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Induction In Vitro of Primary Cytotoxic T-Lymphocyte Responses with DNA Encoding Herpes Simplex Virus Proteins

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Vaccines which successfully protect against virus infections usually need to induce a broadly reactive immune response which includes the induction of cytotoxic T lymphocytes (CTL). In this study, we have used a convenient in vitro approach to investigate if plasmid DNAs encoding proteins of herpes simplex virus (HSV) are capable of inducing primary CD8$^+$ CTL. Dendritic cells or macrophages were transfected with either plasmid DNA encoding glycoprotein B or DNA encoding the immediate-early protein ICP27. These antigen-presenting cells (APC) were then used to stimulate enriched populations of naive T cells in microcultures for 5 days in vitro. Antigen-specific CD8$^+$ CTL which reacted both with specific protein-expressing targets and with syngeneic targets infected with HSV could be demonstrated. Dendritic cells, as APC, generated the maximal responses, but such cells needed to be transfected with DNA in the presence of a cationic lipid. However, macrophages could act as APC when they were exposed to purified DNA. HSV-primed splenocytes were also shown to generate specific CTL responses when they were stimulated with purified DNA encoding ICP27. The novel approach described in this paper promises to be extremely useful, since defining immunogenicity profiles and identifying epitopes on viral proteins should be easier and more convenient when working with DNA and investigating variables in vitro. This is particularly the case with complex viruses such as HSV, most of whose encoded proteins have yet to be isolated in sufficient quantity or purity to perform in vivo immunological studies.

The recent observation that protective immunity can be induced following genetic immunization with DNA (7, 21–23, 26) opens up a new approach to vaccination. This novel immunization strategy promises some advantages, such as resistance to neutralization by any existing antibody, gene expression in host cells so that broadly reactive immunity should be induced, and the potential ease of generating combination vaccines. However, many parameters require detailed analysis before genetic immunization becomes an acceptable procedure. These include defining the proteins and peptides to encode, particularly when aiming to achieve optimal immunity against complex viruses, such as herpes simplex virus (HSV), that expresses multiple proteins (17). Other issues include the evaluation of means of administration that will optimally induce components of immunity that best correlate with protection.

With many viruses, the attainment of protective immunity usually means that an effective response of cytotoxic T-lymphocytes (CTL) must be induced (6, 19). Such responses occur only if antigens are appropriately processed by antigen-presenting cells (APC), and this may fail to occur with certain types of vaccines, in particular with those which are inactivated (4, 9, 20). As regards the role of antigens and presentation systems that result in CTL induction, it is more convenient to perform studies in vitro, particularly since approaches found effective in the more demanding in vitro system are invariably also successful in vivo (5, 10, 14). Accordingly, in the present communication, we have used a primary in vitro CTL induction system (12–14) to establish conditions under which preparations of DNA that encode HSV proteins can induce virus-specific CTL. We demonstrate that two proteins, especially if expressed in dendritic cells (DC) as antigen-presenting cells (APC), induce potent protein and virus-specific CTL.

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MATERIALS AND METHODS

Mice. Female 7- to 8-week-old C57BL/6 (*H-2^b*) and BALB/c (*H-2^d*) mice were obtained from Harlan Sprague-Dawley, Indianapolis, Ind., or from Sasco, Omaha, Nebr. In conducting the research described in this article, the investigators adhered to the guidelines proposed by the Committee on the Care of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

