Sensitivity of Real Time Viral Detection by an Optical Biosensor System Using a Crude Home-Made Antiserum against Measles Virus as a Ligand

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Virus identification in clinical materials during acute phase infections could give necessary information for the treatment of infections by human immunoglobulin (hIg) or interferon (IF). But because of a lack of information, most virus infections have not been treated. We have tried to develop a real time detection system for viruses in general using an optical biosensor and a model virus, Herpes simplex virus Type 1 (HSV-1), and have proved that the HSV-1 virus propagated in Vero cells and diluted in minimum essential medium (MEM) with 10% fetal bovine serum (FBS) could be detected in high sensitivity close to 10 infectious units (50% tissue culture infective dose [TCID₅₀] units) using purified cellular receptor molecules as the ligand because the receptor could be the most specific ligand. However, because ligands available for this system to identify various viral infections in general are limited, we also tested this system using a purified polyclonal antibody which contained many other antigens as the ligand, and produced sensitivity comparable to that using the receptor as a ligand. In this paper, we tested the sensitivity by this system under the worst condition. That is, we used a crude home-made rabbit antiserum against measles virus with host cell debris as a ligand. It was found that less than 500 infectious (TCID₅₀) units of virus were required for detection in a 100 μ l solution, and that the efficacy of the commercially available hIg was also estimated by this system. This result suggested that this real time viral detection and titration system was applicable for the diagnosis of all viral infections even under difficult conditions without requiring any complex skills, with high enough sensitivity for clinical purposes. The efficacy of hIg preparations could also be evaluated by this system at the same time of the clinical material sampling.

Key words viral infection; real time identification; optical biosensor; human immunoglobulin; measles virus

Diagnosis of acute viral infections has mostly been based on their clinical symptoms, and the serological diagnosis to confirm infection was only available after curing or under chronic infections. Recently, polymerase chain reaction (PCR) system was applied to viral detection, but it takes time and requires a special skills. Therefore, viral detection has not been used for treatment in most cases. As a result, viral detection studies were not paid for by the health insurance system in Japan. An exceptional case was measles infection. For this infection, formerly mother's serum and now human immunoglobulin (hIg) were used to protect against further viral multiplication and the appearance of symptoms.

Although hIg, interferons (IF) and some anti-viral chemotherapeutics are now available for the treatment of viral infections, most viral infections other than a few exceptional cases have not been treated properly for two reasons related to the absence of rapid viral detection systems within the acute phase. That is, an easy and reliable system for viral detection in real time has not been available for clinical cases, and the effectiveness of anti-viral drugs could not be evaluated without the detection system. As a result, hIg and IF were not allowed to be used for most of the viral infections by the health insurance system in Japan, although specific antibodies could neutralize most of the viremia and suppress a further spread of viruses in the body to stop the acute phase, and IF could suppress intracellular multiplications of most of the RNA viruses. Earlier treatments, such as administration the same day as material sampling, could provide better effectiveness of hIg and IF.

An optical biosensor system was developed for real time monitoring of the reaction between two molecules. In this system, one of the two molecules was immobilized on a cu-

vette, and the binding of the other molecule in the liquid phase to the immobilized one was monitored by the change in the reflection angle of an irradiated laser beam to the bottom of cuvette.²⁾ The reaction rate was monitored in real time with high sensitivity. However a detectable area for the reaction was announced to be about 500 nm from the irradiated point (manual from manufacturer), and viruses were expected to be larger in size. We tried to apply this system to monitor the interactions between virus-cell receptor molecule and then virus-cell interaction, and had successful results.^{3,4)}

We estimated the sensitivities of viral detection under various experimental conditions to confirm the usefulness of this system for the diagnosis of viral infections.

We first selected Herpes simplex virus Type 1 (HSV-1) because the cellular receptor molecule for this virus is available,⁵⁾ the purified monoclonal as well as polyclonal antibody is easily obtained commercially,^{3,4)} and this belongs to the large size viruses which are expected to be difficult to be detected under the measureable field of the optical biosensor system. Under these conditions, either the antibody or receptor molecule could be immobilized on a cuvette, as well as a virus in the liquid. The crude HSV-1 virus propagated in Vero cells was used for detection after filtration through a membrane filter. As a real time biomolecular interaction assay system, an optical biosensor (IASys; Fisons Applied Sensor Technology, Cambridge, U.K.) was used. Heparan sulfate (HS) was immobilized on the cuvette as a cellular receptor for HSV-1, and the reaction was monitored after serially diluted viral solutions were poured into the cuvette. The same viral solutions were added on the cultured Vero cell monolayers, and the virus titers were estimated as 50% tissue culture infective doses (TCID₅₀) by the conventional cyto-

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pathic effect (CPE) on Vero cells. It was found that less than 10 TCID₅₀/100 μ l sample solution was required for detection (and at the same time, identification) by this system.³⁾

Receptor molecules are not yet known for most viruses. The available ligand for viruses could be specific antibodies. We had IgG polyclonal antibody for HSV-1 as a ligand. A polyclonal anti-HSV-1 antibody was immobilized on the cuvette, and the reactions were monitored after serially diluted viral solutions were poured onto the cuvette. It was found that less than 20 TCID₅₀/100 μ l was required for detection by this system.⁴⁾

However, purified anti-sera are only available for important viruses, and only crude home-made anti-sera with cell debris are available for viruses in general. In order to generalize this real time detection and titration system of viruses in clinical materials, we should prove the sensitivity of detection by this system under the most difficult conditions.

