Antigenic Sequences of Poliovirus Recognized by T Cells: Serotype-Specific Epitopes on VP1 and VP3 and Cross-Reactive Epitopes on VP4 Defined by Using CD4$^+$ T-Cell Clones

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A panel of poliovirus-specific murine CD4$^+$ T-cell clones has been established from both BALB/c (H-2^d) and CBA (H-2^k) mice immunized with Sabin vaccine strains of poliovirus serotype 1, 2, or 3. T-cell clones were found to be either serotype specific or cross-reactive between two or all three serotypes. Specificity analysis against purified poliovirus proteins demonstrated that T-cell clones recognized determinants on the surface capsid proteins VP1, VP2, and VP3 and the internal capsid protein VP4. Panels of overlapping synthetic peptides were used to identify eight distinct T-cell epitopes. One type 3-specific T-cell clone recognized an epitope within amino acids 257 and 264 of VP1. Three T-cell epitopes corresponding to residues 14 to 28, 189 to 203, and 196 to 210 were identified on VP3 of poliovirus type 2. The remaining four T-cell epitopes were mapped to an immunodominant region of VP4, encompassed within residues 6 and 35 and recognized by both H-2^d and H-2^k mice. The epitopes on VP4 were conserved between serotypes, and this may account for the predominantly cross-reactive poliovirus-specific T-cell response observed with polyclonal T-cell populations. In contrast, T-cell clones that recognize epitopes on VP1 or VP3 were largely serotype specific; single or multiple amino acid substitutions were found to be critical for T-cell recognition.

The generation of virus-specific neutralizing antibody is considered the major mechanism of protection against poliovirus infection, and the success of poliovirus vaccines has been directly attributed to the ability of the vaccines to generate humoral immunity (5, 21). Consequently, the antigenic structure of the virus recognized by antibody is well characterized, three serologically distinct serotypes (1, 2, and 3) having been defined (21, 22). At least four neutralizing antibody epitopes have been identified (21) and located on the three-dimensional structure of the icosahedral virus particle, which consists of 60 copies each of the four capsid proteins VP1, VP2, and VP3 and the wholly internal protein VP4 (4, 6). However, the recognition of poliovirus by T cells and their possible role in the protective immune response have received little attention.

T cells have important functions in immune protection against viral diseases (16). Major histocompatibility complex (MHC) class II-restricted CD4$^+$ T cells proliferate upon stimulation by antigen and secrete cytokines involved in the regulation of immune responses, including the activation of B cells to produce antibody (30). Although CD8$^+$ MHC class I-restricted T cells have largely been associated with cytotoxic T-lymphocyte (CTL) function (41), recent evidence from cloned murine T cells suggests that a subpopulation of CD4$^+$ T cells, termed T-helper 1 (Th1), that secrete interleukin-2 (IL-2) and gamma interferon (IFN-γ) are also involved in direct cell-mediated immunity, including lysis of virally infected target cells (3, 23, 26). Another population, termed Th2, release IL-4, IL-5, and IL-6 and are considered to be mainly responsible for providing helper function for antigen-specific antibody production (3, 23).

In studies using polyclonal T-cell populations, we and others have previously demonstrated that immunization of

mice with poliovirus in alum, in Freund's adjuvant, or without adjuvant can generate broadly cross-reactive poliovirus-specific T-cell responses (10, 12, 39). The findings also suggested that a component of the T-cell response was serotype specific. However, the use of polyclonal T-cell populations in these studies precluded specificity analysis at the amino acid level. A detailed study of T-cell fine specificity and function is possible only with the use of T-cell clones.

In this report, we describe the generation of a panel of poliovirus-specific murine CD4$^+$ T-cell clones. Our findings demonstrate that these clones either were specific for poliovirus of a single serotype or were cross-reactive between two or all three serotypes. Specificity analysis against isolated capsid proteins revealed that a proportion of T-cell clones recognized either VP1, VP2, or VP3, but the majority (10 of the 18) recognized the internal capsid protein VP4, hitherto not implicated in the immune response to poliovirus. Using panels of overlapping synthetic peptides, we have identified eight distinct T-cell epitopes and determined the amino acid residues critical for their recognition.

MATERIALS AND METHODS

Virus preparations. Attenuated live poliovirus vaccine strains of Sabin types 1, 2, and 3 were used throughout. Viruses were propagated in Hep2c cells, and purified concentrates were obtained as described by Minor (20). Briefly, infected tissue culture fluid was clarified by low-speed centrifugation (3,000 × g), precipitated by using 0.4 g of ammonium sulfate per ml, and purified through a 15 to 45% sucrose gradient. Virus-bearing fractions were pooled and centrifuged at 30,000 × g for 14 h; the pellets were resuspended in phosphate-buffered saline (PBS) and stored as aliquots at −70°C. Virus infectivity was determined by using the standard plaque assay (20), and protein concentrations

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were estimated by using the Bio-Rad protein assay kit 1 (Bio-Rad Laboratories Ltd., Watford, Hertfordshire, United Kingdom).

