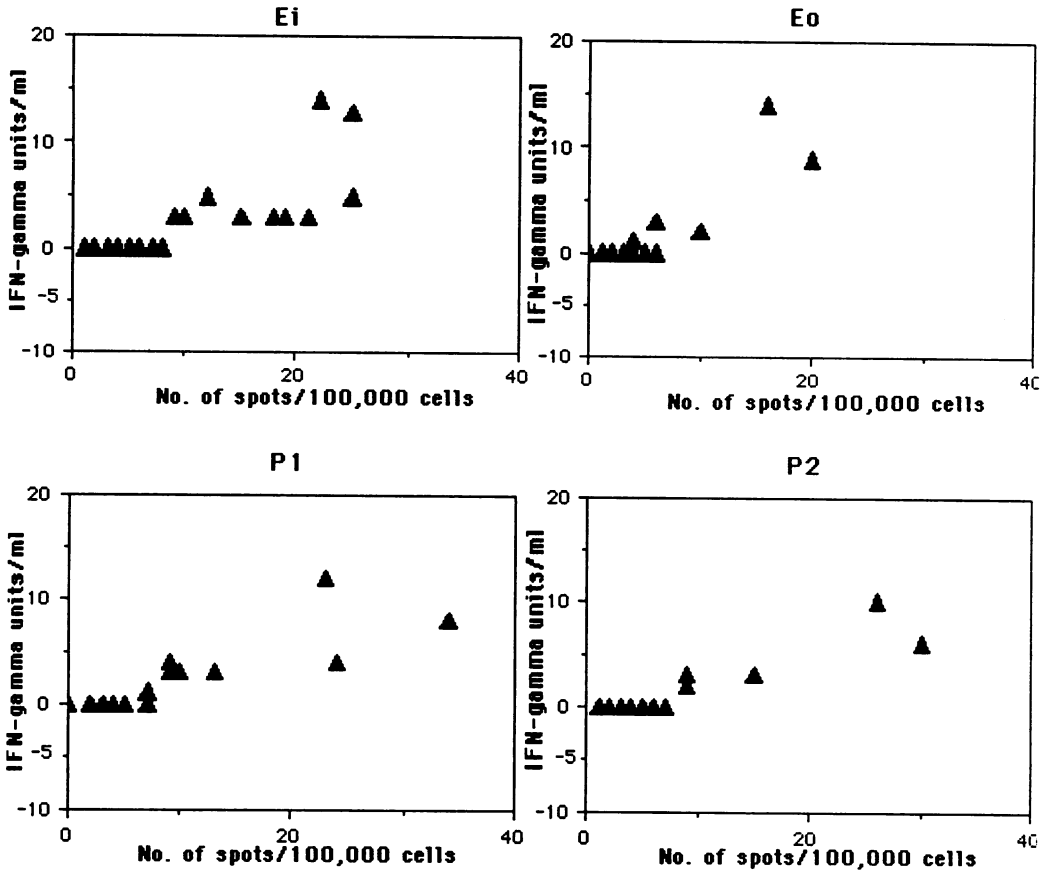

FIG. 1. Comparison of the number of IFN-γ-secreting cells (ELISPOT) and the amount of IFN-γ secreted into the culture supernatant (ELISA) by cells from *P. falciparum*-primed donors in response to crude malarial antigen (E_i), uninfected-erythrocyte control antigen (E_o), and Pf155/RESA peptides P1 (EENVEHDAEENVEHDAEENVEENV) and P2 (TVAEEHVEEPTVAEE). The IFN-γ spots represent the mean values from duplicate wells with 10^5 T cells per well, corrected by subtracting the background values (spots in antigen-free cultures [Table 1]).

a significant correlation between the two assays for all antigens tested. However, the ELISPOT assay was more sensitive, since low numbers of IFN-γ-producing cells could be detected in the absence of significant levels of IFN-γ in culture supernatants.

In conclusion, the present study confirms our previous findings that immunodominant T-cell epitopes from the invariant repeat regions of Pf155/RESA can induce production of IFN-γ in vitro. The results of the ELISPOT assay and the ELISA were significantly correlated, but the ELISPOT technique was more sensitive. We believe that both assays have direct applications for large-scale epidemiological studies of malaria immunity. In addition, the ELISPOT assay makes it possible to analyze T-cell responses to malarial antigens in terms of both absolute numbers of responding cells and the functional heterogeneity of responding clones in individuals primed to the disease through natural infection.

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