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Immunological Cross-Reactions Between Two Low-Molecular-Weight Polypeptides from A Murine Type C Virus

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Two low-molecular-weight type-specific virion polypeptides from the Kirsten strain of Murine leukemia virus, polypeptides p10 and p12, are immunologically related by radioimmunoassay competition techniques.

Mammalian type C viruses have been isolated from a wide variety of species (8, 12). It has been possible to define with purified polypeptides from such type C virus isolates three kinds of immunological reactivities. Group-specific reactions have been described in which purified polypeptides from different isolates of type C virus from a given animal species share antigenic reactivities (4, 18); interspecies reactions also have been described in which a given purified polypeptide from type C viruses of different species shows cross-reactions (3, 15, 17, 23). More recently, type-specific reactivities have been described for purified polypeptides in which strains of type C virus within a given species can be distinguished by immunological assays using purified polypeptides (6, 22). These type-specific reactivities have been attributed to low-molecular-weight polypeptides from murine, feline, and primate type C viruses (6, 22, 26). During our work on the low-molecular-weight proteins of the Kirsten strain of murine type C virus, we were able to confirm the observations that the p12 low-molecular-weight protein contained predominantly type-specific reactivities (22). However, we constantly noted cross-reactions between two of the low-molecular-weight proteins of the Kirsten strain of murine leukemia virus (MuLV). The present work was undertaken to investigate the serological relationships between the individual low-molecular-weight proteins of the Kirsten strain of MuLV in comparison to the low-molecular-weight proteins from other strains of murine leukemia viruses.

The N-tropic Kirsten strain of MuLV grown in NIH 3T3 cells (10), which was obtained from Janet Hartley, National Institutes of Health and originally described by Somers and Kirsten (21), has only been passaged in murine cells. This virus was kindly reidentified by J. Hartley as belonging to the AKR-Gross neutralization group late in the course of these studies in order to insure that no cross-contamination had taken place. Its identity was confirmed by MuLV type-specific competition radioimmunoassays to be described. Virions were disrupted (5) and the isolated polypeptides were separated by gel chromatography in guanidine hydrochloride (GuHCl) (1, 2, 5). In addition to the p30 (group specific [gs]) and higher-molecular-weight polypeptides, two low-molecular-weight viral polypeptides were detected in significant amounts by electrophoresis of GuHCl column fractions in 0.1% sodium dodecyl sulfate (SDS), pH 7.2, 10% polyacrylamide gels (PAGE). Column fractions containing these respective polypeptides were pooled, dialyzed, and concentrated by lyophilization. These proteins were difficult to solubilize completely after GuHCl denaturation (5, 6).

When such lyophilized column fractions were reconstituted and examined by PAGE (Fig. 1), the isolated polypeptides were at least 90% pure. Similarly, further analysis of the purified polypeptides indicated that they migrated as a single band in polyacrylamide gels (pH 8.7), indicating the absence of a mixture of differently charged polypeptides. In comparison with molecular weight standards in SDS-PAGE, molecular weights for these polypeptides were 12,000 and 9,500 (p12 and p10, respectively). In detailed studies using SDS-PAGE analysis, the Kirsten p12 and p10 co-migrated with the p12 and p10, respectively, from the Moloney strain of MuLV; both of the Kirsten polypeptides were significantly smaller than the Moloney p15, and they eluted at a higher V_e/V_o from GuHCl chromatography than would be expected for a polypeptide of 15,000 daltons.

