CHAPTER IV

Discussion

This prospective clinical and immunologic study of recipients of equine rabies immunolobulin (ERIG) was aimed to evaluate the value of allergic skin testing in the diagnosis of allergic reaction to horse protein and to evaluate the incidence of serum sickness following ERIG administration as well as to evaluate the value of heterophile antibody, C_3 and circulating immune complex (CIC) levels in the diagnosis of serum sickness. Two sets of ERIG recipients were included into this study. The first was 131 consecutive patients who were followed from the day that ERIG was given to 2 weeks following the administration of ERIG. The second set of patients was an additional 16 patients who reported back to our Rabies Clinic with symtoms and signs suggestive of serum sickness following ERIG administration at our Clinic. This additional set of patients was prospectively collected during a 12 month period and they were typical serum sickness patients, not different from the 2 patients identified from the systematic prospective study as shown in table IV,V and VI. Therefore, for statistical purpose, the ERIG-induced serum sickness patients from both groups were combined as a single group so that the laboratory measurements in the serum sickness group could be compared

with the ERIG-treated non-serum sickness group. For IgE skin testing, 19 of 131 patients (14.5 %) had positive immediate skin test to ERIG, 4 (3.05%) had borderlined results and 108 (82.4%) had negative skin test. This rate of skin test positivity is different from those reported in other series (2, 3, 21, 22 ,23, 24). Percent of skin test positivity varied from 5% to 23% in one's study to the other studies. These different percentage of skin test positivity might be due to.

a) The different types of vaccines used in skin test such as snake antivenin,antirabies vaccines,botulinal antitoxin.

b) Vaccines are from different companies.

c) The dilution of antiserum used in the test. Undiluted serum can cause local reaction.

d) Any local trauma may produce some wheals in some subjects e.g. in dermatographia.

e) Injection of quite innocuous substances may produce reaction of various kinds.

f) Faulty procedure can cause trauma and lead to local reaction.

g) Certain flushes around the test area may be due to a cresolic preservative.

However, the clinical relevance of this positive skin test is doubful since there was no occurence of either immediate or delayed type I hypersensitivity reactions

among any skin test positive or negative recipients. Our results confirm those of the others that skin testing is unreliable in predicting adverse reactions to equine protein administration (2, 21-25). Some skin test negative patients may develop anaphylaxis (4, 21, 23). Our study also indicates that it is unnecessary to substitute HRIG for ERIG in skin test positive individuals.

HRIG is 10 times more expensive than ERIG (3) and there fore,not generally affordable by people of developing countries where canine rabies still remains a serious public health problem (2). Therefore, ERIG remains an essential component for post-exposure rabies treatment (26). Nevertheless, skin testing prior to the administration of heterologous proteins is still mandatory in practice. It gives healthcare providers an additional level of precaution warning.

Our study showed that 2 of 131 ERIG recipients (1.5 %) developed serum sickness. The incidence rate is considered minimal if compared to the previous reports that the occurence of ERIG induced serum sickness may be as high as 46 % (3). Our study, however, confirms that of Wilde et al that the incidence of Pasteur ERIG- induced serum sickness was as low as 0.87 % (3). The reasons for the low incidence rate of serum sickness in the recent

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studies of equine gamma globulin may be due to the improved purification process,good manufacturing techniques and the low protein content of the final product. It is also well known that the larger the quantity of foreigh protein given, the greater is the chance of developing serum sickness (27). However, the study by Struan et al (1979) gave the first evidence that the larger the quantity of foreign proteins given, the greater is the chance of developing immediate allergic reactions (29). This finding may be explained by the anticomplementary activity of the product which leads to anaphylactoid reactions (28).

Serum sickness is a clinical diagnosis based on the history of foreign protein or antigen administration which is followed by a constellation of symtoms and signs suggestive of immune complex diseases (5, 16, 17). Laboratory comfirmations are needed in some patients, particularly in those who have incomplete clinical manifestations. Several indirect immunologic parameters have been used. These include heterophile antibody, circulating immune complex (CIC) level, complement levels either total hemolytic complement activity (CH_{50}) or individual complement proteins such as C_3 (15, 32). However, sequential changes of these parameters starting from day 0 have rarely been studied We have chosen heterophile antibody, C_3 and CIC as our laboratory

parameters to prospectively follow 102 recipients of ERIG from day 0 to day 14 and to correlate the changes of these parameters with the development of clinical serum sickness.

