Arch Virol (1992) 123: 13–27

Archives of Virology

Localization of herpes simplex virus type 1 in sebaceous glands of mice

K. Moriyama[1], S. Imayama[2], S. Mohri[3], T. Kurata[4], and R. Mori[1]

Departments of [1] Virology and of [2] Dermatology, and [3] Laboratory Animal Center
School of Medicine, Kyushu University, Fukuoka
[4] Department of Pathology, National Institute of Health, Tokyo, Japan

Accepted August 19, 1991

Summary. The distribution of HSV-1 during the development of zosteriform skin lesions in SCID mice was analyzed by immunofluorescence and electron microscopy. The virus initially appeared within certain keratinocytes, sometimes surrounded by keratinocytes whose surfaces were also positive for the antigens, in the lower epidermal layers including the hair follicles, and then extended upward to the entire epidermis and downward to the sebaceous glands 1–2 days later, when no macroscopic skin lesion was seen. The affected epidermal cells subsequently degenerated and lost their viral antigens within a day, when the zosteriform lesion then became evident. This was followed by a degeneration of the dermis. The sebaceous glands eventually degenerated in 10 days, but some glands in the necrotic skin areas preferentially retained HSV-1. The horizontal spread of the virus in the epidermis beyond the first invaded dermatome occurred much later. In mice passively immunized with specific immune serum, viral antigens were observed even 20 days after the infection in sebaceous glands in necrotized areas. Therefore, HSV-1 appears to spread first via the extracellular fluid among the keratinocytes after being shed from nerve endings, and then produces a successive degeneration of the affected keratinocytes which may prevent any further extension of horizontal viral spread. The pilosebaceous apparatus is possibly acting as a site not only for the replication of HSV-1 with a delayed cytopathic effect, but also as an area that is temporarily sheltered from host defense mechanisms.

Introduction

Herpes simplex viruses (HSV), family *Herpesviridae*, genus *Simplexvirus*, enter the peripheral terminals of the neurons at an early stage of primary lytic infection with and/or without proliferation at the primary site, and then travel with the axonal flow to the ganglia where they proliferate. Thereafter, they again travel

down the axon to produce zosteriform lesions of the dermatome. This process has been well demonstrated in mouse models by both macroscopic observation and virus titration [15, 18, 19]. However, few studies on acute cutaneous infection have identified the precise sites of viral proliferation; HSV appeared in the epidermis, which suggests a shedding of the virus from the peripheral nerve endings with a successive viral spread into the dermis [19].

Along with the latent ganglionic infection [20], the persistence of HSV in the skin has been well demonstrated in animals [16]. Moreover, some investigators have reported details of cutaneous latency in mice and guinea pigs [5, 9]. Therefore, a more detailed histological analysis of these cutaneous lesions would also be valuable for a better understanding of the pathogenesis as well as the clinical manifestations of HSV-1.

Murine skin consists of the epidermis, pilosebaceous appendages, and the dermal connective tissue containing vascular and neural networks. Epidermal basal cells proliferate and differentiate to produce either a keratinized layer of the skin surface or pilosebaceous structures. Recent reports describe the localization of stem cells in the bulge area of hair follicles; derivatives of these dormant cells are presumed to regulate the hair cycle [7]. Fibroblasts are present in the connective tissue which forms a physical barrier against extracorporeal organisms [10, 11]. Nevertheless, it is not completely understood how these structures and cell functions are related to the pathogenesis of HSV infection.

Mice with severe combined immunodeficiency (BALB/c C57BL/Ka-Igh-1^b/Icr, SCID mice) carry no detectable immunoglobulin [2]. Therefore, clearer results can be obtained by the immunohistological examinations of tissues from this strain. Syngeneic murine immunoglobulins (Ig) can also be traced histologically when administered to such mice. In addition, we have preliminarily reported that the *scid* mutation did not influence the proliferation of HSV-1 [14]. Thus, because of defects in the development of T- and B-cells (for reviews, see [4]), one would expect to observe the in vivo behaviour of viruses under conditions in which no specific immune response takes place.

In the present study, using SCID mice, we demonstrated that the histological localization of HSV-1 in skin during the development of the zosteriform skin lesion was unexpectedly different from the corresponding morphological changes, and that HSV-1 was predominantly localized in the sebaceous glands, sometimes until after a total cutaneous degeneration had occurred.

Materials and methods

Virus

HSV-1, strain 7401H, isolated from the vesicular skin lesions of an adult patient with herpes labialis and formerly designated as Hayashida strain [15], was passaged 7 times in Vero cells. The virus was propagated by infecting the Vero cells at 0.1 PFU per cell. The infected cultures were incubated at 37 °C for 3 days until a complete cytopathic effect (CPE) was observed. The cells and medium were then harvested, sonicated at 150 W for 5 min (Insonator MR 590, Kubota, Tokyo, Japan) to release the cell-associated virus and centrifuged at

500 × **g** for 5 min. The supernatant was then poured off and stored at − 80 °C. Serial 10-fold dilutions of virus fluid were plated in Vero cell monolayers cultured in MEM with 5% calf serum in wells 25 mm in diameter. The cultures were then fixed with 10% formaldehyde 72 h after infection, stained with crystal violet, and the plaques were counted.