DNA preparation and protein expression. The cDNA clone of the HSV type 1 (KOS) (HSV-1) gB gene in pSR175 (designated gBpSR175) was kindly provided by Martin Muggeridge. The 3.7-kb gB cDNA was cut from gBpSR175 by using *Hin*dIII (5′) and *Bam*HI (3′) and was subsequently subcloned into the plasmid vectors pUC19 and pcDNAI (Invitrogen). The 2.4-kb ICP27 gene was cloned into M13mp18 as described previously (3) and was subsequently subcloned into the pcDNAI vector (*Hin*dIII-*Xba*I sites) oriented 5′→3′ to the plasmid cytomegalovirus promoter. The vector pcDNAI was chosen because it contains a cytomegalovirus promoter and enhancer for gene expression in eucaryotic cells and a ColE1-like high-copy-number plasmid origin of replication, allowing the preparation of large amounts of recombinant plasmid DNA. Recombinant plasmids containing the gB or ICP27 gene of HSV-1 were used to transform *Escherichia coli* (strain MC1061) under ampicillin and tetracycline selection. Recombinant plasmid DNA was isolated from transformed bacterial

colonies and was prepared by standard techniques using alkaline lysis, two purifications on CsCl gradients, and ethanol precipitation (18). The purity and concentration of DNA were analyzed by A_{260} and A_{280} and by agarose gel electrophoresis and ethidium bromide staining.

Expression of the cloned gB and ICP27 genes in vitro was confirmed by transfecting splenic macrophages (Mϕ) and DC (5×10^6 cells) from naive BALB/c female retired breeders with 5 μg of recombinant plasmid DNA and 15 μg of the cationic lipid DOTAP (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Transfected cells were lysed with 0.5 ml of RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 50 mM Tris [pH 8.0]). Cell lysates were immunoprecipitated with either mouse anti-ICP27 monoclonal antibody (MAb) (1:500) or mouse anti-gB polyclonal antibody (1:500) and protein A Sepharose (Pierce). The precipitates were analyzed by gel electrophoresis under reducing conditions on 10% gels with a 29.2:0.8 acrylamide-to-bisacrylamide ratio. Proteins were visualized by Western blotting (immunoblotting) using goat anti-mouse immunoglobulin conjugated to alkaline phosphatase (Bio-Rad).

The relative efficiencies of transfection of ICP27 DNA in Mϕ and DC were determined by comparing relative amounts of ICP27 protein expressed to input DNA. Cells (2×10^6) were transfected with either 5 μg of vector control DNA or 1, 5, 25, or 50 μg of ICP27 DNA using DOTAP as described previously. After overnight transfection, cells were lysed and proteins were processed by immunoprecipitation, SDS-polyacrylamide gel electrophoresis (PAGE), and immunoblotting. Alternatively, Mϕ and DC were incubated in the presence of exogenous ICP27 DNA in culture medium overnight in the absence of cationic lipid, and ICP27 protein expression was analyzed as described above. Protein band intensities on nitrocellulose blots were analyzed by scanning densitometry using Molecular Analyst software (Bio-Rad).

Virus and peptides. HSV-1 strain KOS was propagated on Vero cell monolayers and stored as infectious cell preparations at −70°C. Viral titers for HSV-1 KOS were expressed as 50% tissue culture infectious doses. The *H-2^b*-specific peptide (amino acids 498 to 505 [SSIEFARL]) was synthesized on resin to have unblocked (free) amino and carboxyl ends (Research Genetics, Birmingham, Ala.).

APC and responder T cells. Splenocytes obtained from naive C57BL/6 or BALB/c female mice were treated with ammonium chloride-Tris buffer for 3 min to deplete erythrocytes. Splenocytes (3 ml) at 2×10^7 cells per ml were layered over a 2-ml metrizamide gradient column (Nycomed Pharma AS, Oslo, Norway; analytical grade, 14.5 g added to 100 ml of phosphate-buffered saline [PBS], pH 7.0) and were centrifuged at $600 \times g$ for 10 min. Cells from the interface were collected, and analysis with a fluorescence-activated cell sorter (FACS) showed on average 60 to 70% DC (MAb 33D1; kindly provided by Ralph Steinman, The Rockefeller University) (15), 1% Mϕ (MAb F4/80) (2), 15 to 20% T cells, and 10 to 12% B cells. The pellet was resuspended and allowed to adhere for 1 h. More than 75 to 80% of the adherent population was identified as Mϕ by FACS analysis, with approximately 5% lymphocytes and <5% DC. B cells were separated from the nonadherent population by panning on anti-immunoglobulin-coated plates. The separated cell population, which consisted of >80% T lymphocytes by FACS analysis, was used as responder naive T cells.

