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Immunological Relationship Between the Structural Proteins of Intracisternal A-Particles of *Mus musculus* and the M432 Retrovirus of *Mus cervicolor*

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A specific immunological relationship has been demonstrated between the main structural protein (p73) of intracisternal A-particles and a major internal protein (p24) of the M432 retrovirus.

Intracisternal type A particles (IAPs) are retrovirus-like entities endogenous to *Mus musculus* but genetically and structurally distinct from the type C and type B viruses of this species (9, 15–17, 23, 24, 26, 27). Recently (10), IAP genomic RNA was found to have partial sequence homology with RNA of an endogenous retrovirus, M432, previously isolated from the Asian species *Mus cervicolor* (2, 3). Reciprocal hybridizations between the IAP and M432 viral RNAs and their respective complementary DNAs indicated a 20 to 30% homology (10), with the heterologous hybrids showing a reduction in thermal stability (ΔT_m about 7°C) commensurate with the evolutionary divergence of the two *Mus* species (1, 4, 21). As described below, a comparative immunological study has now revealed a specific antigenic relationship between the major structural proteins of IAPs and the M432 virus.

IAPs were prepared from BALB/c myeloma MOPC-104E (11) or from the N4 neuroblastoma tissue culture line (14) of A/Jax origin. The main 73,000-dalton structural protein (p73), which carries group-specific antigenic determinants (9, 17) and is coded for by the IAP high-molecular-weight RNA (20), was purified as previously described (17) or by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. Rabbit antiserum was prepared (9) by repeated injections of emulsified gel slices containing p73. This antiserum (ANTI-p73) was specific for p73 and several structurally related proteins (17, 18) of the IAP inner shell. Coat glycoproteins have not been identified in purified IAPs by staining or labeling techniques (9, 17).

The M432 virus, originally isolated from *M. cervicolor* spleen cells (2), was grown in NIH/3T3 cells and purified from culture media (3). The virus contains two glycosylated outer coat proteins, gp65 and gp32, and three principal internal structural proteins, p24, p16, and p12 (2, 3). The major component, p24, carries a group-specific antigen. Rabbit antisera were prepared against total M432 virion proteins (ANTI-M432 virion) and against p24 (ANTI-p24) isolated by chromatography in guanidine hydrochloride (3).

Antisera against IAP p73 and M432 proteins were tested for homologous and cross-reactivities as shown in Fig. 1. Partially purified IAPs from [^{35}S]methionine-labeled N4 cells contained a number of electrophoretic components (Fig. 1a) from among which ANTI-p73 bound the major labeled species, p73, and two other proteins present in lesser amounts (Fig. 1b). High-molecular-weight IAP components immunologically related to p73 have been previously described (17). Interestingly, ANTI-M432 virion and ANTI-p24 showed this same binding specificity for the IAP proteins (Fig. 1c and d), although the reactions were much weaker at equivalent serum levels.

In the reciprocal set of reactions, ANTI-M432 virion reacted strongly with the M432 internal proteins p24 and p16 and to a lesser extent with the other virion components (compare Fig. 1f and h), whereas ANTI-p24 gave a weaker but apparently more specific reaction (Fig. 1i). The ANTI-p73 serum gave a strong and remarkably specific reaction with M432 p24 (Fig. 1g). Only small amounts of other virion-associated proteins were bound by ANTI-p73, most notably a minor 45,000-dalton component and traces of a 73,000-dalton protein that was not visualized in the whole virus pattern.

We have recently found that p73 is strongly labeled when prepared from neuroblastoma cells that have been cultured in medium containing [^{32}P]orthophosphate (J. Fewell and E. Kuff, unpublished data). Therefore, it was of particular interest to determine whether this protein is immunologically related to p12, the major phosphorylated species of the M432 virion (3). Since none of the antisera gave clear-cut reactions with ^{35}S-labeled p12 (Fig. 1), they were further tested against M432 virus and IAPs prepared