Mice

CB-17 inbred strain, BALB/c C57BL/Ka-Igh-1^b/Icr (N17F34), mice homozygous for the SCID mutation, provided by Dr. Melvin J. Bosma (Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA, U.S.A.) were maintained in a specific pathogen-free breeder. For the experiment, 6- to 9-week-old mice were housed under partial barrier conditions and given sterilized water and food. Under these circumstances, staphylococci can be isolated from the normal skin surface at any time. To confirm that our mice had severe combined immunodeficiency [3], Ig levels in the serum obtained from a tail vein 1 week before virus inoculation were quantified by a sandwich enzyme-linked immunosorbent assay (ELISA). Microplates pre-coated with goat anti-mouse Ig (MBL, Tokyo, Japan) overnight at 4 °C were incubated for 1 h at 37 °C with dilutions of SCID serum or the standard and washed 5 times with PBS containing 0.05% Tween 20. Alkaline phosphatase-conjugated goat anti-mouse Ig (Cappel, Organon, West Chester, PA, U.S.A.) was then added. After 1 h of incubation at 37 °C, the plates were washed 5 times and p-nitrophenyl phosphate was then added to develop the color. The absorbance at 405 nm was quantified using a microplate reader (Microelisa MR 590, Dynatech, Alexandria, VA, U.S.A.). Pooled mouse serum of a known Ig concentration was used as the standard. Mice which had less than 1 μg/ml serum Ig were used for further experiments.

Inoculation of mice

A chemical depilation agent, Hair Remover (Shiseido, Tokyo, Japan) was applied to the left midflank area of SCID mice for 3 min and then was washed off with warm sterile water. Seven days later, the naked skin was scratched 3 times over an area of about 4 mm using a 26-gauge needle and 10 μl of the HSV-1 suspension containing 5×10^4 PFU was applied to the abraded area. The mice were then released into cages after holding them for 60 s.

Histological examination and detection of HSV-1 antigens in herpetic lesions

The mice were sacrificed at various times after inoculation. The skin lesions were immediately resected and fixed in 10% formalin-PBS. Tissues were embedded in paraffin, sectioned at 2 μm and stained with hematoxylin and eosin (HE). The method used for the indirect immunofluorescence staining of formalin-fixed specimens has been described previously [12]. In brief, serial sections were placed on slide glasses, treated with PBS containing 0.25% trypsin and 0.002% $CaCl_2$, reacted with primary antibody and then with FITC-labeled secondary antibody. The specimen, overlaid with 20% glycerin and a cover slip, was examined with a BH-2 fluorescence microscope (Olympus, Tokyo, Japan) and photographed with an Olympus exposure control unit (C-35AD, PM-CBAD, and BH2-RFL-T2) using ASA 100 reversal film (Fuji, Tokyo, Japan). Rabbit anti-HSV-1 antiserum obtained by infection with the TMK strain of HSV-1 was used as the primary antibody. FITC-conjugated anti-rabbit IgG (Miles, Weizmann, Israel) was used as a secondary antibody. Naive rabbit serum and BALB/c mice serum were sometimes used as first antibodies to exclude any cross-reactivity. Some of the specimens used in the immunofluorescence examination above were further processed for light microscopic examination through PBS washing and HE staining, while others were processed for electron microscopy as described below.

Specificity and sensitivity of indirect immunohistological examination for virus antigen

Since some host cells, gE of HSV-1 and some species of bacteria are known to possess Fc-binding activity, and since cross-reactivity may occur in this method, every antibody used in this study was compared with the sera from naive animals. When both normal and HSV-1-infected skin were reacted with naive rabbit serum and visualized with FITC-labeled secondary antibody, no specific fluorescence was observed except for round microorganisms on the surface of exsudative ulcers of herpetic lesions. These specimens were not used in any further examinations. No cross-reaction was observed when the FITC-labeled anti-rabbit IgG antibody was used alone.

Transmission electron microscopy

For electron microscopy, the remaining specimens which had been examined as described were deparaffinized, fixed with 1% osmium tetroxide in PBS for 120 min, dehydrated with graded dilutions of ethanol, and embedded in epoxy resin (Epon 812, TAAB, Berkshire, U.K.). Previous immunohistological findings enabled us to trim the possible portions in which HSV-1 presented. Thin sections were stained with uranyl acetate and lead citrate [10] and then examined with a JEOL JEM-100C electron microscope (Tokyo, Japan).

Transfer of antibodies

To observe the predisposition of the viral antigens in advanced-stage herpetic lesions, 4 mice were passively immunized with syngeneic HSV-1-specific antisera. The hyperimmune antiserum was obtained by inoculating BALB/c mice intraperitoneally with 1×10^4 PFU of a laboratory strain Ska of HSV-1 [23] 3 times at 1-week intervals. The antiserum was stored at $-30\,^{\circ}C$ until use. Each 400 µl of diluted serum with neutralizing antibody titer of 1 : 256 for 80% plaque reduction in Vero cell-monolayer was injected into the peritoneal cavity 72 h after infection.

Results

Morphological changes and localization of HSV-1 antigens during the development of zosteriform lesions

Virus antigens were detected initially in a few cells in the epidermal basal layer 48 h after infection when no morphological change was evident either macroscopically or on light microscopy (Fig. 1). When studied in detail, cells adjacent to the positive cells were also found to harbor fluorescence along the cell membrane, but not in the nucleus or cytoplasm (Fig. 1 B and C).

Zosteriform skin lesions characterized by linearly arranged, oedematous papules either with or without crusts, developed 72 h after the infection (Fig. 2 A). In the cross-sectional view of early papular lesions, fluorescence labeled HSV-1 was evident throughout the epidermis, except for the horny layer, as well as in the hair follicles and sebaceous glands (Fig. 2 B). The papule then developed into a vesicle, which was produced by the ballooning degeneration of the epidermal keratinocytes at the center of the lesion where a crust

