CHAPTER 5

Discussion

Although the incidence of serum sickness following the use of heterologous serum has been markly decreased, in current medical practice due probably to the infrequent use of foreign serum and to the use of more purified material, it is still frequently encountered (1,3,4). Heterologous antiserum is still occasionally used in the treatment and prophylaxis of botulism, gas gangrene, rabies and snake bites (5,70,71,72). In the present study, serum sickness was found in 2 of the 104 patients (1.9%) who received equine rabies immune globulin (ERIG) manufactured by the Pasteur Institute. Our study confirms that of Wilde et al. who found only 0.8%, 3.58% and 6.19% of Thai patients receiving ERIG manufactured by the Pasteur Institute, the Swiss Serum and Vaccine Institute and SCLAVO respectively developed serum sickness (20,21,22). This low incidence is in contrast to the study by Hosty et al. and Karliner et al. who found that the incidence of serum sickness following anti-rabies horse serum was 15.6% and 16.3% respectively (17,19).

Both human and experimental forms of serum sickness are caused by the antibody response to the

administered heterologous proteins that elicits a brisk IgG and IgM antibody responses (2,6,33,34). Heamagglutinating antibody against horse serum can be demonstrated in serum sickness patients (29,59). Precipitaing IgG antibodies are found less consistently, but may be presented in more severe cases (3).

In this study, a sensitive enzyme linked immunosorbent assay (ELISA) was developed to detect IgG, IgM and IgE antibodies to horse gamma globulin. Of the 104 patients, IgG antibody was present in 2(1.9%), 5(4.8%) and 31(29.8%) patients on day 0, 7, and 14 respectively. Of the 2 patients who had IgG antibody prior to treatment with ERIG had no documented pre-exposure to heterologous sera and skin tests were negative. This antibody reactivity could be absorbed by horse gamma globulin and thus representing pre-existing antibody rather than a non-specific reactivity by ELISA. The prospective study showed that they also had IgG anti-horse gamma globulin on day 7 and 14 but with higher titers (Figure 2). None developed serum sickness. This is in contrast to the study by Reisman et al. who reported that patients with pretreatment antibody titer of 1:200 or greater are at an increased risk of developing serum sickness (59).

Of the 2 serum sickness patients, one had IgG antibody on day 7 and 14 while her symptom started on day 6. The other had IgG antibody on day 14 only, while his symptom of serum sickness started on day 9. It is likely that the IgG antibody was found while there were signs and symptoms of serum sickness.

Of 102 asymptomatic patients, 4(3.9%) and 29 (28.4%) had IgG antibody on day 7 and 14 respectively. The presence of IgG antibody and the absence of signs and symptoms of serum sickness is probably due to the good and effective immune clearance system and/or the improper size of immune complex that cannot deposit at the vessel wall and/or the IgG antibody response in these patients did not belong to the subclass that can fix complement.

Because there were only 2 patients with serum sickness in our prospective study, we included a group of 27 serum sickness patients who were not systematically followed from day 0 resulting in a total of 29 serum sickness patients. When the serum sickness and asymptomatic groups were compared, it was found that on day 7, 21 (95.5%) of the 22 serum sickness patients and 4 (3.9%) of the 102 asymptomatic patients had IgG antibody whereas on day 14, all of the 21 (100%) serum sickness patients and 29 of the 102 (28.4%) asymptomatic patients had IgG antibody (Table 11 and Table 12). The differences

were statistically significant on both days implying the pathogenic roles of the IgG antibody in serum sickness. It was shown that IgG antibody could be demonstrated in all serum sickness patients when the signs and symptoms occured as one serum sickness patient whose symptoms started on day 9 did not have IgG antibody on day 7 but did on day 14.

By comparing the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of the IgG antibody assay against horse gamma globulin at different cut-off levels both on day 7 and day 14, it became evident that IgG anti-horse gamma globulin on day 7 had more diagnostic value for serum sickness than that on day 14 (Table 14 and 15, Figure 4 and 5).

Tatum et al. (27) reported the diagnostic value of IgG anti-horse gamma globulin by ELISA test in complicated serum sickness following anti-thymocyte globulin treatment. They found good correlation between serum sickness and IgG anti-horse gamma globulin with high sensitivity, specificity, positive predictive value, negative predictive value and efficiency. (86%, 100%, 100%, 91% and 94% respectively) whereas those were 100%, 71.56%, 42%, 100% and 76.42% respectively. The reason for the high specificity and the high positive predictive value in the study of Tatum et al. may be due to the

immunosuppression conferred by antithymocyte globulin or other drugs that prevent renal allograft rejection. As the consequence, asymptomatic patients could not form IgG antibody whereas in the present study, 29 or 28.4% of the 102 asymptomatic patients developed IgG antibody since they did not receive any immunosuppressive drugs. This assumption is likely to be correct as Arbesman et al. (29) also reported a significant rise in passive hemagglutinating antibody titer to horse immune globulin in many recipients of equine tetanus antitoxin even without any evidence of serum sickness although the ones with serum sickness had titers greater than 1:2000.

