Interspecies Determinants of Friend Leukemia Virus Antigens Involved in Cytolysis of Virus-Producing Cells

JEFFREY J. COLLINS, LISA D. BOLIO, TIMOTHY P. DENNY, AND DANI P. BOLOGNESI

Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710

Received for publication 30 December 1975

The cytolytic reactivity of a complex goat anti-feline leukemia virus (FeLV) antiserum for mouse cells (Eveline) releasing large quantities of Friend leukemia virus (FLV) was analyzed by the sensitive [14C]nicotinamide release microcytotoxicity assay. Whereas this interspecies killing reactivity could be blocked by absorption of the goat anti-FeLV serum with a preparation of disrupted FLV, absorption with purified FLV gp71, the major envelope glycoprotein, had no effect. Subsequent serum absorptions with the individual FLV structural polypeptides revealed that the lysis of the Eveline cells by the goat anti-FeLV serum is mediated by antibodies recognizing the interspecies determinant of p30, the major internal capsid protein. The expression of this internal viral component at the surface of virus-producing cells is discussed further. The results also demonstrated that removal of ~70% of the carbohydrate portion of gp71 with a preparation of glycosidases did not affect the integrity of its interspecies determinant; these results are in agreement with an earlier study (Bolognesi et al., 1975) that examined primarily the group- and type-specific sites.

The structural components of the mammalian type C viruses have been shown to contain one or more of the three major classes of antigenic sites, defined as the type (unique to an individual virus strain), group (shared by viruses of the same species), and interspecies (shared by viruses of different species) determinants (1). The relative contribution of these determinants in various immunological phenomena is of great interest, particularly with respect to the utilization of the purified viral antigens as possible vaccines against virus-induced neoplasms.

The feasibility of this approach was demonstrated by the successful immunoprophylaxis of virus-induced murine leukemia by active immunization with Friend leukemia virus (FLV) gp71, the major virus envelope glycoprotein, or by passive serum therapy with goat anti-gp71 antiserum (10). However, since protection was demonstrated only in the homologous system (i.e., against challenge with FLV), it is unclear which gp71 determinants were involved. Recent experiments utilizing immunization of various mouse strains with FLV gp71 have not yet demonstrated protection against murine leukemias induced by or associated with murine type C viruses other than FLV (J. N. Ille et al., Virology, in press). However, of particular relevance to the present report are preliminary studies indicating that in vivo protection against viremia can be achieved in the feline system on the basis of the interspecies determinants of FLV structural components (W. Schäfer et al., VII Int. Symp. Comp. Leuk. Res. Related Dis., in press). Elimination of virus is especially important in cats since leukemia induced by feline leukemia virus (FeLV) appears to be horizontally transmitted (8, 13).

To better understand the possible mechanisms of this in vivo protection directed against the viral interspecies determinants, we used a sensitive in vitro microcytotoxicity assay to examine the characteristics of killing of mouse cells releasing FLV by antiserum raised against FeLV. In addition, we extended our earlier immunochemical studies of FLV gp71 (2) by investigating the possible involvement of carbohydrate in the determinants responsible for this interspecies cytotoxic reaction.

MATERIALS AND METHODS

Purification of virus and viral components. The preparation and purification of FLV by velocity and equilibrium density gradient centrifugation were described previously (2, 7). Viral components were subsequently prepared from the purified virions, with gp71 released by osmotic shock followed by density gradient centrifugation, affinity chromatography on concanavalin A-agarose columns, and gel filtration on Sephadex G-150 (14). The other FLV components (p30, p15(E), p12, and p10) were purified by gel filtration in guanidine hydrochloride (7, 15).

Antiserum. The goat anti-FeLV antiserum was raised against Tween-ether-degraded FeLV and was
described previously in detail (7). This is a complex antiserum that reacts strongly with most of the isolated FeLV polypeptides and the analogous FLV proteins bearing interspecies determinants [e.g., gp71, p30, and p15(E)]. The serum was used in the present experiments after absorption with normal cat cells, normal cat plasma, fetal calf serum, and sheep erythrocytes and was heat-inactivated at 56°C for 30 min.

