Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)] plates at 30°C, and each colony was picked onto duplicate NZY-ampicillin plates for incubation at 30°C and 42°C. Plasmid DNA was purified from FS101 clones that did not grow at 42°C, and DNA was introduced into *E. coli* DH5α. Transformants were plated at 30°C and 42°C. We used DH5α, which contains the wild-type RNase P RNA gene, to ensure that failure to complement the FS101 phenotype was not the result of a temperature-sensitive plasmid function. We identified the mutations in RNase P RNA genes present on plasmids that did not complement FS101 but successfully transformed DH5α at 42°C by sequencing the genes. Mutant plasmids that did not complement FS101 were subjected to a second round of hydroxylamine mutagenesis and reintroduced into FS101. We selected revertants by plating the transformants at 42°C. Plasmid DNA from colonies that grew at 42°C were introduced again into FS101 and tested for their ability to complement the temperature-sensitive phenotype. Revertant genes were sequenced.

11. The term "pseudoknot" describes the structure that results from pairing (helix formation) between nucleotides contained in a loop and nucleotides outside of that loop. A pseudoknot, therefore, is a topological (rather than secondary or tertiary) structure composed of two equivalent helices and their interconnections [C. W. A. Pleij, K. Reitveld, L. Bosch, *Nucleic Acids Res.* **13**, 1717 (1985)].
12. Unless otherwise indicated, numbering is based on the *E. coli* sequence. Helix designations are based on the numbering of the nucleotides of each strand of the helix. For example, the helix that is formed by the pairing of nucleotides 260 to 265 with nucleotides 285 to 290 is referred to as helix 260-265/285-290.
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Antibody-Mediated Clearance of Alphavirus Infection from Neurons

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Humoral immunity is important for protection against viral infection and neutralization of extracellular virus, but clearance of virus from infected tissues is thought to be mediated solely by cellular immunity. However, in a SCID mouse model of persistent alphavirus encephalomyelitis, adoptive transfer of hyperimmune serum resulted in clearance of infectious virus and viral RNA from the nervous system, whereas adoptive transfer of sensitized T lymphocytes had no effect on viral replication. Three monoclonal antibodies to two different epitopes on the E2 envelope glycoprotein mediated viral clearance. Treatment of alphavirus-infected primary cultured rat neurons with these monoclonal antibodies to E2 resulted in decreased viral protein synthesis, followed by gradual termination of mature infectious virion production. Thus, antibody can mediate clearance of alphavirus infection from neurons by restricting viral gene expression.

ACCORDING TO THE CLASSIC PARADIGM, the clearance of infectious virus from primary sites of replication results from major histocompatibility complex (MHC) class I–restricted lysis of virally infected cells by $CD8^+$ cytotoxic T lymphocytes. This paradigm can theoretically explain viral clearance from nonneuronal cellular targets in the central nervous system (CNS), but it cannot account for viral clearance from neurons. Neurons are not induced to express class I molecules in vivo in response to cytokine stimulation and viral infection (*1*) and therefore may escape immune recognition by virus-specific $CD8^+$ cells. Furthermore, it seems unlikely that terminally differentiated cells incapable of replication would use a cytolytic mechanism for recovery from viral infection; this hypothesis is supported by histopathologic findings in lymphocytic choriomeningitis virus infection where viral clearance from neurons occurs without evidence of cellular lysis (*2*). Thus, a non-MHC–restricted, noncytotoxic immunologic mechanism for the termination of viral infection of neurons must exist; yet the nature of such a mechanism is poorly understood.

To investigate the primary immunologic mechanism responsible for the clearance of infectious virus from neurons, we used a SCID (severe combined immunodeficient) mouse model of Sindbis virus (SV) infection. The SCID mice lack functional mature B and T lymphocytes because of a defect in T cell receptor and immunoglobulin gene rearrangement (*3*). SV is a single-stranded message-sense RNA virus that causes fatal encephalomyelitis in suckling mice and acute, clinically silent encephalomyelitis in weanling mice (*4*). It is the prototypic member of the alphavirus genus (family Togaviridae), which includes the human pathogens Eastern, Western, and Venezuelan equine encephalitis viruses. In contrast to many other neurotropic viruses that have multiple cellular targets in the CNS, SV replicates predominantly in neurons (*5, 6*). The neuronal specificity of SV ensures that investigation of the mechanism of viral clearance from neurons is not complicated by potentially different mechanisms of viral clearance from other cell populations in the CNS.

