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Identification of a T-Cell Epitope Adjacent to Neutralization Antigenic Site 1 of Poliovirus Type 1

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Proliferative T-cell responses to poliovirus in various strains of mice have been analyzed by using either killed purified virus or capsid protein VP1 synthetic peptides. Following immunization of mice with inactivated poliovirus type 1 (PV1), a specific proliferative response of their lymph node CD4$^+$ T cells was obtained after in vitro stimulation with purified virus. In mice immunized with PV1, PV2, or PV3, a strong cross-reactivity of the T-cell responses was observed after in vitro stimulation with heterologous viruses. By using various strategies, a dominant T-cell epitope was identified in the amino acid 103 to 115 region of capsid polypeptide VP1, close by the C3 neutralization epitope. The T-cell response to VP1 amino acids 103 to 115 is *H-2* restricted: *H-2*^{d} mice are responders, whereas *H-2*^{k} and *H-2*^{b} mice do not respond to this T-cell epitope. Immunization of BALB/c (*H-2*^{d}) mice with the uncoupled p86–115 peptide, which represents VP1 amino acids 86 to 115 and contains both the T-cell epitope and the C3 neutralization epitope, induced poliovirus-specific B- and T-cell responses. Moreover, these mice developed poliovirus neutralizing antibodies.

Specific antibodies capable of neutralizing virus infectivity are considered to be the major mechanism of protection against poliovirus infection. Therefore, many studies have been performed to define the antigenic structures involved in poliovirus neutralization and have resulted in the identification of several neutralization epitopes. The protein capsid of poliovirus is composed of 60 copies each of four polypeptides VP1, VP2, VP3, and VP4. Its three-dimensional structure has been solved at a resolution of 0.29 nm (14). Four major neutralization antigenic sites have been identified on the three major capsid proteins of polioviruses (23, 25). Site 1 is composed of residues from VP1, whereas the structures of the other antigenic sites appear to be more complex, involving residues from VP1, VP2, and/or VP3 (23, 25).

A continuous neutralization epitope (C3 epitope) of site 1 of poliovirus type 1 (PV1), recognized by neutralizing monoclonal antibody C3, is expressed on native (D) and heat-treated (C) viral particles as well as on isolated capsid polypeptide VP1 (2, 15). By using various strategies, investigators have localized this epitope within the region of VP1 from amino acids 93 to 104 (29, 30). According to the three-dimensional structure of the capsid, this region forms an exposed loop (BC loop), located around the fivefold axes of the virion, and is flanked by two β-strands corresponding to VP1 residues 82 to 95 and 105 to 109. Most interestingly, for poliovirus type 2 (PV2), the amino acid sequence of VP1 from residues 93 to 104 is also involved in mouse neurovirulence and is an important determinant of the poliovirus host range (20, 24). Protein conjugates or peptides containing the region of VP1 from amino acids 93 to 103 have been shown by several groups to induce poliovirus-specific antibodies and a low level of neutralizing antibodies in rabbits (8, 15) or rats (4). More recently, recombinant hepatitis B surface antigen (HBs) particles or bacteria expressing the region of VP1 from amino acids 93 to 103 have also been shown to induce antiviral as well as neutralizing anti-poliovirus antibodies in mice and rabbits (3, 5, 6, 18, 28).

These studies indicate that neutralization antigenic site 1 of PV1 contains an important B-cell epitope. Moreover, our preliminary results on the immunogenicity of free synthetic peptides containing the amino acid 93 to 103 epitope as well as residues from the flanking β-strands suggest that this region could also contain a dominant T-cell epitope (27a). The T-cell responses to poliovirus antigens have not been studied so far. Therefore, the present study was undertaken to analyze these T-cell responses and has permitted the identification of a dominant T-cell epitope adjacent to neutralization antigenic site 1 of PV1.

MATERIALS AND METHODS

Mice. Female BALB/c (*H-2*^{d}), BALB.B (*H-2*^{b}), DBA/2 (*H-2*^{d}), C57BL/6 (*H-2*^{b}) and C3H/He (*H-2*^{k}) mice, 6 to 8 weeks old, were bred in the animal facilities of the Pasteur Institute. Female BALB.K (*H-2*^{k}) mice, 6 to 8 weeks old, were obtained from CSEAL-CNRS, Orléans, France.

Virus growth, purification, and inactivation. PV1 (Mahoney), PV2 (MEF 1), PV3 (Saukett), and vΔ9, a derivative of PV1 in which the region of capsid protein VP1 from amino acids 94 to 102 has been deleted by site-directed mutagenesis of the PV1 cDNA (12), were grown in suspension on HeLa (S3) cells. Cells were infected at a multiplicity of infection of 5 to 30 PFU/cell and harvested at 6 to 7 h postinfection. Cells were broken by three cycles of freezing and thawing, and virus stocks were obtained after removal of the cell debris by centrifugation for 10 min at 3,000 × *g* at 4°C. For purification of the viruses, the virus stocks (10^{10} to 10^{11} PFU) were made 2 mM with EDTA and centrifuged for 15 min at 15,000 rpm at 4°C in a 70Ti rotor (Beckman). The supernatants were added with sodium dodecyl sulfate (SDS) up to 0.5% and centrifuged for 3 h at 40,000 rpm at 15°C through a 30% sucrose cushion (1 ml in 10 mM Tris [pH 7.5], 0.1 M NaCl, 1 mM EDTA, and 0.5% SDS) in the SW41 (Beckman) rotor of the ultracentrifuge. The viruses in the pellet were resus-

