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Natural Antibodies to BLV gp51 Are Reactive Against the Carbohydrate Moiety of the Glycoprotein

D. Portetelle, C. Bruck, M. Mammerickx, and A. Burny

A. Introduction

Infection of animals (cattle, sheep, goats, pigs, rabbits, etc.) with bovine leukemia virus (BLV), the etiological agent of enzootic bovine leukosis, induces a rapid and strong humoral antibody response directed against all structural proteins of the virus (Burny et al. 1980). This humoral reaction does not arrest BLV multiplication in the infected host; surprisingly enough, anti-gp51 antibody titer increases steadily and inexorably until the animal's fatal outcome in the tumor phase of the disease. Failure of the immune system to eliminate an established BLV infection does not prove, however, that adequate vaccination of animals at risk would be unseccessful. From recent experiments by Mammerickx et al. (to be published), we know that passive antibody provided to sheep by colostrum feeding prevented BLV take, irrespective of the route of virus administration. The latter observations reinforced our decision to thoroughly investigate the antigenic site of BLV gp51 exposed at the cell membrane of virus producing cells and recognized as target by sera exhibiting strong cytolytic activity towards these cells (Portetelle et al. 1978, to be published).

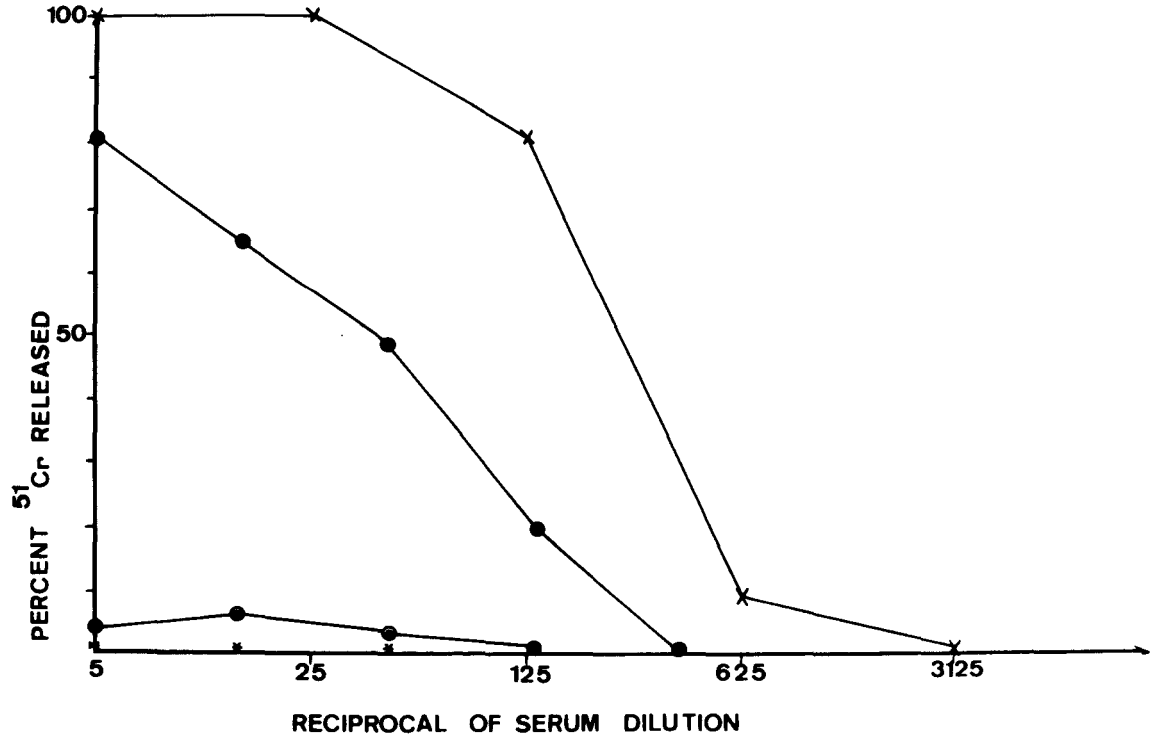

Fig. 1. Susceptibility of FLK-BLV cells to lysis by bovine serum 67 (X—X), bovine serum 351628 (●—●), normal bovine serum (* — *), and rabbit serum 167 (◉—◉). ^{51}Cr labeled FLK-BLV cells were incubated with dilutions of the serum to be tested and with a 1:8 final dilution of rabbit complement

The series of experiments summarized here showed that cytotoxic antibodies present in BLV-infected animals were mostly directed against the carbohydrate part of the gp51 molecule. On the contrary, monospecific immune sera prepared in the rabbit by injection of purified BLV gp51 are directed against determinants of the polypeptide backbone of the antigen.