Isolation of capsid proteins. Capsid proteins VP1, VP2, VP3, and VP4 were isolated from sodium dodecyl sulfate (SDS)-polyacrylamide gels in a form suitable for use in T-cell assays as described by Katrak et al. (9). Briefly, disrupted whole virus was loaded onto the center lanes and 10 μl of Rainbow protein molecular weight markers (Amersham International plc, Little Chalfont, Buckinghamshire, United Kingdom) were loaded onto the outer lanes of an SDS–15% polyacrylamide gel, and the gel was run for 16 h at 5 mA. Bands corresponding to VP1, VP2, VP3, and VP4 were then cut directly from the gel after location by alignment with the visible molecular weight markers and by comparison with a silver-stained test lane. Proteins were eluted by soaking the gel fragments overnight at 4°C in 50% (vol/vol) PBS-distilled water. This solution was then chilled on crushed ice, the bulk of the SDS was removed by centrifugation, and the eluted proteins were concentrated through either a Centricon 10 or Centricon 30 microconcentrator (Amicon Corp., Beverly, Mass.). The purity of separate fractions was assessed by subjecting a sample of the eluted material to SDS-polyacrylamide gel electrophoresis and demonstrating that it was homogeneous (9). Eluted capsid proteins were then coupled to latex microspheres (9), which were sterilized by exposure to UV irradiation prior to use in T-cell proliferation assays.

Synthetic peptides. Synthetic peptides (12-mers overlapping by eight amino acid residues) corresponding to entire sequences of VP1 of poliovirus type 3 and VP2 of type 2 (35) were synthesized by using the multipin cleavable peptide synthesis system (Cambridge Research Biochemicals, Northwich, Cheshire, United Kingdom) by the method of Maeji et al. (14). This set of peptides was synthesized with a carboxyl-terminal β-alanine diketopiperazine group and cleaved into neutral buffer. A further 48 synthetic peptides corresponding to the entire sequence of VP3 of type 2 (15-mers overlapping by 8 residues) and to VP4 of type 2 (15-mers overlapping by 10 or 11 residues) were synthesized commercially (Cambridge Research Biochemicals) according to the sequence described by Toyoda et al. (35).

Mouse immunizations. Eight- to ten-week-old female BALB/c (H-2^d) or CBA (H-2^k) mice were used for immunization. Each mouse was injected intraperitoneally with 10^8 PFU of virus (equivalent to approximately 1 μg of protein), administered either without adjuvant or precipitated with alum. Fourteen days after primary immunization, the mice received a booster immunization of an identical preparation.

Establishment of poliovirus-specific T-cell clones. T-cell clones were established from the spleens of individual BALB/c or CBA mice. Spleens were taken from mice 14 days after the in vivo boost, and cells were cultured at 2 × 10^6 ml^{-1} for 4 to 5 days in RPMI 1640 medium (supplemented with 8% fetal calf serum) with 2 × 10^7 PFU of poliovirus of the appropriate serotype ml^{-1}. Surviving cells were recultured for 7 days in fresh medium with autologous irradiated spleen cells (2 × 10^6 ml^{-1}) as feeders. T-cell lines were established by maintaining these cultures at 10^5 cells ml^{-1} in a 4-day feed/7-day starve cycle, using alternatively 2 × 10^7 PFU of virus ml^{-1} with 2 × 10^6 irradiated syngeneic spleen cells ml^{-1} as antigen-presenting cells (APC) or feeders alone. Cloning of T-cell lines was performed by limiting dilution at 0.5 cell per well in 200-μl volumes in 96-well plates in the presence of APC (2 × 10^6 ml^{-1}), virus (2 × 10^7 PFU ml^{-1}), and 5% IL-2-containing supernatant (the latter

TABLE 1. Serotype specificity of T-cell clones[a]

Clone	H-2 haplotype	Immunizing serotype	Proliferative response (10^3 cpm) to poliovirus			
			No virus	Type 1	Type 2	Type 3
Pol 2c-1	d	2	1.3	1.2	6.8	0.5
CB2.2-3	k	2	0.5	0.9	45.0	0.9
CB2.2-5	k	2	0.1	0.2	5.8	0.2
2KB-3	k	2	0.3	0.4	3.4	0.4
2KB-6	k	2	0.4	0.3	123.3	0.8
3N2s5.1	d	3	0.2	0.2	0.6	5.9
CB2.2-10	k	2	0.2	0.2	16.1	20.0
IN5.1	d	1	0.1	15.8	13.6	12.7
IN8.44	d	1	0.1	82.5	103.3	84.2
CB2.1-3	k	2	0.3	74.6	87.0	64.5
CB2.1-6	k	2	0.2	17.0	10.5	11.3
CB2.2-4	k	2	0.2	12.4	69.2	66.1
CB2.2-8	k	2	0.2	5.7	14.4	11.9
2KA-1	k	2	0.2	7.4	40.8	47.8
2KA-2	k	2	0.7	24.5	47.5	29.3
2KA-5	k	2	0.2	5.0	4.8	7.3
2KB-4	k	2	0.2	14.4	12.8	10.8
3KC-1	k	3	2.9	50.4	54.2	44.3

[a] T-cell clones were cultured with polioviruses at dilutions ranging from 10^{-6} to 10^{-9} PFU ml^{-1} for 2 to 3 days and then pulsed for 4 to 6 h with [^{3}H]thymidine. Results are expressed as mean counts per minute for triplicate cultures at the optimum antigen concentration.

prepared from concanavalin A-activated rat spleen cells). A further 25 μl of IL-2-containing supernatant was added to cultures 5 days later. The clones were restimulated after a further 7 days of incubation and progressively expanded to 25-cm³ flasks. Clones were maintained by restimulation at an initial concentration of 10^5 cells ml^{-1} with virus and APC every 10 days, with IL-2-containing supernatant and feeders added 4 days after antigen stimulation. The specificity of T-cell clones was tested in a proliferation assay.