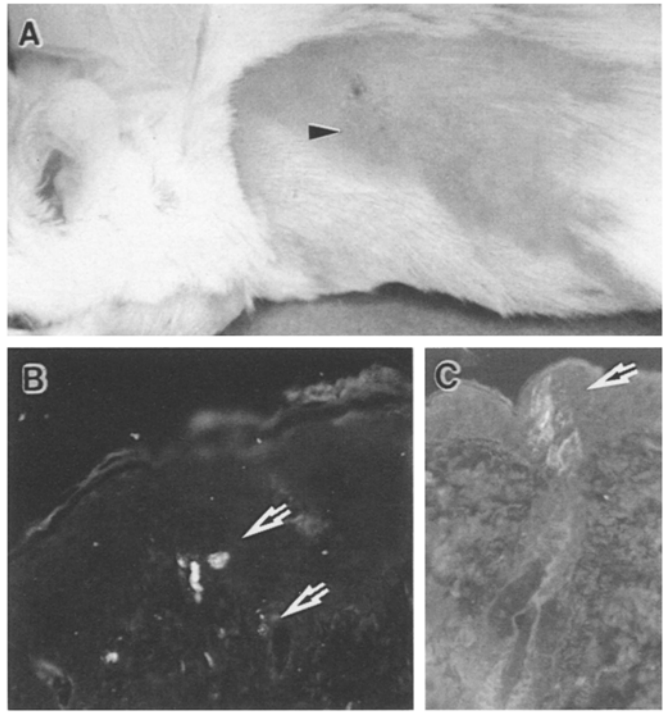
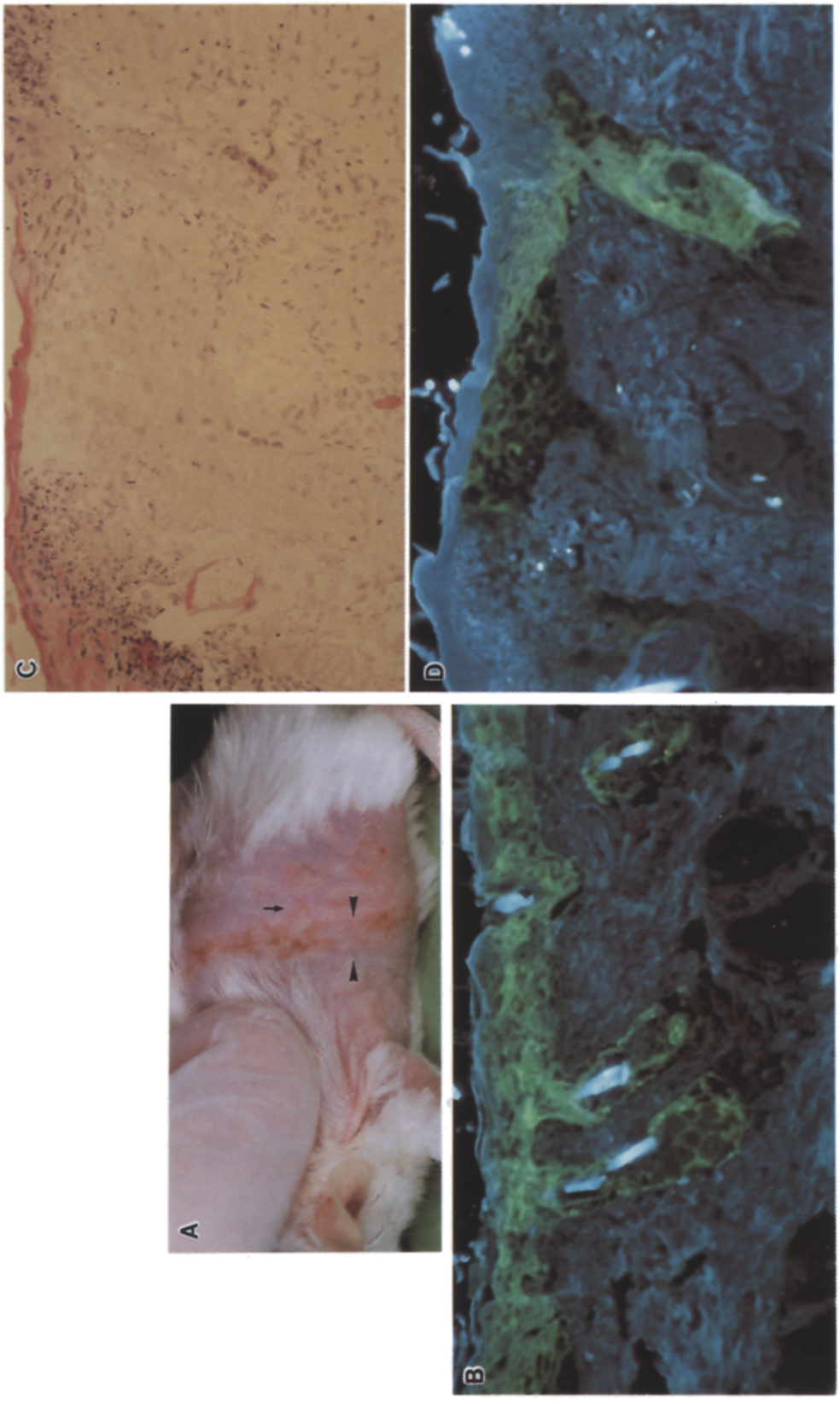


Fig. 1. Macroscopic findings and immunofluorescence for HSV-1 in cutaneous lesions 48 h post-infection. A round local lesion is present at the site of primary inoculation with HSV-1 7 days after treatment with a chemical depilation agent (**A**). A discrete part of the dermatome showing no macroscopic change (arrowhead) was removed and its sections were incubated with the HSV-1-specific antiserum. Specific fluorescence first appeared in a cell of the basal layer of the epidermis (**B**). HSV-1 antigen along the border of several adjacent keratinocytes implies humoral spreading of the virus along the interspaces of the keratinocytes (**C**)

sometimes associated (Fig. 2 C). The degenerated keratinocytes lost their fluorescence, and only the peripheral keratinocytes and the pilosebaceous appendages with a normal appearance remained positive for HSV-1-specific antibody (Fig. 2 D).