We infected 4- to 6-week-old CB17 and congenic *scid*/CB17 mice with wild-type SV (strain AR339) to determine the natural history of SV infection in immunocompetent and immunodeficient mice. After intracerebral inoculation of 10^3 plaque-forming units (pfu) of SV, CB17 mice cleared infectious virus from the brain and spinal cord in 8 days, as measured by plaque-assay titrations of freeze-thawed tissue homogenates (Fig. 1A). Clearance of infectious virus was temporally correlated with the appearance of serum antibody to SV detected by enzyme-linked immunosorbent assay (ELISA). In contrast to the CB17 mice, SCID mice developed persistent SV infections of brain and spinal cord that lasted for the entire 30-day study period; viral titers ranged between 10^4 and 10^6 pfu per gram of tissue (Fig. 1A). The SCID mice had no detectable amounts of serum antibody and no detectable T lymphocytes in the spleen, lymph nodes, or peripheral blood, as measured by flow cytometry analysis. Despite ongoing viral replication and the lack of specific humoral or cellular immune responses, there was no evidence of neurologic disease in the SCID mice.

After establishing that SCID mice develop persistent SV infections, we investigated the effect of transferred immune T lymphocytes and SV hyperimmune serum on the clearance of infectious virus from neural tissue.

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Adoptive transfer experiments were performed on day 7 after infection when both viral titers (Fig. 1A) and the number of SV-infected cells determined by in situ hybridization (Fig. 2) had reached a plateau. Adoptive immunotherapy at this time therefore reflects the role of specific immune effectors on the clearance phase of neuronal infection, rather than on earlier stages of viral pathogenesis (for example, initial entry or spread in the CNS). Treatment of persistently infected SCID mice with 0.2 ml of SV hyperimmune serum resulted in clearance of infectious virus from brain and spinal cord within 48 hours after intraperitoneal administration (Fig. 1B). The effective component of hyperimmune serum was antibody because protein G affinity column–purified immunoglobulin fractions (but not nonimmunoglobulin fractions) mediated viral clearance (Table 1). In contrast, no effect on SV replication in brain or spinal cord was observed after the transfer of 10^6 nylon wool–purified T lymphocytes (7) harvested from lymph nodes of SV-sensitized CB17 mice (Fig. 1B). Transfer of unfractionated lymph node cells resulted in viral clearance 6 days after transfer, which correlated with the appearance of recipient serum antibody to SV (*8*).

These adoptive transfer experiments establish that antibody, in the absence of specific cell-mediated immunity, mediates the clearance of infectious SV from the brain and spinal cord. To determine if the mechanism of antibody-mediated clearance involves antibody-dependent complement or cell-mediated cytotoxicity, we eliminated host cytolytic effectors before the administration of SV hyperimmune serum. We treated SCID mice with cobra venom factor (CVF) to deplete the third component of complement or with a high dose of cyclophosphamide to suppress natural killer cell function (*9*). Neither complement depletion nor cyclophosphamide treatment altered the ability of SV hyperimmune serum to terminate viral replication in the brain or spinal cord (Table 1), suggesting that antibody-mediated viral clearance occurs through a mechanism distinct from classical antibody-dependent cell-mediated cytotoxicity or complement-dependent lysis. This conclusion is also consistent with the histological absence of neuronal destruction and the clinical absence of neurologic disease in mice recovering from SV infection.

