Characterization of Poliovirus-Specific T Lymphocytes in the Peripheral Blood of Sabin-Vaccinated Humans

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Poliovirus-specific cellular immune responses were identified in the peripheral blood mononucleocytes of Sabin-immunized human donors by using a proliferation assay. Complement depletion and monoclonal antibody inhibition studies suggest that the effector population is the major histocompatibility complex (MHC) class II-restricted CD4⁺ T-helper cell. Immune lymphocytes proliferated to polyacrylamide gel purified-capsid proteins VP1, VP2, and VP3 and, in some individuals, to synthetic VP4, indicating the presence of T-cell epitopes in each of these proteins. Using synthetic peptides, T-cell epitopes have been mapped to specific regions in VP1 which lie near previously identified neutralizing antibody recognition sites. Human leukocyte antigen (HLA) typing of the donor individuals indicated that no MHC class II molecule was held in common between all four donor individuals. Thus, the positive responses observed with peptides p182-201 and p244-261 in three of four and four of four donors suggest that these peptides contain epitopes presented by at least two different MHC molecules. Antibody-blocking experiments suggest that an epitope within VP1 residues 244 to 264 is presented by HLA DQ3.

Accurate definition of the immune effector mechanisms, which act on an invading pathogen, requires the examination of responses seen in a susceptible but immune host. In some cases this is relatively straightforward, because immunologically malleable rodents are adequate models for virus-infected hosts. However, the host ranges for some pathogens are restricted to humans and/or primates. Thus, it is important to determine whether the results that have been obtained in earlier rodent studies accurately reflect what is seen in a permissive host.

Extensive studies in rodents have identified neutralizing antibody epitopes to the capsid region of poliovirus by sequence analysis of neutralizing monoclonal antibody escape mutants and by raising neutralizing sera to synthetic peptides (reviewed in reference 20). The presence of poliovirus-specific T-cell responses has been demonstrated in virus-inoculated mice (1, 12, 13, 32, 33). These responses have been further clarified by the recent identification of murine T-cell epitopes within capsid protein VP1 (17-19). These studies of inoculated rodents suggest a colocalization of the poliovirus T- and B-cell epitopes on the poliovirus capsid.

The immunological significance of these observations is difficult to assess because rodents lack a specific cellular receptor for poliovirus and are resistant to poliovirus infection. Epidemiological characterization of naturally occurring poliovirus strains and Pepsan studies of sera from human vaccinees suggest that the neutralization antigenic sites defined in mice are similar to those recognized in humans (15, 27). Poliovirus-specific cellular responses have been observed in human peripheral blood monocytes (PBMcs) (2). However, the nature of this response was uncharacterized, and T-cell epitopes have not been defined.

This report demonstrates the presence of poliovirus-specific CD4⁺ T-helper lymphocytes in the peripheral blood of immunized humans. All structural proteins of the virus

contain epitopes recognized by this lymphocyte population. Using synthetic peptides, several epitopes were mapped to specific regions of VP1. Peptides which surround residues that contribute to antibody neutralization site 2 appear to be recognized by multiple donors. HLA typing and antibody blocking studies suggest that these regions contain more than one T-helper epitope.

MATERIALS AND METHODS

Human subjects. Sabin-vaccinated volunteers were recruited. Healthy donors ranging from 21 to 39 years of age with poliovirus serum neutralizing titers of $>10^{-2}$ were chosen for further study. Although donors were immunized with the attenuated vaccine (Sabin strain), all donors had been exposed to the serotype 1 Mahoney strain. In addition, one donor (donor B) was initially vaccinated with the inactivated vaccine (Salk vaccine). Neutralizing titer determinations were performed as previously described (7). Human leukocyte antigen (HLA) typing of Epstein-Barr virus-immortalized cell lines from donors A and B was performed at the tissue typing laboratory at Brigham and Women's Hospital (Boston, Mass.). Peripheral blood from donors C and D was typed by the Red Cross (Dedham, Mass.). Cord blood lymphocytes, a source of naive cells, were a gift from Francis Ennis (University of Massachusetts Medical Center, Worcester, Mass.).

