Distinction Between Envelope Antigens of Murine Xenotropic and Ecotropic Type C Viruses by Immunoelectron Microscopy

By L. S. OSHIRO†, J. A. LEVY*, J. L. RIGGS AND E. H. LENNETTE

Viral and Rickettsial Disease Laboratory, California State Department of Health, Berkeley, California 94704 and *Department of Medicine and Cancer Research Institute, University of California School of Medicine, San Francisco, California 94122

(Accepted 8 December 1976)

SUMMARY

The indirect ferritin-labelled antibody technique was used to determine the reactivity of an antiserum prepared against the NZB xenotropic virus with three murine xenotropic viruses, a feline xenotropic virus and a murine ecotropic virus. The envelope antigens of the xenotropic type C viruses isolated from the NZB, NIH Swiss and C57L mice were tagged with ferritin. The feline RD114 virus was not. Gross murine leukaemia virus was tagged, but only at high serum concentrations. The cross-reactivity titre of Gross virus to anti-NZB serum was removed by a serum dilution which was still reactive to xenotropic viruses. This difference in reactivity titres between a xenotropic and an ecotropic virus was sufficient to distinguish one from the other in doubly infected cultures. Specific tagging of membranes of cells infected by xenotropic virus was also observed.

INTRODUCTION

Murine xenotropic viruses are endogenous type C viruses which productively infect heterologous but not mouse cells (Levy, 1973). They may represent viruses common to all house mice (Levy, 1973, 1974, 1975). They can be distinguished serologically from murine ecotropic (mouse-tropic) viruses (Levy, 1974) which infect and replicate only in mouse and rat cells. Endogenous viruses, whether from the same host cell or from different host cells, may vary in their antigenic relationships (Aoki & Todaro, 1973; Stephenson et al. 1974). Recently, xenotropic viruses from the NIH Swiss (Todaro et al. 1973; Levy, 1973) and from the C57 Leaden (Arnstein et al. 1974) strains of mice have been isolated. The antigenic relationship of these two xenotropic viruses to the xenotropic virus recovered from NZB mice (Levy & Pincus, 1970) and to the endogenous feline RD114 virus (McAllister et al. 1972) was examined using ferritin-labelled antibodies. In addition, a study of the antigenic relationship between a xenotropic and an ecotropic virus was carried out by determining the reactivity of the Gross murine leukaemia virus (G-MuLV) with antiserum prepared against the xenotropic NZB virus.

† To whom reprint requests should be addressed.
METHODS

Viruses, cells and media. The xenotropic type C viruses, NZB (Levy, 1973), AT124 (Todaro et al. 1973), C57L (Arnstein et al. 1974) and RD114 (McAllister et al. 1972), were propagated in D17 dog cells growing on coverslips in a medium consisting of Eagle's minimal essential medium (EMEM) in Earle's balanced salt solution fortified with 10% foetal bovine serum. The D17 cells (uncloned) were derived from a canine osteosarcoma. The NZB virus and the ecotropic G-MuLV (Gross, 1951) were also propagated on normal rat kidney (NRK) cells grown on coverslips in the same maintenance medium. The NRK cell line was originally provided by Dr Robert Ting, Rockville, Maryland, and has been propagated in the laboratory of Dr Jay Levy. A clone of NRK cells was used for these studies.

Ferritin labelling and electron microscopy. Ferritin-labelled antibodies were prepared by coupling goat anti-rabbit gamma globulins to horse spleen ferritin using xylylene di-isocyanate as the coupling agent (Singer, 1959; Rifkind, Hsu & Morgan, 1964). Ferritin-tagging experiments were performed by the indirect method on virus-infected cells grown on coverslips. The antiserum prepared in rabbits against the NZB xenotropic virus has been described (Levy et al. 1975). Briefly, supernatant fluids from co-cultures of NZB embryo and NRK-Harvey cells were cleared of cellular debris by centrifuging for 20 min at 5000 rev/min. The supernatant fluids containing xenotropic viruses were then centrifuged at 52000 g for 2 h. The pellet was resuspended in 0.5 m-tris-NaCl, pH 7.4, and used as the source of antigen for rabbit immunization. A sample (0.5 ml) of a 1:2 dilution of antiserum was absorbed with a 0.1 ml mixture of packed NRK and D17 cells which had been frozen and thawed three times. The serum-cell mixture was incubated for 1 h at 37 °C and for 3 h at 4 °C. The cells were removed by centrifugation at 2000 rev/min for 30 min and the procedure repeated. The serum was filtered through a 0.45 μm Millipore filter and appropriate dilutions made in phosphate buffered saline (PBS), pH 7.2.

The cells were covered with a few drops of the antiserum and incubated at 37 °C for 30 min. They were then washed with several changes of PBS and treated with the ferritin-labelled goat anti-rabbit gamma globulin for 20 min at 22 °C. The cells were again washed in several change of PBS and were briefly fixed in 1% glutaraldehyde solution and scraped off the coverslips in a continuous motion to form a compact aggregate of cells. The cells were then fixed in glutaraldehyde for an additional hour, processed and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope.

