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Immunology

In vitro activity of CD4$^+$ and CD8$^+$ T lymphocytes from mice immunized with a synthetic malaria peptide

(*Plasmodium yoelii*/liver stage/circumsporozoite protein)

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ABSTRACT **In previous work, a T-helper epitope was mapped within the circumsporozoite protein of the murine malaria parasite *Plasmodium yoelii*. A 21-mer synthetic peptide corresponding to this epitope (amino acid positions 59–79; referred to as Py1) induced a specific T-cell proliferation in BALB/c and C57BL/6 mice and provided help for the production of antibodies to peptides from the repetitive region, (Gln-Gly-Pro-Gly-Ala-Pro)$_n$, of the *P. yoelii* circumsporozoite protein when mice were immunized with the Py1 peptide conjugated to the repetitive peptide. Experiments were then designed to study the *in vitro* antiparasite efficacy of T cells elicited *in vivo* by peptide immunization. T-cell activity was evaluated on cultured hepatic stages of *P. yoelii*. Peptide immunizations led to the preferential activation of CD8$^+$ T cells in BALB/c mice and of both CD4$^+$ and CD8$^+$ T cells in C57BL/6 mice. Parasite elimination was mediated directly by these cells and did not seem to be dependent on lymphokine secretion. These data suggest that peptide-primed CD4$^+$ T cells as well as CD8$^+$ T cells could be cytolytic for the hepatic phase of malaria parasites. The fact that the same peptide could activate different lymphocyte populations, depending on the strain of mouse, highlights the importance of a better understanding of the fine mechanisms behind the immune responses to synthetic peptides being tested for malaria vaccine development.**

Malaria infection begins with the injection of sporozoites during a mosquito blood meal. Sporozoites then invade parenchymal liver cells in which they differentiate and multiply, subsequently releasing merozoites capable of initiating a blood-stage infection. Immune response to the parasite is a complex phenomenon, involving both nonspecific (1, 2) and specific components. Although antibodies have been shown to play a role in protection against malaria sporozoites (3, 4), it is clear that the host's immune response involves other mechanisms; this was demonstrated as early as 1977 by Spitalny *et al.* (5) and Chen *et al.* (6) and recently has been confirmed and extended. Thus there is loss of immunity conferred by irradiated sporozoites after *in vivo* depletion of CD8$^+$ cytotoxic T cells in certain mouse strains (7, 8). Passive transfer of some circumsporozoite (CS) protein-specific CD8$^+$ T-cell clones protects mice against a sporozoite challenge (9). *In vivo* and *in vitro* results using T-cell clones suggest a possible involvement of cytotoxic CD4$^+$ T cells in protection against sporozoite infection (refs. 10–12; L.R., M.S.M., D.G., G.D.G., and D.M., unpublished results).

In this study, we investigated the *in vitro* antiparasitic activity of T-cell populations of lymph node cells (LNC) from different mouse strains immunized with the synthetic peptide Py1. This corresponds to a nonrepetitive segment of the *Plasmodium yoelii* CS protein and contains identifiable T-cell epitopes (13). We used freshly prepared LNC to avoid the selective overgrowth or functional alteration of lymphocyte populations that might result from prior *in vitro* stimulation with antigen and/or interleukin (IL) 2 (14, 15). We also measured the *in vivo* protection conferred by immunization with the peptide.

MATERIALS AND METHODS

Parasites. Sporozoites of the 265 BY strain of *P. yoelii yoelii* and of the ANKA strain of *Plasmodium berghei* were obtained from infected salivary glands of *Anopheles stephensi* mosquitoes. After aseptic dissection, salivary glands were homogenized in a glass grinder and diluted in culture medium or sterile phosphate-buffered saline (PBS).

Synthetic Peptides. Py1 peptide from a nonrepetitive segment of the CS protein of *P. yoelii* (amino acids 59–79) was synthesized (13) according to the sequence reported by Lal *et al.* (16) and de la Cruz *et al.* (17).

