Specific Deposition of Passively Transferred Monoclonal Antibodies against Herpes Simplex Virus Type 1 in Rat Brain Infected with the Virus

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Abstract: The kinetics of human monoclonal antibody (anti-gB) to herpes simplex virus type 1 (HSV-1) were investigated after intravenous injection of anti-gB into an HSV-1 encephalitis animal model. Immunohistochemical study revealed specific deposition of passively transferred anti-gB in the hippocampus and thalamus of the infected rat brain, and it bound to the same neurons in which HSV-1 antigen was positively stained. To examine the macroscopic distribution of anti-gB in the infected brain, we undertook an 125I-labeled anti-gB injection study, and the same distribution of 125I-labeled anti-gB deposition was observed by brain semimicroautoradiography as in the immunohistochemical study. These results suggest that anti-gB easily permeates the capillary wall and is deposited in the inflammatory site where HSV-1-specific antigen is detectable. The use of radioisotope-labeled anti-gB injection and external brain imaging could lead to a noninvasive diagnostic tool for the early detection of HSV-1 antigen in cases of suspected HSV-1 encephalitis.

Key words: Glycoprotein B (gB), Herpes simplex virus type 1 (HSV-1), Human monoclonal antibody

Herpes simplex virus type 1 (HSV-1) is one of the most important causes of sporadic encephalitis in humans because of its resulting high mortality and severe mental and physical sequelae in survivors (2). With the recent development of effective antiviral drugs (49, 50), the disease can now be more favorably treated. Despite recent advances in such therapy and progress in useful diagnostic methods such as the CT scan (52) and MRI (41), however, management of HSV-1 remains difficult. In particular, a major limitation lies in the difficulty of establishing an accurate diagnosis in the early stage of the disease (51). This problem thus prevents the initiation of effective therapy in the initial stage of HSV-1 infection.

At present, brain biopsy and HSV-1 identification is the only sufficiently accurate method of diagnosis (28, 30), but the necessity for brain biopsy has not been widely accepted (44). Although many methods for the early diagnosis of HSV-1 encephalitis have been proposed, for example, those detecting specific HSV-1 antigens (5) or antibodies (46, 48) or viral DNA (42) in the cerebrospinal fluid (CSF), establishment of a rapid, sensitive, specific, and noninvasive method for the early diagnosis of HSV-1 encephalitis is still urgently being sought.

Recently, we carried out a series of studies on the use of human monoclonal antibodies to HSV (anti-gB) (12, 25) for passive immunotherapy of humans against HSV infection (22, 23). In an effort to address this diagnostic need, we undertook a study to clarify the kinetics of anti-gB in the brain with HSV-1 infection, which was established as an animal model of HSV-1 encephalitis (38), and we chose the HF strain of HSV-1 (29) which provokes mild inflammatory changes in

Abbreviations: anti-C, human anti-cytomegalovirus monoclonal antibody; anti-gB, human anti-herpes simplex virus type 1 monoclonal antibodies; anti-P, human anti-Pseudomonas aeruginosa monoclonal antibody; BBB, blood-brain barrier; Bq, Becquerel; Ci, Curie; CMV, cytomegalovirus; CSF, cerebrospinal fluid; CT, computed tomography; DAB, 3, 3'-diaminobenzidine; dpm, disintegrations per minute; ELISA, enzyme-linked immunosorbent assay; gB, glycoprotein B; HSV, herpes simplex virus; Ig, immunoglobulin; NT, neutralizing titer; PAP, peroxidase-anti-peroxidase; PCR, polymerase chain reaction; PFU, plaque-forming unit.
experimental animals. This was done to examine the usefulness of such antibodies as the initial step in confirming the cause and indicating the lesion site of early, mild HSV-1 encephalitis.

Materials and Methods

Viruses. The HF strain of herpes simplex virus type 1 (HSV-1) (29) was kindly provided by Dr. Yukimasa Kazuyama, Kitasato University, Tokyo. A stock pool of HF-strain virus (5.6 x 10^{6} PFU/ml) was prepared from infected Vero cells and stored at -80°C. As a viral control, the JHM strain (clone 2) of murine coronavirus (26) provided by Dr. Fumihiro Taguchi was selected, because it induces acute encephalitis in rodents with a similar distribution of lesions as in HSV-1 infections.

HSV-1 in the blood and in homogenized brain and liver tissues from infected animals was titrated on monolayers of Vero cells in rotary tubes at 37.0°C.