RESULTS

Ferritin-labelling of viruses and cell membranes

The xenotropic viruses isolated from the NZB (Fig. 1), NIH Swiss (Fig. 2) and the C57L (Fig. 3) mice were heavily tagged with ferritin-labelled antibodies when treated with a 1:20 dilution of the rabbit antiserum prepared against the xenotropic NZB virus. When ‘tailed’ forms were observed, tagging occurred only on the virus envelope and was not seen on the membranous ‘tailed’ segments (Fig. 1 and 2). The xenotropic feline virus, RD114, was not tagged (Fig. 4) and provided a good control virus for the specificity of the reactions. Membrane tagging (Fig. 5) was also observed on the dog cells infected with all three murine xenotropic viruses. No tagging of uninfected dog cells or dog cells infected with RD114 was seen.
Ferritin-labelled xenotropic viruses

Fig. 1 to 3. The murine xenotropic viruses, NZB (Fig. 1), Swiss (Fig. 2) and C57L (Fig. 3) tagged with ferritin-labelled antibodies after treatment with anti-NZB virus serum.

Fig. 4. The feline xenotropic virus, RD114, not tagged after treatment with anti-NZB virus serum.

Fig. 5. Cell surface membrane tagging of a dog cell infected with xenotropic NZB virus.

Fig. 6. Gross murine leukaemia viruses are not tagged after treatment with a 1:16 dilution of anti-NZB virus serum.
Fig. 7 to 9. Normal rat kidney cells simultaneously infected with Gross leukaemia and xenotropic NZB viruses and treated with anti-NZB virus serum. Tagged (Fig. 7), untagged (Fig. 8), and a mixture of both (Fig. 9) are illustrated.
Ferritin-labelled xenotropic viruses

Distinction between xenotropic and ecotropic viruses

To determine the antigenic relationship between a xenotropic and an ecotropic virus, the reactivity of the anti-NZB virus serum with G-MuLV was determined. Since the G-MuLV could not be propagated in dog cells, the xenotropic NZB and the G-MuLV viruses were grown on NRK cells. After 7 days the cells were treated with anti-NZB virus serum at dilutions of 1:8, 1:16, and 1:32. At the 1:8 serum dilution the NZB viruses were heavily tagged. Tagging also occurred on the Gross ecotropic virus. This apparent cross reaction with G-MuLV was not evident at the 1:16 dilution (Fig. 6), which still effectively tagged the xenotropic NZB viruses. At the 1:32 dilution tagging was not evident on either virus.

The results obtained with the 1:16 dilution suggested that a mixture of xenotropic and ecotropic viruses replicating within the same cell line could be distinguished from each other. Accordingly, NRK cells chronically infected with xenotropic viruses were super-infected with Gross ecotropic viruses and treated with the rabbit anti-NZB antiserum at dilutions of 1:8, 1:16 and 1:32. Random examination of sections on several grids revealed that treatment with the 1:16 dilution resulted in tagging of approx. 50% of the particles. The xenotropic viruses which were represented by the tagged particles were observed in large numbers, suggesting a concentration much greater than the number of particles which was observed in the control cultures chronically infected with xenotropic virus alone. Fields of particles were either tagged (Fig. 7) or untagged (Fig. 8), but on occasion tagged and untagged particles could be seen in the same field (Fig. 9). Treatment with the 1:8 and 1:32 dilutions resulted in serum reactivity similar to the tagging experiments on cultures infected with a single virus type.

DISCUSSION

The capacity of rabbit anti-NZB serum to distinguish between ecotropic and xenotropic viruses as tested by the ferritin-labelled antibody technique confirmed previously reported studies illustrating differences in the type specificities of ecotropic and xenotropic viruses (Aaronson & Stephenson, 1974; Arnstein et al. 1974; Lee et al. 1974; Levy, 1974, 1975; Hanna et al. 1975; Hartley & Rowe, 1976; Hino, Stephenson & Aaronson, 1976). Our studies further demonstrated the applicability of the ferritin-labelled antibody technique in distinguishing morphologically similar but antigenically dissimilar viruses replicating within the same cell line. Antigenic differences between endogenous viruses replicating in the BALB/3T3 cell systems have previously been detected with ferritin-labelled antibodies (Aoki & Todaro, 1973) and with ferritin-labelled concanavalin A (Stewart & Maizel, 1975). An identification of the different virus classes, however, was not made. More xenotropic virus particles were observed in cultures chronically infected with xenotropic viruses and super-infected with ecotropic virus than in the control cultures chronically infected with xenotropic viruses alone. Previous studies have suggested that superinfection of cells producing xenotropic virus with an ecotropic virus may enhance xenotropic virus production (Levy et al. 1975).

The cross-reactivity seen in our experimental system could be removed by serum dilution, suggesting either a difference in the affinity of the rabbit anti-NZB virus antibodies for ecotropic and xenotropic viruses or the existence of antibodies to both group- and type-specific determinants. This difference in tagging at a 1:16 dilution was sufficient for distinguishing an ecotropic from a xenotropic virus in tissue culture and provided an effective means of identifying the two classes of MuLV in mixed infection. The pattern of tagging
in the mixed cultures suggested certain cells predominantly produced either the xenotropic or ecotropic virus. In some fields tagging of all particles was observed, while in others no tagging occurred despite the presence of ferritin within the vicinity of a group of viruses. However, since thin sections of cells may only reveal a small portion of the whole cell, one cannot discount the possibility of certain areas on one cell producing a single virus type while another area was producing a virus of another type. Other methods, such as double staining with fluorescein and rhodamine, should be used to distinguish cells containing a single antigen from those containing a mixture of both (Cremer, Taylor & Lennette, 1969).

This investigation was supported by Public Health Service grants AIo8200, NO1CP43209 and CA13086 from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute, National Institutes of Health. Dr Levy is a recipient of Research Career Development Award no. 5 KO4 CA 70990 from the National Cancer Institute.

We thank Marion L. Davison, Dr Osman H. Luis and Koichi Nakamura for technical assistance.

REFERENCES


Ferritin-labelled xenotropic viruses


(Received 8 October 1976)