Antigenic Comparison of Bovine Type C Virus with Murine and Feline Leukemia Viruses

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INTRODUCTION

SUMMARY

Bovine Serum 27-125 (spontaneous regression case of leukemia), which contains antibodies to a type C virus found in bovine cultures [New Bolton Center (NBC) cell lines], did not react in immunofluorescence tests with rat cells transformed by and releasing abundant quantities of murine leukemia viruses. In addition, these rat cells did not remove the immunofluorescence activity of Serum 27-125 for virus-containing NBC cells.

Negative results were also obtained when 3 rat antisera containing antibodies to most, if not all, the known antigens of the murine leukemia-sarcoma viruses, including the group-specific (gs) antigens, were tested by immunofluorescence on NBC cultures showing relatively high proportions of cells with the bovine virus immunofluorescence antigen. Two anti-feline leukemia virus sera, with strong anti-gs antigen activity, also failed to react with the NBC cultures. Repeated absorption with large numbers of disrupted NBC cells did not remove the immunofluorescence activity of one of the anti-feline leukemia virus sera for gs-3 antigen contained in a rat lymphoma.

Immunodiffusion experiments with the anti-murine leukemia virus and anti-feline leukemia virus sera also failed to reveal the gs antigens in Tween-ether-treated and concentrated preparations of cells and/or pellets of culture fluids from the virus-producing NBC cell lines. These preparations did not remove the anti-gs-precipitating activity of an anti-murine leukemia virus serum either.

Thus, the results of both immunofluorescence and immunodiffusion studies indicate that the type C virus of the NBC cell lines is an indigenous bovine virus which is antigenically unrelated to the known mammalian type C viruses. These findings suggest the possibility that leukemia in cattle and perhaps in other species may be caused by a family of type C viruses different from that of the presently known leukemia viruses.

INTRODUCTION

Immunological methods have contributed significantly to studies on the characterization and interrelationships of type C viruses, particularly those known to induce leukemia in mice. With the use of mouse antisera, type-specific antigens located at the cell surface and/or in the envelope of the virus were first identified in the murine leukemia system (20). Subsequently, antisera prepared in inbred rats by immunization with syngeneic or histocompatible tumors proved to be of particular value in these studies since, while being practically free of hetero- or isoantibodies, these reagents react strongly with antigens not recognized by the mouse (7, 11, 13, 24). Indeed, it was through the use of these rat antisera that soluble antigens shared by all the MuLV2 were discovered (11, 13). These group-specific (gs) antigens are internal constituents of the virus and occur also in the infected cells where they can be detected by immunodiffusion (11), complement fixation (13), and, as shown more recently, by immunofluorescence (3, 15, 18). Geering et al. (9, 10) demonstrated that one of these group-specific antigens, termed gs-3, is also present in the type C viruses of the cat, hamster, and rat.

The gs-3, or interspecies group-specific antigen, has proven to be one of the most satisfactory markers for the presence of the virus or its genome. In fact, this antigen has been found in cases in which neither infectious virus nor type-specific viral antigens were detectable (14, 16). Aside from this practical contribution, the discovery of gs-3 antigen has obvious far-reaching implications in studies on the viral etiology of leukemia in other species, since it suggests a common origin for all the mammalian type C tumor viruses.

The presence of type C virus in long-term cultures of lymphoid cells from leukemic cows (NBC cell lines) as well as in short-term cultures of buffy coat cells from cows with leukemia or persistent lymphocytosis has now been well established (4, 8, 19, 23). However, in view of the widespread distribution of MuLV and FeLV and evidence that MSV-MLV replicates in bovine cultures (25) it was essential to determine whether the type C virus found in bovine cultures represents a contaminant. In the present work this possibility has been examined by: (a) testing for the presence of the previously described (5) immunofluorescence viral antigen of the bovine type C virus in cells heavily infected with murine leukemia-sarcoma viruses; and (b) searching for the antigens associated with the murine and feline type C viruses, and

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particularly for gs-3 antigen, in cells infected with the bovine virus and in the bovine virus itself.