Antibodies for passive transfer. In the immunohistochemical and radioisotopic studies, we used two types of human anti-HSV-1 monoclonal antibodies (anti-gB), both of which were classified as IgG-1: anti-gB-2 (12) and anti-gB-3 (12) (Teijin). Both anti-gBs not only have strong neutralizing activities (NT: 2^a <) against HSV-1 and HSV-2, but also have the ability to recognize antigenic epitopes on glycoprotein B (gB), a molecule common to both HSV-1 and HSV-2.

For a control study of anti-gB, we used mouse γ-globulin, human anti-cytomegalovirus (CMV) monoclonal antibody (anti-C-23, Teijin: IgG-1) (47), and human anti-Pseudomonas aeruginosa monoclonal antibody (40) (anti-P-8, Teijin: IgG-2) (40). The former monoclonal antibody recognizes a specific antigen of CMV gB, which does not cross-react with the gB of HSV-1; and latter recognizes a specific antigenic lipopolysaccharide on P. aeruginosa.

In vivo infection. Crl:CD(SD)BR female rats (Charles River, Japan) were inoculated at the age of 21 days (45 g - 55 g) with 50 µl of the stock HSV-1 (2.3 x 10^{5} PFU) or murine coronavirus (1 x 10^{4} PFU), and were raised in an air-conditioned compartment. The former virus was injected into the anterior chamber of the right eye (38), and the latter was directly injected into the brain by the craniopuncture method under anesthesia (26). In the radioisotope studies, the rats to be inoculated with HSV-1 were given a 0.1% KI solution as drinking water for thyroid blocking.

Antibody injection. At 72 hr after virus inoculation, 1 mg of anti-gB-2, anti-gB-3, anti-C-23, or anti-P-8 was injected into the caudal vein of each rat. One to 24 hr after the injection of a given antibody, 1.5 mg of pentobarbital was administered into the peritoneal cavity as an anesthetic, and 500 ml of 4% paraformaldehyde in 0.12 M phosphate-buffered solution (pH 7.3) was then perfused slowly into the left ventricle of each rat.

Histological studies: Immunohistochemistry. The brains and the vertebrae of the rats were cut vertically into three parts. Each part of the brain was sectioned serially with a cryostat (Damon) calibrated to 10 µm and stored at -80°C. Antibodies were detected by the peroxidase-anti-peroxidase (PAP) method using rabbit anti-
human γ-globulin (Dako: cooled overnight under dark conditions), swine anti-rabbit γ-globulin (Dako: allowed to start at 90 min at room temperature), rabbit anti-peroxidase-peroxidase complex (Dako: 90 min at room temperature), and 0.01% 3,3'-diaminobenzidine (DAB: Dottit) in 0.1 M Tris-HCl buffer solution (pH 7.6) containing 0.03% hydrogen peroxide for 15 min. We used normal rabbit serum and performed PAP according to a previously described method for the serum of negative controls.

Double-staining for anti-gB and HSV-1 was performed with biotinylated goat anti-human γ-globulin (Zymed), rabbit anti-HSV-1 γ-globulin (Denka: cooled overnight under dark conditions), and peroxidase-conjugated avidin (Vector Lab.: 90 min at room temperature), in a solution of 0.01% DAB and 0.03% hydrogen peroxide, with the final step being application of the PAP method using swine anti-rabbit γ-globulin, rabbit anti-peroxidase-peroxidase complex, and 0.02% 4-chlor-1-naphthol (Dako) in 0.05 M Tris HCl buffer solution (pH 7.6) containing 0.03% hydrogen peroxide for a 15-min period. Double-staining for anti-gB and neurofilament was undertaken using peroxidase-conjugated goat anti-human γ-globulin (Zymed: 90 min at room temperature) in a solution of 0.01% DAB and 0.03% hydrogen peroxide, with the second staining being done with mouse anti-rat-neurofilament monoclonal antibody (Sigma: cooled overnight under dark conditions), biotinylated horse anti-mouse γ-globulin (Vector Lab.: 90 min at room temperature), and peroxidase-conjugated avidin in a solution of 0.02% 4-chlor-1-naphthol and 0.03% hydrogen peroxide. Another double-staining for anti-gB and monocyte was performed with peroxidase-conjugated goat anti-human γ-globulin and mouse anti-rat-monocyte monoclonal antibody (Serotec). Single-staining was carried out as for the controls for double-staining between anti-gB and HSV-1, anti-gB and neurofilament, and anti-gB and monocyte.