Absorption of the goat anti-FeLV serum with soluble antigens was carried out as described previously (2). Briefly, one part of antigen was mixed with nine parts of appropriately diluted serum (see below; diluent was Eagle minimal essential medium without fetal calf serum) and incubated overnight at 4°C. The mixture was then centrifuged at 8,000×g for 3 h, and the absorption was repeated with one part of fresh antigen. Control unabsorbed serum was treated in parallel with minimal essential medium (without fetal calf serum) in place of antigen. The protein concentrations of the antigen preparations used for serum absorptions were as follows (milligrams/mL milliliter): gp71, 0.71; p30, 0.17; p15(E), 0.5; p12, 0.44; p10, 0.48; and disrupted FLV, 64. The FLV preparation was disrupted by five cycles of freeze-thawing (dry ice-ethanol/37°C).

Enzyme treatments. Treatment of the gp71 and the disrupted FLV preparations with the glycosidase mixture, neuraminidase, trypsin, and Pronase were carried out as described in reference 2, which also provides the source and/or the preparation of the enzymes.

Serological assays. Both the double-antibody direct radioimmunoassay (RIA) and the [14C]nitrocellulose ([14C]NA) release microcytotoxicity assay were described in detail previously (2). The target cell in the microcytotoxicity assay was the Eveline mouse cell line (17), which are FLV-infected STU mouse embryo cells releasing very large amounts of FLV (9, 16, 17) and expressing all of the viral antigens [i.e., gp71, p30, p15(E), p12, and p10] at the cell surface, as determined by cytotoxicity assay (2, 9; J. J. Collins et al., submitted for publication).

RESULTS
Lysis of the Eveline cells by goat anti-FeLV serum. The cytotoxic reactivity of the goat anti-FeLV antiserum for the FLV-producing Eveline mouse cells, as measured by the [14C]NA release assay, is presented in Fig. 1. On the basis of this titration, which indicated a marked prozone and a 50% end point of 1:346, we decided to use a dilution of 1:64 for the subsequent absorption studies. Note that the reactivity of this serum, which detects only viral interspecies determinants on the Eveline cells, is considerably weaker than the anti-Eveline activity seen in other experiments with goat anti-FLV gp71 antiserum (50% end point of >1:4,000) (2). The goat anti-FeLV antiserum did not have any activity on uninfected STU mouse embryo cells (data not shown).

Recognition of viral antigens on the Eveline cells by goat anti-FeLV serum. To demonstrate that the killing of the Eveline cells by the goat anti-FeLV serum actually reflected the activity of antibodies directed against viral antigens, this serum was diluted 1:64 (in minimal essential medium without fetal calf serum) and absorbed with disrupted FLV. Moreover, to examine the contribution of protein and carbohydrate in whatever viral component(s) might be involved, parallel absorptions were carried out with the various preparations of enzymatically treated disrupted FLV. A control of unabsorbed goat anti-FeLV serum (incubated with medium alone) was also included. When these absorbed serum samples were tested by the [14C]NA release assay on the Eveline cells, the results demonstrated that the disrupted FLV preparation could completely neutralize this cytotoxic reactivity (Fig. 2). Furthermore, whereas treatment with either of the proteases completely removed the ability of the disrupted FLV to block the cytotoxicity reaction, treatment with the glycosidase mixture or neuraminidase had little or no effect on the absorptive capacity of this preparation. These results indicate that the interspecies antigenic determinants being detected by the goat anti-FeLV antiserum on the Eveline cells that are involved in the cytotoxicity reaction do not require the presence of extensive sugar moieties.

