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Induction of Antigen-Specific Cytotoxic T Lymphocytes in Humans by a Malaria DNA Vaccine

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CD8$^+$ cytotoxic T lymphocytes (CTLs) are critical for protection against intracellular pathogens but often have been difficult to induce by subunit vaccines in animals. DNA vaccines elicit protective CD8$^+$ T cell responses. Malaria-naïve volunteers who were vaccinated with plasmid DNA encoding a malaria protein developed antigen-specific, genetically restricted, CD8$^+$ T cell–dependent CTLs. Responses were directed against all 10 peptides tested and were restricted by six human lymphocyte antigen (HLA) class I alleles. This first demonstration in healthy naïve humans of the induction of CD8$^+$ CTLs by DNA vaccines, including CTLs that were restricted by multiple HLA alleles in the same individual, provides a foundation for further human testing of this potentially revolutionary vaccine technology.

During 1990–1994, the administration of "naked" plasmid DNA encoding a specific protein antigen was shown to induce expression of the protein in mouse myocytes (*1*), to elicit antibodies against the protein (*2*), and to manifest protection against influenza (*3*) and malaria (*4*) that was dependent on CD8$^+$ T cell responses against the expressed protein. Hundreds of publications have now reported the efficacy of DNA vaccines in small and large animal models of infectious diseases, cancer, and autoimmune diseases (*5*).

DNA vaccines elicit antibodies and CD4$^+$ T cell responses in animals, but their major advantage at the immunological level has been their capacity to induce antigen-specific CD8$^+$ T cell responses, including CTLs, which is a major mechanism of protection against intracellular pathogens. Important to our method of developing a malaria vaccine is the induction of CD8$^+$ T cell responses against *Plasmodium falciparum* –infected hepatocytes (*6*). The lysis of cells in a standard chromium release assay was used as a surrogate for antihepatocyte responses, because it has been established that CD8$^+$ CTLs, which recognize peptide-pulsed target cells, also recognize and eliminate parasite-infected hepatocytes (*6*). On the basis of our work with rodents (*4*, *7*) and our work and that of others with rhesus monkeys (*8*, *9*), we have developed a plan for manufacturing and testing the efficacy of a multigene *P. falciparum* liver-stage DNA vaccine in humans (*10*). This has been contingent on establishing that DNA vaccination of humans is safe and induces antigen-specific, genetically restricted, CD8$^+$ T cell–dependent CTLs. Recently, the presence of CTL responses in human immunodeficiency virus (HIV)–infected individuals after vaccination with plasmid DNA encoding the *nef*, *rev*, or *tat* genes or the *env* and *rev* genes of HIV was reported (*11*). Interpreting these results is difficult because of the concurrent HIV infection, which has been demonstrated to prime individuals for a CTL response that is independent of immunization.

Accordingly, 20 healthy, malaria-naïve adults were recruited and randomized into four dosage groups of five individuals. Three injections of 20, 100, 500, or 2500 μg of plasmid DNA encoding the *P. falciparum* circumsporozoite protein (PfCSP) (*12*) were administered at 4-week intervals in alternate deltoids (*13*). The details of recruitment, safety, and tolerability were reported elsewhere (*14*). To assess CTL responses, we collected peripheral blood mononuclear cells (PBMCs) from each volunteer before vaccination, 2 weeks after the second immunization, and 2 and 6 weeks after the third immunization. These cells were either assayed while fresh for recall antigen-specific CTL responses (*15*) or were frozen (*16*) for subsequent study. In parallel, CTL assays were carried out with PBMCs from nonimmunized control volunteers. Cytolytic activity was assessed after both primary and secondary in vitro restimulation against HLA-matched and HLA-mismatched PfCSP-specific and control targets. The percent lysis and the percent specific lysis were determined as described (*15*). The most sensitive and specific method (*17*) for demonstrating the presence of CTLs was with effector cells that were expanded in vitro by exposure to cells infected with canary pox (ALVAC) expressing the PfCSP (*18*) and with target cells that were sensitized with PfCSP-derived synthetic peptides (*19*). There was no apparent difference between the primary and secondary assays (*20*) or between the fresh and frozen specimens (*21*).