Radioisotope studies: Method of 125I-labeling. For radioiodination of anti-gB-2 and anti-P-8, the iodogen method (17) was used. The labeling was accomplished rapidly with 125I-Nal (Amersham, Japan) at 0–2 C, pH 7.5. After 125I-labeling of anti-gB-2 and anti-P-8, Sephadex G-50 was used to eliminate the free 125I. 125I-labeled anti-gB-2 also had high neutralizing activity (NT: 2).

125I-labeled antibodies and measurement of radioactivity. 125I-labeled anti-gB-2 was adjusted to 4.98 × 10^6 Bq/ml (134.48 μCi/ml) and 125I-labeled anti-P-8 was adjusted to 5.29 × 10^6 Bq/ml (142.86 μCi/ml) with each isotope-free antibody. Seventy-two hours after HSV-1 inoculation, 0.5 ml of each 125I-labeled antibody was injected into the caudal vein of each rat. Twenty-four hours after each antibody injection, blood was drawn, and the brain, liver, and gastrocnemius muscle were immediately excised. 125I-radioactivity was measured in the blood and in homogenates of brain, liver, and gastrocnemius muscle with an autowell counter (Aloka). After counting, F-values were calculated for comparison of the different doses of each 125I-labeled antibody per body weight of rats.

F-values were defined as follows:

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F-value = \frac{\text{Radioactivity in sample (dpm)/sample weight (g or ml)}}{\text{Administered dose (dpm)/body weight (g)}} \times 100.
\]

Semimicroautoradiography. Twenty-four hours after 125I-labeled antibody injection, the rats were decapitated, the brains were removed under cooled conditions and immediately placed into liquid nitrogen for freezing, and then cut into frontal sections 15 μm in thickness with a microtome (Leitz) (17). After freeze-drying, each section was exposed to X-ray film (Sakura) and developed with Sakura Konidal solution for 4 min. The films were rinsed and fixed with Sakura Konifix as specified by the manufacturer.

Results

Virus Titer in Infected Animals

HSV-1 was titrated in the brain, liver, and blood of three infected rats every 24 hr for 7 days (Fig. 1). On the second day after HSV-1 inoculation, HSV-1 was successfully isolated from the brain. On the fourth day after inoculation, the amount of HSV-1 in the brain of all rats reached its peak, whereas the liver and the blood
exhibited no count. By the seventh day after HSV-1 inoculation, the amount of HSV-1 in the brain had declined to the initial level in surviving rats. The mortality of the infected rats was 58.3% (14/24) on the seventh day after HSV-1 infection.

**Histology and Immunohistochemistry**

On the third day after HSV-1 infection, when the rats were injected with the desired antibody, pathological changes in all rat brains were observed to be mild but distinct. Small necrotic foci were scattered mainly in the hippocampus and thalamus (Fig. 2, A and B), but petechial hemorrhage was not present in this early stage of the infection.

One hour, and 3, 6, 12, and 24 hr after the injection of 1 mg of human anti-HSV-1 monoclonal antibody (anti-gB-2 or anti-gB-3) into each of 36 animals that had been inoculated with HSV-1 three days previously, specific deposition of passively transferred anti-gB was detected in all rat brains (Table 1), especially in the hippocampus and thalamus. These depositions were observed not only in the pericapillary region, but also in parenchymal
cells that appeared like grossly intact neurons (Fig. 3C). When 1 mg of anti-P-8 or anti-C-23 was injected into the HSV-1-infected rats, the former produced staining in only one case, in the pericapillary region but not in parenchymal cells; but the latter was undetectable in the brain (Table 1).

By means of double-staining for anti-gB and HSV-1 antigen in two HSV-1-infected animals that had been injected with 1 mg of anti-gB-3, passively transferred anti-gB-3 was shown to bind to the same cells that were stained positively for HSV-1 antigen in both cases (Fig. 3, A and B). When 1 mg of anti-gB-2 and mouse γ-globulin was administered to an HSV-1-infected rat, HSV-1 antigen and only anti-gB-2 were stained in the same cells, but mouse γ-globulin was not found in the brain (data not shown). Furthermore, we detected passively transferred anti-gB in the same cells that were also stained positively for neurofilaments (Fig. 3E) or for monocyte antigen (Fig. 3F) by the method of double-staining in the above-mentioned three cases. On the other hand, when HSV-1-infected rats were injected with 1 mg of anti-P-8 or anti-C-23, only HSV-1 antigen was detected in the infected cells; i.e., there were no double-stained cells (Fig. 3D). Nor could we detect any monocytes containing either monoclonal antibody in the HSV-1-infected rat brain (data not shown).

