Temperature-sensitive HVJ (Sendai virus) with altered P polypeptide derived from persistently infected cell lines

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Temperature-sensitive HVJ (Sendai Virus) with Altered P Polypeptide Derived from Persistently Infected Cell Lines

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SUMMARY

HVJ isolated from culture fluids of G2, THEL and GM2 cells persistently infected with HVJ (G2-HVJ, THEL-HVJ and GM2-HVJ) were characterized in comparison with wild-type HVJ (HVJo). Viral structural proteins were analysed by 10% SDS–polyacrylamide gel electrophoresis and it was found that only the P polypeptides of all the HVJ clones isolated from G2-HVJ cells had a smaller size mol. wt. of 77 000 (77K), than that of HVJo with a mol. wt. of 79000 (79K). One of six clones from THEL-HVJ cells and one of ten clones from GM2-HVJ cells exhibited the same migration pattern of P polypeptide as that of the clones from G2-HVJ cells. However, the other structural proteins were not different from those of the wild-type virions. All the clones from these carrier cultures were temperature-sensitive and were blocked in early step(s) required for RNA synthesis. These results indicate that some mutations(s) associated with P polypeptide could occur during the course of HVJ persistent infection in cell cultures.

Many studies have already been reported on characterization of mutants recovered from paramyxovirus carrier cultures. As concerns mutations of viral structural proteins among them, M proteins of *ts* mutants from BHK cells persistently infected with HVJ were larger in mol. wt. than that of the wild-type virus (Kimura *et al.*, 1979; Yoshida *et al.*, 1979). Similar findings in measles virus carrier cultures were reported by Wechsler *et al.* (1979). In this communication we describe studies on mutants with altered P protein isolated from different HVJ persistently infected cell lines.

Two carrier cultures, G2 (from human giant cell tumour)-HVJ cells and THEL (from golden hamster embryonic lung)-HVJ cells, which were persistently infected with wild-type HVJ, had been passaged *in vitro* for 12 years. GM2 (from 3-methylcholanthrene-induced fibrosarcoma of inbred golden hamster)-HVJ cells were recently established by infecting with wild-type HVJ purified through plaque isolation at 39 °C. These cell lines were cultured at 34 °C in Eagle's minimal essential medium (MEM) containing 10% calf serum and 100 μg/ml kanamycin. Cultures of LLCMK2 (from rhesus monkey kidney) cells were done with a similar medium containing 5% calf serum. The Nagoya-1-60 strain of HVJ wild-type (HVJo) and HVJ mutants isolated from the carrier cultures were propagated by allantoic inoculation of 10-day-old embryonated eggs at 37 °C for 3 days and at 32 °C for 7 days respectively. Plaque formation of HVJ on LLCMK2 cell monolayers was carried out by addition of 1 μg/ml *N*-acetyltrypsin (Sigma) to the agar overlay medium (Sugita *et al.*, 1974). Haemagglutinin was titrated by a microtitre technique according to the procedure of Sever (1962). The method of fluorescent antibody staining was performed as described previously (Ogura *et al.*, 1980). Labelling of virus particles was carried out by adding 10 μCi/ml [^{35}S]methionine (750 Ci/mmol; The Radiochemical Centre, Amersham) to virus-infected LLCMK2 cells at 48 h after infection. The culture medium was harvested at 72 h after infection and labelled virus was purified by ultracentrifugation. SDS–polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (1970) and subsequent

