BIOLOGICAL CONTROL OF RICE-FIELD RATS: RAT CYTOMEGALOVIRUS (RCMV) FOR THE DEVELOPMENT OF AN IMMUNO-CONTRACEPTIVE VACCINE

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Introduction

Rats have been viewed as important pest that cause a significant damage in rice fields and oil palm plantation. The major rat species include *Rattus argentiventer* in rice-field, *Rattus rattus tiomanicus* in cocoa plantation and *Rattus rattus diardii* in oil palm plantation (Lam, 1980). Damages inflicted are related to substantial pre-harvest loss to crops. Besides, wild rats can carry and disseminate various zoonotic diseases. Methods include chemicals such as warfarin and brodifocum, and mechanical instruments, which are expensive and of limited use. Alternatively, lethal disease agents can be used to control a target population (Fenner and Ratcliffe, 1965). Immunological sterilisation has been widely considered more humane. Target animals could be immunologically induced to destroy their own essential reproductive proteins e.g., sperm and zona pellucida (Shagli et al. 1990). The objective of this study was to identify the most highly prevalence virus in rice-field rats and its geographical distribution in the rice-field.

Materials and Methods

Rice-field rats were trapped at Parit, Seberang Prai, Tanjung Karang, Besut and Alor Setar. Serum samples obtained were screened by immunoflourescence technique for 13 viruses: mouse adenoviruses (strains MadV-FL and MAdV-K87), new rat parvovirus, rat coronavirus, Theiler's virus, lymphocytic choriomeningitis virus, vaccinia, Hantaan virus, Seoul virus, pneumonia virus, conventional rat parvovirus, reovirus, and Sendai virus. Serum samples were tested by an indirect ELISA technique for the presence of antibodies against RCMV. Exogenous RCMVs strains Dutch and English were used as antigens. Salivary glands, kidney and lymph nodes were collected and processed for virus isolation. Viruses isolated were studied for their growth properties, electron microscopic structure, immunohistochemistry staining, DNA fingerprinting, immunogenic protein profiles, host specificity as well as pathogenicity in laboratory animals.

Results and Discussion

More than 280 serum samples were positive serologically for viruses tested except LCMV, vaccinia, Hantaan, Seoul, Pneumonia and Sendai viruses. Excluding RCMV, coronavirus was highly (30% in Parit) prevalence but its geographical distribution was not consistent. In contrast, approximately 50% of serum samples were positive for RCMV and this was geographically consistent. Two RCMVs were isolated from salivary gland and kidney respectively. The two virus isolates were indistinguishable in an electron microscope, their growth in cell culture or by immunohistochemistry techniques. These viruses grow slowly in rat embryo fibroblast. Cytopathic effects started to appear on day 4 after virus inoculation. Maximum virus titres could only be obtained by day 7 following virus inoculation. Viruses obtained were highly cell- associated to indicate a typical characteristic of cytomegaloviruses. Upon staining in H&E, eosinophilic inclusion bodies were observed in cytoplasm and nucleus. This was confirmed by immunofluorescence and immunoperoxidase tests. Most infected cells contained numbers of viral nucleocapsids with the average size of 120nm in diameter. These viruses failed to grow in cell cultures of non-rat origin. At least 8 major and 10 minor protein species were identified by SDS-PAGE technique. The size of major viral proteins varied from 220kD to 32kD. At least 10 immunogenic proteins with the size ranging 21kD to 95kD were identified by Western Blot technique. Some of these proteins were not detected in exogenous RCMVs. Generally, at least five common immunogenic proteins identified among RCMVs. Such antigenic sharing is believed to be responsible for the high antibody cross-reaction among RCMVs. DNA fingerprinting by means of restriction endonuclease enzyme digestion and PCR techniques showed some differences among RCMVs. Results showed that local RCMVs are different from exogenous strains. By PCR, primers prepared based on the immediate-early gene-2 (IE-2) of RCMV English strain failed to amplify local RCMVs. This is attributed to differences in their DNA sequences. It has been suggested that IE-2 gene is suitable for the insertion and expression of foreign genes (Beisser et al. 1998). Since IE-2 gene is not essential for the virus growth, its modification would not affect the overall virus infectivity and immunogenicity in the host. In a different study, the gene that encodes zona pellucida protein-3 (ZP3) has been identified in female *Rattus argentiventer*. Rats employed were first treated with a regime of folicullar stimulating hormone (FSH). The size of the target fertility gene (ZP3) mRNAs was approximately between 1.2Kb and 1.3Kb. This gene is being manipulated and would be recombined into the IE-2 gene locus of a selected local RCMV. The recombinant virus could be disseminated via the release of infected rat colonies or by baiting. The virus would be remained persistence in infected rat population. This will ensure their rate of reproduction and population expansion is reduced to the minimum at all time.

Conclusions

RCMVs are host specific and highly prevalence in rats population in the wild. In most cases, the virus does not cause clinical disease in rats. The virus was found to be suitable for the development of an immunocontraceptive vaccine. Such vaccine could be used to immunosterilise and control the rat species. It is the aim of this study to exploit the present valuable findings towards the development of recombinant RCMV-IE2/ZP3.

References

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