On the third day after murine coronavirus infection, when we injected 1 mg of anti-gB-2 or anti-C-23 for a viral control study, we observed a mild inflammation in the brain of all infected rats, but could not detect the specific deposition of either antibody (Table 1). Double-staining for monocytes and anti-gB-2 or anti-C-23 also failed to detect specific antibody deposition (data not shown).

When 1 mg of anti-gB-2, anti-gB-3, or anti-P-8 was injected into several healthy rats, none of the nonencephalitic rat brain sections treated with human monoclonal antibodies were stained by the procedure, even in the pericapillary region (Table 1).

Semimicroautoradiography and comparison of F-values. At 24 hr following 125I-labeled antibody administration, we observed a significant difference in brain section semimicroautoradiographs between those from rats administered 125I-labeled anti-gB-2 (Fig. 4, A and C) and 125I-labeled anti-P-8 (Fig. 4B). In anti-gB-2 imaging, a patchy granular pattern of dense precipitates was seen throughout the brain, especially in the periventricular thalamus and hippocampus, in the subthalamic area, and in the temporal cortex. In contrast, there were only a few deposits of 125I-labeled anti-P-8 in brain semimicroautoradiographs imaged 24 hr after 125I-labeled anti-P-8 administration.

The ratio of the F-value of 125I-labeled anti-gB-2 (14.62) to that of 125I-labeled anti-P-8 (7.67) in the brain was 1.91. However, the same ratio for the blood was only 1.1 (472.43 vs. 430.09), for the liver 1.19 (59.88 vs. 50.28), and for the gastrocnemius muscle 1.23 (101.03 vs. 82.28). The mean ratio of F-values in the blood, liver, and gastrocnemius muscle was 1.17±0.1.

Discussion

The blood-brain barrier (BBB) is composed of three parts: the BBB between capillaries and brain, between capillaries and meninges, and between meninges and brain (6–8). Although large molecular substances, for example, albumin, γ-globulins, lipoprotein, hormones, or drugs, have difficulty passing through the BBB in the normal state, the barrier becomes permeable when inflammation (13) or extensive burns (43), or brain tumor (27) is present, or drugs are administered for osmotic opening of the BBB (1), for example, between capillaries and brain (1) as demonstrated in our study, between capillaries and meninges (10, 32), or between meninges and brain (27). Recently, some reports have noted that cationized bovine serum albumin (45) or α 1-acid glycoprotein (20) was actively transported through the BBB and that hormones (33) or drugs (34) bound to these substances easily passed into the rat brain. In addition, another group reported active and specific

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Table 1. Ratio (positive/examined) of passively transferred monoclonal antibody staining in Crl:CD(SD)BR rat brain

<table>
<thead>
<tr>
<th>Rat</th>
<th>Virus</th>
<th>Antibody</th>
<th>Dose (mg)</th>
<th>1 hr</th>
<th>3 hr</th>
<th>6 hr</th>
<th>12 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalitic</td>
<td>HSV-1</td>
<td>Anti-gB-2</td>
<td>1</td>
<td>6/6</td>
<td>4/4</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-gB-3</td>
<td>1</td>
<td>6/6</td>
<td>4/4</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-P-8</td>
<td>1</td>
<td>1/6</td>
<td>0/4</td>
<td>0/4</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-C-23</td>
<td>1</td>
<td>0/6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-encephalitic</td>
<td>Corona</td>
<td>Anti-gB-2</td>
<td>1</td>
<td>0/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-C-23</td>
<td>1</td>
<td>0/5</td>
<td></td>
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</tbody>
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* Stained only in the pericapillary region.

