Syncytium-induction Inhibition Test with Complement for Detection of Antibodies Against Bovine Leukemia Virus

Y. Kono, W. Irishio and H. Sentsui*

ABSTRACT

A modified syncytium-induction inhibition test which is more sensitive than the immunodiffusion test, was developed using rabbit complement. In this test, fetal lamb kidney cells continuously infected with bovine leukemia virus were used as effector cells, and the CC81 cat cells transformed with murine sarcoma virus, were used as indicator cells. The syncytium-induction inhibition effect of anti-bovine leukemia virus serum was enhanced significantly by the addition of rabbit complement. The syncytium-induction inhibition titers had a statistically significant correlation with the immunodiffusion titers and were four to 64 times higher than immunodiffusion titers. In 12 experimentally infected cattle, the syncytium-induction inhibition test detected the antibodies earlier than the immunodiffusion test and continuously detected them when immunodiffusion antibody changed to negative. In the 81 sera from naturally infected herds, 35 (43.2%) were positive by the immunodiffusion test and 55 (67.9%) by the syncytium-induction inhibition test.

Key words: Bovine leukemia virus, syncytium-induction inhibition test, immunodiffusion test, antibodies to bovine leukemia virus.

RESUMÉ

Cette expérience consistait à développer, en se servant du complément de lapin, une épreuve d'inhibition de la formation de syncytioms, plus sensible que celle de la précipitation en milieu gélifié. Dans cette épreuve, on utilisait des cellules rénales d'embryons ovins, continuellement infectées par le virus de la leucémie bovine, comme cellules médiaterices; la lignée CC81 de cellules félines transformées par le virus du sarcome murin fournissait par ailleurs les cellules indicatrices. L'addition du complément de lapin conféra à l'antisérum de la leucémie bovine une intensification appréciable de son pouvoir d'inhibition de la formation de syncytioms. Les titres de l'épreuve d'inhibition de la formation de syncytioms affichèrent une corrélation statistique significative avec ceux de l'épreuve de précipitation en milieu gélifié et se révèlèrent de quatre à 64 fois plus élevés. Chez 12 bovins infectés expérimentalement, l'épreuve d'inhibition de la formation de syncytioms décéla les anticorps plus tôt que celle de la précipitation en milieu gélifié et elle continua à les détecter, même après que cette dernière eut cessé de le faire. Par ailleurs 35, i.e. 43.2% des 81 échantillons de sérum provenant de sujets appartenant à des troupeaux aux prises avec une infection naturelle par le virus de la leucémie bovine, donnèrent des résultats positifs avec l'épreuve de précipitation en milieu gélifié, comparativement à 55, i.e. 67.9%, avec celle de l'inhibition de la formation de syncytioms.

Mots clefs: virus de la leucémie bovine, épreuve d'inhibition de la formation de syncytioms, épreuve de précipitation en milieu gélifié, anticorps contre le virus de la leucémie bovine.

INTRODUCTION

Diagnosis of bovine leukemia virus (BLV) infection has been done mainly by the immunodiffusion (ID) test with the glycoprotein (gp) antigen of BLV (1). This method is superior to other diagnostic methods in simplicity but inferior to radioimmunoassay (2-5) and the viral neutralization test (3-5) in sensitivity. The enzyme-linked immunosorbent assay, which is generally accepted as one of the most sensitive methods, is reported to be as sensitive as the ID test (7-9). Bovine leukemia virus and BLV-infected cells induced syncytium formation in various types of cells (10-12). Since this syncytium formation is inhibited by anti-BLV antibody, a syncytium-inhibition test has been used for detection of BLV antibody (7,13,14). It has been reported that when BLV-infected cells are used as effector cells this test is less sen-
sitive than the ID test (13) but equally as sensitive as the ID test if cell-free BLV is employed (7). Recently it was observed that addition of rabbit complement to the syncytium-inhibition test significantly enhances the inhibitory activity of antiserum (15). In this paper we describe the detection of BLV antibodies by the syncytium-induction inhibition (SII) test with complement, in a modification from the previously reported method (15).

MATERIALS AND METHODS

CELL CULTURE

Fetal lamb kidney (FLK) cells persistently infected with BLV (16,17), FLK-BLV, were kindly supplied by Dr. M. Onuma, Hokkaido University, Japan. The cat cell line, CC81, containing the murine sarcoma virus genome (18), was a gift of Dr. P. Fischinger, National Cancer Institute, USA. Both of the cell lines were grown in Eagle's minimal essential medium (MEM) with 10% bovine serum and 10% tryptose phosphate broth.

SERUM

Three groups of sera were tested. The first group was collected periodically from 12 cattle infected experimentally with BLV. The second group (81 sera) was collected from clinically normal cattle which were kept in herds heavily infected with BLV. The third group (20 sera) was harvested on an island (Ie Island, Okinawa, Japan) on which BLV-positive reactors have never been found. A BLV antibody-negative control serum was collected from a steer that was kept in our institute for several years and found to be BLV free by repeated serological and virological tests.

COMPLEMENT

Fresh rabbit serum which has been shown to have no inhibitory effect on syncytium formation in CC81 cells by FLK-BLV was used as complement, at a 1:2 dilution in the medium.