B. Sera

Sera 2146 and 2152 were prepared in the rabbit by injection of BLV-infected bovine lymphocytes. Sera 67 and 82 were from cattle in the tumor phase of enzootic bovine leukosis. Serum 3162 was from a animal in persistent lymphocytosis and serum 351628 from an asymptomatic BLV carrier. Rabbit serum 167 was raised by injection of purified BLV gp51.

C. Results

Complement-dependent antibody-mediated immune cytolysis was previously shown to recognize mostly, if not solely, gp51 as the target molecular structure (Portetelle et al. 1978). Data presented in Fig. 1 illustrate the striking difference between natural sera (serum 67 X——X and serum 351628 ●—●) and rabbit serum 167 (◉——◉) prepared against purified gp51.

To confirm that cytolytic and noncytolytic antibodies recognized separate sites on the gp51 molecule, a competition radioimmunoassay was performed between bovine serum 67 and rabbit sera 2146 (Fig. 2).

Advantage was also taken of a solid phase radioimmunoassay. The results obtained indicated that: (1) natural sera and rabbit serum 167 reacted with different antigenic regions of gp51, and (2) the antigenic site recognized by natural sera was most probably unique (Fig. 3).

Importance of the carbohydrate moiety of gp51 was finally assessed by glycosidase treatment of ^{125}I-labeled antigen followed by addition of a variety of antisera (Fig. 4). Sera 2152, 3162, and 67 behaved similarly: removal of the carbohydrate moiety of the antigen resulted in almost complete loss of reactivity with the three sera. The behavior of serum 167 was strikingly different. Glycosidase digestion

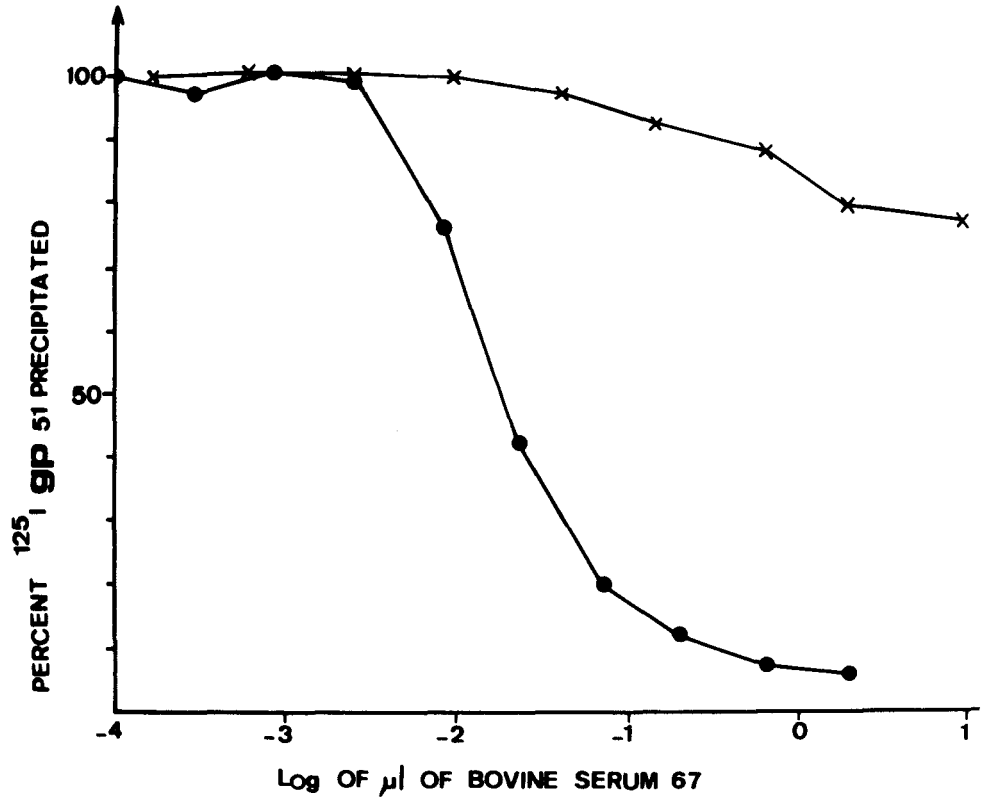

Fig. 2. Competition by bovine serum 67 of the immune precipitation of ^{125}I gp51 by rabbit sera 167 (X——X) and 2146 (●—●). Labeled antigen (0.5 ng) and a limiting rabbit antibody dilution (1/40.000 for serum 167; 1/75,000 for serum 2146) were incubated with increasing amounts of bovine serum 67. After a 72 h incubation, anti-rabbit Ig coated cellulose was added to adsorb immune complexes involving rabbit antibody. Pelleted and washed immunosorbent was counted in a γ counter

