

Poliovirus-specific CD4⁺ Th1 Clones with Both Cytotoxic and Helper Activity Mediate Protective Humoral Immunity against a Lethal Poliovirus Infection in Transgenic Mice Expressing the Human Poliovirus Receptor

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Summary

The current understanding of the function of CD4⁺ T helper (Th) cells in immunity to infectious diseases is that Th1 cells, which secrete interleukin (IL)-2 and interferon- γ , induce cellular immune responses, whereas Th2 cells, which secrete IL-4, IL-5, IL-6, and IL-10, provide helper function for humoral immunity. We have used a panel of poliovirus-specific murine CD4⁺ T cell clones and mice transgenic for the human poliovirus receptor to evaluate the role of Th cell subpopulations in protective immunity to poliovirus. The majority of T cell clones, as well as polyclonal T cells generated from mice infected or immunized with poliovirus, secreted IL-2 and interferon- γ , but not IL-4, IL-5, or IL-10, a profile typical of Th1 cells. The Th1 clones displayed major histocompatibility complex class II-restricted cytotoxic T lymphocyte activity against specific poliovirus peptide-pulsed target cells, but also provided help for antipoliovirus neutralizing antibody production. To examine the mechanism of immunity in vivo, we have used poliovirus receptor-transgenic mice on a BALB/c (H-2^d) background. These animals developed a poliomyelitis-like disease when challenged intravenously with a virulent wild-type strain of poliovirus, but not with an attenuated vaccine strain. Furthermore, mice immunized with the vaccine strain were protected against a subsequent challenge with wild-type virus. Using an adoptive transfer technique, we demonstrated that it was possible to confer protection with primed B cells in the presence of polyclonal poliovirus-specific T cells, but not when transgenic mice received either B cells or T cells alone. Furthermore, protection was observed when mice received primed B cells in the presence of a VP4-specific Th1 clone. The findings demonstrate that Th1 cells can mediate a protective immune response against poliovirus infection in vivo through helper activity for humoral immunity and that CD4⁺ T cells, specific for the internal poliovirus capsid protein, VP4, can provide effective help for a protective antibody response directed against surface capsid proteins.

CD4⁺ T cells have been divided into two populations termed Th1 and Th2 on the basis of the profile of cytokines secreted after antigen stimulation in vitro (1, 2). The Th1 subpopulation secretes IL-2, TNF- β , and IFN- γ , whereas the Th2 subpopulation secretes IL-4, IL-5, IL-6, and IL-10. A current immunological paradigm holds that the nature of an immune response is governed by the dichotomy that Th1 cells mediate cellular immunity, delayed type hypersensitivity, and inflammatory responses, and possibly direct lysis of virus-infected cells, whereas Th2 cells are considered

to be mainly responsible for the provision of specific T cell help for antibody production by B cells (1, 2).

Poliomyelitis is an acute paralytic disease caused by infection of the central nervous system by virulent strains of poliovirus, a member of the enterovirus genus of the picornaviridae. The introduction of an inactivated poliovaccine (3) and of a live attenuated oral polio vaccine, developed by the late Albert Sabin (4), has led to a dramatic reduction in the occurrence of the disease. The resolution of the structure of the virus (5) and the elucidation of the complete nucleotide

sequence (6) have allowed study of the relationships governing virulence and attenuation (reviewed by P. D. Minor [7]). Virus-specific neutralizing antibody has been considered the major mechanism of protection against poliovirus infection. Consequently, the antigenic structure of the virus recognized by antibody has been well characterized. At least four neutralizing antibody epitopes have been identified and located on the three-dimensional structure of the virus particle (5, 7). In contrast, the possible role of T cells in the protective immune response to poliovirus has received little attention. However, recent studies of T cell recognition of poliovirus have identified a number of CD4⁺ T cell epitopes, using polyclonal T cell populations (8–10). Furthermore, we have used a panel of poliovirus-specific CD4⁺ T cell clones to characterize T cell epitopes on each of the four capsid proteins of poliovirus and to identify amino acids critical for T cell recognition (11). These and other studies on heterotypic priming (12) provide circumstantial evidence of a role for T cells in immunity to poliovirus. If immunity against poliovirus is mediated through antibody responses, and, in particular, if secretory IgA in the gut plays a role in protection, it might be predicted that Th2 would be involved in the protective mechanism.