T-cell proliferation assay. T-cell clones were rested for 10 days after stimulation with virus before assay. T-cell clones (2 × 10^4) were cultured with antigen (viruses, isolated virus capsid proteins, or synthetic peptides) in the presence of 4 × 10^5 feeder cells as APC in 200-μl volumes in flat-bottom 96-well microtiter plates. Controls included medium alone or a lysate prepared from noninfected Hep2C cells. Cultures were incubated for 2 or 3 days and pulsed for the last 4 to 6 h with 0.5 μCi of [^{3}H]thymidine. Cells were harvested onto filter paper by using an automated cell harvester, and [^{3}H]thymidine incorporation was measured by liquid scintillation counting. Results are expressed as mean counts per minute for triplicate cultures.

RESULTS

Serotype specificity of poliovirus-specific T-cell clones. A panel of poliovirus-specific CD4$^+$ T-cell clones was established by limiting dilution of bulk cultures or T-cell lines derived from spleen cells of individual BALB/c or CBA mice immunized with the Sabin strain of poliovirus serotype 1, 2, or 3. All clones studied were of the Thy 1$^+$ CD4$^+$ CD8$^-$ phenotype (data not shown). The serotype specificities of 18 T-cell clones derived from nine mice were examined by testing their proliferative responses against the three different poliovirus serotypes (Table 1 and Fig. 1) and shown to fall into three specificity groups (summarized in Table 2). Five T-cell clones (Pol 2c-1, CB2.2-3, CB2.2-5, 2KB-3, and 2KB-6) were specific for poliovirus type 2 but failed to
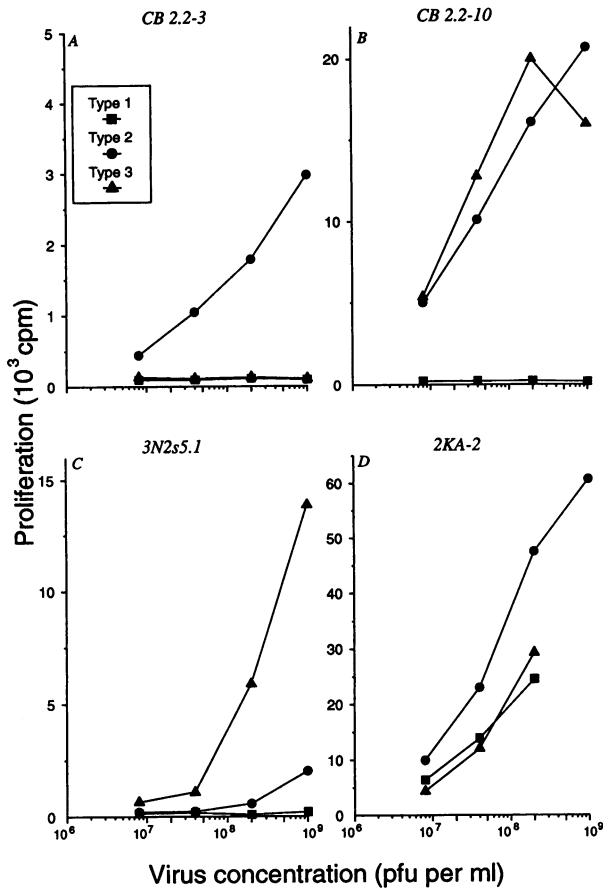
FIG. 1. Proliferative responses of the indicated poliovirus-specific T-cell clones to Sabin strains of poliovirus serotypes 1, 2, and 3.

TABLE 2. Poliovirus capsid protein specificity of T-cell clones

Clone	Specificity[a]						
	Serotype			Capsid protein			
	1	2	3	VP1	VP2	VP3	VP4
Pol 2c-1	−	+	−	+	−	−	−
CB2.2-3	−	+	−	−	−	+	−
CB2.2-5	−	+	−	−	−	+	−
2KB-3	−	+	−	−	−	+	−
2KB-6	−	+	−	−	−	+	−
3N2s5.1	−	±	+	+	−	−	−
CB2.2-10	−	+	+	−	−	+	−
IN5.1	+	+	+	−	−	−	+
IN8.44	+	+	+	−	+	−	−
CB2.1-3	+	+	+	−	−	−	+
CB2.1-6	+	+	+	−	−	−	+
CB2.2-4	+	+	+	−	−	−	+
CB2.2-8	+	+	+	−	−	−	+
2KA-1	+	+	+	−	−	−	+
2KA-2	+	+	+	−	−	−	+
2KA-5	+	+	+	−	−	−	+
2KB-4	+	+	+	−	−	−	+
3KC-1	+	+	+	−	−	−	+

[a] Positive (+), intermediate (±), and negative (−) proliferative responses correspond to stimulation indices of >10, 2.5 to 10, and <2.5, respectively. Results are the summarized from three independent experiments.

proliferate upon stimulation with type 1 or type 3 over a range of virus concentrations (Fig. 1A). T-cell clone CB2.2-10 responded fully to poliovirus types 2 and 3 but failed to respond to type 1 (Fig. 1B), whereas clone 3N2s5.1 responded strongly to type 3, failed to recognize type 1, and responded to type 2 only at high antigen concentrations (Fig. 1C). The remaining 11 T-cell clones were fully cross-reactive between each serotype (Table 1 and Fig. 1D).