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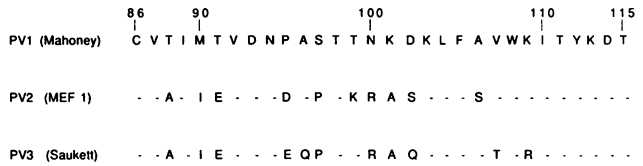

FIG. 1. Amino acid sequence of capsid polypeptide VP1 in the region of neutralization antigenic site 1 of PV1 Mahoney (17), PV2 MEF1 (our unpublished results) and PV3 Saukett (our unpublished results). Amino acid numbering is according to the PV1 sequence.

pended and further purified in CsCl (1.33 g/ml) gradients prepared in 10 mM Tris (pH 7.5)–10 mM NaCl–1 mM EDTA–1% Brij 58. Centrifugation was overnight at 50,000 rpm at 15°C in the VTi65 (Beckman) rotor of the ultracentrifuge. The gradients were fractionated from the bottom, and the optical density of each fraction was determined at 260 nm. The virus-containing fractions were pooled and dialyzed overnight at 4°C against phosphate-buffered saline (PBS). The concentration of purified virus was estimated by determination of the optical density at 260 nm (8 optical density units are equivalent to 1 mg of virus per ml). Purified virus was inactivated by incubation for 48 h at 37°C in the presence of Formalin (1:10,000).

Immunogens and antigens. Ovalbumin (grade V) was obtained from Sigma Laboratories, St. Louis, Mo. Synthetic peptides consisting of VP1 amino acid residues 90 to 101 (p90–101), 95 to 106 (p95–106), 100 to 111 (p100–111), 103 to 116 (p103–116) and 105 to 116 (p105–116) were synthesized by Jean-Paul Briand, IBMC, Strasbourg, France. Other synthetic peptides representing PV1 neutralization antigenic site 1 (Fig. 1) were synthesized by Neosystem, Strasbourg, France. p95–104 consists of amino acid residues 95 to 104 of capsid protein VP1, flanked by additional Tyr-Gly-Cys-Gly residues at the N terminus and by Gly-Cys residues at the C terminus. $p(93–103)_3$ is a trimer of the VP1 sequence from amino acids 93 to 103. p86–115 consists of VP1 amino acid residues 86 to 115 followed by Gly-Cys residues at the C terminus. p79–103 and p93–116 consist of VP1 amino acid residues 79 to 103 and 93 to 116, respectively.

p120–145, consisting of amino acid residues 120 to 145 of hepatitis B virus pre-S antigen, was synthesized by Neosystem and used as a negative control.

Assays for proliferative responses of antigen-specific T cells. Mice were injected at the base of the tail with 0.1 ml of Freund's complete adjuvant emulsion containing the antigen. Fourteen days later, the animals were killed and the inguinal lymph nodes (LN) were removed aseptically. A single-cell suspension of LN cells was prepared in RPMI 1640 medium (Seromed) containing 2 mM L-glutamine, antibiotics, 5×10^{-5} M 2-mercaptoethanol, 1.5% fetal calf serum, and 0.5% normal mouse serum. The cells were cultured in 0.2 ml in the presence of the antigens at a final concentration of 3.75×10^6 cells per ml in 96-well flat-bottom culture plates (Nunc, Roskilde, Denmark). Each test was performed in triplicate. After 3 days of culture at 37°C under 7.5% CO_2, the cells were pulsed for 18 h with [^{3}H]thymidine (NEN, Boston, Mass.; 25 Ci/mmol = 37 GBq/mmol; 2.5 μCi/ml) per ml and then harvested on fiber glass filters (Titertek; Flow Laboratories) with a cell harvester (Skatron, Lier, Norway). Incorporated radioactivity was measured in a liquid scintillation counter (Betamatic Kontron). Results are expressed as background-subtracted geometric means. Standard deviations of triplicate cultures were less than 15% of the mean. Background values of control cultures were less than 10^4 cpm.

Monoclonal anti-CD4 and anti-CD8 antibodies. The L3T4 (CD4)-specific rat anti-mouse hybridoma GK1-5 was originally produced by Dialynas et al. (7). The CD8-specific rat anti-mouse hybridoma H35.17.2 was obtained by Pierres et al. (27). Ascitic fluid preparations of monoclonal antibodies were used in in vitro experiments. To obtain ascitic fluids, nude mice were pristane primed and injected with 10^6 hybridoma cells. Antibodies were prepared by precipitation with ammonium sulfate. The quantity of protein was assessed by measurement of the optical density at 280 nm.

Detection of antibodies. (i) Antipeptide antibodies. Serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of antipeptide antibodies. Microtiter plates were coated with 500 ng of the various free synthetic peptides per well and incubated overnight at 37°C.

