NOTES

Lentiviruses Are Naturally Resident in a Latent Form in Long-Term
Ovine Fibroblast Cultures

VÉRONIQUE BARBAN,1 GILLES QUÉRAT,1 NICOLE SAUZE,1 PIERRE FILIPPI,1 ROBERT VIGNE,1* PIERRE
RUSSO,2 AND CHRISTIAN VITU2

Laboratoire de Virologie, Faculté de Médecine Nord, 13326 Marseille Cedex 15,1 and Laboratoire de Pathologie des
Petits Ruminants, Ministère de l’Agriculture, 06000 Nice,2 France.

Received 14 March 1984/Accepted 12 July 1984

Long-term ovine fibroblast cultures contain replicative-competent lentiviruses in a latent form. This in vitro
phenomenon, never described previously for lentiviruses, was clearly demonstrated by activating the
expression of latent viruses with various inducing cell treatments, some of which were efficient in inducing
endogenous retroviruses or latent herpesviruses. Activated lentiviruses were highly lytic in ovine fibroblasts
(type I), or they established persistent infections (type II) as described previously for field isolates from sheep

A common property of ovine and caprine lentiviruses is their persistence in animals for years despite a strong
immune response, due to the fact that they reside in an intracellular form without producing significative amounts
of free viral particles (6, 9). This low expression of lentiviruses in animals (in vivo) seems to be the consequence of an
intrinsically restricted virus replication at the cellular level (1-3).

The in vivo restriction of virus replication can be overcome by explantation of tissues from infected sheep, which
allows the cell-associated virus to initiate its extracellular production and induce cytopathic effects on the naturally
infected cells, within an average period of 1 month after the beginning of explantation (6, 9). However, we have recently
observed that ovine cell cultures generated from tissue explants could be induced to produce lentiviruses after
several months of cell cultivation, suggesting that the restriction of lentivirus expression can be strongly preserved in
tissue culture (10). We therefore suspected that long-term ovine cell cultures used in various laboratories worldwide
could contain maedi-visna virus information in a silent form (latent form).

To activate latent lentiviruses eventually present in long-term ovine fibroblast cultures, several cell lines were treated
by the following modes of induction, some of which were found to be efficient for inducing endogenous retroviruses or
latent DNA herpesviruses (5, 11): maintenance of the cells in serum-poor medium for a long period of time, treatment
of the cells for 24 h with 100 µg of BuDR per ml or 100 mM 5-azacytidine, physical stress on the cells by successive freezings
in Eagle minimal essential medium containing 30% fetal calf serum and 20% dimethyl sulfoxide as well as in Eagle
minimal essential medium supplemented with 10% lamb serum thawings, coculture with heterologous rodent cells
(NRK cell line), and superinfection by an exogenous persistent lentivirus such as the American strain of caprine arthri-
tis-encephalitis virus (USA-CAEV). The cell cultures tested for the presence of lentiviruses were fetal ovine cell lines
from various organs or ovine choroid plexus (OCP) fibro-
blasts established from adult animals from France, Federal
Republic of Germany, Iceland, and the United States.

The success of the induction mode was monitored by the appearance of typical cytopathic effects of lentivirus replica-
tion, i.e., cell fusion and accumulation of refractile dying
cells (6, 7, 9).

Table 1 shows that one American ovine fetal cornea cell
line (USA-OFcF) was highly sensitive to all of the induction
methods. In contrast, some cell lines were sensitive only to a
particular treatment, e.g., the French cell lines F-OCP2 and
F-OCP4, which were induced to produce lentiviruses only
after the 5-azacytidine treatment and the repetitive freeze-
thaw dimethyl sulfoxide method, respectively. Several cell
lines were resistant to all of the induction treatments
presently used. This was the case with F-OCP1 cells, which we
used in all of our virus production experiments. Similarly, an
Icelandic cell line, ICE-OCP1, was not able to produce
lentiviruses, nor were two American fetal cell lines, USA-
OFcF and ovine fetal turbinate (USA-OFTuF). However, our failure to induce viruses from these cell lines does not
necessarily mean that they do not contain latent viral
information.

As in the case of virus isolation from lung explants from
French sheep with pneumonia, two types of viruses were
induced from cell lines: those that very efficiently killed
cells, such as the Icelandic visna virus strain K1514 (type I
viruses), and those that caused chronic infections, such as
the new type of ovine lentivirus, which we have recently
isolated from ovine lung fibroblasts from a French sheep
with progressive pneumonia (type II viruses) and which was
found to be closely related to USA-CAEV (10).