Specificity of T-cell clones for poliovirus capsid proteins. Using a technique developed for the preparative separation of proteins in a form suitable for T-cell assays (9), poliovirus capsid proteins VP1, VP2, and VP3 and the internal capsid protein VP4 were isolated and used for specificity analysis with the panel of T-cell clones. The results summarized in Table 2 indicate that the partially or fully serotype-specific T-cell clones recognized VP1 or VP3. One cross-reactive T-cell clone, IN8.44, responded to VP2, while the remaining 10 of the 11 fully cross-reactive clones recognized VP4. Thus, 10 of 18 of the T-cell clones derived from six of nine H-2^d or H-2^k mice studied responded to VP4, suggesting that this polypeptide may be immunodominant for murine T cells.

Specificity of T-cell clones for synthetic peptides. From a total of 18 clones that have been mapped to distinct poliovirus capsid proteins, 16 were tested for specificity against overlapping peptides corresponding to the entire sequence of the protein recognized. Peptides corresponding to VP1 of type 2 were not available at the time of testing with clone Pol

2c-1. T-cell clone CB2.2-8, specific for VP4, was difficult to propagate, which precluded peptide specificity analysis. One fully cross-reactive T-cell clone, IN8.44, which was generated from a type 1-immunized mouse and recognized purified VP2, failed to respond to any of the 12-mer peptides synthesized according to the sequence of VP2 of poliovirus type 2.

Serotype-specific epitopes on VP1. Clone 3N2s5.1, derived from a BALB/c (H-2^d) mouse immunized with poliovirus type 3, recognized peptide VP1 257-268 and also responded, but not as strongly, to the overlapping peptide, VP1 253-264 (Fig. 2). This result suggests that the T-cell epitope is centered on residues Arg-257 to His-264. This T-cell clone responded weakly to poliovirus type 2 and not at all to type 1 (Fig. 1C). An examination of the sequences of the corresponding regions of poliovirus type 1 or type 2 (Table 3) demonstrates that the substitution of Ile-258 with Val in type 2 substantially reduces stimulation of T-cell clone 3N2s5.1 and that a second substitution, Met-260 to Leu, in poliovirus type 1 completely abrogates T-cell recognition.

Serotype-specific epitopes on VP3. T-cell clone CB2.2-10, derived from a CBA (H-2^k) mouse, responded to a single peptide, VP3 189-203 of poliovirus type 2 (Fig. 3A), over a range of antigen concentrations (Fig. 4A). The amino acid sequence of this peptide and of the corresponding regions of poliovirus types 1 and 3 are shown in Table 4. The specificity pattern of this T-cell clone for virus variants indicated that a single substitution (Val → Ile) at position 198 in type 3 is tolerated, whereas a second change (Met → Val) at position 192 in type 1 completely abrogates the T-cell response (Table 4). Another H-2^k T-cell clone, 2KB-6, specific for poliovirus type 2, recognized the overlapping peptide VP3 196-210 (Fig. 3B and 4B). A comparison of the amino acid sequences of the three Sabin strains of poliovirus in this region (Table 4) shows considerable variation, with two sequence changes in type 1 and five changes in type 3.

Three further clones, CB2.2-3, CB2.2-5, and 2KB-3, derived from CBA (H-2^k) mice, recognized a single peptide corresponding to residues 14 to 28 of VP3 of poliovirus type
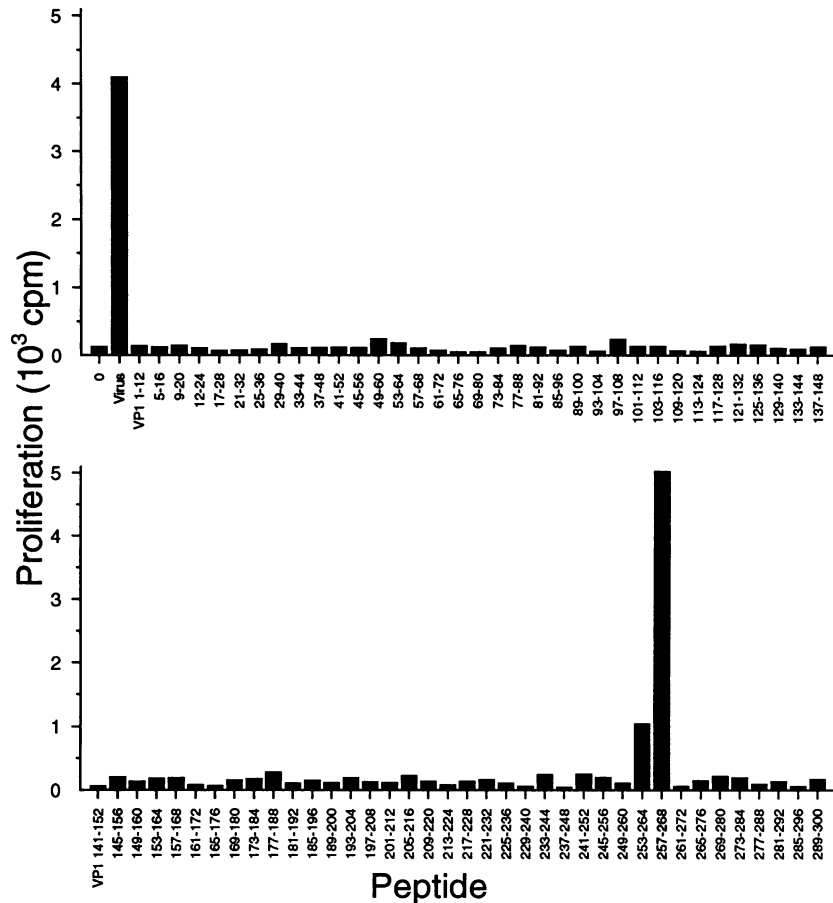
FIG. 2. Proliferative responses of T-cell clone 3N2s5.1 against overlapping synthetic peptides corresponding to the sequence of VP1 of poliovirus type 3. Peptides and whole virus were tested at a concentration of 1 µg ml^{-1}.

