

RE

AD-A283 953

Form Approved
OMB No. 0704-0188Public reporting burden for
gathering and maintaining
collection of information, if
Data Highway, Suite 1204,including the time for reviewing instructions, searching existing data sources,
tion. Send comments regarding this burden estimate or any other aspect of this
in Services, Directorate for Information Operations and Reports, 1215 Jefferson
Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		1994		REPORT TYPE AND DATES COVERED Journal Article																					
4. TITLE AND SUBTITLE Complete protection against Plasmodium yoelii by adoptive transfer of a CD8+ cytotoxic T-cell clone recognizing sporozoite surface protein 2				5. FUNDING NUMBERS PE - 61102A PR - 001.01 TA - BFX WU - 1431																					
6. AUTHOR(S) Khusmith S, Sedegah M, Hoffman SL																									
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Medical Research Institute Commanding Officer 8901 Wisconsin Avenue Bethesda, Maryland 20889-5607				8. PERFORMING ORGANIZATION REPORT NUMBER NMRI 94-27																					
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Naval Medical Research and Development Command National Naval Medical Center Building 1, Tower 12 8901 Wisconsin Avenue Bethesda, Maryland 20889-5606				10. SPONSORING / MONITORING AGENCY REPORT NUMBER DN244530																					
11. SUPPLEMENTARY NOTES Reprinted from: Infection and Immunity 1994 July Vol.62 No.7 pp.2979-2983																									
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.				12b. DISTRIBUTION CODE																					
13. ABSTRACT (Maximum 200 words)																									
<table border="1"> <tr> <td colspan="2">Accession For</td> </tr> <tr> <td>NTIS CRA&I</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>DTIC TAB</td> <td><input checked="" type="checkbox"/></td> </tr> <tr> <td>Unannounced</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Justification</td> <td></td> </tr> <tr> <td colspan="2">By _____</td> </tr> <tr> <td colspan="2">Distribution / _____</td> </tr> <tr> <td colspan="2">Availability Codes</td> </tr> <tr> <td>Dist</td> <td>Avail and/or Special</td> </tr> <tr> <td>A-1</td> <td>20</td> </tr> </table>						Accession For		NTIS CRA&I	<input checked="" type="checkbox"/>	DTIC TAB	<input checked="" type="checkbox"/>	Unannounced	<input type="checkbox"/>	Justification		By _____		Distribution / _____		Availability Codes		Dist	Avail and/or Special	A-1	20
Accession For																									
NTIS CRA&I	<input checked="" type="checkbox"/>																								
DTIC TAB	<input checked="" type="checkbox"/>																								
Unannounced	<input type="checkbox"/>																								
Justification																									
By _____																									
Distribution / _____																									
Availability Codes																									
Dist	Avail and/or Special																								
A-1	20																								
14. SUBJECT TERMS malaria; cytotoxic T lymphocytes; immunology; plasmodium yoelii sporozoite surface protein 2				15. NUMBER OF PAGES 5																					
				16. PRICE CODE																					
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited																						

DTIC
SELECTE
SEP 01 1994
S G D

Complete Protection against *Plasmodium yoelii* by Adoptive Transfer of a CD8⁺ Cytotoxic T-Cell Clone Recognizing Sporozoite Surface Protein 2

SRISIN KHUSMITH,¹* MARTHA SEDEGAH,^{1,2} AND STEPHEN L. HOFFMAN¹*

*Malaria Program, Naval Medical Research Institute, Bethesda, Maryland 20889-5607,¹
and Pan American Health Organization, Washington, D.C. 20037²*

Received 14 February 1994/Returned for modification 25 March 1994/Accepted 1 April 1994

BALB/c mice immunized with irradiated *Plasmodium yoelii* sporozoites produce antibodies and cytotoxic T lymphocytes against the circumsporozoite protein and against a 140-kDa protein, sporozoite surface protein 2 (PySSP2). Approximately 50% of mice immunized with P815 cells transfected with the gene encoding PySSP2 are protected against malaria, and this protection is reversed by in vivo depletion of CD8⁺ T cells. To determine if CD8⁺ T cells against PySSP2 are adequate to protect against malaria in the absence of other malaria-specific immune responses, we produced three CD8⁺ T-cell clones by stimulating spleen cells from mice immunized with irradiated *P. yoelii* sporozoites with a mitomycin-treated P815 cell clone transfected with the PySSP2 gene. Adoptive transfer of clone TSLB7 protected 100% of mice against *P. yoelii*. The second clone protected 58% of mice, and the third clone provided no protection. Clone TSLB7 protected even when administered 3 h after sporozoite inoculation at a time when sporozoites had entered hepatocytes, suggesting that it is recognizing and eliminating infected hepatocytes. These studies demonstrate that cytotoxic T lymphocytes against PySSP2 can protect against *P. yoelii* sporozoite challenge in the absence of other parasite-specific immune responses.

