JOURNAL OF VIROLOGY, July 1974, p. 187-189

Vol. 14, No. 1
Printed in U.S.A.

Structural Proteins of Mammalian Oncogenic RNA Viruses: Murine Leukemia Virus Neutralization by Antisera Prepared Against Purified Envelope Glycoprotein

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Received for publication 18 March 1974

Goat and rabbit antisera prepared against a purified Rauscher murine leukemia virus glycoprotein (gp69/71) rapidly neutralized spleen focus-forming virus in Rauscher and Friend virus preparations. Absorption studies revealed that most of the neutralizing activity of goat anti-Rauscher virus gp69/71 serum was directed against type- and group-specific determinants.

The major glycoprotein constituent of Rauscher murine leukemia virus (MuLV) has been purified and shown to be composed of two glycopeptides of apparent molecular weights of 69,000 and 71,000 (gp69/71) (5). Analysis of the purified protein by radioimmunoassay has shown it to contain predominantly type-specific and group-specific antigenic determinants with a minor component of inter-species antigens (6). As gp69/71 comprises the major component of the virion glycoproteins and contains type-specific determinants, it has been suggested that it is a component of the virion envelope (6).

Further evidence for the envelope localization of these glycopeptides has now been obtained by analysis of virus neutralization by monospecific antiserum prepared against purified Rauscher MuLV gp69/71. Such antiserum effectively neutralized the spleen focus-forming virus (SFFV) component (4) of Friend and Rauscher virus complexes, whereas antiserum similarly prepared against Rauscher MuLV p30 did not neutralize SFFV. The virus-neutralizing activity of an anti-Rauscher MuLV gp69/71 serum was absorbed weakly by a leukemia virus of another species (cat), moderately by distantly related leukemia and sarcoma viruses of mice, and completely by Rauscher MuLV itself.

Purification of Rauscher MuLV gp69/71 and p30 was achieved by phosphocellulose column chromatography and Sephadex gel filtration, and goat and rabbit antisera were prepared against these purified proteins as described previously (5). The Friend and Rauscher virus complexes were NB-tropic (2); they were harvested from, and their residual activity assayed in, young adult (4- to 8-week-old) BALB/c mice by methods described previously (1, 3).

Initial testing was conducted with Friend virus complex, because the higher titer of SFFV in this preparation made it more suitable for studying the kinetics of neutralization by antisera of unknown potency. Antiserum and virus (5×10^4 focus-forming units/ml) were each mixed at a final dilution of 1/10 and incubated at 37 C. At various times thereafter, samples were diluted in ice-cold phosphate-buffered saline (PBS) and injected intravenously into groups of seven mice. From the spleen focus counts obtained 9 days later, the results are presented as neutralization kinetics curves (Fig. 1). The goat and rabbit anti-Rauscher MuLV p30 sera failed to neutralize Friend SFFV, whereas the anti-Rauscher MuLV gp69/71 sera were both potent. The rabbit serum at a 1/10 dilution neutralized over 99% of the virus in 10 min and required another fivefold dilution to obtain an accurate neutralization constant (K). Gamma globulin, purified from the goat anti-Rauscher MuLV gp69/71 serum and diluted 10-fold to a serum-equivalent level, neutralized (at a final dilution of 1/100) Friend SFFV as effectively as whole serum.

To analyze the properties of the antigenic determinant active in the neutralization of Friend SFFV by the goat anti-Rauscher MuLV gp69/71 serum, we tested the effects of absorbing the serum with various leukemia and sarcoma viruses (Table 1). Absorption with Thielen feline leukemia virus removed very little neutralizing activity, whereas absorption with Gross MuLV and Kirsten murine sarcoma virus removed some activity and absorption with Rauscher MuLV removed essentially all activ-

ity. This suggests that most of the determinants involved in the neutralization of Friend SFFV by this antiserum are group-specific as well as type-specific.

The role of type-specific determinants was confirmed by comparing the potency of unabsorbed goat anti-Rauscher MuLV gp69/71 serum with SFFV from Friend and Rauscher virus preparations (Table 2). Rauscher SFFV was neutralized significantly more rapidly than Friend SFFV by antiserum diluted 1/10, but the rate of Rauscher SFFV neutralization was too fast to be determined precisely. Comparison of the rate by which these viruses were neutralized in antiserum diluted 1/100 showed that Rauscher SFFV was neutralized about fivefold faster than Friend SFFV.