MATERIALS AND METHODS

Cell Cultures and Tumors. The cell cultures established from leukemic cattle (NBC cell lines) have been described previously (8). Cell line 78 A1, established by Bernard et al. (2) with embryo Wistar rat cells transformed in vitro by MSV-M, was brought to our laboratory by Dr. Bernard Guillemain and is maintained as a monolayer in E20FSI. This line continuously releases abundant quantities of MSV and MLV (2, 3). Lymphoma LW-27 was induced by neonatal injection of RadLV into W/Fu inbred rats. This tumor is maintained by serial transplantation into preirradiated syngeneic rats. It contains abundant type C particles as well as high concentrations of both specific cell surface antigens (6, 7) and group-specific antigens (1).

Sera. Details on the origin of our bovine sera have been given in the preceding paper (5). The rat anti-RadLV serum, prepared in our laboratory, and the anti-GLV serum, kindly supplied by Dr. L. J. Old and by Miss G. Geering (Sloan-Kettering Institute, New York, N. Y.), were produced in W/Fu X BN F1 hybrid rats by repeated immunizations with W/Fu lymphomas induced by the corresponding virus. These antisera contain: (a) high titers of antibodies specifically cytotoxic for lymphomas induced by RadLV or GLV in mice or rats; (b) virus-specific neutralizing antibodies; and (c) precipitating, complement-fixing, and fluorescent antibodies to the intraspecies (gs-J) and interspecies (gs-3) group-specific antigens of MuLV (1, 7, 11, 13, 15). Anti-MSV-M serum, prepared in Fischer rats bearing syngeneic tumors induced by this virus, was supplied by Huntington Laboratories (Baltimore, Md.) through the courtesy of Dr. Wilna Woods (National Cancer Institute). The anti-gs activity of this antiserum has been reported (27) and confirmed in our laboratory. In addition, we found that the antiserum is specifically cytotoxic for MLV leukemias and has strong neutralizing activity for MSV-M. A goat anti-FeLV serum (IS-8) and a normal goat serum (OS-142) were also obtained from Huntington Laboratories. Two antisera with strong anti-gs activity were kindly supplied by Dr. F. Noronha and Dr. C. Rickard (Cornell University, Ithaca, N. Y.). One of these antisera was obtained from a goat immunized with disrupted FeLV and the other from a rabbit immunized with a more purified preparation of FeLV-gs antigen. Before use, all of the anti-FeLV sera were absorbed with lyophilized fetal calf serum and with disrupted normal rat lymphoid cells. One ml of antiserum was mixed with 20 mg of powder and incubated for 2 hr at room temperature and then at 4°C overnight. Antiserum was recovered by centrifugation at 2,000 X g for 20 min and then absorbed with an equal volume of packed and disrupted normal rat lymphoid cells, following the procedure described below.

Immunofluorescence Tests. These tests as well as the preparation of FITC-conjugated goat γ-globulin anti-bovine 7S γ-globulin have been fully described in the previous paper (5). FITC-conjugated goat antiserum to rat 7S γ-globulin was purchased from Hyland Laboratories (Los Angeles, Calif.) and used at 1:10 dilution without previous adsorption. FITC-conjugated rabbit antiserum to goat 7S γ-globulin and FITC-conjugated goat antiserum to rabbit 7S γ-globulin were also supplied by Hyland Laboratories and used at a 1:10 dilution after adsorption with acetone-extracted rat organ powder (100 mg of powder per ml).

Absorption Tests. Cells were washed twice, counted, and packed by centrifugation at 2,000 X g for 20 min. The supernatant fluid was removed and the packed cells were disrupted by 3 cycles of freezing and thawing. One volume of serum was mixed with 2 volumes of packed, disrupted cells. The mixtures were incubated for 2 hr at room temperature with periodic resuspensions and then at 4°C overnight. The serum was recovered after centrifugation at 2,000 X g for 20 min and further clarified by another centrifugation at 15,000 X g for 30 min.

Antigen for Immunodiffusion. Cells were pelleted at 2,000 X g for 20 min, resuspended in small volumes of distilled water, and mixed with pellets obtained by ultracentrifugation (56,000 X g, 90 min) of the corresponding culture fluids. This material was subjected to 3 cycles of freezing and thawing and mixed first with 1 volume of a Tween 80 solution (2 mg/ml) and then with 20 volumes of peroxide-free ether. After continuous agitation for 2 hr at room temperature, the mixture was centrifuged for 15 min at 1,000 X g. The H2O phase was removed and bubbled with nitrogen to eliminate any residual ether. This material was then clarified twice at 15,000 X g for 30 min, the pellets were discarded, and the supernatant fluid was concentrated approximately 500 times (with respect to the original volume of the culture) by vacuum dialysis against phosphate-buffered saline. The same procedure was also used to prepare antigen from either cells or pellets of culture fluids.