For logistical reasons, fresh PBMCs were studied only before vaccination and after the second immunization in the 20- and 100-μg-dosage groups but were studied before vaccination and after all immunizations in the 500- and 2500-μg-dosage groups, with the exception of one individual (*13*). For 14 individuals, adequate amounts of frozen PBMCs were available for further analysis. A typical pattern of CTL responses is presented in Fig. 1A. These responses were peptide-specific and genetically restricted because there was little or no recognition of autologous targets that were incubated with the control peptide or of HLA class I–mismatched targets that were incubated with the specific peptide. This activity was shown to be CD8$^+$ T cell–dependent by restimulating

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the effector cells and repeating the assay after the depletion of T cell subsets (Fig. 1B) (*22*). The simultaneous assessment of coded frozen PBMCs that were collected before and after vaccination (Fig. 1C) confirmed that these CTLs were induced after vaccination with a plasmid DNA and after subsequent translation of the encoded PfCSP.

The CTL responses with fresh PBMCs from 9 of 20 volunteers and with frozen PBMCs from 6 of 14 volunteers met our criteria for positivity. Eleven of 20 volunteers were shown to have antigen-specific, genetically restricted CTL activity. The effect of T cell subset depletion was studied in fresh cells that were acquired from volunteers in the 500- and 2500-μg-dosage groups after the third immunization. In the five responders tested, $CD8^+$ T cell depletion eliminated the CTL activity (Fig. 1B), and $CD8^+$ T cell dependence was demonstrated for all 10 peptides, except peptide $B35_{353}$. Volunteer 33 was the only volunteer who expressed HLA-B35. Accordingly, peptide $B35_{353}$ was tested only once [at 2 weeks after immunization (*13*)]—at which time peptide-specific, genetically restricted CTLs were detected, but $CD8^+$ dependence was not assessed. The presence of $CD4^+$ CTLs could not be completely excluded, because (in some cases) there was a reduction in cytolytic activity upon the depletion of $CD4^+$ T cells. However, this reduction was minor in relation to the effect of $CD8^+$ T cell depletion (Fig. 1B).

In nine volunteers, CTLs could not be detected in the three assays that were conducted after immunization. In three of these volunteers, the lack of response could not be attributed to a failure to respond to the vaccine, because these individuals did not express any of the HLA alleles restricting the peptides under study (volunteers 8 and 19, 20 μg; volunteer 20, 500 μg). Four of the other six nonresponders were in the two lower dosage groups.

The CTL responses of all volunteers to all peptides after immunization are summarized in Table 1. The representative data for each responder to each peptide are presented in Fig. 2. The responses after immunization (80 of 341

Table 1. Summary of overall CTL responses as assessed by vaccinia-stimulated effectors and peptide-sensitized targets with fresh and frozen PBMCs. Numbers separated by slashes without parentheses indicate the number of positive peptides out of the number of tested peptides; numbers separated by slashes in parentheses indicate the number of positive assays out of the number of assays. A positive assay result is defined as a percent specific lysis after vaccination that is >10% for at least two E:T ratios in the same assay, with a percent specific lysis before vaccination of <10%. In volunteers 7, 5, 13, and 37, CTLs against some peptides were detected before vaccination; these peptides were excluded from analysis for the respective individuals. nt, not tested.