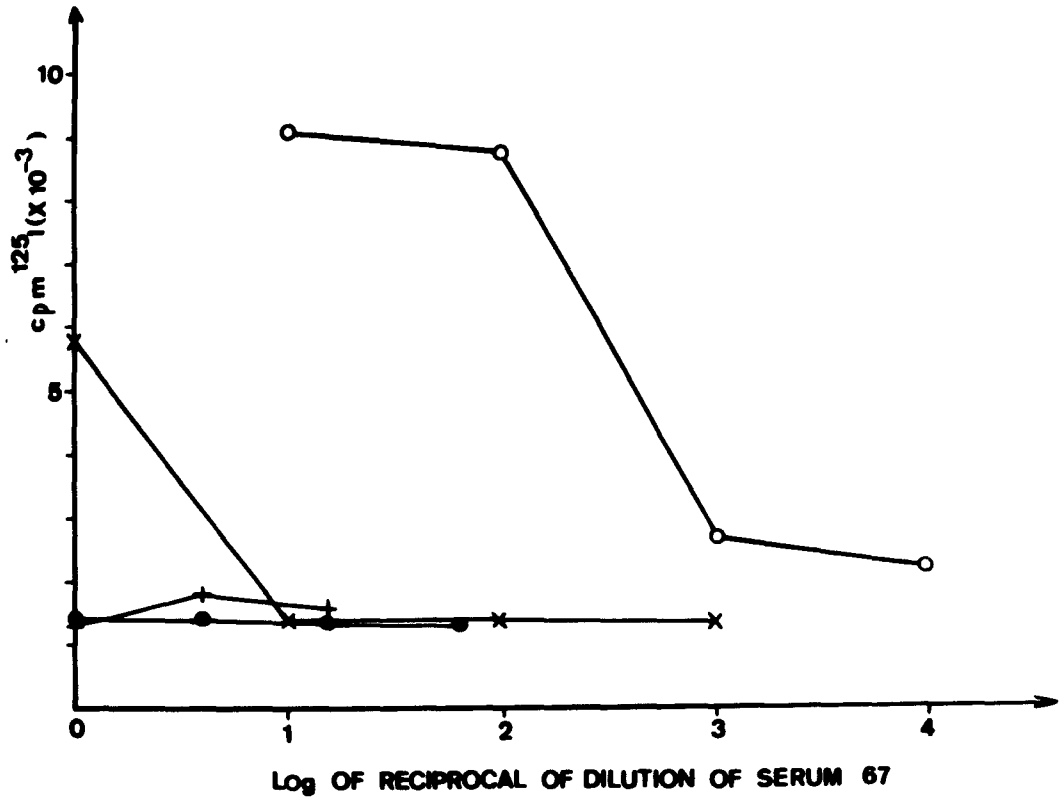

Fig. 3. Solid phase radioimmunoassay. Reagents were added to the wells in the following order: (1) Ig82-gp51-serum 67-^{125}I gp51 (X——X), (2) Ig82-gp51-normal bovine serum-^{125}I gp51 (●—●), (3) Ig rabbit 167-gp51-serum 67-^{125}I gp51 (○—○), and (4) Ig rabbit 167-gp51-normal bovine serum-^{125}I gp51 (+——+). At each step an excess reagent was added, incubation was performed, and nonadsorbed excess protein was washed away. After completion of the last step, the wells were separately counted in a γ counter

