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# Use of a New Synthetic-Peptide-Derived Monoclonal Antibody To Differentiate between Vaccine and Wild-Type Venezuelan Equine Encephalomyelitis Viruses

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**We have prepared a murine monoclonal antibody (MAb) capable of distinguishing between wild-type Venezuelan equine encephalomyelitis (VEE) virus and the TC-83 vaccine derivative. This MAb, 1A2B-10, was derived from immunization with a synthetic peptide corresponding to the first 19 amino acids of the E2 glycoprotein of Trinidad donkey VEE virus. The MAb reacts with prototype viruses from all naturally occurring VEE subtypes except subtype 6 in an enzyme-linked immunosorbent assay. It does not react with TC-83 virus or members of the western and eastern equine encephalitis virus complex or with Semliki Forest virus. This antibody will also differentiate between TC-83 and Trinidad donkey VEE virus in indirect immunofluorescence assays with virus-infected Vero cells.**

Venezuelan equine encephalomyelitis (VEE) virus is the etiologic agent of a severe encephalitis in horses and humans. The virus can be serologically divided into six subtypes (subtypes 1 through 6), with subtype 1 containing five varieties (1AB, 1C, 1D, 1E, and 1F) (14). The disease can occur in an epizootic or enzootic form. VEE epizootics have been associated with members of the 1AB or 1C variety (1). The other subtype viruses are responsible for enzootic VEE transmission. The last documented VEE epizootic occurred from 1969 to 1971 in northern South America, Central America, and the southern United States (2). The source of this epizootic has never been determined. After this epizootic subsided, the epizootic form of VEE virus has not been isolated (13). The apparent disappearance of epizootic VEE virus has led to a number of hypotheses regarding the source of this virus (4). One such hypothesized source was incompletely inactivated VEE virus vaccine. The development of a live attenuated VEE virus vaccine (TC-83) solved the possible problems caused by incompletely inactivated virus; however, severe side effects in some TC-83 vaccinees indicated possible in vivo reversion to virulence of the TC-83 vaccine (5). This problem, coupled with the ability of TC-83 to be transmitted from vaccinated animals to susceptible mosquitoes, makes unambiguous differentiation of the TC-83 vaccine from wild-type VEE virus very important (6). Standard serologic differentiation of these viruses is impossible because of the indistinguishable antigenic structure of the TC-83 vaccine and its virulent parent, Trinidad donkey (TRD) virus.

We have been studying the antigenic structure of VEE viruses. We have previously identified a unique epitope ($E2^a$) on the E2 surface glycoprotein of TC-83 virus by using monoclonal antibodies (MAbs) (9, 11). Unfortunately, we were unable to identify a reciprocal antibody reagent capable of unambiguously identifying the wild-type TRD virus parent. A subsequent study identified a MAb capable of differentiating epizootic subtype viruses (1AB and 1C) from enzootic subtype viruses (1D, 1E, and 1F) (7). In our studies with synthetic peptides derived from the deduced amino acid sequences of the envelope proteins (E1 and E2), we observed that peptides derived from the amino-terminal 25 amino acids of TC-83 and TRD viruses (VE2pep01) were able to elicit virus-specific responses in peptide-immunized BALB/c mice (3). In this study we report the isolation of a monoclonal antipeptide antibody capable of differentiating naturally occurring prototype VEE viruses from the TC-83 VEE virus vaccine derivative.

The synthetic peptide antigen used in preparing this MAb corresponded to the amino-terminal 19 amino acids of the deduced sequence of the TRD virus E2 glycoprotein. The sequence of this peptide is STEELFKEYKLTRPYMARC. The sequence of the same region on TC-83 virus differs only at residue number 7 (K→N substitution). Male BALB/c mice were immunized twice with 50 μg of peptide in Freund complete adjuvant at biweekly intervals. Four days after the final immunization, spleens were harvested and fused to Sp2/0-Ag14 myeloma cells to prepare hybridomas as previously described (8). After 14 days in culture, supernatants were screened with an enzyme-linked immunosorbent assay (ELISA) on free peptide, TC-83, and TRD viruses (3, 9). Hybridoma cells from two wells contained antibody reactive with peptide and TRD virus. These cells were cloned in soft agar, and two cell lines were established. One cell line (1A2B-10) was further characterized. The MAb secreted by this hybridoma did not neutralize virus infectivity, block viral hemagglutination, or fix complement (data not shown). The MAb subclass was determined to be immunoglobulin G1 in an isotyping ELISA (Amersham Corp., Arlington Heights, Ill.).

