Influence of Peptide Acylation, Liposome Incorporation, and Synthetic Immunomodulators on the Immunogenicity of a 1-23 Peptide of Glycoprotein D of Herpes Simplex Virus: Implications for Subunit Vaccines

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A peptide corresponding to residues 1 to 23 of glycoprotein D of herpes simplex virus type 1 was chemically synthesized and coupled to a fatty acid carrier by standard Merrifield synthesis procedures. The resulting peptide-palmitic acid conjugate (acylpeptide) exhibited enhanced immunogenicity in mice as compared with that exhibited by the free form of the peptide. Incorporation of the acylpeptide into liposomes further increased the immunogenicity of the peptide, while inclusion of the immunomodulators muramyl tripeptide phosphatidylethanolamine and monophosphoryl lipid A into the same liposome stimulated the strongest response. The humoral immune responses induced by the acylpeptide-liposome construct were greater than those induced by peptide in Freund complete adjuvant, and cellular responses were equal. The acylpeptide-immunomodulator-liposome formulation also induced significant levels of protective immunity, although the immunity was less than that induced by herpes simplex virus infection. Acylated peptides, especially in liposomes, were taken up more effectively by draining lymph nodes, which possibly accounts in part for the enhanced immunogenicity of the peptides. Since the acylpeptide-immunoliposome formulation used was nontoxic, it could represent a useful way to enhance immunogenicity of subunit peptides used for vaccine purpose in humans and animals.

Immunity to herpes simplex virus (HSV) entails aspects of both humoral and cellular immunity, and optimal responses of both types seem to require exposure to live virus (11, 21, 24, 25, 27, 30). However, living vaccines will probably be unacceptable because of the lingering uncertainty about the oncogenicity of HSV DNAs and their possible disease-producing activity especially in immunologically compromised individuals. Synthetic peptides representing protective antigen provide an alternative and theoretically ideal approach, providing ways can be found to attain acceptable immunogenicity. Several groups have investigated HSV-specific synthetic peptides for possible vaccine use (8, 10, 12, 15, 32), with the first 23 amino-terminal amino acids of HSV glycoprotein D (gD) 1 and gD 2 receiving the most attention. Antibody responses to these peptides react with the native virus and will neutralize its infectivity (8). In addition, certain types of cell-mediated responses can be induced with a peptide corresponding to residues 1 to 23 of HSV gD (gD 1-23). As is usually the case with peptides, gD 1-23 is a weak immunogen and potent unacceptable adjuvants have usually been employed to achieve immunity (6, 22). Moreover, it is necessary to couple the peptide to a carrier molecule, a procedure which also may prove unacceptable for practical use (3).

One vehicle of immune potentiation, which has few if any toxicity problems, is the liposome. It appears that the immunogenicity of nearly any protein can be enhanced by association with liposomes (2) and that liposomes of the appropriate composition are biodegradable and nonimmunogenic (29). In addition, immunomodulatory drugs can simultaneously be delivered in liposomes, thus further potentiating the immune response. The high efficiency of liposome-associated MDP (*N*-acetylmuramyl-L-alanyl-D-isoglutamine),

a lipophilic derivative of MDP (muramyl tripeptide phosphatidylethanolamine [MTP-PE]), or lipid A at augmenting the immunogenicity of antigen is well documented (5, 19, 26). These synthetic immunomodulators, especially a new form of lipid A, monophosphoryl lipid A (MPL) (28), and the lipophilic derivative of MDP, MTP-PE (7), exhibit little or no toxicity in animals. Moreover, an added advantage of liposomes to enhance immune response is that they are readily taken up by cells involved in antigen presentation and processing. In this report, we have evaluated the immunological consequences of palmitilization of a synthetic peptide of gD. Our results show that peptide association is more stable and that superior levels of humoral and cellular immunity against HSV resulted from exposure to palmitilized peptide in liposomes. However, maximum immunogenicity was only achieved by the additional incorporation of synthetic immunomodulators.