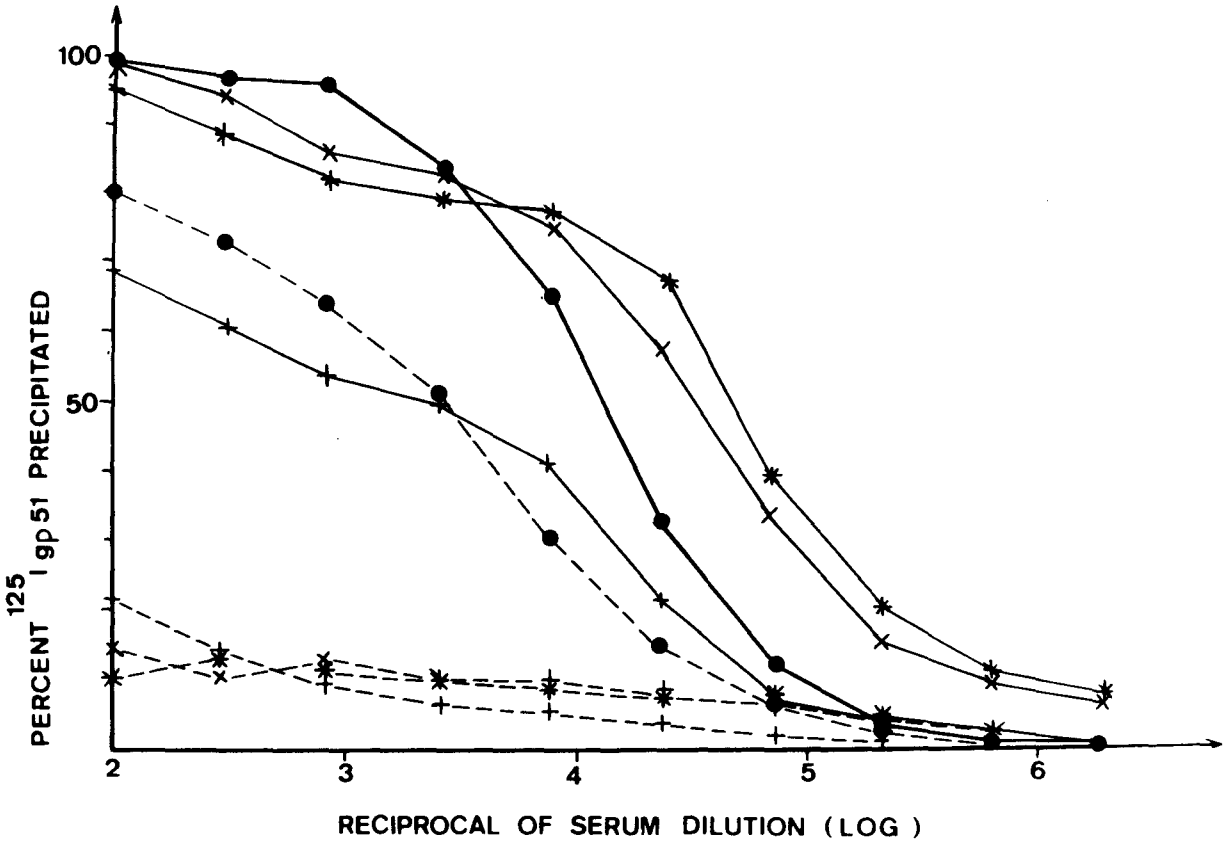

Fig. 4. Effect of glycosidase digestion of ^{125}I gp51 on direct radioimmunoassay with sera of different origins. Precipitation curves obtained with sera from rabbit 167 (●—●), cow 3162 (X——X), cow 67 (*——*), and rabbit 2152 (+——+) are taken as controls. The precipitation curves obtained with the same sera after glycosidase treatment of the antigen are depicted as interrupted lines

lowered maximal precipitability of gp51 by only 20%. Consequently, apparent antibody titer was also slightly lowered.

That the polypeptide backbone of gp 51 was the target for serum 167 reactivity was later demonstrated by protease treatment of the antigen (data not shown). Finally, SDS-PAGE analysis of native and glycosidase-treated BLV gp51 showed that the enzyme treatment reduced the apparent molecular weight from 51,000 to about 30,000 (Portetelle et al., to be published).

D. Conclusion

In conclusion, a probably unique carbohydrate antigenic site belonging to BLV envelope gp51 is strongly immunogenic for naturally infected animals or for rabbits injected with BLV lymphocytes. On the other hand, when purified gp51 is used as immunogen, it induces synthesis of antibodies directed to the protein skeleton of the glycoprotein antigen. From a very practical point of view it follows that purified BLV gp51 should not be used as a vaccinal preparation against BLV infection. Results very similar to those reported here have been obtained by Schmerr et al. (1980), personal communication).

References

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