The recent development of transgenic mice bearing the human poliovirus receptor (PVR)¹ (13–15) and the establishment of poliovirus-specific Th clones (11) has provided us with a convenient model to study the mechanism of immunity operating in a permissive host *in vivo*. The findings of the present investigation demonstrate that the predominant cytokines secreted by poliovirus-stimulated spleen cells from either transgenic mice infected with attenuated poliovirus, immunized normal mice, or by poliovirus-specific CD4⁺ T cell clones were IL-2 and IFN- γ , a response that is characteristic of the Th1 subpopulation. Although Th1 clones displayed cytotoxic activity, adoptive transfer experiments demonstrated that poliovirus-specific Th1 cells could confer protective immunity *in vivo* through the stimulation of neutralizing antibody production. The findings suggest that Th1 cells can act as true helper cells in immunity to viruses *in vivo*.

Materials and Methods

Mouse Immunizations and Challenge. Mice of the BALB/c (H-2^d) and CBA (H-2^k) strains were maintained as inbred colonies and used at 8–12 wk old. Transgenic mice which expressed the human poliovirus receptor and H-2^d MHC class II genes, termed BALB/c^{PVR}, were derived from ICR mice transgenic for the PVR. Normal BALB/c mice were crossed with ICR PVR Tg1 mice (13, 15) and F1 hybrids expressing the PVR gene backcrossed with BALB/c. The progeny of the second generation were screened, using the polymerase chain reaction and restriction fragment polymorphism analysis, for the presence of the H-2^d MHC class II and PVR genes. Transgenic mice were challenged intravenously with the virulent poliovirus type 3 Leon strain at doses between 1 \times 10⁵ and 1 \times 10⁸ PFU/mouse. A concentration of 1 \times 10⁷ PFU/mouse of the type 3 Leon strain, >10 \times LD₅₀ dose, was rou-

tinely used for challenge. All mice were examined daily for 28 d after challenge and scored for paralysis. Mice which developed paralysis in more than one limb were killed. In contrast, transgenic mice challenged by oral inoculation failed to show signs of paralysis.

In active immunization experiments, normal BALB/c or BALB/c^{PVR} mice received two intraperitoneal injections of 1 \times 10⁸ PFU Sabin type 3 poliovirus formulated in alum, 28 d apart. Alternatively, BALB/c^{PVR} mice were infected intravenously with 1 \times 10⁷ PFU of the attenuated Sabin type 3 vaccine strain.

MHC-restricted Poliovirus-specific T Cell Clones. T cell clones were established from the spleens of individual BALB/c or CBA mice immunized with the Sabin vaccine strains of poliovirus, as previously described (11). The MHC restricting element for each clone was defined in proliferation assays, using APC from congenic mice and syngeneic APC preincubated with monoclonal antibodies specific for distinct class II molecules (16).

Analysis of Cytokine Production. Spleen cells (2 \times 10⁶/ml) from immunized BALB/c or infected BALB/c^{PVR} mice, or CD4⁺ T cell clones (1 \times 10⁵/ml) and irradiated syngeneic spleen cells (2 \times 10⁶/ml) were cultured with 1 \times 10⁸ PFU/ml poliovirus of the appropriate serotype or with medium alone as control. Supernatants were removed after 24 h to determine IL-2 and, after 72 h, to determine IFN- γ , IL-4, IL-5, and IL-10. IL-2 release was assessed by the ability of culture supernatants to support the proliferation of the IL-2-dependent cell line CTLL-2 as described (17), except that test samples were preincubated for 45 min with 25 μ l of the anti-IL-4 monoclonal antibody 11-B-11 (National Cancer Institute, Frederick, MD) at a final concentration of 1.0 μ g/ml to ablate responses to murine IL-4. Control cultures of the CTLL-2 line with IL-4 in the presence of 11-B-11 showed complete abrogation of proliferation. Unitage was determined by reference to the CTLL proliferative responses obtained with a standard preparation of IL-2. Results are expressed as units per milliliter. The concentrations of murine IL-4, IL-5, IL-10, and IFN- γ were determined by ELISA using commercially available antibodies (PharMingen, San Diego, CA). Concentrations were determined by comparing the OD₄₉₂ for test samples with a standard curve for recombinant cytokines of known potency and concentration.

In Vitro CTL Assay. T cell clones were tested for *in vitro* CTL activity using a standard technique (18) modified as follows: T cell clones were harvested 10–12 d after antigen stimulation, washed, and counted. The target cells, A20.2J (H-2^d) and CH1 (H-2^k), were labeled with sodium [⁵¹Cr]-chromate (Amersham International, Little Chalfont, UK) and incubated with peptides (0.4–10 μ g/ml), corresponding to the sequence of Sabin type 3 poliovirus VP1:257–268 or VP4:16–30. Labeled target cells (1 \times 10⁴) were incubated with varying numbers of T cells to give effector/target ratios of 20:1–1:1. The percentage of specific lysis was determined after 6–8 h, as described (18). Spontaneous release usually ranged between 10 and 20% of maximum release, and assays, in which, the spontaneous release exceeded 20% of the maximum release were discarded. Results presented are the arithmetic means of three cultures.