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a) Stained only in the pericapillary region.
Fig. 3. (A, B): When double-staining for HSV-1 antigen (using rabbit anti-HSV-1 γ-globulin) and injected anti-gB-3 (using goat anti-human γ-globulin) is performed, passively transferred anti-gB-3 (stained brown) and HSV-1 antigen (purple) are seen in the same neurons (long arrows in Fig. 3, A and B). A neuron that is HSV-1 antigen positive only (arrowhead in Fig. 3A) and a non-staining neuron (curved short arrow in Fig. 3A) are also observed. Scale bar = 100 μm. (C): Passively transferred anti-gB-2 is detectable by the PAP method (using rabbit anti-human γ-globulin) in neurons that appear grossly intact (long arrows) as well as in the pericapillary region (arrowheads) of the HSV-1 encephalitic rat brain. Scale bar = 100 μm. (D): Double-staining for HSV-1 antigen and passively transferred anti-P-8 (using goat anti-human γ-globulin) in the infected rat brain tissue reveals only HSV-1 antigen in the infected neurons, with no anti-P-8 detected. Scale bar = 100 μm. (E): By the method of double-staining, passively transferred anti-gB-2 (stained brown using goat anti-human γ-globulin) and neurofilaments (purple using mouse anti-rat neurofilament monoclonal antibody) are detected in the same cell of HSV-1-infected rat brain (long arrow). Pericapillary exudation positive for only anti-gB-2 (arrowhead) and a cell positive for only neurofilaments (curved short arrow) are also seen. Scale bar = 100 μm. (F): A monocyte (stained purple using mouse anti-rat monocyte monoclonal antibody) containing passively transferred anti-gB-2 (brown) is shown in HSV-1-infected rat brain (long arrow). A monocyte devoid of anti-gB-2 is also seen (curved short arrow). Scale bar = 100 μm.
Fig. 4. (A, C): When brain semimicroautoradiographs of passively transferred $^{125}$I-labeled anti-gB-2 are examined, patchy precipitation of $^{125}$I-labeled anti-gB-2 is seen throughout these serial frontal sections of an HSV-1-infected rat brain, especially in the periventricular thalamus and hippocampus (arrowhead), in the subthalamic area (arrow), and in the temporal cortex (arrow), at 24 hr after injection. (B): Only a few deposits of passively transferred $^{125}$I-labeled anti-P-8 are seen in a semimicroautoradiograph of HSV-1-infected rat brain at 24 hr after injection.
transport of γ-globulin (18) across the BBB. These reports suggest that whenever the subject is under physiologically and/or chemically reactable conditions, large molecular substances can be passively or actively transported across the BBB to the brain.

Although passively transferred anti-γB-2 and anti-γB-3 as well as anti-P-8, anti-C-23, and mouse γ-globulin were able to penetrate into the pericapillary area during the early stage of HSV-1 encephalitis, the anti-γBs were the only antibodies in our immunohistochemical study proven to bind to the parenchymal cells, the morphology of which is similar to that of grossly intact neurons. We also observed several cases of positive double-staining for anti-γB and HSV-1 antigen, and for anti-γB and neurofilaments in the same cells of HSV-1-infected brain tissue. These results indicate that both anti-γBs were specifically deposited in infected neurons because of their considerable ability to combine with the γB of HSV-1. Conversely, although anti-P-8, anti-C-23, and mouse γ-globulin could permeate into the pericapillary area, they were not detected in the neurons in the immunohistochemical studies. Since they could not bind to the specific antigens, they were widely spread in the infected brain along their permeability gradient, and therefore their deposits could not be detected. By the method of double-staining for monocytes and anti-γB or anti-C-23, we detected monocytes containing anti-γB in HSV-1 encephalitis, but could not detect those containing anti-γB-2 in coronavirus encephalitis or those containing anti-C-23 in either type of encephalitis. These double antigen (HSV-1 and anti-γB)-positive monocytes in the HSV-1-infected brain could be derived from monocytes that had phagocytosed immune complexes comprising HSV-1 antigen and HSV-1-specific antibody, or from those monocytes infected by HSV-1. Non-specific binding of transferred antibody to host cells including monocytes is negligible as was shown in the control experiments (Table 1), probably due to the dilution of the transferred IgG by the abundant IgG concentration in the host serum.

In the radioisotope studies, a comparison of F-values revealed a large difference between the value for passively transferred 125I-labeled anti-γB-2 and that for 125I-labeled anti-P-8 in the infected brain. Since the background level of the radioisotope in non-infected rat brain is usually 2% below the total administration dose of the radioisotope (17, 31), the F-value of 125I-labeled anti-γB-2 in the infected rat brain was higher than the expected F-value in non-infected rat brain. Conversely, there was no difference between the F-value of 125I-labeled anti-P-8 in the infected rat brain and the expected F-value in non-infected rat brain. Furthermore, the ratios of the F-values between 125I-labeled anti-γB-2 and 125I-labeled anti-P-8 in the blood, liver, and gastrocnemius muscle did not differ.