Vaccination with radiation-attenuated sporozoites protects mice, monkeys, and humans against malaria (reviewed in reference 7). In some mice, this immunity is eliminated by depletion of CD8⁺ T cells (24, 29), indicating that cytotoxic T lymphocytes (CTL), probably directed against malaria antigens on the surface of infected hepatocytes (8, 28), are required for this immunity. Mice (10, 23, 28) and humans (12) immunized with irradiated sporozoites produce CD8⁺ CTL against the major sporozoite surface protein, the circumsporozoite protein (CSP). Such CTL against the *Plasmodium yoelii* CSP (PyCSP) eliminate malaria-infected hepatocytes in culture (28), and CTL clones against defined epitopes on the *Plasmodium berghei* (23) and *P. yoelii* (20, 27) CSPs adoptively transfer protection. Accordingly, the CSP is a target for vaccine development (7). However, vaccines based on the CSP alone have not provided protection comparable to that found after immunization with irradiated sporozoites, and there has been a search for additional targets of protective immunity (reviewed in reference 7).

Mice immunized with irradiated *P. yoelii* sporozoites produce antibodies (2) and CTL (9) against a 140-kDa protein called sporozoite surface protein 2 (PySSP2). We have reported the nucleotide sequence of the gene encoding the 826-amino-acid PySSP2 (6, 22) and that 33 to 67% of mice immunized with P815 cells (mastocytoma cells) transfected with a 1.5-kb gene fragment of PySSP2 were protected against sporozoite challenge (9). As with the irradiated sporozoite vaccine, the immunity induced by immunization with this PySSP2 vaccine was eliminated by in vivo depletion of CD8⁺ T

cells. The current studies were undertaken to determine if CD8⁺ CTL against PySSP2 can protect against malaria in the absence of other parasite-specific immune responses.

MATERIALS AND METHODS

Mice. Six-week-old female BALB/cByJ mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. The experiments reported herein were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*.

Parasite. *P. yoelii* 17X, nonlethal strain (clone 1.1), was used for all experiments. For in vitro and in vivo infections, salivary glands of *Anopheles stephensi* were dissected by hand. For immunizations, sporozoites were isolated by discontinuous gradient centrifugation (16) from infected mosquitoes that had been exposed to 10,000 rads from a ¹³⁷Cs source. The dose of this parasite that infects 50% of mice is usually between two and eight sporozoites (9).

PySSP2 transfectant. PySSP2-transfected P815 cell clone 3.9 (PySSP2-P815 3.9) (9) was used in all experiments. This clone was obtained by cotransfecting the mouse tumor cell line P815 (H-2^d mastocytoma) by calcium phosphate precipitation with *P. yoelii* DNA including a 1.5-kb fragment of the gene encoding PySSP2 cloned into expression vector pcEXV-3 and pSV2neo DNA as described previously (9). The clones were grown in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum, 8 mM L-glutamine, 4 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), and 50 U of penicillin and streptomycin per ml (complete DMEM medium) plus 200 µg of G418 (Geneticin; Gibco) per ml.

T-lymphocyte clones. BALB/c mice were immunized intravenously three times at 2- to 4-week intervals with live irradiated (10,000 rads; ¹³⁷Cs) sporozoites (5 × 10⁴, 3 × 10⁴, and 3 × 10³ sporozoites). Two weeks after the last immunization, T-cell lines were initiated by stimulating 5 × 10⁶ spleen cells

* Corresponding author. Mailing address: Malaria Program, Naval Medical Research Institute, 12300 Washington Ave., Rockville, MD 20852. Phone: (301) 295-0026. Fax: (301) 295-6171.

† Present address: Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand.