PBMC isolation. After obtaining informed consent, whole blood was drawn from volunteers into 15-ml heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, N.J.). After being diluted 1:1 in RPMI 1640 with L-glutamine (GIBCO, Grand Island, N.Y.), blood was layered on Histopaque-1077 (Sigma Chemical Co., St. Louis, Mo.) and centrifuged at $900 \times g$ for 20 min. The buffy coat was isolated, washed extensively, resuspended in culture medium (RPMI 1640 plus 10% human AB serum [Sigma], penicillin [50 U/ml], streptomycin [50 µg/ml] [GIBCO]) and counted. (The human AB serum in the PBMC culture medium contained $\sim 10^{-2}$ neutralizing titer to poliovirus.

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The presence of these antibodies may aid in antigen presentation through antibody-mediated antigen uptake by Fc receptor-positive antigen-presenting cells.) Cells not immediately used in proliferation assays were cryopreserved in RPMI 1640 plus 20% fetal calf serum plus 10% dimethyl sulfoxide (DMSO).

Antigens. Poliovirus type 1 (Mahoney strain) stocks were derived from an infectious cDNA clone, grown in HeLa cells, and purified by CsCl gradient ultracentrifugation by using standard procedures (28). Virion concentration was determined by optical density at 260 nm, using an extinction coefficient of 7.7 optical density units per mg per ml (29). Heat-inactivated virus was generated by incubating purified virus at 65°C for 10 min. Capsid proteins VP1, VP2, and VP3 were isolated from sodium dodecyl sulfate-polyacrylamide gels (7), and protein concentrations were determined using a BCA-based protein assay (Pierce, Rockford, Ill.). Synthetic capsid protein VP4 (68 amino acids, corresponding to the sequence of serotype 1, Mahoney strain) was synthesized by the Massachusetts Institute of Technology Biopolymer Laboratory on an Applied Biosystems 430A automated peptide synthesizer using standard t-BOC chemistry and double coupling of each residue. The unmyristoylated protein was shown to be approximately 85% pure by high-pressure liquid chromatography (HPLC) and amino acid analysis. Synthetic peptides corresponding to the sequence of type 1 (Mahoney strain) capsid protein VP1 were synthesized with a Beckman 990b peptide synthesizer. These peptides were used earlier to identify neutralizing antibody epitopes in VP1 (8). Additional peptides p7-24 and p110-120 were synthesized by the Massachusetts Institute of Technology Biopolymer Laboratory and were purified to greater than 95% by preparative HPLC. Bovine serum albumin (fraction V) was purchased from Sigma.

Immunological reagents. Anti-human CD4 (clone Edu-2) and anti-human CD8 (clone UCH-T2) antibodies were purchased from Pierce. Anti-human CD3 (clone UCH-T1) antibody was purchased from Oncogene Science (Manhasset, N.Y.). Anti-I-A^k antibody (clone 14V.18) with cross-reactivity to human HLA DR (11) and rabbit complement were purchased from Cedarlane Laboratories Ltd. (Westbury, N.Y.). W6/32, a monoclonal antibody which binds to human major histocompatibility complex (MHC) class I antigens, was kindly provided by Herman Eisen, Massachusetts Institute of Technology. The monoclonal antibodies to HLA DR and DQ molecules were a gift of the Brigham and Women's Hospital Tissue Typing Laboratory (Boston, Mass.) and have been described earlier (16).

Proliferation assays. Assays were performed in 96-well U-bottom microtiter plates (Costar 3799, Cambridge, Mass.). Antigens, in 10 μ l of phosphate-buffered saline (PBS), were added to 90 μ l of culture medium. To this, PBMCs (2×10^5 /100 μ l of culture medium) were added and cultures were incubated at 37°C in 7% CO₂ for 6 days. At 8 h prior to harvesting, 1.25 μ Ci of [³H]thymidine (specific activity, 2 Ci/mmol; New England Nuclear, Boston, Mass.) in 50 μ l of medium was added to each well. Cells were subsequently harvested on glass fiber filters, using a Skatron semiautomatic cell harvester (Sterling, Va.). Incorporated ³H label, determined by scintillation counting, is reported as the mean ($\pm$ standard deviation) of triplicate cultures. Stimulation indexes (SI) were determined by the ratio of ³H counts in cultures incubated with and without antigen, with SI > 2 interpreted as positive proliferation responses.