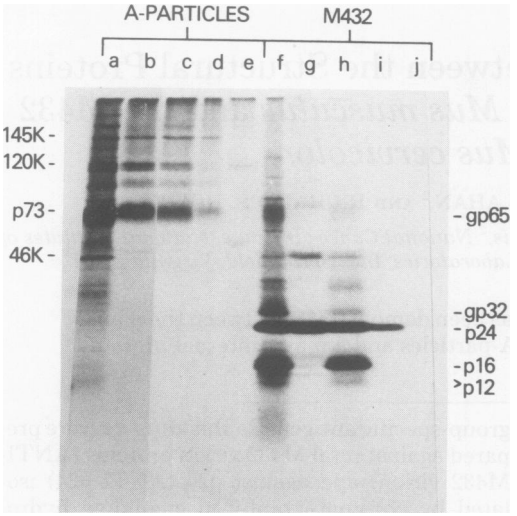

FIG. 1. *Immunoprecipitation of [^{35}S]methionine-labeled IAP and M432 virion proteins with antisera against the two types of particle proteins. Particles were labeled by incubation of N4 cells or M432-infected NIH/3T3 cells in methionine-free medium supplemented with 100 μCi of [^{35}S]methionine per ml (600 to 900 Ci/mmol, Amersham). Portions of partially purified IAPs (14) or banded M432 virus (2) were heated for 3 min at 100°C in 0.5% sodium dodecyl sulfate–1 mM dithiothreitol and then treated with 2 to 4 μl of undiluted antiserum in 0.5-ml reaction mixtures containing 10 mM Tris·hydrochloride (pH 7.4), 0.01% Triton X-100, and 0.03% sodium dodecyl sulfate. After 15 min at 20°C, the mixtures were cooled to 5°C and antibody-antigen complexes were bound to Staphylococcus aureus (50 μl of 10% suspension, prepared as described elsewhere [8]). The bacteria were washed four times at 5°C with a solution of 50 mM Tris·hydrochloride (pH 7.4),0.15 M NaCl, 5 mM EDTA, and 0.05% Nonidet P-40. Then the labeled proteins were eluted with sodium dodecyl sulfate (8) and analyzed by electrophoresis on sodium dodecyl sulfate-containing 5 to 15% polyacrylamide gels and autoradiography (20). Lanes a and f, portions of the whole IAP and M432 preparations, respectively; b and g, immunoprecipitations with ANTI-p73 (2 μl); c and h, ANTI-M432 virion (2 μl); d and i, with ANTI-p24 (4 μl); e and j, with nonspecific rabbit serum (anti-goat). Lanes a and b were printed from a 4-day autoradiograph; c to j were printed from a 12-day exposure. Molecular weights were established by reference to radioactive standards electrophoresed simultaneously (but not included in the figure). Known IAP and M432-virion specific proteins are identified per convention (p73, p24, gp65, etc.). Several other proteins are indicated by their apparent molecular weights.*

from cultures labeled with [^{32}P]orthophosphate. ANTI-M432 virion reacted strongly with its homologous phosphorylated protein and weakly (as expected from the results in Fig. 1) with IAP p73. On the other hand, ANTI-p73, although reacting strongly with the ^{32}P-labeled p73, gave no evidence whatever of binding with the phosphorylated M432 protein. Thus, the immunological studies of in vivo-labeled preparations, with the antisera currently available, reveal a specific cross-reactivity limited to the major structural proteins, p73 and p24, of the two types of particles.

The nature of this cross-reactivity was further explored by using competitive radioimmunoassays of ^{125}I-labeled particle and viral proteins. Details of antigen labeling and immunoprecipitation are given in the legend of Fig. 2. Preliminary studies showed that ANTI-p73 bound the same proportion of ^{125}I-labeled M432 p24 as did the homologous ANTI-M432 virion serum (65% of total input) and that the concentrations of ANTI-p73 required for half-maximal binding of ^{125}I-labeled p24 and ^{125}I-labeled p73 were not greatly different (antiserum dilutions of 1:3,000 and 1:10,000, respectively). In the competitive radioimmunoassay based on the homologous reaction between ANTI-p73 and ^{125}I-labeled p73 (Fig. 2A), 50% displacement was achieved with 4 ng of competing p73 and 10 ng of whole IAP protein. Only 70% displacement was reached with as much as 10 μg of competing M432 p24; the reaction curve shows that this antigen competed with reduced affinity for many—but not all—of the antibody binding sites directed against p73. Whole M432 virus may have contained additional competing antigenic sites, but complete displacement of ^{125}I-labeled p73 from its homologous antiserum was not achieved even with 50 μg of viral protein (Fig. 2A).

In the heterologous reaction between ANTI-p73 and the ^{125}I-labeled M432 p24 (Fig. 2B), both p73 and whole IAP proteins were more effective competitors than p24 itself. The amount of p73 required for 50% displacement of ^{125}I-labeled p24 was consistent with that required in the homologous competition assay (Fig. 2A), when prorated for the differing amounts of antisera used. Therefore, the antigenic sites which compete with ^{125}I-labeled p24 are group properties of p73 and are not confined to a small fraction of the molecules.

Competitive radioimmunoassays were also carried out with ANTI-M432 virion and the same two labeled antigens (data not shown). Unlabeled p73 competed effectively and completely in the heterologous reaction (ANTI-M432 virion versus ^{125}I-labeled p73), but very poorly in the homologous system. The latter observation is consistent with the weak (although specific) reaction between ANTI-M432 virion and the in vivo-labeled IAP proteins (Fig. 1c).

