Biochemical evidence that MCF murine leukemia viruses are envelope (env) gene recombinants  
(glycoprotein/tryptic peptide analysis/AKR viruses)

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Contributed by Wallace P. Rowe, August 9, 1977

ABSTRACT  Recently, a novel class of murine type C virus (MCF), some strains of which are highly oncogenic in the AKR acceleration test, has been isolated from premalignant and malignant thymuses of AKR mice. The biology of these viruses suggested that MCFs are the product of recombination between endogenous ecotropic and xenotropic viruses and, further, that the recombination has taken place within the envelope (env) gene which encodes the surface glycoprotein (gp70) of the virion. We have compared, by tryptic peptide analysis, the gp70s of four MCF isolates with the gp70s of various possible parental viruses. In addition, we have compared the tryptic peptides of the gag gene products p30 and p15 from several of these viruses. The results allow the following conclusions: (i) the gp70s of the MCF viruses are not identical to one another and are different from the gp70s of the possible parental viruses tested; (ii) the MCF virus gp70s have tryptic peptides in common with xenotropic virus gp70s as well as with ecotropic virus gp70s; and (iii) the gag region protein, p30, of the MCFs tested is identical to p30 of AKR ecotropic virus (Akv-1 or Akv-2) and distinct from p30 of xenotropic viruses, suggesting that the S' end of the recombinant viruses is of Akv origin. The findings are discussed with respect to the possible role a recombinant virus might play in leukemogenesis in AKR mice.

Recent studies have demonstrated the appearance of a unique class of murine leukemia viruses (MuLVs) in AKR mice during the late preleukemic period (1, 2). These viruses, referred to as mink cell focus-inducing (MCF) strains because they produce characteristic alterations in a mink lung cell culture line, show characteristics of both the ecotropic and xenotropic MuLVs. In particular, they show envelope-determined properties of both viruses—namely, host range, interference, and serum neutralization—and it has been postulated that they represent genetic recombinants.

The major protein component of the oncornaviral envelope is a glycoprotein (gp70) with a molecular size of 70,000 daltons (3, 4) that is encoded by a gene (env) located about 1 kb from the 3' end of the viral RNA (5). In the mouse germ line there are a number of proviruses that encode MuLV gp70, which is often expressed without coordinate synthesis of other components of complete virions (6-9). For example, some major glycoproteins of serum, cell membranes, sperm, and epithelial cell secretions are encoded by these proviruses (7, 9-11). Recently we completed an analysis, by the tryptic digest fingerprint technique, of more than 50 different MuLV-specific gp70 molecules representing either viral envelope components or cellular gp70 molecules expressed as cells proceed through their various differentiation programs (12). From our analysis it was clear that the proviruses encoding gp70 form a multigene family of considerable complexity. We have now used this technique to study the envelope gp70s of several of the MCF MuLVs and have found that they are distinct from other gp70s of AKR origin but appear to be hybrid molecules with elements characteristic of both ecotropic and xenotropic type gp70s. These findings provide further support for the concept that MCF viruses arose by genetic recombination in the env gene and suggest that aberrant expression of viral gp70 molecules in the cell membranes may be involved in the pathogenesis of spontaneous murine lymphoma.

MATERIALS AND METHODS

The virus strains used in this study are described in Table 1.

Procedures for testing MuLVs capable of infecting heterologous cells as well as details of culture conditions and fluorescent antibody procedures have been described (1). Methods for propagation and quantitation of MCF viruses have been outlined (2). Details of virus purification procedures are described elsewhere (12).

The gp70s of the virus isolates were purified by immunofinity chromatography (12, 14), iodinated with 125I, and analyzed by tryptic peptide analysis as described (12). For analysis of the gag region proteins p30 and p15, the proteins from each of the viruses were separated on 5-17% sodium dodecyl sulfate/polyacrylamide slab gels (15) and the individual proteins were cut out of the gel and radioiodinated directly within the gel slice for tryptic peptide analysis (16). Briefly, after the proteins were located by staining with Coomassie blue, slices containing the proteins were taken from the slab gel and washed overnight in 10% methanol. The slices were then dried and the following reagents were added sequentially: 20 μl of 0.5 M sodium phosphate buffer (pH 7.5), 1 mCi of 125I (Amersham; specific activity, 17 Ci/mmol) in 5 μl, and 5 μl of chloramine-T (1 mg/ml). The slices were allowed to absorb the liquid for 30-60 min, at which time 200 μl of sodium bisulfite (1 mg/ml) was added to stop the reaction. The slices were washed with 10% acetic acid and then with 10% methanol until background in the wash solution was approximately 5% of the total incorporation. The proteins were then trypsinized from the gel slice, and tryptic peptide analysis was performed as previously described (12).

