Yellow fever virus envelope protein has two discrete type-specific neutralizing epitopes

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Two monoclonal antibody neutralization resistant (MAbR) variants of the yellow fever (YF) 17D-204 vaccine virus strain were selected using YF type-specific MAb B39. These B39R variants were compared with the variant virus selected by Lobigs et al. (Virology 161, 474–478, 1987) using a second YF-type specific MAb (2E10) which mapped to amino acid position 71/72 in the envelope (E) protein. Neutralization assays with a panel of MAbs suggested that these two YF-type-specific epitopes are located in two discrete regions of the folded E protein. Each of the B39R variants had a single nucleotide mutation which encoded an amino acid substitution at either position E-155 or E-158. Thus, YF type-specific epitopes map to both domain I (B39) and II (2E10) of the YF virus E protein. The B39 defined epitope represents the first flavivirus neutralizing epitope localized to this region of domain I of the E protein.

Yellow fever (YF) virus is the prototype of the genus Flavivirus, family Flaviviridae. The flaviviruses are a group of single-stranded, positive-sense RNA viruses, which include several human pathogens of major significance (Monath, 1986). The mature virion is composed of a capsid surrounded by a lipid envelope with two membrane-associated proteins, the membrane (M) and envelope (E) proteins (Chambers et al., 1990). The E-protein is the major structural protein of the virus and is believed to mediate several important virus functions, such as host cell receptor-binding, membrane fusion activity and virion assembly (Heinz & Mandl, 1993). It is also the major antigen, responsible for induction of neutralizing antibodies and protective immunity. The antigenic structure of the flavivirus E protein has been proposed by Mandl et al. (1989) using tick-borne encephalitis (TBE) virus as a model. Three non-overlapping antigenic domains, designated A, B and C, were identified which comprise multiple overlapping epitopes and exhibit different functional activities and serological specificities. Subsequently, Rey et al. (1995) determined the crystallographic structure of the ectodomain of the TBE virus E protein and identified three domains (I, II and III) which appear to correspond to antigenic domains C, A and B, respectively.

Molecular analyses of the antigenic domains and epitopes of the flavivirus E protein have been performed in order to assess the effect of individual amino acid substitutions on biological function. To this end, MAb neutralization resistant (MAbR) variants have been isolated and characterized for several flaviviruses: TBE (Holzmann et al., 1990), Japanese encephalitis (Cecilia & Gould, 1991; Hasegawa et al., 1992), louping ill (Jiang et al., 1993), dengue (DEN) type-2 (Lin et al., 1994), Murray Valley encephalitis (McMinn et al., 1995) and YF (Lobigs et al., 1987) viruses. Single amino acid E protein mutations affecting pathogenicity were observed in a number of cases and were predominantly associated with domain III of the E protein (Holzmann et al., 1990; Cecilia & Gould, 1991; Hasegawa et al., 1992; Jiang et al., 1993).

To date, only one YF virus E protein epitope has been characterized (Lobigs et al., 1987). This epitope was localized in domain II of the E protein. In this study, we define a YF type-specific, neutralizing epitope on the E protein. In addition, we have compared this epitope with the YF type-specific epitope previously localized by Lobigs et al. (1987) and investigated the role of these epitopes in virus virulence for mice.

A neutralizing MAb (B39) was used to select antigenic variants of YF virus in vitro. MAb B39 represents one of a panel of E protein-reactive MAbs raised against the 17DD strain vaccine virus manufactured in Brazil (17DD-Braz), characterized previously as a YF type-specific MAb (Ledger et al., 1992). In plaque reduction neutralization tests (PRNTs),

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performed in monolayers of SW13 cells as described by Barrett et al. (1989). MAb B39 neutralized the World Health Organization secondary seed lot of the 17D-204 substrain vaccine virus (17D-204-WHO) to high titre (4 log_{10} units), but failed to neutralize any of the other YF virus strains tested, including 17DD-Braz (Barrett et al., 1989). In this study, two MAbR variants of the 17D-204-WHO virus, resistant to neutralization with MAb B39, were plaque-purified as described by Ledger et al. (1992). The variants were designated B39R WHO-1 and B39R WHO-2. The authenticity of the MAbR variants selected was assessed by indirect immunofluorescence (IIF) tests and PRNTs (Ledger et al., 1992), which revealed loss of binding and neutralization (Figure 1) by the selecting MAb, and thus confirmed the loss of the corresponding B39-defined E protein epitope. The 2E10v2 variant, characterized previously by Lobigs et al. (1987), was selected using another YF type-specific MAb (2E10) which neutralized 17D-204 substrain vaccine virus obtained from the ATCC (17D-204-ATCC) to a titre greater than 5 log_{10} units.

A panel of five YF type-specific MAbs was employed in IIF tests and PRNTs to compare the E protein antigenic composition of the B39R variants with their parent virus, 17D-204-WHO, and also with the 2E10v2 variant and its parent virus, 17D-204-ATCC (Fig. 1). Although MAb B39 bound to 17D-204-ATCC, no neutralization of 17D-204-ATCC or its derivative 2E10v2 was observed with this MAb. MAb 2E10, however, was found to neutralize both the 17D-204-WHO parent virus and its B39R variants to an equal extent. Therefore, loss of one MAb-defined epitope had no conformational influence on neutralization involving the other epitope, indicating that these two YF type-specific epitopes are located on topologically distinct sites on the E protein. The concomitant loss of neutralization of 2E10v2 by MAbs 2E10 and H33 suggests that these MAbs define structurally overlapping epitopes. A similar phenomenon may operate in the case of the B39R variants where loss of neutralization by MAb B39 is coincident with appearance of neutralization by MAb G23. Thus, the YF type-specific MAbs investigated here bind epitopes clustered in two antigenically discrete regions.