Pulsing of APC with DNA preparations. DC or Mϕ were exposed to purified DNA preparations either without or in the presence of the cationic lipid DOTAP. For transfection with this cationic lipid, DNA preparations (50 to 75 μg) were reacted with 15 μg of DOTAP in 200 μl of PBS (pH 7.2) for 10 min at room temperature in a 4-ml polystyrene tube (Falcon, Lincoln Park, N.J.). The APC (5×10^6 cells) were transfected with a DNA-DOTAP mixture in 1 ml of RPMI–5% fetal calf serum (FCS) medium at 37°C for 3 h with occasional shaking. At the end of the incubation, the cells were washed and used as stimulators. The procedure was not visibly toxic to APC, as determined by trypan blue staining.

Induction of primary CTL in vitro. Purified T cells (1.0×10^7 cells per ml) from naive BALB/c or C57BL/6 mice were cultured with DC or Mϕ (1.0×10^5 cells per ml) to give responder-to-stimulator ratios ranging from 100:1 to 6.25:1 in 200 μl of LDA medium (NCTC 109 and RPMI 1640 [1:1]), supplemented with 10% heat-inactivated FCS, 10 mM L-glutamine, 1 mM oxalacetic acid, 0.2 U of bovine insulin per ml, and 50 μl of 2-mercaptoethanol in 96-well U-bottom plates. The plates were incubated at 37°C under 5% CO_2 for 5 days. CTL assays were performed on the 5th day by taking 100 μl of medium from each 200-μl microculture well and adding 100 μl of target cells (10^4). Alternatively, T cells (5×10^6 cells per ml) and APC (2×10^5 cells per ml) were cultured in LDA in 96-well U-bottom plates to give a responder-to-stimulator ratio of 25:1. After 5 days, the cells were used as effectors in a standard 4-h ^{51}Cr release assay.

Restimulation of HSV-primed effectors with DNA. Spleens from mice immunized with infectious HSV three times at 2-week intervals were collected 10 to 14 days after the last immunization, and bulk cultures were set up in six-well plates (Corning Glass Works, Corning, N.Y.) as described elsewhere (3). Briefly, 1.25×10^7 splenocytes were cultured per well in 5 ml of LDA medium. The splenocytes were restimulated with DNA at a concentration of 5 μg/ml for 5 days at 37°C.

Target cells for cytotoxicity assays. EMT6 cells (*H-2^d* [mammary adenocarcinoma cells provided by Ed Cantin, City of the Hope National Medical Center, Duarte, Calif.]), EL4 cells (*H-2^b* lymphoma [American Type Culture Collection]), and YAC-1 cells (*H-2^a*, lymphoma) were all cultured in Dulbecco modified Eagle medium (Gibco, Grand Island, N.Y.) supplemented with 10% heat-inactivated FCS. EMT6 cells were transfected with the recombinant plasmid pJF24, containing the HSV-1 ICP27 gene (EMT6-27), by Frank Jenkins, Uniformed Services University, Bethesda, Md. This plasmid contains the ICP27 gene and its own promoter as well as the G418 resistance gene under the control of the HSV thymidine kinase promoter. These cells were grown in Dulbecco medium with 10% FCS containing 300 μg of G418 per ml. EMT6 cells were also transfected with pcDNAgB by using DOTAP as described previously. These cells were transfected overnight prior to ^{51}Cr labeling and were used as targets.

Cytotoxicity assays. All target cells were labeled with ^{51}Cr by reacting 2×10^6 cells in 500 μl of RPMI 1640 plus 10% FCS with 100 μCi of $Na_2{}^{51}CrO_4$ for 90 min at 37°C. The cells were washed three times prior to addition to effector cells. To detect HSV-specific lysis, target cells were infected with HSV (at a multiplicity of infection of 5) at the same time as ^{51}Cr labeling.