Specificity of the lysis of Eveline cells by goat anti-FeLV serum. For a number of reasons, a prime candidate for the FLV antigen involved in the lysis of the Eveline cells by the goat anti-FeLV serum was the major envelope glycoprotein, gp71. These reasons include its high concentration at the surface of the Eveline cells (16), the ease with which these cells are
lysed by heterologous anti-gp71 serum (2, 6, 9; Collins et al., submitted for publication), the presence of an interspecies determinant on the molecule (1), and the fact that FeLV-producing cat cells can be lysed with anti-FLV gp71 antiserum (9; J. J. Collins and P. Bolognesi, unpublished data). Thus, similar absorptions of the goat anti-FeLV serum at a dilution of 1:64 were carried out with untreated FLV gp71 and the various preparations enzymatically treated gp71. The results indicated that none of the gp71 preparations was capable of absorbing the cytotoxic reactivity of the serum, including the untreated glycoprotein (Fig. 3).

To rule out the possibility that the absorptions with the gp71 preparations were incomplete, the residual reactivity of the absorbed goat anti-FeLV serum samples for 125I-labeled FLV gp71 was examined by RIA, a reaction which measures only recognition of the gp71 interspecies determinant. The absorption with untreated gp71 completely neutralized the ability of the goat anti-FeLV serum to precipitate 125I-labeled FLV gp71 (Fig. 4a).

Furthermore, treatment of the purified gp71 with the glycosidase or neuraminidase enzymes did not affect the ability of this antigen to absorb, whereas treatment with either protease (trypsin or pronase) completely eliminated its absorptive capacity. These absorption results confirm the lack of involvement of carbohydrate in the interspecies determinant of FLV gp71 and are in accord with preliminary data demonstrating that treatment of FLV gp71 with glycosidase did not affect its precipitation by the goat anti-FeLV serum in direct RIA (see Fig. 5 in reference 2).

The residual RIA reactivities for FLV gp71 of the goat anti-FeLV serum samples absorbed with the disrupted FLV preparation (untreated or enzyme-treated) are presented for comparison in Fig. 4b. The results indicate clearly that an FLV antigen other than gp71 is involved in the interspecies lysis of the Eveline cells, since both the gp71 and disrupted FLV absorptions removed the anti-gp71 antibodies; however, only absorption with the virus preparation eliminated cytotoxic reactivity.

To determine which viral antigen(s) (other than gp71) might be involved in the lysis of the Eveline cells by the goat anti-FeLV serum, similar absorptions of the serum at a dilution of 1:64 were carried out with all of the major FLV structural polypeptides, including gp71, p30, p15(E), p12, and p10. Only absorption with the major internal capsid protein, p30, removed the cytotoxic reactivity of the anti-FeLV serum, demonstrating this to be the specific target for the interspecies cell-killing reaction (Fig. 5).

**DISCUSSION**

Our results demonstrate that antibodies specific for the interspecies determinant of the major internal capsid protein, p30, are responsible for the lysis of FLV-releasing mouse Eveline

---

**Fig. 2. Effect of enzyme treatment on the ability of disrupted FLV to block the killing of Eveline target cells by goat anti-FeLV antiserum.**Enzyme treatments and serum absorptions were carried out as described in the text and previously (2). Results are expressed as percent specific release based on control of medium plus C' (specific release ≥20% is taken to reflect significant killing). Abbreviations: Unabs., unabsorbed; Glyc., glycosidase mixture; Nase, neuraminidase; Tryp., trypsin; Pron., Pronase; No Enz., no enzymes.

---

**Fig. 3. Effect of enzyme treatment on the ability of FLV gp71 to block the killing of Eveline target cells by goat anti-FeLV antiserum.** Enzyme treatments and serum absorptions were carried out as described in the text and previously (2). Results are expressed as percent specific release based on control of medium plus C' (specific release ≥20% is taken to reflect significant killing).
cells by a complex goat anti-FeLV antiserum. Despite the ability of this serum to react with the interspecies determinants of FLV gp71, the major envelope glycoprotein, and p15(E), a membrane-associated protein, its cytotoxic reactivity for the Eveline cells was not directed against these virus components. Thus, whereas absorption with FLV p30 (or disrupted FLV) neutralized the cytotoxic reactivity of the goat anti-FeLV serum, absorption with FLV gp71 or p15(E) had no effect (Fig. 3 and 5). Nevertheless, the absorption with gp71 was shown by RIA to have removed the antibodies capable of precipitating 125I-labeled FLV gp71 (Fig. 4a). Thus, its failure to affect the cytotoxic reactivity for Eveline cells was not due to an incomplete absorption or to an inability to absorb cytotoxic antibodies (2).