Unfortunately, we had no home-made antiserum for HSV-1. But we did have a crude home-made rabbit antiserum against crude measles viruses propagated in Vero cells, and measles virus is as the same size as HSV-1. Because of wide spread vaccination, measles have not been seen recently. But measles was one of the prevalent infections in this country, and hIg was effective in protecting against the appearance of its symptoms. We selected the measles virus and a crude home-made anti-serum for this experiment because this antiserum contained an antibody against not only the virus but also cell debris, and to remove non-specific reactions by the antibody against cell debris, excess cell debris was added to this anti-serum.

MATERIALS AND METHODS

1. Measles Virus AIK-C strain was used. This strain is commercially available as a vaccine from Kitasato Institute. The virus was kindly supplied by Dr. T. Nakayama of Kitasato Institute and propagated on Vero cells in Eagle's minimum essential medium (MEM, Nissui Pharmaceutical, Tokyo) with 5% fetal bovine serum (FBS).

2. Virus Titration by the Conventional Biological Method Serially diluted viral solutions were poured into each of 10 wells of Vero cells grown up to half a monolayer in MEM with 2% FBS and cultured in a CO₂ incubator at 37 °C, and their CPE was observed. The minimal concentration which caused CPE in 50% of the wells was given as 1 TCID₅₀. For measurement by an optical biosensor, the viral solution was UV-irradiated, and fully inactivated virus was used.

3. Crude Home-Made Antiserum for Measles Hyperimmunized rabbit serum with measles virus grown on Vero cells was kindly supplied by Dr. T. Nakayama of Kitasato Institute. Since this virus was grown in Vero cells, the antigens used for immunization could contain Vero cell debris. To this antiserum solution was added enough Vero cell debris to block the binding of Vero cell antigens to the antibody against Vero cells in serum solution before use.

4. Procedure IAsys (Fisons Applied Sensor Technology, Cambridge, U.K.) was used as an optical biosensor. The aminosilane cuvette was activated with a BS3 [bis(sulfosuccinimidyl)suberate] linker, the crude home-made antiserum was immobilized on the cuvette, and it was then blocked with

1 M ethanolamine, pH 8.5. After being washed with Dulbecco's phosphate buffered saline solution without metal salt (PBS(-); Nissui Pharmaceutical, Tokyo), 200 μ l of serially diluted viral MEM solution was poured into the cuvette, the reactions were monitored at 25 °C, and the dose of the virus was calculated from the levels of maximal reactions vs. virus dilutions. The cuvette was regenerated by washing with 200 μ l of 20 mM HCl and then 200 μ l of PBS(-). The antibody immobilized on the cuvette was stable after this treatment. The virus free MEM solution was also tested for the reaction, and this amount was deducted as zero reaction from the measured reactions. After maximal binding of virus, the cuvette was used to measure the binding of Vero cells to bound viral particles.

5. hIg A commercially available sulfonated hIg preparation (Venillon-I®; Teijin, Tokyo) was used for the measurement of an antibody titer against the measles virus. Two-and-a-half grams of preparation were dissolved with distilled water to 25 ml, and 2.5 ml portions were kept at -80 °C, and used just after lysis and dilution by PBS(-). Dissolved hIg was serially diluted with PBS(-), and 100 μ l of the diluted solutions and 100 μ l of Vero cell suspension (10⁵/ml) were poured onto the cuvette. The rate of inhibition of the reactions was estimated, and the antibody titer against measles virus was calculated.

