VI SUMMARY

During the 1980 and 1987 epidemic seasons two genetic subtypes (IIIa and IIIb) of dengue serotype 2 virus co-circulated in the Bangkok metropolitan area. Based on the phylogenetic comparison of nucleotide sequence encoding the envelope glycoprotein, the subtype IIIa represented as many as 3 out of 5 (60%) and 18 out of 19 (94.7%), respectively, of the serotype 2 viruses studied in the years 1980 and 1987. In order to determine the temporal change in the proportion of these two dengue subtypes in Bangkok, five pairs of oligonucleotide probes were designed for use in the dot blot hybridization analysis of the serotype 2 viruses present in the year 1994. The design was based on the presence of six non-synonymous nucleotide substitutions that were fixed in the two subtypes between 1980 and 1987. By determining the envelope gene sequence of four serotype 2 viruses isolated from patients' sera at different time points of 1994, we established that the six differentiating nucleotide substitutions remained fixed among serotype 2 viruses of the epidemic year 1994, indicating that the probes should be useful in subtyping of viruses present in 1994. When the probes were labeled and hybridized against the PCR products derived from serotype 2 viruses with known envelope gene sequence from the years 1980 and 1987, specific hybridization was quantitated and normalized for differential binding of the PCR products to the nylon membrane by densitometric analysis. Two ratios, the maximum optical density ratio and the adjusted volume ratio, derived from the densitometric analysis served as the basis for determining the sensitivity and specificity of the probes. When the lowest levels of adjusted volume ratio obtained from the hybridization

analysis of 24 subtype IIIa viruses and 5 subtype IIIb viruses derived from the 1980 and 1987 epidemic seasons were employed as the cutoff points, the sensitivity of the probes 141-IIIa, 203-IIIa, 308-IIIa, 484-IIIa and 491-IIIa for detecting the subtype IIIa viruses was all 100%. Similarly, the sensitivity of the probes 141-IIIb, 203-IIIb, 308-IIIb, 484-IIIb and 491-IIIb for the subtype IIIb viruses was also 100%. At this high level of sensitivity, the specificity of most probes were 100%. Only one probe, 141-IIIa exhibited the specificity of 80%. On the other hand, when the lowest levels of maximum optical density ratio were employed as the cutoff points, the specificity was detected at the 100% level with only seven probes. The remaining three probes exhibited low levels of specificity by this criterion: 141-IIIa (80%), 203-IIIa (60%), and 308-IIIa (80%). When both ratios were considered, two pairs of the oligonucleotide probes (484-IIIa, 484-IIIb, 491-IIIa and 491-IIIb) appeared to give the highest sensitivity and specificity for detecting the two subtypes of dengue serotype 2 viruses by the hybridization analysis.

Dengue viruses may undergo random mutational change (genetic drift) and the resulting base substitution at the probe binding sequences may alter the sensitivity and specificity of oligonucleotide probes. To accommodate such possible changes, all five pairs of oligonucleotide probe were used in the analysis of 24 dengue serotype 2 viruses derived from the 1994 epidemic season. When the lowest adjusted volume ratio value obtained from the hybridization analysis of known subtype IIIa viruses and subtype IIIb viruses derived from the 1980 and 1987 epidemic seasons were used as the cutoff points, four pairs of oligonucleotide probes typed all serotype 2 viruses from 1994 as subtype IIIa. Only one probe, 491-IIIa, failed to identify one (out of 20) strain as the subtype IIIa strain. Identical results were obtained when the lowest maximum optical density ratio values was employed as cutoff points. For four viruses of which

nucleotide sequences of the envelope region were available, the hybridization pattern matched perfectly with the sequence data. Thus, 24 out of 24 dengue serotype 2 viruses which circulated in Bangkok in 1994 belonged to the subtype IIIa.

During the 1987 epidemic season in Bangkok, circulating subtype IIIa viruses shared a novel non-synonymous nucleotide substitution (C1036→T, His346→Tyr) which was absent in the subtype IIIa viruses found in 1980 and other known dengue serotype 2 viruses. This base substitution may serve as a marker for determining the relationship between the subtype IIIa viruses of the years 1987 and 1994 in Bangkok. For this purpose a probe, 346-IIIa-87, was designed and employed in the hybridization analysis. To obtain the specificity level of 100%, the highest adjusted volume ratio and the highest maximum optical density ratio derived from the hybridization with the subtype IIIa strains and IIIb strains which contained C1036 were used as cutoff points. The resulting sensitivity of probe 346-IIIa-87 for detecting the C1036→T substitution among the subtype IIIa strains from 1987 was only 85% and 90%, respectively. Even with these low level of sensitivity, the probe 346-IIIa-87 hybridized strongly to all dengue serotype 2 subtype IIIa strains found in Bangkok in 1994. Thus, the subtype IIIa strains found in Bangkok in 1994 might be direct descendants of the 1987 precursors rather than being introduced from other localities.