The antigen-coated plates were washed and incubated for 1 h with serial dilutions of serum samples. The wells were then washed and treated with a goat anti-mouse immunoglobulin G peroxidase conjugate for 1 h. Fifteen minutes after the addition of a substrate solution, the reaction was stopped by the addition of 50 μl of 12% H_2SO_4. The A_{492} of each well was measured with a Titertek Multiskan ELISA reader (Flow Laboratories). The negative control consisted of pooled normal mouse serum. Individual titers are expressed as the $\log_{10}$ of the maximal dilution giving an absorbance twice as high as the negative control. Titers ± standard errors are calculated as the arithmetic mean of $\log_{10}$ titers.

(ii) Anti-poliovirus antibodies. The reactivity of the serum samples with viral particles was determined by immunoprecipitation in the presence of activated *Staphylococcus aureus* (immunoglobulin G sorbent; The Enzyme Center Inc.) of [^{35}S]methionine-labeled, infectious PV1 (Mahoney) virions (D particles) or of virions heated for 1 h at 56°C (C particles). Reactivity with C particles was expressed as the percentage of immunoprecipitated radioactivity obtained by using the different serum samples at a 1:10 dilution. For the determination of anti-D-particle antibody titers, immunoprecipitations were carried out by serial dilutions of the serum samples. The antibody titers, defined as the reciprocal of the dilution of serum giving 50% immunoprecipitation, were calculated from exponential regression curves established by the least-squares method.

Neutralizing antibody titers were measured on HeLa cell monolayers in a plaque reduction assay. The titers were defined as the reciprocal of the endpoint dilution of serum which neutralized 50% of one dose (100 PFU) of PV1 (Mahoney).

RESULTS

T-cell proliferative responses of BALB/c mice to poliovirus. BALB/c mice were immunized with 1 μg of inactivated PV1 in Freund's complete adjuvant, and 2 weeks later their LN cells were stimulated in vitro with the homologous inactivated virus. A strong proliferative response of LN lymphocytes to inactivated poliovirus was obtained in PV1-primed mice, whereas LN cells from ovalbumin-primed mice did not respond to the same virus preparation (Fig. 2). This poliovirus-specific proliferative response was due to the stimulation of the $CD4^+$ T-cell subset, since the poliovirus-specific proliferation of LN cells from PV1-primed BALB/c mice was totally inhibited by the addition to the culture of anti-$CD4^+$ monoclonal antibodies but was not affected by

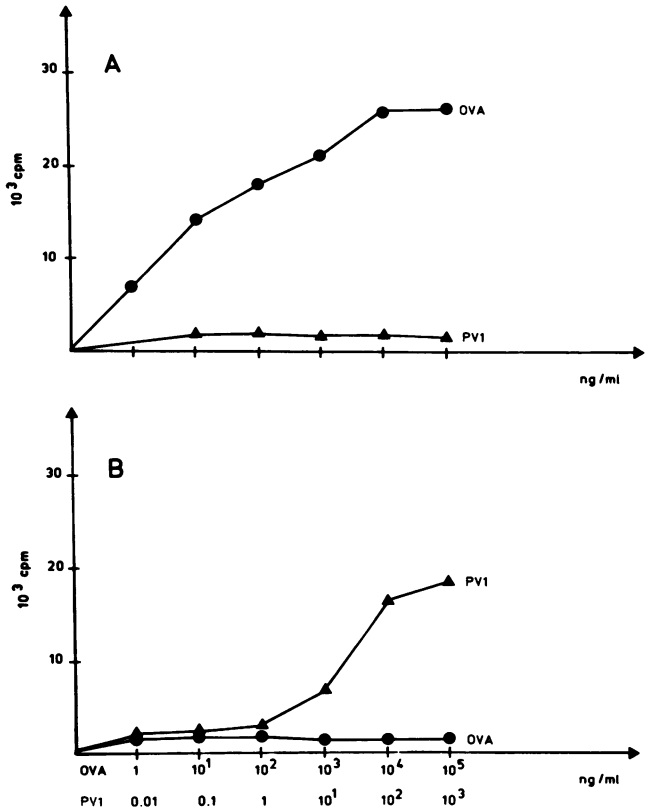

FIG. 2. Poliovirus-specific T-lymphocyte proliferation. BALB/c mice were immunized either with 100 μg of ovalbumin (OVA) (A) or with 1 μg of inactivated PV1 (B). Two weeks later, the mice were killed and the proliferative responses of their LN cells to ovalbumin (●) or PV1 (▲) were assayed.

