

## Two Outbreaks of Herpes Simplex Virus Type 1 Nosocomial Infection among Newborns

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**Two outbreaks of herpes simplex virus type 1 (HSV-1) infection occurred in three newborns at each of two hospitals, and two of the infants in each case died of disseminated HSV-1 infection. Restriction endonuclease profiles of HSV-1 DNAs isolated from the three in each instance were essentially identical, indicating that they were epidemiologically related. In the first instance, each of three infants born in the same hospital at intervals of approximately 2 years or 1 year was infected with HSV-1. In this case, it was suggested that periodically reactivated HSV-1 strains from hospital personnel had been transmitted to three newborns. In the second instance (infants G-36, G-37, and G-38), the radiant warmer that had been occupied by infant G-36, who was infected with HSV-1, was used for infant G-37. Infant G-38 was in another radiant warmer 2 m from the radiant warmer occupied by infant G-37. Therefore, it was suggested that the virus had possibly been transmitted via radiant warmer and by hospital personnel in the two instances.**

The variability in restriction endonuclease (RE) cleavage profiles of herpes simplex virus type 1 (HSV-1) isolates (5, 16, 17) has been used to trace the spreading of the virus from one individual to another (2, 4, 8-10, 18). These methods are based on the observations that no two epidemiologically unrelated isolates of HSV were identical in RE profiles and that, conversely, if the viruses isolated from different individuals could not be differentiated from each other, then the infection could be concluded to have been caused by transmission of the virus from one individual to the other. Using the RE cleavage analysis, Buchman et al. (2) and Adams et al. (1) showed that isolates from personnel and patients in a hospital could be categorized into two different strains, indicating transmission from patients to personnel.

We have recently established a practical method for the differentiation of strains by using unlabeled DNA from HSV-infected cells (23) and shown that RE profiles of paired isolates obtained from siblings were essentially identical (20). Using this method, we analyzed RE profiles of HSV-1 isolates obtained from three newborn infants at each of two hospitals and presented the evidence that the isolates were epidemiologically related.

In the first instance, at intervals of 2 years and 3 months or 1 year and 6 months from 1980 to 1983, the occurrences of herpetic diseases in three newborns (infants 1, 2, and 3) were observed in association with the Perinatal Hospital (PNH) in Sapporo, Japan.

**Case 1.** Infant 1 was born at PNH on 9 February 1980. Because of fever, she was transferred to the Sapporo City General Hospital on day 3 of birth. On day 10 of life, hepatosplenomegaly and bleeding diatheses developed. On day 11, infant 1 expired with a disseminated herpetic infection. HSV-1 was isolated postmortem from the liver.

**Case 2.** Two years and 3 months after infant 1 expired, infant 2 was born at PNH. Because of fever, he was transferred to the Sapporo City General Hospital on day 4

after birth. After developing hepatomegaly, seizures, and lethargy, he also died of a disseminated form of herpes infection on day 13 of life. HSV-1 was isolated from a throat swab 3 days before his death and from postmortem samples of the lung, liver, and adrenal gland.

**Case 3.** Infant 3 was also born at PNH 1 year and 6 months after infant 2 expired. Because of poor feeding and fever, he was transferred to another hospital on day 6 of life. On day 7, infant 3 had evidenced skin vesicles in the neck, back, and gluteal sites. On day 21 of life, the skin lesions had completely disappeared. Viral cultures of vesicular fluid of the neck and gluteal sites were positive for HSV-1.

The mothers of these newborn infants were not related and their residences were very distant from each other. After the time of deliveries, vaginal swabs of these mothers were negative for the cultivation of HSV. It was not clear whether personnel who contacted these newborns in the PNH had herpetic lesions.

In the second instance, in January 1983, each of three neonates (G-36, G-37, and G-38) was born at different hospitals in Aichi Prefecture, Japan, and was transferred to the Neonatal Intensive Care Unit in Aichi Prefecture because of respiratory distress syndrome.

