JOURNAL OF VIROLOGY, Nov. 1995, p. 7315–7318 Vol. 69, No. 11
0022-538X/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Identification of a Major T-Cell Epitope within VP3 Amino Acid Residues 24 to 37 of Theiler's Virus in Demyelination-Susceptible SJL/J Mice

ROBERT L. YAUCH, KAYE KEREKES, KESHMA SAUJANI, AND BYUNG S. KIM*

Departments of Pathology and Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois 60611

Received 10 April 1995/Accepted 10 August 1995

Intracerebral inoculation of susceptible strains of mice with Theiler's murine encephalomyelitis virus (TMEV) results in a chronic, immunologically mediated demyelinating disease that shares many features with human multiple sclerosis. $CD4^+$ T lymphocytes play a critical role in the pathogenesis of virus-induced demyelinating disease. We have identified a region within amino acid residues 24 to 37 of the VP3 capsid protein of TMEV ($VP3_{24-37}$) that is recognized by T lymphocytes from the demyelination-susceptible SJL/J strain of mice. The T-cell response to $VP3_{24-37}$ represents a predominant Th-cell response against the virus from either TMEV-immunized or TMEV-infected SJL/J mice, and viral epitopes $VP1_{233-250}$, $VP2_{74-86}$, and $VP3_{24-37}$ account for most of the Th-cell response to TMEV.

Epidemiological studies have suggested an infectious etiology for human multiple sclerosis (1, 8). In addition, experimental animal models have supported a potential role for viruses as the causative agent for demyelination (7). In particular, intracerebral injection of Theiler's murine encephalomyelitis virus (TMEV) into susceptible strains of mice results in a chronic demyelinating disease that shares many features with human multiple sclerosis (7, 29). Immunologic, histopathologic, and genetic evidence has strongly supported an immunologically mediated mechanism in the pathogenesis of TMEV-induced demyelinating disease (TMEV-IDD) (4, 5, 25, 30). These findings closely parallel those of human multiple sclerosis and thus make TMEV-IDD a strong infectious animal model for studying human multiple sclerosis.

Susceptibility and/or resistance to TMEV-IDD has been associated with several genes, including the *D* region of the *H-2* complex and a gene mapping near the β chain of the T-cell receptor (5, 17, 25, 28). Class I-restricted cytotoxic T-lymphocyte responses have been detected in TMEV-IDD (22, 26), although recent evidence suggests that such class I-restricted T-cell responses are necessary for protection, rather than the pathogenesis of demyelinating disease (2, 9, 27). Immunization of TMEV-infected mice with UV-irradiated TMEV accelerates the disease, suggesting that immunity to TMEV structural proteins plays an important role in the pathogenesis of demyelination (6). Several lines of evidence support the involvement of virus-specific T lymphocytes in the immunologically mediated tissue damage induced by TMEV. Demyelinating lesions are characterized by an inflammatory cell infiltrate, which includes $CD4^+$ T cells (21), and the course of disease correlates with the development of a virus-specific, class II-restricted T-cell response (4, 5, 11, 13, 33). Direct evidence comes from the finding that treatment of mice with antibodies to the $CD4^+$ subset of T cells, but not the $CD8^+$ subset, can significantly suppress demyelination induced by TMEV (13, 33). Therefore, identification of the fine epitopes recognized by TMEV-specific, $CD4^+$ T lymphocytes is necessary to determine the mechanisms involved in T-cell-mediated demyelination.

As with most picornavirus infections, the cellular immune response is directed primarily at the proteins which form the icosahedral structure of the virus (14, 24, 31, 32). TMEV is composed of four capsid proteins, VP1, VP2, VP3, and VP4, of which VP1, VP2, and VP3 are external and VP4 is internal. T-lymphocyte responses against the VP1 and VP2 capsid proteins of Theiler's virus have been characterized the best. Dominant T-cell epitopes have been identified within amino acid residues 233 to 244 of VP1 (35) and residues 74 to 86 of the VP2 capsid protein (12, 14, 35) in the demyelination-susceptible SJL/J strain of mice infected with TMEV. However, there is very little information regarding T-cell responses against the VP3 capsid protein. Previous reports from other investigators have shown only minimal, if any, T-cell response to the VP3 capsid protein of TMEV (14, 31). In contrast, other studies analyzing T cells from humans and mice vaccinated with poliovirus, a closely related picornavirus, have identified determinants in the VP3 capsid protein (24, 32). Furthermore, humoral immune responses against VP3 in TMEV-IDD have been detected (16, 19). Therefore, we investigated the cellular immune response to VP3 in virus-infected SJL/J mice.