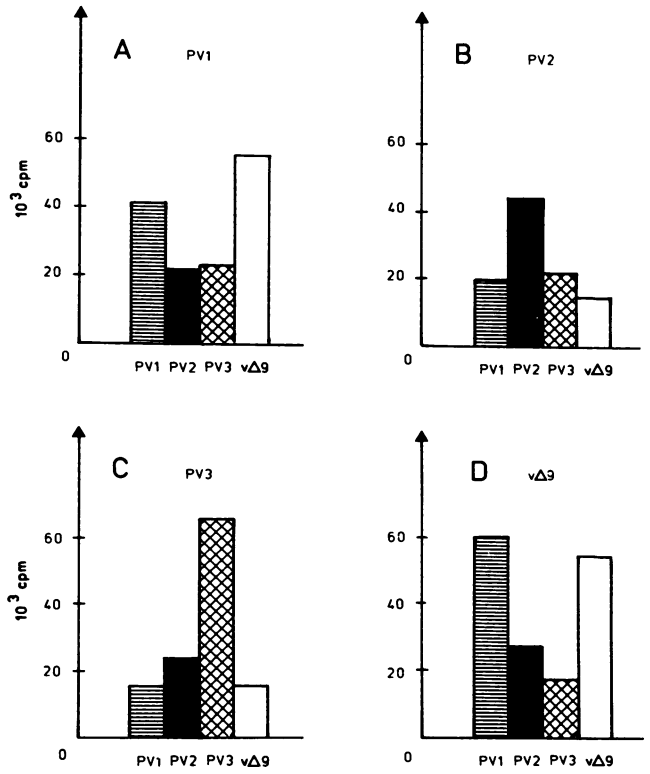

FIG. 4. Proliferative responses to heterologous polioviruses. BALB/c mice were immunized with 1 μg of inactivated PV1 (A), PV2 (B), PV3 (C), or vΔ9 (D). Two weeks later, LN cells were stimulated in vitro with 0.1 μg of PV1, PV2, PV3, or vΔ9.

coculture with anti-CD8$^+$ monoclonal antibodies even at high doses (Fig. 3).

To examine the serotype specificity of poliovirus-specific T-cell proliferative responses, we immunized BALB/c mice with 1 μg of inactivated PV1 (Mahoney), PV2 (MEF 1), PV3 (Saukett), or vΔ9, a derivative of PV1 in which the 94–102-amino-acid region of capsid protein VP1 has been deleted by site-directed mutagenesis of the PV1 cDNA (12). Two weeks later, we stimulated LN cells of the mice in vitro with various doses of inactivated PV1, PV2, PV3, or vΔ9. The best proliferative responses of these cultures were obtained with 0.1 μg of the various viruses and are shown in Fig. 4. In all cases, the highest levels of proliferation were obtained in response to the homologous virus, but strong cross-reactiv-

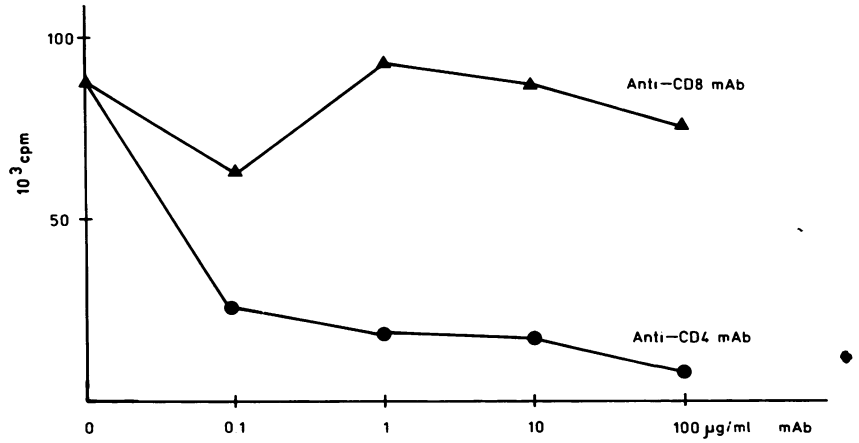

FIG. 3. Poliovirus-specific proliferation of CD4$^+$ T lymphocytes. BALB/c mice were immunized with 1 μg of inactivated PV1. Two weeks later, the mice were killed and the proliferative responses of their LN cells to PV1 (1 μg/ml) were assayed in the presence of anti-CD8 (▲) or anti-CD4 (●) monoclonal antibodies (mAb).