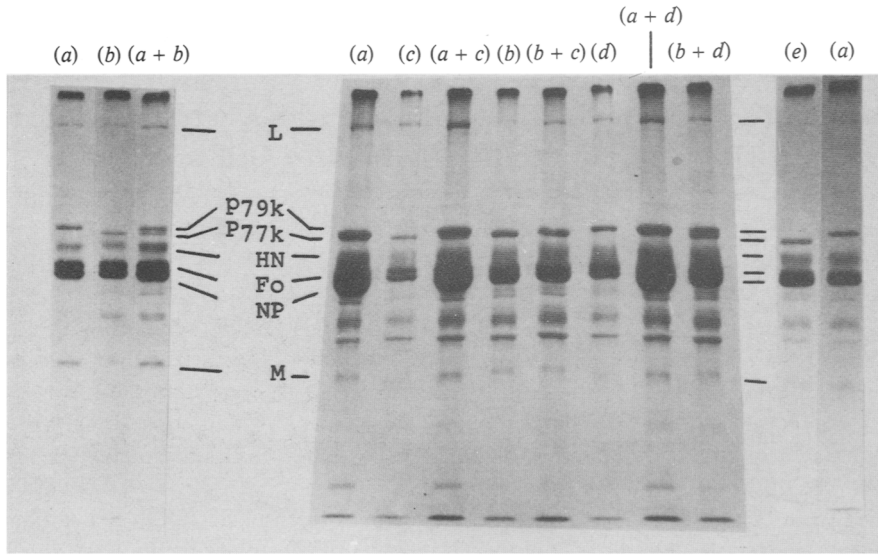

Fig. 1. SDS–PAGE of: track *a*, HVJo; track *b*, HVJpiG cl.14; track *c*, HVJpiT cl.27; track *d*, HVJpiT cl.15; track *e*, HVJpiGM cl.5006. Infected LLCMK2 cells were incubated at 32 °C for 48 h and then labelled with [^{35}S]methionine (10μCi/ml) for 3 h. At 24 h after labelling, virus released into medium was harvested and purified. The virus samples were subjected to electrophoresis and processed for autofluorography. The designation for virus polypeptides was the one described by Scheid & Choppin (1974). Migration is from top to bottom.

detection of [^{35}S]methionine in gels was carried out by autofluorography as described by Bonner & Laskey (1974), using dimethyl sulphoxide and 2,5-diphenyloxazole. The recording was done on Kodak X-Omat film. Analysis of virus-specific RNA synthesis was performed according to the method described by Portner & Kingsbury (1972).

Viruses in the culture fluids from confluent monolayers of each carrier culture were cloned by three successive isolations of plaques. Isolated clones from G2-HVJ, THEL-HVJ and GM2-HVJ cells were designated as HVJpiG, HVJpiT and HVJpiGM respectively. Viral polypeptides of the clones were analysed by 10% SDS–PAGE of [^{35}S]methionine-labelled virions from lytically infected LLCMK2 cells. As shown in Fig. 1, only the P polypeptide of HVJpiG cl.14 (Fig. 1, track *b*) migrated faster than that of HVJo (Fig. 1, track *a*) and their apparent mol. wt. were estimated to be 77000 for HVJpiG cl.14 and 79000 for HVJo. All five clones isolated from G2-HVJ cells showed the same electrophoretic patterns. In six clones from THEL-HVJ cells, P polypeptide of HVJpiT cl.27 alone (Fig. 1, track *c*) was from the result of the mixture of the both samples (Fig. 1, track *a* + *c* and track *b* + *c*) to be smaller in mol. wt., and to the same degree as HVJpiG cl.14. No significant differences in electrophoretic patterns could be observed between the other five clones of HVJpiT, such as HVJpiT cl.15 (track *d* in Fig. 1) and HVJo. HVJpiGM cl.5006 (track *e* in Fig. 1), one of ten clones isolated from the culture fluid at the 50th passage of GM2-HVJ cells, showed similarly faster migration of P protein as HVJpiG. However, no mutants with altered P polypeptide could be isolated from the culture fluids at the 12th and the 31st passage of GM2-HVJ cells.

By partial digestion with either *Staphylococcus aureus* V8 protease or chymotrypsin, altered P protein of these mutants yielded patterns closely similar to that of HVJo. The peptide patterns obtained from HN as well as other major proteins (Fo, NP and M) also showed no detectable variations (data not shown).

In addition to the biochemical alterations, the biological properties of these clones were studied compared with the parent HVJ wild-type. Monolayer cultures of LLCMK2 cells were

