A Haemagglutination Assay for the Primate Syncytium-forming (Foamy) Viruses

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SUMMARY

Preparations of highly concentrated simian foamy virus type 1 (SFV1) agglutinate guinea pig red blood cells. The agglutination occurs both at 22 °C and 37 °C. It is inhibited by specific antisera against SFV1 but also by antisera prepared against several other types of simian foamy viruses.

Syncytium-forming or 'foamy' viruses have been isolated from several mammalian species, namely hamsters, cats, cows and various primates, including New World and Old World monkeys and apes (Hooks & Gibbs, 1975). Like other retroviridae (Dalton et al. 1974), these viruses contain an RNA genome, an RNA-dependent DNA polymerase (reverse transcriptase), and have a buoyant density in sucrose of approx. 1.16 g/ml. Following infection of cultured cells in vitro, foamy viruses typically bud into intracisternal vesicles leading to dilation of the endoplasmic reticulum, and ultimately, to giant cell formation and cell lysis. There are several reports stating that foamy viruses lack haemagglutination activity (Johnston, 1961, 1971; Stiles, Bittle & Cabasso, 1964; Riggs et al. 1969; Hooks et al. 1972, 1973; Fabish, Takemoto & Hruska, 1973). In this communication, however, we describe the haemagglutinating activity of a simian foamy virus (type 1) using guinea pig erythrocytes.

A syncytium-forming virus was isolated by co-cultivating cells from the placenta of a normal rhesus monkey with a continuous line of canine thymus cells (FCf2th, Naval Bio-medical Research Laboratories). Serial propagation in canine cells of this isolate, designated M11, led to the rapid appearance of reverse transcriptase activity in supernatant fluids of infected cultures and the ready detection of typical intracellular foamy viruses by electron microscopy. By selection of virus-infected cells, a clone of cells was obtained which was resistant to cytopathic effects but still produced high levels of virus, as determined by supernatant reverse transcriptase assays. The virus released by this clone produced readily-detected lytic c.p.e. in the parent canine thymus cells, and was neutralized specifically by standard reference antisera to simian foamy virus type 1 (SFV1). For most of our experiments, virus was centrifuged from culture supernatant fluids and isopycnically banded at a density of 1.16 g/ml in sucrose (Parks et al. 1971). Concentrated virus stocks contained about 10^12 virus particles/ml as determined by electron microscopy.

Several different type C viruses also propagated in canine thymus cells and concentrated to 10^11 to 10^12 particles/ml by the same methods were employed as controls. These viruses included: the endogenous baboon virus, M7 (Benveniste et al. 1974); the simian sarcoma-associated virus (SSAV) isolated from a woolly monkey (Theien et al. 1971); and a murine xenotropic virus induced from a subclone of BALB/3T3 cells (Callahan, Lieber & Todaro, 1975).

Tests for haemagglutination (HA) were performed in microtitre 'U' disposable plastic plates. Serial twofold dilutions of the virus stocks were prepared in 0.025 ml normal saline (0.85 % NaCl). Four wells were used for each dilution. An equal volume of a 0.75 % erythrocyte suspension, also in saline, was added to each cup. The plates were shaken, held
Table I. *Haemagglutination of guinea pig RBCs by simian foamy virus type I*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle count* (virions/ml)</th>
<th>Viron/RBC ratio</th>
<th>Haemagglutination titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simian foamy virus type I (SFV I)‡</td>
<td>$8 \times 10^{11}$</td>
<td>133</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Low-speed clarified supernatants§ of SFV I cultures</td>
<td>$1 \times 10^8$</td>
<td>0.016</td>
<td>32</td>
</tr>
<tr>
<td>High-speed pelleted SFV I virus§</td>
<td>$9 \times 10^9$</td>
<td>1.5</td>
<td>128</td>
</tr>
<tr>
<td>Rauscher murine type C virus isopycnically banded</td>
<td>$1 \times 10^{12}$</td>
<td>167</td>
<td>0</td>
</tr>
<tr>
<td>Baboon type C virus‡ isopycnically banded</td>
<td>$7 \times 10^{11}$</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>Simian sarcoma associated type C virus‡ isopycnically banded</td>
<td>$5 \times 10^{11}$</td>
<td>83</td>
<td>0</td>
</tr>
</tbody>
</table>