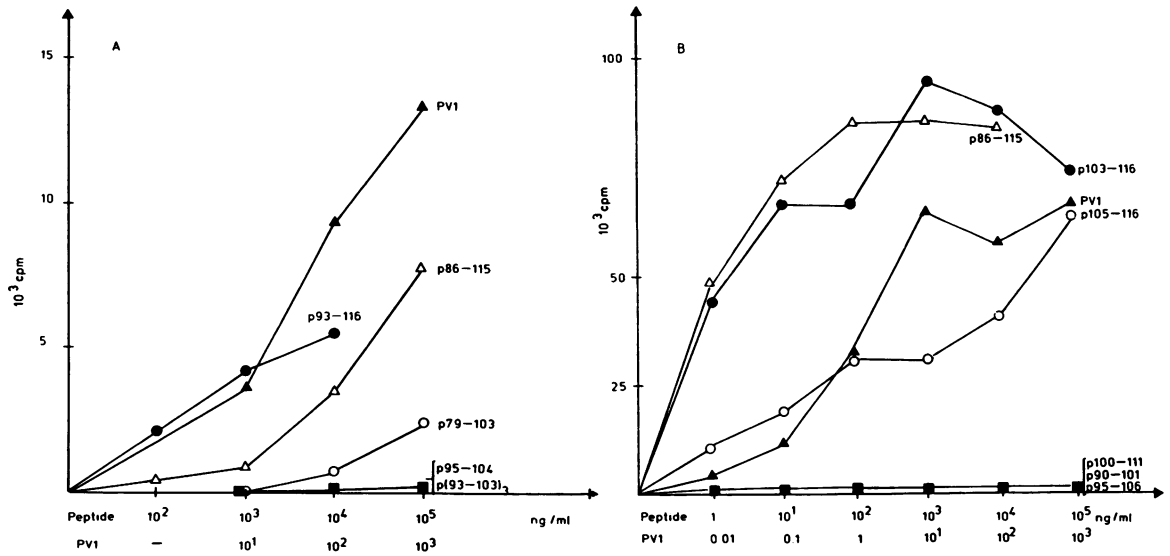

FIG. 5. Proliferative responses of poliovirus-primed LN cells from BALB/c mice to synthetic peptides representing neutralization antigenic site 1 of PV1. BALB/c mice were immunized with 1 μg of PV1. Two weeks later, LN cells were stimulated in vitro with PV1, p93–116, p86–115, p79–103, and p(93–103)$_3$ (experiment A) or PV1, p103–116, p86–115, p105–116, and p100–111, p90–101, or p95–106 (experiment B).

ity could also be observed. LN cells from mice immunized with PV1, PV2, PV3, or vΔ9 responded very well to in vitro stimulation with PV1, PV2, PV3, or vΔ9.

Proliferative responses of PV1-primed lymphocytes to synthetic peptides from PV1 neutralization antigenic site 1. Lymphocytes from PV1-primed BALB/c mice were stimulated in vitro with various synthetic peptides representing PV1 neutralization antigenic site 1. Peptides of various length, corresponding to the region of amino acid residues 79 to 116 of VP1, were tested (Fig. 1). No significant proliferative responses were obtained following stimulation of LN cells with p95–104, p(93–103)$_3$ or p79–103 (Fig. 5A). In contrast, these cells did proliferate when incubated with p93–116 and p86–115, indicating that the VP1 region of amino acids 93 to 115 could contain a T-cell epitope. To localize the T-cell epitope more precisely, we analyzed proliferative responses to a series of peptides corresponding to the region of amino acids 90 to 116 of VP1 (Fig. 5B). LN cells from PV1-primed mice did not proliferate with p90–101, p95–106, or p101–111, whereas significant proliferative responses were obtained following stimulation with p103–116 and, to a lesser extent, with p105–116. These observations indicate that the T-cell epitope is located between residues 103 and 116 of VP1. Moreover, LN cells from vΔ9-primed BALB/c mice responded as well to p86–115 as did the cells from PV1-primed mice (Fig. 6). Since the region from amino acids 94 to 102 has been deleted in vΔ9, this confirms that the putative T-cell epitope is located between residues 103 and 115 of VP1. Interestingly, LN cells from PV2-primed mice responded well to p86–115 (approximately half the response of the PV1-primed lymphocytes), whereas LN cells from PV3-primed mice responded only weakly. It should be noticed that the VP1 sequences from amino acids 103 to 115 of PV1 and PV2 differ only by one amino acid residue, whereas those of PV1 and PV3 differ by two residues (Fig. 1).

***H-2* restriction of proliferative response to p86–115.** Various inbred strains of mice, BALB/c (*H-2*^{d}), DBA/2 (*H-2*^{d}), C57BL/6 (*H-2*^{b}), and C3H/He (*H-2*^{k}), were immunized in vivo with 1 μg of inactivated PV1, and 2 weeks later their LN cells were stimulated in vitro either with PV1 or with p86–115. Various doses of antigens were used. Figure 7 shows the best proliferative responses obtained in a representative experiment. A proliferative response to PV1 was observed in all strains of mice tested, although the LN-cell response of the C57BL/6 mice was usually weaker than that

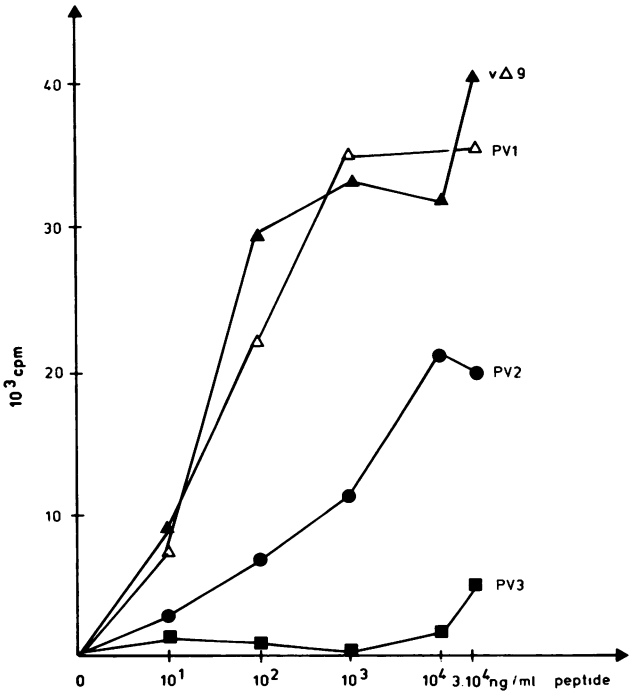

FIG. 6. Proliferative responses to p86–115 of LN cells from BALB/c mice immunized with various polioviruses. BALB/c mice were immunized with 1 μg of inactivated PV1, PV2, PV3, or vΔ9. Two weeks later, LN cells were stimulated in vitro with p86–115.