Complement depletion assays. Freshly isolated PBMCs were treated with monoclonal antibodies and complement

before being tested for specific proliferative responses. Specifically, 1×10^6 to 2×10^6 cells in 50 μ l of RPMI were added to 100 μ l of RPMI containing a 1:15 dilution of antibody and incubated on ice for 30 min. After the cells were washed with cold PBS, cells were resuspended in 100 μ l of RPMI, added to 200 μ l of RPMI containing a 1:3 dilution of rabbit complement, and incubated at 37°C for 1 h. Cells were washed in PBS and resuspended in 0.6 ml of RPMI plus 10% human AB serum, and aliquots of this cell suspension (100 μ l per well) were distributed into wells containing poliovirus antigen or no antigen. Proliferative responses were analyzed as described above.

Monoclonal antibody blocking assays. Monoclonal antibodies to MHC class I and class II molecules (20 μ g/ml) were added to triplicate cultures of 2×10^5 PBMCs incubated with poliovirus (1 μ g/ml). After 6 days in culture, wells were pulsed with [³H]thymidine and harvested as described above.

Peptide proliferation assays. Lyophilized peptides were resuspended in DMSO at 10 mg/ml and stored in glass vials at -20°C. On the day of the assay, peptide antigens were added to RPMI plus 10% human AB serum and media containing control antigen (2 μ g of poliovirus per ml) or no antigen were adjusted to 0.2% DMSO. Antigens (100- μ l aliquots) were added to 96-well U-bottom plates. PBMCs from freshly drawn peripheral blood (2×10^5 cells in 100 μ l of RPMI plus antibiotics) were added to give a final concentration of 10 μ g of peptide per ml (or 1 μ g of poliovirus per ml) in RPMI-5% serum-0.1% DMSO. Cells were pulsed with [³H]thymidine after 4.5 days in culture and harvested as described above.

RESULTS

Human lymphocytes specifically proliferate in response to poliovirus. To assess whether poliovirus-specific T cells were present in poliovirus-vaccinated humans, PBMCs from seropositive donors were cultured in the presence of different concentrations of poliovirus serotype 1 Mahoney strain (Fig. 1). PBMCs exposed to poliovirus specifically proliferated above a background of bovine serum albumin-stimulated or unstimulated cultures. These responses decreased with decreasing antigen concentrations and were independent of virus infection, because both native and heat-inactivated poliovirus stimulated thymidine incorporation (data not shown).

Several observations suggest that these proliferative responses were due to prior exposure of the donors to poliovirus. All donors tested had neutralizing serum titers to poliovirus and contained PBMCs which proliferate in response to poliovirus addition. Plaque assays of the PBMC culture supernatants showed no demonstrable poliovirus titers. This indicated that no viral replication is occurring in these cultures. Therefore, the poliovirus-specific thymidine incorporation observed in these cultures was not a result of cellular injury caused by replicating virus. Finally, lymphocytes prepared from human cord blood was used as a source of naive lymphocytes to provide a negative control. As expected, poliovirus and poliovirus antigens (at the highest antigen concentrations used in the donor PBMC cultures) did not stimulate proliferation in these cultures (data not shown). Thus, the positive proliferative responses observed were not due to the *in vitro* culture conditions.

Immunological characterization of poliovirus-specific PBMCs. Antibody-mediated complement depletion of the donor PBMC cultures identified the subpopulation of cells

FIG. 1. Poliovirus induces lymphocytes to proliferate in vaccinated humans. Lymphocytes from three Sabin-vaccinated individuals (donors A, B, and E) were cultured in vitro in the presence of poliovirus (■) or bovine serum albumin (▨) as the stimulating antigen or with no antigen (□). The induced proliferative responses were measured by [3 H]thymidine incorporation after 6 days. The mean $\pm$ standard deviation of triplicate cultures is reported. Positive stimulation indexes (>2) are reported above the corresponding bars.

necessary for proliferative responses to poliovirus (Fig. 2A). Proliferation was reduced or eliminated in cultures depleted of CD3 $^+$ (T-cell) or CD4 $^+$ (T-helper) lymphocytes. No inhibition of proliferation was observed upon removal of the CD8 $^+$ (T-cytotoxic) cells. In addition, depletion of HLA DR $^+$ cells (B cells and monocytes) reduced poliovirus-specific proliferation in these cultures, suggesting that antigen-presenting cells are necessary for T-cell proliferation. Although the data are displayed for only one donor, similar results were observed for all donors tested. Thus, the effector cells are members of the T-helper population.

