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Epidemiological study on hantavirus infection by capture ELISA and generation of serotyping diagnostic antigen by using recombinant hantavirus nucleocapsid protein

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Recombinant nucleocapsid proteins (rNP) of Hantaan (HTN), Seoul (SEO) and Puumala (PUU) viruses in the genus Hantavirus were prepared using a baculovirus expression system. An rNP-based capture enzyme linked immunosorbent assay (ELISA) was established and applied for a serological survey of sera from healthy residents in Hokkaido and from patients showing hematuria and pneumonia.

Truncated recombinant nucleocapsid protein (trNP), which possessed both the capture antibody (MAb E5/G6) binding site and HTN or SEO serotype-specific epitopes but lacked the common antigenic sites was applied for indirect fluorescent antibody assay (IFA) and ELISA to develop a serodiagnostic procedure that distinguished between HTN and SEO infections. The results obtained were as follows.

1. The sensitivity and specificity of the ELISA that utilized rNP-HTN, SEO and PUU were the same as those of IFA and Western blotting.

2. A total of 1,000 sera from healthy residents in Hokkaido, 25 sera from pneumonia patients and 38 sera from hematuria patients were examined for the antibody prevalence rates to HTN and PUU by rNP-based capture ELISA. About 5% of sera showed absorbance values slightly higher than the cutoff values (mean \pm 2SD) but none of them were confirmed as

positive by IFA and Western blotting.

3. Truncated rNP, which retained the HTN-specific antigenic site (trNP-HTN155), or SEO-specific antigenic site (trNP-SR155) with the capture antibody binding site, was expressed by a recombinant baculovirus vector.

4. Insect cells (High Five cells) expressing trNP-HTN155 or trNP-SR155 were applied for serotyping in IFA. The immune sera to HTN and SEO, and patient sera with HTN or SEO infection showed stronger IFA intensity in response to the homologous antigen than those obtained with heterologous antigen.

5. The trNP-HTN155 antigen had a slight reaction to serum with HTN infection by capture ELISA. It seemed that trNP-HTN155 antigen retained less antigenicity than trNP-SR155 antigen.

6. Capture ELISA, that utilized trNP-SR155 antigen reacted strongly to patient sera with SEO infection but no reaction was observed in patient sera with HTN infection. These results indicated that capture ELISA with trNP-SR155 was useful to discriminate between HTN and SEO infections.

The combination of rNP- and trNP-based ELISA could provide rapid, sensitive, safe, and useful serological diagnosis for hantavirus infection