Mice and Immunization Schedule. Seven- to 12-week-old female BALB/c, C3H/HeJ, and C57BL/6 mice, purchased from Iffa Credo (Lyon, France), were immunized at the base of the tail with a 100-μl volume containing 30 μg of Py1 peptide emulsified 1:1 (vol/vol) in complete Freund's adjuvant (Difco). Mice were boosted twice at the base of the tail with 30 μg of peptide emulsified in incomplete Freund's adjuvant (Difco) 2–3 weeks after the priming and then 1 week later. Control mice received only the adjuvants.

Reagents. Ascites of H-35.17.2, a rat IgG2b anti-CD8 monoclonal antibody (mAb) (18), and the supernatant of RL172.4, a rat IgM anti-CD4 mAb (19), were used. Both mAbs were titrated to define optimal concentration for complement-mediated T-cell depletion. Supernatants of two anti-L^d mAbs, 30-5-7, a mouse IgG2a (20), and 64-3-7, a mouse IgG2b (21), were a gift of M. Kreiss (Institut de Recherche Sur le Cancer, Villejuif, France). Purified mouse IgG2a anti-I-E^d, 14-4-4S (20), was a gift of A. Bandeira (Institut Pasteur, Paris). Ascites of TIB 120, a rat IgG2b anti-I-A^{b,d,q}/anti-I-E^{d,k} (22); ascites of TIB 93, a mouse IgG2b anti-I-A^k; and ascites of H82-B, a rat IgG3 anti-I-A^{d,k}/anti-I-E^{d,k}, were a gift from M. Pierres (Marseille-Lumigny, France). R4-6A2, a purified rat IgG1 anti-mouse interferon γ (IFN-γ) (23) (hybridoma ATCC HB 170) was a gift of G. Milon (Institut

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Abbreviations: CS, circumsporozoite; LNC, lymph node cell(s); IFN-γ, interferon γ; IL, interleukin; CsA, cyclosporin A; MHC, major histocompatibility complex; mAb, monoclonal antibody; Py1, 21-mer synthetic peptide corresponding to the CS protein of *Plasmodium yoelii*.

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Pasteur). Murine recombinant IFN-γ was a gift of C. Damais (Institut National de la Santé et de la Recherche Médicale, Unité 313, Paris); 6B4, a rat IgG1 anti-mouse IL-6 (24) and recombinant murine IL-6 were a gift from J. Van Snick (Ludwig Institute, Brussels). Cyclosporin A (CsA) (Sandimmune i.v., Sandoz, Basel) was dissolved in ethanol at a concentration of 10 mg/ml and was used at a final concentration of 1 μg/ml in culture medium.

Effector Lymphocytes. T lymphocytes were obtained from draining periaortic and inguinal lymph nodes of normal or immunized mice 1 week after the last boost. Lymph nodes were excised and processed in a tissue grinder. The resulting tissue suspension was passed through nylon mesh, washed twice by centrifugation at 300 × *g*, and resuspended in Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/ml), and streptomycin (100 μg/ml) (GIBCO/BRL). LNC were incubated overnight in culture flasks coated with 2 ml of 10% FCS. Nonadherent cells were recovered by rinsing the flask four times with supplemented MEM. B cells were then removed by panning into flasks coated with 2 ml of goat anti-mouse immunoglobulin (Nordic, Tilburg, The Netherlands) diluted 1:20 in PBS (pH 7.2). Cells were incubated at 4°C for 1 h, and nonadherent cells were removed by washing three times with medium. These were centrifuged and resuspended in MEM supplemented with 10% FCS. B-cell depletion was analyzed by flow cytometry and was always >95%.

$CD4^+$ or $CD8^+$ T-Cell Depletion. The T-cell-enriched populations were incubated 15 min with titrated amounts of anti-CD4 or anti-CD8 mAbs at 37°C. Freshly prepared complement (Low-Tox rabbit complement, Cedarlane Laboratories, Hornby, ON) was added at a 10% final concentration. After a 45-min incubation at 37°C, cells were washed twice in supplemented MEM. Depletion was analyzed by flow cytofluorometry and was always >90%.