Determination of Poliovirus-specific Antibody Titers. The titers of poliovirus-specific neutralizing antibody were determined by microneutralization assay, as described (19). Each test was performed in quadruplicate and the results taken as the reciprocal of the final dilution that totally inhibited a viral cytopathic effect. Results are expressed as geometric mean titers of at least two titrations. The IgG subclass of poliovirus-specific antibody was determined, using an immunoassay. Purified poliovirus-specific rabbit IgG diluted 1/1,000 in PBS was added (50 μ l/well) to Maxisorp-Immunoplates (Gibco, Ltd., Paisley, UK). Plates were then incubated overnight

¹ Abbreviation used in this paper: PVR, poliovirus receptor.

at 4°C in a sealed humidified container. Excess capture antibody was discarded, and the plates washed four times in 0.1% (vol/vol) Tween 20 in PBS before the addition of 200 µl/well 5% (wt/vol) milk powder in PBS and incubation at 35°C in a humidified incubator for 1 h. Plates were then washed six times before incubation for 3 h at 35°C with 50 µl/well poliovirus at ~0.5 µg/ml and of matched serotype to the specificity of the capture antibody. Unbound virus was then discarded, and the plates washed six times before incubation for 2 h with serum samples at various dilutions. Plates were again washed six times before a 30-min incubation with 50 µl/ml of 1/1,000 diluted horseradish peroxidase-conjugated detecting antibodies specific for murine IgG subclasses. Plates were washed a further six times and incubated for 45 min with 50 µl/well of freshly prepared 1 mg/ml *o*-phenylenediamine (Sigma Chemical Co., Poole, UK) in citrate-phosphate buffer supplemented with 1 µl/ml H₂O₂. The reaction was stopped by the addition of 50 µl/well 1 M H₂SO₄, and the OD₄₉₂ measured using a Multiskan + plate reader (ICN Flow, High Wycombe, Bucks, UK). Control wells in which either capture antibody, virus, sample, or detecting antibody had been systematically omitted showed only background readings at OD₄₉₂.

Isolation of Polyclonal T and B Cell Populations. Enriched B cells were prepared from the spleens of poliovirus-immunized BALB/c mice. Spleen cells were washed once (5-min centrifugation at 300 g), and the pellet resuspended at 1 × 10⁷ cells/ml in filter-sterilized GK1.5 tissue culture supernatant, to which sterile anti-Thy 1.2 ascitic fluid had been added (final dilution 1/200). After incubation on ice for 45 min, the cells were washed three times in serum-free medium before resuspension at 1 × 10⁷ cells/ml in agarose-absorbed guinea-pig complement (Cedarlane Laboratories Ltd., Hornby, Canada) diluted to 1/16 in serum-free medium. The cells were incubated for 1 h at 37°C and then washed three times in complete medium.

T cell purification was performed using a T cell isolation kit (IsoCell; Pierce Chemical Co., Rockford, IL) or T cell isolation columns (Immulan; Biotex Laboratory Inc., Houston, Texas), as described by the manufacturers. Briefly, columns were charged with an avid anti-mouse Ig reagent bound to an inert support, the columns were washed, and spleen cell preparations loaded. An enriched T cell population was obtained from the column by elution with PBS, either directly (Immulan), or after a 1-h incubation at room temperature (IsoCell). The purity of B and T cell preparations was monitored by FacScan® analysis of 50-µl samples of the cell preparations before and after treatment, using goat anti-mouse Ig-FITC (B cells) or anti-mouse Thy 1.2 (T cells). All preparations used contained <1% contamination of the depleted population.

Determination of T Cell Helper Function. The ability of poliovirus-specific T cell clones or primed polyclonal T cell populations to provide help for poliovirus-specific antibody production in vivo was determined using an adoptive transfer technique. Recipient BALB/c, CBA, or BALB/c^{PVR} mice were irradiated with a sublethal dose of 6 Gy total body irradiation by exposure to a Gammacell 2,000 ¹³⁷Cs source (Molsgaard Medical, Horsholm, Denmark). 18 h later, the irradiated mice were selectively reconstituted with combinations of 1 × 10⁷ primed or naive B cells and 1 × 10⁷ primed or naive T cells or 1 × 10⁶ T cell clones in either the presence or absence of virus. Control mice were reconstituted with spleen cell preparations from naive mice. 14 d later, mice were killed, and the serum was collected and assayed for poliovirus-specific antibody.