Rauscher SFFV is thought to be closely related to but antigenically distinguishable from Friend SFFV, apparently due to an antigenic difference between their repective helper viruses, LLV-R and LLV-F(1). A Rauscher MuLV typing serum (called Rich antiserum [1]) neutralized Rauscher SFFV almost four times more rapidly than Friend SFFV. Proteins of these viruses also showed different competitive binding efficiencies in the radioimmunoassay (6). This difference in type between Friend and Rauscher virus preparations was demonstrated here by the neutralizing capacity of an anti-

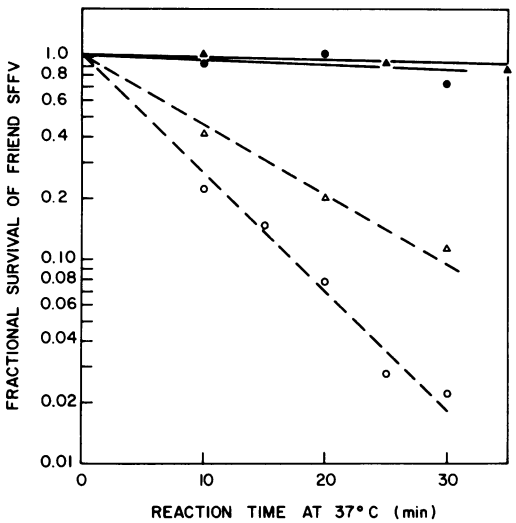

FIG. 1. *Neutralization kinetics of Friend SFFV incubated with goat and rabbit antisera prepared against purified Rauscher MuLV gp69/71 and p30, Symbols: ▲, rabbit anti-Rauscher MuLV p30, final dilution 1/10, K = 0.04 ± 0.01; ●, goat anti-Rauscher MuLV p30, final dilution 1/10, K = 0.10 ± 0.10; △, rabbit anti-Rauscher MuLV gp69/71, final dilution 1/50, K = 3.31 ± 0.23; ○, goat anti-Rauscher MuLV gp69/71, final dilution 1/10, K = 1.38 ± 0.10.*

TABLE 2. *Comparison of Friend SFFV and Rauscher SFFV by the kinetics of their neutralization by goat anti-Rauscher MuLV gp69/71 serum*

Antiserum dilution	Neutralization constant ($K \pm$ S.E.)	
	Friend SFFV	Rauscher SFFV
1/10	1.38 ± 0.10	>2
1/100	1.31 ± 0.35	6.8 ± 0.7

TABLE 1. *Absorption of goat anti-Rauscher MuLV gp69/71 serum by viruses sharing type, group, and interspecies determinants with Rauscher MuLV*

Absorbing virus[a]	Determinants shared with Rauscher MuLV gp69/71[b]			Neutralization constant against Friend SFFV[c] ($K \pm$ S.E.)
	Type	Group	Interspecies	
None	–	–	–	1.38 ± 0.10
Thielen FeLV	–	–	+	1.14 ± 0.02
Gross MuLV	–	+	+	0.38 ± 0.02
Kirsten MuSV	–	+	+	0.28 ± 0.04
Rauscher MuLV	+	+	+	0.02 ± 0.02

[a] Protein (400 μg) of absorbing virus was added to 0.1 ml of goat anti-Rauscher MuLV gp69/71 and kept at 4 C for 12 h. After centrifugation to sediment any precipitate, the volume was made up to 1 ml (i.e., serum diluted 1/10) in PBS. Abbreviations used are: FeLV, feline leukemia virus; MuLV, murine leukemia virus; MuSV, murine sarcoma virus.

[b] The determinants of gp69/71 shared by different viruses are classified as previously described (6); type determinants are those unique to a given type of virus; group, those shared by different viruses of the same group; and interspecies, those shared by viruses of different species.

[c] The neutralization constant (K) is a measure of the neutralizing potency of the antiserum, and is determined from the equation: $K = (D/t)Log_e (V_o/V_t)$, where D = serum dilution, V_o = original virus titer, and V_t = virus titer at reaction time t (3).

serum against purified Rauscher MuLV glycoprotein 69/71.

We thank Vernon Grant for technical assistance.

This research was supported in part by Public Health Service grants CA 14529-02 from the National Cancer Institute and GM 113-1-11 from the National Institute of General Medical Sciences, and by Public Health Service contract 71-2251 within the Special Virus Cancer Program of the National Cancer Institute.

LITERATURE CITED

1. Eckner, R. J., and R. A. Steeves. 1972. A classification of the murine leukemia viruses: neutralization of pseudotypes of Friend spleen focus-forming virus by type-specific murine antisera. J. Exp. Med. **136**:832-850.
2. Lilly, F., and T. Pincus. 1973. Genetic control of murine viral leukemogenesis. Advan. Cancer Res. **17**:231-277.
3. Steeves, R. A., and A. A. Axelrad. 1967. Neutralization kinetics of Friend and Rauscher leukemia viruses studied with the spleen focus assay method. Int. J. Cancer **2**:235-244.
4. Steeves, R. A., R. J. Eckner, M. Bennett, and E. A. Mirand. 1971. Isolation and characterization of a lymphatic leukemia virus in the Friend virus complex. J. Nat. Cancer Inst. **46**:1209-1217.
5. Strand, M., and J. T. August. 1973. Structural proteins of oncogenic ribonucleic acid viruses. Interspec II, a new interspecies antigen. J. Biol. Chem. **248**:5627-5633.
6. Strand, M., and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses. Multiple antigenic determinants of the major internal protein and envelope glycoprotein. J. Virol. **13**:171-180.