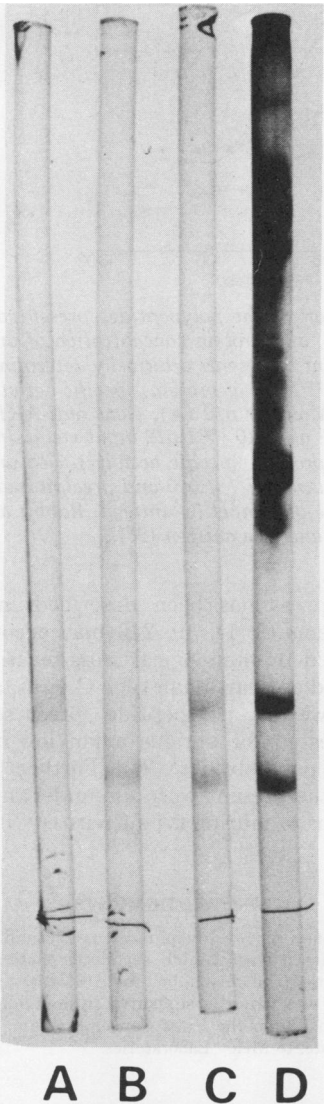

FIG. 1. *SDS, 0.1%; (pH 7.2), 10% polyacrylamide gel electropherograms of GuHCl purified polypeptides from the Kirsten strain of MuLV. Nine-centimeter gels contained: (A) 14 μg of p12; (B) 16 μg of p10; (C) 14 μg of p12 and 16 μg of p10; (D) 190 μg of ether-disrupted, acetone-extracted Kirsten MuLV. Proteins were determined by the method of Lowry, with bovine serum albumin as a standard (13). Wires projecting through gel at the lower end mark the peak migration of bromophenol blue dye marker. Molecular weights of 12,000 were determined for the polypeptide in gel A and 9,500 in gel B. These figures were determined by comparison with the following molecular weight standards—RNase A (Pharmacia), mol wt 13,700, hemoglobin (Pentex), mol wt 15,500, lysozyme (Calbiochem), mol wt 14,300, chymotrypsinogen A (Pharmacia), mol wt 25,000, aldolase (Pharmacia), mol wt 40,000, and bovine serum albumin (Armour), mol wt 69,000 (28) and are in good agreement (±5%) with molecular weight determinations from GuHCl gel chromatography.*

Even when mixed, the GuHCl column purified p10 and p12 from the Kirsten strain of MuLV could be clearly separated in SDS-PAGE (Fig. 1). These different-molecular-weight entities corresponded to two bands present in disrupted whole virus preparations (gel D). The absence of a detectable 15,000-dalton polypeptide is unexplained; however, we noted a 15,000-dalton polypeptide in preparations of the Moloney strain of MuLV grown in the same cell line. When the p10 and p12 KiMuLV-purified polypeptides were iodinated by the chloramine T method as described (7, 16, 20), they were precipitated with goat antisera against disrupted intact AKR strain of MuLV virions and not with sera from unimmunized goats, all kindly supplied by Roger Wilsnack, Huntington Laboratories, Baltimore, Md. In studies not shown, this serum had a comparable titer against both polypeptides and suggested the possibility of an immunological cross-reaction.

Competition assays were carried out using a serum concentration which precipitated 35 to 50% of the labeled protein. With I^{125}-labeled Kirsten p12, competition between the p12 Kirsten polypeptide and the p10 Kirsten polypeptide yielded essentially identical displacement curves in terms of sensitivity of detection (1 to 3 ng/assay), slope of curve, and final degree of displacement (Fig. 2A). Unlabeled p12 from the AKR strain of MuLV competed comparably with the p10 and p12 Kirsten polypeptides as measured by the slope and percent of displacement; however, the sensitivity (~15 ng/assay) of detection is significantly higher. This suggests that AKR p12 is highly related to the low-molecular-weight polypeptides of the Kirsten strain, but that they may not be identical. In contrast, the p12 polypeptide from the Moloney strain of MuLV displaces labeled Kirsten p12 only partially even at 40 to 75 ng of protein; at even higher polypeptide concentrations, the p12 from Moloney MuLV displaces only 14% of the labeled protein, suggesting a more distant relationship for this strain. This confirms the type specificity of the low-molecular-weight polypeptides reported by others (6, 22, 26).