The lack of a specific role for cytotoxic T cells or antibody-dependent cytolytic mechanisms in recovery from SV encephalomyelitis suggests that clearance of infectious virus results from the direct suppression of intracellular viral replication. To test this hypothesis, we performed in situ hybridization on all brain sections from killed CB17, SCID, and antibody-treated SCID mice with an S^{35}-labeled RNA probe representing the structural region of the SV genome. The number of cells with detectable SV RNA was calculated per unit area of brain by computerized quantitative image analysis (Fig. 2). SV RNA was no longer detectable in the brains of CB17 mice by day 20 after infection but persisted at constant levels in SCID mice throughout the 30-day study period. A dramatic reduction in the number of cells expressing detectable SV RNA was observed in SCID mice within 6 days after antibody transfer, and by day 20 after antibody transfer, no SV RNA–positive cells were present. The reduction of viral RNA to amounts below the sensitivity of in situ

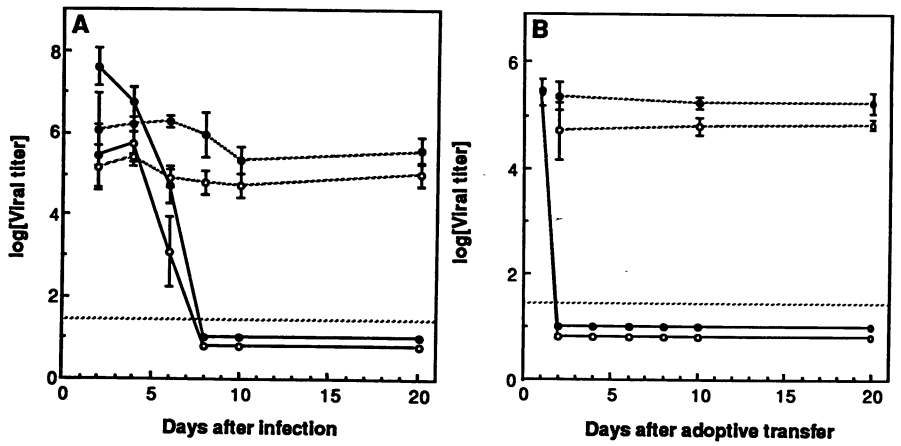

Fig. 1. In vivo clearance of infectious SV from mouse CNS. Specific pathogen-free CB17 mice (Institute for Cancer Research, Fox Chase, Pennsylvania) and *scid*/CB17 (Imdyne, San Diego, California) that were 4 to 6 weeks old were inoculated intracerebrally with 10^3 pfu of wild-type SV (strain AR339). Mice were killed at serial time points after inoculation. The amount of virus present in freeze-thawed tissue homogenates of brain and spinal cord was determined by plaque-assay titration on BHK-21 cells (*20*). Each point represents the geometric mean of viral titers (in plaque-forming units per gram of tissue) from three mice ± SEM. Horizontal dashed line represents the lower limit of detection (1.4). (**A**) Growth curves of SV in brain (●) and spinal cord (○) of CB17 (solid line) and congenic *scid*/CB17 mice (dashed line). (**B**) Growth curves of SV in brain (●) and spinal cord (○) of SCID mice after adoptive transfer of T lymphocytes (dashed line) and SV hyperimmune serum (solid line). Adoptive transfer therapy was performed on day 7 after intracerebral inoculation. Mice treated with T lymphocytes were injected intraperitoneally with 10^6 purified T cells harvested from the spleen and lymph nodes of CB17 mice that were immunized with 10^3 pfu of SV footpad inoculation 10 days earlier. We obtained purified T cell preparations by reacting them twice with rabbit antibody to mouse immunoglobulin (Dako, Carpinteria, California) and then by separating them with nylon wool. Flow cytometry analysis of lymphocytes obtained by this method and stained with a fluorescein isothiocyanate–labeled goat antibody to mouse IgM (Calbiochem) revealed greater than 99% depletion of B cells. Mice treated with SV hyperimmune serum received 0.2 ml of serum intraperitoneally, obtained from BALB/c mice immunized with 10^3 pfu of SV by means of footpad inoculation 21 and 7 days before they were killed [log[ELISA] = 3.9; plaque-reduction neutralization titer = 3.07 (the log of the largest dilution reducing 50 SV plaques on BHK-21 cells by 50%)]. Mean ELISA and neutralization titers for CB17 mice on day 8 after SV infection were 3.9 and 3.22, respectively.