Presence of a major T-cell epitope(s) on VP3. To investigate T-cell reactivity to the VP3 capsid protein of TMEV, we produced a fusion protein containing the entire VP3 capsid protein following glutathione *S*-transferase (GST). The VP3 coding sequence was amplified from the full-length BeAn cDNA clone (3) by PCR using primers containing a *Bam*HI site plus the 5′-end sequence (AGTCCTATTCCAGTCACAGTG) and an *Xho*I site plus the complementary 3′-end sequence (TTG TGGAACCCACTTGGTGGG) of VP3. The amplified VP3 product was inserted at the *Bam*HI-*Xho*I site of plasmid pGEX-5X-1 (Pharmacia Biotech, Uppsala, Sweden) and expressed as described previously for the production of GST-

* Corresponding author. Mailing address: Department of Microbiology-Immunology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611. Phone: (312) 503-8693. Fax: (312) 503-1339. Electronic mail address: bskim@casbah.acns.nwu.edu.

VP1 (35). We subsequently utilized this GST-VP3 fusion protein to determine whether T cells from virus-immunized or virus-infected SJL/J mice react with the VP3 capsid protein. The BeAn 8386 strain (3×10^6 PFU) was used to infect SJL/J mice intracerebrally. To induce a virus-specific T-cell response, mice were immunized with 30 to 50 μg of purified, UV-inactivated TMEV (23) in complete Freund's adjuvant (Difco, Detroit, Mich.). Lymph node cells from TMEV-immunized mice were able to mount a strong proliferative response to the fusion protein containing the VP3 capsid protein to a level similar to its response to the VP1 fusion protein. Furthermore, splenocytes from TMEV-infected mice were also able to proliferate in response to the VP3 fusion protein (data not shown). Although the response of splenocytes from TMEV-infected mice to GST-VP3 was weaker than that against GST-VP1, proliferative responses against GST-VP3 were consistently detectable. These results indicate that T cells from both virus-immunized and infected mice recognize an epitope(s) on the VP3 capsid protein of TMEV.

Localization of the VP3 T-cell epitope within amino acid residues 24 to 37. To define the VP3 T-cell epitope(s) on TMEV, we utilized T-cell hybridomas derived from TMEV-infected SJL/J mice. Figure 1A demonstrates the reactivity of one representative hybridoma clone, TV-C13.3, to either the native VP3 capsid protein, purified by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (experiment 1), or the recombinant fusion protein containing the entire VP3 capsid protein (experiment 2). As shown in Fig. 1A, this representative hybridoma clone is reactive to TMEV, as well as the VP3 capsid protein but not to GST, since it produced interleukin 2 (IL-2) when stimulated only with either the native or recombinant VP3 capsid protein. Furthermore, this representative hybridoma clone did not respond to other fusion proteins containing either the VP1, VP2, or VP4 capsid protein (data not shown). These results suggest that the T-cell reactivity measured with the fusion protein represents recognition of a VP3 epitope on native VP3.

To further define the epitope region reactive with T cells, we initially examined the reactivity of a VP3-specific hybridoma (TV-C13.3) against a synthetic peptide consisting of amino acids 24 to 37 of VP3 ($VP3_{24-37}$) that contains a previously identified antibody epitope (16). As shown in Fig. 1B, this hybridoma clone produced IL-2 upon stimulation with $VP3_{24-37}$ but reacted with neither another VP3 peptide ($VP3_{215-232}$) nor a nonspecific control peptide corresponding to residues 34 to 45 of chicken egg lysozyme. Therefore, this representative T-cell hybridoma is specific for $VP3_{24-37}$, a region identical to that recognized by antibodies from mouse strains C57BL/6, BALB/c, and SJL/J (16).