Group data	2 weeks after second immunization	2 weeks after third immunization	6 weeks after third immunization	Total	Range of percent specific lysis
		Group 1 (20 μg)			
Volunteer code					
7	2/4 (2/8)	4/7 (4/7)	0/7 (0/7)	5/7 (6/22)	10.0–33.7
8	0/1 (0/2)	0/1 (0/1)	0/1 (0/1)	0/1 (0/4)	
12	0/2 (0/4)	nt	1/2 (1/2)	1/2 (1/6)	12.4–22.6
13	0/5 (0/8)	0/6 (0/6)	0/6 (0/6)	0/6 (0/20)	
19	0/1 (0/2)	0/1 (0/1)	0/1 (0/1)	0/1 (0/2)	
Overall	2/13 (2/24)	4/15 (4/15)	1/17 (1/17)	6/17 (7/54)	10–22.6
Percentage of positive peptides (percentage of positive assays)	15.4% (8.3%)	26.7% (26.7%)	5.9% (5.9%)	35.3% (13.0%)	
		Group 2 (100 μg)			
Volunteer code					
5	0/4 (0/4)	3/6 (3/6)	1/6 (1/6)	4/6 (4/16)	10.0–16.1
6	2/4 (2/8)	nt	nt	2/4 (2/8)	10.0–22.9
18	0/2 (0/4)	0/2 (0/2)	0/2 (0/2)	0/2 (0/8)	
22	0/4 (0/4)	0/5 (0/5)	0/5 (0/5)	0/5 (0/14)	
26	0/4 (0/8)	0/6 (0/6)	0/1 (0/1)	0/6 (0/15)	
Overall	2/18 (2/28)	3/19 (3/19)	1/14 (1/14)	6/23 (6/61)	10.0–22.9
Percentage of positive peptides (percentage of positive assays)	11.1% (7.1%)	15.8% (15.8%)	7.1% (7.1%)	26.1% (9.8%)	
		Group 3 (500 μg)			
Volunteer code					
20	0/1 (0/2)	0/1 (0/2)	0/1 (0/2)	0/1 (0/6)	
23	0/4 (0/8)	0/5 (0/8)	4/5 (4/13)	4/5 (4/29)	10.8–26.7
27	0/3 (0/6)	2/4 (2/8)	4/4 (4/11)	4/4 (6/25)	10.7–51.9
36	0/4 (0/8)	6/7 (12/14)	6/7 (6/17)	7/7 (18/39)	10.9–55.4
40	0/4 (0/8)	0/4 (0/8)	0/5 (0/10)	0/5 (0/26)	
Overall	0/16 (0/32)	8/21 (14/40)	14/22 (14/53)	15/22 (28/125)	10.7–55.4
Percentage of positive peptides (percentage of positive assays)	0% (0%)	38.1% (35.0%)	63.6% (26.4%)	68.2% (22.4%)	
		Group 4 (2500 μg)			
Volunteer code					
21	0/2 (0/4)	0/2 (0/4)	0/2 (0/4)	0/2 (0/12)	
29	1/1 (1/2)	0/1 (0/2)	0/1 (0/2)	1/1 (1/6)	16.6–28.5
33	4/4 (4/8)	nt	nt	4/4 (4/8)	10.1–31.1
37	1/4 (1/8)	1/6 (3/16)	3/7 (6/17)	3/7 (9/41)	10.0–67.73
39	4/4 (6/8)	5/5 (6/10)	5/5 (7/14)	5/5 (19/32)	10.5–37.9
Overall	10/15 (12/30)	6/14 (9/32)	8/15 (13/37)	13/17 (33/99)	10.0–67.73
Percentage of positive peptides (percentage of positive assays)	76.9% (40%)	42.9% (28.1%)	53.3% (35.1%)	76.5% (33.3%)	