To further characterize the specificity of this reagent, we compared the 1A2B-10 ELISA reactivity on purified alphaviruses with three other previously characterized MAbs, 5B4D-6 (TC-83 specific), 2A2C-3 (alphavirus group reactive), and 6B6C-1 (flavivirus group reactive) (9–12). These results indicated that MAb 1A2B-10 was not only reactive with TRD virus, but also reacted with prototype VEE viruses (with the exception of VEE subtype 6 virus Ag80-663) representing all other naturally occurring subtypes and varieties (Table 1). A similar analysis using indirect immu-

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TABLE 1. ELISA reactivity of MAbs[a]

| Virus complex[b] | Subtype (virus) | Titer of MAb | | | |
|---|---|---|---|---|---|
| | | 5B4D-6 | 1A2B-10 | 6B6C-1 | 2A2C-3 |
| VEE | 1AB (TC-83) | ≥512,000 | ≤4,000 | ≤4,000 | 256,000 |
| | 1AB (TRD) | 16,000 | ≥512,000 | ≤4,000 | 128,000 |
| | 1C (P676) | 32,000 | ≥512,000 | ≤4,000 | 128,000 |
| | 1D (V209A) | ≤4,000 | ≥512,000 | ≤4,000 | 256,000 |
| | 1E (Mena II) | 8,000 | ≥512,000 | ≤4,000 | 128,000 |
| | 1F (78V-3531) | 4,000 | ≥512,000 | ≤4,000 | 64,000 |
| | 2 (Fe3-7c) | 8,000 | ≥512,000 | ≤4,000 | 64,000 |
| | 3 (Mucambo) | 8,000 | ≥512,000 | ≤4,000 | 128,000 |
| | 4 (Pixuna) | ≤4,000 | 25,600 | ≤4,000 | 128,000 |
| | 5 (Cabassou) | ≤4,000 | ≥512,000 | ≤4,000 | 64,000 |
| | 6 (Ag80-663) | ≤4,000 | ≤4,000 | ≤4,000 | 128,000 |
| WEE | McMillan | ≤4,000 | ≤4,000 | ≤4,000 | 128,000 |
| EEE | NJ-60 | ≤4,000 | ≤4,000 | ≤4,000 | 128,000 |
| SFV | Original | ≤4,000 | ≤4,000 | ≤4,000 | 128,000 |

[a] ELISA procedures have been previously published (3, 9). The antibody sources were MAbs purified from mouse ascitic fluid by protein A-Sepharose chromatography and standardized to a concentration of 1 mg/ml.

[b] Abbreviations: VEE, Venezuelan equine encephalomyelitis; WEE, western equine encephalitis; EEE, eastern equine encephalomyelitis; SFV, Semliki Forest virus.

TABLE 2. IFA reactivity of MAbs[a]

| MAb | Titer of MAb to the following antigen: | |
|---|---|---|
| | TC-83 | TRD |
| 5B4D-6 | 2,560 | 40 |
| 1A2B-10 | <10 | 32,000 |
| 6B6C-1 | <10 | <10 |
| TRD HIAF | ≥1,000 | ≥1,000 |
| NMAF | <10 | <10 |

[a] IFA procedures were previously published (12). The antibody sources were mouse ascitic fluids.

nofluorescence (IFA) with virus-infected Vero cells (12) also demonstrated TRD virus specificity of MAb 1A2B-10 (Table 2).

The use of a synthetic peptide derived from a region on the E2 of TRD virus known to differ in sequence from the TC-83 vaccine derivative has enabled us to derive a wild-type VEE virus-reactive MAb. This MAb when used in conjunction with the previously characterized TC-83 specific MAb, 5B4D-6, should permit rapid ELISA or IFA differentiation of VEE vaccine from wild-type strains for the first time. In the face of another VEE epidemic or epizootic, epidemiologists and diagnostic laboratory personnel should be able to accurately determine the source and nature of the infecting VEE virus agent.

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