Presence of single major T-cell epitopes on VP1, VP2, and VP3. As a means of comparing the levels of T-cell responses to the three major T-cell epitopes of TMEV, we determined the T-cell proliferative responses of either virus-immunized or infected mice to peptides containing these epitope regions. As shown in Fig. 2A, lymph node cells from TMEV-immunized SJL/J mice produced strong proliferative responses against peptides containing the major T-cell epitopes, $VP1_{233-250}$, $VP2_{74-86}$, and $VP3_{24-37}$. At the higher molar peptide concentrations, the proliferative response to $VP3_{24-37}$ was comparable to the proliferative response to $VP2_{74-86}$. The proliferative responses against the peptides could be blocked by antibodies specific for $I\text{-}A^S$ or CD4 (14, 35; data not shown), demonstrating that these T-cell responses are $CD4^+$ and major histocompatibility complex class II restricted. Splenocytes from virus-infected mice also mounted strong proliferative responses against the three major epitopes of TMEV (Fig. 2B), further

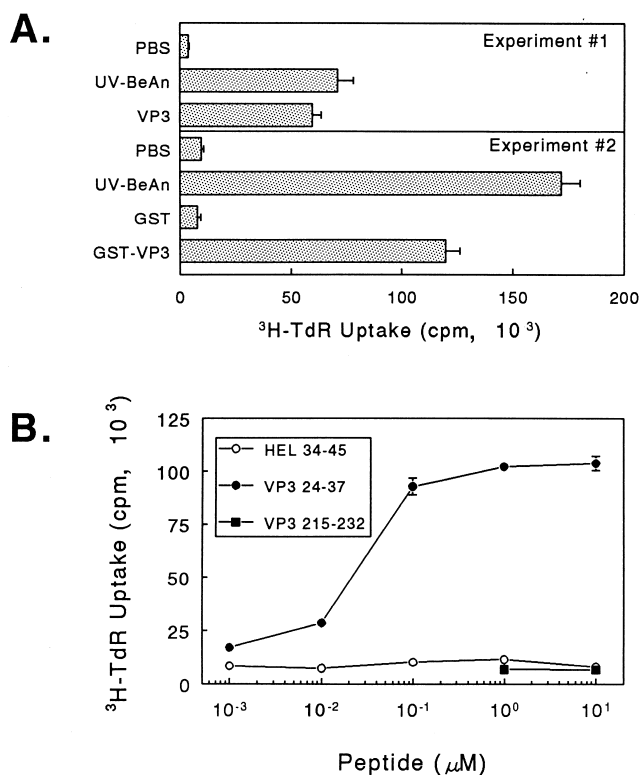

FIG. 1. Analysis of the fine specificity of a representative T-cell hybridoma clone, TV-C13.3, with native capsid protein VP3, fusion protein GST-VP3, and synthetic VP3 peptides. Hybridoma cells (10^5) were tested for antigen specificity on the basis of IL-2 production after antigen stimulation in the presence of 5×10^5 irradiated, syngeneic splenocytes as antigen-presenting cells. IL-2 production by antigen-specific hybridoma cells was determined on the basis of the ability of culture supernatants to support the growth of an IL-2-dependent cell line, CTLL-2. (A) IL-2 production after stimulation of hybridoma cells with native capsid protein VP3 (experiment 1) or fusion protein GST-VP3 (experiment 2). UV-irradiated BeAn was used as a positive control, and either phosphate-buffered saline (PBS) or GST was used as a negative control. The results represent IL-2 production after stimulation of hybridoma cells with 5 μg of the appropriate antigen. (B) IL-2 production upon stimulation of hybridoma cells with various concentrations of $VP3_{24-37}$ (PIYGKTISTPSDYM). A synthetic peptide representing residues 34 to 45 (FESNFNTQATNR) of chicken egg lysozyme or a peptide containing residues 215 to 232 of VP3 (GDDFTLRMP ISPTKWVPQ) was used as a negative control. The results shown are expressed as [^{3}H]thymidine incorporation (mean counts per minute ± the standard error of the mean) by an IL-2-dependent cell line, CTLL-2, in the presence of antigen-stimulated hybridoma culture supernatants (18) and are representative of several experiments. TdR, thymidine.

demonstrating that these epitopes are recognized by T cells generated upon virus infection. In addition, the response against $VP3_{24-37}$ in these virus-infected mice is comparable to the response against the other previously defined T-cell epitopes of TMEV. Taken together, these data demonstrate that $VP3_{24-37}$ is also a dominant epitope recognized by T cells induced following either immunization or infection with TMEV.

