Structural Heterogeneity in p30 Molecules of Type C Viruses

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SUMMARY

Tryptic digests of p30 proteins from mouse type C viruses were subjected to cation-exchange chromatography. Structural heterogeneity of p30 molecules was seen in two specific areas of the peptide elution profiles. These hypervariable regions of p30 proteins were used to discriminate representative ecotropic (N- and B-tropic), xenotropic (alpha and beta) and amphotropic viruses.

The aetiological associations between the expression of endogenous type C virus genomes and naturally occurring diseases of inbred mice are a complex problem due, in part, to the polymorphism among certain type C virus proteins (Elder et al. 1977; Schindler et al. 1977; Boiocchi & Nowinski, 1978; Albino et al. 1979). Polymorphism probably reflects the response of proviral sequences to genetic recombination, non-lethal mutations and other as yet unknown selective pressures (Barbacid et al. 1977, 1978; Benade et al. 1978).

One rigorous and sensitive approach to the study of polymorphism is the comparison of the primary structure of virus proteins utilizing the technique of cation-exchange chromatography of tryptic peptides. In a previous study of 3H- and 14C-labelled gag gene proteins of endogenous mouse type C viruses, it was shown that the p30 proteins of typical xenotropic, N-tropic, amphotropic and dual-tropic viruses were distinguishable, as were the p15, p12 and p10 proteins (Albino et al. 1979). Further, we observed one peptide of the p30 molecule which represented a highly type-specific portion of its primary structure.

We now report the following observations: (i) the p30 proteins from an N-tropic virus from the NZW strain and from a B-tropic virus from the C57BL strain were clearly distinguishable, as were the p30 proteins from NZB beta and BALB/c: virus-2 (BV-2) alpha xenotropic viruses; we have previously shown that the p30 proteins from NZB and NIH Swiss AT124 viruses were distinguishable; (ii) the p30 proteins from C57BL B-tropic virus and from AT124 beta xenotropic virus had identical peptide maps; and (iii) the p30 protein from BV-2 was closely related to N-tropic virus p30 proteins.

Detailed knowledge of primary sequences could provide a basis for identifying hypervariable regions which are likely candidates for species- and/or type-specific reactivities which may have evolved specific functions. For example, one hypervariable region of the p30 molecule has been linked in an as yet unknown way to Fv-1 gene mediated tropism (Pincus et al. 1971; Hopkins et al. 1977; Schindler et al. 1977; Gautsch et al. 1978). While the number of p30 species analysed in this work is small and cannot yet be used to group type C viruses, the data presented here will aid in the eventual discrimination of the many possible virus subtypes activated in mouse cells.

Initial stocks of the following cell lines infected with cloned virus were obtained from P. V. O'Donnell of the Sloan-Kettering Institute (cell line given first followed by virus type): SC-1 cells, derived from a feral mouse embryo, lack host range restrictions for murine leukemia viruses (Hartley & Rowe, 1975); SC-1/amphotropic-4070A, a virus originally isolated from an outbred mouse exhibiting dual-tropism (Hartley & Rowe, 1975, 1976); SC-1/BL/6-7(B) CL D3, a B-tropic virus isolated from C57BL/6 spleen (O'Donnell & Stockert, 1976); mink lung (CCL-64)/AT124, a beta xenotropic virus of NIH Swiss mice.
isolated from human rhabdomyosarcoma cells after transplantation into immunosuppressed NIH Swiss mice (Todaro et al. 1973; Henderson et al. 1974; Callahan et al. 1975); mink lung/BALB: virus-2 (BV-2), an alpha xenotropic virus induced from BALB/c cells by iododeoxyuridine (Stephenson & Aaronson, 1972). BV-2 was not cloned prior to analysis. The following viruses were isolated in this laboratory (uninfected cells used for propagation were obtained from P. V. O’Donnell); mink lung/NZB-X, a xenotropic virus spontaneously produced by NZB embryo cells; SC-1/NZW-N, an N-tropic virus induced from NZW embryo cells by 5-bromodeoxyuridine (Lowy et al. 1971). NZB and NZW mice are from specific pathogen-free colonies maintained at this institute. All cells were maintained with Dulbecco’s modified Eagle medium (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Microbiological Associates) and grown in a 5% CO$_2$ atmosphere.