Zosteriform skin lesions developed a relatively firm, adherent crust 5 days after infection (Fig. 3 A). HE staining of the lesions showed a superficial coagulation necrosis involving the upper dermis (Fig. 3 B). Fluorescence for HSV-1 was positive only for the pilosebaceous appendages, when the upper half of which was included within the necrotized layer. Some sebaceous glands within the necrotic areas of the dermis retained viral antigens (Fig. 3 B and C). It was of interest that the fluorescence for HSV-1 was more prominent in the sebaceous glands than in the outer root sheaths surrounding the hair shaft. No fluorescence was seen in the hair germ (Fig. 3 D and E).

No significant infiltration of mononuclear cells into the dermis was observed in any specimen of naive SCID mice until their death on days 7–10. A large ulcer spanning the dermatome was observed in some mice and was presumably produced by scratching with the hind limb.


C
D
A
B

Localization and frequency of virus particles

To confirm whether the above immunohistological results reflected the multiplication of HSV-1 as well as to localize the precise distribution of the virus particles, an electron microscopic examination was performed using the tissue specimens which had previously been used for the fluorescence study. Ultrathin sections of the border zone between the necrotic and intact areas of the epidermis, and the pilosebaceous apparatus showing predominant fluorescence were examined. As shown in Fig. 4, the necrotic keratinocytes, characterized by condensed cytoplasm and irregularly aggregated tonofilaments, contained only a small number (less than 2 per section of a cell) of evaluable particles. In contrast, keratinocytes with a relatively normal appearance at the necrotic-intact border zone contained numerous 100 nm capsids in the nucleus (20–50 per section of a nucleus) and some had 180 nm virions in the cytoplasm (10–20 per section). Crystalline arrays of the nucleocapsids were rare.

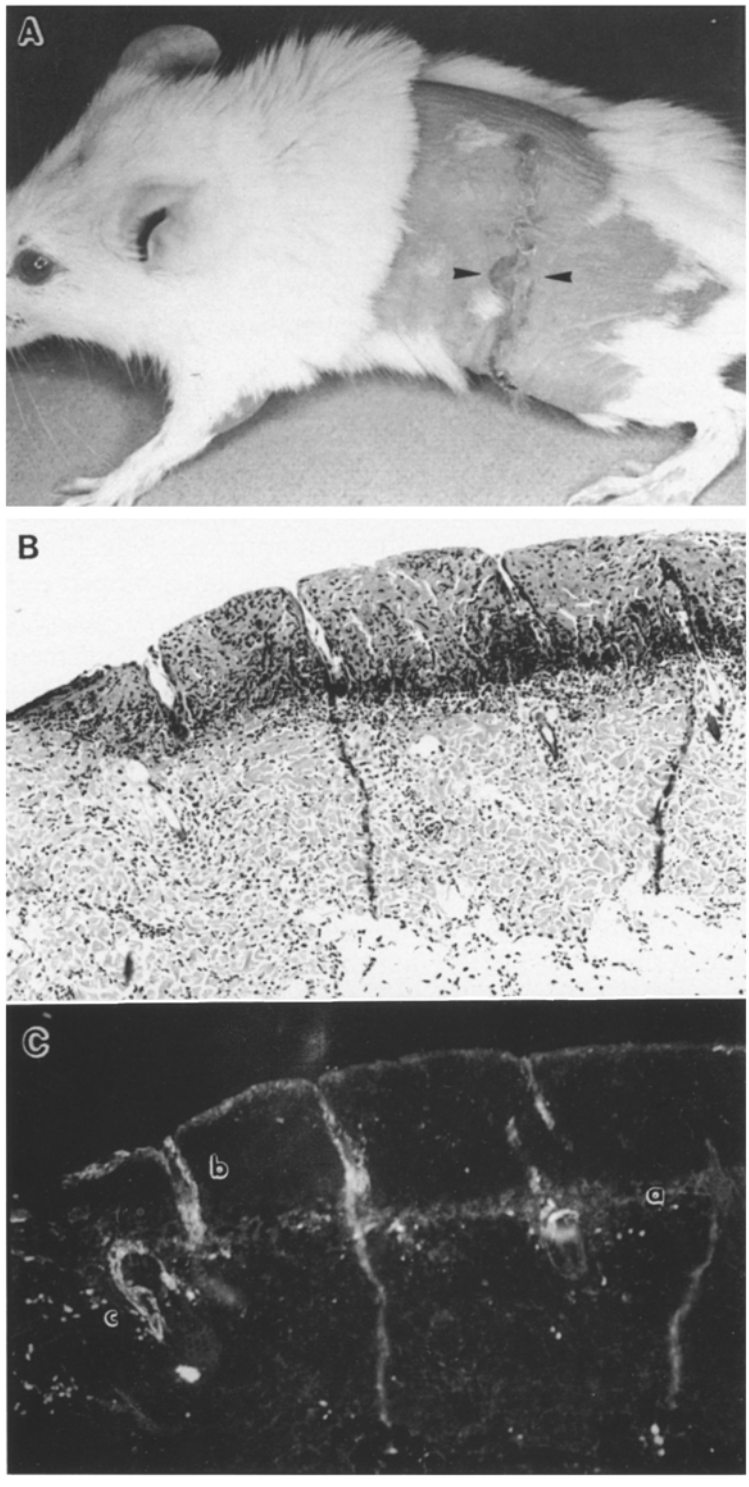
In the sebaceous cells 72 h post-infection, approximately 20 nucleocapsids per nucleus section were observed (Fig. 5). Some unenveloped particles were present in the cytoplasm of these cells at up to 10 per section, whereas enveloped virions were rarer at up to 5 per cell. Some viruses preserved their structures in degenerated cells even at the late exocrine stage. We failed to detect any free virus particles in lipid droplets. No evidence of bacterial infection was obtained in these specimens.