94-28350



68

from these mice with 2×10^6 SSP2-P815 3.9 cells previously treated with 50 μ g of mitomycin per ml for 30 min in 2 ml of DMEM complete medium containing 5×10^{-5} M 2-mercaptoethanol for 7 days. Viable cells were separated from dead cells by density gradient centrifugation with lymphocyte separation medium (Organon Teknika, Durham, N.C.) and then restimulated with mitomycin-treated SSP2-P815 3.9 cells for 7 days in complete medium containing 5×10^{-5} M 2-mercaptoethanol and 50 U of human recombinant interleukin-2 (rIL-2; Cetus Corp., Emeryville, Calif.) per ml. At 3- to 4-day intervals, the medium was replaced with fresh medium containing 50 U of human rIL-2 per ml. After the third in vitro stimulation, T cells were cloned by limiting dilution at 10, 3, and 1 cells per well in 96-well plates (Costar, Cambridge, Mass.) in the presence of irradiated, syngeneic, unprimed spleen cells as feeders and mitomycin-treated SSP2-P815 3.9 cells as stimulators. Cells from wells with signs of growth were expanded in the DMEM complete medium containing 20 U of human rIL-2 per ml and then tested for CTL activity against ^{51}Cr -labeled SSP2-P815 3.9 cells. Recloning was done once by limiting dilution. Once established, the CTL clones were maintained and stimulated on the schedule described above. Before each assay, viable cloned T cells were isolated by density gradient centrifugation. Clones were tested for phenotype by microfluorometry.

CTL assay. After in vitro stimulation for 6 to 7 days, cytolytic activity of the effector cells was measured as described previously (9) by using a 6-h assay with ^{51}Cr -labeled targets. The CD8^+ T-cell clones were mixed at effector-to-target (E/T) cell ratios of 10:1, 3.3:1, 1.1:1, and 0.3:1 with 5,000 targets, namely, ^{51}Cr -labeled PySSP2-P815 3.9 cells, ^{51}Cr -labeled pSV2neo-transfected P815 cells, and ^{51}Cr -labeled P815 cells. Cultures were incubated for 6 h at 37°C in an atmosphere of 5% CO_2 . Supernatants were harvested by using the SCS system (Skatron, Inc., Sterling, Va.), and released ^{51}Cr was detected by a gamma counter (Clinigamma-1272; LKB, Gaithersburg, Md.). Experimental wells were reproduced in triplicate. The percent specific ^{51}Cr release was calculated as $100(\text{experimental release} - \text{spontaneous release})/(\text{maximum release} - \text{spontaneous release})$. Maximum release was determined from supernatants of cells that were lysed by the addition of 5% Triton X-100. Spontaneous release was determined from target cells incubated without effector cells.

Adoptive transfer of T-cell clones. PySSP2-specific CTL clones, harvested 7 days after restimulation with PySSP2-P815 3.9 and feeder cells, were adoptively transferred to irradiated 8- to 10-week-old BALB/c mice (500 rads; ^{137}Cs) by intravenous (i.v.) injection. Immediately before transfer, the CTL clones were washed and resuspended in 0.5 ml of DMEM medium without serum and containing 10^3 U of human rIL-2. A second dose (10^3 U) of rIL-2 was administered intraperitoneally 20 h after cell transfer. The mice were challenged 24 h after cell transfer by i.v. injection of *P. yoelii* sporozoites. A final dose of 10^3 U of rIL-2 was administered i.v. 24 h after the sporozoite challenge. As controls, mice were injected i.v. with either the human immunodeficiency virus (HIV)-specific CTL clone RT-1 (26) or with spleen cells from normal, uninfected BALB/c mice. Prior to administration, the RT-1 cells (2×10^5 cells) were stimulated in vitro every 2 weeks with BALB/c 3T3 cells (5×10^5 cells) expressing HIV gp160 (transfected cell line 15-12) (26) pretreated with 100 μ g of mitomycin per ml for 40 min at 37°C in medium containing 10 U of rIL-2 per ml. Thin blood films were prepared on days 7, 10, and 14 after challenge, stained with Giemsa, and examined by light microscopy to detect parasitized erythrocytes. Protection was defined as absence of parasitemia.

