Thesis Title	Production and Characterization of Monoclonal Antibodies to Dengue 2 Virus.
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ABSTRACT

A set of 15 monoclonal antibodies (MAbs) against dengue virus has been successfully established. The hybridomas had been prepared by fusion of cells from mice immunized with the 16681 strain of dengue 2 virus. Eight MAbs are of IgG1, 5 of IgG2a and 2 of IgG2b subclasses; all are of kappa light chain. None of the 15 MAbs was dengue 2 strain specific. The MAbs could be classified broadly into three groups: the first group with specificity directed against the non-structural NS1 product of the virus , the second which had been classified as anti-virion and the third with still undefined specificity, directed against either the NS2a or the PrM proteins.

Majority of the MAbs (11 MAbs) were specific for the non-structural NS1 protein. All the anti-NS1 MAbs except 1B2 (that reacted with all 4 serotypes) were dengue 2 specific. Based on the results of western blot, the anti-NS1

MAbs could be classified into 3 groups. The largest group consisted of 6 MAbs (4A6, 5C5, 4D2, 4D3, 4A2 and 5D3) which are specific for both the monomeric and dimeric conformational epitopes of the dengue 2 NS1 protein. This is in accord with the data obtained by others that most of the MAbs against NS1 protein were type specific and were directed against conformational epitopes (59) and (60). The second group consisted of three MAbs (1A4, 1D5 and 3D1). Their specificities were directed to only the dimeric form of the dengue 2 NS1. The final two MAbs (1B2 and 4D4) were specific for linear epitopes which exist on both the monomeric and dimeric forms of the NS1. Of particular interest is the fact that 1B2 is the only one MAb against NS1 which is cross-reactive to all the four serotypes and is reactive to all the 57 dengue isolates collected in Thailand, spanning a period from 1986 to 1992. This indicates that the epitope recognized by 1B2 is a common epitope on the surface of the NS1 molecule, both of monomeric and dimeric forms, shared by all 4 dengue serotypes; the epitope is well conserved within the different isolates found in Thailand. This epitope is probably unique for dengue virus and does not exist on the JE virus. 1B2 is one of the rare anti-NS1 MAbs that could be used in further study about the nature of dengue NS1.

Three MAbs (1D3, 1C2 and 5A1) were classified as anti-virion (structural) MAbs by the virtue of their IF staining pattern and neutralizing properties. They were not reactive with any protein band in the western blot. Within the three anti-virion MAbs, one (5A1) was dengue 2 specific, one (1C2) bound to an epitope shared by all the four serotypes except dengue 4, and the last one (1D3) was cross-reactive against all other dengue serotypes and also with 57 dengue isolates (that were isolated in Thailand from 1986-1991) suggesting that 1D3 recognizes a stable and common epitope that is shared by all 4 dengue serotypes. Due to this broad cross reactivity, the MAb could be used to capture the structural proteins in capturing ELISA.

The last MAb (4C1) which is non neutralizing, reacted with dengue 1 and 2 in ELISA and with a MW 21 kDa protein band, which probably corresponds to either the PrM or the NS2a proteins as both proteins migrate at the 22-24 kDa range. However the IF pattern of 4C1 was similar to the IF pattern stained by anti-PrM antibody as described by Westaway and Goodman in 1987 (72). Therefore 4C1 is more likely to be anti-PrM MAb.