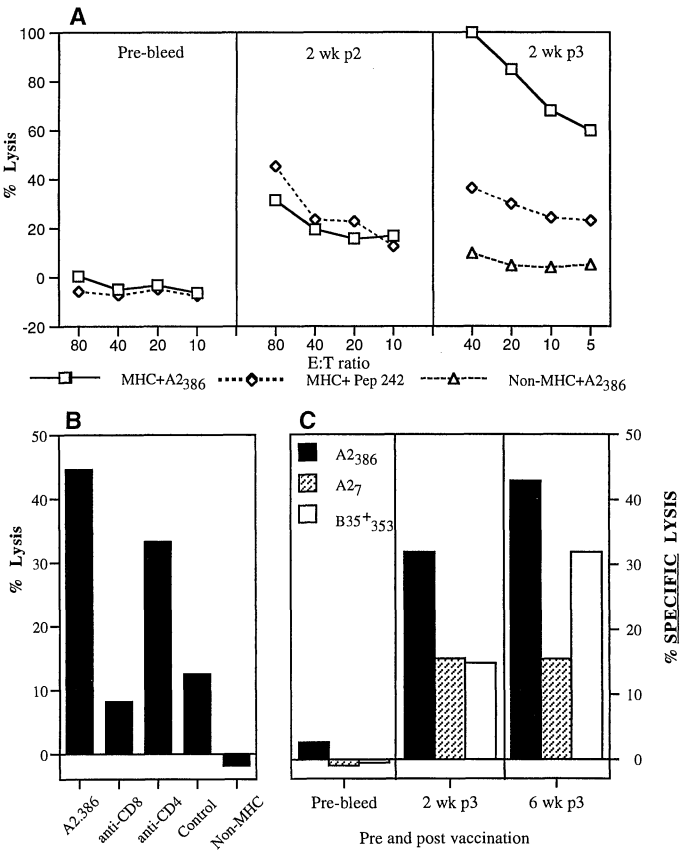

Fig. 1. (**A**) Peptide-specific recall CTL response to vaccination with plasmid DNA. Fresh PBMCs from volunteer 37 (2500-μg-dosage group) [which were collected before vaccination (pre-bleed), 2 weeks after the second immunization (2 wk p2), and 2 weeks after the third immunization (2 wk p3)] were stimulated in vitro with ALVAC expressing the PfCSP for 7 days and then were assayed against HLA class I–matched or HLA class I–mismatched PfCSP peptide–pulsed or control peptide 242–pulsed targets in a conventional chromium release assay (*15*). MHC, major histocompatibility complex. (**B**) DNA-induced CTL responses are $CD8^+$ T cell–dependent. ALVAC PfCSP–stimulated effector cell populations from volunteer 37 were depleted in vitro of $CD8^+$ or $CD4^+$ T cells (*22*) immediately before the chromium release assay. Results of a 13-day assay at an E:T ratio of 20:1 are presented. (**C**) DNA-induced CTL responses with frozen PBMCs (*16*). Coded frozen PBMCs from volunteer 37 were assayed for peptide-specific CTLs. The % specific lysis in a 7-day assay at an E:T ratio of 20:1 is presented.

assays, 23.4%) were significantly greater than those before immunization (6 of 139 assays, 4.3%) ($P = 0.0000007$, chi-squared test). Two of the 82 assays (2.4%) that were conducted with PBMCs from control (nonimmunized) volunteers were positive, which is significantly less than those assays that were conducted with PBMCs after immunization ($P = 0.000015$).

An apparent positive response was noted with one peptide for volunteers 5 (1 of 12 assays), 7 (1 of 10 assays), and 13 (1 of 8 assays) and with three peptides for volunteer 37 (3 of 11 assays) in secondary but not primary assays of fresh PBMCs that were collected before immunization. However, CTL responses after vaccination were significantly enhanced in relation to the levels before vaccination. Furthermore, no activity was detected when the assay was repeated with frozen PBMCs (*21*). Nevertheless, in accordance with our conservative definition of positivity, all peptides with ≥10% specific lysis before vaccination were excluded from subsequent analysis for the respective individual.

Peptide-specific, genetically restricted, and $CD8^+$ T cell–dependent CTL responses were induced by as little as two 20-μg doses of DNA (Table 1). The induction of CTLs after a single immunization was not tested. CTL responses were detected in two of five volunteers immunized with 20 μg of DNA or 100 μg of DNA, in three of five volunteers immunized with 500 μg of DNA, and in four of five volunteers immunized with 2500 μg of DNA. Data indicate that immunization with either 500 or 2500