**Infant G-36.** At the time of delivery, the mother of infant G-36 had herpetic lesions at her genital site. On day 6 after birth, infant G-36 was moved to a radiant warmer (tentatively designated as radiant warmer I). He was kept in radiant warmer I until he died of a disseminated herpes infection on day 8 after birth. HSV-1 was isolated postmortem from the liver, spleen, adrenal gland, and brain.

**Infant G-37.** Infant G-37 was born 2 days after infant G-36 expired. Sixty-three hours after infant G-36 expired, infant G-37 was moved to the same and uninfected radiant warmer I that had been used for G-36. On day 12 of life, infant G-37 also expired with disseminated herpes infection. HSV-1 was isolated postmortem from the liver and adrenal gland.

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TABLE 1. Newborn infants of each instance and HSV isolates obtained from them

Infant	Sex	Date of isolation	Site of isolation	Subtype of HSV-1 <sup>a</sup>	Outcome
First instance					
1	Female	20 February 1980	Liver	G	Expired
2	Male	11 May 1982	Adrenal gland	G	Expired
3	Male	1 November 1983	Gluteal vesicle	G	Survived
Second instance					
G-36	Male	13 January 1983	Liver	A	Expired
G-37	Male	27 January 1983	Liver	A	Expired
G-38	Male	2 February 1983	Pharyngeal swab	A	Survived

<sup>a</sup> Subtypes of HSV-1 were determined by criteria published previously (19).

**Infant G-38.** On his day of birth, infant G-38 was kept in radiant warmer II, which was located 2 m from radiant warmer I, which G-37 had occupied. The day of birth of G-38 was hospital day 7 for G-37. On day 11 after birth, G-38 evidenced vesicles in the left scapular region, the left neck, and both sides of the cheek and also had a vesicle on the tongue. Two days later, viral cultures of both pharyngeal swab and vesicular fluid were positive for HSV-1. None of the personnel in contact with the infants at the Neonatal Intensive Care Unit had herpetic lesions.

#### MATERIALS AND METHODS

**Viruses, isolates, and cells.** Strain F of HSV-1 and strain G of HSV-2 were used as standard laboratory strains. Two batches of three isolates from three newborns are shown in Table 1. The isolates were obtained by using Vero cells (Flow Laboratories, Rockville, Md.).

**Preparation of infected-cell DNA.** Confluent cultures of Vero cells were infected with each isolate at a multiplicity of infection of 5 to 20 PFU per cell and incubated at 36°C for 18 to 24 h. When the cells showed the maximum cytopathic changes, they were harvested for infected-cell DNA. Infected-cell DNA was prepared as previously described (19, 20, 23).

**REs and agarose gel electrophoresis.** *Bam*HI, *Kpn*I, *Sal*I, *Hind*III, and *Eco*RI were purchased from Takara Shuzo Co., Kyoto, Japan. Infected-cell DNA was digested with 5 U of each restriction enzyme per µg of DNA at 37°C for at least 8 h. Other details were the same as described previously (19, 20), except that the *Hind*III reaction buffer contained 20 mM Tris hydrochloride (pH 7.5), 7 mM MgCl<sub>2</sub>, 60 mM NaCl, and 2 mM 2-mercaptoethanol and the *Eco*RI reaction buffer contained 100 mM Tris hydrochloride (pH 7.5), 10 mM MgCl<sub>2</sub>, and 50 mM NaCl.

**Southern blot hybridization.** To verify the fragments of HSV-1 isolates, clones of recombinant plasmids containing HSV-1 (F) *Bam*HI fragments (15) were used as probes. The clones were donated by Bernard Roizman, the Marjorie B. Kolver Oncology Laboratories, the University of Chicago, Ill. The methods for the transfer of electrophoretically separated DNA fragments to nitrocellulose and the hybridization with <sup>32</sup>P-labeled plasmid probe were reported elsewhere (7).