To determine which MHC antigen presented poliovirus antigens to the T lymphocytes, monoclonal antibodies to MHC class I or class II antigens were added to triplicate cultures stimulated with poliovirus (Fig. 2B). In agreement with the complement depletion data, antibodies to class II and not class I MHC antigens inhibited the proliferative response elicited by poliovirus. Therefore, the poliovirus-specific effector cell population in these PBMC cultures is the classic CD4 $^+$, MHC class II-restricted T-helper lymphocyte.

T-cell epitopes exist in capsid proteins VP1, VP2, VP3, and VP4 and map to specific regions of VP1. T-cell responses to the individual capsid proteins of poliovirus were assessed by using gel-purified viral (VP1, VP2, and VP3) or synthetic (VP4) capsid proteins (Fig. 3). PBMCs from each donor

FIG. 2. Poliovirus-specific proliferation is dependent on the presence of CD4 $^+$ MHC class II-restricted T cells. (A) Depletion of CD4 $^+$ lymphocytes removes poliovirus-specific proliferation. PBMCs from donor B were treated with specific cell surface monoclonal antibodies (to CD3, CD4, CD8, or I-A k , with cross-reactivity to HLA DR) plus complement before being cultured with (■) and without (□) poliovirus. (B) Monoclonal antibodies to MHC class II antigens inhibit proliferation to poliovirus. PBMCs from donor C incubated with 20 μ g of monoclonal antibodies to MHC class I (W6/32) per ml or class II (I-A k) antigens were stimulated with 1 μ g of poliovirus per ml and assayed for thymidine incorporation. The mean $\pm$ standard deviation of triplicate cultures is reported.

showed positive simulation indexes to capsid proteins VP1, VP2, and VP3, indicating the presence of T-cell epitopes in these capsid proteins. Synthetic VP4 induced positive proliferation responses in three of the four donors. The lack of proliferation in one of the donors was reproducible and probably reflected the inability of VP4-specific epitopes to be presented by this individual. This is not surprising, given the small size of the VP4 antigen (68 amino acids). In addition, the proliferative profiles observed in some donors reproducibly favored one of the capsid proteins. This suggested that a higher proportion of the poliovirus-specific T-cell epitopes recognized by that individual may reside within that capsid protein.

Positive SI were observed in all PBMC cultures upon exposure to VP1 capsid protein. Synthetic peptides were used to map more precisely the T-cell epitopes within this protein (Fig. 4). Specific VP1 peptides stimulated PBMCs to proliferate. However, in general, proliferative responses observed for any given peptide were not strong and varied between experiments. This is in contrast to the reproducibly strong responses obtained from poliovirus-sensitized murine lymphocytes upon in vitro stimulation with these VP1 peptides (17). This variation most likely arises due to factors within the human donors that affect the specific immune status of an individual and cannot be easily controlled, such as natural exposure to other infectious agents, dietary fluctuations, and/or stress levels. To compensate for this variability, human donors were tested on several occasions. (Figure 4 displays the results of one of these experiments.) The SI from two (donor A) or three (donors B, C, and D) separate lymphocyte preparations were averaged to generate Table 1. From this analysis, several peptides which contain T epitopes were identified: two, corresponding to residues 182 to 201 and 244 to 264 (p182-201 and p244-264, respectively) stimulated positive proliferation responses in three of four and four of four donors, respectively. A third, p161-181, stimulated positive proliferation responses in two of four donors. The remaining positive peptides (p91-109 and p202-

FIG. 3. Proliferative responses to poliovirus can be identified to capsid proteins VP1, VP2, VP3, and VP4 (donors A, B, C, and D, respectively). Lymphocytes were cultured with polyacrylamide gel-purified capsid proteins VP1 (■), VP2 (□), VP3 (▨), synthetic VP4 (▤), or no antigen (▩). The mean $\pm$ standard deviation of triplicate cultures is reported.

221) appeared to elicit donor-specific responses. The strength of proliferative responses observed for peptides that test positive for proliferation ($SI > 2$) decreased with decreasing peptide concentrations. In addition, none of these positive peptides induced positive proliferative responses when used to stimulate cord blood lymphocytes (data not shown).