Protection Studies in Transgenic Mice. In active immunization experiments, transgenic mice were injected intravenously with two doses of 1 × 10⁷ PFU/mouse of the attenuated Sabin type 3 strain of poliovirus at weeks 0 and 4. 2 wk later, a small quantity of blood

was removed for serum antibody analysis, and mice were challenged with the virulent Leon type 3 strain. In a passive immunization study, groups of BALB/c^{PVR} mice received 0.2 ml of high titer neutralizing serum by intravenous injection. The serum was isolated from BALB/c mice which had previously received two immunizations of Sabin type 3 poliovirus in alum. Control mice received 0.2 ml serum derived from naive mice. Mice were challenged 6 h later with a lethal dose of virulent poliovirus and followed for signs of paralysis.

In initial adoptive transfer experiments designed to define the role of lymphocyte subpopulations in protection, BALB/c^{PVR} mice were irradiated, reconstituted after 24 h with either immune spleen cells or combinations of immune T and B cells, and challenged 6 h later. These mice all developed paralysis. In subsequent experiments, lethal challenge was delayed by 7 d to allow the development of a protective immune response. Transgenic mice were reconstituted with B and T cells, purified from the spleens of immune mice as described above, together with 1 × 10⁶ PFU/mouse of Leon type 3 virus, which had been heat inactivated at 56°C for 40 min. 7 d later, all animals were challenged with a lethal dose of Leon type 3 poliovirus after the collection of a small sample of serum for analysis of neutralizing antibody titers. Mice were examined daily and scored for paralysis.

Results and Discussion

Lack of expression of the PVR by nonprimate species has precluded the use of a small animal model for studies of immunity to poliomyelitis. However, the recent development of transgenic mice bearing the human poliovirus receptor (13–15) has provided us with a convenient small animal model for studies of immunity against poliovirus in a permissive host. Transgenic mice expressing the PVR and homozygous

Table 1. Active or Passive Immunization Protects BALB/c^{PVR} Transgenic Mice against a Lethal Poliovirus Challenge

Immunization*	Neutralizing Ab titer [†]	Challenge	Onset paralysis (d) [‡]	Survival
–	<1/10	Sabin 3	–	4/4
–	<1/10	Leon 3	3–4	0/6
Active	1/1,448	Leon 3	–	3/4
	1/64		–	
	1/1,448		–	
	1/11		4	
Passive	1/1,280	Leon 3	–	6/6

* Groups of BALB/c^{PVR} transgenic mice were either untreated, immunized with 1 × 10⁸ PFU of Sabin type 3 poliovirus in alum 14 and 42 d before challenge, or received 0.2 ml serum from naive or immune mice 6 h before challenge.

[†] Neutralizing antibody titer was determined either on blood samples taken at the time of challenge, or on the serum samples used for passive immunization.

[‡] Results are expressed as the mean day of onset of paralysis within the mice showing signs of paralysis for each group.

^{||} Mice received either no pretreatment, or were injected with serum from normal naive BALB/c mice.

Table 2. Cytokine Secretion by Poliovirus-specific CD4⁺ T Cell Clones and T Cells from Immunized BALB/c or Infected BALB/c^{PVR} Mice

T cells	MHC restriction	Specificity*	Cytokines†				
			IL-2	IFN-γ	IL-4	IL-5	IL-10
Clones			U/ml	ng/ml	pg/ml	pg/ml	pg/ml
3N2s5.1	I-E ^d	VP1:257–264	<0.05	2	500	150	180
CB2.2–5	I-A ^k	VP3:14–28	1.92	47	<50	<50	<70
2KB-3	I-A ^k	VP3:14–28	2.07	55	<50	<50	<70
2KB-6	I-E ^k	VP3:196–210	3.15	90	230	>10 ³	150
CB2.2–3	I-A ^k	VP3:14–28	2.05	100	<50	<50	<70
CB2.2–10	I-E ^k	VP3:189–203	0.83	51	<50	<50	<70
1N5–1	I-A ^d	VP4:16–25	2.20	53	<50	<50	<70
1N8.44	ND	ND	<0.05	45	240	120	140
CB2.1–3	I-E ^k	VP4:21–35	1.94	80	<50	<50	<70
CB2.1–6	I-E ^k	VP4:21–35	1.66	32	<50	<50	<70
CB2.2–4	I-A ^k	VP4:5–20	<0.05	49	200	210	100
2KA-1	I-A ^k	VP4:11–25	3.02	100	<50	<50	<70
2KA-2	I-E ^k	VP4:21–35	1.26	66	<50	<50	<70
2KA-5	I-E ^k	VP4:21–35	1.17	52	<50	<50	<70
2KB-4	I-A ^k	VP4:11–25	0.93	68	<50	<50	<70
3KC-1	I-A ^k	VP4:11–25	0.51	100	<50	<50	<70
Spleen cells [‡]							
BALB/c	H-2 ^d	Polyclonal	3.2	>100	<50	100	<70
BALB/c ^{PVR}	H-2 ^d	Polyclonal	1.1	>100	<50	<50	<70

* The Poliovirus serotype, capsid protein, and peptide specificity of the T cell clones have previously been described (11).