**Determination of HSV-1 subtypes.** Subtypes of HSV-1 were determined by criteria described previously (19).

#### RESULTS

**RE analysis of infants 1, 2, and 3.** The comparisons between standard strain F of HSV-1 and the isolates from

infants 1, 2, and 3 from the first instance are shown in Fig. 1 and 2. RE profiles of infected-cell DNA showed that all three isolates from the first instance were HSV-1 (11) and were classified into subtype G of HSV-1 (19).

The shared characteristics of RE profiles in the three isolates from the first instance were as follows (Fig. 1 and 2; see Fig. 4), leading to the conclusion that these isolates were essentially identical. In the *Bam*HI digestion, three common

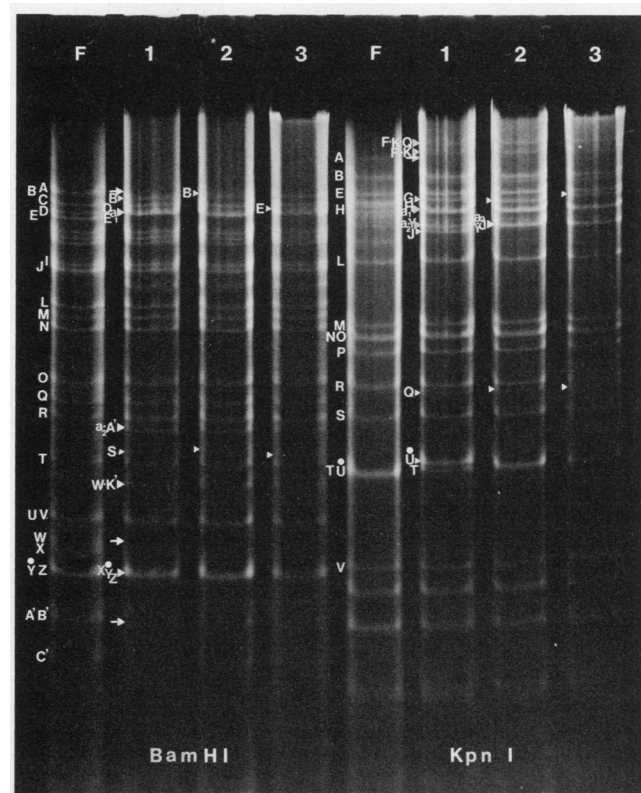


FIG. 1. RE profiles (with *Bam*HI and *Kpn*I) of standard laboratory strain F and the isolates from infants 1, 2, and 3 (lanes F, 1, 2, and 3, respectively). Each infected-cell DNA (20 µg) was cleaved with restriction enzymes as described in Materials and Methods. The fragment nomenclature is that of strain F (11). Triangles indicate shared and characteristic fragments for these isolates. Larger triangles indicate fragments in the unique regions, whereas the smaller triangles indicate fragments containing portions of or entire repeated regions of genome. Arrows indicate the fragments existing in strain F but missed in the other three strains. Small letters show the fragments generated by the new cleavage sites on the relevant fragments. Also, the white circle indicates a 2 M fragment.