Immunodiffusion Tests. The double diffusion (Ouchterlony) tests were performed with 2% Noble agar (Immunoplate Pattern C, Hyland). Wells were filled 3 times with the reagents and the plates were incubated at room temperature in a humidified chamber. Plates prepared by incorporating varying amounts of polyethylene glycol (M.W., 6000; J. T. Baker Chemical Co., Phillipsburg, N. J.) into 1.5% Noble agar gel made in 0.9% NaCl solution were used in some tests in order to enhance the precipitin reaction.

RESULTS

Immunofluorescence Studies. In the 1st series of experiments, serum from a spontaneous regression case (27-125) of bovine leukemia and the serum from a normal cow were tested by the immunofluorescence technique against rat cells transformed by, and also abundantly releasing, MSV and MLV as well as against a rat lymphoma induced by and infected with RadLV. As reported previously, RadLV lymphomas are antigenically indistinguishable from those induced by GLV (7). Since in preliminary tests it was found that sera from both normal and leukemic cows stained most normal rat lymphoid cells, the bovine sera used in all subsequent tests were

1In these tests, control smears incubated only with FITC-conjugated goat-anti-γ-globulin also showed fluorescent cells (possibly immunoglobulin-containing lymphoid cells), but in much smaller

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subjected first to an in vivo absorption procedure. For this, 4 ml of serum were injected i.p. into a young female rat and recovered 16 hr later by exsanguinating the animal. As shown in Table 1 the in vivo absorbed Serum 27-125 gave a strong fluorescent reaction with a relatively large proportion of the NBC-6 and NBC-13 cells. On the contrary the virus-releasing as well as the normal rat cells were negative. Furthermore, experiments summarized in Table 2 showed that, while disrupted NBC-13 cells grown in E20FSI significantly removed the fluorescent activity of Serum 27-125 for NBC-6 cells, disrupted LW-27 cells did not. Lymphoid cells obtained from the lymph node of a normal cow were also negative. These results indicate that the viral immunofluorescence antigen of the bovine type C virus is not present in the murine leukemia-sarcoma viruses.

In another series of experiments, samples of NBC-6 and NBC-13, containing a relatively large proportion of cells with type C virus particles and virus-specific antigen, were examined by immunofluorescence with 3 potent and broadly reactive sera prepared in rats against murine leukemia-sarcoma viruses. As shown in Table 3, while the 3 antisera reacted strongly with the control cells infected with murine type C viruses, NBC-6 and NBC-13 cells were completely negative. Since it has been shown that prolonged incubation with the antiserum was needed for detecting gs antigen by immunofluorescence in nonproducing Rous sarcoma cells (17), the tests summarized in Table 3 were repeated, this time with the cells and antiserum being allowed to react for 90 rather than 40 min. Again, in no instance did the NBC cells show a staining reaction with the rat antiserum. The cross-reactivity of the RadLV lymphoma with the MSV-MLV-infected cells found in these tests is probably due to the gs antigens with which the rat antiserum used react strongly. Experiments in this laboratory, to be reported elsewhere, indicate that these cells also cross-react with rat antiserum in in vitro cytotoxic and absorption tests.

Negative results were also obtained when the same samples of cell lines NBC-6 and 13 were tested by the indirect immunofluorescence method with 2 anti-FeLV sera with potent anti-gs activity. On the other hand, these antisera reacted strongly against the rat cells releasing murine leukemia-sarcoma viruses, but not with normal rat lymphocytes (Table 4). As recently shown this interspecies reaction is due to the gs-3 antigen shared by MuLV and FeLV (15).

In order to evaluate more critically whether the NBC cells contain gs-3 antigen, the ability of these cells to remove the immunofluorescence activity of a goat anti-FeLV serum for the RadLV lymphoma LW-27 was examined. The absorption procedure with NBC-13 cells was carried out in 2 steps. One volume of the antiserum diluted 1:10 was first absorbed with 1.8 X 10⁹ cells following the procedure described in "Materials and Methods." The antiserum was then recovered and absorbed again with an equal volume of packed and disrupted cells (approximately 0.6 X 10⁹) from another pool of NBC-13 cultures. The NBC-13 cultures used in this experiment contained a relatively large proportion of virus-infected cells (as judged by immunofluorescence with

\[\text{numbers. Thus, it is quite possible that the reaction between the normal rat lymphocytes and the bovine sera is due to naturally occurring anti-rat antibodies in these sera.}\]