To identify both types of viruses, analyses of their structural
proteins expressed in infected cells were performed as
previously described (13). Briefly, ovine cell cultures were
labeled with [35S]methionine for 24 h, either at the time of
the induction of their own latent lentiviruses or after exoge-
nous infection by stocks of the new ovine lentiviruses.
Intracellular labeled proteins were immunoprecipitated by
antisera raised against two major internal proteins of Icelan-
dic visna virus strain K1514 (p30 and p16) and analyzed by
polyacrylamide gel electrophoresis. Examples of these anal-
yses were shown in Fig. 1. As previously described (13),
cells infected by highly lytic viruses, e.g., Icelandic visna
TABLE 1. Lentiviruses can be induced by several treatments in long-term passaged ovine cells lines

<table>
<thead>
<tr>
<th>Cell lines*</th>
<th>Ovine tissue origin</th>
<th>Passage no.*</th>
<th>Maintenance in sernum poor medium</th>
<th>BUdR</th>
<th>5-Azacytidine</th>
<th>Freeze-thawing (DMSO)</th>
<th>Coculture with NRK</th>
<th>Superinfections by USA-CAEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>With inducible virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA-OFC1</td>
<td>Cornea (embryo)</td>
<td>40</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F-OCP2</td>
<td>Choroid plexus (adult)</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F-OCP3</td>
<td>Choroid plexus (adult)</td>
<td>20</td>
<td>-</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>F-OCP4</td>
<td>Choroid plexus (adult)</td>
<td>15</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>USA-OFTU1</td>
<td>Choroid plexus (adult)</td>
<td>Not known</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>NT</td>
</tr>
</tbody>
</table>

| Without inducible virus |
| F-OCP1 | Choroid plexus (adult) | 30 | - | - | - | - | - | - |
| F-OCP1 | Choroid plexus (adult) | 15 | - | - | - | - | - | - |
| ICE-OCP1 | Choroid plexus (adult) | 10 | - | - | - | - | - | - |
| USA-OFC2 | Cornea (embryo) | 20 | - | - | - | - | - | - |
| USA-OFTU1 | Turbine (embryo) | 15 | - | - | - | - | - | - |

*Cell line symbols and country of origin: F, French; G, German; USA, American; ICE, Icelandic. Laboratory of origin: G-OCP1, O. Narayen (at that time, Giessen, Germany); USA-OFC, USA-OFC1, USA-OFC2, and USA-OFTU1, R. Cutlip (Ames, Iowa); ICE-OCP1, G. Petursson (Reykjavik, Iceland). All of the French cells were generated in laboratories in Nice (F-OCP1) or Marseille (F-OCP2, F-OCP3, F-OCP4, F-OCP5) from sheep from slaughterhouses.

*Passage number of the cell lines at the time of induction experiments.

*Details on treatments used to induce viral expression in cells are described in the text. Induced viruses were detected by the appearance of cytopathic effects typical of lentiviruses. DMSO, Dimethyl sulfoxide; NT, not tested.

virus K1514, contain both proteins p30 and p16 and a common polypeptide precursor to those proteins, Pr55gag (lanes G and G'). Polypeptide intermediates smaller than Pr55gag (P50 and P35) and Pr150gag-pol were also detected but will be excluded from our comparative study. Similarly, F-OCP1 cells infected exogenously with type I lentivirus isolated from USA-OFC1 cells contain type I lentivirus-specific antigens, p30 and p16, and their precursor, Pr55gag.

Analyses of viral proteins of cells infected by persistent lentiviruses were also performed. As described previously (10), a type II virus recently isolated from a lung explant of a sheep with pneumonia shares with type I viruses lentivirus-specific p30 antigen (lane A) but not type I-specific p16 antigen (lane A'). The antigen properties of this first ovine type II virus are also characteristic of one new persistent lentivirus, induced from the cell line F-OCP1 (lanes B and B') and characteristic of the caprine lentivirus USA-CAEV, in goat synovial membrane cells (10) and in ovine cell lines (lanes C and C'). The particular example of a type I virus induced by superinfection of USA-OFC1 cells with USA-CAEV is shown in lanes D and D' (see Table 1).

To confirm our classification of the induced viruses, a rapid analysis of unintegrated linear proviral DNA present in infected cells was performed by using the Southern blotting technique and type I virus-specific cDNA hybridization as previously described (10). All of the highly lytic viruses replicate through an unintegrated proviral DNA of 9.25 kilobase pairs which is easily detectable by a type I virus-specific cDNA. Moreover, efficient hybridization of this probe to all restriction fragments generated from the complete genome of type I viruses suggests that all of the type I viruses, including Icelandic visna virus, are closely related all along their genome (data not shown). In contrast, proviral DNA of type II viruses were poorly detected by type I virus-specific cDNA, as previously described for the prototype of type II ovine maedi-visna viruses [F-OMVV-(I)(II)] (10).

Table 2 summarizes the types of lentivirus that were induced from five latently infected long-term ovine cell cultures. We should note that four of the five cell lines gave rise to only one type of lentivirus. F-OCP2 was a unique cell line which produced both types of viruses in two independent experiments. This last result suggests that sheep may be infected by both types of virus; thus, the isolation of only one type of virus from most of the cell lines does not necessarily prove that the other type of virus was not present in those cell cultures. Further experiments in lentivirus activation should help settle this question.