***In Vitro* Assay of Liver-Stage Parasite Elimination.** Mouse hepatocytes were prepared as described with minor modifications (25). Cells were isolated by collagenase perfusion of liver fragments and were further purified on a discontinuous Percoll gradient. Hepatocyte purity and viability was >95% as assessed by trypan blue dye exclusion. Cells (6×10^4) were cultured in eight-chamber plastic Lab-Teck slides (Miles) in supplemented MEM and incubated in 5% CO_2 at 37°C for 24 h. After removal of medium from the culture chambers, 6×10^4 sporozoites were added in 100 μl of fresh medium. Three hours later, medium was replaced by LNC suspended in fresh supplemented MEM. Cultures were incubated for 45 h with a change of 50 μl of medium 24 h after sporozoite infestation. In some experiments, cultures were stopped 24 h after infection. Experimental wells were set up in triplicate. Schizont numbers were assessed by immunofluorescence using hyperimmune sera recognizing either *P. yoelii* or *P. berghei* liver stages. The role of lymphokines was studied by adding anti-cytokine antibodies at the same time as the LNC. As a control, 10^3 units of IFN-γ and IL-6 were added to hepatocyte cultures 3 h after infection. The ability of CsA to inhibit parasite elimination in the presence or absence of LNC was also tested.

Sporozoite Challenge. BALB/c and C57BL/6 mice immunized three times with peptide in adjuvant or with adjuvant alone (see above) were challenged i.v. with 4000 *P. yoelii* sporozoites. Parasitemia was monitored from 3 to 12 days after challenge.

RESULTS

***In Vitro* Activity of LNC from Pyl-Immunized Animals.** Purified LNC from Pyl-immunized C57BL/6 and BALB/c mice (but not C3H/HeJ mice) substantially decreased the number of liver-stage parasites developing in culture. This effect was not observed with LNC from normal mice or mice receiving only adjuvant (Fig. 1). When experiments were carried out with LNC not depleted of adherent cells, a strong nonspecific cytotoxicity (up to 40% inhibition) was observed with preparations from control BALB/c mice (i.e., not immunized or injected with adjuvant alone). This phenomenon was not seen with LNC from C57BL/6 mice (data not shown). As expected from our previous results with Pyl-specific T-cell clones (13), the inhibitory effect of Pyl-primed LNC from both BALB/c and C57BL/6 mice was specific for *P. yoelii* sporozoites; there was no effect on *P. berghei* parasites (data not shown). Experiments were then performed to determine the time required by T cells to exert their antiparasitic effect. For this, (*i*) T cells were added 3 h after sporozoite infection and cultures were stopped either 21 or 45 hours later; (*ii*) T cells were added 24 h after the sporozoites and cultures were examined 24 h later (Fig. 2). Parasite inhibition was observed when T cells and infected hepatocytes were coincubated for 45 h, the time that is required for complete parasite development. LNC of both BALB/c and C57BL/6 mice did not eliminate liver schizonts in a 21-h incubation during the early phase or in a 24-h incubation during the late phase of parasite development.

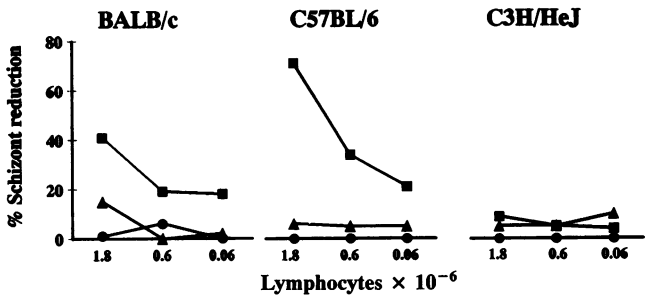

FIG. 1. LNC from BALB/c, C57BL/6, or C3H/He mice immunized with Pyl peptide in complete Freund's adjuvant (■) or with complete Freund's adjuvant alone (▲) or from nonimmunized controls (●) were layered on syngeneic hepatocyte cultures 3 h after sporozoite inoculation. Cultures were stopped 45 h later. Data are presented as the mean reduction in parasite numbers from triplicate counts due to the different lymphocyte populations and represent one of three separate experiments. Parasite number reduction was calculated by counting numbers of 48-h schizonts in culture in the presence or absence of LNC. The number of schizonts in control wells was 153 ± 5 (BALB/c), 178 ± 11 (C57BL/6), and 57.5 ± 2 (C3H/HeJ).