RESULTS

MuLV gp70s of Young AKR Mice. The molecular characteristics of the recombinant viruses of AKR mice are best appreciated by reference to the parental viruses from which they are evidently derived. Our structural analysis of virion-assoc-
Table 1. Description of murine leukemia virus strains

<table>
<thead>
<tr>
<th>Class</th>
<th>Strain</th>
<th>Source</th>
<th>Cells used for propagation of virus studied</th>
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</thead>
<tbody>
<tr>
<td>Ecotropic</td>
<td>Akv-1-566</td>
<td>Isolated in Sc-1 cells from tail extract of 4-wk-old NIH Swiss mouse congenic for Akv-1*</td>
<td>Sc-1</td>
</tr>
<tr>
<td></td>
<td>Akv-2-603</td>
<td>Isolated in Sc-1 cells from tail extract of 5-wk-old NIH Swiss mouse congenic for Akv-2*</td>
<td>Sc-1</td>
</tr>
<tr>
<td>Xenotropic</td>
<td>AKR-Th-6</td>
<td>Isolated in mink lung cells from thymus cells of normal 2-mos-old AKR/J</td>
<td>Mink lung (ATCC CCL 64)</td>
</tr>
<tr>
<td></td>
<td>AT-124</td>
<td>Isolated by Todaro et al. (13) from NIH Swiss mouse</td>
<td>Mink lung (ATCC CCL 64)</td>
</tr>
<tr>
<td>MCF</td>
<td>AKR-247</td>
<td>Isolated in mink lung cells from thymus cells of 6-mos-old AKR/J; cloned by limiting dilution</td>
<td>Sc-1</td>
</tr>
<tr>
<td></td>
<td>AKR-13</td>
<td>Isolated in mink lung cells from thymus cells of 3-mos-old AKR/J that had received thymus graft from 6-mos-old AKR/J at 2 mos of age; cloned by limiting dilution</td>
<td>Sc-1</td>
</tr>
<tr>
<td></td>
<td>Akv-1-36</td>
<td>Isolated in mink lung cells from spontaneous thymoma in Akv-1 congenic mouse; cloned by limiting dilution</td>
<td>Sc-1</td>
</tr>
<tr>
<td></td>
<td>Akv-2-34</td>
<td>Isolated in mink lung cells from spontaneous lymphoma in Akv-2 congenic mouse; cloned by limiting dilution</td>
<td>Sc-1</td>
</tr>
</tbody>
</table>

* NIH Swiss mice partially congenic for one or the other of the two ecotropic virus-inducing loci of AKR/J, Akv-1 and Akv-2.

The tryptic peptides of constitutively expressed free and virion-associated gp70 molecules expressed in young AKR mice are shown in Fig. 1. Three distinctive gene products have been identified

(i) Akv-1 and Akv-2 are unlinked loci of AKR mice that each yield an ecotropic virus. The gp70s of these two viruses, as recovered from mice made congenic for the respective loci, were indistinguishable (Fig. 1 A and B).

(ii) A xenotropic virus has been recovered from tissues of normal young AKR mice. The virion yielded a second distinctive AKR gp70 (Fig. 1C).

(iii) A third distinctive gp70 (Fig. 1D) was present in the serum of AKR, as well as many of other mouse strains. Only in the case of a xenotropic virus of NZB mouse origin is this gp70 found in a virion envelope (12).

MCF Viruses Appear to be Recombinants between Genes Encoding AKR Ecotropic Virus and Those Encoding a gp70 Related to Xenotropic Viruses. The tryptic peptides of the gp70s of two MCF viruses from AKR mice are shown in Fig. 2. Several points are important. First, the tryptic peptides of the gp70s from both isolates differ in structure from the putative parental viruses shown in Fig. 1. Second, both gp70s contain a tryptic peptide that has been encountered only in xenotropic viruses (brackets), as well as peptides unique to ecotropic virus gp70 (arrows). Third, although the gp70s of the two MCF isolates are similar, they are not identical, indicating that, for different isolates, recombination has taken place at different positions within the env cistron. Alternatively, this lack of identity could occur if the different recombinants arose from different parental proviruses.

gp70s of MCF Strains from Akv Congenic Mice. MCF strains of AKR origin have also been recovered from hematopoietic neoplasms in NIH Swiss mice congenic for the Akv-1 or Akv-2 ecotropic virus-inducing loci (13). These congenic strains provide, in principle, possible approaches to such questions as whether the entire genome of the MCF viruses is present in the Akv loci or only the ecotropic portion, whether the MCF viruses show characteristics of the xenotropic MuLV strains endogenous to the strain carrying the Akv loci, and whether MCF viruses derived from one Akv locus are different from those derived from the other.

Fig. 3 shows the tryptic peptides of the gp70s of xenotropic virus (AT 124) from NIH Swiss and MCF strains from Akv-1 and Akv-2 congenic mice. The NIH Swiss xenotropic gp70 was similar, but not identical, to that of the AKR xenotropic virus (Fig. 1C). The gp70s of the two MCFs were similar to one of the AKR MCFs (MCF-13) shown in Fig. 2, and both showed peptides characteristic of both ecotropic and xenotropic MuLVs. Unfortunately, it is not possible to gain any impression as to whether the xenotropic component of these two MCF isolates is of AKR or NIH origin.