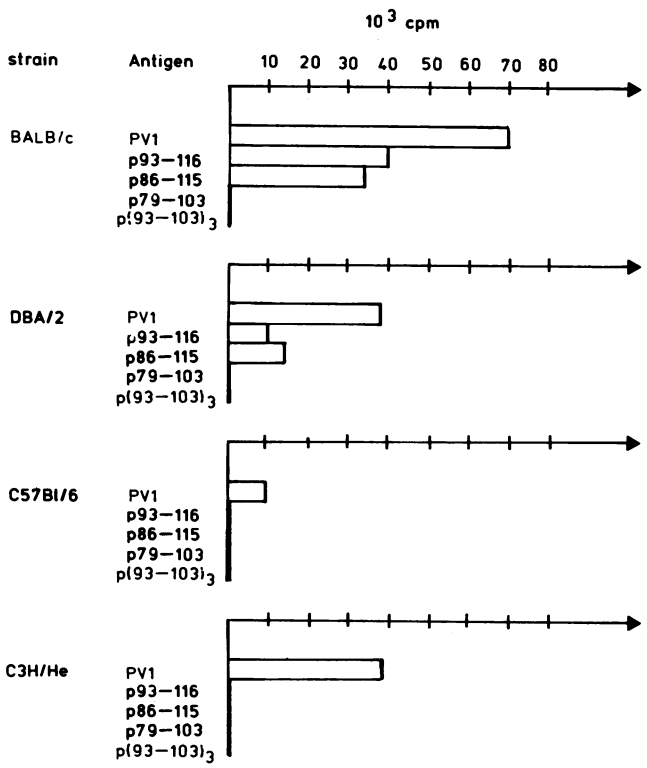

FIG. 7. Specific proliferative responses of poliovirus-primed LN cells from various strains of mice to synthetic poliovirus peptides. Groups of three or four mice of the indicated strains were immunized with 1 μg of inactivated PV1. Two weeks later, their LN cells were stimulated in vitro with 1 μg of PV1 per ml or 10 μg of the synthetic poliovirus peptides per ml.

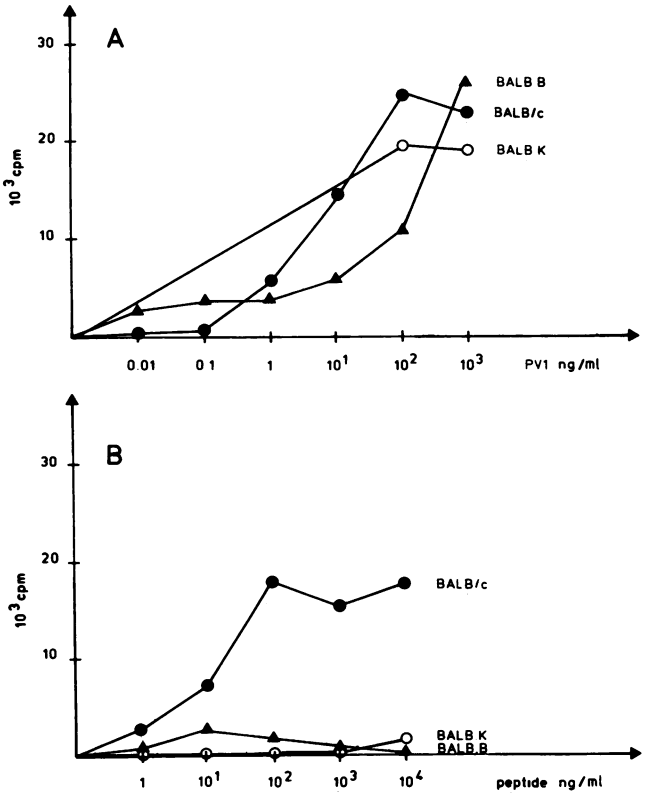

FIG. 8. Poliovirus-specific proliferative responses of *H-2* congenic mice. BALB/c, BALB.B, and BALB.K mice were immunized with 1 μg of PV1. Two weeks later, their LN cells were stimulated in vitro either with PV1 (A) or with p86–115 (B).

of the other strains of mice. None of these mice responded to p79–103 or $p(93–103)_3$. C57BL/6 and C3H/He LN cells did not proliferate when incubated with p93–116 or p86–115. In contrast, a proliferative response to these peptides was observed with LN cells from BALB/c and DBA/2 mice primed with PV1. These data could indicate that PV1 contains a T-cell epitope included in VP1 residues 103 to 115 and recognized by $H\text{-}2^d$ but not by $H\text{-}2^b$ or $H\text{-}2^k$ mice. To confirm this result, we repeated the experiment with *H-2* congenic strains of mice. LN cells from PV1-primed BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), or BALB.K ($H\text{-}2^k$) mice responded equally well to in vitro stimulation with inactivated PV1 (Fig. 8). In contrast, only LN cells from BALB/c mice proliferated in response to p86–115. Therefore, p86–115 contains a T-cell determinant recognized in the context of the $H\text{-}2^d$ haplotype but not in that of the $H\text{-}2^b$ or $H\text{-}2^k$ haplotype.