(A preliminary account of this work has been presented elsewhere [4].)

MATERIALS AND METHODS

Virus production and titration. HSV type 1 (HSV-1) strains BK and KOS were grown in monolayers of HEp-2 cells. After three freeze-thaw cycles, the cellular debris was removed ($1,000 \times g$ for 10 min) and the virus was divided and stored at -70°C . Samples were titrated in quadruplicate with Vero cell monolayers grown in microdilution plates. After 4 days, the plates were washed in phosphate-buffered saline (PBS), stained with crystal violet, and examined for cytopathic effects. The results were expressed as tissue culture infective dose (50% endpoint).

Mice. C3H/HeN mice were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Ind.

Peptide synthesis. The 1-23 peptide, corresponding to the amino-terminal end of HSV-1 gD, was synthesized by solid-

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phase methods with alpha amino groups protected with *tert*-butyloxycarbonyl groups. Half of the complete peptide was removed from the resin, while the other half was derivatized with hydrophobic palmitic acid at the amino-terminal lysine by the anhydride method, exactly as the method used for amino acid coupling. Briefly, an 8 M excess of palmitic anhydride was added to peptide-containing resin in the presence of 10 ml of dichloromethane. The peptide has an amino-terminal lysine with two free amino groups, but the internal amino groups were blocked. After being stirred for 2 h, the resin was washed twice in dichloromethane and dried under high vacuum. The resulting diacylpeptide was cleaved from the resin by the standard *tert*-butyloxycarbonyl method (20). This dipalmitic derivative was used to facilitate a stable incorporation of the 23-mer into the liposomal bilayer. The crude peptides were purified by gel filtration on silica gel C-18 columns. Homogeneity of the peptides was demonstrated by thin-layer chromatography after hydrolysis of the peptide and by negative ninhydrin analysis.

Liposome preparation. Egg phosphatidylcholine, cholesterol, and phosphatidylserine were dissolved in methanol-chloroform (1:4) and mixed at a molar ratio of 7:3:1. This mixture was dried to a thin film by rotating the vial in warm water under a stream of nitrogen and was then further dried in a vacuum desiccator. The lipophilic MTP-PE, MPL, and palmitic acid-peptide (acylpeptide) were incorporated into the liposome structure by inclusion in the original lipid preparation at molar ratios of 0.02:1 (MTP-PE:phosphatidylcholine), 0.05:1 (MPL:phosphatidylcholine), and 0.3:1 (peptide:phosphatidylcholine). The dry mixture was rehydrated with PBS and vortexed at high speed for 6 min to produce large multilamellar vesicles (MLVs). Unincorporated material was removed by washing three times in PBS (30,000 $\times$ g for 10 min) and suspending in PBS at a final concentration of 10 μ mol of lipid per ml. The efficiency of incorporation of palmityl-peptide and immunomodulators into the membranes of MLVs was determined by using tracer radiolabeled derivatives of each. The final capture efficiencies of liposome-associated [3 H]MTP-PE, [125 I]acylpeptide, and [125 I]free peptide were calculated from the radioactivity present in the original volume of liposome and were approximately 90% for MTP-PE, 55% for acylpeptide, and 5% for free peptide. The initial concentrations of acylpeptide and free peptide were manipulated to obtain a final lipid to peptide molar ratio of 30:1 in both preparations. Better immune responses were seen with MLVs containing cholesterol, which stabilized the liposomes *in vivo*, as opposed to those seen with liposomes composed of only phosphatidylserine and phosphatidylcholine (data not shown). MTP-PE was manufactured by CIBA GEIGY Corp. and was kindly supplied to us by Biosine Corp., Emeryville, Calif.; MPL was purchased from the RIBI Corporation, Hamilton, Mont.