IgM anti-horse gamma globulin was absent prior to ERIG administration but was present in 4 patients 7-14 days after ERIG administration. Two had IgM antibody on both day 7 and day 14 whereas one each had IgM antibody only on day 7 or day 14 (Figure 6). The patient who had IgM antibody only on day 7 was one of the 2 serum sickness patients in our prospective study. When the larger group of serum sickness patients was analysed, 36.4% (8/22) and 19% (4/21) had IgM antibody on day 7 and 14 respectively as compared to 2% (2/102) and 2.9% (3/102) respectively in the group without serum sickness (Table 16 and 17). The difference was statistically significant (Table 16 and 17). One results indicate that IgM antibody

to horse gamma globulin may play an important role in the pathogenesis of serum sickness due to its early occurence (i.e., more on day 7 than on day 14) and its efficient complement activation property.

However, as compared to IgG antibody, IgM antibody was found in a smaller proportion of serum sickness patients. This may be due to several possibilities. First, the level of IgM antibody may be very low thus, escape from our test sensitivity. Second, the onset of IgM antibody response may start earlier than 7 days after ERIG anministration or may rapidly disappear during the interval of our serum collection regimen, i.e., between day 7 and day 14. The rapid decrease of IgM antibody was confirmed by the data obtained from the 14 serum sickness patients who had paired sera (Figure 8). Five patients had IgM antibody on day $\pm$ 7 (35.7%) but the antibody disappeared in 5 when re-examined 7 days later. No other studies have reported the IgM antibody response to horse gamma globulin in serum sickness.

When kinetic of IgG and IgM responses were compared, it was evident that IgM antibody appeared and disappeared earlier than IgG antibody in the course of serum sickness (Figure 9). This chronologic change of IgM and IgG antibodies is compatible with any known primary antibody responses and indirectly implies the pathogenic

role of the IgG anti-horse gamma globulin in serum sickness.

By comparing the sensitivity, specificity, positive predictive value, negative predictive value and efficeincy of IgM antibody assay aganist horse gamma globulin at various cut off levels, it is evident that the test has to be further modified in order to improve its sensitivity and specificity (Table 18 and 19).

In this study, ELISA test for IgE anti-horse gamma globulin was developed and compared with the skin test. Of the 131 patients, 15 (11.5%) and 19 (14.5%) had IgE anti-horse gamma globulin by ELISA and skin test respectively (Table 20). Of the 19 skin test positive patients, 12 (63.2%) had IgE antibody whereas 3 (2.8%) of the 108 skin test negative patients had positive results. None of the patients with boderlined skin test had IgE antibody (Table 20). By chi-squar test, correlation was found between ELISA and skin tests (Table 21). However, none of the skin test-positive or IgE antibody-positive patients developed anaphylaxis or type I allergic reaction following ERIG administration.

Our results confirm those of Black et al and Malasit et al that allergic skin test to horse proteins does not provide any reliable clues of anaphylaxis following the administration of botulinal antitoxin and

anti-snake venom respectively (11,13). This agreed with Wilde et al. who had reported that none of the skin test-positive patients had any immediate hypersensitivity after receiving ERIG under close observation (21).

Conclusion

1. IgG and IgM antibodies to horse gamma globulin could be detected in both asymptomatic and serum sickness patients following ERIG administration. However, the prevalence and the mean antibody titers were significantly higher in serum sickness patients indicating these IgG and IgM antibodies might play a role in the pathogenesis of serum sickness as well as might be used as the diagnostic tool for serum sickness.

2. The presence of specific IgE antibody by ELISA correlated very well with skin test reactivity to ERIG but neither IgE nor the skin test correlated with anaphylactic reaction following ERIG administration.

Further study

It will be of interest to determine the subclasses of IgG anti-horse gamma globulin which occur after the administration of equine immune globulin. The differences

in IgG subclass response may explain why some patients will develop serum sickness and some will not depending on its complement fixing activity.

Research advantage

1. The study has developed quick, sensitive, specific, inexpensive and noninvasive tests for anti-horse gamma globulin antibodies.

2. The specific IgG and IgM antibodies were proved to be reliable diagnostic aids for serum sickness.

3. The study has also established the fact that although IgE antibody to horse gamma globulin correlated well with the immediate skin test results but neither of them was reliable in predicting anaphylactic reaction to horse gamma globulin.