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# Genetic Approaches Toward Elucidating the Mechanisms of Type-C Virus-Induced Leukemia

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## A. Introduction

Type-C RNA viruses may be horizontally transmitted as infectious cancer-inducing viruses or vertically transmitted from one generation to the next, often in an unexpressed form, within the host genome (for review see Aaronson and Stephenson 1976). To date the translational products of three leukemia viral genes have been identified (Baltimore 1975). The *gag* gene product is a polyprotein precursor that undergoes cleavage to form the major nonglycosylated viral structural proteins, which for mammalian type-C viruses are p30, p15, p12, and p10. It has been possible to determine that the information within the murine viral *gag* gene is arranged 5′-p15-p12-p30-p10-3′ (Barbacid et al. 1976). The products of the *pol* and *env* genes are, respectively, the viral reverse transcriptase and a precursor protein containing the major envelope glycoproteins, gp70 and p15E. Evidence primarily from the avian system indicates that the type-C viral genes are ordered 5′-*gag-pol-env*-3′ (for review see Wang et al. 1976).

The lack of an in vitro transformation assay for type-C helper leukemia viruses has so far impaired efforts to elucidate the mechanisms by which these viruses cause tumor development in the animal. Thus, it is not known whether there exists a discrete viral gene that codes for a product that transforms a specific target cell population or whether the malignant potential of these viruses is exerted through a more indirect mechanism. The present report reviews biologic approaches currently underway within our laboratory to elucidate the mechanisms by which murine type-C viruses induce leukemia.

## B. Results

### I. Lymphoid Cell Targets for Transformation by Rauscher and Moloney MuLV Strains

In an effort to study target cells for leukemia induction by murine leukemia virus (MuLV), we analyzed the kinetics of tumor formation and the histopathology of tumors induced by clonal strains of two oncogenic replication-competent mouse type-C viruses, Rauscher and Moloney MuLV. The susceptibility of newborn NIH/Swiss mice to tumor induction by each virus was comparable. Gross enlargement of spleen and lymph nodes occurred as early as 10–11 weeks following inoculation of $5 \times 10^4$ XC pfu of either virus. As few as $5 \times 10^2$ XC pfu of each virus were capable of causing 50% mortality within 23 weeks. With Rauscher MuLV affected organs invariably included the spleen and (less frequently) liver and peripheral or visceral lymph nodes. Thymic involvement was not detected in 50 tumor-bearing animals examined. In contrast, Moloney MuLV caused gross evidence of tumor involvement of the thymus in the majority of animals. Other lymphoid organs were also affected at high frequency. Histopathologic analysis of tumors revealed no obvious differences in the morphology of the neoplastic lymphoid cells.

### II. Distribution of T and B Cell Markers Associated with MuLV-Induced Tumors

As seen in Table 1, Moloney MuLV induced tumors and lymphoma cell lines exhibited Thy. 1 antigen in the absence of detectable Fc or C3 receptors, indicating their T cell origin. Rauscher MuLV primary tumors and lymphoma

**Table 1.** T and B lymphoid markers on cell membranes of cultured Rauscher MuLV and Moloney MuLV-induced lymphoma cell lines[a]

| Lymphoma cell line | % Positive cells | | |
|---|---|---|---|
| | Thy.1 antigen | Fc receptor | C'3 receptor |
| *Rauscher MuLV induced* | | | |
| 6E | <1 | 95 | <2 |
| 13-2-6 | <1 | 89 | <2 |
| 13-1 | <1 | 94 | <2 |
| *Moloney MuLV induced* | | | |
| 19-1-2 | >99 | <1 | <2 |
| 19-1-5 | >99 | <1 | <2 |
| P1798 | >99 | <1 | <2 |
| L691 (spontaneous) | >99 | <1 | <2 |
| 129J (X-ray) | >99 | <1 | <2 |
| Normal spleen | 45 | 47 | 42 |

[a] The assays for Thy. 1 antigen and Fc and C'3 receptors were performed as described previously (Reddy et al. 1980). In the Fc receptor assay, SRBC alone did not yield rosettes. In the C'3 receptor assay, SRBC alone or SRBC coated with SRBC antibody did not give rosettes

cell lines of the same mouse strain, however, invariably exhibited Fc receptors in the absence of Thy.1 antigen, suggesting that these tumors were of the B lymphoid lineage. The pattern of immunoglobulin synthesis by individual Rauscher MuLV tumor cell lines was determined by both biosynthetic and radioimmunologic techniques. Rauscher MuLV lymphoma lines generally expressed immunoglobulin heavy ($\mu$) chain in the absence of detectable light (●——● or $\lambda$) chains. These findings established that the target of neoplastic transformation in response to Rauscher MuLV is an immature cell within the B lymphoid lineage (Burrows et al. 1979; Siden et al. 1979).

## III. Generation of Recombinants Between Oncogenic and Nononcogenic Mouse Type-C Viruses in Tissue Culture

An important approach toward defining viral genes required for transformation might result from the generation of recombinants between oncogenic and nononcogenic type-C viruses or between oncogenic viruses with different targets for transformation. We initially set out to obtain recombinants between a prototype ts mutant of the oncogenic Rauscher strain of MuLV (Stephenson and Aaronson 1973) and the endogenous xenotropic BALB:virus-2. Use of these viruses permitted the design of a protocol for recombinant virus isolation based upon specific virus growth requirements (Aaronson and Barbacid 1980). By assay at 39°C on NIH/3T3 cells, the replication of the ts mutant and xenotropic parental viruses, respectively, was effectively blocked. However, recombinants possessing Rauscher MuLV envelope functions and BALB:virus-2 sequences in genes affected by the ts lesions might be expected to grow efficiently in mouse cells at the nonpermissive temperature.