The present results show that some long-term ovine fibroblast cultures obtained from various laboratories contain, in a latent form, replicative-competent lentiviruses. This in vitro phenomenon, never described previously for lentiviruses, was clearly demonstrated by activating the expression of latent viruses with inducing cell treatments and by characterizing the induced viruses. For example, one embryonic cell line (USA-OFC1) was induced many times to produce viruses under several induction treatments. In contrasts, several cell lines were not activated to produce lentiviruses, in particular, one Icelandic (ICE-OCP1) and two fetal American cell lines (USA-OFC2 and USA-OFTU1). Our failure to isolate Icelandic virus is a priori not surprising, since maedi and visna diseases have been eradicated from Iceland for two decades (8). Similarly, embryonic cells should be frequently virus negative, since transmission of lentiviruses has been shown to occur mainly through the milk after the birth of the kid (4, 12). However, we should point out that cell lines which were not induced to produce virus during our assays may nevertheless contain latent lentiviruses which are not easily inducible, as previously described for endogenous retroviruses (for a review, see reference 14). More efficient treatments should be investigated to conclusively answer this question.

Long-term ovine cell cultures contain replicative-competent lentiviruses which are either highly lytic or persistent. By analyzing their protein and nucleic acid contents, we have shown that highly lytic ovine viruses [OMVV(I)] are quite similar to the Icelandic strains of visna virus and to the American strains of progressive pneumonia virus, which are the causative agents of progressive encephalitis and pneumonia. Persistent ovine viruses [OMVV(II)] are highly related to CAEV, which is responsible for the goat arthritis-encephalitis syndrome, and to a persistent ovine lentivirus isolated in our laboratory from a sheep with pneumonia (10).
Latent lentiviruses were not detected before activation by hybridization or immunoprecipitation techniques (data not shown), suggesting that lentiviruses may be present in a low proportion to the cells in the cell cultures. Further experiments, such as cloning of infected cells and in situ hybridization at the level of a single cell, will be necessary to determine the latent form of the viral information. Such experiments should help to ascertain whether the in vitro latent lentiviruses are really restricted at the cellular level, as suggested by others for lentiviruses during their in vivo persistence (1–3, 7), or whether they correspond to unrestricted viruses which replicate very slowly in long-term cell cultures.

ACKNOWLEDGMENTS

We thank J. Tamalet for support during this work, L. Gazzolo and N. Pourreua for reading the manuscript, P. Antonini for typing the manuscript, and R. Cullip, O. Narayan, and G. Petrusson for providing cell lines. This work was supported by grants from the Association du Développement de la Recherche sur le Cancer (Villejuif), Fondation de la Recherche Médicale (Paris), Institut de la Santé et de la Recherche Médicale (CRL 812007), and Ministère de l’Agriculture (Direction de la Qualité et Ministère de l’Éducation Nationale (Mission de la Recherche).

LITERATURE CITED


FIG. 1. Intracellular protein expression of induced lentiviruses. [35S]methionine-labeled viral proteins, extracted from cells infected with various viruses, were immunoprecipitated by monospecific sera raised against two major internal proteins of the Icelandic strain K1514 of visna virus, p30 (lanes A through G) and p16 (lanes A’ through G’). Ovine type I virus-infected cells are as follows: (A and A’) F-OCP, cells infected by F-OMVV-I(II) recently isolated from a lung explant of a sheep with naturally occurring progressive pneumonia (explant coculture 2150-81) and (B and B’) F-OCP, chronically infected by its own 5-azacytidine-induced latent virus. USA-CAEV-infected cells are as follows: (C and C’) F-OCP, cells infected by USA-CAEV and (D and D’) F-OCP, cells infected by USA-CAEV and USA-OMVV(I) induced from USA-OFC cells. Ovine type I virus-infected cells are as follows: (F and F’) F-OCP, cells infected by USA-OMVV(I) induced from USA-OFC cells and (G and G’) F-OCP, cells infected by the Icelandic strain K1514 of visna virus. On the right of the figure, marks indicate the position of p30 and p16 of the prototype strain K1514 of visna virus and of their common precursor, Pr55sup and Pr150sup, pol. Normal cells are as follows: (E and E’) F-OCP, cells.

Structurally related to pathogen agents, activated lentiviruses could be responsible for slow virus diseases. To answer this important question, experimental infections of sheep and goats with activated lentiviruses should be performed.

TABLE 2. Latent lentiviruses activated from long-term ovine cell cultures are either highly lytic or persistent

<table>
<thead>
<tr>
<th>Cell line*</th>
<th>Type of activated lentivirus*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Highly lytic (type I)</td>
</tr>
<tr>
<td>USA-OFC1</td>
<td>+</td>
</tr>
<tr>
<td>G-OFC1</td>
<td>+</td>
</tr>
<tr>
<td>F-OCP2</td>
<td>-</td>
</tr>
<tr>
<td>F-OCP4</td>
<td>-</td>
</tr>
<tr>
<td>F-OCP5</td>
<td>-</td>
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</table>

* See footnote a, Table 1 for explanations of the cell line symbols.

* All of the new lentiviruses were typed I or II, with regard to their replicative properties and their protein or DNA contents or both.