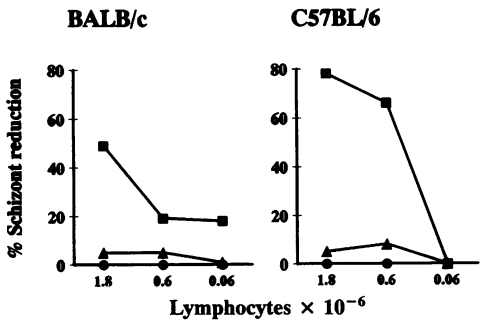

FIG. 2. Timing of the antiparasite effect of Pyl-primed LNC. Pyl-primed LNC were layered on cultures 3 h or 24 h after sporozoite inoculation. Three experiments were performed: (*i*) LNC were deposited 3 h after sporozoite inoculation, and cultures were stopped 21 h later (▲); (*ii*) LNC were added 24 h after the sporozoites, and cultures were examined 24 h later (●); (*iii*) LNC were added 3 h after sporozoite infection and cultures were stopped 45 h later (■). Results are expressed as in Fig. 1. The number of schizonts in control wells was 104.5 ± 5.5 (BALB/c) and 216.5 ± 4 (C57BL/6).

Table 1. Effect of T-cell subset depletion on parasite elimination by LNC

Mouse strain	Antibody	% schizont reduction		
		Exp. 1	Exp. 2	Exp. 3
BALB/c	None	50 ± 5	29.5 ± 5	39 ± 5
	Anti-CD4	ND	28 ± 1	38 ± 2
	Anti-CD8	0	8 ± 1	13 ± 2
C57BL/6	None	58.5 ± 2	38 ± 5	54.5 ± 2
	Anti-CD4	17 ± 6	13 ± 1	17.5 ± 2
	Anti-CD8	32.5 ± 2	ND	35 ± 3

LNC (1.8×10^6) from Pyl-immunized mice were incubated with either anti-CD8 mAb (1:100 dilution) or with anti-CD4 mAb (1:4 dilution) followed by complement. Washed cells were added to hepatocyte cultures 3 h after sporozoite inoculation and incubated a further 45 h. The percent reduction in schizont numbers (mean ± SE) by comparing numbers in experimental and control wells without LNC. ND, not done.

Lymphocyte Subset Activation. To define further which Pyl-primed lymphocyte populations were involved in the elimination of differentiating parasites, immune LNC were pretreated with an anti-CD8 or anti-CD4 mAb plus complement. Pretreatment of BALB/c LNC with the anti-CD8 mAb removed the inhibitory activity of the whole-cell preparation; depletion with the anti-CD4 mAb did not have any effect. Depletion of $CD4^+$ T cells from C57BL/6 LNC suppressed the inhibitory effect on parasite development to a greater degree than that achieved by depletion of $CD8^+$ cells (Table 1).

Major Histocompatibility Complex (MHC) Control of Parasite Elimination. The question was then addressed of whether T-cell-mediated parasite elimination was MHC controlled. LNC were thus layered either on syngeneic- or allogeneic-infected target cells (Fig. 3). Pyl-stimulated LNC from BALB/c and C57BL/6 mice induced a reduction in the number of schizonts only when cultured in the presence of infected MHC-matched hepatocytes. The role of MHC restriction was confirmed by adding anti-class I or anti-class II antibodies to cultures (Table 2). Anti-L^d mAbs specifically inhibited parasite elimination from BALB/c mouse hepatocytes, while anti-class II molecules antibodies had no effect. On the other hand, anti-I-A^b determinant antibodies diminished parasite elimination from C57BL/6 mouse hepatocytes.