IFN- γ , IL-2, IL-4, and IL-5 production by the CTL clones. Cloned T cells (10^6) prestimulated in vitro with PySSP2-P815 3.9 cells were washed twice with DMEM medium and restimulated in vitro with irradiated SSP2-P815 3.9 cells as described previously in complete DMEM medium without human rIL-2. After 48 h of incubation, the supernatant was harvested and assayed for gamma interferon (IFN- γ), interleukin-2 (IL-2), IL-4, and IL-5 by standard methods (4, 15, 25).

RESULTS

In vitro characterization of CTL clones. To isolate T-cell clones, mice were immunized with irradiated sporozoites, and their spleen cells were stimulated in vitro with PySSP2-P815 3.9 cells. Ten clones were derived by limiting dilution and tested for CTL activity against PySSP2-P815 3.9. All 10 CTL clones lysed PySSP2-P815 3.9 in vitro (data not shown). The three most active CTL clones, TSLB7 (Fig. 1a), TSL14.18 (Fig. 1b), and TSL10.21 (Fig. 1c) were selected for further study. All were CD8^+ and CD4^- . All had greater than 50% cytolytic activity at an E/T cell ratio of 3.3:1 (Fig. 1). All three clones produced IFN- γ (TSLB7, 2.7 ng/ml; TSL14.18, 1.2 ng/ml; TSL10.21, 0.7 ng/ml), but none produced detectable IL-2, IL-4, or IL-5.

In vivo activity of CD8^+ CTL clones. When mice are immunized with PySSP2-P815 3.9, about 50% are consistently protected against malaria, and the protection is completely eliminated by in vivo depletion of CD8^+ T cells (9). Our previous data demonstrated a requirement for SSP2-specific CTL in SSP2-P815 3.9-induced protective immunity but did not prove that CTL against SSP2 were adequate to protect on their own. SSP2-specific CTL clones were therefore produced to determine if CTL against SSP2 could protect against malaria independent of any other antigen-specific acquired immune responses. The data presented in Table 1 indicate that this is the case. Adoptive transfer of CTL clone TSLB7 consistently protected 100% of recipients, while transfer of CTL clone TSL14.18 protected 50 to 67% of recipients. Transfer of clone TSL10.21, which has the same apparent cytolytic activity as the other two clones (Fig. 1) but produces much less IFN- γ , did not protect (Table 1).

Having established that two of the CTL clones provide protection after adoptive transfer to naive, irradiated mice, we wondered where in the life cycle this activity was occurring. Infected hepatocytes are the logical target for such activity (7, 8, 19, 28). Within several minutes after i.v. inoculation, most sporozoites enter hepatocytes. To determine if the CTL were acting after the sporozoites had entered the hepatocytes, we adoptively transferred CTL clone TSLB7 3 h after sporozoite inoculation; TSLB7 provided 83% protection (Table 2). In contrast, passive transfer of NYS1, a highly protective monoclonal antibody against the CS protein (3) that must interact with sporozoites prior to the invasion of hepatocytes to protect, does not have any protective activity when transferred 5 min after sporozoite inoculation (1).

DISCUSSION

These studies establish that CD8^+ CTL against PySSP2 can protect against malaria in the absence of other parasite-specific immune responses. Since CTL against PySSP2 provide protection against malaria, even when administered as much as 3 h after sporozoites have invaded hepatocytes, these studies suggest that these CTL recognize PySSP2 epitopes on the surface of infected hepatocytes. Thus, like the CSP (28), PySSP2 is also a liver stage antigen and CD8^+ CTL against both the PyCSP