In toto, the results support the concept that MCF isolates are env gene recombinants between ecotropic and xenotropic proviruses and that recombination takes place at different positions in the env gene.

The gag Gene of MCF-247 Is of Akv Origin. The above data showed that the MCF virus has an env gene derived by recombination. To determine whether other viral genes differ between the parental and recombinant viruses, we studied the tryptic peptides of the gag gene products p30 and p15 from Akv-1, Akv-2, MCF-247, and AKR and NZB xenotropic viruses (Fig. 4). The gag gene products p30 and p15 from these viruses were highly conserved relative to their gp70s. However, the p30
of MCF was indistinguishable from that of Akv-1 and Akv-2 and subtly different from that of the xenotropic virus, suggesting that in MCF-247, the 5′ end of the genome was of Akv origin. The differences in peptide maps of different p30s were small, and therefore mixing experiments were performed to ensure that the p30 of MCF was of Akv origin. When tryptic peptides derived from Akv and MCF p30s were mixed and separated, no additional spots were seen (Fig. 5). By contrast, additional spots appeared when tryptic peptides of Akv and the xenotropic virus were mixed (brackets). Thus, the gag gene product p30 of MCF-247 appears to be of AKR ecotropic virus origin.

**DISCUSSION**

Initially suggested by the biological properties of the MCF viruses, the hypothesis that they are recombinants within the env gene is strongly supported by the studies of their gp70 molecules. The tryptic digests are distinct from those of the gp70s from AKR ecotropic and xenotropic viruses and from the xenotropic-like serum gp70. Furthermore, the MCF gp70s show peptide spots that appear to be otherwise unique to ecotropic and to xenotropic gp70s; the patterns are not compatible with mixtures of ecotropic and xenotropic gp70s in that some spots of each are missing.

The biochemical data confirm the biological indications that the MCF viruses are a heterogeneous class rather than a single agent (J. W. Hartley, unpublished data). Of the four strains studied here, no two showed identical gp70 tryptic digests. However, the strains did fall into two groups on the basis of the overall patterns. These two groups did not correspond to the two Akv congenic mice were in the same group. Rather, the data are more compatible with the concept that the MCF viruses are not determined by a preexisting genetic locus but are recombinants that arise de novo.

Recently, it has been recognized that certain of the more highly oncogenic laboratory strains of MuLVs and sarcoma viruses are recombinant in nature. For example, the Kirsten and Harvey sarcoma viruses are recombinants between ecotropic MuLVs and an endogenous rat type-C virus (18, 19), whereas the defective Friend spleen focus-forming virus represents a recombinant between ecotropic and xenotropic MuLVs (20, 21) as does the Moloney MuLV-derived HIX virus (22, 23). In recent studies Troxler et al. (24) found that, like MCF-247, the Friend spleen focus-forming virus and a HIX-type virus derived from Moloney virus appear to be env gene recombinants. The temporal association between the emergence of MCF virus and the onset of lymphoma in AKR mice (1, 2), the presence of MCF type viruses in all other high lymphoma strains studied (ref. 1; unpublished data), and the lymphomagenic activity of several of the MCF isolates in the AKR acceleration test (unpublished data) provide strong evidence that these naturally occurring recombinant viruses are involved in the pathogenesis
of spontaneous viral lymphoma. The evidence that MCF viruses are 
env gene recombinants thus suggests that aberrant MuLV gp70 expression in cell membranes may be directly involved in the mechanism of viral lymphomagenesis. Possible mecha-
nisms include an inappropriate display of cell surface differ-
entiation signals resulting in a disturbance in intercellular
growth control mechanisms, a change in properties of cell
membranes resulting in loss of regulation of some crucial
membrane-bound enzyme, or disturbance of a cytoskeletal membrane interaction. However, an indirect effect is also possible, such as the altered viral envelope extending the tissue tropism of MuLV to involve thymic lymphocytes.

In light of the multiplicity of endogenous MuLV genomes in the mouse (12, 25), the evidence that MCF viruses are a heterogeneous group of env gene recombinants carries the implication that there may be an immense diversity of spontaneously arising env variants. These viruses thus provide a rich source of genetic variants of MuLV that may be useful for genetic analysis of viral envelope functions and their role in lymphomagenesis. An additional important inference of this diversity is that it provides a mechanism whereby each individual within inbred mouse strains could generate unique tumor cell surface antigens.

This is publication no. 42 from the Department of Cellular and Developmental Immunology and publication no. 1376 from Scripps Clinic and Research Foundation, La Jolla, CA. This research was supported by Contract NOI CP71018 within the Virus Cancer Program of the National Cancer Institute.