To detect ICP27-specific lysis, EMT6-27 cells were used. To detect gB-specific lysis, two systems were used. For *H-2^d*-specific responses, EMT6 cells were transfected with pcDNAgB in the presence of DOTAP, whereas in the *H-2^b* system, gB killing was detected by adding the CTL epitope peptide (amino acids 498 to 505 [SSIEFARL]) (12) to ^{51}Cr-labeled EL4 cells (10^5/ml) at a concentration of 10 μg/ml to 2×10^6 cells.

All ^{51}Cr release assays were run for 4 h at various effector-to-target cell ratios in 96-well V-bottom plates. Prior to harvesting, the plates were centrifuged and 100 μl of the

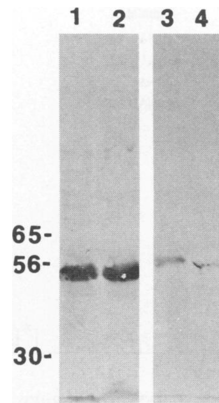

FIG. 1. Expression of gB and ICP27 in splenic Mϕ and DC. Lysates from cells transfected with recombinant plasmids were immunoprecipitated with anti-gB or anti-ICP27 antibody and were analyzed by SDS-PAGE and Western blotting. Truncated gB in Mϕ (lane 1) and DC (lane 2) and full-length ICP27 expressed in Mϕ (lane 3) and DC (lane 4) at 24 h posttransfection are shown.

culture supernatant was collected to measure radioactivity. Percent specific release was computed as (experimental release − spontaneous release)/total release × 100.

Each assay was performed in triplicate, and only experiments with spontaneous release values of 20% or less were reported.

In vitro antibody depletions. Effector cells generated by stimulating naive T cells with DC pulsed with pcDNAgB or pcDNAICP27-DNA-DOTAP at a responder-to-stimulator ratio of 25:1 were depleted of either T cells, $CD8^+$ cells, or $CD4^+$ cells by using anti-Thy-1.2, anti-CD8, and anti-CD4 MAbs along with complement, respectively. Rat anti-Thy and anti-CD8 and rabbit Low-tox-m complement were obtained from Cedarlane Laboratories (Accurate Chemical and Scientific, Westbury, N.Y.). The GK1.5 (anti-CD4) hybridoma cell line was obtained from the American Type Culture Collection (Rockville, Md.) and was used as ascites fluid (40 to 50 μg/5 × 10^6 cells). Effectors were incubated with the required volume of MAb at 4°C for 1 h. The cells were washed twice and were resuspended in complement at a concentration which did not cause lysis of the cells when they were treated with complement alone. After 60 min of incubation at 37°C, the cells were washed and used as effectors in the CTL assay.

RESULTS

Expression of HSV proteins. Following transfection of both Mϕ and DC with DNA encoding gB and ICP27, viral proteins were detectable, as shown by SDS-PAGE and Western blotting (Fig. 1). The ICP27 protein appeared as full length in both cell types, whereas gB was truncated to a 55-kDa protein rather than the normal 110-kDa protein. It is not uncommon to detect truncations of gB, since this protein is highly sensitive to proteolytic cleavage (25). Densitometer analysis of ICP27 protein expression after transfection of Mϕ and DC did not reveal major differences (within 20%) in the total amount of protein made by the two cell types (data not shown). In addition, Mϕ exposed to 25 μg of purified pcDNAICP27 in the absence of transfecting agent expressed a small but detectable amount of ICP27 protein; however, DC similarly exposed to pcDNAICP27 DNA failed to express detectable protein.