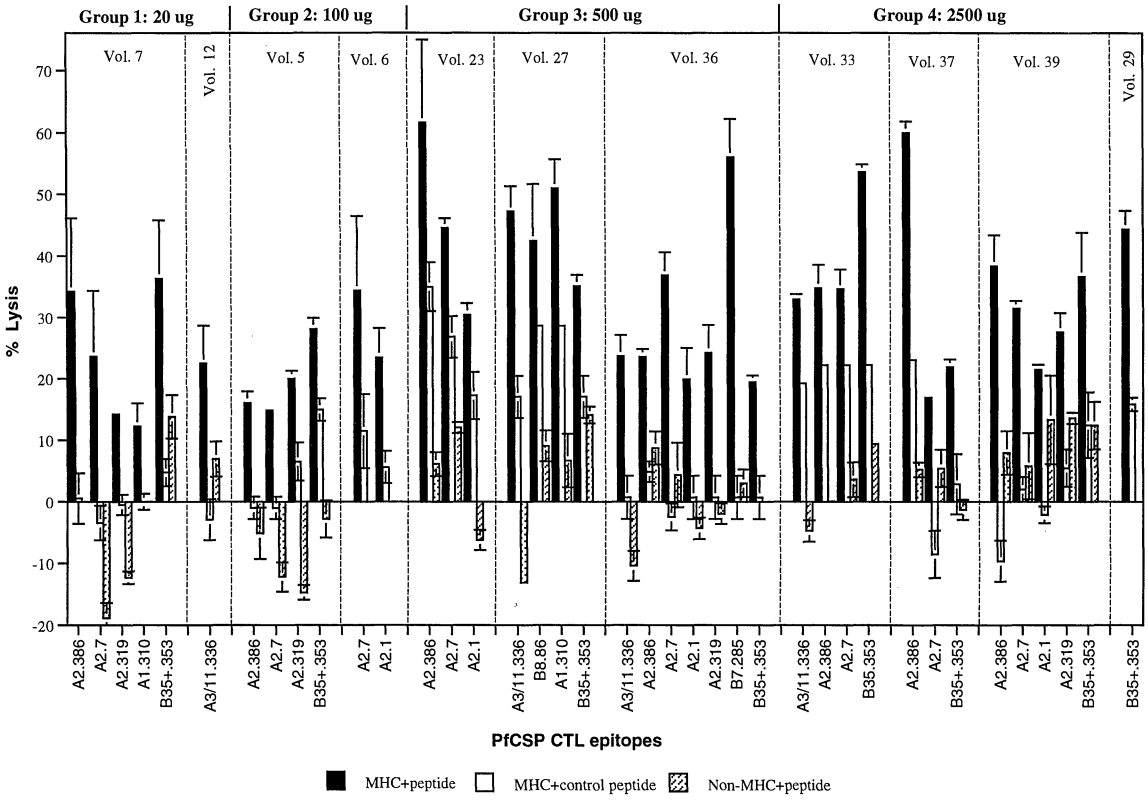

Fig. 2. Representative data of positive (difference between the percent lysis of target cells pulsed with experimental or control peptides ≥10%) CTL responses for each volunteer for each peptide. Fresh or frozen PBMCs, taken at the same or different time points, were assayed for peptide-specific, genetically restricted CTLs as described in the caption of Fig. 1A. Shown is the percent lysis (mean ± SEM) for each peptide with its simultaneously assessed controls at a single E:T ratio. Error bars indicate SEM.

μg of DNA induced a significantly better CTL response in comparison with either 20 or 100 μg of DNA ($P \leq 0.0003$). There was no significant difference between 500- and 2500-μg dosages overall or after the third immunization ($P \geq 0.53$), but a significantly higher frequency of response was induced with 2500 μg of DNA in comparison to 500 μg of DNA after the second immunization ($P = 0.000001$). There was no significant difference between 20- and 100-μg dosages at any time ($P \geq 0.43$).

With regard to the immunization schedule, overall, the rate of positive assays 2 weeks after the third immunization (31 of 106) was significantly greater than the rate after the second immunization (19 of 114) ($P = 0.026$); there was no significant difference between the 2- and 6-week time points after the third immunization (31 of 106 versus 30 of 121) ($P = 0.45$) (Table 1).

The frequency and magnitude of the CTL responses to specific peptides are summarized in Table 2. Overall, 5 of the 11 responders recognized 100% of the peptides studied, 3 responders recognized 60 to 70% of the peptides, and 3 others recognized 43 to 50% of the peptides.

The DNA-induced CTLs were genetically restricted by multiple HLA alleles (Table 2). Representative data are presented in Fig. 3. There was no apparent hierarchy in terms of allele-specific recognition. The magnitude of the induced CTL responses to defined epitopes varied between volunteers. Overall, the best response was detected for the HLA-A2 restricted epitope, $A2_{386}$ (Table 2). This response was not substantially different than the responses that were noted for the peptides $A1_{310}$, $A2_{7}$, $A3/11_{336}$, $B7_{285}$, $B35_{353}$, and $B35+_{353}$. Induction by subunit vaccines of $CD8^+$ T cell–dependent immune responses of multiple HLA restrictions in the same individual has, to our knowledge, not previously been reported for any infectious agent in humans. This has been a major obstacle to vaccine development and will be critical to the success of a malaria vaccine, because T cell responses to individual epitopes are genetically restricted and there is a substantial allelic variation of CTL epitopes among *P. falciparum* isolates in nature (*23*). Indeed, it has been demonstrated that the irradiated *P. falciparum* sporozoite vaccine, which confers potent protective immunity in humans (*6*), induces CTL responses that are restricted by multiple HLA alleles in genetically diverse individuals (*24*).