2. As clones CB2.2-3 and CB2.2-5 were derived from the same animal and displayed identical specificity patterns, only the results for clones CB2.2-3 and 2KB-3 are shown (Fig. 3C and D). Each of the T-cell clones responded to poliovirus type 2 but not to type 1 or 3. An examination of the amino acid sequences in the region of peptide 14-28 revealed that substitutions at position 19 and at either

TABLE 3. CD4$^+$ T-cell epitope on VP1 of poliovirus type 3 recognized by T-cell clone 3N2s5.1

Antigen	Sequence[a]	Response of T-cell clone 3N2s5.1[b]
Poliovirus	249 272	
Type 3	PTKVTSKVRIYMKPKHVRVWCPRP	+
Type 2	--RL---I-V--------------	±
Type 1	-------I-V-L----I-------	−
Peptide VP1		
249-260	------------	−
253-264	------------	+
257-268	------------	+
261-272	------------	−

[a] Amino acid sequences of poliovirus types 1, 2, and 3 (35) and of the overlapping peptides of type 3 are shown in single-letter code (amino acid numbering according to the type 3 sequence), with residue substitutions within the minimal T-cell determinant indicated by underlining.

[b] Results summarized from Table 1, Fig. 1, and Fig. 2.

position 16 or position 25 in types 1 and 3, respectively, abrogates recognition by these T-cell clones (Table 5).

Cross-reactive epitopes on VP4. Nine of the ten cross-reactive T-cell clones that recognize VP4 were tested against twelve overlapping 15-mer peptides corresponding to the entire sequence of VP4 of poliovirus type 2. T-cell clone CB2.2-4, derived from a CBA (H-2^k) mouse, proliferated in response to a single peptide, VP4 6-20 (Fig. 5A). Three further H-2^k clones, 2KA-1 and 2KB-4, derived from type 2-immunized mice, and clone 3KC-1, derived from a type 3-immunized mouse, proliferated in response to one peptide corresponding to residues 11 to 25 of VP4 (Fig. 5B and C). Clone IN5.1, derived from a BALB/c (H-2^d) mouse, also responded to this peptide, but in addition recognized an overlapping peptide VP4 16-30 (Fig. 5D). This finding suggests that T-cell clone IN5.1 recognized an overlapping epitope encompassed within residues Ser-16 and Ile-25. The remaining four T-cell clones, CB2.1-3, CB2.1-6, 2KA-2, and 2KA-5, all responded to the peptide corresponding to residues 21 to 35, indicating the presence of at least one further epitope in this region of VP4 (Fig. 5E and F). As T-cell clones 2KA-2 and 2KA-5 are derived from the same animal and exhibit identical specificity, and likewise clones CB2.1-3 and CB2.1-6, only the results for 2KA-5 around CB2.1-3 are shown in Fig. 5. A single amino acid substitution within residues 6 and 35 in poliovirus type 3 (Arg-34 to Lys) does
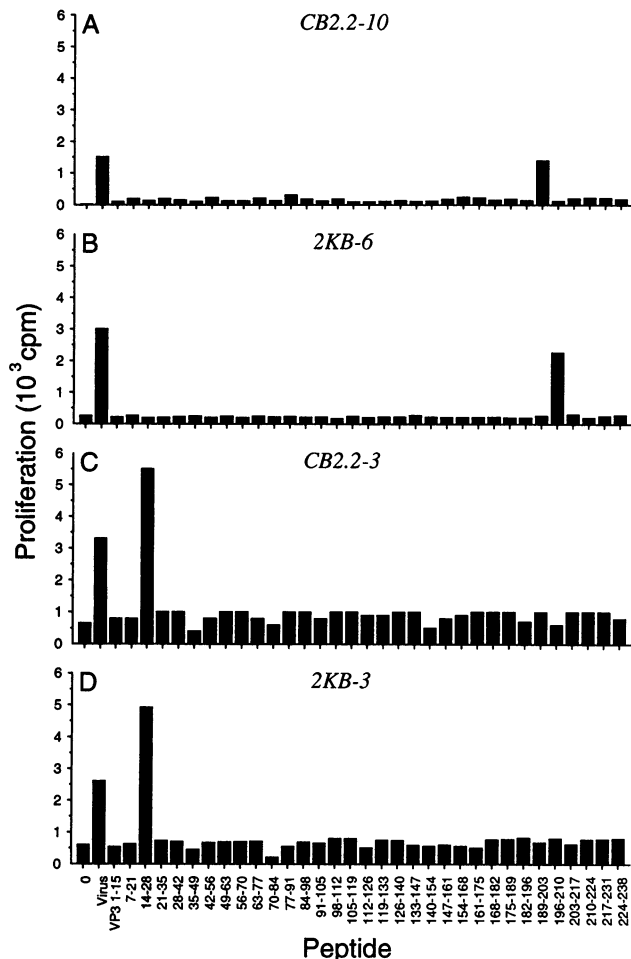
FIG. 3. Proliferative responses of the indicated T-cell clones against overlapping synthetic peptides corresponding to the sequence of VP3 of poliovirus type 2. Peptides and whole virus were tested at a concentration of 1 μg ml^{-1}.