† Cytokine levels were assayed in supernatants of T cell clones stimulated with poliovirus in the presence of irradiated syngeneic APC. Results are the mean of triplicate assays, performed in duplicate.

‡ Spleen cells were derived from BALB/c mice immunized with Sabin type 3 virus in alum or BALB/c^{PVR} mice infected with Sabin type 3 virus.

for Ia^d genes developed a poliomyelitis-like disease when challenged intravenously with a virulent wild type strain of poliovirus (Leon type 3), but not with the attenuated Sabin type 3 strain (Table 1). Furthermore, PVR transgenic mice immunized with the vaccine strain formulated in alum were protected against a subsequent challenge with wild type virus (Table 1). Thus, the rules governing virus attenuation and the development of a protective immune response appear to be similar in both primates (20) and transgenic mice (13–15).

The transgenic mice used in the present study were derived from outbred ICR PVR Tg1 mice (13, 15) which were backcrossed with BALB/c mice for two generations and individual offspring screened for the expression of BALB/c MHC and poliovirus receptor genes. Consistent with previous findings in the PVR transgenic mouse models (13–15), it was not possible to reproducibly demonstrate infection in the gut after oral inoculation. However, the choice of intravenous challenge was relevant to the induction of viremia in poliomyelitis and resulted in consistent development of paralysis and death of transgenic mice. This was found to be the most con-

sistent and unambiguous end point of infection in a nonimmune host. The limited number of mice available precluded studies on the kinetics of viral replication. Nevertheless, the development of single MHC haplotype PVR transgenic mice and the generation of MHC-compatible poliovirus-specific T cell clones has allowed us to examine for the first time the role of T cells in immunity to poliovirus, which had been impossible in previous animal models of poliomyelitis.

Since antipoliovirus antibodies are considered to be critical in protective immunity against poliovirus infection, it might be predicted that Th2 cells could play an important role in the protective mechanism. However, our study demonstrated that poliovirus-specific murine CD4⁺ T cells induced by either immunization of normal BALB/c mice or infection of transgenic mice are predominantly of the Th1 profile (Table 2). An examination of the cytokines released by a panel of MHC class II-restricted CD4⁺ T cell clones, specific for defined epitopes on poliovirus capsid proteins VP1, VP3, or VP4 (11), revealed that 12 out of 16 clones produced IL-2 and IFN-γ, but not IL-4, IL-5, or IL-10 after specific an-

tigen stimulation (Table 2). Three poliovirus-specific clones, 2KB-6, 1N8.44, and CB2.2-4, did not conform to either the Th1 or Th2 classification. These clones consistently secreted IL-4, IL-5, IL-10, high levels of IFN- γ , and in the case of clone 2KB-6, IL-2, and may therefore belong to the Th0 subpopulation (1). Clone 3N2s5.1 secreted IL-4, IL-5, and IL-10, but not IL-2, and only low levels of IFN- γ , an atypical cytokine profile. A number of parameters, including the choice of adjuvant, dose, and route of immunization and the form of the antigen (particulate versus soluble, replicating versus killed), may influence the induction of distinct Th cell subpopulations (21–23). However, the predominant cytokines detected in the supernatants of poliovirus-stimulated spleen cells derived from either transgenic mice infected with Sabin type 3 virus or normal BALB/c mice immunized with inactivated virus, with or without alum, by systemic or intranasal routes, were IL-2 and IFN- γ (Table 2 and data not shown). The selective induction of Th1 cells in mice is not compatible with the perceived importance of mucosal antibodies in immunity to poliovirus in man. However, it is possible that oral immunization with attenuated poliovirus vaccine, which is known to induce an antipoliovirus mucosal IgA response in humans (24), may favor the induction of Th2 cells. Nevertheless, our findings suggest that inactivated poliovirus vaccines, which are also highly effective at conferring protective immunity against poliovirus infection in humans (25), may preferentially generate Th1 cells.