TABLE 1. Primary induction of CTL with plasmid DNA-transfected APC[a]

APC	Stimulant	Percent specific lysis			
		EMT6-27	EMT6 HSV	EMT6	EL4 HSV
DC	None	<1	<1	<1	<1
DC	pcDNAICP27	46 ± 4	11 ± 1.5	<1	1 ± 1
DC	pcDNA	<1	<1	<1	<1
Mϕ	pcDNAICP27	27 ± 2	<1	<1	<1
Mϕ	pcDNA	<1	<1	<1	<1

[a] Naive enriched T cells from BALB/c mice were cultured for 5 days in vitro with syngeneic APC (Mϕ or DC) that had been transfected with plasmid DNA via the DOTAP procedure (see Materials and Methods). Various responder-to-stimulator ratios (100:1 to 6.25:1) were used, but the data shown in the table were for the ratio 50:1. Background release from targets during the 4-h ^{51}Cr release assay was 15% or less.

Primary CTL induction with APC transfected with DNA. Splenic Mϕ and DC were purified from young immunologically naive BALB/c mice and were transfected with either plasmid DNA or plasmid DNA encoding ICP27. Following transfection, washed cells were cultured at various stimulator-to-responder ratios with naive enriched T cells for 5 days. At the end of culturing, the cytolytic activities of the cells were tested against a range of target cells. As is evident in Table 1, both APC types exposed to pcDNAICP27 induced protein-specific primary CTL responses. However, responses were higher when DC were used as the APC. In addition, when DC were used as APC for naive T cells, cytolytic activity was detectable by using HSV-infected syngeneic target cells.

Further experiments were performed to test the specificity of CTL induced in vitro. For this purpose, DC were transfected with either pcDNAICP27 or pcDNAgB in the presence of DOTAP, and these cells were used as APC. Cultures were performed at optimal responder-to-stimulator ratios (25:1). The results, depicted in Table 2, clearly show that both gB-specific and ICP27-specific CTL were generated. Thus, gB-induced CTL that were cytotoxic for gB but that were not ICP27-expressing targets. Similarly, the ICP27-specific cells failed to kill gB-expressing targets. In this same experiment, control uninfected EMT6 cells and the NK cell target YAC-1 cells were not killed. In other experiments, primary gB-specific CTL generated by induction with pcDNAgB-transfected DC were shown to kill syngeneic but not allogeneic HSV-infected targets cells (data not shown). In Table 3, the data show that the cytotoxicity generated in the primary in vitro induction system was mediated by $CD8^+$ T cells.

TABLE 2. Primary induction of CTL in vitro with DC transfected with plasmid DNA[a]

Stimulant	E:T[b]	Percent specific lysis		
		EMT6-27	EMT6-gB	YAC-1
None	50:1	<1	<1	1 ± 1
pcDNAICP27	80:1	28 ± 2	<1	2 ± 1
pcDNAICP27	20:1	15 ± 1	<1	1 ± 1
pcDNAgB	80:1	<1	45 ± 2	0 ± 1
pcDNAgB	20:1	<1	10 ± 1	1 ± 1
pcDNA	80:1	<1	<1	2 ± 1
pcDNA	20:1	<1	<1	1 ± 1

[a] DC as APC from BALB/c mice were transfected with plasmid DNA in the presence of DOTAP and were cultured with syngeneic naive enriched T cells for 5 days at a responder-to-stimulator ratio of 25:1. On day 5, effectors were pooled and cytotoxicity assays were set up at various effector-to-target cell ratios.

[b] E:T, effector-to-target cell ratio.

TABLE 3. Evidence that CTL generated in primary induction by DNA are CD8+ T cells[a]

Treatment	Percent specific lysis EMT6-27		
	75:1	37:1	16:1
C′	16 ± 1.8	12 ± 3	3 ± 2
Anti-Thy-1 + C′	<1	<1	<1
Anti-CD8 + C′	<1	<1	<1
Anti-CD4+ C′	15.5 ± 1	ND	ND

[a] DC from BALB/c mice were transfected with pcDNAICP27 in the presence of DOTAP and were used to induce primary CTL in naive syngeneic T-cell cultures. In an experiment of similar design, in which effector cells from T-cell cultures were stimulated with DC transfected with pcDNAgB in the presence of DOTAP, the percent specific lysis of syngeneic HSV-infected target effector cells of 18% (effector-to-target cell ratio, 50:1) was abolished by treatment with anti-CD8 + C′. C′, complement; ND, no data.