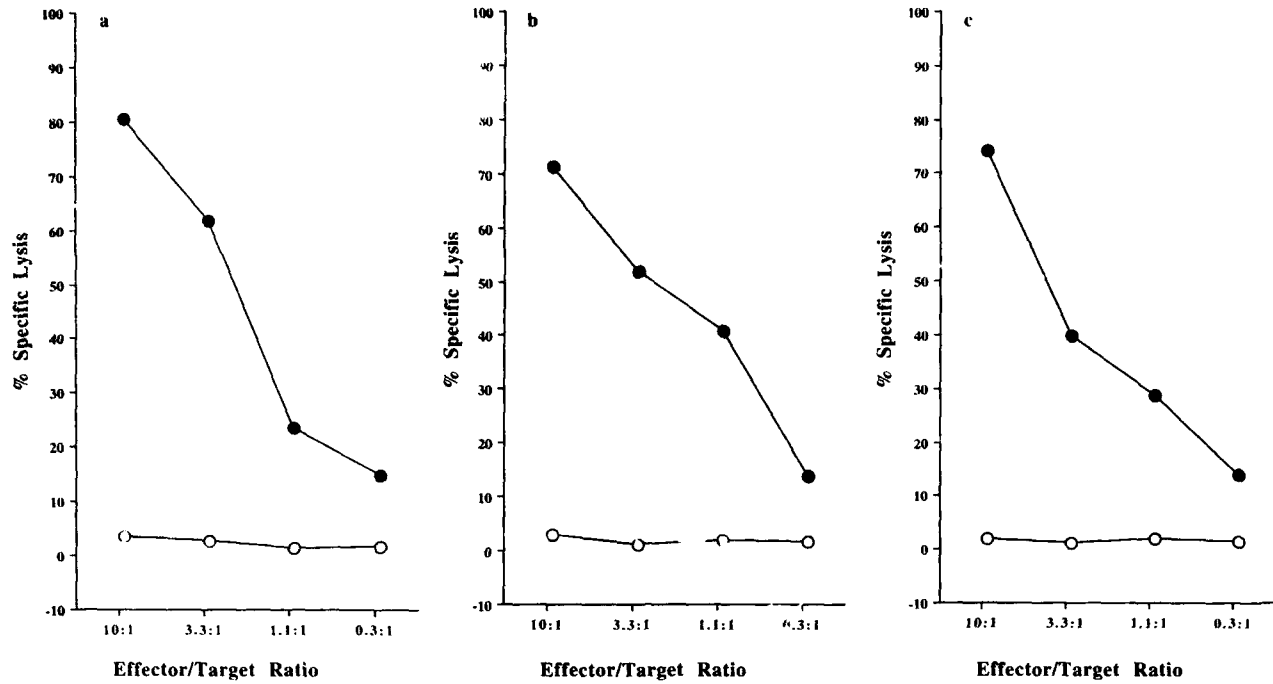


FIG. 1. In vitro lytic activity of CD8⁺ SSP2-specific CTL clones generated by immunization with irradiated *P. yoelii* sporozoites. The CTL clones (TSLB7 [a], TSL14.18 [b], and TSL10.21 [c]) stimulated in vitro by SSP2-P815 3.9 cells were incubated with ⁵¹Cr-labeled SSP2-P815 3.9 (●) at different E/T cell ratios, and cytolytic activity was measured in a 6-h chromium release assay. The control targets were pSV2neo-transfected P815 cells (○).

and PySSP2 almost certainly protect against malaria by eliminating malaria-infected hepatocytes.

The mechanism of this killing, the epitopes recognized by the CTL clones, and the major histocompatibility complex restriction elements of these clones remain to be established. These CTL-like protective anti-PyCSP CTL (27) clones produce IFN-γ. IFN-γ eliminates malaria-infected hepatocytes from culture and, when administered i.v., provides some protection against sporozoite-induced malaria (5, 11, 13, 14). The anti-PySSP2 and anti-PyCSP CTL may directly lyse infected hepatocytes. Alternatively, the T cells could be activated by recognition of parasite antigen on the surface of the

infected hepatocyte and then release IFN-γ or other cytokines that induce the hepatocyte to produce nitric oxides that destroy the parasite (13). It is interesting to note that in a single experiment with the three clones, there was a direct correlation between the quantity of IFN-γ released in vitro and the protective activities of the clones (TSLB7, 100% protection and 2.7 ng of IFN-γ released per ml; TSL14.18, 50 to 67% protection and 1.2 ng/ml; TSL10.21, no protection and 0.7 ng/ml). Further work is required to determine the meaning of this finding. Protective and nonprotective CTL clones against the CSP have been described in the *P. berghei* (23) and *P. yoelii* (19) models. In addition to production of IFN-γ, specificity of

TABLE 1. Protection against sporozoite-induced malaria by adoptive transfer of CD8⁺ T-cell clones against SSP2