Immunogenicity of peptide p86–115. Since p86–115 contains the C3 epitope (amino acids 93 to 103) as well as a T-cell epitope, we inferred that immunization with this peptide would induce poliovirus-specific B- and T-cell responses. We examined the ability of p86–115 to induce T-cell responses in congenic strains of mice. BALB/c, BALB.B, and BALB.K mice were immunized in vivo with 30 μg of p86–115, and LN cells were stimulated in vitro either with inactivated PV1 or with p86–115. Control BALB/c mice were immunized either with PV1 or with an unrelated hepatitis B virus pre-S peptide, p120–145 (Fig. 9). LN cells from BALB/c mice primed with p86–115 responded very well to in vitro stimulation with the peptide. Moreover, a significant response to inactivated PV1 could also be demonstrated. As expected from the previous experiment, LN cells from p86–115-primed BALB.B or BALB.K mice responded neither to the peptide nor to PV1. Therefore, in $H\text{-}2^d$ mice, a poliovirus-specific T-cell response can be induced by immunization with the free p86–115 peptide. To determine whether a poliovirus-specific B-cell response could be induced with the same peptide, we examined the ability of p86–115 to induce a poliovirus-specific antibody response in BALB/c (responder) or C3H/He (nonresponder) mice. In BALB/c mice only one injection of the peptide was sufficient to induce high antibody titers against either the C3 epitope or the p86–115 peptide (Table 1). Moreover, after two injections, the serum of these mice did recognize the infectious (anti-D) or heat-treated (anti-C) virions. Four of five mice also developed poliovirus neutralizing antibodies. In contrast, even after two injections of peptides, serum from immunized C3H/He mice recognized neither the peptides nor the virus.

DISCUSSION

The present study demonstrates that immunization with inactivated, purified poliovirus induces a specific $CD4^+$ T-cell response. Moreover, the use of various strategies permitted the identification of a dominant T-cell epitope in the amino acid 103 to 115 region of capsid polypeptide VP1.

A high degree of cross-reactivity of T-cell proliferative responses to poliovirus was observed for PV1, PV2, and PV3 serotypes, the strongest cross-reactivity occurring be-

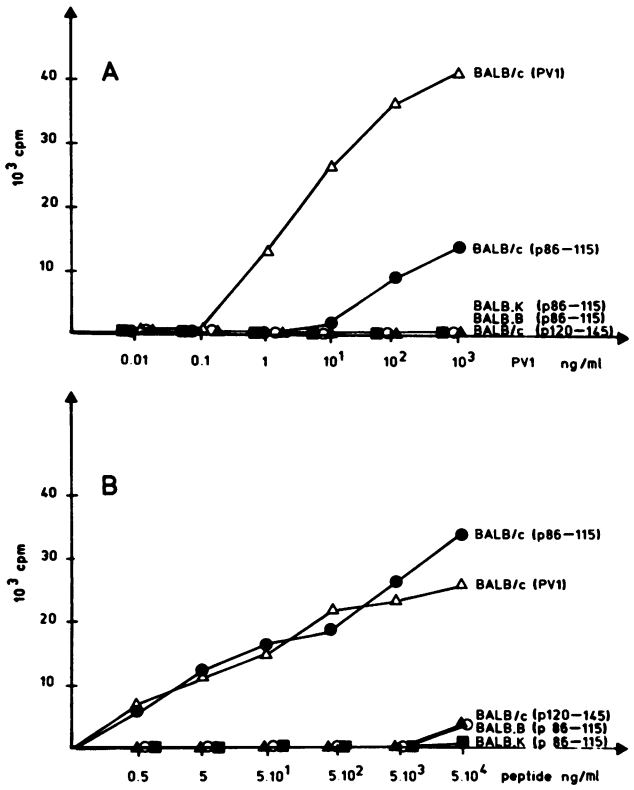

FIG. 9. Poliovirus-specific proliferative responses of LN cells from mice primed with p86–115. BALB/c (●), BALB.B (■), or BALB.K (▲) mice were immunized with 30 μg of p86–115. Control BALB/c mice were immunized either with 1 μg of PV1 (△) or with 100 μg of a hepatitis B virus pre-S peptide, p120–145 (○). Two weeks later, their LN cells were stimulated in vitro either with PV1 (A) or with p86–115 (B).