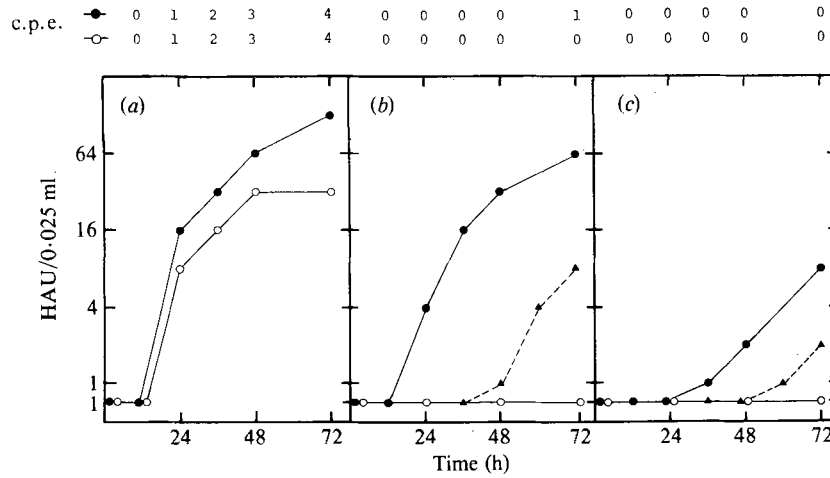

Fig. 2. One-step growth curves for the wild-type virus, HVJo and the *ts* mutants in LLCMK2 cells at 32 °C and 39 °C. Haemagglutinin (measured as haemagglutinating units) was assayed in the culture medium. (*a*) HVJo; (*b*) HVJpiG cl.14; (*c*) HVJpiT cl.27. ●, 32 °C; ○, 39 °C; ▲, shift down from 39 °C to 32 °C at 24 h after infection. The extent of c.p.e. was scored as 0, 1, 2, 3 or 4 (degeneration involving: 0, 25%, 25 to 50%, 50 to 75% or 75 to 100% of the cells respectively).

infected with HVJo and the clones at an input multiplicity of approx. 5 p.f.u./cell and were incubated in serum-free Eagle's MEM at 32 °C or 39 °C. At the appropriate times after infection, culture fluids were harvested and assayed for haemagglutinin. HVJo grew equally well at both temperatures (Fig. 2*a*). Replication of HVJpiG cl.14 and HVJpiT cl.27 proceeded more slowly at 32 °C than HVJo, especially in HVJpiT cl.27, but at 39 °C no detectable haemagglutinin in the culture fluids could be found. When the incubation temperature was shifted down from 39 °C to 32 °C at 24 h after infection, haemagglutinin was first detected after an additional 24 h for HVJpiG cl. 14 and after 36 h for HVJpiT cl.27 following the temperature shift (Fig. 2*b*, *c*). These results suggest that HVJpiG cl.14 and HVJpiT cl.27 are temperature-sensitive (*ts*) mutants and that *ts* defects may reside at early step(s) of replication. The other clones derived from the three carrier cultures were also the similar type of *ts* mutants. In addition, the infection of LLCMK2 cells with these *ts* mutants exhibited little or no obvious cytopathic effects (c.p.e.) at either temperature but differed greatly from HVJo-infected cells (Fig. 2).

By fluorescent antibody staining or radioimmunoprecipitation and SDS–PAGE, it was confirmed that no viral proteins could be synthesized in the cells infected with each clone of the *ts* mutants at the non-permissive temperature. The analysis of virus-specific RNA synthesis proved that incorporation of [^{3}H]uridine into 18S and 50S RNA in the *ts* mutant-infected cells was rapidly stopped at the non-permissive temperature. This analysis also showed reduced amounts of RNA synthesis by the *ts* mutants even at permissive temperature when compared with that of HVJo (data not shown). The above results indicate that these *ts* mutants are defective in functions required for virus-specific RNA synthesis in replication.

Some of the HVJ clones isolated from the three carrier cultures showed faster P protein mobility in SDS–PAGE compared with that of HVJo. The altered migrations of P proteins in this study were the result of viral genetic mutations since all these clones were isolated by plaque formation. Mutants with slow mobility of M protein were reported to be isolated from HVJ carrier cells (Kimura *et al.*, 1979; Yoshida *et al.*, 1979). Wechsler *et al.* (1979) observed altered mobilities in three (H, NP and M) of the four major viral proteins of measles virus in persistently infected cells. Our findings show that P protein also could mutate in mol. wt.

during the course of persistent infection in cell cultures. In addition to this biochemical difference, the *ts* mutants were unable to synthesize viral RNAs at the non-permissive temperature. Moreover, these viruses showed slow growth, weak cytopathogenicity and reduced amounts of RNA synthesis even at the permissive temperature.