Expt	T-cell clone	No. of cells transferred	No. of sporozoites injected	No. of mice infected/ no. of mice challenged	% Protection
1	TSLB7 (SSP2)	27.5 × 10 ⁶	200	0/6	100
	TSL14.18 (SSP2)	25.8 × 10 ⁶	200	2/6	66.7
	TSL10.21 (SSP2)	30.0 × 10 ⁶	200	6/6	0
	RT-1 (HIV)	17.5 × 10 ⁶	200	6/6	0
	NSC ^a	30.0 × 10 ⁶	200	6/6	0
	None	0	200	6/6	
	None	0	40	6/6	
	None	0	8	4/6	
	None	0	1.6	4/6	
2	TSLB7 (SSP2)	29.2 × 10 ⁶	200	0/6	100
	TSL14.18 (SSP2)	30.0 × 10 ⁶	200	3/6	50
	RT-1 (HIV)	30.0 × 10 ⁶	200	6/6	0
	None	0	200	6/6	
	None	0	40	6/6	
	None	0	8	6/6	
	None	0	1.6	4/6	

^a NSC, normal spleen cells.

TABLE 2. Protection against malaria by adoptive transfer of CD8⁺ T-cell clones 3 h after sporozoite inoculation^a

T-cell clone	No. of cells transferred	No. of sporozoites injected	No. of mice infected no. of mice challenged ^b	% Protection
TSLB7 (SSP2)	30 × 10 ⁶	200	1/6	83%
RT-1 (HIV)	30 × 10 ⁶	200	6/6	0
None	0	200	6/6	0

^a Cells were transferred to irradiated, naive mice 3 h after challenge with sporozoites. Experimental and control mice received 10⁷ U of rIL-2 by i.v. injection at the time of adoptive transfer of the CTL clones and 24 and 48 h after transfer.

^b Six of six naive control mice injected with eight sporozoites became infected.

epitopes, the pattern of cytokine production, homing patterns, and cell surface expression of adhesion proteins could all play a role in modulating protective activity. It will be of interest to determine whether the cell surface expression of CD44 correlates with protection as it has with other anti-PyCSP clones (19).

One of the major obstacles faced by malaria vaccine developers is that the malaria parasite changes its character as it passes through the different stages of its life cycle. If a single parasite escapes from a specific protective immune response directed against a single target, it is possible that infection will proceed unencumbered, since that target may not be present in the next stage of the parasite life cycle. Antibodies against the CSP (1a, 3, 17), CD8⁺ CTL against SSP2 and CSP (19, 20, 23, 27), and CD4⁺ T cells against the CSP (18) are each independently protective against malaria, and immunization with a combination of PyCSP and PySSP2 provides additive protection (9). The *Plasmodium falciparum* homolog of PySSP2 has been identified (23), and B epitopes (unpublished data) and CTL epitopes (30) on this protein have been defined. Work is now in progress to develop human vaccines that protect by inducing antibodies and T cells directed against multiple, short, defined B- and T-cell epitopes from CSP and SSP2 expressed on the surface of sporozoites and the surface of infected hepatocytes.

ACKNOWLEDGMENTS

We thank Jay Berzofsky for providing the HIV-specific CTL clone RT-1 and 3T3 cells expressing HIV gp160 and for manuscript review, Jeffrey K. Actor for assaying IFN-γ and IL-5 levels, Patricia Caspar for assaying IL-2 and IL-4 levels, and the Cetus Corporation for providing human rIL-2.

This work was supported by the Naval Medical Research and Development Command work unit nos. 611102A.S13.00101.BFX.1431 and 612787A.870.00101.EFX.1432 and by Office of Naval Research grant N0001489-J-1856 to the Pan American Health Organization (M.S.). This work was done while Srisin Khusmith was a National Research Council Senior Resident Research Associate at the Naval Medical Research Institute, Bethesda, Md.

