Location of Feline Leukemia-Sarcoma Group-Specific Antigen in Infected Human Tissue Culture Cells

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Feline leukemia-sarcoma group-specific antigens were located in human embryo cells infected with feline leukemia and feline sarcoma viruses. This was done by using the fluorescent-antibody and enzyme-labeled antibody techniques at both light and electron microscopic levels. The antigens were found to be exclusively intracytoplasmic, diffuse, and located in discrete punctate foci.

Leukemia and sarcoma in cats are often associated with C-type ribonucleic acid (RNA) (8) viral particles. These viruses are morphologically similar to, and have many characteristics in common with, the agents of avian and murine leukemia and sarcoma (4, 14). Like avian and murine leukemia and sarcoma viruses, feline leukemia and sarcoma particles possess at least two kinds of antigens: type-specific antigen, presumably located at their surfaces, and group-specific (gs) antigens which are situated within the virus particles. Although there are different type specific antigens for various isolates of feline leukemia (FeLV) and feline sarcoma viruses (FeSV) (7), the gs antigens have been shown to be the same for FeLV and FeSV (11). The same gs antigens have been demonstrated in feline leukemia cells as well as in tissue culture cells infected with these viruses, where they can be detected by various immunological techniques (10).

In the present study, it was attempted to locate the gs antigens of FeLV and FeSV in infected tissue culture cells of human origin, which have been shown to be susceptible to infection and to support the growth of FeLV and FeSV (9). The techniques used were the fluorescent-antibody (FA) and peroxidase-labeled antibody (PLA) techniques (5) which allow localization of antigens at both light and electron microscopic levels.

MATERIALS AND METHODS

Viruses. Two viruses were used. An FeLV (Rickard strain) was originally isolated in our laboratory from the neoplastic thymus tissue of a spontaneous case of leukemia in a cat (8). This isolate was purified and stored in liquid nitrogen. The GA isolate of FeSV (3) was passed for several passages in feline embryo cell cultures and then purified and stored as the FeSV. Both viruses had been maintained in continuous cultures of feline embryo cells for more than 30 passages in the 6 months preceding the experiment. For infection, 1-ml amounts of tissue culture supernatant fluid (clarified by centrifugation at 5,000 rev/min for 20 min and 10,000 rev/min for 10 min in a Beckman type 30 rotor) were used. Each represented more than 10^4 tissue culture infectious doses.

Preparation of labeled antibody. FeLV gs antiserum was produced in goats by hyperimmunization with highly purified and physically disrupted FeLV of the same strain, propagated in tissue culture as previously described (12). Separation of the globulin fraction from the serum, as well as conjugation with fluorescein isothiocyanate and horseradish peroxidase, have been reported elsewhere (12, 13). Serum from goats hyperimmunized with reovirus (mamalian type I) was conjugated to fluorescein and horseradish peroxidase (HRP) by the same procedures.

Tissue cultures. Human embryo cells (kindly provided by P. S. Sarma) were grown in 200-ml screw-capped plastic bottles with 20-ml amounts of culture medium consisting of 50% McCoy's A and 50% Leibowit L 15 (Grand Island Biological Co., Grand Island, N.Y.) plus 15% heat-inactivated fetal calf serum and antibiotics (penicillin, 250 units/ml; streptomycin, 250 µg/ml) and incubated at 37°C. Fifty per cent monolayers were infected with the viral suspensions described above. At 3-day intervals, cells were trypsinized (12) and serially transferred into replicate subcultures at 1:3 split ratio. After seven passages, carried out to increase the amounts of viral antigen in the tissue culture cells, infected and uninoculated control cell cultures were collected for immunoenzymatic studies at the electron microscopic level. At the same time, infected and control culture cells were seeded in Leighton tubes containing cover slips and collected 3 hr later for immunofluorescent and immunoenzymatic studies in light microscopy.

Immunofluorescent and immunoenzymatic techniques in light microscopy. Infected and control cells on cover slips were fixed and stained with FA- and HRP-conjugated antibody against FeLV according to techniques previously described (13). Parallel infected and control cells on cover slips were stained...
with FA- and HRP-conjugated antibody against reovirus as a control. Some infected cells on cover slips were also exposed (1 hr at 37°C) to unconjugated anti-FeLV and anti-reovirus sera, before staining with FA- and HRP-conjugated antibody against FeLV, for specific staining inhibition control. After completion of the FA reaction, cells on cover slips were washed in saline, mounted in buffered glycerol, and observed by dark-field illumination with an HB 200-w Osram mercury vapor lamp, a BG 12 excitor filter, and a 510-μm barrier filter. Immunoenzymatically reacted cells on cover slips were mounted in balsam and observed with an ordinary light microscope.

Immunoenzymatic studies at the electron microscopic level. On the same day the cover slips were collected, and infected and control cells were obtained from the plastic bottles by gentle scraping and separated from the culture medium by low-speed centrifugation. Cells were subsequently washed in saline and processed for immunoenzymatic staining with HRP-conjugated antiserum by using a technique previously described (13). Infected and control cells were also reacted with anti-reovirus HRP-conjugated antibody for specific stain control. Immunoenzymatically reacted cells were dehydrated, embedded in Epon-Araldite mixture, and incubated in the oven for 36 hr at 60°C. Prepared specimens were sectioned with a Porter-Blum MT-1 microtome equipped with a diamond knife. Sections, collected on carbonized, Formvar-coated grids, were double stained with uranyl acetate and lead citrate and examined with an RCA-EMU-3G electron microscope.