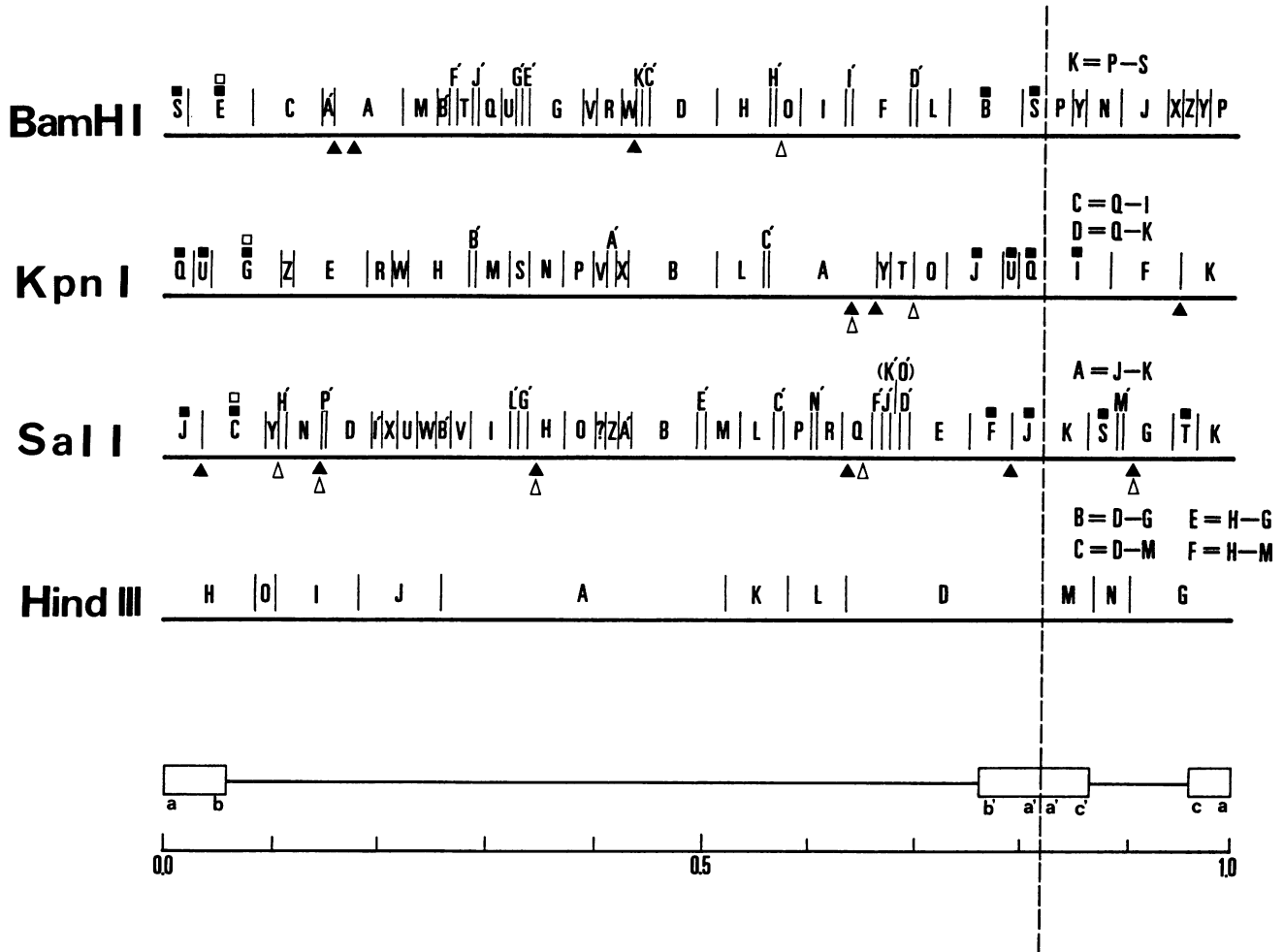


FIG. 4. The *Bam*HI, *Kpn*I, *Sall*I, and *Hind*III cleavage map of HSV-1, strain F. The *Bam*HI, *Kpn*I, and *Sall* map was based on one by Locker and Frenkel (11) and Post et al. (15). The *Hind*III map was based on the *Hsu*I map of strain F by Roizman and Buchman (17). Triangles below the each map identify the position of the gain or loss of cleavage sites that show the variation of three HSV-1 isolates of the first instance (▲) or the second instance (△) compared with strain F. Squares above the each map indicate the fragments that show the size variation among three isolates of the first instance (■) or the second instance (□).

isolates from the second instance were classified as HSV-1 subtype A. Because of the low incidence of subtype G HSV-1 isolates obtained in Japan (19), it was suggested that the three subtype G isolates were possibly epidemiologically related.