REFERENCES

- Charoenvit, Y. Unpublished data.
- Charoenvit, Y., W. E. Collins, T. R. Jones, P. Millet, L. Yuan, G. H. Campbell, R. L. Beaudoin, J. R. Broderson, and S. L. Hoffman. 1991. Inability of malaria vaccine to induce antibodies to a protective epitope within its sequence. *Science* 251:668-671.
- Charoenvit, Y., M. F. Leef, L. F. Yuan, M. Sedegah, and R. L. Beaudoin. 1987. Characterization of *Plasmodium yoelii* monoclonal antibodies directed against stage-specific sporozoite antigens. *Infect. Immun.* 55:604-608.
- Charoenvit, Y., S. Mellouk, C. Cole, R. Bechara, M. F. Leef, M. Sedegah, L. F. Yuan, F. A. Robey, R. L. Beaudoin, and S. L. Hoffman. 1991. Monoclonal, but not polyclonal, antibodies protect against *Plasmodium yoelii* sporozoites. *J. Immunol.* 146:1020-1025.
- Curry, R. C., P. A. Kiener, and G. L. Spitalny. 1987. A sensitive immunochromatographic assay for biologically active Mu IFN-gamma. *J. Immunol. Methods.* 104:187-142.
- Ferreira, A., L. Schofield, V. Enea, H. Schellekens, P. Van der Meide, W. E. Collins, R. S. Nussenzweig, and V. Nussenzweig. 1986. Inhibition of development of exoerythrocytic forms of malaria parasites by gamma-interferon. *Science* 232:881-884.
- Hedstrom, R. C., J. R. Campbell, M. L. Leef, Y. Charoenvit, M. Carter, M. Sedegah, R. L. Beaudoin, and S. L. Hoffman. 1990. A malaria sporozoite surface antigen distinct from the circumsporozoite protein. *Bull. W. H. O.* 68(Suppl.):152-157.
- Hoffman, S. L., E. D. Franke, W. O. Rogers, and S. Mellouk. 1993. Preerythrocytic malaria vaccine development, p. 149-167. *In* M. F. Good and A. J. Saul (ed.), *Molecular immunological considerations in malaria vaccine development*, vol. 6. CRC Press, Inc., Boca Raton, Fla.
- Hoffman, S. L., D. Isenbarger, G. W. Long, M. Sedegah, A. Szarfman, L. Waters, M. R. Hollingdale, P. H. VanderMeide, D. S. Finblom, and W. R. Ballou. 1989. Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. *Science* 244:1078-1081.
- Khushmith, S., Y. Charoenvit, S. Kumar, M. Sedegah, R. L. Beaudoin, and S. L. Hoffman. 1991. Protection against malaria by vaccination with sporozoite surface protein 2 plus CS protein. *Science* 252:715-718.
- Kumar, S., L. H. Miller, I. A. Quakyi, D. B. Keister, R. A. Houghten, W. L. Maloy, B. Moss, J. A. Berzofsky, and M. F. Good. 1988. Cytotoxic T cells specific for the circumsporozoite protein of *Plasmodium falciparum*. *Nature (London)* 334:258-260.
- Maheshwari, R. K., C. W. Czarniecki, G. P. Dutta, S. K. Puri, B. N. Dhawan, and R. M. Friedman. 1986. Recombinant human gamma interferon inhibits simian malaria. *Infect. Immun.* 53:628-630.
- Malik, A., J. E. Egan, R. A. Houghten, J. C. Sadoff, and S. L. Hoffman. 1991. Human cytotoxic T lymphocytes against the *Plasmodium falciparum* circumsporozoite protein. *Proc. Natl. Acad. Sci. USA* 88:3300-3304.
- Mellouk, S., S. J. Green, C. A. Nacy, and S. L. Hoffman. 1991. IFN-γ inhibits development of *Plasmodium berghei* exoerythrocytic stages in hepatocytes by an L-arginine-dependent effector mechanism. *J. Immunol.* 146:3971-3976.
- Mellouk, S., R. K. Maheshwari, A. Rhodes Feuillette, R. L. Beaudoin, N. Berbiguier, H. Matile, F. Miltgen, I. Landau, S. Pied, J. P. Chigot, R. M. Friedman, and D. Mazier. 1987. Inhibitory activity of interferons and interleukin 1 on the development of *Plasmodium falciparum* in human hepatocyte cultures. *J. Immunol.* 139:4192-4195.
- Mosmann, T. R., and T. A. T. Fong. 1989. Specific assays for cytokine production by T cells. *J. Immunol. Methods* 116:151-158.
- National Institutes of Health. 1985. Guide for the care and use of laboratory animals. NIH publication 86-23. U.S. Government Printing Office, Washington, D.C.
- Pacheco, N. D., C. P. A. Strome, F. Mitchell, M. P. Bawden, and R. L. Beaudoin. 1979. Rapid, large scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. *J. Parasitol.* 65:414-417.
- Potocnjak, P., N. Yoshida, R. S. Nussenzweig, and V. Nussenzweig. 1980. Monovalent fragments ("Fab") of monoclonal antibodies to a sporozoite surface antigen ("Pb 44") protect mice against malarial infection. *J. Exp. Med.* 151:1504-1513.
- Renia, L., D. Grillot, M. Marrussig, G. Corradin, F. Miltgen, P. H. Lambert, D. Mazier, and G. D. Giudice. 1993. Effector functions of circumsporozoite peptide-primed CD4⁺ T cell clones against *Plasmodium yoelii* liver stages. *J. Immunol.* 150:1471-1478.
- Rodrigues, M., R. S. Nussenzweig, P. Romero, and F. Zavala. 1992. The in vivo cytotoxic activity of CD8⁺ T cell clones correlates with their level of expression of adhesion molecules. *J. Exp. Med.* 175:895-905.
- Rodrigues, M. M., A. Cordey, G. Arreaza, G. Corradin, P. Romero, J. L. Maryanski, R. S. Nussenzweig, and F. Zavala. 1991. CD8⁺ cytolytic T cell clones derived against the *Plasmodium yoelii*