Role of Lymphokines in Liver-Stage Parasite Elimination. When 1000 units of IFN-γ or IL-6 was added 3 h after sporozoite inoculation, parasite development was inhibited 56% by IFN-γ and 35% by IL-6. These effects could be reversed by adding defined concentrations of specific mAbs. When these mAbs were added with the LNC, they had no effect on the parasite elimination (Fig. 4). These results are in agreement with the finding that hepatocyte culture supernatants collected 45 h after the addition of LNC to hepatocyte cultures (48 h after sporozoite infection) and added diluted

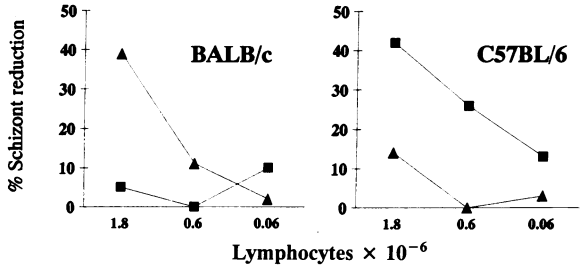

FIG. 3. MHC restriction of parasite elimination. LNC from Pyl-immunized mice were added to MHC syngeneic or allogeneic hepatocytes 3 h after sporozoite inoculation. Cultures were stopped 45 h later. Results are expressed as in Fig. 1. ■, C57BL/6 hepatocytes; ▲, BALB/c hepatocytes.

Table 2. Effect of anti-MHC class I and class II mAbs on parasite elimination by Pyl-immune LNC

Mouse strain	Antibody	Specificity	% schizont elimination
BALB/c	None	—	48.5 ± 0.5
	30-5-7	L^d	12 ± 2
	64-3-5	L^d	13.5 ± 3
	14-4-4S	I-E^d	40.5 ± 8
	H82-B	I-A^d/E^d	38.5 ± 5
C57BL/6	None	—	48.5 ± 3.5
	TIB 120	L-A^b	18 ± 1
	TIB 93	I-A^k	46.2

LNC (1.8×10^6) from Pyl-immunized mice were deposited on hepatocyte culture 3 h after sporozoite infestation. mAbs against class I antigens 30-5-7 (1:30 dilution) and 64-3-7 (1:30 dilution) and mAbs against class II antigens 14-4-4S (5 μg/ml), H82-B (1:100 dilution), TIB 120 (1:100 dilution), and TIB 93 (1:100 dilution) were added at the same time. Cultures were stopped 45 h later, and the percent schizont elimination was determined by comparing schizont numbers in experimental and control wells. None of these mAbs was toxic for the parasite.

1:2 to other cultures had no effect on parasite development (data not shown). To further assess the importance of lymphokines, we analyzed the ability of LNC to mediate antiplasmodial effects in infected hepatocytes in the presence or absence of CsA, an agent known to interfere with the production of lymphokines such as IL-2, IL-3, and IFN-γ (26, 27). As shown in Fig. 5, CsA did not interfere with parasite elimination mediated by immune Pyl-primed LNC.

Sporozoite Challenge. Taken together, these data strongly suggested that immunization with the Pyl peptide activated heterogeneous T-cell populations having antiparasitic effects *in vitro*. However, after active immunization of responder mice with the Pyl peptide, 90% of immunized BALB/c and C57BL/6 mice (18 out of 20) developed a patent parasitemia as compared to 100% (20 out of 20) of control nonimmunized mice.

DISCUSSION

We have shown that it is possible to induce polyclonal T cells able to kill the liver stages of *Plasmodium in vitro* by prior immunization of donor mice with a synthetic peptide corre-

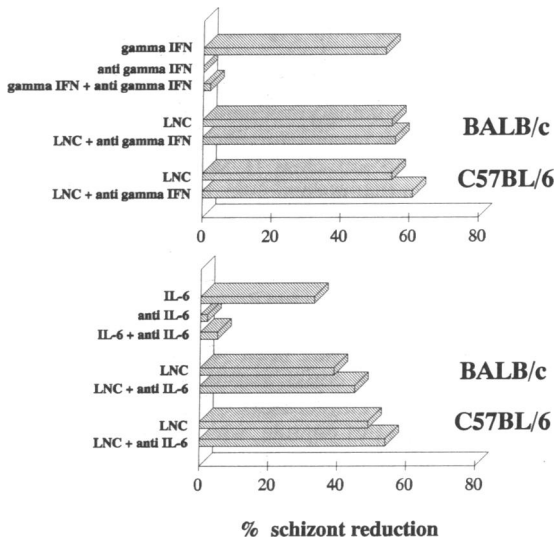

FIG. 4. Effect of anti-lymphokine antibodies on LNC-mediated inhibition. LNC from Pyl-immunized mice (1.8×10^6) were added with anti-lymphokine antibodies to *P. yoelii*-infected hepatocytes 3 h after sporozoite inoculation. Cultures were stopped 45 h later.