The viruses used in this study were biologically characterized in this laboratory with respect to host range, interference groups and neutralization patterns except for the BL/6 B-tropic virus which was characterized by O'Donnell & Stockert (1976). Viruses so defined were labelled in cell cultures with radioactive amino acids, purified, chromatographed by
Table 1. Grouping of mouse type C viruses on the basis of structural heterogeneity of p30 molecules

<table>
<thead>
<tr>
<th>Area I (Fractions 15-22)</th>
<th>Pattern I</th>
<th>Pattern II</th>
<th>Pattern III</th>
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<tbody>
<tr>
<td>NZW N-tropic</td>
<td>C57BL B-tropic</td>
<td>NZB xenotropic</td>
<td></td>
</tr>
<tr>
<td>W/B F1 N-tropic*†</td>
<td>AT124 xenotropic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WN1802 N-tropic*‡</td>
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<tr>
<td>MCF-247 dual-tropic*</td>
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<tr>
<td>Amphotropic 1504A*</td>
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<tr>
<td>Amphotropic 4070A</td>
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<td></td>
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<tr>
<td>BV-2 xenotropic</td>
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<table>
<thead>
<tr>
<th>Area II (Fractions 62-78)</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZW N-tropic</td>
<td>Amphotropic 1504A*</td>
<td></td>
</tr>
<tr>
<td>W/B F1 N-tropic*†</td>
<td>Amphotropic 4070A</td>
<td></td>
</tr>
<tr>
<td>WN1802 N-tropic*‡</td>
<td>C57BL BL/6 B-tropic</td>
<td></td>
</tr>
<tr>
<td>MCF-247 dual-tropic*</td>
<td>NZB xenotropic</td>
<td></td>
</tr>
<tr>
<td>BV-2 xenotropic</td>
<td>AT124 xenotropic</td>
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</tbody>
</table>

* For details see Albino et al. (1979).
† From (NZW x NZB)F1 mouse.
‡ From BALB/c mouse.

It has been established that the technique of cation-exchange chromatography yields peptide maps that are reproducible and can detect subtle differences in the primary structure of proteins (Albino et al. 1979). This technique showed the strong conservation of the p30 molecule but clearly defined two areas of structural heterogeneity, the first eluting at low normality (fractions 15 to 22), termed Area I, and the second eluting at intermediate normality (fractions 62 to 78), termed Area II.

Fig. 1 (a) compares the peptide maps of the p30 proteins from NZB N-tropic virus and C57BL BL/6 B-tropic virus. The patterns are very similar but show (i) the lack of co-migration of a major peptide in Area I and (ii) the presence of a unique peptide in Area II of each of the virus p30 proteins. Fig. 1 (b) indicates that the p30 peptide maps of the BL/6 B-tropic virus and the AT124 beta xenotropic virus are identical in all areas. Fig. 1 (c) compares the peptide maps of the p30 proteins from NZB beta xenotropic and BV-2 alpha xenotropic viruses. The patterns differ in (i) the lack of co-migration of the major peptide in Area I and (ii) the presence of a unique peptide in Area II of each of the virus p30s.

Fig. 1 (d) compares the p30 proteins from BV-2 and wild-mouse amphotropic virus isolate 4070A. The maps are identical except for the presence of a unique peptide in Area II of each of the viruses. A previous comparison of the p30 proteins from AT124 and NZB viruses showed that they differed only in the lack of co-migration of the major peptide in Area I (Albino et al. 1979).