Preservation of HSV-1 antigens in sebaceous glands in necrotic lesions of SCID mice passively immunized with HSV-1-specific antiserum

When anti-HSV-1 antiserum was administered 72 h after infection to 4 mice, oedematous zosteriform skin lesions appeared 5–6 days later and were followed by brownish crusted lesions. All 4 mice died 18–21 days following infection without any healing of the lesions. Histological localization of the HSV-1 antigens in the early oedematous lesion was similar to that in naive mice 72 h after infection (shown above) concerning the epidermis and sebaceous cells. However, the upper and lateral spreads in the middle and outer layers of the epidermis were less than that observed in the naive mice. No morphological changes occurred in the subcutaneous tissue before death. Viral antigens were rarely seen in the dermis even in areas of degeneration. Virus antigens were detected in the sebaceous cells at death 20 days after the infection, when the

---

Fig. 2. Zosteriform skin lesion 3 days after infection. Slightly elevated oedematous macules are arranged linearly. Some are already associated with a brownish crust on their surface (**A**). The oedematous part of the lesion (between the arrowheads in **A**) exhibits viral spreading throughout the malpighian layer of the epidermis and pilosebaceous appendages (**B**). Both an HE-stained and an immunofluorescent continuous section obtained from the border of an advanced lesion (arrow in **A**) show that viral antigens are present only in the morphologically intact epidermis, not in the degenerated epidermal area (**C** and **D**)


A
B
C
b
a
c

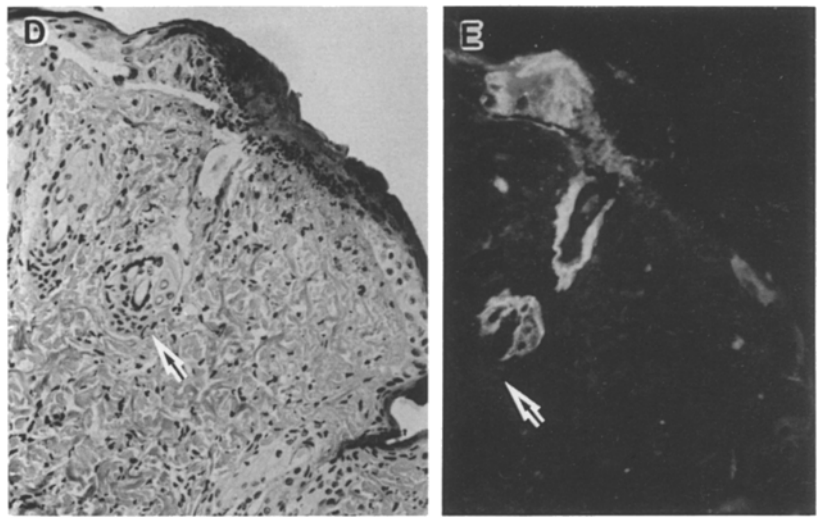
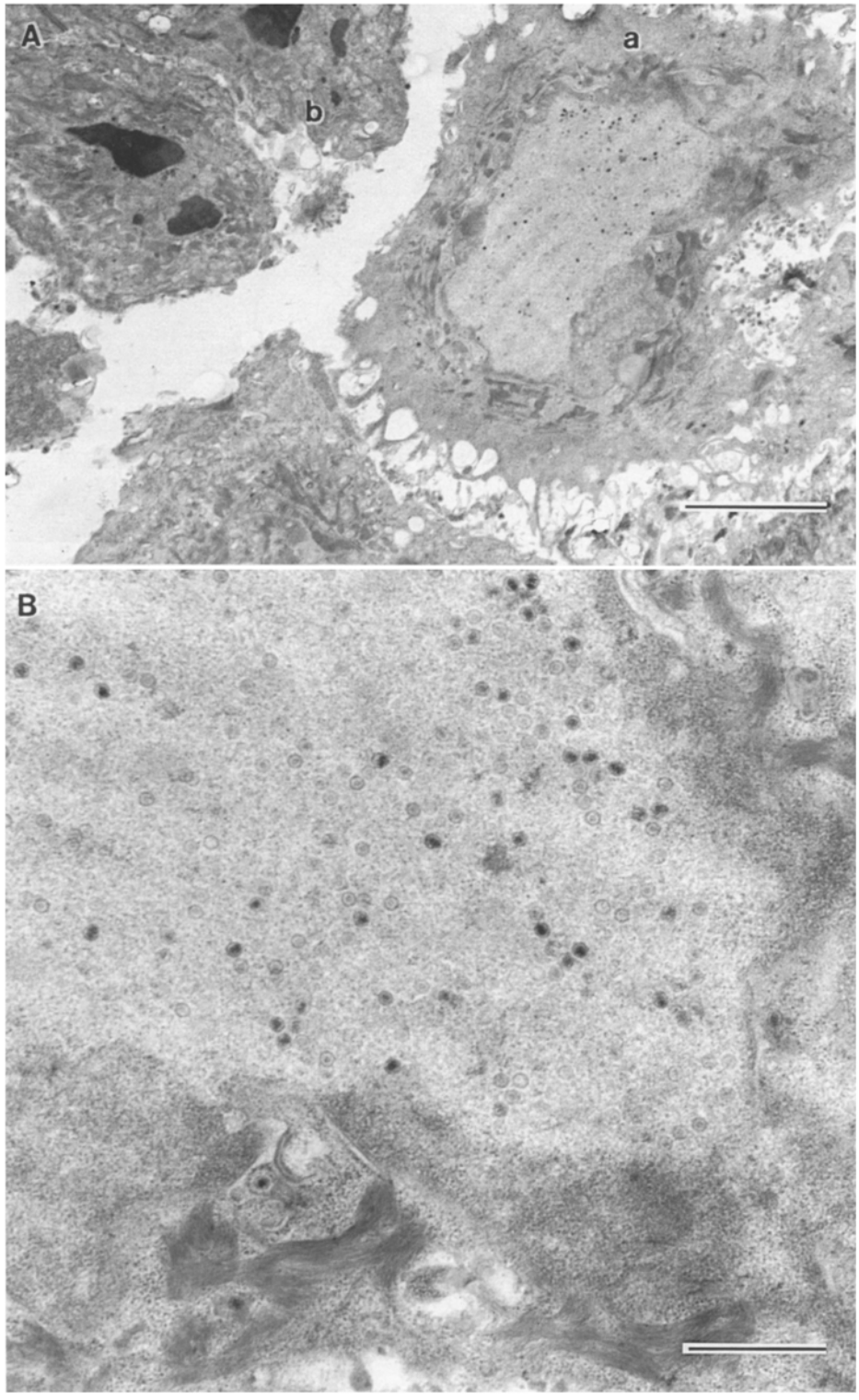