An examination of the function of the CD4⁺ poliovirus-specific T cell clones in vitro revealed that clone 1N5.1 and all other Th1 clones were capable of lysing MHC-matched, but not mismatched, target cells pulsed with specific poliovirus peptides (Fig. 1 A). Significant lysis of target cells was observed at an effector to target ratio of 1:1 using a peptide concentration of 10 μ g/ml (Fig. 1 C). However, CTL activity could not be demonstrated for clone 3N2s5.1 (Fig. 1 B). This finding is consistent with other studies which have associated Th1 cells with classical cellular immune responses (1, 2). However, our study demonstrated that the poliovirus-specific Th1 cells could also provide help for B cell production of antibody. Polyclonal poliovirus-specific T cells, which secreted predominantly IL-2 and IFN- γ (Table 2), stimulated antipoliovirus antibody production by immune B cells in vivo (Fig. 2) and in vitro (data not shown). Furthermore, adoptive transfer techniques revealed that both Th1 and Th0 clones could provide help in vivo for the production of poliovirus-specific neutralizing antibody production (representative examples shown in Fig. 2). In other murine systems, the cytokines IL-4, IL-5, and IL-6 have been shown to play a role in the provision of help to B cells, in particular for IgG1, IgG3, IgA, and IgE production by controlling Ig class switching and B cell differentiation (1, 26). However, in vitro studies with B cells stimulated with mitogens or model antigens have also shown that IFN- γ can augment an IgG2a response (27). In the present study, the predominant subclass of poliovirus-specific IgG produced through the helper function of Th1 cells and in the serum of poliovirus-immunized normal mice or infected transgenic mice was IgG2a (Fig. 3).

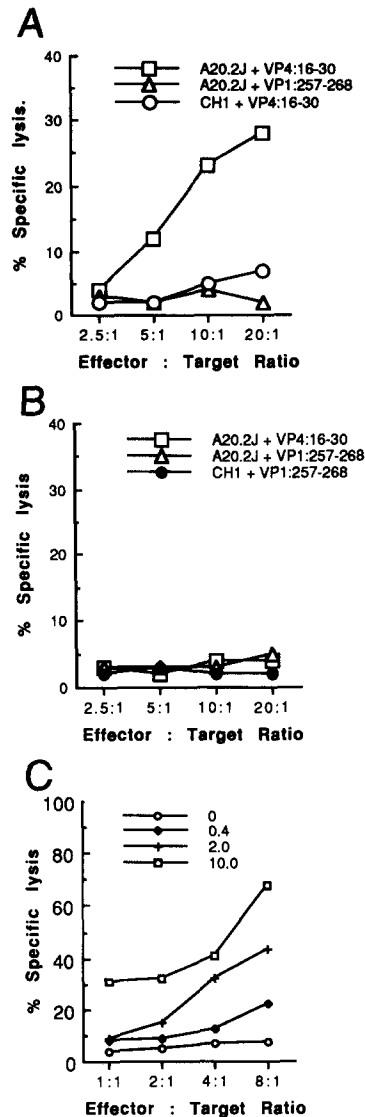


Figure 1. Cytotoxic activity of poliovirus-specific Th1 clones. The CTL activity of MHC class II-restricted T cell clones 1N5.1 (A) and 3N2s5.1 (B) was determined in vitro against MHC-matched A20.2J(H-2^d) and mismatched CH1(H-2^k) target cells incubated with peptides (1 μ g/ml), corresponding to the sequence of Sabin poliovirus type 3 VP4:16–30 or VP1:257–268. (C) CTL activity of clone 1N5.1 against MHC-matched A20.2J target cells incubated with either no peptide, or with 0.4, 2.0, or 10.0 μ g/ml of peptide VP4:16–30.

Furthermore, an increased antipoliovirus antibody response was observed when primed B cells were cultured with IFN- γ . Therefore, our findings demonstrate that Th1 cells, through the secretion of IFN- γ , may mediate T cell help for a protective antipoliovirus IgG2a antibody response. Although the consensus view has been that Th1 cells mediate cell-mediated immunity, while Th2 cells mainly stimulate humoral immunity, our demonstration of helper function by Th1 cells in vivo is supported by a study of an enveloped virus, influenza virus, which also demonstrated that Th1 clones could provide help for B cell production of antibody (28).