* Virus particles were counted following the pseudoreplica technique (Smith, 1967).
† Reciprocal of the highest virus stock dilution showing a detectable haemagglutination.
‡ Viruses were grown in FCEFTh cells and concentrated as described (Parks et al. 1971).
§ Supernatant fluids were harvested when c.p.e. attained ++++ and centrifuged at 1800 g for 10 min.
|| Cell debris was removed from the medium and the virus was pelleted through a glycerol cushion at 30000 rev/min (90 min, 4 °C) in a Type 30 Beckman rotor.

at the desired temperature, and scored for HA by the pattern method (Rosen, 1964). Titres were recorded as the reciprocal of the highest virus stock dilution causing haemagglutination in at least two out of the four wells used per dilution. Red blood cells (RBCs) were obtained from Microbiological Associates, Bethesda, MD, and from Flow Laboratories, Rockville, MD, U.S.A.

Haemagglutination inhibition (HI) tests were performed as described by Rosen (1964). Reference antisera to simian foamy viruses employed to ascertain specificity were supplied by the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute, Bethesda, MD. Another antiserum to SFV I was obtained from Flow Laboratories, Rockville, MD. Sera from normal, non-immunized animals (horse, goats, rabbits) were purchased from different commercial suppliers. Since preliminary studies revealed that most sera, even from non-immunized animals, inhibited HA induced by M I I virus preparations, the sera were heated at 56 °C for 30 min and absorbed with a 25 % suspension of acid-washed kaolin in saline (w/v). This procedure eliminated non-specific inhibitors while the inhibiting activities of specific antisera were retained. RBC agglutinins present in sera were removed by absorption with a 50 % suspension of fresh indicator erythrocytes. After the absorptions, sera were twofold serially diluted in saline and distributed in 0.025 ml samples into the cups of a microtitre ‘U’ plate. Four units of M I I haemagglutinin contained in a volume of 0.025 ml were added. The mixtures were allowed to stand for 2 h at room temperature, then 0.025 ml of erythrocyte suspension were added and the plates were incubated at 37 °C. The titres of sera were taken as the highest dilutions that completely inhibited agglutination.

SVF I virus preparations were tested for HA activity at various temperatures (4, 22 and 37 °C) with RBCs collected from several avian and mammalian species: chicken (adult and newborn), duck, turkey, pigeon, goose, man, rhesus monkey, green monkey, dog, cat, cow, horse, burro, sheep, hamster, rat, mouse, and guinea pig. Crude clarified culture fluids and virus concentrated about 100-fold by centrifugation always gave negative results. By contrast, when the banded virus preparations were employed, guinea pig RBCs were agglutinated at
Table 2. Inhibition of MII haemagglutination by specific simian foamy viruses antisera

<table>
<thead>
<tr>
<th>Serum</th>
<th>Species</th>
<th>Source*</th>
<th>HI titre†</th>
<th>Homologous neutralization titre‡</th>
<th>Heterologous neutralization titre§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-simian foamy virus type 1</td>
<td>Horse</td>
<td>National Cancer Inst. (8CDE-1010)</td>
<td>80</td>
<td>128</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Pre-immune serum</td>
<td>Horse</td>
<td>National Cancer Inst. (8A-101)</td>
<td>&lt; 10</td>
<td>&lt; 4</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 1</td>
<td>Goat</td>
<td>Flow Laboratories (81004)</td>
<td>40</td>
<td>64</td>
<td>ND</td>
</tr>
<tr>
<td>Pre-immune serum</td>
<td>Goat</td>
<td>Flow Laboratories (E81003N)</td>
<td>&lt; 10</td>
<td>&lt; 4</td>
<td>ND</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 2</td>
<td>Horse</td>
<td>National Cancer Inst. (2DEGF1100)</td>
<td>320</td>
<td>256</td>
<td>64 (against SFV Type 7)</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 4</td>
<td>Horse</td>
<td>National Cancer Inst. (6DEF-1310)</td>
<td>40</td>
<td>32</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 6</td>
<td>Rabbit</td>
<td>National Cancer Inst. (151)</td>
<td>40</td>
<td>64</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 6</td>
<td>Rabbit</td>
<td>National Cancer Inst. (161)</td>
<td>80</td>
<td>1024</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Anti-simian foamy virus type 7</td>
<td>Rabbit</td>
<td>National Cancer Inst. (68-1600)</td>
<td>320</td>
<td>512</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Normal</td>
<td>3 horses, 2 goats, 2 rabbits</td>
<td></td>
<td>&lt; 10</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Numbers in brackets indicate catalogue references.
† Reciprocal of the highest dilution that completely inhibited haemagglutination.
‡ Reciprocal of the highest dilution that completely inhibited c.p.e. induced by the homologous simian foamy virus (Johnston, 1971).
§ Reciprocal of the highest dilution that completely inhibited c.p.e. induced by an heterologous simian foamy virus (Johnston, 1971).
| ND indicates that the test was not done.