### Table 1

**Indirect immunofluorescence tests with bovine Serum 27-125 on cells releasing murine leukemia-sarcoma viruses**

<table>
<thead>
<tr>
<th>Cells tested</th>
<th>No. of fluorescent cells/No. of cells examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBC-6 (grown in E20FSI)</td>
<td>102/410 (25)(^a)</td>
</tr>
<tr>
<td>NBC-13 (grown in E20FSI)</td>
<td>34/217 (15)(^b)</td>
</tr>
<tr>
<td>LW-27 (rat RadLV lymphoma)</td>
<td>0/514 (0)</td>
</tr>
<tr>
<td>78 A1 (MSV-MLV-infected embryo rat cells)</td>
<td>0/456 (0)</td>
</tr>
<tr>
<td>Normal rat lymphoid cells</td>
<td>0/580 (0)</td>
</tr>
</tbody>
</table>

\(^a\) Control smears incubated with normal bovine serum (BI-038) previously absorbed in vivo (see text) and the conjugate or incubated with the conjugate alone showed less than 1% fluorescent cells.

\(^b\) Numbers in parentheses, % of fluorescent cells.

### Table 2

**Induction immunofluorescence tests with bovine Serum 27-125**

<table>
<thead>
<tr>
<th>Cells used for absorption(^a)</th>
<th>Immunofluorescence reactivity of absorbed serum(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>No. ((X 10^9))</td>
</tr>
<tr>
<td>NBC-13 (grown in E20FSI)</td>
<td>1.6 (18/363) (4.9)(^d)</td>
</tr>
<tr>
<td>LW-27 (rat RadLV lymphoma)</td>
<td>1.9 (95/421) (23)</td>
</tr>
<tr>
<td>Normal bovine lymphoid cells</td>
<td>1.7 (91/354) (25)</td>
</tr>
<tr>
<td>None</td>
<td>75/360 (20)</td>
</tr>
</tbody>
</table>

\(^a\) Serum diluted 1:10 and incubated with the indicated number of packed, frozen, and thawed (3 times) cells (1 volume of diluted serum per 2 volumes of packed cells).

\(^b\) Tested against NBC-6 cells grown in E20FSI.

\(^c\) Numbers in parentheses, % of fluorescent cells.

\(^d\) Obtained from the right prefemoral lymph node removed surgically from a cow (BI-371) in a leukemia-free herd.

Serum 27-125. Absorption with disrupted 78 A1 cells or with disrupted normal rat lymphoid cells was carried out in only 1 step and according to the standard procedure ("Materials and Methods"). The results of these absorption tests (Table 5) fully confirmed the results of the indirect immunofluorescence tests. In fact, while 78 A1 cells completely removed the anti-gs-3 immunofluorescence activity of the FeLV antiserum, NBC-13 cells did not, even when the antiserum was incubated with a large number of these cells.

**Immunodiffusion Studies**. As a 1st step the 5 antisera used in the immunofluorescence experiments plus another antiserum against disrupted FeLV were tested for precipitating activity against a TE-treated pellet obtained by ultracentrifugation of plasma from W/Fu rats bearing transplants of the RadLV-induced LW-27 lymphoma. The results of a representative test (Table 5) fully confirmed the results of the indirect immunofluorescence tests. In fact, while 78 A1 cells completely removed the anti-gs-3 immunofluorescence activity of the FeLV antiserum, NBC-13 cells did not, even when the antiserum was incubated with a large number of these cells.
Table 3

Indirect immunofluorescence tests on NBC cell lines with antisera against murine leukemia-sarcoma viruses

<table>
<thead>
<tr>
<th>Cells tested</th>
<th>Anti-RadLV serum</th>
<th>Anti-GLV serum</th>
<th>Anti-MSV-M serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBC-6 (grown in E20FSI)</td>
<td>0/650e (0)</td>
<td>0/589 (0)</td>
<td>0/515 (0)</td>
</tr>
<tr>
<td>NBC-13 (grown in E20FSI)</td>
<td>Not done</td>
<td>0/615 (0)</td>
<td>0/503 (0)</td>
</tr>
<tr>
<td>LW-27 (rat RadLV lymphoma)</td>
<td>215/265 (82)</td>
<td>220/245 (91)</td>
<td>276/345 (80)</td>
</tr>
<tr>
<td>78 Al (MSV-MLV-infected embryo rat cells)</td>
<td>197/212 (92)</td>
<td>215/226 (95)</td>
<td>263/294 (89)</td>
</tr>
</tbody>
</table>

a Sera diluted 1:2. Control smears incubated with the conjugate only or with a normal rat serum and the conjugate showed less than 1% fluorescent cells.
b Same samples of NBC cultures tested in experiments of Table 1. Between 15 and 20% of the cells in these cultures showed type C virus particles.
c No. of fluorescent cells/no. of cells examined.
d Numbers in parentheses, % of fluorescent cells.