RESULTS AND DISCUSSION

As reported in our first paper,³⁾ HSV-1 was propagated in Vero cells cultured in MEM with 10% FBS. After partial purification, the virus was diluted with PBS(-) or MEM and used for detection. As a real time biomolecular interaction assay system, an optical biosensor (IAsys; Fisons Applied Sensor Technology, Cambridge, U.K.) was used. Ten microliter of 1 mg/ml HS solution was immobilized on the carboxymethylated dextran cuvette via a C6 linker as a cellular receptor for HSV-1, and the reaction was monitored after the serially diluted viral solution was poured into the cuvette. The same viral solutions were added on the cultured Vero cell monolayers, and the viral titers were estimated as TCID₅₀ by conventional CPE on Vero cells. It was found that less than 10 TCID₅₀/100 μ l was required for detection by this system (Fig. 1).³⁾

Receptor molecules are not known for most viruses. The available ligand for viruses could be specific antibodies. Purified monoclonal and polyclonal antibodies for popular viruses are commercially available. In our second paper,⁴⁾ we used an IgG polyclonal antibody for HSV-1 as a ligand. This antibody solution contained a high amount of IgG molecules not specific for HSV-1, and this excess protein on the cuvette surface could interfere with specific molecular interactions, or the binding of contaminated cell debris in a viral solution to non-specific IgG could raise non-specific reactions. A hundred microliter of a (100:100) goat polyclonal anti-HSV-1 antibody (Catalog No. AB1054, Chemicon Instrumental, CA, U.S.A.) were immobilized on an aminosilane cuvette via a BS3 linker, and the reactions were monitored after serially diluted viral solutions were poured onto the cuvette. It was found that less than 20 TCID₅₀/100 μ l was required for detection and identification by this system (Fig. 1),⁴⁾ that is, the polyclonal antibody required only about twice as much virus

Fig. 1. Evaluation of Response Rate Constants between Virus and the Receptor or the Antibody

Responses (extent of change) in a receptor immobilized (●) or antibody immobilized (○) cuvette are plotted against the concentration of HSV-1 (viral dilutions and the viral concentrations estimated by the conventional TCID₅₀). The viral titer and sensitivity were calculated from the curve by the viral dilution at extent 0 and by the units TCID₅₀.

Fig. 2. Evaluation of Response Rate Constant between the Measles Virus and the Antiserum

An aminosilane cuvette with the immobilized antiserum against measles virus was used in a Fisons IAsys at 25 °C. The reactions were carried out through the following steps. Two hundred microliter of serially diluted viral solutions were poured into the cuvettes, and the binding reaction between the virus and antiserum was monitored as the extent of change (arc seconds). Responses are plotted against the concentration of measles virus (viral dilution). Then the viral concentration estimated by the TCID₅₀ was given under the dilution scale to estimate sensitivity.

as the purified receptor.

But purified anti-sera are only available for popular viruses, and only crude home-made anti-sera with cell debris are available for viruses in general. These anti-sera could contain anti-sera against the cell debris of host cells in which the virus was propagated, and these antibodies should be blocked by excess cell debris to reduce virus non-specific reactions. Thus, the surface of the cuvette was covered with excess protein, and specific viral bindings were reduced. In order to generalize the real time detection and titration of viruses in clinical materials, we must prove the sensitivity of this system under the most difficult condition, as described above.

Because of wide spread vaccination, measles have not been seen recently. But measles were very prevalent infection

Fig. 3. Evaluation of the Response Inhibiting Rate Constants of hIg against Virus

The response rates are plotted against the concentrations of hIg. The titer of the neutralizing antibody against measles virus was calculated from the curve by means of the concentrations of hIg at the extent of the serum free control (50 Arc s).

in this country, and hIg was effective in protecting against the appearance of symptoms. We selected the measles virus and a crude home-made antiserum to estimate the sensitivity under the above conditions which could give the lowest sensitivity for virus detection in our optical biosensor system. The amount of binding of the virus to the immobilized antiserum was shown in Fig. 2. Although the reaction by viral-free MEM was deducted, a high background reaction was observed, and low dose virus titration was difficult. About 500 viral infectious units/100 μl were found to be required for the detection and identification of measles virus under this condition.

After being washed with PBS(-), 100 μl of serially diluted hIg solution and then 100 μl of Vero cell suspension (10⁵/ml) were poured into the virus fixed cuvette, and the reaction was monitored. The reactions were found to be reduced depending on the concentration of hIg added (Fig. 3), and the calculated anti-measles virus neutralizing titer in hIg was 5 × 10² infectious units/ml or 5 infectious units/mg hIg.

These results suggested that 10–20 viral infectious units in 100 μl solution could be detected in real time by our optical biosensor for popular viruses, and that about 500 viral infectious units in 100 μl solution could be detected for general viruses for which only the crude and contaminated ligands were available. These results indicated that all acute viral infections which gave over 100 μl sample solution of 5 × 10³/ml viral infectious units such as blood serum, sputum, exudate and fecal material could be detected and titrated in real time by our system at the first day of material sampling.

The efficacy of the commercially available hIg against this infection could also be estimated at the same time. It was also proved that the binding of the virus to the immobilized antibody and the inhibition of binding of the virus by hIg were quantitatively detectable.

This system could be applied for all viral infections to identify the causative viruses because of the specificity of the reaction between an anti-serum and the causative virus and titrations of the viruses. At the same time, we could also test the efficacy of the hIg preparation for the treatment.

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