- circumsporozoite protein protect against malaria. *Int. Immunol.* **3**:579-585.
21. **Rogers, W. O., A. Malik, S. Mellouk, K. Nakamura, M. D. Rogers, A. Szarfman, D. M. Gordon, A. K. Nussler, M. Aikawa, and S. L. Hoffman.** 1992. Characterization of *Plasmodium falciparum* sporozoite surface protein 2. *Proc. Natl. Acad. Sci. USA* **89**:9176-9180.
 22. **Rogers, W. O., M. D. Rogers, R. Hedstrom, and S. L. Hoffman.** 1992. Characterization of the gene encoding sporozoite surface protein 2, a protective *Plasmodium yoelii* sporozoite antigen. *Mol. Biochem. Parasitol.* **53**:45-52.
 23. **Romero, P., J. L. Maryanski, G. Corradin, R. S. Nussenzweig, V. Nussenzweig, and F. Zavala.** 1989. Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria. *Nature (London)* **341**:323-325.
 24. **Schofield, L., J. Villaquiran, A. Ferreira, H. Schellekens, R. S. Nussenzweig, and V. Nussenzweig.** 1987. Gamma interferon, CD8⁺ T cells and antibodies are required for immunity to malaria sporozoites. *Nature (London)* **330**:664-666.
 25. **Schumacher, J. H., A. O'Garra, B. Schrader, A. van Kimmenade, M. W. Bond, T. R. Mosmann, and R. L. Coffman.** 1988. The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme immunosorbent assays. *J. Immunol.* **141**:1576-1581.
 26. **Takahashi, H., J. Cohen, A. Hosmalin, K. B. Cease, R. A. Houghten, J. L. Cornette, C. DeLisi, B. Moss, R. N. Germain, and J. A. Berzofsky.** 1988. An immunodominant epitope of the human immunodeficiency virus envelope glycoprotein gp160 recognized by class I major histocompatibility complex molecule-restricted murine cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA* **85**:3105-3109.
 27. **Weiss, W., J. A. Berzofsky, R. Houghten, M. Sedegah, M. Hollingdale, and S. L. Hoffman.** 1992. A T cell clone directed at the circumsporozoite protein which protects mice against both *P. yoelii* and *P. berghei*. *J. Immunol.* **149**:2108-2109.
 28. **Weiss, W. R., S. Mellouk, R. A. Houghten, M. Sedegah, S. Kumar, M. F. Good, J. A. Berzofsky, L. H. Miller, and S. L. Hoffman.** 1990. Cytotoxic T cells recognize a peptide from the circumsporozoite protein on malaria-infected hepatocytes. *J. Exp. Med.* **171**:763-773.
 29. **Weiss, W. R., M. Sedegah, R. L. Beaudoin, L. H. Miller, and M. F. Good.** 1988. CD8⁺ T cells (cytotoxic/suppressor) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl. Acad. Sci. USA* **85**:573-576.
 30. **Wizel, B., W. O. Rogers, R. A. Houghten, D. E. Lanar, J. A. Tine, and S. L. Hoffman.** *Eur. J. Immunol.*, in press.