Immunizations. Six-week-old C3H/HeN mice were immunized intraperitoneally with solutions containing 50 μ g of peptide in its various forms: MLVs with acylpeptide with or without immunomodulators; MLVs with free peptide with or without immunomodulators; keyhole limpet hemocyanin (KLH)-peptide in complete Freund adjuvant (CFA); acylpeptide in CFA; free peptide in CFA; or free peptide. Controls included live virus, MLVs and immunomodulators, and PBS. The immunomodulator doses given with each injection were 50 μ g of MPL and 20 μ g of MTP-PE.

Antibody measurements. HSV-1-specific antibodies were measured with an indirect enzyme-linked immunosorbent assay (ELISA) and a microneutralization assay as previously described (23).

Lymphoproliferation. Draining lymph node cells or spleen cells were obtained 14 days after the last immunization with peptide or virus. Cells (5×10^5) were cultured with X-irradiated syngeneic spleen cells (5×10^5) infected with UV-irradiated virus (multiplicity of infection of 5 before inactivation) for 5 days in U-bottom microdilution plates. On the last day of culturing, 0.5 μ Ci of tritiated thymidine was added to each well. The incorporation of the tritiated label in the cellular DNA after the final 18 h of incubation was determined by liquid scintillation counting and was used as a measure of lymphocyte proliferation.

Viral clearance assay. Mice were inoculated with 20 μ l of virus containing 10^4 or 10^6 PFU of HSV-1 (KOS) on the dorsal side of the ear pinnae. After 3 days, infected pinnae were removed and stored at -70° until required. Ear pinnae were homogenized, and their infectious virus titers were determined in a microtitration assay on Vero cells as described previously (23).

Assessment of IL-2 production. On day 3 of the lymphoproliferation assay, supernatant fluids from the wells were collected and assayed for the presence of IL-2 by measuring tritiated thymidine uptake by an IL-2-dependent cell line, CTLL-20. The cells were incubated for 24 h in the presence of twofold serial dilutions of the supernatant fluids or known concentrations of recombinant IL-2 as standards. The lower limit of IL-2 detection in this assay was 0.5 U/ml.

Peptide retention *in vivo*. Free peptide and acylpeptide were iodinated by the Iodo-Gen method (14) and were injected into mice. Both forms of the peptide were given free and incorporated into liposomes in the hind footpads of mice at a volume of 50 μ l per footpad. Retention of peptide was determined by measuring radioactivity in the draining lymph nodes at the various times of infection. Results are expressed as percentage of input radioactivity at day zero.

RESULTS

Palmitilization and immunomodulators in liposomes enhance immunogenicity. C3H/HeN mice were immunized on four occasions with 50 μ g of gD 1-23 peptide (per injection) in various formats, and ELISA antibody titers against HSV were measured at weekly intervals. Although free peptide was barely immunogenic by itself, in liposomes, or when given with CFA, the immunogenicity was enhanced (up to fourfold) when a carrier (KLH) was attached to the peptide and given with CFA (Table 1). To facilitate incorporation into liposomes, gD 1-23 was palmitilized by adding two palmitic acid residues onto the terminal lysine. This procedure led to a considerable improvement in the efficiency of incorporation into liposomes with about 5% of free peptide and 50 to 55% of acylpeptide incorporated. Acylpeptide incorporated into liposomes was also more stable than free peptide-associated liposomes (Table 2). Incorporation data were used to ensure that the mice received equal amounts of free and derivatized peptide at the same lipid to protein ratio (30:1). By increasing the amount of free peptide or decreasing the amount of acylpeptide or both in the incorporation procedure, equal ratios of lipid to peptide were obtained.

