Subtyping of Hepatitis B Antigens in Indigenous and Foreign Populations in Southeast Asia

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OBJECTIVE: To develop the capability of subtyping Hepatitis B Antigen (HB—Ag) by the agar gel diffusion and other methods and to use these methods to investigate the subtypes of HB—Ag found in Southeast Asia in both indigenous and foreign populations.

BACKGROUND: The heterogeneity of HB—Ag was originally described by Blumberg and associates and has been further investigated by Le Bouvier and Bancroft, et. al., among others. This antigenic heterogeneity has been investigated using the Ouchterlony agar gel diffusion technique. Essentially, three major sets of antigenic determinants have been described. The first is a single determinant, "a" which appears to be common to all antigens. The second is one of a pair of determinants y or d which have been considered to be mutually exclusive alleles and the third also contained one of a pair of mutually exclusive alleles which have been labelled w and r. Thus antigens described by these means are ayw, adw, adr and most recently ayr.

Observations as to distribution have shown geographical areas of prevalence of these subtypes. In the Far East ad has predominated and adr has been the major subtype detected. Recent investigation has also demonstrated the ayr subtype in samples originating from the Far East and it has been suggested that this subtype might emerge through mutation or recombination of separately infecting viral genomes. The unique opportunity in Thailand of studying both indigenous and foreign populations and the admixture of these two led to an effort to perfect methods of subtyping hepatitis B antigen in Thailand.

METHOD:

Sera: Over the past several years serum samples have been acquired from a number of different US and Thai groups for both point prevalence studies of HB—Ag in populations and for identification of HB—Ag positive hepatitis. These samples have been tested for HB—Ag by several different methods (see elsewhere in this report) and those that were found to be positive were submitted for subtyping by the agar gel diffusion method.

Immunodiffusion Test: Alcohol—cleaned 1 x 3 inch microscope slides were flooded with 3.0 ml of hot 0.8% agarose (Seakem) in 0.01 M tris EDTA buffer, pH 7.6. The agarose was allowed to solidify and then a pair of 7—well patterns were punched into it, forming wells which were 2 millimeters in diameter and 5 millimeters apart arranged in a hexagonal pattern around the central seventh well. The slides were stored in a moist chamber until used.

Sera to be tested were concentrated with Lyphogel. Concentration from five to ten fold was accomplished by placing 0.3 ml of serum in a tube with two granules of Lyphogel and incubating at room temperature for two hours. Concentration was helpful for serum in which antigen titered $< 1:16$ by complement fixation. Reference antisera adw and adr were obtained from blood donors at the Phra Mongkut Klao Hospital in Bangkok. Reference antigen ayw and rabbit antisera against all three subtypes were kindly provided by

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LTC, William H. Bancroft of Dept. of Virus Diseases, Walter Reed Army Institute of Research. The sera were arranged on the slides placing each unknown serum next to a reference antigen so that lines of identity or partial identity could be easily detected. One of the antisera was placed in the central well.

Slides were incubated in a moist chamber at 37°C for 24 hours and then at 21°C for a further 48 hours or more. Slides were read at 24 hour intervals. Results were read as complete identity when test sera and reference antigen produced precipitation lines which fused without spurs. The presence of spurs was read as partial identity.

PROGRESS: Table 1 shows the results of subtyping of antigens found in both American and Thai populations. Out of a total of 198 antigens detected in all groups, subtyping was possible in 109 or 55%. In 14 of these (13%) only a and d determinants could be detected; w and r determinants could not be defined. In 39, however, three sets of determinants were identified. Among these ayw made up 23%, adw made up 10% and adr made up 67%. No ayr subtypes were found among American or Thai sera in this study. On repeated testing, two antigens from the US troops in the Republic of Vietnam appeared to have four determinants. They clearly contained the common "a" determinant and the "w" determinant. The d and the y determinant, which previously had been thought to be mutually exclusive, were both present (see elsewhere in this report).

DISCUSSION: These data can be analysed in two ways. First, subtypes obtained from hepatitis patients may be compared with subtypes obtained from carriers detected on point prevalence studies. It is of note that the percentage of subtypable antigens in those subjects with hepatitis was much lower than those detected in point prevalence studies. This reflects largely the titer of the antigen in the serum, which is generally higher in carriers than in individuals suffering from disease.