Table 4

Indirect immunofluorescence tests on NBC cell lines with antisera against FeLV

<table>
<thead>
<tr>
<th>Cells tested</th>
<th>Goat anti-FeLV serum</th>
<th>Rabbit anti-FeLV serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBC-6 (grown in E20FSI)</td>
<td>0/615e (0)</td>
<td>0/539 (0)</td>
</tr>
<tr>
<td>NBC-13 (grown in E20FSI)</td>
<td>0/450 (0)</td>
<td>0/580 (0)</td>
</tr>
<tr>
<td>LW-27 (rat RadLV lymphoma)</td>
<td>210/245 (85)</td>
<td>189/193 (92)</td>
</tr>
<tr>
<td>78 Al (MSV-MLV-infected rat embryo cells)</td>
<td>ND</td>
<td>167/190 (88)</td>
</tr>
<tr>
<td>Normal rat lymphoid cells</td>
<td>0/480 (0)</td>
<td>0/415 (0)</td>
</tr>
</tbody>
</table>

a Sera preabsorbed with lyophilized fetal calf serum and with normal rat lymphoid cells. Used at dilution of 1:2. Control smears incubated with the conjugate only or with a normal goat serum and the conjugate showed less than 1% fluorescent cells.
b Supplied by Huntington Laboratories through the courtesy of Dr. W. Woods.
c Supplied by Dr. F. Noronha and Dr. C. Rickard.
d Same samples of NBC cultures tested in experiments of Tables 1 and 3.

Six different antigens prepared from either cells and culture fluid pellets, cells only, or culture fluid pellets only, of cell lines NBC-6 or NBC-13, were tested with each of the 6 anti-gs sera. Cultures of these cell lines in E20FSI, usually showing 10% or more cells with virus particles and/or viral immunofluorescence antigen, were used in preparing these antigens. No precipitin reactions were obtained in any of these tests. Subsequently, 4 of the NBC antigen preparations were pooled; this pool was concentrated 10 more times and then reacted against all the anti-gs sera. Again in no instance were precipitin lines observed.

Another series of immunodiffusion tests were conducted with the use of plates made in 1.5% agar containing 2% polyethylene glycol which has been shown to enhance precipitin reactions (12). Negative results were also obtained in most of these tests. In 3 tests, however, one of the antigen preparations from NBC-13 cells formed a very faint, unstable band with the RadLV antiserum. This band appeared 4 or 6 hr after the wells were filled and then, moving toward the antiserum well, gradually disappeared. At no time did this band show a reaction of identity with any of the bands formed between the RadLV antiserum and the reference gs antigen preparation.

In attempts to reproduce the band formed by the NBC-13 antigen preparation with RadLV antiserum, variations in the relative concentration of the reactants were tested in agar preparations of different ionic strengths and with several different concentrations of polyethylene glycol. All these attempts yielded negative results.

Immunodiffusion tests in which agar containing various concentrations of polyethylene glycol was used also failed to elicit precipitation reactions between antigen preparations from NBC cell lines and the other anti-gs sera.

The ability of the antigens prepared from the NBC cell lines to absorb the precipitating activity of the RadLV antiserum would be of considerable interest. Therefore, it is hoped that the NBC-13 antiserum will be used for the absorption experiments.
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for gs antigens was also investigated. The antiserum diluted 1:2 was mixed with 2 volumes of the antigen preparation and incubated at room temperature for 2 hr and overnight at 4°C. Serum was recovered by centrifugation and tested against the reference gs antigen. None of the 2 NBC antigen preparations (including that forming the weak and unstable band with the RadLV antiserum) tested by this procedure showed absorption activity. As shown in the representative experiment of Fig. 2, the gs-precipitating activity of the RadLV antiserum (Well 1) remained unchanged after incubation with the NBC-13 antigen (Well 3). On the contrary the RadLV-gs antigen preparation completely removed this activity (Well 2). (Excess of RadLV-gs antigen in Well 2 must be responsible for the precipitating line formed between this well and Wells 1 and 2.)

