Hemagglutination by herpes simplex virus type 1

Brief Report

E. Trybała, Z. Larski, and J. Wiśniewski

Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Academy of Agriculture and Technology, Olsztyn, Poland

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Summary. The McIntyre and HSZP strains as well as clinical isolate of herpes simplex virus type 1 were found to agglutinate C57Bl/10su and CBA mouse red blood cells. The hemagglutinating activity was inhibited by antisera that neutralized the infectivity of the virus.

Several members of the family Herpesviridae have so far been reported to possess hemagglutinating (HA) activity, i.e., equine abortion virus [3], infectious laryngotracheitis virus of chickens [4], feline rhinotracheitis virus [1, 8] and bovine herpesvirus type 1 [2, 7]. Quite recently, in 1989, HA activity of Aujeszky’s disease (pseudorabies) virus was described by Tetsu et al. [6] and by Trybała [9]. This latter report also contains brief information about such property in herpes simplex virus type 1 (HSV-1), and here we present some more details.

The HSV-1 strains used were McIntyre strain kindly provided by Prof. Z. Łuczak, Medical Academy, Warsaw, Poland and syncytial HSZP strain [5] kindly supplied by Prof. A. Inglot, Institute of Immunology and Experimental Therapy, Wrocław, Poland. Moreover, clinical isolate “Eye” of HSV-1 (B. Litwińska, unpublished data) courteously furnished by Prof. M. Kańtoch, National Department of Hygiene, Warsaw, Poland, was used.

Vero, CV-1, GMK (African green monkey kidney) and RK-13 (rabbit kidney) cells were grown in equal parts of Eagle’s minimum essential medium and medium 199 supplemented with 10% calf serum. Confluent cell monolayers were infected with the appropriate viral strain at an input multiplicity of infection of about 0.1 TCID₅₀ per cell and maintained in serum free medium 199 at 37 °C for the required periods of time. Extracellular hemagglutinins (E preparation) were determined in supernatant fluid of infected cells, whereas the total amount
of hemagglutinins (T preparation) were assayed in culture fluid of cells subjected to three freezing and thawing cycles. Both these preparations were clarified by centrifugation at $4,000 \times g$ for 20 min before being tested for HA activity. Uninoculated cultures were processed in an identical manner to serve as control material.

Unless otherwise stated, hemagglutination and hemagglutination-inhibition (HI) assays were carried out as described for bovine herpesvirus type 1 by Trépanier et al. [7] with some modifications. Briefly, blood from C57Bl/10su mice were collected into a 3.8% solution of sodium citrate and immediately washed three times with an isotonic 0.02 M phosphate buffer (PBS), pH 7.0. Both assays were performed in V-bottom microtiter plates (Plastomed, Poland). For hemagglutination, 50 μl portions of 0.4% suspension of red blood cells (RBC) in PBS containing 0.2% bovine serum albumin (Biomed, Cracow, Poland) (PBS-BSA) were added to 25 μl volumes of serial 2-fold dilutions of virus in PBS-BSA. After shaking, the mixtures were left undisturbed for 2 h before the HA titers were read.

Sera used in the HI assay were previously heated at 56 °C for 30 min. Then, to remove non-specific HA inhibitors 150 μl of serum, diluted 1:2.5 in PBS-BSA, were mixed with an equal volume of 25% suspension of kaolin, kept for 30 min at room temperature under moderate agitation and then centrifuged. To adsorb heterohemagglutinins 150 μl volume of the resulting supernatant was mixed with 75 μl of packed RBC and incubated for 1 h at 37 °C with occasional manual shaking, and for 30 min at room temperature without shaking. The suspension was then centrifuged at 1,000 × g for 20 min and the supernatant, diluted by then 1:5, was used in HI assay. For this test, 4 HA units of McIntyre strain in 25 μl portions were added to equal volumes of serial 2-fold dilutions of serum in PBS-BSA, and the mixtures were incubated for 20 h at 20 °C. Subsequently, 50 μl volumes of 0.4% suspension of RBC were added and after shaking the plates were kept at room temperature for 2 h before the results were read.

Virus neutralization (VN) assay was performed as follows: 50 μl portions of serial 2-fold dilutions of serum in medium 199 supplemented with 5% calf serum were mixed with equal volumes of McIntyre strain suspension (100 TCID₅₀) made in the same medium. Following incubation at 37 °C for 1 h, 30 μl portions of each dilution were transferred into three separate wells of microtiter F plate (Plastomed, Poland). Subsequently, 150 μl portions of RK-13 cell suspension in the same medium as used for serum dilution were added, and the cells were checked for cytopathic effect after incubation at 37 °C in a 5% CO₂ atmosphere for 5 days. The titer was calculated according to Reed and Muench's formula and expressed as VN₅₀.

In the preliminary experiment [9] we found that McIntyre strain of HSV-1 agglutinated C57Bl/10su mouse RBC. No HA was found with horse, cattle, sheep, pig, dog, rabbit, guinea pig, chicken, chick embryo, goose, turkey, and human RBC. The ability of McIntyre and HSZP strains to agglutinate RBC