HLA D antigen expression and poliovirus peptide presentation. Donor lymphocytes were typed for HLA D antigens to define which MHC class II molecules might be presenting epitopes found within the positive peptides (Table 2). No MHC class II molecule was held in common between all donors. Because p244-264 was recognized by all donors and p182-201 was recognized by three of the four donors tested, it appears that p244-264 and p182-201 each contain T-cell epitopes that can be presented by at least two different MHC molecules. It is interesting to note that although donors C and D express the same HLA D antigens, donor C recog-

FIG. 4. Proliferative responses to synthetic peptides derived from the capsid protein VP1 sequence. Lymphocytes were cultured with 10 μ g of VP1 peptides per ml (8), 1 μ g of poliovirus (PVM) per ml, or no antigen (No Ag). Mean $\pm$ standard deviation of triplicate cultures is reported. SI (>2) are reported above the corresponding bars. Variability between assays is observed in the responses to a given peptide. To indicate this, peptides with SI of <2 (for the assay displayed in this figure) but which over several assays have an average SI of >2 (Table 1) are underlined. Because the average SI was greater than 2, these peptides are considered to be proliferation positive. Conversely, peptides with SI of >2 in this assay, but with an average SI of <2 , are considered to be proliferation negative and are indicated in parentheses.

nizes p182-201 and not p161-181, whereas donor D recognizes p161-181 and not p182-201. Because these individuals are not related, it is possible that this disparate pattern of epitope recognition arose from molecular differences between the HLA antigens that were not detected by the typing antisera (3). In addition, antigen processing and presentation differences in donors C and D may also account for this disparity of recognition.

Due to the limited number of defined antibodies which block MHC presentation of antigen to human T cells, it has been difficult to identify the specific MHC-presenting molecule for the positive peptides. However a monoclonal anti-

TABLE 1. Proliferative responses of PMBC cultures to VP1 peptides

VP1 peptide	Avg SI for indicated donor ^a			
	A	B	C	D
p7-24				
p24-40				
p61-80				
p86-103				
p91-109	2.39			
p100-109				
p110-120				
p121-141	2.63			
p141-156				
p161-181		2.58		3.04
p182-201	2.26	5.29	2.84	
p202-221		4.65		
p211-228				
p220-237				
p222-241				
p231-251				
p244-264	2.61	3.43	3.07	2.23
p270-287				
p286-302				
Poliovirus (1 µg)	32.35	46.60	24.91	11.19

^a The average SI of several proliferation experiments for the donors were calculated. SI of >2 are presented.

body specific for HLA DQ3 (anti-DQ3) appeared to inhibit the proliferative response to p244-264 but not that to p182-201 in donor B (Fig. 5). Thus, HLA DQ3 appears to present a T epitope located in p244-264 but not in p182-201. Although donor A was not available for further studies, because donor A also expresses the DQ3 molecule, it is likely that p244-264 is also being presented by the DQ3 molecule in this donor. The absence of DQ3 in donors C and D suggests that another class II molecule is presenting an epitope located in p244-264.

DISCUSSION

In this study, poliovirus-immunized human donors were analyzed for virus-specific cellular immune responses. PBMCs from Sabin-vaccinated donors specifically proliferated upon exposure to intact poliovirus particles and isolated viral capsid proteins. In contrast, lymphocytes isolated from cord blood, an immunologically naive population, did not proliferate in response to these antigens. Complement depletion and antibody-blocking experiments identified the CD4⁺ MHC class II-restricted T-helper lymphocytes as being necessary for the proliferative response. Using synthetic peptides which correspond to residues of the VP1 capsid protein, two T-epitope regions (residues 182 to 201

TABLE 2. HLA D antigen expression

HLA DR and DQ antigen expressed by donor ^a :			
A	B	C	D
DR4	(DR3)	(DR1)	DR1
DRw53	DR9	DR7	DR7
DQ3	DRw52	DQw1	DQw1
	DRw53	DQw2	DQw2
	DQ3		

^a HLA typings that are indeterminate are in parentheses.

FIG. 5. Antibodies to HLA DQ3 inhibit the proliferation to p244-264. PBMCs from donor B were incubated with p182-201 (10 µg/ml; ■), p244-264 (10 µg/ml; ▨), poliovirus (1 µg/ml; □), or no antigen (□) in the presence (DQ3) or absence (No Ab) of a 1/40 dilution of monoclonal antibody to HLA DQ3. After 5 days, cultures were assayed for thymidine incorporation. The mean ± standard deviation of triplicate cultures is reported. SI are presented above the corresponding bars.

and 244 to 264) induced lymphocyte proliferation in three of four and four of four donors tested. HLA typing of the human donors showed no common HLA class II determinants for all donors, which recognized a specific positive peptide. Thus, cross-reactive proliferative responses to a peptide by HLA-mismatched donors suggest that the peptide contains more than one T-cell epitope.