Fig. 3. **A** Zosteriform lesion with adherent crust 5 days after infection. **B** HE-staining of the lesion (arrowhead) shows a wide, thick coagulation necrosis extending to the upper dermis. **C** Fluorescence for HSV-1 is seen faintly along the horizontal border between the necrotic and intact dermis (*a*), but is prominent in the hair follicles of the necrotic area (*b*) and in the sebaceous cells (*c*) beneath the necrotic area. **D** and **E** Hair germ cells (arrow) are preserved and emit no fluorescence

remaining cutaneous lesions had already lost their antigens (Fig. 6), and infectious viruses were isolated from homogenates of the zosteriform lesions. The number of morphologically definable sebaceous glands were 5–10 per tissue specimen of 7 mm-width zosteriform lesions, and those of HSV-1 antigen-positive glands were 2–3 per specimen.

Discussion

The first appearance of HSV-1 in the epidermal basal cells implies a viral shedding from the peripheral nerve endings, consistent with the report by Simmons and Nash [18]. Subsequent spreading of the virus among the neighbouring keratinocytes including the outer hair sheath seems to be due to an extension via the extracellular fluid between the epidermal cells, since the fluorescent area extended rapidly within the entire epidermis up to 1 day, and viral extension was blocked, in part, by passive immunization with HSV-1-specific antibodies. In comparison, a gradual extension of the virus toward the sebaceous glands, continuous to the epidermis and lacking innervation [13], was presumed to be due to cell-to-cell infection by the virus rather than via fluid spreading between the narrow interspaces in the sebaceous glands. The small number of nerve endings and the relatively sparse cells in the connective tissue must lead to a slow spread in the dermis. This may be attributable to a tentative blockade of HSV-1 by the basal lamina separating the epidermis from the dermis, and cell-to-cell infection mechanisms among the sparse fibroblasts.


A
a
b
B

In addition, the self-limiting nature of this skin lesion, which rarely extended beyond the primary dermatome, was observed even in our immunodeficient mice. This restriction of the pathogen to a small zone surrounding the site of viral shedding may be due to extensive cell death with progressive necrotic coagulation resulting from the viral infection rather than from cytopathic effects. The degradative changes demonstrated by electron microscopy would support this hypothesis. Moreover, this observation also indicates a segregation in the spinal cord of the infected nerve fibers from the contacting fibers of next ganglion which is responsible for the adjusting dermatome.

Several reports indicate that functional natural killer cells and macrophages are present even in SCID mice [1, 6], and that both cell populations play a role in protecting against HSV-1 [8, 21]. However, we did not observe apparent infiltrations of NK cells or macrophages immunohistologically in the skin lesions of the SCID mice.

The order in which the viral antigens disappeared differed from that in which they initially occurred. The antigens disappeared from the keratinocytes within 1 day and faded from the underlying dermis in 2 to 3 days. On the other hand, some sebaceous glands preserved the viral antigens and maintained their structural integrity until host death on days 7–10. These delayed cytopathic changes may be due to the slower cell kinetics of the sebaceous gland, compared with that of the epidermal keratinocytes [22]. These findings would not be produced by a modification of the immune response to HSV-1, because of the use of the SCID mouse [4]. The mice which had been passively immunized with a specific antibody retained the viral antigens in their sebaceous glands much longer, up to 20 days after infection. This might not be due to the immunological containment itself, but might rather result from the prolongation of a lethal encephalitis by the antibodies.

The sequence of viral spreading including sebaceous glands during the development of zosteriform lesions in both BALB/c and BALB/c nude mice was essentially the same as those observed in SCID mice. In BALB/c mice, however, non-specific cellular response of both mononuclear and polymorphonuclear cells, possibly associated with cytokines, were evident from the early stage of disease. Complications by early and advanced areas within a specimen were also evident. In nude mice, ulcerations of the skin lesion were severe, probably due to its thinner epidermis, and bacterial infections, which complicate the

---

Fig. 4 A, B. Electron micrographs of HSV-1-infected keratinocytes. The specimen block used in the previous light microscopic and immunofluorescence studies was deparaffinized, post-fixed with OsO_4, and embedded in epoxy resin, then trimmed for EM study in accordance with the immunohistological findings. **A** Both morphologically normal (*a*) and degenerated (*b*) keratinocytes harbor round particles. Bar: 4 μm. **B** Nucleocapsids (100 nm in diameter) in the nucleus and enveloped virions in the cytoplasm are evident at a high magnification. Bar: 1 μm