The results also extend our earlier observations that the serological determinants of FLV...
gp71 depend primarily, if not completely, on the integrity of the protein portion of the molecule. It is now clear that this holds true for the gp71 interspecies determinant, as well as for the previously investigated group- and type-specific sites (2). Results to be reported separately demonstrate that the carbohydrate portion is not intimately involved in several of the biological properties of FLV gp71, although it is required for its hemagglutinating capacity (14a).

Given that the specific antigen responsible for the cytotoxic reactivity of the goat anti-FeLV serum for Eveline cells is the non-glycosylated p30 capsid protein, it is not surprising that treatment of the disrupted FLV preparation with neuraminidase or the glycosidase mixture did not affect its ability to absorb this lytic activity. Nevertheless, it is somewhat unexpected that the killing activity would be directed against the internal viral capsid component rather than the major envelope glycoprotein, gp71, present in such large concentration on the Eveline cells (16). This may partially reflect the higher anti-p30 reactivity of the complex goat anti-FeLV serum relative to its anti-gp71 activity (unpublished data). It is also possible that the interspecies determinant of gp71 is not as accessible to antibody as the other gp71 antigenic sites when the molecule is situated on the cell membrane. It should be noted that the lysis of FeLV-producing cat cells by goat anti-FLV gp71 antiserum is quite weak despite the high interspecies titer of this serum against FeLV (9; Collins and Bolognesi, unpublished data). However, alternative hypotheses can be raised for the failure of the goat anti-FeLV serum to lyse the Eveline cells on the basis of gp71 reactivity, including low-avidity antibodies for the gp71 interspecies determinant or an inability of such antibodies to mediate C'-dependent cell lysis.

It has been suggested that with cells producing large amounts of virus, such as the Eveline cell line, internal viral antigens (e.g., p30) may be passively absorbed to the cell surface, since these antigens could be detected by cytotoxicity and immunoelectron microscopy on Eveline cells, but not on virus-infected nonproducer or low-producer cells (9, 16; Collins and Bolognesi, unpublished data). That passively adsorbed p30 can act as a target for cytotoxic antibody was shown previously (5, 6), and its release in a soluble form from growing Eveline cells was also demonstrated (3). Thus, the lysis of Eveline cells by anti-p30 antibodies in the goat anti-FeLV serum may also partially reflect the passive adsorption of significant levels of this internal viral component to the cell surface. Nevertheless, it should be noted that mouse cells releasing much lower levels of virus, and even nonproducer cells, have also been reported to express murine leukemia virus p30 at the cell surface (6, 12, 18).

Preliminary experiments to test the involvement of the interspecies determinants of FLV structural components in protecting against FeLV-induced leukemia in cats by passive therapy with specific sera have thus far focused on FLV gp71 (Schafer et al., in press). The present results, however, suggest that it would be worthwhile to examine the role of p30 in influencing the course of virus infection in the feline system. It is intriguing that significant levels of natural antibody to p30 were reported recently to be present in cat sera (4), whereas the autogenous humoral response in mice appears to be directed primarily against the surface-associated viral components gp71, gp45, and p15(E) (11).

ACKNOWLEDGMENTS

We are grateful to W. Schafer and F. de Noronha for providing the goat anti-FeLV antiserum and to P. Atkinson for the preparation of glycosidases.

This work was supported by Public Health Service contract NCI NO1 CP 33008 from the Virus Cancer Program of the National Cancer Institute, American Cancer Society grant VC-161, and Damon Runyon-Walter Winchell grant DRG-1285. J. J. C. is a Special Fellow of the Leukemia Society of America. D. P. B. is a recipient of an American Cancer Society Faculty Research Award.

LITERATURE CITED