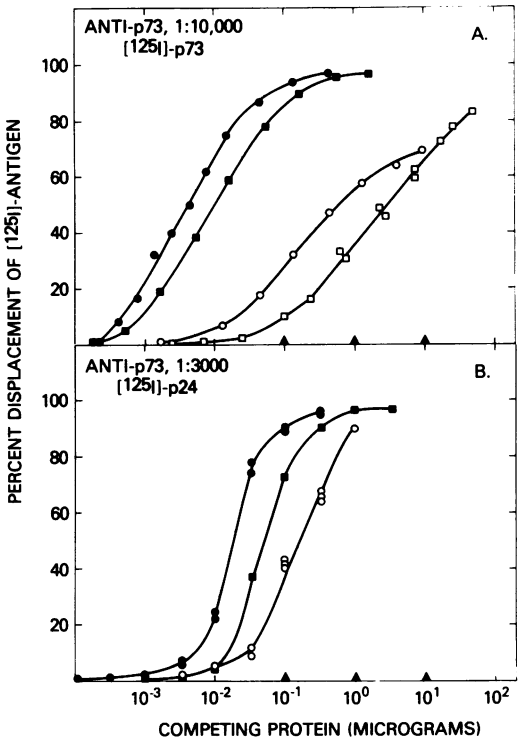

FIG. 2. *Competitive radioimmunoassays for IAP and M432 virus proteins with antiserum against IAP-p73 (ANTI-p73) and, as ^{125}I-labeled antigen, either IAP-p73 (A) or M432 p24 (B). Nonradioactive competing antigens are as follows: ●, IAP p73; ■, whole MOPC-104E IAP; ○, M432 p24; □, whole M432 virus; ▲, MMTV (mammary tumor virus from milk of M. musculus strain RIII mice). Sodium dodecyl sulfate-treated inner shells (17) from MOPC-104E IAPs and samples of whole M432 virus were reduced with β-mercaptoethanol in sodium dodecyl sulfate (100°C, 3 min) and alkylated with iodoacetamide or iodoacetic acid. The dialyzed proteins, in portions of 3 to 40 μg, were then labeled by reaction for 1 min at 25°C with 0.5 mCi of Na^{125}I and 25 μg of chloramine T (7). After addition of sodium meta-bisulfite (62.5 μg), the mixtures were passed over G50 Sephadex. All steps were carried out in 0.01 to 0.1% sodium dodecyl sulfate. Individual proteins were separated by electrophoresis of ^{125}I-labeled preparations in sodium dodecyl sulfate-polyacrylamide gels followed by elution in pH 7.4 buffer containing 0.1% sodium dodecyl sulfate. Radioimmunoassay mixtures (0.5 ml) contained 1.25 μl of normal rabbit serum, 100 μl of diluted ANTI-p73, and 0.3 to 0.5 ng (6×10^3 to 10×10^3 cpm) of ^{125}I-antigen; the buffer was 10 mM Tris·hydrochloride (pH 7.4), 0.01% Triton X-100, and 0.004% sodium dodecyl sulfate. Tubes were held at 5°C for 18 h; then, antibody-bound ^{125}I-antigen was determined with S. aureus (8). In noncompeted reactions, the maximum bindings of ^{125}I-labeled p73 and ^{125}I-labeled p24 by ANTI-p73 were 75 and 65% of total input, respectively. For competitive radioimmunoassays, ANTI-p73 was diluted (as shown) to give about half the maximum binding. Competing antigens were treated at 100°C for 3 min with 0.5% sodium dodecyl sulfate and 1 mM dithiothreitol and then diluted into the reaction mixtures. Mixtures with antibody and competing protein were kept at 20°C for 30 min before addition of ^{125}I-antigen. Protein concentrations were determined according to Lowry et al. (13).*

Mouse mammary tumor virus proteins were completely inactive in any of the homologous and heterologous competition assays (Fig. 2 and unillustrated data), as were the proteins of a mammary tumor virus (22) from the milk of feral *M. cervicolor popaeus*, a bromodeoxyuridine-induced guinea pig retrovirus (5), type D squirrel monkey retrovirus, and Rauscher murine leukemia virus (most of the virus preparations generously supplied by J. Schlom, National Cancer Institute). These findings support other observations that M432 virus and *M. musculus* IAP are unrelated to previously defined classes of retroviridae (3, 9, 15, 25, 26).

Thus far, we have no evidence for other biochemical or antigenic relationships between the proteins of IAPs and M432 virus. An antiserum known to inhibit the M432 DNA polymerase (3) was without effect on the activity of the IAP-associated enzyme (23; S. H. Wilson, personal communication), and the ANTI-M432 virion serum has not revealed additional protein components in IAPs beyond those seen with ANTI-p73 (Fig. 1). The latter antiserum did not react with M432 p16 and p12 even though it is likely, both by analogy with other retroviruses (6) and on the basis of direct preliminary evidence, that they are generated from the same precursor polypeptide as p24. And finally, when the acidic tryptic peptides generated from ^{125}I-labeled p73 and p24 were analyzed by chromatography on Aminex A-5 (Bio-Rad) cation-exchange columns (19), we found no correspondence between the elution patterns of the two samples.

A limited protein relationship is in keeping with the partial and divergent sequence homology found for the RNAs of the two types of particles (10). Since IAP-related sequences are quite broadly represented in the *M. cervicolor* cellular DNA (10), they could have contributed structural protein information to the M432 viral genome through some recombinational event in the evolutionary past. Alternatively, sequences coding for the cross-reactive portions of the major internal proteins could represent highly conserved regions of two otherwise more divergent retrovirus genomes. The precise nature of this relationship may be clarified by mapping studies of the M432 and IAP genomes, now in progress.

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