Because it is conceivable that measurement of T-cell proliferative levels does not reflect the T-cell repertoire specific for the epitopes, the precursor frequency of epitope-specific T cells from TMEV-immunized mice was determined by limiting-dilution analysis as described previously (35), with a minor modification: antigen-containing cultures were considered positive if the incorporated [^{3}H]thymidine level was greater than the mean counts per minute plus 3 standard deviations of naive lymph node cell cultures with the respective antigen. The

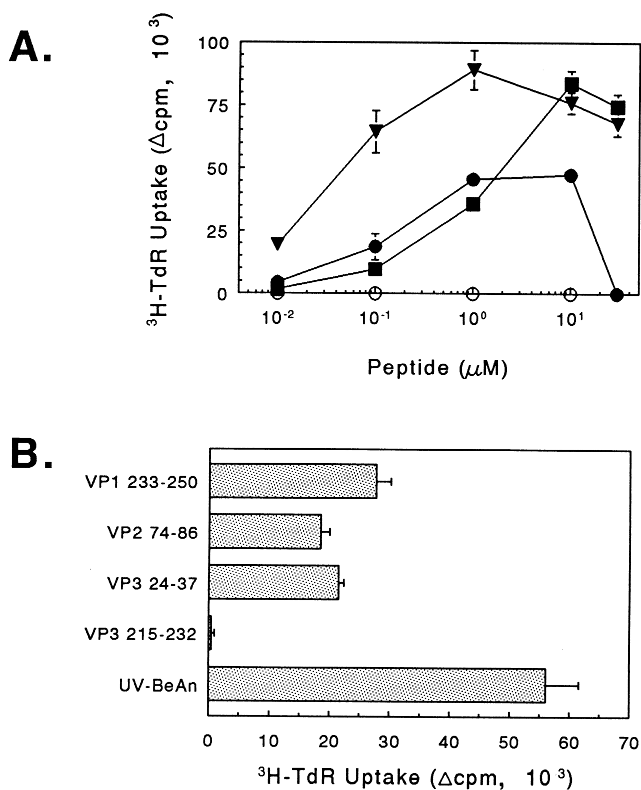

FIG. 2. Comparison of proliferative responses of SJL/J mice immunized (A) or infected (B) with TMEV to peptides containing various TMEV T-cell epitopes. (A) Ten days after immunization with 30 μg of UV-irradiated BeAn, draining lymph node cells were cultured in triplicate in 96-well plates (Costar, Cambridge, Mass.) with various concentrations of VP1$_{233-250}$ (●), VP2$_{74-86}$ (▼), VP3$_{24-37}$ (■), or VP3$_{215-232}$ (○). The amino acid sequences of VP1$_{233-250}$ and VP2$_{74-86}$ are SASVRIRYKKMKVFCPRP and QEAFSHIRIPLPH, respectively. [^{3}H]thymidine incorporation by the cultures was determined after 96 h as described previously (35). The positive control proliferative response to 5 μg of UV-irradiated BeAn was 160,866 ± 10,545 cpm. (B) At 34 days after viral infection, the pooled splenocytes (5 × 10^{5}) from three mice were cultured in triplicate with 10 μM peptide antigen or 2.5 μg of UV-irradiated BeAn for 4 days. Chicken egg lysozyme amino acids 34 to 45 were used as a negative control. The results shown are the mean counts per minute of antigen-stimulated cultures minus the mean counts per minute of cultures stimulated with chicken egg lysozyme amino acids 34 to 45 ± the standard error of the mean and are representative of several experiments. TdR, thymidine.

precursor frequency analysis based on χ^2 minimization indicates that immunization of SJL/J mice with TMEV generates VP3$_{24-37}$-specific T cells at a frequency (1/57,468) similar to that of T cells specific for VP1$_{233-250}$ (1/52,520) or VP2$_{74-86}$ (1/34,872). The combined frequencies of T cells specific for these predominant epitopes were equivalent to the frequency (1/16,975) of T cells reactive to the whole virus. Therefore, these results strongly suggest that VP3$_{24-37}$-specific T cells are a dominant population of TMEV-specific T cells in virus-immunized mice.