The experiments recorded in Tables 1 to 3 all demonstrate primary CTL responses with APC transfected with DNA in the presence of DOTAP. However, as shown in Table 4, cytotoxic responses could be obtained in a naive T-cell population by using APC exposed to purified DNA (in the absence of DOTAP). Thus, splenic Mϕ were exposed to pcDNAgB or pcDNAICP27 in microcultures for 24 h, after which they were added to naive T-cell and DC cultures (responder-to-stimulator ratio, 25:1). After 5 days of incubation, the cytotoxicities against different target cells were measured. As is evident in Table 4, this procedure resulted in the generation of specific CTL in both the gB and the ICP27 systems. Similar results were obtained in three other experiments. In the case of Mϕ exposed to purified pcDNAgB, the C57BL/6 system was used and the target peptide for gB-specific CTL (amino acids 498 to 505) (12) was used to detect specific lysis. Not shown is the observation that when DC were similarly exposed to purified DNA without the cationic lipid, CTL responses were not induced. As mentioned previously, although Mϕ exposed to purified DNA in the absence of DOTAP do produce some detectable protein, such protein could not be detected in DC.

Restimulation of HSV-primed splenocytes. Unfractionated spleen cells from mice previously infected with HSV-1 were cultured in bulk cultures for 5 days with purified pcDNAICP27 or with control pcDNA, and the cells were tested for cytotoxicity against antigen-expressing target cells. As is evident in Fig. 2, potent cytotoxic activity against ICP27-expressing target cells was demonstrated with the pcDNAIPC27-exposed splenocytes. Normal unfractionated splenocytes exposed to pcDNAICP27 under the same bulk culture conditions failed to generate detectable CTL activity (data not shown).

TABLE 4. Generation of primary CTL from naive cells by Mϕ exposed to purified DNA[a]

Expt	Stimulant to Mϕ	Percent specific lysis			
		EL4-498-505	EL4	EMT6-498-505	EMT6-27
1	pcDNA gB	28 ± 1	<1	1.2	ND
1	pcDNA	<1	<1	<1	ND
1	None	<1	<1	<1	ND
2	pcDNA ICP27	ND	ND	<1	15 ± 1
2	pcDNA	ND	ND	<1	<1
2	None	ND	ND	<1	<1

[a] Mϕ (5×10^5 cells per ml) were treated with purified DNA (5 μg/ml) for 24 h, and then DC microcultures of T cells (responder-to-stimulator ratio of 25:1) were added. CTL assays were done after 5 days of culture. In experiment 1, Mϕ were exposed to pcDNAgB in the *H-2^b^* system (C57BL/6 mice), while in experiment 2, *H-2^d^* Mϕ were exposed to pcDNAICP27 (BALB/c mice). In experiment 3, Mϕ exposed to pcDNAgB generated gB-specific CTL in T cells (DC cultures in the *H-2^d^* [BALB/c] system). ND, no data.

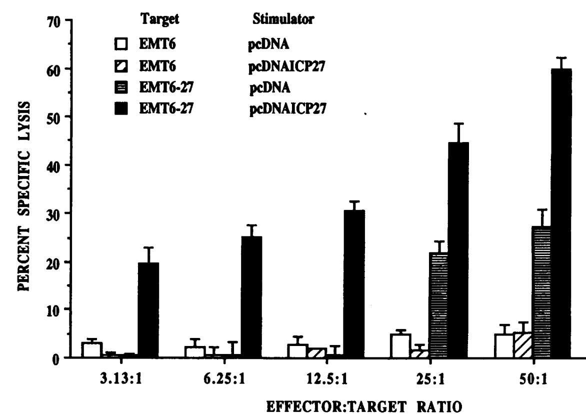

FIG. 2. Cytotoxicity resulting from stimulation in vitro of HSV-primed splenocytes with purified DNA. Not shown are values of lysis for unstimulated cells primed against EMT6 and EMT6-27 targets. Levels were approximately the same as those shown for pcDNA-stimulated cells.