tween PV1 and PV2. The VP1 regions of PV1 and PV2 between amino acids 103 and 115 differ only by one amino acid residue, and priming by PV2 induced a strong T-cell response against a synthetic peptide containing the VP1 103 to 115 region of PV1. The identification of a common T-cell site on these two poliovirus serotypes could therefore explain this strong T-cell cross-reactivity. In contrast, a two-amino-acid difference exists between PV1 and PV3 in this region, and PV3-primed T cells responded only weakly to the PV1 p86–115 peptide. However, these cells proliferated in response to purified PV1 and PV2. These data indicate that at least a second dominant T-cell site, which could be common to all three serotypes, must exist on VP1, VP2, VP3, or VP4. Evidence for a cross-reacting T-cell epitope common to several enteroviruses was recently reported (1). Such a putative T-cell epitope(s) could be identified by comparing the sequences of the four capsid polypeptides of the three polioviruses and of other enteroviruses. These results could be important for designing a synthetic vaccine of broad specificity.

This study demonstrates that neutralization antigenic site 1 of PV1 contains both B and T-helper (Th) epitopes. Such short peptides containing B and T epitopes have been described for other viruses such as hepatitis B virus (22) and foot-and-mouth disease virus (10, 11). This could indicate that the immunodominance of a B-cell epitope depends upon the existence of a strong T-cell epitope in the vicinity. The reverse hypothesis, i.e., that T-cell immunodominance could be influenced by the proximity of a dominant B-cell epitope, was excluded by the experiment showing that the vΔ9 poliovirus, lacking the C3 epitope, induces a high T-cell response against p86–115. Obviously, other factors are critical for B-cell immunogenicity, as shown, for instance, by our recent results on the immunogenicity of the C3 epitope presented at the surface of *Escherichia coli* by genetic insertion into the outer membrane protein LamB (28). In that study, it was shown that insertion of the C3 epitope together with its flanking sequences (including the C3 Th site) decreased its immunogenicity compared with insertion of the C3 epitope alone. The conformation of the epitope should therefore remain the major factor for B-cell immunogenicity.

Immunization of mice with the vΔ9 derivative of PV1 also induced a strong proliferative response against the three purified viruses and against p86–115. Poliovirus chimeras have been constructed by replacement of cDNA corresponding to antigenic site 1 by synthetic oligonucleotides encoding foreign antigenic determinants such as human immunodeficiency virus (9) or human papillomavirus 16 (16) epitopes. The identification of a dominant helper T-cell site located close to this site could indicate that this T-cell epitope plays

TABLE 1. Antipoliovirus responses of mice immunized with synthetic peptide p86–115[a]

Mouse strain	Antipeptide antibody (Log_{10} ELISA titer) for:				Antipoliovirus secondary antibody responses		
	Primary responses		Secondary responses		Anti-C (%)	Anti-D titer	Virus neutralization titer
	p93–103	p86–115	p93–103	p86–115			
BALB/c (*H-2*^{d})	3.04	4.03	4.79	5.43	97	72	128
	3.47	4.50	4.71	5.06	95	50	<16
	2.70	3.70	5.67	5.29	96	82	16
	2.39	3.56	4.11	6.15	97	334	32
	3.73	4.43	5.44	4.45	98	1,496	8,192
C3H/He (*H-2*^{k})	<2	<2	<2	<2	1	<10	<16
	<2	2.06	<2	<2	6	<10	<16
	<2	2.01	<2	2.64	0	<10	<16
	<2	2.21	<2	<2	4	<10	<16
	<2	<2	<2	<2	6	<10	<16

[a] On day 0, BALB/c, or C3H/He mice (five mice per group) were injected subcutaneously with 30 μg of p86–115 in Freund's complete adjuvant. Three weeks later, they received an intraperitoneal injection of 30 μg of p86–115 in Freund's incomplete adjuvant. They were bled on days 20 (primary responses) and 31 (secondary responses).

a major role in the immunogenicity of the C3 epitope. Therefore, it could be suggested that to confer optimal immunogenicity to the epitopes grafted in antigenic site 1, the chimeras have to be built without deleting the amino acid region (amino acids 103 to 115) containing the C3 Th site.

Immunization with the uncoupled p86–115 peptide induced poliovirus-specific B- and T-cell responses in the responder strain of mice ($H\text{-}2^d$) but not in nonresponder strains such as $H\text{-}2^k$. Moreover, 80% of the $H\text{-}2^d$ mice immunized with this peptide developed poliovirus-specific neutralizing antibodies. This clearly demonstrates that the T-cell epitope at amino acids 103 to 115 is a T-helper site and that the induction of an antibody response against the C3 epitope by the free p86–115 peptide requires the responsiveness of T cells to the peptide. High neutralizing-antibody titers were also obtained in rabbits immunized with free peptides representing VP1 amino acids 82 to 116 or 93 to 115 (27a). The C3 Th site could therefore also function as a T-helper epitope in rabbits. It has been previously demonstrated that synthetic vaccines could be constructed by appropriate selection and organization of B- and T-cell determinants (13, 19, 21, 26). The present study demonstrates the feasibility of such an approach for poliovirus. Moreover, it is also possible that a vaccine protecting against the three poliovirus serotypes could be designed by the selection of appropriate B- and T-cell cross-reactive or common epitopes.

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