Since neutralization resistance could result from changes in the M or the E protein, both genes were sequenced to determine the molecular basis of MAb neutralization escape and locate the B39-defined, YF type-specific epitope on the deduced primary amino acid sequence. For nucleotide sequencing analysis, viral RNA was extracted directly from infected cell culture supernatant and amplified in two overlapping fragments covering the entire M and E protein-encoding genes by RT–PCR as described previously (Jennings et al., 1993).

Previous comparison of the nucleotide sequence of the E protein gene of 17D-204-ATCC (Rice et al., 1985) and the 17D-204-WHO virus has identified a single amino acid substitution at position E-153 (Post et al., 1992). Comparative sequence analyses of the B39R variants revealed no nucleotide changes in the M protein gene, while each variant differed from the 17D-204-WHO parent virus by only a single nucleotide change and corresponding amino acid substitution in the E protein (Fig. 2a): B39R WHO-1 mutated from C to U at nt 1446 (Thr to Ile at E-158); B39R WHO-2 from A to G at nt 1437 (Asp to Gly at E-155). It is speculated that these single amino acid mutations changed the conformation of the MAb binding site by altering the hydrophobicity, as found for the majority of flavivirus MAbR variants isolated to date (Holzmann et al., 1990; Hasegawa et al., 1992; Jiang et al., 1993; McMinn et al., 1995; Lobigs et al., 1987).

Identification of these amino acid substitutions mapped the YF type-specific B39-defined epitope within domain I of the E protein. Conversely, substitutions responsible for loss of the YF type-specific epitope recognized by MAb 2E10 were previously identified at E-71 or E-72 (Lobigs et al., 1987) in domain II. These findings further substantiate the conclusion that there are at least two discrete type-specific neutralizing epitopes on the surface of the YF virus E protein.

The number and position of potential N-linked glycosylation sites in flavivirus E proteins vary, although similarities are noted among members of the same subgroup. The 17D-204-WHO-derived and 17DD vaccine virus substra...
YF type-specific E protein epitopes

Fig. 2. Alignment of currently available flavivirus E protein sequences across domain I. Viruses are grouped according to antigenic complex and are compared to the representative virus in their serogroup: (a) YF viruses which are serologically distinct from other flaviviruses; (b) JE serocomplex; (c) DEN serocomplex; (d) TBE serocomplex. The first and last amino acid is numbered from the amino terminus of the E protein. Dots (.) represent conserved amino acids, dashes (–) represent ‘detected’ amino acids and potential N-linked glycosylation sites are underlined. Amino acid substitutions found in the B39R variants are shown in bold and their position is indicated by arrows. Amino acid sequences are adapted from Marin et al. (1995).

unique among YF virus strains sequenced to date in that they are glycosylated in domain I at Asn residues, E-151 and E-153, respectively (Post et al., 1992). We propose that this structural similarity is significant for the binding and neutralizing properties of MAb B39, since neutralization by this MAb appears to be glycosylation-dependent (Ledger et al., 1992). This suggestion is supported by the observation that the carbohydrate side-chain moiety has a role in stabilizing conformation-independent, sequential epitopes in domain I of TBE virus (Guirakhoo et al., 1989). However, the B39R variants retain a potential N-linked glycosylation site at E-153 and therefore, unless a conformational change induced by the amino acid substitutions identified abrogates glycosylation, as shown for DEN-2 Jamaica (Johnson et al., 1994), presence of a carbohydrate side-chain is not solely responsible for neutralization by this MAb.

In order to assess the virulence characteristics of the MAbR variants in vivo, groups of seven to nine female mice, 4 weeks old and weighing 20–25 g (outbred strain T0 or NIH Swiss, Harlan Olac) were inoculated intracerebrally (i.c.) with 10⁷ p.f.u. of virus and average survival times (AST) were calculated. Mice inoculated i.c. with either the parent 17D-204-WHO virus strain or the B39R variants died with no significant difference in AST, although strain T0 mice were found to be slightly less sensitive than NIH Swiss mice (data not shown). Similarly, all mice died with no difference in AST when inoculated i.c. with either variant 2E10v2 or its parent virus 17D-204-ATCC. Therefore, none of the variants were attenuated for mouse neurovirulence. Variant B39R WHO-2 inoculated intraperitoneally into 1-week-old mice or intranasally into weaning mice was found to be as avirulent as its parent virus, confirming that this variant did not have increased neuroinvasive properties. Therefore, neither B39 nor 2E10 YF type-specific epitopes contribute to YF virus virulence for mice and glycosylation of E-153 does not apparently contribute to mouse neurovirulence of YF 17D vaccine viruses. In contrast, E protein glycosylation in domain I of TBE (Pletnev et al., 1992) and DEN-4 (Kawano et al., 1993) viruses has been closely linked to virulence of these viruses in mice.

The B39-defined epitope is the first flavivirus E protein epitope mapped to this region of domain I. Previously, only one other flavivirus epitope has been identified in domain I at position E-171 in TBE virus (Holzmann et al., 1990). Comparison of the flavivirus E protein sequences currently available across this region (see Marin et al., 1995 for flavivirus sequences) indicates that, although there is a striking degree of homology among members of the same serological group, there is a relatively high degree of diversity between different subgroups (Fig. 2). In the YF virus E protein, an eight residue ‘deletion’ is observed compared to the Japanese encephalitis subgroup viruses (Fig. 2b) when amino acid sequence alignment is performed, possibly reflecting a difference in the antigenic structure of YF virus E protein in this region. The amino acid mutations which facilitated MAb neutralization escape in the B39R variants are positioned on either side of this deletion. Thus, we speculate that the B39-defined YF type-
specific epitope is created by bringing together a unique group of amino acid residues and that this region may also be type-specific in other flaviviruses.

References


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