We observed the same distribution of passively transferred 125I-labeled anti-γB-2 in the infected brain by semimicroautoradiography as was revealed in the immunohistochemical studies. 125I-labeled anti-γB-2 was specifically deposited in the periventricular thalamus and hippocampus, in the subthalamic area, and in the temporal cortex where the inflammation was severe and γB of HSV-1 was abundant; interestingly, this same distribution of pathological changes is observed in cases of human HSV-1 encephalitis (2).

Since γB is one of the important viral substances essential for facilitating HSV-1 penetration into cells (21), and since a large amount of antibodies to γB from outside can easily pass from the capillary into the infected brain and be deposited in specific sites in the early stage of the disease, the combination of new effective anti-HSV drugs and anti-γB is more efficacious than their single administration for treatment of HSV-1 encephalitis (9, 24).

Attempts at early diagnosis of HSV-1 encephalitis can be roughly divided into four categories. The first group includes the detection of HSV-1 antigens in the brain or CSF. Recently, HSV-1-specific glycoproteins, such as γB or γD, have been detected in the CSF of some HSV-1 encephalitis patients (19), but their reliability for the early diagnosis of HSV-1 encephalitis is uncertain.

The second group includes the detection of specific antibodies to HSV-1 in the serum and CSF. Although some measurement methods, for example, enzyme-linked immunosorbent assay (ELISA) (14) or radioimmunoassay, have been used in attempts to detect elevation in titer of specific antibodies to HSV-1 (15, 16), which not only include IgG but also IgM (11), early diagnosis of HSV-1 encephalitis remains difficult by this means.

The third group includes a detection method of viral-specific DNA. With recent improvements in HSV-DNA detection techniques, for example, in situ hybridization (3) and the polymerase chain reaction (PCR) (36, 37), early diagnosis of HSV-1 encephalitis in the CSF may be possible in the future. It should be noted, however, that at present these techniques pose risks of giving false-negative or false-positive results. Since detection of HSV-DNA in the CSF is possible only after the BBB between the brain and meninges has broken down, a few false-negative results cannot be prevented (4). And, in some cases, some possibility still exists of detecting HSV-DNA derived from inapparent infection not directly related to the disease.

The last category is the radioisotope-labeled sub-
stance injection method. Radioisotopic imaging of chemical substances in an animal model of HSV-1 encephalitis has been investigated (35, 39). Imaging of such substances might not reflect the proliferation of HSV-1 in neurons, but it does show the degree of HSV-1-induced changes in the permeability of the capillaries in the brain to these substances. On the other hand, the radioisotope-labeled anti-gB administration method may prove more useful because it makes possible not only the detection of specific antigens of HSV-1, a virus is known to proliferate in neurons, but also imaging of accumulated radioisotope-labeled anti-gB in the form of insoluble immune complexes in the pericapillary area, in the interstitial space, and in monocytes. Anti-gB proved to be stable for a long period in the infected animal body because it was detectable immunohistochemically from one to 24 hr following antibody injection and at 24 hr following 125I-labeled anti-gB-2 injection. Brain image, which are then examined serially during the one- to 24-hr period following radioisotope-labeled anti-gB administration, and comparison of the radioisotopic distribution of each brain imaging could be more useful than single imaging of the brain for confirming the early diagnosis of HSV-1 encephalitis.

When we inject anti-gB into human subjects to facilitate the diagnosis of HSV-1 encephalitis, steps must be taken to avoid a number of problems. For example, to reduce the possibility of harmful effects that might arise from antigen-antibody reactions, we can reduce the total administration volume of anti-gB and use a harmless substance conjugated to anti-gB and some other imaging system to avoid radiation injury.

Despite possible identification of other unresolved points, we think that trials evaluating the anti-gB administration method for diagnosis and treatment of HSV-1 encephalitis should be continued. We speculate that an image analysis system using a specific antibody labeled with a harmless substance may well provide clinicians with a diagnostic aid for some infections for which early diagnosis is presently difficult to confirm, for example, HSV-1 encephalitis.

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