DISCUSSION

Our results demonstrate that CTL responses can be generated in vitro from naive or memory T cells upon exposure to APC transfected with plasmid DNA encoding HSV genes driven by a cytomegalovirus promoter. Primary CTL responses were investigated with two proteins, the glycoprotein gB and the immediate-early protein ICP27. The responses generated were not only protein specific but were also reactive against HSV-infected target cells. From the genotype of the target cells killed and that of the effector cells, the responses being induced were mediated by major histocompatibility complex class I-restricted CD8+ CTL. However, the possibility that other functional aspects of T-cell immunity were also induced was not fully evaluated, and this aspect is under further investigation.

The approach that we have described appears to be novel. It promises to be extremely useful, since defining immunogenicity profiles and identifying component epitopes on viral proteins should be easier and more convenient when working with DNA. This is particularly the case when studying complex viruses such as HSV, most of whose encoded proteins have yet to be isolated in sufficient quantity or purity for immunological studies to be performed. Moreover, since there is now a lively interest in genetic vaccines (7, 21–23, 26), screening candidate DNA constructs for various aspects of immunogenicity by in vitro analyses is likely to be a more efficient approach than extensive in vivo studies. Interestingly, both of the HSV protein-encoding plasmid DNA constructs that we have described were, in preliminary studies, immunogenic in vivo when administered in an appropriate fashion (17a).

In most of the experiments described in the present communication, plasmid DNA was transfected into APC by using the cationic lipid DOTAP. Moreover, as observed with approaches in which proteins are introduced into APC with, for example, pH-sensitive liposomes (13, 14), DC were invariably superior to Mϕ as APC in primary in vitro CTL responses from naive T responder cells (10, 11, 13, 14). To obtain induction of CTL with DC, it was necessary to transfect with DNA that formed a complex with a cationic lipid, and APC activity for

primary CTL responses has yet to be obtained when DC were exposed to purified DNA. Interestingly, however, this was not the case with Mϕ as APC. Accordingly, Mϕ exposed to purified DNA without a cationic lipid were shown to express APC activity for both primary and secondary CTL responses. How Mϕ take up purified DNA and subsequently express protein in such a way that it becomes available for MHC class I processing and CTL recognition remains to be established. It is curious that naked DNA injected in vivo seems to be best expressed when it is delivered to muscles (1, 24). Muscle cells are not generally considered as APC for the immune system; however, genetic vaccines injected intramuscularly may induce effective protective immunity that appears to correlate with the induction of the CTL response (22). Conceivably, muscle cells may take up DNA and express protein; however this material or some metabolic fragment may be subsequently taken up by APC such as DC which then, in turn, express it in a form appropriate for CTL induction. We have preliminary evidence from in vitro studies that Mϕ may take up naked DNA and release peptides that can be presented by DC to achieve CTL induction (13a). There is also evidence from several in vivo studies that Mϕ and DC may interact during antigen presentation (8, 16). Thus, for example, we have observed that the APC activity of DC after in vivo administration of liposomal antigen failed to materialize when Mϕ in the recipient were previously inactivated by exposure to the selective Mϕ-inactivating agent dichloromethylene disphosphonate (13). It will be important to establish the role of different types of APC and how best to deliver antigen to them in order to achieve optimal T-cell response with genetic vaccines.

In conclusion, our data show that primary in vitro CTL induction can be achieved with plasmid DNA encoding viral proteins. This approach should be valuable to monitor the immunogenicity of proteins and peptides encoded by plasmid DNA constructs and should help guide the development of genetic vaccines that optimally induce long-term protective immunity in vivo.

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