titres of from 1/32 to 1/128 both at 22 and 37 °C (Table 1). Under the same conditions, all the other RBCs gave negative results. Preparations of type C viruses grown in the same cell line as the MII virus, and concentrated by similar methods, showed no HA activity using guinea pig RBCs or RBCs from each of the other species.

The highest apparent titres and clearest HA patterns with SFV1 were obtained using freshly collected and washed guinea pig erythrocytes. There were no significant differences between HA titres when dilutions and tests were made in saline, phosphate-buffered saline, or in veronal-gelatine solution at pH from 6.0 to 8.2. The agglutination patterns were stable when the plates were shifted from 37 to 4 °C, but HA was reversed after more than 9 h at 37 °C. The HA activity disappeared after heating the virus stock at 56 °C for 30 min or after treatment with trypsin following the technique of Corbo & Cunningham (1959) or with ether according to Buckland & Tyrrell (1963).

To determine if HA by the MII isolate of SFV1 was inhibited by antisera to viruses of this group, HI tests were performed using various reference antisera. The results in Table 2 indicate that HA was inhibited by two different antisera which specifically neutralize SFV1, while pre-immunization sera from the same animals had no effect. Several normal horse, rabbit and goat sera failed to block HA. Other antisera prepared against simian foamy
viruses types 2, 4, 5, 6, and 7 also inhibited SFV1 HA, suggesting a broad pattern of cross-reactivity in haemagglutination.

Our results indicate that haemagglutinating activity may be detected when highly concentrated preparations of M11 simian foamy virus type I are used. Previous workers (Johnston, 1961; Stiles et al. 1964; Riggs et al. 1969; Johnston, 1971; Hooks et al. 1972, 1973; Fabish, et al. 1973) may have failed to detect HA since they probably used less concentrated virus stocks. Using the methods that we describe, supernatant fluids from and cell extracts of foamy virus-infected cultures showed no detectable haemagglutinating activity. The need for a threshold virus concentration before HA can be detected has been reported for many other viruses, including certain murine type C viruses (Witter et al. 1973) and some strains of the chicken infectious bronchitis virus (Bingham, Hilary Madge & Tyrrell, 1975).

The M11 isolate was neutralized specifically by reference antiserum to SFV1 and not by antisera raised against the other primate syncytium-forming viruses. By contrast, while inhibition of haemagglutination was observed using antiserum to SFV1, antisera to several other foamy virus types also inhibited the haemagglutinating activity of the M11 isolate. Control experiments showed that neither pre-immunization sera from the same animals nor normal sera from various species blocked the haemagglutinating activity of high titre virus preparations. Moreover, several other viruses also propagated in the canine cells and concentrated by similar methods failed to show haemagglutinating activity. Taken together, the results show that guinea pig RBCs may be agglutinated by a component present in simian foamy virus preparations. The virus components responsible for haemagglutination may not be identical to the virus antigens that react with neutralizing antibodies. The components of the virion responsible for haemagglutination, like the complement-fixing antigens of foamy viruses (Stiles et al. 1964), may be expressed by various strains of simian syncytium-forming viruses. If adequate virus concentration is available HA and HI assays for the primate foamy virus group should provide a new test for exposure of primates, including man, to this group of viruses.

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REFERENCES


* On leave of absence from the Institut de Recherches sur les Leucémies, Paris, France.
Short communications


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