It was possible to demonstrate for one donor (donor B) that monoclonal antibodies to DQ3 inhibited lymphocyte proliferation to p244-264 but not to p182-201, suggesting that epitopes found in p244-264 are presented by the DQ3 HLA molecule. Although donor A was not available for further studies, the recognition of this peptide by donor A lymphocytes (who shares HLA DQ3 in common with donor B) suggests that the same epitope within p244-264 is being presented by HLA DQ3 for this individual as well. However, because DQ3 is not found in donors C and D and p244-264 stimulates proliferation of lymphocytes from donors C and D, this peptide must contain other epitopes that can be presented by a non-DQ3 molecule. Interestingly, both donors C and D express the HLA DR1 molecule. Recently, a peptide-binding motif for HLA DR1 has been reported (5). It contains a positive-charged or large hydrophobic residue at position 1, a hydrogen bond donor at position 1+5, and a hydrophobic residue at position 1+9. Examination of the p244-264 sequence does not reveal the presence of this HLA DR1 motif. Thus, it is likely that in donors C and D this peptide is being presented by a non-DR1 molecule.

A DR1 motif is found in the broadly recognized p182-201, with Phe at position 1, Thr at 1+5, and Ile at 1+10. Consistent with the presence of this motif, PBMCs from donor C (who expresses the DR1 molecule) recognize and proliferate in response to p182-201. However, the response by PBMCs from donor D, which express the DR1 molecule but fail to recognize p182-201, is problematic. As suggested above, there may be molecular differences in the polymorphic β-chains of DR1 between donors C and D that are not detected by the typing antisera. These differences may account for this discrepancy. However, because all HLA DR alleles contain a monomorphic α-chain, the residues making up half of the peptide-binding groove will be invariant in HLA DR molecules (4). Therefore, broad recognition

TABLE 3. Human/murine comparison of immune responses upon poliovirus exposure

VP1 peptide	Responding individuals ^a		Neutralization site ^b
	Human donor	Mouse	
p7-24		<i>H-2^d</i> , <i>H-2^k</i>	
p24-40		<i>H-2^k</i>	
p61-80		<i>H-2^b</i>	
p86-103		<i>H-2^k</i>	1
p91-109	A		1
p100-109		<i>H-2^d</i>	1
p121-141	A		
p141-156		— ^c	1
p161-181	B, D		
p182-201	A, B, C	<i>H-2^k</i>	
p202-221	B	<i>H-2^b</i> , <i>H-2^d</i>	
p211-228		—	2
p220-237		—	2
p222-241		<i>H-2^b</i> , <i>H-2^d</i> , <i>H-2^k</i>	2
p231-251		—	
p244-264	A, B, C, D	<i>H-2^k</i>	
p270-287			
p286-302			

^a Individuals (human donors or mouse *H-2* haplotypes tested) are indicated where peptides elicit stimulation indexes of >2. Mouse data were taken from reference 17.

^b Determined by sequence of mutant viruses which escape neutralizing monoclonal antibodies (nomenclature from reference 22).

^c —, not determined.

of the p182-201 peptide (donors A, B, and C) may reflect a general affinity of the DR1 motif for the invariant DR α -chain which is modified by the polymorphic DR β -chains. In addition, the HLA molecules that present epitopes from p182-201 and other peptides remain to be determined for all donors. It is possible that these peptides are presented by HLA DP molecules or by other nonclassical type II restriction elements that are held in common between these individuals. These issues remain to be resolved.

Studies of poliovirus-specific T-cell epitopes in mice have identified both MHC class II-restricted T-helper and class I-restricted T-cytotoxic epitope regions in VP1 (17–19). General observations from these studies were that the majority of these T epitopes clustered near two previously identified neutralizing antigenic sites (sites 1 and 2) and these regions are recognized by mice of different MHC haplotypes. The natural poliovirus host range is narrow (only humans and Old World primates); in general, mice are nonpermissive hosts for poliovirus infection. Thus, it was important to assess whether the apparent clustering of B and T epitopes observed in the rodent studies reflected the immune response observed in poliovirus-permissive hosts (Table 3). In contrast to findings in the rodent studies, peptides surrounding neutralization antigenic site 1 failed to stimulate proliferation of the human lymphocytes in all but one of the donors. This may reflect the strain differences between the immunizing antigen (Sabin) and the stimulating peptides (Mahoney). The majority of amino acid differences in the capsid proteins (four of seven) observed between the Sabin 1 and Mahoney strains occur between residues 88 and 106 of VP1, residues that overlap neutralization site 1 (21). However, one individual (donor B) has an early immunization history with the Salk vaccine (i.e., Mahoney strain) and all of the donors have been exposed to the Mahoney strain. In addition, donor A's lymphocytes recognize a site 1