In mice, immunization with a *P. yoelii* circumsporozoite protein DNA vaccine elicits a substantially greater CTL response than does immunization with irradiated sporozoites (*4*). We did not simultaneously compare CTL responses in our vaccine recipients with CTL responses in individuals who were immunized with irradiated sporozoites or naturally exposed to malaria. However, as in the rodent model (*4*), the magnitude of the CTL response that was seen in some of the volunteers (Fig. 1) was considerably higher than the response that is generally seen in humans exposed to irradiated sporozoites or to natural infection (*24–27*).

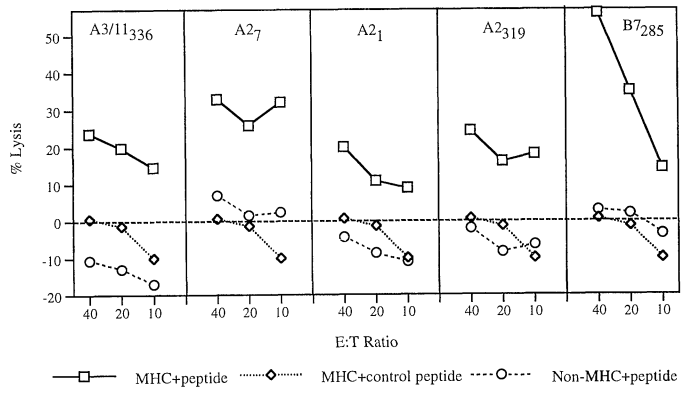

Fig. 3. DNA-induced CTL responses are restricted by multiple HLA alleles. Fresh PBMCs from volunteer 36 (500-μg-dosage group), who expressed the alleles HLA-A2, A3, and B7, were assayed for antigen-specific, genetically restricted CTLs (*15*). The assay was repeated with coded frozen PBMCs (*16*) that were collected before and after vaccination; the results confirmed that the peptide-specific (the same five peptides), genetically restricted CTLs were induced by vaccination with plasmid DNA (*21*).

Table 2. HLA restriction and magnitude and frequency of CTL responses for each of the 10 peptides studied.

Peptide	HLA restriction	Percent specific lysis range	Number of positive assays/total assays (%)	Number of responders/ number tested
Al_{310}	A1	12.3–18.0	3/16 (18.8)	2/4
$A2_{386}$	A2 supertype	10.2–67.7	15/42 (35.7)	7/12
$A2_{7}$	A2 supertype	10.0–36.9	15/46 (32.6)	7/12
$A2_{1}$	A2.1	10.8–23.2	7/32 (21.9)	4/11
$A2_{319}$	A2.1	10.5–24.6	11/39 (28.2)	5/11
$A3/11_{336}$	A3/11 supertype	10.0–51.9	8/27 (29.6)	4/7
$B7_{285}$	B7	10.2–55.4	3/12 (25.0)	1/3
$B8_{86}$	B8	11.7–14.0	2/9 (22.2)	1/2
$B35_{353}$	B35	23.1–31.4	1/2 (50.0)	1/1
$B35_{+353}$	B35 plus	10.0–37.9	9/61 (14.8)	7/18

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12. The full-length PfCSP gene [1194 base pairs (bp)] from the *P. falciparum* clone 3D7 [J. R. Campbell, *Nucleic Acids Res.* **17**, 5854 (1989)] was cloned into the eukaryotic expression vector VR1020 [C. J. Luke, K. Carner, X. Liang, A. G. Barbour, *J. Infect. Dis.* **175**, 91 (1997)] as an in-frame fusion with the human tissue plasminogen activator leader peptide to create Vical Clinical plasmid VCL-2510. Clinical supplies and a qualification of this construct were produced under good manufacturing practices (S. E. Parker *et al.*, in preparation). Four vaccine formulations were stored as 1.0-ml doses at −20°C and then thawed at room temperature for 30 min before injection. In vitro expression and in vivo immunogenicity of VCL-2510 in rodents and nonhuman primates have been reported elsewhere (*8*) [R. C. Hedstrom *et al.*, *Int. J. Mol. Med.* **2**, 29 (1998).
13. The study was a dose-escalating phase I safety and immunogenicity trial in healthy adult volunteers with informed consent. A total of 28 healthy, malaria-naïve volunteers were selected for the study on the basis of negative serologic studies for PfCSP, HIV, hepatitis B virus, hepatitis C virus, and smallpox. The volunteers were between 20 and 29 years old, and 61% were male. Complete class I and class II HLA typing profiles were obtained (*21*). Eight volunteers did not receive the PfCSP DNA vaccine and served as assay controls. Volunteer 33 (2500-μg-dosage group) was withdrawn from study after the second immunization because of an unplanned pregnancy.
14. T. P. Le *et al.*, in preparation.
15. PBMCs were cultured in RPMI 1640 supplemented with 10 mM Hepes, 2 mM L-glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml) (Life Technologies, Grand Island, NY), and 10% heat-inactivated type AB human serum (ICN Biomedical, Costa Mesa, CA). To generate effector cells, we infected 20% of the total PBMCs with ALVAC expressing the PfCSP (vCP182) (*18*) at 5 plaque-forming units per cell for 90 min at 37°C; these PBMCs were washed twice, combined with the remaining PBMCs, and cultured at 3×10^6 cells per 2 ml in 24-well plates for 7 days. Recombinant human interleukin-2 (rIL-2) (Cetus, Emeryville, CA) was added after 48 hours (20 U/ml). For secondary stimulation (13-day assay), rIL-7 (Peprotech, Rocky Hill, NJ) was added at the