Our studies revealed that 50 of 102 subjects (49%) developd a four fold rise in heterophile antibody titer during the 14 days of follow-up as compared to the baseline titer on day 0 (Figure 2). 45 of these 50 screening heterophile antibody positive sera or 90 % were proved to be antibody of serum sickness by Davidsohn's diffenential test (36). Therefore, it can be concluded that 45 of 102 recipients of ERIG or 44.1 % developed true serum sickness type of heterophile antibody. However, the presence of specific heterophile antibody did not correlate with the development of serum sickness probably because of too few cases of serum sickness in this prospective study, i. e., only 2 out of 102 ERIG recipients.

However, in another set of 16 ERIG induced serum sickness patients who did not have pretreatment sera for analysis 12 or 75 % had specific heterophile antibody within 14 days after ERIG administration. When these 16 patients were combined with the 2 patients from the prospective study, specific heterophile antibody had non significent correlation with serum sickness table XIV),i.e, 12 out of 18 or 66.7 % were specific heterophile antibody

positive. This probably represent the first study of the clinical usefulness of specific heterophile antibody in a relatively large group of serum sickness patients. However, the test may be made more sensitive by replacing sheep red cells with horse red cells both before and after the differential absorption. (30, 31).

When the titers of the heterophile antibody before differential absorption were compared between the group with serum sickness and the group without serum sickness, the titers in the former were significantly higher than those in the latter group (Figure 10). This again indirectly points out the clinical significance of heterophile antibody in confirming the diagnosis of serum sickness (15, 32). Heterophile antibody negative in these 2 patients might be owing to :-

a) Their less intense heterophile antibody responses produced by these two patients until they could not be detected in the change of antibody level.

b) Sheep red cell might be older than one weak which yielded less sensitivity of the test.

c) Paul Bunnel Davidsohn (PBD) sheep cell test was done. The positive rates were much lower in comparison with results obtained when sera were examined by PBD horse cell test (31). The use of sheep cells for agglutination

indicators in PBD test produced quite inferior results(31). Because horse red cells are over sheep red cells in greater sensitivity (30).

Unlike the findings of Lawley, T.J et al that Complement activation occured in as much as 100% of recipients of equine antithymocyte globulin. We found no significant change in mean C_3 levels as far as 2 weeks after ERIG adminstration (Figure 3,). Similarly, no significant difference in mean C_3 levels was observed between the group of 18 patients with overt serum sickness and the 100 asymptomatic ERIG recipients although there was a trend for C_3 to be lower in the symptomatic group (Figure 4). if C_3 level below 1 standard deviation from the mean of normal value (i.e., day 0) was used as the cut-off value, abnormal (low) C_3 levels were not correlated with clinical serum sickness (Table 8). Even if positive heterophile antibody was used as "gold standard" of overall serum sickness, either symptomatic (clinical) or asymptomatic (subclinical), no correlation with C_3 levels was found either (Figure 5, Table IX).

Our results thus indicate that complement activation in serum sickness, if occurs, is very subtle. This is probably due to

a) low level of activation of complement system.

b) the wide normal ranges of C_3.

c) inappropriate timing for C_3 determination because of the brisk complement activation.

d) the rapid synthetic rate as compared to the rate of immune consumption.

Therefore, determination of CH_{50} which is more sensitive in measuring the complement change than C_3 or the measurement of complement breakdown products such as C3d may be better than C_3 measurement.

Similarly, there was no significant change in the circulating immune complex levels either with time following ERIG administration or when serum sickness evolved (Figure 7,) . The reasons for the failure to detect CIC in our patients with serum sickness may be due to the relatively insensitive CIC assay or the rapid clearance of CIC by the reticuloendothelial system. Our findings were similar to those reported by Umetsu et al(37). Their patients with serum sickness had low normal CH_{50} but normal C_3, C_4 and CIC levels. Therefore, our studies indicate that serum sickness is a clinical diagnosis based on the history of drug or heterologous protein administration, followed in 7-14 days by fever, skin rash,arthralgia,lymphadenopathy and proteinuria (16). Routine immunologic tests for immune complex diseases,

such as complement and circulating immune complex levels are generally not helpful in the diagnosis of serum sickness as described by others(17,22,37). This may be due to the minimal changes of C_3 or CIC, the wide normal range of C_3, the insensitivity of the currently used CIC assays, the rapid clearance of CIC or the rapid resynthesis of C_3 etc.

Although Paul-Bunnel-Davidsohn differential heterophile antibody test was first developed to diagnose serum sickness (32), it was rarely described as diagnostic tool for serum sickness (32,39), Our studies indicate that it probably is the most reliable diagnostic laboratory tool for serum sickness.