A relative frequency of T cells reactive to the major T-cell epitopes was verified by analyzing the epitope specificity of a panel of 17 TMEV-specific T-cell hybridoma clones derived from the spleens of TMEV-infected SJL/J mice. T-cell hybridomas were derived from the spleens of TMEV-infected SJL/J mice at 35 days post viral infection following in vitro stimulation of spleen cells (6 × 10^{6}/ml) with UV-inactivated TMEV (25 μg/ml) for 4 days as described previously (34, 35). Hybridomas reactive to TMEV were selected and subsequently tested for reactivity with peptides containing previously defined T-cell epitopes VP1$_{233-250}$ (35), VP2$_{74-86}$ (12), and VP3$_{24-37}$. Most (88%) of the T-cell hybridomas generated from the spleens of virus-infected mice were specific for one of the three dominant T-cell epitopes: six were specific for VP1$_{233-250}$ (33.3%), three were specific for VP2$_{74-86}$ (16.7%), and six were specific for VP3$_{24-37}$ (33.3%). Of the 17 hybridomas, 2 that were reactive to TMEV were not specific for any of the three major T-cell epitopes of TMEV, suggesting that other minor T-cell epitopes reside on TMEV. Nevertheless, our data collectively suggest that the T-cell response induced after TMEV infection is directed primarily at the three dominant T-cell epitopes (Fig. 3) on the external capsid proteins, VP1$_{233-250}$, VP2$_{74-86}$, and VP3$_{24-37}$.

The identification of a T-cell epitope within residues 24 to 37 of VP3 is very interesting because this region has previously been defined as a site recognized by antibodies induced by immunization or infection of resistant BALB/C or C57BL/6 mice with TMEV (16, 19). Susceptible SJL/J mice are also able to produce antibodies against this region upon immunization with UV-inactivated TMEV. It is less clear whether infected SJL/J mice produce antibodies against VP3$_{24-37}$, since the antibody titer against this region is generally low in virus-infected mice (16, 19). Helper T-cell epitopes located within or near antibody sites have been identified in several viruses, including poliovirus (15, 20) and foot-and-mouth disease viruses (10). Our findings are consistent with these observations: two of the three major T-cell epitopes of TMEV (those on VP1 and VP3) are overlapping or adjacent to sites recognized by antibodies (Fig. 3). However, it is unclear whether there is any functional relationship between the closely positioned B- and T-cell epitopes within capsid proteins VP1 and VP3 of TMEV. It is conceivable that adjacent or overlapping T-cell determinants are able to more efficiently elicit antibody, as well as T-cell, responses.

The role of VP3$_{24-37}$-specific T cells in TMEV-induced demyelination is not known. It has been hypothesized that local production of cytokines by T cells within the central nervous system results in recruitment and activation of macrophages, which nonspecifically destroy myelin via a "bystander" mechanism (5). Immunization of suboptimally virus-infected mice with peptides or fusion proteins containing the VP1$_{233-250}$ and VP2$_{74-86}$ epitopes significantly exacerbates the onset and severity of demyelination (13, 36). Therefore, we speculate that T cells specific for VP3$_{24-37}$ may also contribute to demyelination. We are currently examining the role of VP3$_{24-37}$-specific T cells in the pathogenesis of TMEV-induced demyelination by testing the effect of immunization with T-cell epitope-containing peptides, as well as the adoptive transfer of epitope-specific T-cell lines into suboptimally infected SJL/J mice.

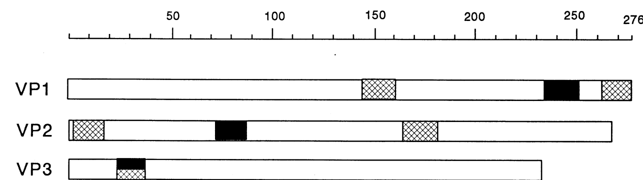

FIG. 3. Immunogenic regions of the BeAn strain of TMEV recognized by demyelination-susceptible SJL/J mice. The diagrams represent the external TMEV structural proteins and linear determinants recognized by antibodies (▩) or T cells (■) generated from TMEV-immunized and/or TMEV-infected SJL/J mice. The scale above the diagrams shows amino acid positions.

We thank Yong-Suk Jang and Atsushi Inoue for the production of several synthetic peptides.

This work was supported by USPHS grants RO1 NS28752 and RO1 NS33008.