FIG. 4. Recognition of overlapping poliovirus VP3 peptides 189-203 and 196-210 by T-cell clones CB2.2-10 and 2KB-6.

not affect recognition by these fully cross-reactive T-cell clones (Table 6).

DISCUSSION

The findings of this study demonstrate that T cells recognize epitopes on each of the four poliovirus capsid proteins, including an immunodominant region of VP4. Fine specificity analysis with a panel of CD4$^+$ T-cell clones revealed that, analogous to monoclonal antibody binding, T-cell recognition of the surface capsid proteins VP1 and VP3 was affected by sequence variation between viruses of different serotypes. In contrast, T-cell clones specific for the conserved internal capsid protein VP4 were cross-reactive between each of the three serotypes. To our knowledge, this study constitutes the first description of poliovirus T-cell specificity at the clonal level and provides the first evidence for a possible role for VP4 in immunity to poliovirus.

A number of studies have alluded to a role for T cells in immunity to poliovirus through their helper function in antibody production (7, 8, 37), and poliovirus-specific T-cell responses have been demonstrated in studies using lymph nodes or spleen cells from immunized mice (10, 12, 39). Furthermore, a single T-cell epitope has been identified on
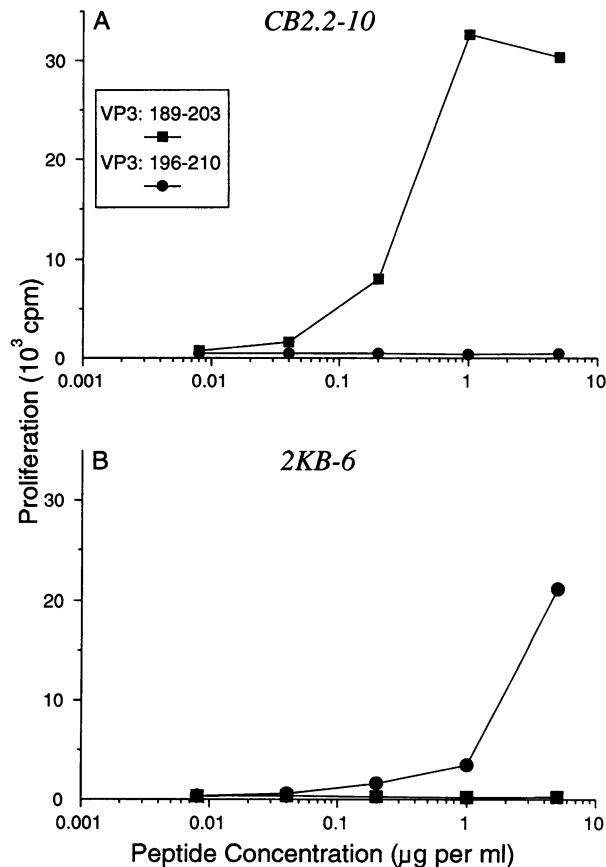
poliovirus VP1 by using a polyclonal T-cell preparation (12). In the present investigation, we used a panel of T-cell clones and overlapping synthetic peptides in a detailed analysis of the antigenic sequences of poliovirus recognized by T cells. From a total of 18 CD4$^+$ T-cell clones characterized, 10 recognized the internal capsid protein VP4. The four epitopes defined on VP4 were all found toward the N-terminal region, encompassed within residues 6 and 35, a sequence which is highly conserved between the three polio-

TABLE 4. Two overlapping CD4$^+$ T-cell epitopes on VP3 of poliovirus type 2 recognized by T-cell clones CB2.2-10 and 2KB-6

Antigen	Sequence[a]		Response of T-cell clone[b]:	
			CB2.2-10	2KB-6
Poliovirus	189	210		
Type 2	YISMFYQTRVVVPLSTPRKMDI		+	+
Type 3	---------I-------KS-SM		+	−
Type 1	---V-----I-------E---		−	−
Peptide VP3				
182-196	--------		−	−
189-203	---------------		+	−
196-210	----------------		−	+
203-217	--------		−	−

[a] As for Table 3 except that overlapping synthetic peptides are based on the sequence and numbering of poliovirus type 2 (35).
[b] Results summarized from Table 1, Fig. 1, and Fig. 3.