initiation of the culture at 100 U/ml. On day 8, 6×10^6 to 10×10^6 responder cells were incubated with 1×10^7 autologous phytohemagglutinin (PHA) blasts infected with vCP182 and were cultured at 1×10^6 cells/ml in a 25-cm^2 flask in the presence of rIL-2 (20 U/ml) and rIL-7 (100 U/ml) for an additional 5 days. PHA blasts were generated by stimulating PBMCs with 0.2% (v/v) PHA (Sigma). Primary CTL assays were performed on day 7. Secondary CTL assays were performed on day 13 after restimulation on day 8. Target cells were autologous or HLA-mismatched PHA blasts that were sensitized overnight with synthetic peptides (10 μg/ml) representing previously identified CTL epitopes on the PfCSP (*19*). Targets were labeled with 100 μCi ^{51}Cr [sodium chromate solution (Dupont New England Nuclear, Boston, MA)] for 90 min at 37°C and washed three times before use. The CTL activity was assessed by a conventional 6-hour chromium release assay, in the presence of a peptide (10 μg/ml). The percent lysis was defined as (experimental release − medium control release)/(maximum release − medium control release) × 100. The percent specific lysis was determined by subtracting the percent lysis of the targets that were sensitized with control peptide 242 from the percent lysis of the targets that were incubated with the experimental peptide. The results were expressed as the mean of triplicate determinations. The CTL responses were considered to be positive only if the percent specific lysis after immunization was ≥10% for at least two effector:target (E:T) ratios in the same assay and if the percent specific lysis before immunization was <10%. Spontaneous release values were always <20%.