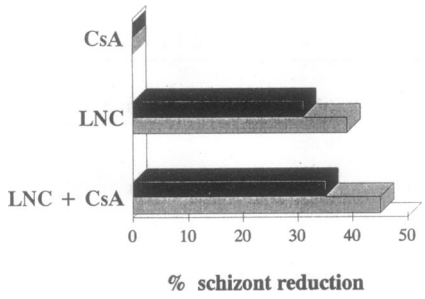

FIG. 5. Effect of CsA on LNC-mediated inhibition. LNC (1.8×10^6) were added to *P. yoelii*-infected hepatocytes with CsA at a concentration of 1 μg/ml 3 h after sporozoite inoculation. Cultures were stopped 45 h later. Black bars, BALB/c hepatocytes; shaded bars, C57BL/6 hepatocytes.

sponding to part of the nonrepetitive segment of the CS protein of *P. yoelii*. No expansion of the cell population or further stimulation *in vitro* was necessary. The effector cells, obtained from lymph nodes, were both $CD4^+$ and $CD8^+$ T cells, but the relative importance of the two subsets varied in different strains of mice. Effector cells obtained from immunized C57BL/6 mice, while including some $CD8^+$ T cells, were predominantly $CD4^+$. The cytotoxic T cells isolated from immunized BALB/c mice were all $CD8^+$. This contrasts with results from our previous study where $CD4^+$ T-cell clones specific to the Py1 peptide and able to eliminate liver stage parasites *in vitro* (refs. 10 and 11; L.R., M.S.M., D.G, G.D.G., and D.M., unpublished results) were obtained from both BALB/c and C57BL/6 mice (13). One could hypothesize that $CD4^+$ T cells were produced in BALB/c mice by immunization with the peptide but were selected and expanded through the cloning procedure.

It is possible that the difference in the activation of $CD4^+$ versus $CD8^+$ T cells in these two strains of mice is a reflection of variation in the handling (i.e., processing or presentation) of this peptide by antigen-presenting cells. Nevertheless, it could be possible that $CD8^+$ T cells have an inhibitory effect on the expansion of the $CD4^+$ T-cell population *in vivo*. Furthermore, the absence of any detectable response in C3H/HeJ mice ($H\text{-}2^k$), while suggesting an MHC control of the induction of antisporozoite T cells after immunization with the Py1 synthetic peptide, is in full agreement with the data already reported by us (13). In fact, in that study both proliferative response to the peptide and its helper activity *in vivo* were always undetectable in this strain of mouse.

The T-lymphocyte-mediated antiparasite effect was MHC restricted, since the elimination of parasites occurred only in the presence of infected syngeneic target cells and was inhibited by both anti-class I and anti-class II MHC antibodies. Several studies have shown that normal human or murine hepatocytes do not express MHC class II molecules on their membranes, and there is little or no expression of class I antigens (28–30). Expression of MHC antigens is nevertheless induced by IFN-γ or hepatitis B virus (31). Hepatocytes bearing hepatitis B virus-induced class II molecules possess accessory cell functions (32). Moreover, it has been reported that intracellular virus-derived peptides could stabilize and induce surface expression of L^d class I molecules (33). One could then suggest that parasite-derived Py1 peptide, which is L^d restricted, could have the same effect on the expression of MHC molecules. Nevertheless, we were unable to detect MHC molecules on the surface of the infected hepatocyte by immunofluorescence using mAbs directed against MHC antigens (data not shown).