Table 1. Effect of immune therapy on SV clearance from persistently infected SCID mouse CNS. The *scid*/CB17 mice were infected with 10^3 pfu of SV intracerebrally on day 0 and killed on day 9; viral titers (per gram of tissue) were measured by plaque assay. Each value represents geometric mean (±SEM) of titers from five mice. Lower limit of detection for plaque assay is 1.4. Treatment A: On day 7, mice were treated intraperitoneally with 0.2 ml of IgG purified by protein G affinity chromatography from SV hyperimmune serum (SV HIS), prepared as described in Fig. 1B. Treatment B: On day 7, mice were treated intraperitoneally with 0.2 ml of the non-IgG fraction of SV HIS (unbound fraction from protein G chromatography). Treatment C: On days 6, 7, and 8, mice were treated with 20 units of CVF intravenously (Diamedix) and with 0.2 ml of SV HIS intraperitoneally on day 7. Mice were bled before the administration of CVF on day 6, as well as on days 7, 8, and 9. Serum C3 levels were assayed by ELISA with a peroxidase-conjugated sheep antibody to mouse C3 (Binding Site, Birmingham, United Kingdom). C3 levels in sera obtained from mice after CVF treatment were >99.9% reduced, as compared with values obtained before CVF treatment. Treatment D: On day 5, mice were treated with 300 mg of cyclophosphamide per kilogram of body mass intraperitoneally and were treated with 0.2 ml of SV HIS intraperitoneally on day 7.

Treatment	log[Viral titer]	
	Brain	Spinal cord
A: SV HIS IgG fraction	<1.4	<1.4
B: SV HIS non-IgG fraction	4.5 ± 0.4	5.1 ± 0.3
C: CVF + SV HIS	<1.4	<1.4
D: Cyclophosphamide + SV HIS	<1.4	<1.4

hybridization, combined with the evidence against antibody-mediated cytolysis, suggests that SV hyperimmune serum directly inhibits SV replication.

To gain further insight into the mechanism of antibody-mediated viral clearance, we identified the critical viral antigenic determinants. Nine monoclonal antibodies (MAbs) to the major epitopes of SV E1 and E2 envelope glycoproteins were administered to SCID mice on day 7 after SV infection. The biological properties of these MAbs are summarized in Table 2. Despite neutralizing activity, the ability to protect against fatal infection with a virulent neuroadapted strain of SV, or both, the MAbs 106, 101, 105, 202, 23 and 208 (*10–12*) to the E1-c, E1-e, E1-f, E2-ab, E2-b and E2-c epitopes had no effect on SCID mouse brain or spinal cord titers. MAb 50 (*13*) to E2-a and MAbs 209 (*10*) and R6 (*14*) to E2-c resulted in clearance of SV from brain and spinal cord within 48 hours after administration. MAb 209 is a member of the immunoglobulin G3 (IgG3) subclass and does not mediate complement-dependent lysis of SV-infected cells (*10*). The lack of a relation between neutralizing activity, protective capacity, complement-dependent lysis, and the ability of MAbs to SV to mediate clearance of established alphavirus infection in vivo indicates that viral clearance involves a different humoral effector mechanism.