Although the acylpeptide without added vehicle or adjuvant was barely more immunogenic than was the free peptide, its incorporation into liposomes was more immunogenic, particularly as measured late in the course of immunization (Table 1). It can also be seen that the addition of 50 μ g of MPL, 100 μ g of MDP, or 20 μ g of MTP-PE to the acylpeptide-liposome preparations stimulated significantly higher antibody titers compared with the other immunogenic

TABLE 1. Mean anti-HSV titers after peptide immunization^a

Immunogen ^b	Mean titers on day:					
	7	14	21	35	42	56
Free peptide	<25	<25	<25	25	25	<25
Peptide CFA/IFA	<25	25	50	100	100	67
Peptide liposome	NT ^c	33	50	133	167	100
KLH-peptide CFA/IFA	<25	25	100	267	400	333
Free acylpeptide	NT	25	50	50	67	NT
Acylpeptide CFA/IFA	25	50	167	333	667	533
Acylpeptide-liposome	25	50	100	200	533	533
Acylpeptide-liposome + MDP	NT	50	333	1,066	1,866	1,600
Acylpeptide-liposome + MTP-PE	25	67	533	2,133	3,200	2,667
Acylpeptide-liposome + MPL	NT	50	200	1,600	2,667	2,667
ALMM	25	100	667	4,267	5,333	4,267
HSV-1	133	1,333	4,267	8,533	8,533	8,533
Mock infected	NT	<25	<25	<25	<25	<25

^a Six-week-old female C3H/HeN mice were immunized on days zero, 5, 10, and 15, and blood samples were taken on the indicated days. ELISA titers represent the mean of three experiments with five mice per group.

^b Peptide in CFA for the first injection (immunization); peptide in incomplete Freund adjuvant (IFA) for subsequent injections.

^c NT, Not tested.

formulations. The incorporation of these immunomodulators into acylpeptide-liposomes was even superior to acylpeptide in CFA. Of the two synthetic muramyl peptide adjuvants, the lipid-soluble form, MTP-PE, proved to be better, presumably in part because of its more stable incorporation into liposomes. The adjuvant effects of MTP-PE and MPL appeared to be additive, since the highest antibody titers were obtained when both immunomodulators were incorporated into the same acylpeptide-liposome. This combination produced 8-fold-higher titers by week 6 than those achieved by the same dose of acylpeptide in CFA and 256-fold-higher titers than those induced by free acylpeptide. In fact, the combination of acylpeptide-liposome-MTP-PE-MPL (ALMM) induced titers approaching those induced by live virus.

Anti-HSV neutralizing antibody titers were examined (Table 3), and a pattern of results similar to those observed for ELISA titers was obtained. The ALMM construct stimulated significantly higher neutralizing antibody responses in mice than did the other immunogenic 1-23 peptide constructs tested, though this response was threefold less than that induced by live virus.

Immunoglobulin G (IgG) isotypes were examined by an Ig

TABLE 3. Serum neutralizing antibody titers following immunization of C3H/HeN mice^a

Immunogen	Titer after experiment			Mean titer
	1	2	3	
HSV-1	256	128	128	171
Free peptide in CFA	4	4	4	4
Liposome peptide	4	8	8	6.7
Liposome peptide:MTP-PE:MPL	8	8	16	10.7
Acylpeptide in CFA	16	16	16	16
Liposome containing acylpeptide	16	16	8	13.3
ALMM	64	64	32	53.3
Mock infected	<4	<4	<4	<4

^a Serum samples collected from mice on day 35 following four immunizations on days zero, 5, 10, and 15. Data of three independent experiments are shown.

isotype-specific ELISA (Table 4) to determine the distribution of immunoglobulins induced by the 1-23 peptide, and the effects of immunomodulators on this distribution were assessed. The form of the antigen appeared to influence the pattern of isotype responses. Accordingly, vaccination with live HSV or acylpeptide-liposome containing MPL elicited predominantly antibodies of the IgG1 isotype, while vaccination with acylpeptide-liposome constructs containing MTP-PE preferentially elicited IgG2a.