SII TEST

Equal volumes (0.05 mL) of undiluted serum or serial twofold dilutions of a serum test, FLK-BLV cell suspension (4 x 10⁴ cells/mL) and complement were mixed in wells of a tissue culture microplate and incubated at 37°C for two hours in a CO₂ incubator. Then, 0.05 mL of CC81 cell suspension (4 x 10⁴ cells/mL) was added to each well and the plates were incubated for a further two days. The culture was then stained with Giemsa and syncytia were counted under a microscope. As a control BLV antibody-negative bovine serum was used instead of a test serum. Three wells were used for each dilution of the test serum and eight wells for the BLV antibody-negative control serum. Serum diluted 1:4 was used for antibody surveys. In this test a few small syncytia having four to seven nuclei were commonly found in an uninoculated control culture. These small syncytia, clearly distinguishable from 70 to 90 BLV-induced syncytia, having 15 or more nuclei, appeared in a positive control culture. A serum dilution or a sample which resulted in 50% or more inhibition of syncytium formation compared to the control cultures was regarded as positive. The SII titer was expressed as the reciprocal of the highest serum dilution that showed a positive reaction.

ID TEST

The ID test with glycoprotein (gp) antigen of BLV was performed as described previously (19).

RESULTS

Enhancement of SII by the addition of complement was first tested. As shown in Table I, SII antibody titers were increased four to eight times when fresh rabbit serum was added to the mixture containing postinoculation sera. However, no inhibitory effect was observed in the mixture containing preinoculation sera.

The reproducibility of the inhibitory effect of the sera was tested three times with each of two ID antibody-positive sera. The inhibitory titers were 256, 256 and 512 for one serum and 64, 128 and 128 for the other.

The correlation between SII and ID titers was tested with 70 serum samples and a high coefficient of correlation (r = 0.798) with a significance level of 1% was observed (Fig. 1).

When the rise and fall of SII and ID antibody titers were examined in 12 cattle experimentally infected with BLV the SII antibody appeared before or simultaneously with the appearance of ID antibody and fluctuated in parallel with the ID titers. The titers were, however, four to 64 times higher than the ID titers. Syncytium-induction inhibition antibody was detected persistently during the course of infection over an observation period of about two years in all the cattle, although ID antibody became undetectable intermittently in one animal (Fig. 2).

Immunodiffusion and SII tests were performed on 31 and 50 serum samples collected, respectively, from a herd in which an

<p>| TABLE I. Effect of Rabbit Complement on Antibody Titers in the Syncytium-Induction Inhibition (SII) Test |
|-------------------------------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Time of serum collection</th>
<th>ID titer</th>
<th>SII titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>B5</td>
<td>Preinoculation</td>
<td>0⁺</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18 MPI⁺</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>B9</td>
<td>Preinoculation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20 MPI⁺</td>
<td>64</td>
<td>256</td>
</tr>
<tr>
<td>B27</td>
<td>128</td>
<td>512</td>
<td>64</td>
</tr>
</tbody>
</table>

*0 means negative
*MPI = Months postinoculation
*Naturally infected
outbreak of enzootic bovine leuko-
sis was reported recently and from a
herd heavily infected with BLV. As shown in Table II, all the ID-
positive sera were also positive in the SII test. Of the ID-negative sera in the first and second herd, 40
and 47%, respectively, were posi-
tive in the SII test. On the other hand, all sera collected in the BLV-
free island showed negative reac-
tion in both tests.

DISCUSSION

Bovine leukemia virus infection induces early syncytium formation in CC81 cells (11,12). This cell line is suitable for the SII test since the syncytia formed are so large and distinct that they can be readily counted under a microscope at a low magnification. This SII test is considered to be highly specific because 1) ID-antibody positive sera consistently inhibited syncytium induction, 2) BLV-antibody negative sera which were collected in a BLV-free area showed no inhibitory effect, 3) antibodies as measured by SII increased and persisted in parallel with ID antibodies in experimentally infected animals and 4) a significant correlation was observed between ID and SII titers.

The syncytium-inhibition test reported previously (7,13) was equally as sensitive as or less sensitive than the ID test. However, the SII test was much more sensitive than the ID test. This high sensitivity may be due to enhanced inhibitory activity caused by addition of complement (Table I) (15). Although the correlation between the SII and ID tests was significant, it remains to be confirmed that the SII test is detecting antibody identical to that resulting in a positive ID test or a different antibody.

Since the SII test is more sensitive than the ID test, the SII test seems to be suitable for more accurate detection of BLV-infected animals, particularly in heavily infected herds (Table II), although the test requires tissue culture facilities, labor and time to count syncytia. In our preliminary tests BLV was isolated from the lymphocytes of cattle that were positive for SII antibody but not for ID antibody.

REFERENCES


| TABLE II. Comparison of the Detection of Antibody-positive Reactors by Immunodiffusion (ID) and Syncytium-induction Inhibition (SII) Tests |
|---------------------------------|-----|-----|-----|-----|
| Cattle kept in                  | No. tested | ID test | SII test | SII+/ID+ |
| Heavily infected herd           | 31   | + 12  | - 12  | - 0  | 100  |
|                                 |      |      |       |       |      |
| 50                              | 19   |      |       |       |      |
|                                 | 23   |      |       |       |      |
| BLV-free island                 | 20   | 0    | 0     | 0    | 0    |
| 20                              | 20   |      |       |       |      |
|                                 | 20   |      |       |       |      |

\[ y = 0.77x + 4.09 \]

\[ r = 0.798 \quad (n=70) \]

Fig. 1. Correlation between syncytium inhibition (SII) and immunodiffusion (ID) antibody titers.
Fig. 2. Sequential antibody titers in cattle after inoculation with bovine leukemia virus.