As also shown in Fig. 1, serum from the regression case, 27-125, did not react in immunodiffusion tests with the gs antigen prepared from RadLV (Well 4). On the other hand, this serum consistently gave 1 distinct precipitin band when tested against the antigens prepared from NBC-6 or NBC-13. Fig. 3 shows a representative experiment. A single precipitin line was formed between Serum 27-125 (center well) and a TE-extracted antigen from cells and supernatant fluid of NBC-13 cultures (Well 1). This line showed reactions of identity with the lines formed with the TE-extracted antigen prepared from cells and culture fluid of NBC-6 (Well 2), NBC-6 cells (Well 3), and pellets of NBC-6 culture fluids (Well 4). TE-extracted antigen prepared from lymphocytes of a normal cow in a leukemia-free herd was negative (Well 5). In other tests, the supernatant fluid obtained after ultracentrifugation of the NBC-6 culture fluids used for the preparation of the antigen in Well 4, was concentrated approximately 300 times and tested. No precipitin band was formed by this preparation, suggesting that the precipitin antigen detected by Serum 27-125 is contained in the virus or at least in the sedimentable fraction of the NBC culture fluid.

DISCUSSION

The data presented in this report indicate that the type C virus found in our NBC cell lines is an indigenous bovine virus which is serologically unrelated to the type C viruses of the mouse and cat. The immunofluorescence studies provide 2

Fig. 1. Center well, TE-treated RadLV; Well 1, rat RadLV serum; Well 2, rabbit anti-FeLV serum; Well 3, rat anti-MSV-M serum; Well 4, serum of spontaneous regression case (27-125) of bovine leukemia; Well 5, goat anti-FeLV serum.

Fig. 2. Center well, TE-treated RadLV; Well 1, rat RadLV antiserum nonabsorbed; Well 2, rat RadLV serum absorbed with TE-treated RadLV; Well 3, rat RadLV serum absorbed with TE-treated antigen preparation from NBC-13 cells.

Fig. 3. Center well, serum of spontaneous regression case (27-125) of bovine leukemia; Well 1, TE-treated antigen preparation from NBC-13 (cells and pellet of culture fluids); Well 2, TE-treated antigen preparation from NBC-6 (cells and pellet of culture fluids); Well 3, TE-treated antigen preparation from NBC-6 (cells only); Well 4, TE-treated pellet obtained by ultracentrifugation of NBC-6 culture fluids; Well 5, TE-treated antigen preparation of lymphoid cells from a normal cow (BI-196) in a leukemia-free herd.
kinds of evidence which indicate that the bovine virus and the murine leukemia-sarcoma viruses are antigenically dissimilar. First, the viral immunofluorescence antigen detected in the NBC cultures by serum from a cow in which leukemia had regressed spontaneously was not found in cells releasing abundant quantities of RadLV or MSV and MLV, even when absorption tests were used. In the interpretation of these observations it is important to keep in mind that RadLV seems to be antigenically indistinguishable from GLV (7) (J. F. Ferrer, unpublished observations) which is the most prevalent naturally occurring type C virus of the mouse (11, 21). Second, 3 potent rat antisera containing antibodies to most, if not all, the known antigens of MuLV failed to react in immunofluorescence tests with NBC cultures containing relatively large proportions of cells with the viral immunofluorescence antigen of the bovine type C virus.

The failure of the same NBC cultures to react in immunofluorescence tests with 2 antisera prepared against FeLV indicates that the virus of these cell lines is also antigenically different from the feline virus.

The fact that all the antisera used in the immunofluorescence experiments contain antibodies against the interspecies group specific or gs-3 antigen deserves particular attention. As is well known, gs-3 antigen is present not only in the virus but also in the virus-infected cells where it can be detected by immunofluorescence (3, 15, 18). Thus, the possibility that the bovine virus does not share gs-3 with the other known mammalian RNA type C viruses* must be considered. It could be argued that direct testing by immunofluorescence is a relatively insensitive method. However, results of absorption tests, considered as one of the most sensitive serological procedures, showed that incubation with large quantities of disrupted, virus-containing NBC cells does not alter the immunofluorescence reactivity of one of the FeLV antisera for gs-3 antigen contained in RadLV-infected cells.