Effect on CMI. Both antigen-induced cell proliferation and IL-2 production were used as measures of cell-mediated immunity (CMI). Immunization with HSV-1 gave rise to significant responses as measured both by stimulation with UV-inactivated HSV and by exposure to the soluble 1-23 peptide (on syngeneic stimulator cells). Mice immunized with acylpeptide incorporated in adjuvant or liposomes with immunomodulators developed significant responses, but neither the soluble peptide formulation nor the acylpeptide in liposome induced significant responses (Table 5). In regard to antigen-induced IL-2 induction, only mice immunized with HSV and the ALMM formulation developed significant responses (data not shown).

Induction of immunity to local and systemic challenge with HSV. To test the effect on antiviral immunity, mice were challenged in the ear pinnae with either 10⁴ or 10⁶ PFU of the virus and their ability to clear such virus was measured 3 days later by measuring the quantity of virus in homogenized pinnae. Only mice immunized with infectious HSV-1 were capable of clearing 10⁶ PFU of virus (Table 6). However, mice given the ALMM immunogen and acylpeptide in CFA could clear 10⁴ PFU, whereas significant levels of clearance were not observed with mice immunized with the other peptide formulations. With regard to protection from sys-

TABLE 2. Stability of peptide and acylpeptide association with liposomes

Peptide form	% Leakage after ^a :		
	6 h	12 h	24 h
MLV-free peptide	36	59	86
MLV-acylpeptide	2	3.5	4.9

^a Liposomes were prepared with ¹²⁵I-labeled peptide and acylpeptide and were incubated in PBS at 37°C for 6, 12, and 24 h. Free peptide was determined by separating the liposomes from leaked material by centrifugation (30,000 × g for 10 min). The radioactivity of the supernatant fluid was compared with the initial radioactivity, and the percentage of leakage was calculated.

TABLE 4. Immunoglobulin subclass profile in mice immunized with different preparations

Immunogen	% Total IgG response with ^a :			
	IgG1	IgG2a	IgG2b	IgG3
HSV-1	61	25	11	3
Acylpeptide-liposome-MTP-PE	25	59	12	3
Acylpeptide-liposome-MPL	50	28	16	5
ALMM	32	50	16	4
Acylpeptide in CFA/IFA ^b	25	50	19	3

^a Sera from mice after four immunizations.

^b Acylpeptide in CFA for the first injection (immunization); acylpeptide in incomplete Freund adjuvant (IFA) for subsequent injections.

TABLE 5. Lymphoproliferation of immune T cells by viral and peptide antigen

Priming antigen ^a	Antigenic stimulation	cpm (³ H]TdR uptake) ^b	S.I. ^c
HSV-1	UV HSV-1	26,539 + 2,826	4.8 ^d
	1-23	14,893 + 1,531	2.7 ^d
	None	5,484 + 1,646	1.0
Free acylpeptide 1-23	UV HSV-1	5,439 + 873	0.9
	1-23	6,341 + 1,760	1.1
	None	5,816 + 1,051	1.0
Acylpeptide 1-23 in CFA	UV HSV-1	10,231 + 1,698	2.1 ^d
	1-23	17,346 + 1,873	3.5 ^d
	None	4,940 + 745	1.0
Acylpeptide 1-23-liposome	UV HSV-1	9,017 + 1,281	1.3
	1-23	12,103 + 2,237	1.7 ^d
	None	6,893 + 937	1.0
ALMM	UV HSV-1	14,118 + 1,854	2.4 ^d
	1-23	18,952 + 2,698	3.4 ^d
	None	5,816 + 1,051	1.0

^a Splenocytes from mice immunized with different forms of the free 1-23 peptide did not exhibit any significant proliferation to viral or peptide antigens.

^b Peptide and virus-immune splenic T cells were prepared as described in Materials and Methods. T cells were stimulated for 5 days with an equal number of X-irradiated syngeneic normal splenocytes (APC) which were preincubated with the 1-23 peptide or UV-inactivated HSV. The final 12 h of incubation was in the presence of 1.0 μ Ci per well of [³H]TdR. cpm, Counts per minute; TdR, thymidine.