It is now generally accepted that a function of the P polypeptide may involve nucleocapsid-mediated mRNA synthesis with the L polypeptide, since several enzymic activities are required for this process (Raghow & Kingsbury, 1976). It is not known what kind of altered RNA synthetic functions might result from the decrease in P protein mol. wt. of our present mutants. However, it may possibly be related to *ts* defectiveness in RNA synthesis or reduced RNA synthesis activity at permissive temperature. Our finding that mutants with an altered P protein could be isolated from three different HVJ carrier cell lines seems to suggest that functions for which P protein is responsible may be variable in the process of establishment of HVJ persistent infection. Wechsler *et al.* (1979) noted that virus persistently infected cells might undergo an extensive genetic change since point mutations usually were not reflected as alterations in electrophoretic mobility of proteins. Kimura (1979) was able to detect some differences in tryptic peptide analysis of the M protein between an M defective *ts* mutant with slow mobility of the M protein and the wild-type HVJ, although we found no differences between normal (HVJo) and altered P proteins by partial protease digestion. However, it is still unclear what kind of altered functions of M protein occur as a result of its variation in mol. wt.

It is of interest that our mutants showed little or no cytopathogenicity and easily established a persistent infection at permissive temperature. It is tempting to speculate about a possible role of reduced RNA synthetic activity in establishing HVJ persistent infection. Major characteristics common to biological mutations of viruses isolated from persistently infected cell cultures of paramyxoviruses by many investigators are temperature sensitivity and weak cytopathogenicity. However, how *ts* characteristics—although the *ts* steps may be different from each other depending on differences in the virus–cell system – are linked to weak cytopathogenicity or establishment and maintenance of persistent infection at permissive temperature still needs further clarification. In addition, it also remains to be shown how virus-specific RNA synthesis and protein synthesis are regulated in persistently infected cells at the permissive temperature without proceeding to c.p.e., and what kind of variations are responsible for the functional defects of *ts* mutants at non-permissive temperature.

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REFERENCES

BONNER, W. M. & LASKEY, R. A. (1974). A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *European Journal of Biochemistry* **46**, 83–88.

KIMURA, Y. (1979). Tryptic peptide analysis of the structural proteins of a temperature-sensitive mutant derived from an HVJ (Sendai virus) carrier culture. *Archives of Virology* **61**, 157–161.

KIMURA, Y., ORVELL, C. & NORRBY, E. (1979). Characterization of the polypeptides synthesized in cells infected with a temperature-sensitive mutant derived from HVJ (Sendai virus) carrier culture. *Archives of Virology* **61**, 23–33.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.

OGURA, H., SATO, H., TANAKA, J., HATANO, M., FUKUDA, S. & MORITA, O. (1980). Relationship between tumor formation and cell-mediated immunity in hamsters with transplanted HVJ (Sendai virus)-carrying tumor cells. *Gann* **71**, 325–332.

PORTNER, A. & KINGSBURY, D. W. (1972). Identification of transcriptive and replicative intermediates in Sendai virus-infected cells. *Virology* **47**, 711–725.

RAGHOW, R. & KINGSBURY, D. W. (1976). Endogenous viral enzymes involved in messenger RNA production. *Annual Review of Microbiology* **30**, 21–39.

SCHEID, A. & CHOPPIN, P. W. (1974). Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. *Virology* **57**, 475–490.

SEVER, J. L. (1962). Application of a microtechnique to viral serological investigations. *Journal of Immunology* **88**, 320–329.

SUGITA, K., MARU, M. & SATO, K. (1974). A sensitive plaque assay for Sendai virus in an established line of monkey kidney cells. *Japanese Journal of Microbiology* **18**, 262–264.

WECHSLER, S. L., RUSTIGIAN, R., STALLCUP, K. C., BYERS, K. B., WINSTON, S. H. & FIELDS, B. N. (1979). Measles virus-specified polypeptide synthesis in two persistently infected HeLa cell lines. *Journal of Virology* **31**, 677–684.

YOSHIDA, T., NAGAI, Y., MAENO, K., IINUMA, M., HAMAGUCHI, M., MATSUMOTO, T., NAGAYOSHI, S. & HOSHINO, M. (1979). Studies on the role of M protein in virus assembly using a *ts* mutant of HVJ (Sendai virus). *Virology* **92**, 139–154.

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