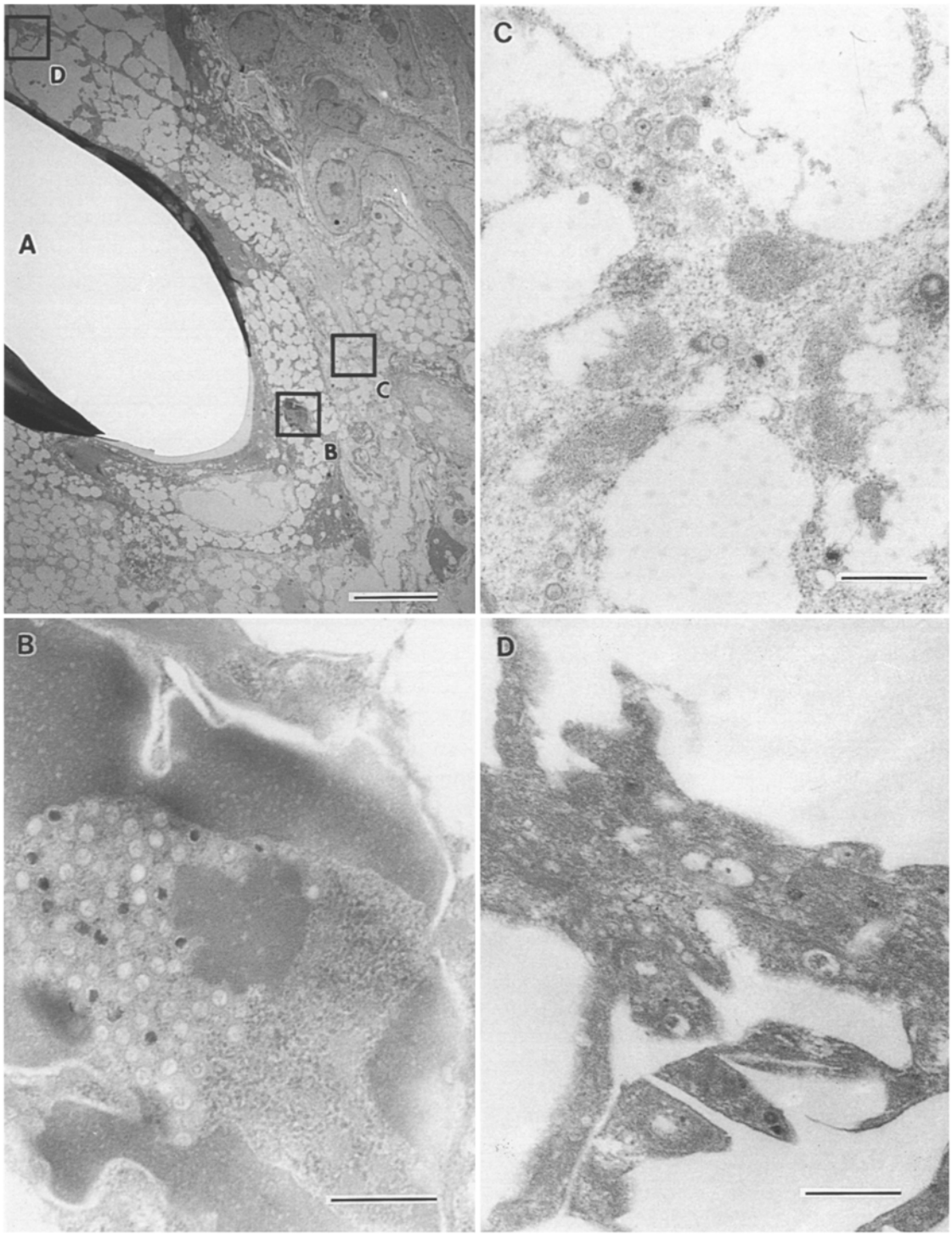


Fig. 5 A–D. Electron micrographs documenting the replication of the virus in sebaceous cells and the presence of virions in senescent cells. **A** Sebaceous cells in various stages of maturation are seen around one hair. Bar: 10 μm. **B** Some viruses exist in the nucleus as nucleocapsids (bar: 500 nm), and **C** others are present in the cytoplasm as either nucleocapsids or virions (bar: 500 nm). **D** Some viruses retain their structures in the degraded exocrine cells. Bar: 500 nm

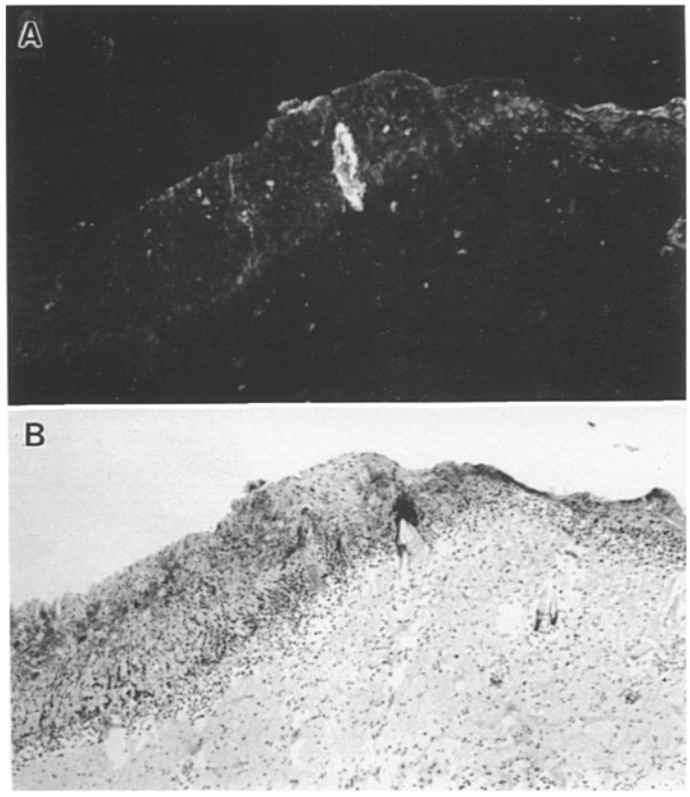


Fig. 6 A, B. Immunofluorescent and light micrographs of the zosteriform lesion 20 days after infection of a SCID mouse which had been passively immunized with HSV-1-specific immune sera 72 h post-infection. **A** Preservation of HSV-1 antigens in sebaceous glands within a necrotic area, demonstrated by HSV-1-specific antibody and FITC-labeled secondary antibody. **B** The corresponding HE-stained preparation is shown

immunohistological evaluation, were also more frequent. In addition, infiltration of polymorphonuclear cells was evident in these mice (data not shown).

