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PSEUDOTYPES OF VESICULAR STOMATITIS VIRUS WITH COAT ANTIGEN OF BOVINE LEUKAEMIA VIRUS – VSV (BLV): ANTIGENIC SURFACE MOSAIC AND THE ROLES OF PRECIPITATING ANTIBODIES AND POLYCATIONS

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Summary. – The pseudotype particles vesicular stomatitis virus (bovine leukaemia virus) [VSV (BLV)] contain a surface antigenic mosaic, composed of both VSV- and BLV-specific antigens, as demonstrated by increased neutralization by anti-VSV serum after addition of complement or of "second antibody". All the pseudotype infectivity was precipitated by VSV-specific antibody: it could be pelleted by low-speed centrifugation and its infectivity recovered without any loss by sonication. Polycations present during adsorption had little effect on infectivity of the pseudotype for Vero cells, but markedly increased its titre for chicken fibroblasts.

Key words: Retrovirus; Rhabdovirus; vesicular stomatitis virus; bovine leukaemia virus; phenotypic mixing;pseudotype; neutralization

Introduction

In the past years, phenotypic mixing between enveloped viruses belonging to unrelated families has been studied in several laboratories. It has been shown to be a rather common phenomenon, but was studied in more detail mostly between rhabdoviruses (usually represented by VSV) and retroviruses. Most of the available information has been reviewed by Závada (1976, 1977) and Weiss (1980).

One of the viral combinations studied in our laboratory was that of VSV with BLV (Závada *et al.*, 1978, 1979). VSV grown in BLV-shedding lamb cells produces a proportion of particles which resist an excess of anti-VSV serum and are neutralized when also anti-BLV serum is added. These are the VSV (BLV) pseudotype particles. Using these, we developed a highly sensitive, rapid and simple test for detecting BLV-specific antibodies in the sera of spontaneously infected cattle. In this work, we came across several additional problems, which will be dealt with in the present paper:

1. What is actually the surface composition of the apparent VSV (BLV) pseudotypes: do they contain only BLV-coded neutralization antigen, or

are they in fact particles with an antigenic mosaic, composed of both BLV- and VSV-specific surface antigens? It was shown that the infectivity of the apparent pseudotype formed between VSV and chicken (Weiss *et al.*, 1975; Weiss and Bennett, 1979) or murine (Witte and Baltimore, 1977) retroviruses decreases by a factor of 10—100, when the pseudotype is treated with anti-VSV serum and subsequently with complement or with "second antibody" (antiserum to IgG of the animal species used for production of anti-VSV antibody). This indicated that 90—99% of apparent pseudotype are in fact mosaic particles, and that only 1—10% might be a "pure pseudotype". Also a statistical consideration indicated (Závada, 1976) that formation of "pure pseudotypes" is an extremely unlikely event.

2. When we harvested infectious culture fluid from cells mixedly infected with BLV and VSV, and added anti-VSV serum, we noticed a rapidly developing faint turbidity. This indicated precipitation of VSV by the antibody. This precipitate could by pelleted by low speed centrifugation. The question was, to what extent this precipitation contributed to the neutralization of the virus.

3. VSV, and still more Chandipura virus, another member of *Rhabdoviridae* family, when grown in BLV-shedding FLS cells and treated with a combination of both anti-rhabdovirus and anti-BLV sera, was not neutralized totally. With VSV (BLV) pseudotype there was 1%, and with Chandipura (BLV) up to 20% of pseudotype infectivity that survived treatment with both antisera. We used the term "noise" for such residual infectivity. The "noise" could mean that *a*) the FLS/BLV cells contain besides BLV an additional donor of coat antigens-possibly a hypothetical endogenous sheep virus or a contaminant enveloped virus, and *b*) part of virus infectivity is protected from neutralizing sera by aggregation of the virions.

4. The VSV (BLV) pseudotype showed the highest infectivity for Vero cells, a lower infectivity for other mammalian cells, and a very low one for chicken cells. Does this low infectivity for chick cells indeed reflect the VSV (BLV) pseudotype, or is it due to possible other causes, mentioned in point 3 above?

Materials and Methods

Antisera. Sheep anti-VSV serum was the same as used by Závada *et al.* (1979). Also anti-BLV sera and corresponding negative bovine sera were selected in experiments described in the same paper. The "second antibody" — RasShIgG — was raised in rabbits immunized with sheep IgG. The latter was purified by combined precipitation with caprylic acid (Steinbuch and Audran, 1969), the resulting supernatant was exhaustively dialyzed against physiological saline buffered with 0.067 M phosphate, pH 7.2 and precipitated with 40% saturated ammonium sulphate, pH 7.2, and finally dialyzed against buffered physiol. saline. The immunization scheme for the rabbit was the same as that for VSV in the sheep. The RaShIgG showed an optimum ratio of 30 : 1 for precipitation with sheep serum as judged from turbidimetry. It showed a virtually monospecific reaction with IgG, when tested in immunodiffusion with whole serum or IgG from sheep serum. From the immune sera (sheep anti-VSV and RaShIgG) IgG was again isolated by the method described above and used in all experiments presented below.

Cells, media and infectivity assays were as described previously (Závada *et al.*, 1977, 1978, 1979).

Treatment of VSV grown in mixed infection with BLV with a combination of anti-VSV serum and "second antibody" or complement. The VSV(BLV) pseudotype is produced in BLV-shedding