Second, a comparison of subtypes obtained from Thai and Americans may be made. The total number of Thai subjects studied was 900 of which 114 or 13% were positive by IEOP. Of these, subtypes were identified in 59 or 52% and in all of these, the subtype was ad. In 3 of these or 5% no further determinants could be detected, however, 51 or 86% of the subtypeable antigens were adr and 5 or 8% were adw. There were no ayw subtypes found among the Thai population studied, confirming the observations of Bancroft, et. al., on smaller numbers of sera, of a predominance of adr in Thailand. The adw subtypes were found in the point prevalence study of a lower socioeconomic Bangkok subdivision (see Annual Report 1971—1972, pages 129—139) and were found in three families, two in each of two families and one in a third family. In three additional carriers in these families, subtyping was not accomplished. All of the carriers in these three families in which subtypes could be identified were adw. This finding tends to support either intrafamilial spread of hepatitis B antigen or a genetic predisposition of families to certain subtypes.

The exclusive subtype detected among Thai adult populations with HB—Ag antigen positive hepatitis presenting at Phra Mongkut Klao Army Hospital was adr. Among this group of 113 individuals, hepatitis both positive and negative for HB—Ag, occurs 2—4 times as commonly in the decade between 20—30 as in any other age group (Annual Report 1971—1972, pages 117—120). This suggests that further investigation might show considerable cross—infection with hepatitis among Thai military personnel similar to that seen with adenovirus respiratory disease in United States military recruits.

In the American groups, on the other hand, 2539 individuals were studied of which 84 or 3% were positive by IEOP for antigens and 5% of these or 50 were subtypeable. The point prevalence study of HB—Ag in United States military personnel in the Republic of Vietnam has been reported elsewhere in this report. Little can be determined from the distribution of subtypes in this population because of the small number of carriers detected and the varied background of the men sampled. It is of note, however, that the two antigens subtyped as "adyw" were found in men who had spent at least one year in the Republic of Vietnam.

The subtyping of antigens obtained from Americans with hepatitis in the Republic of Vietnam is more revealing. 36 or 53% of 68 antigens positive by IEOP were subtypeable. Of these, 18 or 50% were ayw. The remaining 18 were ad with 7 adr, 2 adw and 9 in which only "a" and "d" could be detected. Although we have not yet investigated the subtypes found among the Vietnamese population, it is highly probable that, like the Thais, the subtypes are predominantly ad. If this assumption is correct, the high proportion of ayw subtypes detected in HB–Ag positive disease among Americans in the Republic of Vietnam would indicate considerable cross–infection among American military populations in Vietnam as opposed to acquisition of antigen from the indigenous population. Nine of the antigens isolated from these American hepatitis cases were not fully typed. Investigation of these specimens is continuing to determine whether these may not also show adyw or adyr subtypes.

SUMMARY: HB–Ag subtyping by the agar gel diffusion method has been established in this laboratory. Results of early testing are shown in Table 1. Further investigations of Thai and American hepatitis B antigen positive subjects may shed light on transmission of hepatitis among military populations in Southeast Asia.

Table 1. HB – Ag Subtypes of Thai and American Populations in Southeast Asia

Population	Number Studied	HB – Ag Positive %	With Typeable Antigens	With ADR	With ADW	With AYW	With AD	With Other Patterns
Thai lower socioeconomic Group	687	55(8)	** 40(73)	*** 32(8)	*** 5(12)	0	3(0.8)	
Thai Blood Donors	100	8(8)	8(100)	8(100)	0	0	0	
Thai Adult Hepatitis	113	51(45)	**** 11(28)	11(100)	0	0	0	
Thai Total	900	114(13)	59(52)	51(86)	5(8)	0	3(5)	
US Troops in VN	2365	16(0.6)	16(88)	4(28)	2(14)	4(28)	2(14)	***** 2(14)
US Hepatitis	174	68(39)	36(53)	7(19)	2(6)	18(50)	9(25)	
Total US	2539	84(33)	50(60)	11(22)	4(8)	22(44)	13(26)	
Grand Total	3439	198(6)	109(55)	62(57)	9(8)	22(20)	14(13)	2(2)

* IEOP positive
** Number subtyped (% of total positive)
*** Number of subtypes (% of total subtyped)
**** 40 were submitted to subtype
***** adyw