Further and more fine cleavage analysis of each genome with five REs showed that three isolates of the first or the second instance were essentially identical, indicating that they were epidemiologically related, because of having many shared characteristics in their particular RE profiles. From the frequency of the gain or loss of RE sites (19, 21), for example, it was calculated that the possibility of infection occurring incidentally in triplicate by these strains showing each RE profile of the first or second instance was less than  $10^{-20}$  or  $10^{-15}$ , respectively.

However, among three related isolates, variation in the mobility of *Bam*HI-B, -E, and -S; *Kpn*I-G, -J, -Q, and -U, and *Sall*-C, -F, -J, -S, and -T could be observed. Cleavage maps of HSV-1 strain F DNA (11) indicate that these fragments are located at similar regions of each map and are derived from the region spanning the unique-repeat junction and repeated regions of HSV-1 genome. The sizes of these

fragments are known to vary in recurrent isolates from a single individual (12, 13, 23), in paired isolates from siblings (20), and in plaque-purified stocks of a single virus strain (6, 18). Therefore, size differences of the fragments at the regions should be excluded from the criteria for determining whether isolates are epidemiologically related or unrelated, as proposed previously (20). Also, in the gain or loss of cleavage sites which is essential for strain differentiation, there was no difference among three isolates of each case. From the above facts, we concluded that the three isolates originated from the same source.

The three HSV-1 isolates of the first instance were obtained from each of three newborn infants born in the same hospital (PNH) at intervals of approximately 2 or 1.5 years. The primary route of transmission to the newborns appears to be contact with genital herpes of mothers (9, 14, 22), suggesting the second instance in this paper. However, HSV could not be obtained from the genital tracts of the mothers after the time of delivery. None of the mothers of the infants were related, nor did they have any contact with each other.

Unlike other viruses, HSV can be transmitted to new contacts upon reactivation of HSV carriers, and the virus is

transmitted only by direct contact with herpetic lesions (4, 16, 17). Furthermore, analysis of the RE profile of HSV revealed that isolates from the same individual could be the same except for a few cases (3, 12, 13, 23, 24). Therefore, it was concluded for the first instance that the periodically reactivated HSV-1 strains of a member hospital personnel had been transmitted to three newborns, although it was not clear whether such a hospital employee who had contact with the three newborns had herpetic lesions. Thus, this case seemed to be the transmission of a particular nosocomial infection, for which the lack of attention of medical personnel was held responsible.

Three strains of the second instance were isolated from three newborns infected with HSV-1 in the same room of a hospital within a 1-month period. Since the mother of infant G-36 had herpetic lesions at her genital site at the time of delivery, this maternal infection was considered to be the source for G-36 infection, although samples of virus from the mother were not obtained for study. The same radiant warmer occupied by G-36 was used for G-37 without disinfection 63 h after G-36 expired. Therefore, the possibility was supported that the same strain was transmitted from G-36 to G-37 via a radiant warmer. Since infant G-38 was in another radiant warmer 2 m from G-37, it was supported that a strain had been transmitted from G-37 to G-38 via the personnel of the hospital unit.

Until recently, definite evidence of HSV transmission from person to person could not be obtained since differentiation between epidemiologically related and unrelated isolates was not possible. However, in several recent studies, RE analysis of HSV DNA has provided clear evidence to determine whether infections were epidemiologically related (1-4, 8-10, 17, 18, 20). Here, we showed that the RE profiles of HSV DNA from two outbreaks of HSV-1 nosocomial infection led to the conclusion that the source of infection was a member of the medical staff, an HSV-1 carrier who contacted the neonates, and presented the possible roles of radiant warmers and personnel in virus transmission. Because of the fatal nature of this disease in newborns (14), there should be greater concern about members of the medical staff who are HSV carriers and about HSV transmission to other newborns in the hospital.

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