Immunodiffusion experiments with 6 different anti-gs sera have also failed to detect the gs antigens of MuLV and FeLV in TE-treated preparations from NBC cultures. The significance of the weak band formed between the rat RadLV antiserum and the TE-treated homogenate from NBC-13 in a small percentage of tests is highly questionable. Owing to the weakness, instability, and irreproducibility of this band, it was impossible to study its nature, but these various features suggest that it is due to a nonimmunological phenomenon. Indeed, it has shown that certain tissue extracts may give a line of precipitation with serum albumin and that this reaction is due to electrostatic forces rather than to antigen-antibody interaction (26). However, even if it is assumed that the band represents an immunological phenomenon, it seems very unlikely that it could be due to the known MuLV and FeLV gs antigens. In fact, the band did not show reactions of identity with either of the 2 bands formed between the RadLV antiserum and the reference gs antigen preparation. In addition, the NBC-13 antigen preparation did not remove the gs-precipitating activity of the RadLV antiserum nor was a similar band found in any of the tests in which this antigen preparation was tested with the other anti-gs sera.

Schäfer et al. (22) reported preliminary experiments showing that extracts of PHA-treated buffy coat cultures from a leukemic cow reacted, although weakly, in complement fixation tests with a rabbit anti-gs serum. However, the authors point out that the significance and specificity of this finding has not been definitively established. In the preceding paper (5), we have presented evidence indicating that the type C virus from a similar buffy coat culture is antigenically related to, if not identical with, the NBC cell line virus. Thus, the negative results of all of our attempts to demonstrate gs antigens in the NBC cell lines may indicate that antigens other than these, or nonspecific reactions, are responsible for the preliminary findings of Schäfer et al.

Of course, the possibility that our failure to detect gs-3 antigen in the NBC cell lines is due to the limited sensitivity of the techniques used or to low concentration of the antigen in our preparations cannot yet be completely ruled out. Further investigations on this problem are underway in our laboratory and through collaborative efforts with other investigators. If we assume that the present findings will be confirmed and, in view of the results of our preliminary seroepidemiological survey suggesting a close association between the virus of the NBC cell lines and bovine leukemia (5), it is tempting to speculate that a family of type C viruses antigenically unrelated to any of the presently known leukemia viruses may be responsible for leukemia in cows and other higher species. As a corollary to this, one could also speculate that all the members of this family may share their own internal, group-specific antigen. On the basis of this hypothesis, we are attempting to produce an antiserum to the internal antigens of the bovine type C virus. The antiserum will be used to test the leukemias of humans and other species for the possible presence of such an internal group-specific antigen.

The absence of precipitin reactions between Serum 27-125 and the gs antigen preparation from RadLV is in line with previously discussed results showing that the same serum fails to react in immunofluorescence and absorption tests with rat cells releasing murine type C viruses. The nature of the precipitin antigen of the NBC cell lines is under study. The observation that this antigen is present in the sedimentable fraction of the virus-containing culture fluids suggests that it may be a virus constituent.

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The author thanks Dr. Robert R. Marshak for his interest and support. The author is also indebted to Dr. Bernard Guillenmain for supervising the preparation of antigens for immunodiffusion tests, Miss Jocelyne Laumond and Mrs. Diane M. Bhatt for their excellent technical assistance, and Mrs. Louise L. Wiggins for help in the preparation of the manuscript.

ADDENDUM

A recent study carried out in Dr. L. J. Old's laboratory at the Sloan Kettering Institute for Cancer Research (New York, N. Y.), in

*Recent experiments carried out in our laboratory indicate that the bovine type C virus is also an RNA virus.
collaboration with Dr. William Hardy and Dr. Hisami Ikeda, has also failed to detect MuLV-gs and FeLV-gs antigens in cell line NBC-13. In this study, samples of NBC-13 containing 15 to 20% virus-releasing cells (as evaluated by immunofluorescence with reference serum 27-125) were negative when tested by the indirect immunofluorescence method with potent anti-MuLV-gs and anti-FeLV-gs sera prepared according to procedures previously described (Hardy, W., et al. Science, 166: 1019—1021, 1969). Negative results were also obtained when these samples of NBC-13 cells were tested with the same antisera by the highly sensitive immunofluorescence absorption technique recently developed by Hilgers et al. (15).

REFERENCES


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