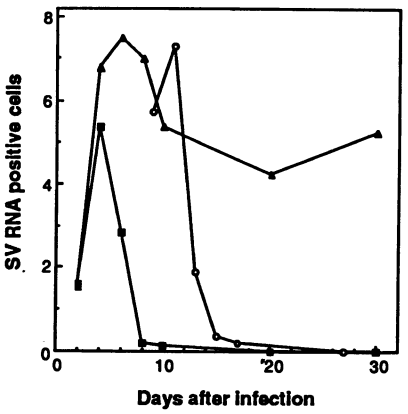

Fig. 2. In vivo clearance of SV RNA from mouse brain. Mice were infected with SV, treated with SV hyperimmune serum, and killed at serial time points as in Fig. 1. In situ hybridization was performed on paraffin-embedded sagittal brain sections as described (*5, 6*) with the following modifications in preparation of the hybridization probe: a pGEM plasmid containing DNA encoding the entire structural region of SV was linearized with Hind III, and minus-strand RNA was transcribed with SP6 polymerase (Riboprobe kit; Promega) in the presence of [S^{35}]cytidine triphosphate (800 Ci/mmol, Amersham). The number of cells expressing Sindbis viral genome RNA per square millimeter of brain was determined with computerized quantitative image analysis. At ×4 magnification, each brain section was divided into nonoverlapping fields. The light threshold of each field image was independently set to define clusters of silver grains over SV RNA positive cells by an observer blinded to the experimental history. The number of clusters of silver grains and the surface area of each field were computed. For clusters of silver grains larger than single cells, we divided the area of each cluster by the maximum area that could be distinguished as a single cell (500 μm) to obtain an estimate of the number of positive single cells. The sum of positive cells of all fields of each section was divided by the sum of the measured areas of all fields to calculate the number of positive cells per square millimeter of brain. Each data point represents the geometric mean of the number of SV RNA positive cells per square millimeter from duplicate brain sections of three mice. Clearance curves of SV plus-strand RNA in brains of CB17 (■), SCID (▲), and SV hyperimmune serum–treated SCID (○) mice are shown.

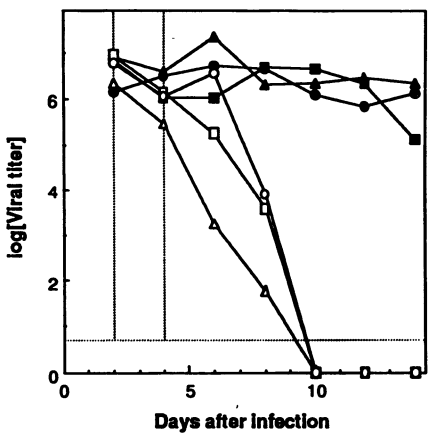

Fig. 3. Effect of MAb therapy on SV viral replication in primary rat neuron cultures. DRG were dissected from 15-day-old embryonic Sprague-Dawley rats (Zivic-Miller) and plated on collagen-coated 35-mm petri dishes at a density of eight ganglia per dish. DRG were cultured in Earle's minimal essential medium containing 10% fetal calf serum, 0.2% glucose, 1.4 mM L-glutamine, 1% penicillin and streptomycin, 2.5*S* nerve growth factor (50 ng/ml), and 10 μM of both FUDR and uridine, which inhibited the growth of all dividing, nonneuronal cells. Six-week-old neuron cultures were infected with a multiplicity of infection of 0.1 SV (3×10^3 pfu per dish). Two days after infection, 10 to 100 μl of SV MAbs were added to the tissue culture medium; the concentration of antibody was adjusted for differences in neutralizing activity so that each culture was treated with the exact amount of antibody that produced a 50% reduction of 50 SV plaques on BHK-21 cells. Two days after MAb treatment, the neuron cultures were washed five times with minimum essential medium and replaced with media that did not contain antibody. We performed ELISA and plaque-reduction neutralization assays on medium from the last wash to screen for remaining antibody. Supernatant samples were collected from each culture dish at 48-hour intervals for a 14-day period, and virus titrations (in plaque-forming units per milliliter of supernatant) were performed on BHK-21 cells. Each data point shown represents the geometric mean of titers from two to three DRG cultures. MAbs that mediated in vivo clearance are represented by the following open symbols: 209 (△), R6 (○), and 50 (□). MAbs that did not mediate in vivo clearance are represented by the following solid symbols: 106 (■) and 202 (●). (▲), Untreated SV-infected control DRG cultures. The area between horizontal broken lines indicates the time period in which antibody was present in culture medium. The horizontal line represents the lower limit of detection (0.7).

We developed an in vitro model to confirm our in vivo observations that antibody directly suppresses viral replication. Embryonic rat dorsal root ganglia (DRG) were explanted and allowed to undergo in vitro differentiation and maturation in the presence of nerve growth factor and an antimitotic agent, fluorodeoxyuridine (FUDR) and uridine, that is cytotoxic to all DRG cells except neurons. Neurons maintained in culture for 6 weeks and then infected with SV developed persistent infection. (Continuous vertebrate cell lines, including neuroblastoma cells, undergo lytic infection within 24 to 48 hours.) In neuron cultures treated with MAbs R6, 50, and 209, which mediated in vivo clearance, production of infectious virus was completely terminated between 2 and 6 days after antibody was removed from the culture medium (Fig. 3). In contrast, cultured neurons treated with equivalent neutralizing concentrations of MAbs 106 and 202, which did not mediate in vivo clearance, continued to produce infectious virus throughout a 14-day study period. By day 14 after infection, neurons treated with MAbs 106 and 202 showed significant cytopathic effects, whereas those treated with MAbs R6, 50, and 209 did not. In all cases, infectious virus was still present at the time of antibody removal from the culture medium, indicating that extracellular neutralization did not significantly contribute to the antiviral effects observed. Thus, these data demonstrate that the same MAbs to E2 that mediate clearance of SV from brain and spinal cord in vivo also shut off viral replication in mature, nondividing neurons in vitro.