TABLE 5. CD4+ T-cell epitope on VP3 of poliovirus type 2 recognized by T-cell clones CB2.2-3 CB2.2-5, and 2KB-3

Antigen	Sequence[a]	Response of T-cell clone[b]:		
		CB2.2-3	CB2.2-5	2KB-3
Poliovirus	7 35			
Type 2	TPGSNQYLTADNYQSPCAIPEFDVTPPID	+	+	+
Type 1	------------F-----L----------	−	−	−
Type 3	---------S--H----------------	−	−	−
Peptide VP3				
7-21	---------------	−	−	−
14-28	---------------	+	+	+
21-35	---------------	−	−	−

[a] As in Table 4.
[b] Results summarized from Table 1, Fig. 1, and Fig. 3.

virus serotypes (35). A single amino acid sequence alteration in this region (Arg-34 to Lys in poliovirus type 3) had no effect on T-cell recognition. Studies with polyclonal T cells (10, 12, 39) have indicated the existence of uncharacterized T-cell sites common to all poliovirus serotypes. The result of the present study suggests that VP4 may be the major target for cross-reactive T cells. Ten T-cell clones derived from six individual H-2[k] or H-2[d] mice recognized the N-terminal region of VP4. T-cell recognition of conserved internal viral
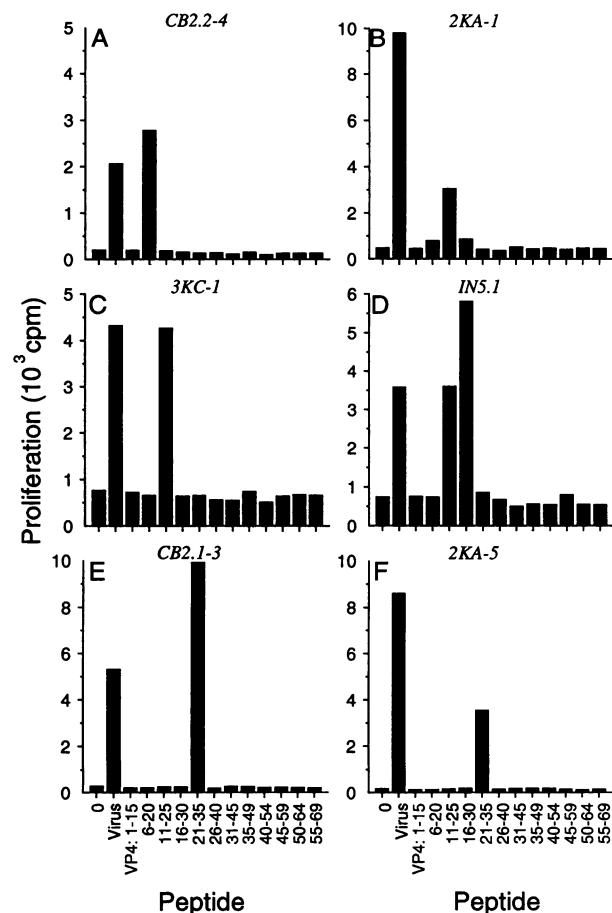

FIG. 5. Proliferative responses of the indicated T-cell clones against overlapping synthetic peptides of VP4 of poliovirus type 2. Peptides and whole virus were tested at a concentration of 1 μg ml⁻¹.

proteins is well established from studies with other viruses such as human immunodeficiency virus and influenza virus, for which the Gag protein and nucleoprotein have been shown to contain both CD4+ and CD8+ T-cell epitopes (16, 18, 25, 33, 34).

In contrast to the fully cross-reactive T-cell responses to VP4, T-cell clones specific for VP1 or VP3 responded only to the immunizing serotype or showed limited cross-reactivity between poliovirus types 2 and 3. One T-cell epitope was defined in a variable region of VP1 or poliovirus type 3 by using clone 3N2s5.1, generated from a type 3-immunized H-2[d] mouse. The minimum epitope recognized by this T-cell clone is encompassed within residues 257 and 264, which on the three-dimensional structure of poliovirus is located on the I strand of VP1, a region of β sheet (4, 6). An Ile-to-Val substitution at position 258 in the corresponding sequence of poliovirus type 2 substantially reduced the recognition of this virus, while a second sequence alteration at position 260 of Met to Leu in the type 1 sequence completely abrogated the proliferative response of this clone. The H-2[d] T-cell epitope previously identified on VP1 of poliovirus strain Mahoney type 1 (12) is adjacent to the main neutralizing antibody site on VP1 (site 1, amino acids 89 to 104). The T-cell epitope on VP1 identified in the present study, although not close in the linear sequence, forms part of an adjacent loop region that includes residue 253, which has been implicated in the monoclonal antibody binding to site 1 (6, 22).

T-cell epitopes identified on VP3 also map to variable regions of the protein. One epitope on VP3 of poliovirus type 2 recognized by three distinct H-2[k] T-cell clones was encompassed within residues 14 and 28, which, by analysis with the structure of Mahoney type 1 (6) and Sabin type 3 (4), forms part of the internal N-terminal tail of VP3. The other two T-cell epitopes were mapped to overlapping sequences in VP3, corresponding to amino acids 189 to 208 and 196 to 210, which form part of the internal H strand and the exposed H-I loop regions. This loop region has also been shown to include residues critical for monoclonal antibody binding to poliovirus type 2 (36). An overlap between T-cell and B-cell epitopes has been described for influenza hemagglutinin; it has been suggested that B cells may function to direct the T-cell response to specific sites on the viral protein (1, 19).