In Fig. 2B similar competition assay results were obtained when the p10 polypeptide was labeled. Kirsten p10 displaced the labeled antigen more efficiently than did p12 Kirsten polypeptide (detected at <1 ng/assay and displace-

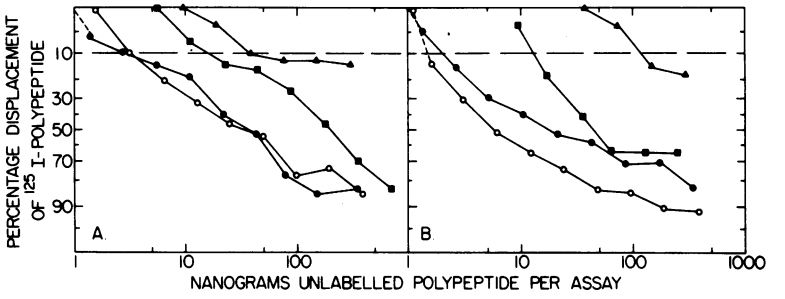

FIG. 2. *Competition radioimmunoassays of Kirsten low-molecular-weight polypeptides presented as the percentage displacement of ^{125}I-labeled polypeptide (on logit scale), and protein concentration of unlabeled competing polypeptide (on log scale). Dashed line at 10% displacement represents arbitrarily determined level of significant competition. (A) competition of [^{125}I]p12 (1.1 μCi of [^{125}I]/μg of protein, specific activity) with unlabeled Kirsten p12 (●), Kirsten p10 (○), AKR p12 (■), and Moloney p12 (▲). Goat anti-AKR serum (25-296) was used at a final dilution of serum which precipitated 30% of 6,410 [^{125}I]p12 input counts/min. (B) competition of [^{125}I]p10 from Kirsten MuLV (2 μCi of [^{125}I]/μg of protein, specific activity) with unlabeled competing proteins as shown in (A). Serum was used at a final dilution of 1:1,000 and precipitated 35% of 10,700 counts/min of [^{125}I]p10 added to each reaction in the absence of competing antigen. Rabbit anti-goat serum was used as the second antibody for the separation of bound and free antigen (20).*

ment to 92%), but they were highly related as judged by this method. Cross-reacting determinants on physically distinct polypeptides were confirmed when the individual labeled polypeptides were used in competition assays with individual fractions from GuHCl chromatography or from PAGE-eluted fractions; it was possible to detect as discrete peaks both the p10 and p12 reactivities with either assay. Similar results were obtained using purified p10 and p12 from three different preparations of Kirsten MuLV grown in NIH 3T3 cells. In other studies, a xenotropic strain of MuLV, S16 Cl 10 isolated from BALB/c 3T3 (25) did not react significantly in either assay. Furthermore, neither p30(gs) nor gp70 from KiMuLV competed in the p12 and p10 competition assays.

The current studies thus show that the p12 and p10 of the Kirsten strain of murine type C virus show two-way immunological cross-reactions and do not react appreciably with either the murine p30 or gp70 from Kirsten MuLV. The evidence suggests that these cross-reactions are not due to cross-contamination of the p12 protein with p10 or the p10 protein with p12; this evidence is based on analysis of the purity of the proteins by both size and charge in acrylamide gels and by quantitative analysis of the immunological cross-reactions noted in radioimmunoassays for the respective proteins. In previous studies on type-C viral low-molecular-weight proteins, there have been reports of immunological (6) or physicochemical (14) relatedness; however, their significance is at present not clear. These studies taken together with the present work suggest that a product-precursor relationship, as has been described in other viral systems (9, 11, 19, 24), may account for the observed immunological cross-reactions. In fact, in studies of an avian type C virus, a large-molecular-weight polypeptide precursor has been suggested for various avian low-molecular-weight polypeptides (27). Further studies to extend the present work are underway using other strains of murine type C virus.

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Dani Bolognesi provided helpful discussions and information concerning polyacrylamide gel electrophoresis procedures and George Todaro supplied the S16 Cl 10 virus.

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