RESULTS

FA. FeLV- and FeSV-infected cells reacted with specific FA antibody exhibited fluorescence which was exclusively cytoplasmic and located in discrete punctate foci (Fig. 1 and 2). The punctate areas of fluorescence seemed to be more prominent in the perinuclear areas. Fluorescence was absent when noninfected cells were reacted with FeLV FA-conjugated antiserum. Likewise, fluorescence was absent when infected cells were reacted with anti-reovirus-conjugated serum. Fluorescence was also prevented by exposure of infected cells to unconjugated FeLV antiserum prior to the immunofluorescence reaction.

Peroxidase-labeled antibody. Cells infected with FeLV and FeSV, when reacted with FeLV HRP-conjugated antiserum, exhibited areas of positive reaction diffuse within the cytoplasm. These areas, characteristic of the PLA technique, were dark brown and very prominent. Areas where the specific reaction did not occur were very light brown and difficult to visualize. The brown areas of specific reaction extended to the whole cytoplasm in heavily positive cells, whereas in other cells more limited areas were involved, sometimes in the perinuclear portion of the cells (Fig. 3 and 4).

Control experiments, similar to those performed with the FA technique, confirmed the specificity of the reaction. At the electron microscopic level, many infected cells showed areas of reaction product accumulation extending to the whole cytoplasm. The reaction product appeared to be uniformly diffuse in the cytoplasm and to be deposited around polysomal aggregates in the form of amorphous clusters. Reaction product stained the mitochondria heavily because of the

Fig. 1. Human whole embryo (HWE) cells 30 days postinfection with FeLV. Diffuse cytoplasmic granular fluorescence. FA preparation X 500.

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endogenous cytochrome-oxidase activity of these organelles, as previously reported by others (1). The cell nuclei did not stain, although they showed affinity for the metal stains used in electron microscopy. The specific stain, granular and amorphous, due to the specific immunoenzymatic reaction was easily distinguishable from the dark clear stain due to the heavy metals. In certain areas of the cytoplasm of infected cells, the deposition of stained reaction product was so intense as to render identification of cytoplasmic structures almost impossible. Polysomes were covered by a diffuse amorphous osmiophilic material. Conversely, the appearance of polysomes in infected cells exposed to anti-reovirus-labeled serum had no reaction product surrounding them. Budding particles in infected cells reacted with FeLV HRP-conjugated antiserum were heavily stained, as was the cytoplasm underneath them (Fig. 6). It was not possible to detect which structure of the virus was involved in the immunoenzymatic reaction since the whole particles were covered by stained reaction product. Particles budding from infected cells, reacted with reovirus-conjugated antiserum, were not stained. The ultrastructural preservation of
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FIG. 4. HWE cells 30 days postinfection with FeSV. Diffuse cytoplasmic areas of antigen localization. PLA preparation $\times 500$.

FIG. 5. HWE cells 30 days postinfection with FeLV, reacted with anti-reovirus PLA. Absence of any reaction. PLA preparation $\times 500$.

the particles was not optimal. However, typical buds could be easily identified (Fig. 6).

DISCUSSION

Cytochemically demonstrable amounts of FeLV and FeSV gs antigen appeared to be limited to the cytoplasm of infected human embryo cells in culture. No evidence of nuclear involvement was found in the present study although a nuclear participation cannot be ruled out with certainty on the sole basis of the techniques used. These results are similar to those obtained when avian and murine leukemia-sarcoma gs antigens are located in infected tissue culture cells. Intracytoplasmic location of FeLV and FeSV gs antigen was also observed in feline cell cultures infected with FeLV (12).

It appears, therefore, that the antigenic expression of the viral genome of FeLV and FeSV follows a common pattern in human as in feline cells. This pattern is similar to that of a variety of oncogenic RNA viruses of various species (2, 4, 6, 14). It has been shown previously that FeLV and FeSV share common gs antigens (11), and it was then expected that these antigens would react with an anti-FeLV gs antiserum. Where localization of the antigen within the cytoplasm of infected cells is concerned, these observations seem to indicate that FeLV and FeSV gs antigens were
Fig. 6. HWE cells 30 days postinfection with FeLV and treated with anti-FeLV PLA. Diffuse reaction involving the whole cytoplasm. PLA preparation × 5,000. Insert above: budding particle from a cell reacted with PLA anti-FeLV. There is reaction product accumulating on the budding particle and in the cytoplasm underneath. × 22,000. Insert below: particles budding from cells exposed to anti-reovirus PLA. Absence of any reaction. × 30,000.

Fig. 7. HWE cells 30 days postinfection with FeSV and reacted with anti-FeLV PLA. Diffuse reaction product extending to the cell cytoplasm. Arrow indicates a budding particle. × 4,000. Insert above: polysomal aggregates in cell infected with FeSV and reacted with anti-FeLV PLA. Reaction product involving the polysomes. Insert below: polysomes in cells infected with FeSV and reacted with anti-reovirus PLA. There is no reaction product accumulation. Inserts × 30,000.

mostly related to polysomes or were free within the cytoplasm. The diffuse punctate localization observed by FA seemed to indicate that the antigen was diffuse within the cytoplasm rather than related to definite cytoplasmic organelles. However, definite evidence could be obtained only by cellular fractionation studies combined with immunological techniques.
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LITERATURE CITED