The mechanism(s) by which T lymphocytes eliminate their targets remains unclear. Our results with mAbs and CsA are against an effect of lymphokines such as IFN-γ or IL-6, which are known to eliminate directly liver stages *in vitro* (34–38). Tumor necrosis factor α seems unlikely to play any role, since tumor necrosis factor α has no antiplasmodial effect when purified hepatocytes are used (37). Different hypotheses can be made: (*i*) T lymphocytes killed their targets through a direct cytotoxic mechanism; (*ii*) parasites were eliminated by an unknown lymphokine(s), the secretion of which is not inhibited by CsA; or (*iii*) the release of lymphokines was polarized, occurring in the region of interactions between T cells and antigen-presenting cells, as shown for the release of the B-cell growth factor by a T-cell clone (39); this localized secretion could limit the effect of anti-lymphokine mAbs. Findings similar to ours have been reported for other infectious agents. For example, during the course of localized infection, certain LNC acquire the ability to activate *Leishmania*-infected macrophages *in vitro* for antiparasite effects by direct cell contact, through an apparently lymphokine-independent pathway (40). Cytotoxic lymphocytes can limit human immunodeficiency virus replication without inducing cell death through an apparently noncytolytic mechanism that does not involve IFN-γ (41).

In our *in vitro* assay, parasite elimination took more than 24 h to achieve (Fig. 2). It seems unlikely that this delay was due to T-cell multiplication, since parasite elimination also occurred in the presence of CsA, which inhibits T-cell proliferation. Using sporozoite-primed spleen cells, Hoffman *et al.* (42) and Weiss *et al.* (43) found that parasite killing could be achieved if lymphocytes were added 24 h after sporozoite inoculation of the hepatocyte cultures. In their study, Hoffman *et al.* (42) used a polyclonal T-cell population from the spleen of animals immunized with irradiated sporozoites. These cells recognized different epitopes from different sporozoite proteins, the CS protein and the protein SSP2 (44), and may have been more effective than cells recognizing only one epitope. On the other hand, Weiss *et al.* (43) used T cells from *in vitro* peptide-stimulated immune spleen cell. This enriched population acted more rapidly. We showed recently that CS-specific $CD4^+$ T-cell clones act also more rapidly (L.R., D.G., M.S.M., G.D.G., and D.M., unpublished results). Frequency of effective lymphocytes *in vitro* and perhaps *in vivo* in contact with the infected hepatocytes might be an important factor in parasite elimination.

An increasing number of reports are appearing showing that $CD8^+$ T cells can be activated after immunization with soluble synthetic peptides and that in some instances this immunization can confer protection against viral infections (45, 46). However, in our model, in spite of the *in vitro* activity of Py1 peptide-primed LNC, immunization of mice with the Py1 peptide in Freund's adjuvant did not protect the mice against a challenge with *P. yoelii* sporozoites. These negative results were not unexpected. On one hand, in our *in vitro* assays, 30–60% of the hepatic-stage parasites were still able to complete their development. On the other hand, one could hypothesize that even multiple immunizations with this peptide did not efficiently prime a sufficient number of $CD8^+$ or $CD4^+$ T cells and/or these cells did not efficiently home to the liver. More complex constructs containing the Py1 sequence might exhibit a better function *in vivo*. Nevertheless, since the immune responses to this stage of the malaria cycle are multifaceted (10), the Py1 induction of protective $CD4^+$ and $CD8^+$ T cells is likely to be an important part of the resistance that develops naturally or is induced artificially with more complex immunogens. Moreover, we have to stress the point that in virus models the number of virus-specific effector cells in peptide-primed mice reached a significant level only after 4 days (45). *P. yoelii* malaria hepatic stages last 2 days in mouse. If the same delay is needed to obtain an effective parasite-specific T-cell level, the parasite has already completed its development and is no longer in the liver but in the blood. This problem might be

overcome in human malaria (e.g., *P. falciparum*) where parasite development in the liver takes 7 days.

Two observations from our experiments are of particular importance. First we have shown that peptide-primed $CD4^+$ as well as $CD8^+$ T cells isolated directly from immunized mice are cytotoxic for infected hepatocytes. Secondly, we have demonstrated considerable variability in the protective responses to a defined peptide. It is vital to take account of this in assessing the potential of such immunogens as candidate vaccines.

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