To define the critical stage of viral replication inhibited by MAb treatment, we performed immune electron microscopy before the shutdown of mature virion production. Compared to uninfected neurons, the perinuclear regions of untreated SV-infected neurons (Fig. 4A) contained numerous cytopathic vacuoles (*15*) and a marked increase in the amount of rough and smooth endoplasmic reticulum (RER and SER). These organelles were labeled intensely by immunogold, indicating their role in the active synthesis of SV structural proteins. Treatment of SV-infected neurons with MAb 209 (Fig. 4B) resulted in a dramatic reduction in

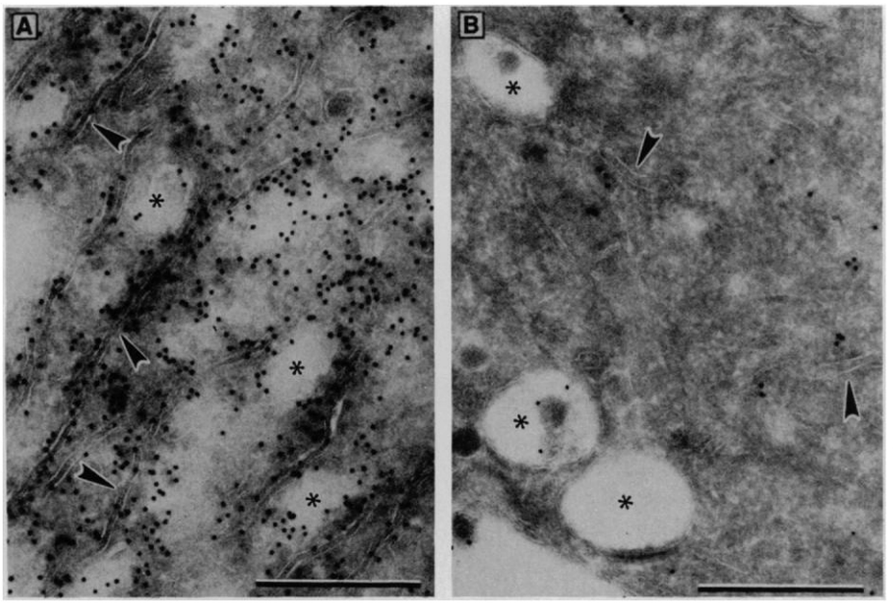

Fig. 4. Electron microscopic analysis of the effects of MAb therapy on SV viral replication in primary rat neuron cultures. Primary rat DRG (neuron-enriched) cultures were prepared as described in Fig. 3. Uninfected neurons, untreated SV-infected neurons, and MAb 209–treated neurons cultures were fixed on day 5 after infection (day 3 after MAb treatment) with 2.5% glutaraldehyde and 4% paraformaldehyde for 24 hours. The ganglia were dissected from the culture dish, cryoprotected with 2.3 M sucrose and 30% polyvinyl pyrrolidone, placed on specimen stubs, and frozen in liquid N_2. Ultrathin cryosections were cut on a Reichardt Ultracut 4E (Cambridge Instruments, Rockville, Maryland) maintained at −110°C. Sections were transferred to electron microscopy grids, stained with a rabbit polyclonal antibody to SV structural proteins (*5*) by immunogold procedures (*21*), and visualized in a Hitachi H-600 electron microscope. (**A**) An untreated SV-infected neuron containing cytopathic vacuoles (asterisks) and RER (arrowheads) that are associated with intense immunogold labeling for SV structural proteins (10-nm gold particles). (**B**) MAb 209–treated neuron containing fewer cytopathic vacuoles (asterisks) and RER (arrowheads) that are associated with a marked reduction in immunogold labeling for SV structural proteins. Scale bars are 0.5 μm.

the number of cytopathic vacuoles, the amount of RER and SER, and the SV structural protein immunoreactivity associated with these organelles. Final stages of replication, such as assembly and virus budding, were intact. These morphologic observations indicate that interruption of SV replication by MAb to E2 occurs at a transcriptional or translational level.

