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Studies of the Post Infectious Transmission of Nucleopolyhedrovirus Associated with the Circulatory System of Silkworms

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Hemocytes of open circulatory system work as immune system and protect them from pathogens. Baculovirus can infect hemocytes, and the infection of hemocytes is possibly playing the important role of systemic infection of this virus. Two systemic infection routes are supposed for the post infectious transmission of Nucleopolyhedrovirus (NPV), via hemolymph and via tracheal lymph systems. Understanding the systemic mechanism of how virions spread around the whole body and how it triggers high and sudden host's mortality is one of the important aspects of study to reduce the death rates.

In the study for chapter 1, GFP expressing recombinant *Bombyx mori* NPV was constructed and used to visualize the infected organs easily. Larvae were infected with GFP recombinant virus subcutaneously or orally, and the tissues and hemolymph were observed along with time course under fluorescent microscope. My observation confirmed previous reports that showed the cells of trachea, fat body and hemocytes are heavily infected. I found slightly earlier infection of hemocytes than tissues, and the distinctive difference of infectivity among hemocytes. Although it was confirmed that all kinds of the hemocytes expressed GFP fluorescence, plasmatocytes showed significantly lower expression of GFP compared to others (Fig. 1).

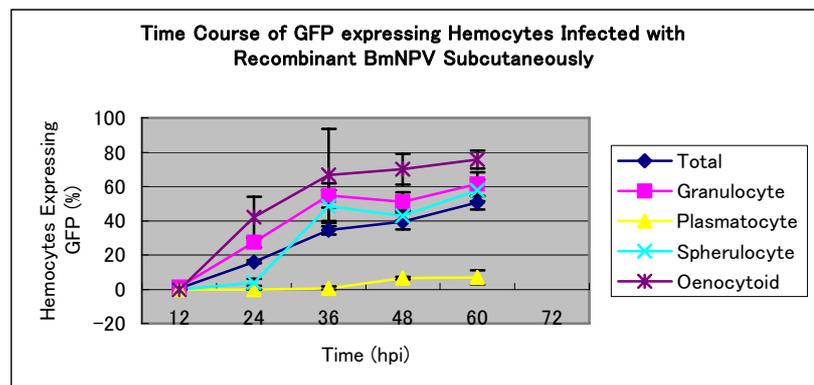


Fig. 1

In the studies for chapter 2, I observed that the latent period of orally infected larvae was elongated and the rate of survivors increased dramatically (from 0% to 60%) when antiserum against NPV was injected into hemocoel (Fig. 2). The level of IgG in hemolymph was checked by Western blotting and Dot Immuno-Binding Assay (DIBA). Although the level of IgG in hemolymph declined gradually, it remained at the detectable level for the whole experimental period. Observation of hemocytes infected with recombinant virus revealed a distinctive difference when anti-NPV serum was applied. The hemocytes of larvae treated with no or unrelated IgG started to express GFP on 2 days post infection. On the contrary, larvae treated with anti-NPV serum suppress the GFP expression (Fig.3). These results suggest that anti-NPV IgG neutralized the virions invaded into hemocoel, and the infection proceeded in only tracheal lymph. In this experiment, I succeeded to block the hemolymph infection conduit, and demonstrated the viral transmission through tracheal lymph system, which has been thought to be the main infection conduit recently. Tracheal system, however, is not enough to attack the host effectively and viral transmission through hemocytes and hemolymph was important in BmNPV infection.

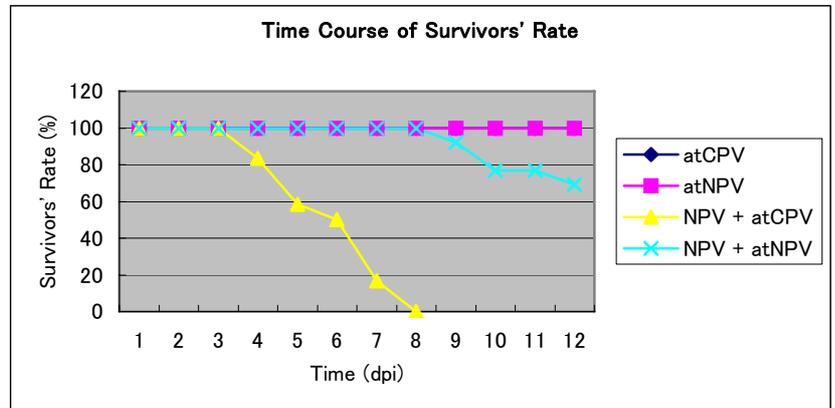


Fig. 2

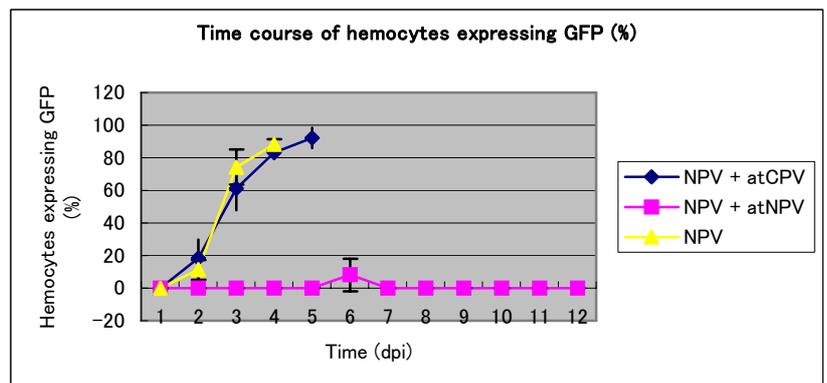


Fig.3