Table 1 summarizes the composite data drawn from this and our previous paper (Albino et al. 1979). Heterogeneity in Area I of the p30 maps was detected by three distinguishable peptide elution patterns. Pattern 1: all viruses which were not restricted by the Fv-Ra allele in mouse cells (i.e. ecotropic, amphotropic and MCF-247 dual-tropic isolates) had an identical peptide elution pattern in Area I, indicating apparent conservation of an N-tropic marker peptide. Gautsch et al. (1978) found, in an extensive study using two-dimensional peptide mapping of 131I-labelled proteins, that N-amphotropic viruses could be distinguished from N-ecotropic viruses by virtue of subtle migrational differences in the apparent
analogous N-tropic marker peptide, indicating either polymorphism of this determinant or some form of biological modification (e.g. glycosylation) not detectable by cation-exchange chromatography. Actual amino acid sequencing of the peptide from representative p30 molecules could clarify this point. We further show that the BV-2 xenotropic virus p30 was also identical to the N-amphotropic virus p30 in Area I, indicating that BV-2 may be responsive to Fv-i gene mediated functions. Pattern II: the p30 protein from BL/6 B-tropic virus could easily be distinguished from N-tropic viruses by the lack of co-migration of the marker peptide in Area I. Buchhagen et al. (1975) were the first to show that the p30 proteins from N- and B-tropic viruses from a BALB/c mouse could be distinguished by non-identical elution patterns in Area I. Gautsch et al. (1978) have also shown that a single peptide could indicate whether a virus is N-tropic or B-tropic. Our data further showed that the p30 proteins of the BL/6 B-tropic virus and the AT124 xenotropic virus were identical in their peptide maps. A similar finding has been made by Gautsch et al. (1978). It has been suggested by Benade et al. (1978) that those portions of B-tropic virus genomes coding for the p12 and p15 proteins in C57BL mice arose by recombination between endogenous N-tropic and inducible xenotropic virus sequences. We have shown (unpublished data) that the p12 peptide patterns from BL/6 B-tropic, BV-2 and several N-ecotropic isolates were closely related to each other and clearly distinct from a second group consisting of the beta xenotropic viruses (NZB and AT124) and wild mouse amphotropic viruses. However, since the p30 proteins of the BL/6 B-tropic viruses are most closely related to beta xenotropic p30 proteins, it would appear that B-tropic viruses from C57BL mice may have acquired genetic information from endogenous beta xenotropic virus sequences as well (Gautsch et al. 1978; Albino et al. 1979). Pattern III: it has previously been shown that NZB p30 has a unique peptide in Area I and, therefore, was distinguishable from AT124 xenotropic virus (Albino et al. 1979). It is unlikely that the unique peptide of the NZB p30 molecule represents a mixture of Pattern I and Pattern II type peptides since cation exchange resins bind peptides by electrostatic forces which are neutralized by titrating with an increasing pH/salt buffer. The association or dissociation of peptides is determined by the equilibrium constant for binding which would be unique for each peptide bound and would not be averaged for two separate species. It is possible that the NZB unique peptide is in fact a mixture of two discrete entities but they would have to be very similar in their binding constants and, therefore, still different from that of either Pattern I or Pattern II peptides.

Heterogeneity in Area II indicated that the p30 molecules examined can be differentiated into two polymorphic groups: Group I: N-tropic, MCF-247 dual-tropic and BV-2 alpha xenotropic viruses; and Group II BL/6 B-tropic, amphotropic and beta xenotropic viruses. Each of the viruses within these groups had an identical peptide structure in Area II. It is not known what role, if any, this hypervariable region has in type C virus function but the discrimination of the region indicates the extreme sensitivity of cation-exchange chromatography of tryptic peptides. Moreover, by varying the type and shape of elution buffer it is conceivable that other previously undetected structural differences will be manifested. One additional point can be noted. While the present study has concentrated on the p30 molecules, we now have data (Albino et al. 1979; unpublished data) which indicates that the p10 protein is the most conserved protein of type C viruses and shows virtual structural identity among a wide range of viruses, including N-tropic, B-tropic, NZB xenotropic and BV-2 xenotropic viruses.

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