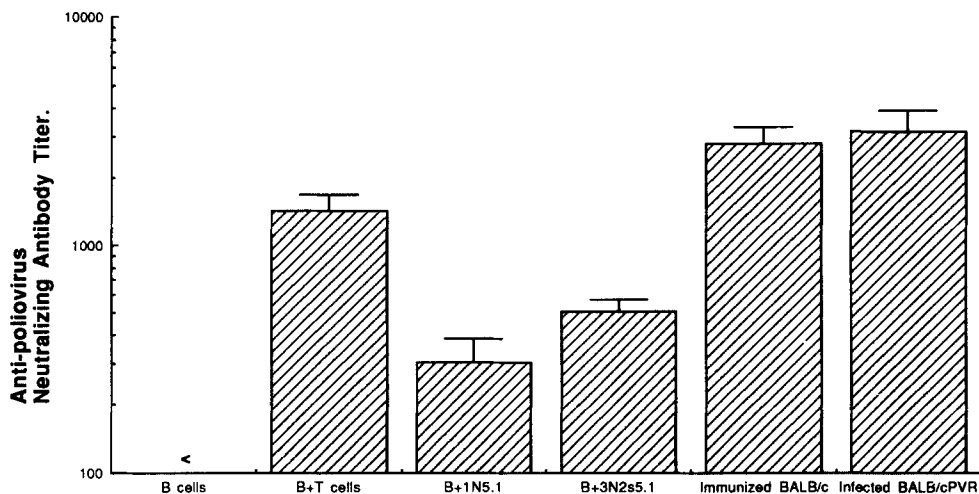


Figure 2. Th1 cells provide helper function in vivo for poliovirus-specific neutralizing antibody production. Irradiated BALB/c mice were reconstituted with primed B cells and inactivated poliovirus, alone, or in combination with either polyclonal immune T cells, T cell clone 1N5.1, or T cell clone 3N2s5.1. The titer of poliovirus-specific neutralizing antibody was determined in the serum 14 d after reconstitution. The neutralizing antibody titers are also shown for serum samples from normal BALB/c mice immunized twice with Sabin type 3 poliovirus in alum and for BALB/c^{PVR} transgenic mice infected with Sabin type 3 poliovirus. <, titer less than 1/100.

The availability of PVR transgenic mice and MHC-compatible, poliovirus-specific, MHC class II-restricted T cell clones allowed us to examine the role of CD4⁺ T cells in protection against a lethal poliovirus challenge in vivo. Our first challenge experiments confirmed the suggestions from studies carried out in primates over 40 yr ago that neutralizing antibodies can protect against poliovirus infection (29). Protection induced by active immunization was found to correlate with the level of neutralizing antibodies in the serum at the time of challenge (Table 1). Furthermore, passive transfer of high titer poliovirus-specific antibody into transgenic mice conferred protection against a lethal challenge with Leon type 3 poliovirus (Table 1). In contrast, adoptive transfer of immune spleen cells into sublethally irradiated transgenic mice, followed by immediate challenge, resulted in mice succumbing to paralysis 3–4 d later (data not shown). However, when challenge was delayed until 7 d after transfer of immune spleen cells (together with an inactivated poliovirus to stimulate the transferred T cells), protection was observed (Table 3). This finding demonstrated that immunity could be transferred using immune spleen cells, but that a period of 7 d was required

to allow the development of the protective immune response. Furthermore, protective immunity was a function of the cells transferred, not a primary response to the inactivated virus, as control mice, which received inactivated virus and either naive spleen cells or no cells, were not protected (Table 3).

The involvement of both memory T and B cells in protective immunity to poliovirus was demonstrated in adoptive transfer experiments using purified or cloned populations of lymphocytes. BALB/c^{PVR} mice that received primed B cells, in combination with either polyclonal immune T cells or a poliovirus-specific I-A^d-restricted T cell clone 1N5.1, developed moderate to high titers of neutralizing antibody and were protected against a lethal infection with the virulent Leon strain when challenged 7 d after cell transfer. In contrast, mice which received T cells or B cells alone were not protected. An examination of poliovirus-specific neutralizing antibody responses in mice that received B cells alone before challenge (Table 3) and on paralysis showed that they mounted a weak or undetectable humoral response. These findings demonstrate the critical role of T cells in providing help for a protective antibody response to poliovirus and also sug-