Our in vivo data demonstrates that CNS clearance of SV in persistently infected SCID mice is mediated by humoral immunity. Our in vitro data provide direct evidence that antibody can restrict SV gene expression in mature neurons in the absence of any antiviral cofactors, except perhaps those substances produced by the neurons themselves. These findings identify an as yet unknown role for antibodies in recovery from viral infection and represent an exception to the widespread belief that cytotoxic T cells are required for viral clearance from tissue sites of replication. The ability of antibody to mediate viral clearance from neurons may reflect a unique immunologic strategy that has evolved to control viral infection in cells that lack surface MHC expression. It is not clear whether this strategy is limited to alphavirus encephalitis or represents a more common host response to neurotropic RNA viruses. A potential role for antibody in CNS viral clearance has been postulated in Theiler's murine encephalomyelitis (*16*), human enteroviral encephalomyelitis (*17*), and in neurally spreading reovirus type 3 (*18*) and street rabies virus (*19*) infections. Such studies, combined with our data, provide a rationale for the future investigation of immunotherapy for viral encephalitis. Furthermore, elucidation of the molecular events involved in antibody-mediated inhibition of alphavirus replication may provide insights into new antiviral strategies for the treatment of neurotropic RNA viruses.

Table 2. Effect of MAb treatment on SV viral clearance in SCID mice. All mice were infected with 10^3 pfu of SV intracerebrally on day 0, treated with 0.2 ml of intraperitoneally on day 7, and killed on day 9. Viral titers (per gram of tissue) were determined by plaque-assay titration. NSV protection refers to the ability of MAb to protect against fatal encephalomyelitis when the MAb is administered prophylactically to mice infected with a neuroadapted strain of SV (*10–12*). ND, not done.

Sindbis MAb	Epitope	Ig Isotype	Neutralizing activity	NSV protection	log[Viral titer]	
					Brain	Spinal cord
106	E1-c	G2b	+	+	5.22 ± 0.30	4.69 ± 0.22
101	E1-e	G2a	−	+	5.08 ± 0.39	4.51 ± 0.08
105	E1-f	G3	+	+	4.23 ± 0.11	4.31 ± 0.15
202	E2-ab	G3	+	+	4.92 ± 0.8	4.57 ± 0.37
50	E2-a	G2a	+	+	<1.4 ± 0.0	<1.4 ± 0.0
23	E2-b	G2a	+	−	5.08 ± 0.11	4.17 ± 0.06
209	E2-c	G3	+	+	<1.4 ± 0.0	<1.4 ± 0.0
R6	E2-c	G2a	+	ND	<1.4 ± 0.0	<1.4 ± 0.0
208	E2-c	A	+	−	5.14 ± 0.24	4.75 ± 0.13

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Selective Depletion in HIV Infection of T Cells That Bear Specific T Cell Receptor V_β Sequences

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The mechanism of T cell depletion during infection with the human immunodeficiency virus (HIV) is unclear. Examination of the repertoire of T cell receptor V (variable) regions in persons infected with HIV revealed the absence of a common set of V_β regions, whereas V_α usage was normal. The lack of these V_β segments did not appear to correlate with opportunistic infections. The selective elimination of T cells that express a defined set of V_β sequences may indicate the presence of an HIV-encoded superantigen, similar to those encoded by the long terminal repeat of the mouse mammary tumor virus.

MOST ANTIGENS ARE RECOGNIZED through their interaction with the variable V portions of the T cell receptor (TCR) α and β chains (*1*). However, T cells recognize another category of ligands, the superantigens, on the basis of the expressed V_β region alone, independently from the other variable TCR segments (*2–4*). Because the murine mammary tumor virus C-type retrovirus has superantigen properties (*5, 6*), HIV may also encode a superantigen that could participate in T helper cell impairment and destruction. This hypothesis suggests that cell depletion must preferentially affect those T cells expressing the V_β elements that can interact with the retrovirus-encoded superantigen (*7*).