Do the sebaceous glands play a role in the persistent infection of skin with HSV-1? If the surrounding damaged tissues are repaired and healed before the glands are destroyed, then these cells could be the foci for the persistence of virus. However, to date, we have not succeeded in detecting any viruses in sebaceous glands in the completely healed skin area.

Because the sebaceous gland is a holocrine organ, sebum together with exsudate from the herpetic skin lesions might also play a role not only in protecting the virus particles against the defense mechanisms within the host but also in their transmission among individuals. It remains to be determined how these glands on such other skin surfaces as the oral cavity and eyelid (Meiboem's gland) [17], are associated with the mucocutaneous lesions of HSV-1 in its natural host.

In addition, the findings that sebaceous glands of the skin can allow the virus to prolong its infection during the acute stage of infection, both with and

without the presence of virus specific antibodies, could provide an explanation for the clinical observation that HSV develops follicle-centered seropapules in the seropositive host during recurrence, while in the primary infection it is manifested as indurated erythema.

Acknowledgements

We thank Dr. M. J. Bosma, Fox Chase Cancer Center, U.S.A., and Dr. T. Watanabe, Institute for Bioregulation, Kyushu University, for providing SCID mice; Dr. S. Handa, Laboratory Animal Center, Kyushu University, Mr. A. Takade, Department of Bacteriology, Kyushu University, and Ms. E. Takayashiki, National Institute of Health, for pertinent advices; and Ms. C. Kaneko, Laboratory Animal Center, Kyushu University, and Ms. R. Masuda, Department of Virology, Kyushu University, for their excellent technical assistance.

References

1. Bancroft GJ, Schreiber RD, Bosma GC, Bosma MJ, Unanue ER (1987) A T-cell independent mechanism of macrophage activation by interferon gamma. J Immunol 139: 1104–1107
2. Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. Nature 301: 527–530
3. Bosma GC, Fried M, Custer RP, Carroll A, Gibson DM, Bosma MJ (1988) Evidence of functional lymphocytes in some (leaky) scid mice. J Exp Med 167: 1016–1033
4. Bosma MJ, Phillips RA, Schuler W (eds) (1989) The Scid mouse. Springer, Berlin Heidelberg New York Tokyo (Current topics in microbiology and immunology, vol 152)
5. Clements GB, Subak-Sharpe JH (1988) Herpes simplex virus type 2 establishes latency in the mouse footpad. J Gen Virol 69: 357–383
6. Dorshkind K, Pollac SB, Bosma MJ, Phillips RJ (1985) Natural killer (NK) cells are present in mice with severe combined immunodeficiency (scid). J Immunol 134: 3798–3901
7. Cotsarelis G, Sun T, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61: 1329–1337
8. Habu S, Akamatsu K, Tamaoki N, Okumura K (1984) In vivo significance of NK cell on resistance against virus (HSV-1) infections in mice. J Immunol 133: 2743–2747
9. Hill TJ, Harbour DA, Blyth WA (1980) Isolation of herpes simplex virus from the skin of clinically normal mice during latent infection. J Gen Virol 47: 205–207
10. Imayama S (1981) Scanning and transmission electron microscope study on the terminal blood vessels of the rat skin. J Invest Dermatol 76: 151–157
11. Imayama S, Braverman IM (1989) A hypothetical explanation for the aging of skin: chronologic alteration of the three-dimensional arrangement of collagen and elastic fibers in connective tissue. Am J Pathol 134: 1019–1025
12. Kurata T, Hondo R, Sato S, Oda A, Aoyama Y, McCormick JB (1983) Detection of viral antigens in formalin-fixed specimens by enzyme treatment. Ann NY Acad Sci 420: 192–207
13. Giacometti L, Montagna W (1969) The innervation of human hair follicles. In: Montagna W, Dobson RL (ed) Advances in biology of skin, vol 9, hair growth. Pergamon Press, Oxford, pp 393–398
14. Mori R, Minagawa H, Sakuma S, Mohri S, Watanabe T (1990) Herpes simplex virus infection in mice with severe combined immunodeficiency (SCID). In: Lopes C, Mori

R, Roizman B, Whitley R (eds) Immunobiology and prophylaxis of human herpesvirus infections. Plenum, New York, pp 191–197
15. Nagafuchi S, Oda H, Mori R, Taniguchi T (1979) Mechanisms of acquired resistance to herpes simplex virus infection as studied in nude mice. J Gen Virol 44: 715–723
16. Scriba M (1977) Extraneural localization of herpes simplex virus in latently infected guinea pigs. Nature 267: 529–531
17. Shimeld C, Hill TJ, Blyth WA, Easty DL (1990) Reactivation of latent infection and induction of recurrent herpetic eye disease. J Gen Virol 71: 397–404
18. Simmons A, Nash AA (1984) Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. J Virol 52: 816–821
19. Steiner I, Spivack JG, Deshmane SL, Ace CI, Preston CM, Fraser NW (1990) A herpes simplex virus type 1 mutant containing a nontransinducing Vmw 65 protein establishes latent infection in vivo in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. J Virol 64: 1630–1638
20. Stevens J, Cook M (1971) Latent herpes simplex virus in spinal ganglia of mice. Science 173: 843–845
21. Stevens J, Cook M (1971) Restriction of herpes simplex virus by macrophages: an analysis of the cell – virus interaction. J Exp Med 133: 19–38
22. Weinstein GD (1974) Cell kinetics of human sebaceous glands. J Invest Dermatol 62: 144–146
23. Yoshino K, Taniguchi S (1969) Isolation of a clone of herpes simplex virus highly attenuated for newborn mice and hamsters. Japan J Exp Med 39: 223–232

Authors' address: K. Moriyama, Department of Virology, School of Medicine, Kyushu University, 3-1-1 Maidashi, Fukuoka 812, Japan.

Received June 28, 1991