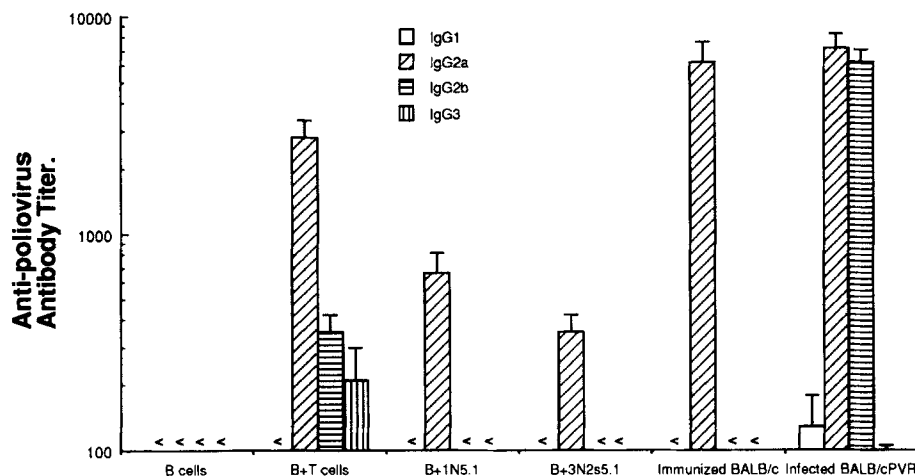


Figure 3. IgG2a is the predominant IgG subclass of antipoliovirus antibody. Irradiated BALB/c mice were reconstituted with primed B cells and inactivated poliovirus, alone, or in combination with either polyclonal immune T cells, T cell clone 1N5.1, or T cell clone 3N2s5.1. The IgG subclass of poliovirus-specific antibody present in serum 14 d after reconstitution was determined by specific ELISA. The isotype of poliovirus-specific antibody present in the serum of normal BALB/c mice immunized twice with Sabin type 3 poliovirus in alum and in serum from BALB/c^{PVR} transgenic mice infected with Sabin type 3 poliovirus are also shown. < denotes a titer less than 1/100.

Table 3. Protection against Poliovirus Challenge in Transgenic Mice after Adoptive Transfer of Th1 Cells in the Presence of Primed B Cells

Cell Transfer*		Neutralizing Ab Titer [†]	Onset Paralysis (d) [§]	Survival
T Cells	B Cells			
—	—	<1/10	5	0/8
Naive	Naive	<1/10	4	0/6
Immune	—	<1/10	7	0/6
Clone 3N2s5.1	—	<1/10	6	0/5
Clone 1N5.1	—	<1/10	5	0/5
—	Immune	<1/10	8	1/6
Immune	Immune	1/350	7	5/6
Clone 3N2s5.1	Immune	1/70	6	4/6
Clone 1N5.1	Immune	1/290	—	6/6

* Irradiated BALB/c^{PV*} transgenic mice were reconstituted with combinations of polyclonal B or T cells purified from spleens of immune mice or poliovirus-specific CD4⁺ T cell clones, together with 1 × 10⁶ PFU heat-inactivated Sabin type 3 poliovirus. Control mice received inactivated virus alone, or inactivated virus and purified B and T cells from naive BALB/c mice.

[†] Neutralizing antibody titers were determined in serum from blood samples taken immediately before challenge.

[§] Results are expressed as the mean day of onset of paralysis within the mice showing signs of paralysis for each group.

gest that neuronal damage observed in poliomyelitis is unlikely to result from T cell-mediated immunopathology. The observation that transfer of T cells in the absence of B cells failed to prevent poliovirus-induced paralysis suggests that direct cellular effector mechanisms alone, such as cyto-

toxicity, are insufficient to confer protective immunity. However, this does not eliminate a role for CTL in viral clearance or for IFN- γ in limiting viral spread. The significance of the demonstrated in vitro cytotoxic function of the poliovirus-specific Th1 clones (Fig. 1) to immunity in vivo is unknown, but may be a mechanism for the downregulation of an active immune response (30).

Our findings indicate that protection can be mediated by a T cell clone specific for an epitope on VP4. Since VP4 is a conserved internal protein which is not known to be a target of the humoral immune response (7), this study demonstrates that Th1 cells can play a critical role in protective immunity against a lethal viral infection through the provision *intermolecular, intrastructural help* (31). Although this phenomenon is not universal (32), it is supported by observations from both the influenza and hepatitis B virus systems (33, 34) and has important implications for the role of cross-reactive T cells in priming the poliovirus-specific immune response (12). For example, it may be possible to exploit this observation to reduce the incidence of vaccine-associated cases of poliomyelitis attributed to the type 3 component of the oral poliovaccine. Immunization with a single poliovirus serotype, or with purified or recombinant VP4, would prime for an anamnestic antibody response to other serotypes after a booster with the trivalent vaccine.

The observation that adoptive transfer of primed B cells with the Th1 clone 1N5.1 conferred 100% protection, whereas clone 3N2s5.1, which secretes low levels of IFN- γ , protected four out of six mice, adds further evidence to the role of IFN- γ in stimulating a protective IgG2a antibody response. Whilst Th1 cells have been largely associated with cellular responses, especially against bacterial pathogens (1, 2, 35), this study clearly demonstrates their role in directing protective humoral immunity against viruses and may explain the IgG2a restriction of murine antibodies elicited by viral infections (36, 37).

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