16. PBMCs were resuspended at a concentration of 10×10^6 cells/ml in 20% fetal calf serum (Sigma) in RPMI 1640, and an equal volume of ice-cold 15% dimethyl sulfoxide in RPMI 1640 was added dropwise, with shaking. All procedures were performed on ice. The cells were transferred to cryotubes at a final concentration of 5×10^6 cells/ml in each vial, and the tubes were placed in a plastic foam container at −80°C overnight before being transferred to liquid nitrogen.
17. The CTL assays were conducted with four E:T combinations: (i) ALVAC PfCSP effectors and Western Reserve (WR) vaccinia PfCSP targets, (ii) ALVAC PfCSP effectors and experimental or control peptide–pulsed targets, (iii) experimental peptide–induced effectors and WR vaccinia PfCSP targets, and (iv) experimental peptide–induced effectors and experimental or control peptide–pulsed targets. All assays with WR vaccinia PfCSP-infected targets were excluded from the analysis because a simultaneous assay of PBMCs from control-naïve volunteers demonstrated an unacceptably high level of positivity (*21*). Assays that were conducted with PfCSP peptide-stimulated effectors against peptide-sensitized targets were not positive.
18. Recombinant pox viruses were produced in collaboration with Virogenetics (Troy, NY) [J. A. Tine *et al.*, *Infect. Immun.* **64**, 3833 (1996); D. E. Lanar *et al.*, *ibid.*, p. 1666]. The ALVAC virus expressing PfCSP (vCP182) was used for the stimulation of CTL effectors. Recombinant vaccinia viruses (WR) encoding PfCSP (vP1255) or PfLSA-1 (vP1253) (control) were used for the infection of target cells.
19. The following peptide sequences and residue numbers are based on the complete PfCSP 3D7 amino acid sequence (residues 1 through 397), and variant residues are indicated in bold and underlined: (i) $A1_{310}$, HLA-A1 restricted, residues 310 through 319, sequence EPSDKHI**KE**Y (*28, 29*); (ii) $A2_{386}$, HLA-A2 supertype, residues 386 through 394, GLIMVLSFL (*24*); (iii) $A2_{7}$, HLA-A2 supertype, residues 7 to 16, ILSVSSFLFV (*24*); (iv) $A2_{1}$, HLA-A2.1, residues 1 through 10, MMRKLAILSV (*30*); (v) $A2_{319}$, HLA-A2.1, residues 319 through 327, YL**N**KI**Q**NSL (*30*); (vi) $A3/11_{336}$, HLA-A3/11 supertype, residues 336 through 345, VTCGNGIQVR (*24*); (vii) $B7_{285}$, HLA-B7, residues 285 through 293, MPN**D**PNRNV (*25*); (viii) $B8_{86}$, HLA-B8, residues 86 through 94, LRKPKHKKL (*25*); (ix) $B35_{353}$, HLA-B35, residues 353 through 360, K**P**-KDELDY (*26*); (x) $B35+_{353}$, HLA-B35 plus (contains a HLA-B35 restricted epitope but also contains one or two additional epitopes of undefined genetic restriction), residues 353 through 375, K**P**KDELDYANDIEK-KICKKMEKCS (*27*); (xi) Pep242, random sequence, RALMSMVLIK. PfCSP-derived synthetic peptides were synthesized by Pasteur-Merieux Connaught Laboratories and were purified through high-performance liquid chromatography. Control peptide 242 was generated by a random scrambling of the HLA-A2 binding peptide, $A2_{1}$. Lyophilized peptides were reconstituted at 20 mg/ml with 100% dimethyl sulfoxide (Sigma) and stored at −80°C until use. The peptide was diluted to 2 mg/ml with RPMI 1640 without serum before use.
20. A comparison of the primary assay (one in vitro restimulation) versus the secondary assay (two in vitro restimulations) gave the following results: The number of positive individuals out of the total number of tested individuals was 10 of 20 versus 6 of 20; the number of positive assays out of the total number of assays was 52 of 218 (22.8%) versus 28 of 123 (23.9%); the range of the percent specific lysis was 10.2 to 67.7% versus 10.1 to 37.91%. Volunteer 33, who was not positive in the primary assay, was only studied at one time point (*13*).
21. R. Wang *et al.*, data not shown.
22. The ALVAC PfCSP–stimulated effector cell populations were depleted in vitro of $CD8^+$ or $CD4^+$ T cells immediately before the assay with anti-$CD4^+$– or anti-$CD8^+$–coated Dynabeads M-450, according to the manufacturer's instructions (Dynal, Great Neck, NY). Flow cytometric analysis confirmed that the cell subset depletion was >95% in all cases (*21*).
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29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Differentiation of Monocytes into Dendritic Cells in a Model of Transendothelial Trafficking

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Essential to the dendritic cell system of antigen-presenting cells are the veiled dendritic cells that traverse afferent lymph to enter lymph nodes, where they initiate immune responses. The origin of veiled cells, which were discovered 20 years ago, is unclear. Monocytes cultured with endothelium differentiated into dendritic cells within 2 days, particularly after phagocytosing particles in subendothelial collagen. These nascent dendritic cells migrated across the endothelium in the ablumenal-to-lumenal direction, as would occur during entry into lymphatics. Monocytes that remained in the subendothelial matrix became macrophages. Therefore, monocytes have two potential fates associated with distinct patterns of migration.

One of the important features of dendritic cells (DCs) is their capacity to migrate from peripheral tissues to lymphoid organs and initiate immunity. DCs gain access to the spleen from the bloodstream and enter lymph nodes by migration through afferent lymphatic vessels (*1*). Bearing soluble proteins (*2*) and particulates (*3*) that they acquire before entry into lymph nodes, DCs localize to the T cell areas where they are ideally positioned to select and activate clones of antigen-reactive T lymphocytes. When afferent lymphatic conduits are severed, immunity to peripherally administered antigen does not develop (*4*). Lymph DCs may also induce peripheral tolerance to self-antigens acquired during the

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