CHAPTER IV

DISCUSSION

This study is the first to look at the immunogenicity of the abbreviated or " 2-1-1 " regimen of purified Vero cell rabies vaccine (PVRV). Our results indicate that the 2-1-1 regimen is at least as immunogenic as the conventional 1x6 full-course regimen. In fact, the neutralizing antibodies generated by the 2-1-1 regimen in our study were significantly higher than the conventional regimen on days 14. This is in contrast to other studies using 2-1-1 regimen which could not demonstrate a higher antibody response as compared to the conventional regimen (94), whereas, our findings confirm those of Baklaić et al (95) and Phanuphak et al (130) that the 2-1-1 regimen was superior to the standard regimen.

Although it was initially expected that the 2-1-1 regimen would result in more rapid antibody response (96), our data as well as other investigators' data (94,95,130) did not lend any support to this expectation. The seroconversion rate of the 2-1-1 regimen on day7 was not higher than the conventional regimen. It may be of interest to extend such investigation to day 9, 11 or 12 in order to see the difference in early antibody response of the 2-1-1 regimen as compared to the conventional regimen.

Antigen-stimulated lymphocyte transformation test (LTT) was used to study the cell-mediated immune response (CMIR) to the vaccine. The test has been widely accepted as an in vitro correlate of CMIR (115). The reaction is antigen specific since no stimulation was seen prior to vaccination. The LTT first became evident on day 14 in both the conventional and the 2-1-1 regimen. The results confirm other previous studies with intramuscular human diploid cell rabies vaccine (90,129) and PVRV (123). Our results could not demonstrate any superiority of the 2-1-1 regimen over the conventional regimen for the cellular immune response. This is in contrary to the results with multisite intradermal regimens where CMIR would develop 1 week sooner than the I.M. regimen (123). This is not very surprising because intradermal (especially multisite) immunization is known to be a better mean to induce CMIR than the intramuscular immunization, even multisite as well (131).

It therefore, appears that the 2-1-1 regimen of PVRV is at least as effective as the conventional full dose I.M. regimen. The 2-1-1 regimen has the advantages over the conventional 1x6 regimen in saving 2 dose of vaccine and 3 clinic visits. All vaccinees had adequate (protective) neutralizing antibody levels up to 6 months. The regimen was well tolerated and no serious side-effects were observed. We thus recommended that the 2-1-1 regimen of PVRV should replace the conventional 1x6 regimen of PVRV in postexposure prophylaxis especially in the developing

countries and in where rabies immunoglobulin is not needed or not available.

Rabies immune globulin (RIG) is recommended for severe (class III) exposure to laboratory proved rabid animals (132). Therefore, before the 2-1-1 regimen can be unquestionably recommended for post-exposure use, it is essential to establish the susceptibility of this regimen to the suppression by exogeneous rabies immune globulin. No study along this line has ever been does. Our results indicate that HRIG and to greater extent, ERIG at dosages of 20 and 40 iu/kg respectively, could significantly suppress the rabies antibody response to the 2-1-1 regimen of PVRV when RIG was simultaneously given at the onset of the vaccination schedule. The HRIG and ERIG used in the study were injected entirely into the buttock area, a different site from the PVRV injection. It is possible that if half of RIG is injected around the bite site and the other half into the buttock as being currently practiced at our Institute, there might be less suppression.

Variables which determine whether HRIG or ERIG will suppress the active antibody response to rabies vaccine include the type and potency of the vaccine, the type and potency of the RIG , the routes and means of vaccine and RIG administration as well as subject variations. In reference to the conventional 1x6 regimen of PVRV, HRIG was found not to suppress the antibody response (124). Similarly, ERIG

was found not to suppress the 2-2-2-0-1 intradermal regimen of PVRV (Phanuphak et al, unpublished results). Therefore, it seems that the suppressibility by RIG is unique for the 2-1-1 regimen of PVRV. The results of this study, if confirmed, will create the major concern for the suitability of the 2-1-1 regimen of PVRV in category III post-exposure rabies prophylaxis. However, one could still argue in favor of the 2-1-1 regimen that even suppressed by HRIG or ERIG, the GMTs were equivalent to the no-RIG, 1x6 regimen (Table 2) and none fell below the arbitrary protective level of 0.5 iu/ml, there might be of no clinical relevance.

The enzyme linked immunosorbent assay for rabies antibody developed by many workers, purified whole virus (Pasteur fixed rabies virus strain) (133), glycoproteine (134), were used as the immunosorbent. Only one study (128) which used the human diploid cell rabies vaccine, HDCV the whole virion as the immunosorbent. Purified Vero cell rabies vaccine (PVRV) was used as the immunosorbent in our study but failed to reproduce the antibody titers correctly and invariably. Reason for this failure is uncertain. It may be due to the type of immunosorbent being used although similar failure was obtained when HDCV was used as the coating antigen. Furthermore, the coated antigen at the dilution used may detach easily and unpredictably from the plate. (Fig.9)

In addition, Perrin et al. (134) have stressed the point that rabies glycoprotein-coated plate gave more reproducible results on ELISA system. However, even with the commercially available ELISA test kit (Platelia® Rage, Diagnostic Pasteur) which use glycoprotein as the immunosorbent, the results obtained were still quite different from the RIFFIT (Khawplod et al, unpublished results). Therefore, although ELISA may offer a more convenient system for rabies antibody determination, a long way is still needed to develop the test especially for its reproducibility and correlation with the neutralizing activity as can be offered by RIFFIT.