peptide (p91-109) containing the Mahoney sequence, confirming this individual's exposure history. Therefore, the lack of T-helper epitopes in site 1 in the other individuals is not likely to be due solely to virus strain differences. The absence of site 1 epitopes may also be due to differences in the immunization and/or exposure routes of the virus between humans and mice. Natural infection or vaccination with the attenuated Sabin strains occurs in humans via an oral route to establish a primary site of infection within the gastrointestinal tract. Studies have shown that trypsin can specifically modify the Sabin poliovirus strains within the gastrointestinal tract by cleavage of VP1 within neutralization site 1 sequences, thus destroying this site while maintaining viral infectivity (9, 10, 25, 26). In the mouse studies, poliovirus particles were injected in complete Freund's adjuvant at the base of the tail. Thus, neutralizing site 1 sequences present on these virions should remain intact. Cleavage of site 1 sequences within primates may alter subsequent antigen processing of the particle and, consequently, may alter the spectrum of antigen fragments available for binding by the class II HLA molecules. In addition, it is possible that epitopes near neutralizing antigenic site 1 and other regions were not observed in the human donors because the peptides used in this study bisect (or subdivide) existing T epitopes. However, consistent with the rodent data, the peptides (p244-264 and p182-201) which stimulated proliferation of lymphocyte populations from a majority of human donors tested are located adjacent to the VP1 residues which contribute to form neutralization antigenic site 2.

It is interesting to note that in both mice and humans, the density of T-helper epitopes localized within neutralizing site 2 sequences is higher than that observed in the site 1 regions. This may explain the serological observation that for the serotype 1 strains, antibodies to neutralizing antigenic site 1 are underrepresented and antibodies to neutralizing antigenic site 2 are more prevalent in both BALB/c mice and humans, irrespective of the immunization conditions (6, 10, 22). This suggests that there may be a functional relationship between neighboring T- and B-cell epitopes; this functional interaction may affect also the populations of T and B lymphocytes that are expanded upon antigen stimulation in vitro (as in these studies) or in vivo (upon infection).

A reproducibly strong proliferative response is elicited in donor B lymphocytes upon in vitro stimulation with VP2. This response may reflect the presence of a high proportion of T epitopes in VP2 which are recognized by this individual. The high proliferative response to VP2 may be related to the antibody specificities observed in the serum from donor B. Although serum from human donors predominantly detects VP1 in Western immunoblots, in contrast to the other donors, donor B's serum clearly contained a strong response also to capsid protein VP2 (data not shown). If one assumes that a robust antibody response requires the physical linkage of B and T epitopes, the level of T-cell proliferation induced by a capsid protein may reflect the density of T epitopes present within the protein and may affect in turn the strength of the antibody response to that capsid protein.

The identification of broadly recognized T-cell epitopes is important for the successful design of subunit vaccines. Efficient, effective neutralizing antibody responses elicited to peptide immunogens require the physical linking of B and T-helper epitopes whether they occur naturally (31) or are created by linking distal B and T epitopes (23). However, because outbred populations (like humans) contain several different MHC alleles, each able to bind a specific spectrum of peptides, a peptide which can be efficiently presented to

T-helper lymphocytes in one individual may fail to be presented in another individual expressing a different set of MHC alleles. Therefore, a single subunit vaccine design may be effective in a limited number of members within a genetically heterogeneous population. This problem may be avoided if one can identify T-epitope-rich regions of an immunogen with the ability to be presented by several different HLA molecules as has been found for a malaria circumsporozoite peptide (14, 30) and tetanus toxin peptide (24). The cross-reactive poliovirus peptide p244-264 may also be highly permissive for MHC class II binding. If further testing bears this out, p182-201 and/or p244-264 may be ideal for providing T-helper epitopes in a poliovirus subunit vaccine.

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