^c S.I. (stimulation index) = means cpm antigen-stimulated cells/means cpm APC only.

^d Statistically significant difference ($P < 0.01$) from the mean of cells stimulated with antigen presenting cells only.

temic challenge (Fig. 1), complete protection was evident in two groups: those given HSV and those immunized with the ALMM immunogen. Partial levels of protection occurred in other groups which received various acylpeptide formulations, but significant protection did not occur in mice immunized with free peptide.

In vivo distribution of acylated and free peptides. To determine the distribution and retention characteristics of various immunogenic preparations, ¹²⁵I-labeled peptides, free and within liposomes, were injected into mice, and at periods thereafter, draining lymph nodes were collected to record levels of radioactivity. The percentage of retention of

TABLE 6. Clearance of local HSV infection by immunized C3H/HeN mice

Immunogen	Clearance ^a after a challenge dose of:	
	10 ⁴ PFU	10 ⁶ PFU
HSV-1	<1.5 ^b	<1.5 ^b
Peptide in CFA/IFA ^c	3.8	5.9
Peptide-liposome	3.8	5.8
Peptide-liposome-MTP-PE-MPL	2.3	4.2
Acylpeptide in CFA/IFA ^d	1.5 ^b	4.3
Acylpeptide-liposome	3.3	5.4
ALMM	<1.5 ^b	3.2
Mock infected (PBS)	4.1	5.9

^a Quantitative analysis of ear clearance ability. On day 28, groups of five immune mice were challenged in the left pinnae with the dose of virus shown. Three days after challenge, the pinnae were removed and the infectious titers determined (log 50% tissue culture infective dose) on Vero cell monolayers. Results are shown as the average titer with a lower limit of detection of 1.5 logs. A similar pattern of results were obtained in two repeat experiments.

^b Significantly below control values.

^c See Table 1, footnote b.

^d See Table 4, footnote b.

A

B

FIG. 1. Protection of mice against lethal challenge by HSV-1 after immunization with acylpeptide in liposomes. Female C3H/HeN mice were immunized four times with gD 1-23 peptide in various forms and then challenged with a lethal dose of HSV-1 (strain BK). Immunizing forms are HSV-1 (○), acylpeptide-liposome (■), acylpeptide-liposome with MPL (△), and PBS (●) (A) and ALMM (◆), acylpeptide-liposome with MTP-PE (×), acylpeptide in CFA/incomplete Freund adjuvant (IFA) (□), and peptide in CFA/IFA (▲) (B).

input material was highest in mice given acylpeptide in liposomes (Table 7). In these mice, more than 10% of the input radioactivity was still present 21 days after initial injection. This value was greater than that observed in mice given free peptide in liposomes when measured 4 days after infection.

DISCUSSION

Our investigations have evaluated liposomes as vehicles to improve the immunogenicity of a synthetic peptide of HSV glycoprotein D (gD 1-23) that is known to induce antibody and T-cell responses that react with the virus itself (12, 30, 31). Our results demonstrate that acylated forms of gD 1-23 in liposomes induced superior antibody responses, although in the absence of adjuvant, neither elicited CMI. Excellent humoral responses occurred when synthetic immunomodulators were included along with the acylated peptide, and these responses exceeded those induced by peptides emulsified in CFA. Whereas liposomes containing acylpeptides failed to induce CMI (unless emulsified in CFA), the addi-

TABLE 7. Retention of labeled peptides following immunization

Test day	% Peptide retained in draining lymph nodes with ^a :			
	Liposome containing acylpeptide	Acylpeptide	Liposome containing free peptide	Free peptide
4	37	24	9	2
7	33	13	3	<1
10	27	8	2	<1
17	16	6	<1	<1
21	12	2	<1	<1

^a Animals were given 50 μ l of inoculum into footpads, and at times given, draining lymph nodes were collected for measurement of radioactivity. Values given represent means of four samples for each time.

tional incorporation of immunomodulators did induce such responses. These liposomes also provided a significant level of protective immunity.