REFERENCES

1. **Allen, I., and B. Brankin.** 1993. Pathogenesis of multiple sclerosis—the immune diathesis and the role of viruses. J. Neuropathol. Exp. Neurol. **52:** 95–105.
2. **Borrow, P., P. Tonks, C. J. Welsh, and A. A. Nash.** 1992. The role of $CD8^+$ T cells in the acute and chronic phases of Theiler's murine encephalomyelitis virus-induced disease in mice. J. Gen. Virol. **73:**1861–1865.
3. **Calenoff, M. A., K. S. Faaberg, and H. L. Lipton.** 1990. Genomic regions of neurovirulence and attenuation in Theiler murine encephalomyelitis virus. Proc. Natl. Acad. Sci. USA **87:**978–982.
4. **Clatch, R. J., H. L. Lipton, and S. D. Miller.** 1986. Characterization of Theiler's murine encephalomyelitis virus (TMEV)-specific delayed-type hypersensitivity responses in TMEV-induced demyelinating disease: correlation with clinical signs. J. Immunol. **136:**920–927.
5. **Clatch, R. J., R. W. Melvold, S. D. Miller, and H. L. Lipton.** 1985. Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease in mice is influenced by the H-2D region: correlation with TMEV-specific delayed-type hypersensitivity. J. Immunol. **135:**1408–1414.
6. **Crane, M. A., R. Yauch, M. C. Dal Canto, and B. S. Kim.** 1993. Effect of immunization with Theiler's virus on the course of demyelinating disease. J. Neuroimmunol. **45:**67–74.
7. **Dal Canto, M. C., and H. L. Lipton.** 1977. Multiple sclerosis. Animal model: Theiler's virus infection in mice. Am. J. Pathol. **88:**497–500.
8. **Dhib-Jalbut, S., and D. E. McFarlin.** 1990. Immunology of multiple sclerosis. Ann. Allergy **64:**433–444.
9. **Fiette, L., C. Aubert, M. Brahic, and C. Peña Rossi.** 1993. Theiler's virus infection of β_2-microglobulin-deficient mice. J. Virol. **67:**589–592.
10. **Francis, M. J., G. Z. Hastings, B. E. Clarke, A. L. Brown, C. R. Beddell, D. J. Rowlands, and F. Brown.** 1990. Neutralizing antibodies to all seven serotypes of foot-and-mouth disease virus elicited by synthetic peptides. Immunology **69:**171–176.
11. **Friedmann, A., G. Frankel, Y. Lorch, and L. Steinman.** 1987. Monoclonal anti-I-A antibody reverses chronic paralysis and demyelination in Theiler's virus-infected mice: critical importance of timing of treatment. J. Virol. **61:** 898–903.
12. **Gerety, S., W. Karpus, A. R. Cubbon, R. Goswami, M. K. Rundell, J. Peterson, and S. Miller.** 1994. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus-induced demyelinating disease. V. Mapping of a dominant immunopathologic VP2 T cell epitope in susceptible SJL/J mice. J. Immunol. **152:**908–918.
13. **Gerety, S., M. K. Rundell, M. Dal Canto, and S. Miller.** 1994. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus-induced demyelinating disease. VI. Potentiation of demyelination with and characterization of an immunopathologic $CD4^+$ T cell line specific for an immunodominant VP2 epitope. J. Immunol. **152:**919–929.
14. **Gerety, S. J., R. J. Clatch, H. L. Lipton, R. G. Goswami, M. K. Rundell, and S. D. Miller.** 1991. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus-induced demyelinating disease. IV. Identification of an immunodominant T cell determinant on the N-terminal end of the VP2 capsid protein in susceptible SJL/J mice. J. Immunol. **146:**2401–2408.
15. **Graham, S., E. C. Wang, O. Jenkins, and L. K. Borysiewicz.** 1993. Analysis of the human T-cell response to picornaviruses: identification of T-cell epitopes close to B-cell epitopes in poliovirus. J. Virol. **67:**1627–1637.
16. **Inoue, A., Y. K. Choe, and B. S. Kim.** 1994. Analysis of antibody responses to predominant linear epitopes of Theiler's murine encephalomyelitis virus. J. Virol. **68:**3324–3333.
17. **Kappel, C. A., M. C. Dal Canto, R. W. Melvold, and B. S. Kim.** 1991. Hierarchy of effects of the MHC and T cell receptor beta-chain genes in susceptibility to Theiler's murine encephalomyelitis virus-induced demyelinating disease. J. Immunol. **147:**4322–4326.