In addition, we found that Arthus type skin reaction using ERIG as skin test antigen was a very useful test for serum sickness. It was present in practically all patients with serum sickness.

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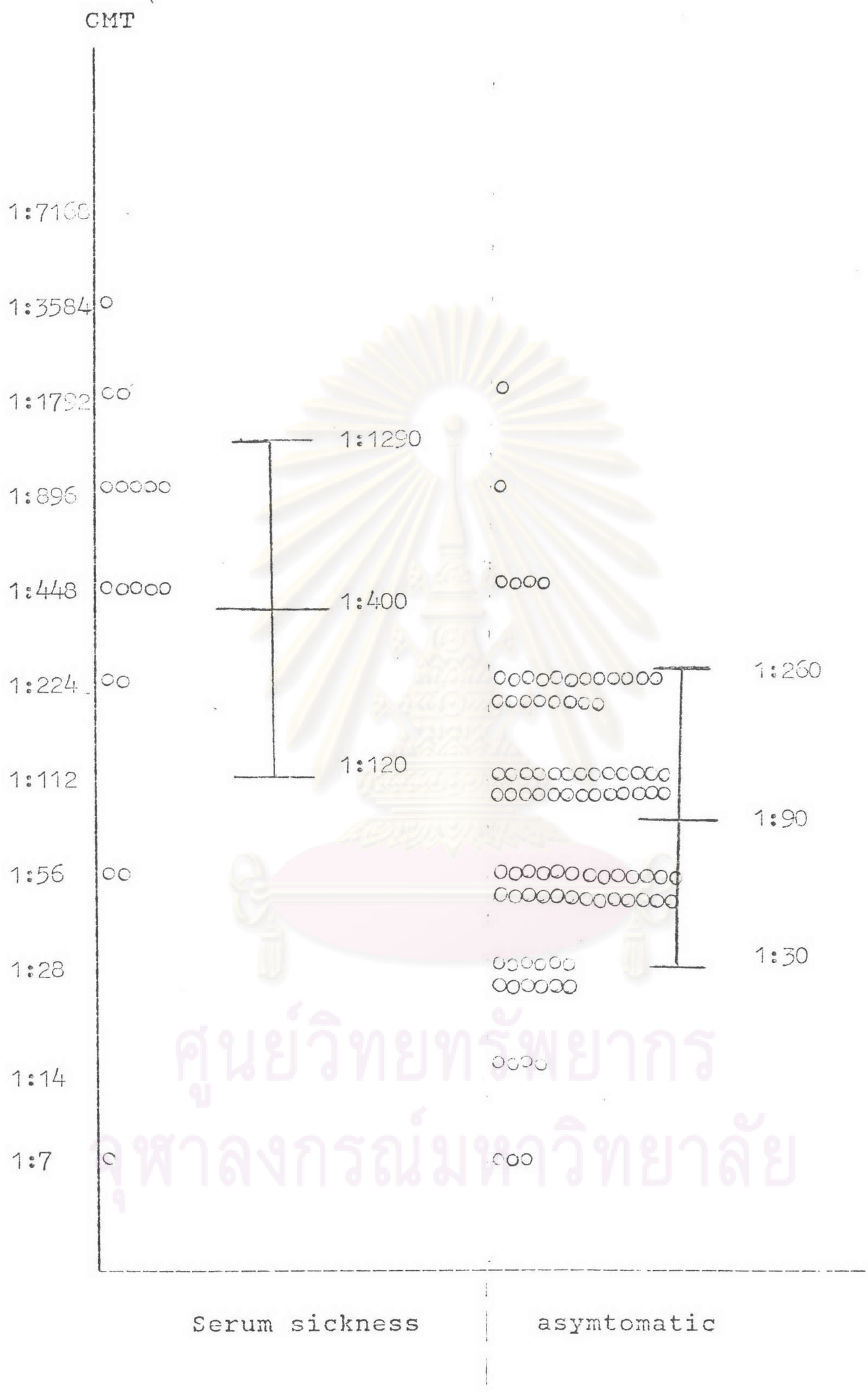

<u>Figure 10</u> Comparative of heterophile antibody between the group with and without serum sickness before differential absorption test.

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Table XIV Chi-square test between heterophile antibody and serum sickness.

Heterophile antibody	serum sickness +	serum sickness −	Total
+	12	45	57
−	6	55	61
Total	18	100	118

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