IMMUNOAUTORADIOGRAPHIC DEMONSTRATION OF VIRUS ANTIGENS IN INFECTED CELLS

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Protein A, a component of the cell wall of *Staphylococcus aureus*, is able to bind with the Fc-fragment of immunoglobulins of most mammals [5]. Because of this property, it is widely used to precipitate immune complexes during radioimmunoassay [2] or when determining the characteristics of cell surface antigens [4].

This paper describes a simple method of determining virus antigens, which consists essentially of the demonstration of immune complexes, formed by treatment of acetone-fixed infected cells with specific immune serum, by means of labeled protein A of *S. aureus*.

Transplantable human HeLa cells, type 1 human adenovirus (AD 1), normal rabbit serum, and specific immune sera, obtained by immunization of rabbits with purified Ad 1 or with hexone Ad 1, according to the usual method [1], and a commercial preparation of protein A of *S. aureus* (from Pharmacia Fine Chemicals, Sweden), labeled with ^{125}I by the chloramine method [3], were used.

HeLa cells were grown in test tubes on Eagle's medium and medium 199 (1:1) with 10% normal bovine serum. After monolayer formation the cells were infected with Ad 1 with a multiplicity of 30-40 PFU per cell. The cells were dispersed with versene 35-48 h after infection, sedimented by low-speed centrifugation, and washed with TN buffer (0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl). Films of the cell suspensions ($5 \cdot 10^3$-$10 \cdot 10^3$ cells) were made on slides. The films were dried in air at room temperature, fixed with acetone for 5-10 min, and washed with TN buffer; the remains of the buffer were removed with filter paper and 1 or 2 drops (25-50 μl) of immune (normal in the control) rabbit serum in a dilution of 1:10 or 1:20 were added; the preparation was incubated for 30 min at 37°C in a humid chamber, after which it was washed in two changes of TN buffer. Traces of buffer remaining were removed with filter paper and 1 or 2 drops of ^{125}I-labeled protein A of *S. aureus* (50,000-100,000 cpm) were added, and the specimen incubated for 30 min in the humid chamber at room temperature, and washed in three changes of TN buffer, after which it was dried in air at room temperature. Autoradiography was then carried out, using ORWO HS 11 x-ray film (East Germany), with overnight exposure (15-18 h).

In the course of this procedure, uninfected HeLa cells and cells infected with Ad 1 were treated in parallel experiments with both immune and normal serum. Considerable darkening of the x-ray film exposed with cells infected with Ad 1 was observed on treatment with immune serum, but only a gray background after corresponding treatment of uninfected cells (Fig. 1a, b). If infected cells and cells not infected with Ad 1 were treated with normal rabbit serum, only a gray background likewise was discovered on film exposed with them (Fig. 1c, d). Identical results were obtained by the use of serum against purified Ad 1 and serum against the basic capsid protein of the adenovirion (hexone). Parallel analysis of the infected cells by a cytomorphological method (staining with acridine orange and luminescence microscopy) revealed characteristic virus-specific inclusions in them. When serum against A2/Hong Kong influenza virus was used as a control, a negative result also was obtained: gray background of film exposed with the specimens.

The authors thus have suggested a simple and highly specific test for demonstrating virus (in this case adenovirus) antigens, which consists of stages of fixing the cells with acetone,

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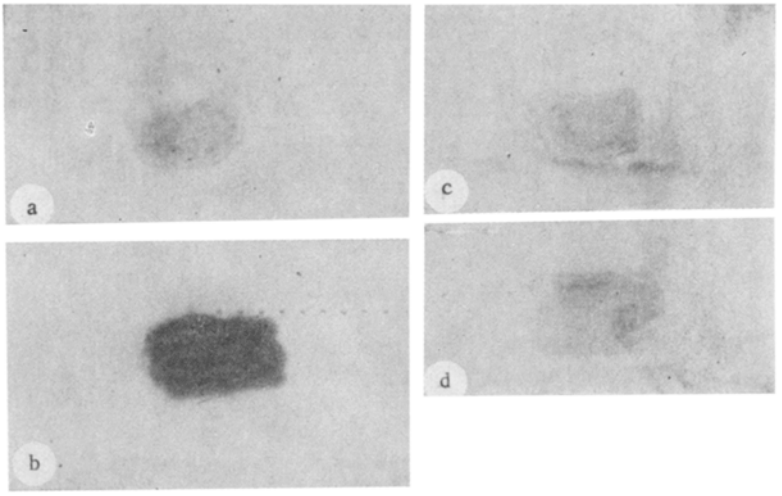

Fig. 1. Demonstration of antigens of adenoviruses in HeLa cells: *a*) treatment of uninfected cells with immune serum to hexone Ad 1, b) treatment of cells infected with Ad 1 with immune serum against hexone Ad 1, c) treatment of uninfected cells with normal rabbit serum, d) treatment of cells infected with Ad 1 with normal rabbit serum.

treating them with immune serum, and demonstrating the immune complex thus formed with the aid of ^{125}I-labeled protein A of *S. aureus*. This method may perhaps be capable of demonstrating any virus antigens, if specific immune sera are used.

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