Although reasons for the greater immunogenicity of acylated peptide were not established, we suspect it occurs because of greater stability and longer retention in cells that serve to present and process antigen. In support of this notion, *in vitro* experiments revealed that antigen leakage from liposomes which contained acylated peptide was far less than occurred with free peptide. Presumably, after *in vivo* administration, liposomes with free peptide lose much material before their engulfment by macrophages involved in antigen presentation. However, there could also be a variable outcome following macrophage uptake. Thus, in comparisons of distribution and retention time of liposomes with free and acylated peptides, a greater percentage of acylated material was trapped in the local lymph node but underwent a slower rate of clearance than occurred with the soluble antigen-containing liposomes. Slower rates of clearance of lipid-soluble components as compared with hydrophilic counterparts in liposomes have been reported in other systems (13).

Enhancement of peptide immunogenicity following acylation was first reported by Hopp with a hepatitis B virus peptide (17), and the approach was also investigated by Heber-Katz and colleagues with the same HSV peptide used in this study (15, 16). In the study by Heber-Katz et al., liposomes were also used, although the value of these vehicles was obscured because the liposome-containing acylpeptide was additionally emulsified in CFA. Accordingly, the adjuvant effect of acylated peptide in liposome could not be independently assessed. Moreover, in marked distinction to our own findings, Watari et al. (30) failed to observe a humoral antibody response but did achieve potent cellular responses. In fact, since the vehicles used by Watari et al. could engender protective immunity, they used these observations to argue that protective immunity against HSV would seem to have a cellular explanation. Our own studies could also be taken to indicate that cellular immunity may be more important than antibody responses, at least as measured by clearance of virus from local sites. Thus, in a model in which the ability of mice to clear a local HSV infection from the ear was tested, only animals immunized with preparations which induced significant T-cell (as well as antibody) responses could clear virus. Moreover, in a protection from lethality model, although many peptide immunogens were capable of inducing antibody and conferred partial protection, the best protection was noted with liposomes which contained acylpeptide and immunomodulators and which also induced a significant CMI response. Indeed, our experiments showed that liposomes which contained

acylpeptide along with lipid-soluble immunomodulators were superior to CFA as vehicles to induce immunity; these liposomes also favored the induction of IgG2a-type antibody, a subclass of IgG that appears to be instrumental in protection against certain infectious agents (1, 9, 18). Moreover, as shown by others, as well as noted in our own studies, the liposome formulation lacks toxicity and may provide a vehicle useful for human use.

Although liposome-containing acylpeptide and lipid-soluble immunomodulators appear to have great promise, our results also indicate that gD 1-23 may not represent an ideal peptide to induce anti-HSV immunity. Accordingly, whereas the best synthetic immunogen preparation could induce protection against a low-dose local challenge, immunity to high-dose challenge only occurred in mice immunized with live virus. Elsewhere, we have argued that immunity to high-dose virus challenge required that numerous aspects of acquired immunity must be induced including the cytotoxic T lymphocytes system (21). Unfortunately, gD appears to have no epitopes that are recognized by murine cytotoxic T lymphocytes, at least in those strains studied so far. Consequently, it will be of interest to perform similar studies on the effects of acylation and liposome incorporation of peptides which are considered part of the repertoire of cytotoxic T lymphocytes. Such studies are currently under way in our laboratory.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI 124762 from the National Institute of Allergy and Infectious Diseases.

The MTP-PE used in these studies was manufactured by CIBA GEIGY Corp., Basel, Switzerland, and was a kind gift of the Biosine Corp., Emeryville, Calif. We greatly appreciate the enthusiastic cooperation of Charles Murphy and his colleagues in the analytical services laboratory at the University of Tennessee and the manuscript preparation skills of Paula Keaton and Audrey Winton.

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