We analyzed the expression of most of the known TCR V_α and V_β genes by polymerase chain reaction (PCR) in peripheral blood cells obtained from six patients affected by the acquired immunodeficiency syndrome (AIDS) (CDC stage IVC1; symptomatic HIV^+ patients with a history of major opportunistic infections and $CD4^+$ lymphocyte counts $<200/mm^3$) and from six healthy HIV^- individuals. Total RNA was prepared from each sample immediately after collection and, at the time of analysis, was reverse-transcribed into cDNA. Aliquots of cDNA were amplified with each of the 22 5′ V_β-specific sense primers and a 3′ C_β-specific antisense primer (*8*) or with each of the 19 5′ V_α-specific sense primers and a 3′ C_α-specific antisense primer (*9*). The expression of each V gene transcript was operationally defined by an optical density value from an enzymatic immunoassay (DEIA), done with a C_β- or a C_α-specific capture probe that mapped to a region internal to the amplified cDNA. The sensitivity and the specificity of the assay are similar to conventional Southern blot (*10*).

There were no major differences between the V_α repertoires of normal individuals and those of AIDS patients (Fig. 1A). Most of the V_α genes were expressed in all samples with no evidence of selective expression. In contrast, comparison of the V_β repertoire of AIDS patients and normal controls revealed differences in the V_β genes expressed by the two groups (Fig. 1B). The V_β14, V_β15,

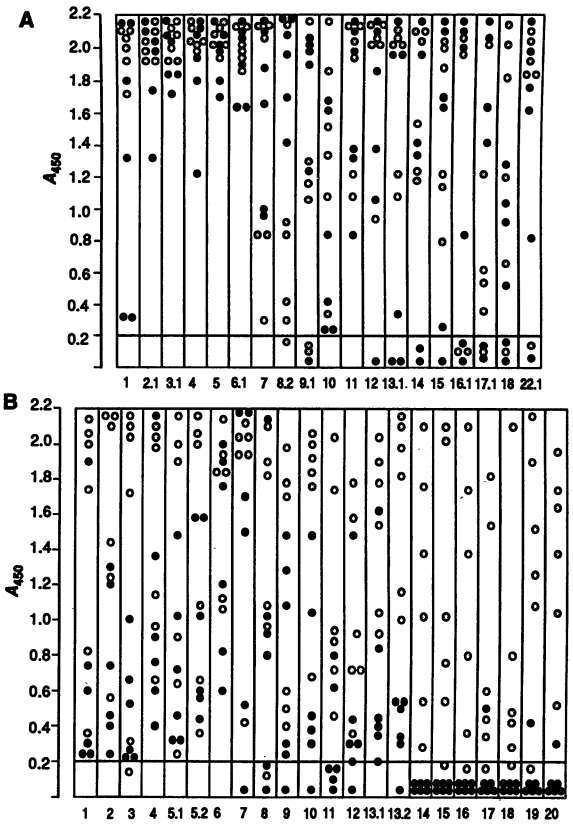

Fig. 1. Expression of TCR (**A**) V_α and (**B**) V_β genes in T cells from AIDS patients (●) and from normal controls (○). The results are expressed as absorbance at 450 nm (A_{450}) from a DEIA test. The cutoff value of 0.20 represents the mean value of ten negative controls ±3 SD. Total RNA was prepared from peripheral lymphocytes by the guanidinium thiocyanate-phenol-chloroform method (*14*). The total RNA (2 μg) preparations were used to synthesize the first strand of cDNA with the Riboclone cDNA Synthesis System (Promega Biotec). Amplification by PCR of the TCR V_α and V_β rearranged genes was as described (*15*), with 19 V_α family-specific (*9*) and 22 V_β family-specific primers (*8*). The specificity of each PCR product was verified by DEIA (*10*) with $C_\beta NH_2$- (ACCCAAAAGGCCACACTGGTGTGCCTGGCC) and $C_\alpha NH_2$- (CAGTGACAAGTCTGTCTGCCTATTCACCGA) specific capture probes, mapping to regions internal to the amplified cDNAs.

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