The proliferative response of clone 2KB.6, which recognized an epitope corresponding to residues 196 to 210 located on the external loop of VP3, was abrogated by the considerable amino acid sequence variation in this region (two amino acid sequence changes in type 1 and five changes in type 3 in comparison with type 2). It has been suggested

TABLE 6. Four overlapping CD4+ T-cell epitopes on VP4

Antigen	Sequence[a]	Response of T-cell clone(s)[b]:			
		CB2.2-4	2KA-1, 2KB-4, 3KC-1	1N5.1	CB2.1-3, 2KA-5, CB2.1-6, 2KA-2,
Poliovirus	6 35				
Type 2	SSQKVGAHENSNRAYGGSTINYTTINYYRD	+	+	+	+
Type 1	-----------------------------	+	+	+	+
Type 3	---------------------------K-	+	+	+	+
Peptide VP4					
6-20	----------------	+	−	−	−
11-25	----------------	−	+	+	−
16-30	----------------	−	−	+	−
21-35	----------------	−	−	−	+

[a] As in Table 4.
[b] Results summarized from Table 1, Fig. 1, and Fig. 5.

that amino acid sequence variation in the external loop regions of poliovirus indicate that these sites are targets for immune recognition (25). However, we have also demonstrated that T-cell responses to epitopes in the internal regions of VP1 and VP3, which are unlikely to be recognized by neutralizing antibodies, are also affected by antigenic variation between poliovirus serotypes. Although the sequence variation in internal proteins may be the result of compensatory changes necessary for the maintenance of the structural integrity of the viral capsid, it is also possible that MHC class II-restricted T cells, like antibodies, play a role in immune selection, resulting in antigenic variation in polioviruses. Studies with lymphocytic choriomeningitis virus (28), human immunodeficiency virus (27), and influenza virus (1, 19) have demonstrated that mutations in internal as well as surface proteins can abrogate virus-specific CD4+ and CD8+ T-cell responses. Amino acid sequence changes within the minimum T-cell epitope may affect T-cell recognition by inhibiting the peptide interaction with the MHC molecule or the T-cell receptor. Alternatively, it is possible that substitutions in flanking sequences prevent T-cell recognition through their effects on antigen processing.

Since there is no evidence that Sabin type 1, 2, and 3 strains of poliovirus can replicate in mice (11, 29), the immunization schedule that we have used in this study is unlikely to generate T cells that recognize antigen processed by the endogenous route. However, as CD4+ T cells normally recognize peptides processed from exogenous antigens, the murine model that we used should provide valuable information on the class II-restricted T-cell repertoire for poliovirus. The functional significance of poliovirus-specific CD4+ T cells in protection against poliovirus infection in humans remains to be defined.

The helper activity of CD4+ T cells is well documented. Studies with murine T-cell clones have shown that the Th2 subpopulation of murine CD4+ T cells secrete the lymphokines IL-4, IL-5, and IL-6, which regulate immunoglobulin G and immunoglobulin A production by controlling immunoglobulin class switching and B-cell differentiation (3, 23). It has been reported that Th cells specific for core antigens of influenza virus and hepatitis B virus can provide helper function for a protective antibody response to the surface proteins (15, 32). We have also suggested that the induction of cross-reactive poliovirus-specific Th cells may allow an enhanced primary neutralizing antibody response to protective epitopes following exposure to either a second serotype or a new virus variant (10). Therefore, CD4+ cells against

conserved epitopes on VP4 may play a role in broadly protective immune defense against poliovirus infection. However, studies with human and simian immunodeficiency viruses have shown that this type of intrastructural intermolecular help does not always operate between surface and internal viral proteins (17).

An alternative role for poliovirus-specific CD4+ T cells may reside in functions associated with the Th1 subpopulation, which secrete IL-2, lymphotoxin, and IFN-γ (23). Th1 cells have been shown to display delayed-type hypersensitivity and in vitro cytotoxic activity (23). Furthermore, an in vivo role for CD4+ MHC class II-restricted CTL specific for lymphocytic choriomeningitis virus has recently been demonstrated (24). Although the highly lytic nature of poliovirus infection of cells in tissue culture argues against a role for CTL in vivo, class I-restricted CD8+ CTL have been demonstrated against other picornaviruses. Virus-specific CTL have been shown to play a role in viral clearance and in immune pathology following infection of mice with coxsackievirus B3 or Theiler's murine encephalomyelitis virus (13, 31, 40). Poliovirus-specific CD4+ Th1 cells could function through IFN-γ production to increase MHC class I or class II expression on the surface of poliovirus-infected cells of the central nervous system such as neurons (38), thus enhancing their susceptibility to lysis by CD8+ or CD4+ CTL. The documented inflammatory response in the form of a mononuclear cell infiltrate in the central nervous system after intracerebral inoculation of mice with mouse-adapted poliovirus (8) provides further circumstantial evidence for the involvement of Th1 cells. The cytokines IFN-γ and lymphotoxin produced by Th1 cells have been implicated in the migration of lymphocytes and macrophages into inflammatory sites (23). Current investigations of the in vitro function and cytokine production by the murine CD4+ T-cell clones described in this report, as well as future experiments on T-cell function in vivo with use of transgenic mice expressing poliovirus receptor gene (29), may help to elucidate the relative roles of T-cell subpopulations in immunity to poliovirus.

ACKNOWLEDGMENTS

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