18. **Kappler, J. W., B. Skidmore, J. White, and P. Marrack.** 1981. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. **153:**1198–1214.
19. **Kim, B. S., Y. K. Choe, M. A. Crane, and C. R. Jue.** 1992. Identification and localization of a limited number of predominant conformation-independent antibody epitopes of Theiler's murine encephalomyelitis virus. Immunol. Lett. **31:**199–205.
20. **Leclerc, C., E. Deriaud, V. Mimic, and S. van der Werf.** 1991. Identification of a T-cell epitope adjacent to neutralization antigenic site 1 of poliovirus type 1. J. Virol. **65:**711–718.
21. **Lindsley, M. D., and M. Rodriguez.** 1989. Characterization of the inflammatory response in the central nervous system of mice susceptible or resistant to demyelination by Theiler's virus. J. Immunol. **142:**2677–2682.
22. **Lindsley, M. D., R. Thiemann, and M. Rodriguez.** 1991. Cytotoxic T cells isolated from the central nervous systems of mice infected with Theiler's virus. J. Virol. **65:**6612–6620.
23. **Lipton, H. L., and A. Friedmann.** 1980. Purification of Theiler's murine encephalomyelitis virus and analysis of the structural virion polypeptides: correlation of the polypeptide profile with virulence. J. Virol. **33:**1165–1172.
24. **Mahon, B. P., K. Katrak, and K. H. G. Mills.** 1992. Antigenic sequences of poliovirus recognized by T cells: serotype-specific epitopes on VP1 and VP3 and cross-reactive epitopes on VP4 defined by using $CD4^+$ T-cell clones. J. Virol. **66:**7012–7020.
25. **Melvold, R. W., D. M. Jokinen, R. L. Knobler, and H. L. Lipton.** 1987. Variations in genetic control of susceptibility to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. I. Differences between susceptible SJL/J and resistant BALB/c strains map near the T cell beta-chain constant gene on chromosome 6. J. Immunol. **138:**1429–1433.
26. **Peña Rossi, C., A. McAllister, L. Fiette, and M. Brahic.** 1991. Theiler's virus infection induces a specific cytotoxic T lymphocyte response. Cell. Immunol. **138:**341–348.
27. **Pullen, L. C., S. D. Miller, M. C. Dal Canto, and B. S. Kim.** 1993. Class I-deficient resistant mice intracerebrally inoculated with Theiler's virus show an increased T cell response to viral antigens and susceptibility to demyelination. Eur. J. Immunol. **23:**2287–2293.
28. **Rodriguez, M., J. Leibowitz, and C. S. David.** 1986. Susceptibility to Theiler's virus-induced demyelination. Mapping of the gene within the H-2D region. J. Exp. Med. **163:**620–631.
29. **Rodriguez, M., E. Oleszak, and J. Leibowitz.** 1987. Theiler's murine encephalomyelitis: a model of demyelination and persistence of virus. Crit. Rev. Immunol. **7:**325–365.
30. **Roos, R. P., S. Firestone, R. Wollmann, D. Variakojis, and B. G. Arnason.** 1982. The effect of short-term and chronic immunosuppression on Theiler's virus demyelination. J. Neuroimmunol. **2:**223–234.
31. **Scheu, A. K., A. Cuesta, and N. Rubio.** 1989. Characterization of the immune response to intracerebral inoculation of Theiler's murine encephalomyelitis virus: fine protein specificity of the splenic T cell and humoral antibody response. Immunol. Lett. **21:**171–176.
32. **Simons, J., M. Kutubuddin, and M. Chow.** 1993. Characterization of poliovirus-specific T lymphocytes in the peripheral blood of Sabin-vaccinated humans. J. Virol. **67:**1262–1268.
33. **Welsh, C. J., P. Tonks, A. A. Nash, and W. F. Blakemore.** 1987. The effect of L3T4 T cell depletion on the pathogenesis of Theiler's murine encephalomyelitis virus infection in CBA mice. J. Gen. Virol. **68:**1659–1667.
34. **White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D. P. Gold, and W. Born.** 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. J. Immunol. **143:**1822–1825.
35. **Yauch, R. L., and B. S. Kim.** 1994. A predominant viral epitope recognized by T cells from the periphery and demyelinating lesions of SJL/J mice infected with Theiler's virus is located within VP1(233–244). J. Immunol. **153:**4508–4519.
36. **Yauch, R. L., and B. S. Kim.** 1995. Unpublished data.