To generate potential recombinant viruses, we utilized a wild mouse embryo cell line, WM-C, which was permissive for replication of certain mouse xenotropic viruses. WM-C cells were first chronically infected with BALB:virus-2 and then superinfected with the Rauscher MuLV ts mutant at the permissive temperature (31°C). The mutant utilized, ts 25, represented a class known to accumulate noncleaved *gag* gene precursors at the nonpermissive temperature, 39°C (Stephenson et al. 1975). To control for mutant leakiness or reversion, WM-C cells were infected with the mutant alone and passaged under identical conditions. After 4 weeks, virus released from each culture was tested for infectivity for NIH/3T3 cells at the restrictive temperature. Only if the infectivity of the virus at 39°C was significantly enhanced by passage of the mutant through WM-C cells replicating the xeno-

tropic virus were individual virus clones selected by the microtiter procedure for further analysis.

## IV. Immunologic Identification of Recombinant Viruses

Type-specific antigenic determinants have been readily demonstrated in the p15 and p12 *gag* gene coded proteins as well as in the reverse transcriptase and envelope glycoprotein (gp70) of mouse leukemia viruses (for review see Stephenson et al. 1977). Furthermore, using appropriate antisera, type-specific determinants have also been demonstrated even in the more broadly immunoreactive proteins such as p30 (Boiocchi and Nowinski 1978) and p10 (M. Barbacid and S. A. Aaronson, unpublished observations).

We submitted clonal viruses isolated according to the above protocol to immunologic analysis. The origin of the viral sequences of each recombinant virus was determined by the ability of its respective gene products to compete in corresponding Rauscher MuLV or BALB:virus-2 homologous radioimmunoassays. The results, summarized in Figure 1, indicate the generation of individual recombinants that contained different amounts of genetic information of each parent. In some cases, as with rec 25e–8, recombination appeared to involve more than one cross-over.

## V. Oncogenicity of Recombinants Between Rauscher MuLV and Balb: Virus-2

We have investigated the biologic properties of some of the recombinant viruses so far generated. Their infectivity in tissue culture for NIH/3T3 cells was found to be similar (data not shown). However we observed striking differences in their ability to infect and replicate in newborn NIH/Swiss mice. As shown in Table 1, the Rauscher MuLV parental virus induced readily detectable levels of MuLV p30 in serum within 5 months with as few as 10 XC pfu inoculated. Rec 25b–14 showed almost comparable infectivity. In contrast, recombinants, including rec 25c–3, rec 25d–22, and rec 25e–8, were markedly less able to induce sustained virus replication in vivo. It should be noted that these viruses each contain substantially more genetic information of the xenotropic parental virus than did rec 25b–14. The ability of a given virus to chronically replicate in vivo was highly predictive of subsequent tumor formation. As shown in Table 2, both Rauscher MuLV and rec 25b–14 induced tumors by 10 months with as few as $10^2$ XC pfu inoculated, whereas each of the other recombi-

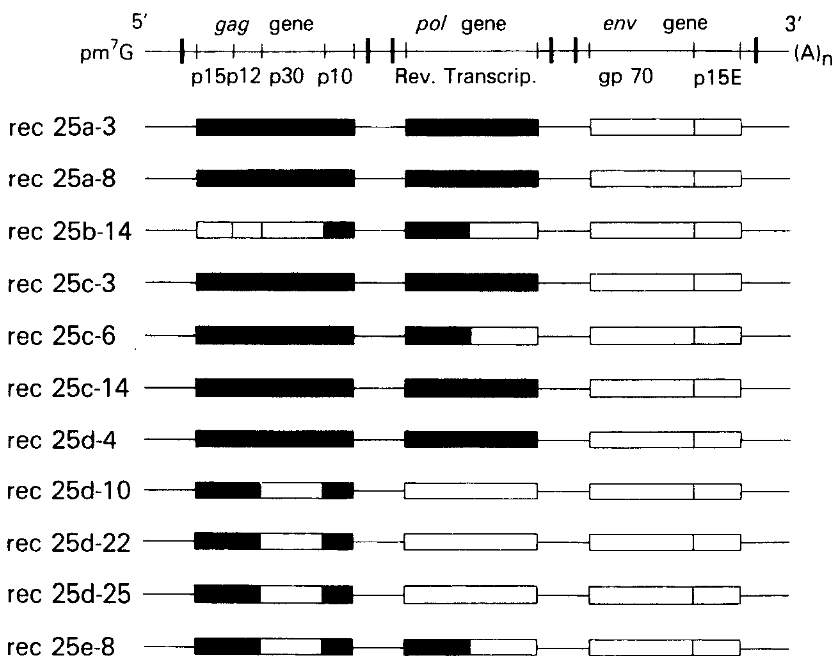


**Fig. 1.** Proteins exhibiting parental Rauscher-MuLV (▭) or BALB:virus-2 (▬) antigenic determinants are indicated. Where intracistronic crossing-overs are depicted, the relative localization and extent of Rauscher-MuLv and BALB:virus-2 derived genetic information have been arbitrarily assigned

**Table 2.** In vivo growth and oncogenicity of recombinants between Rauscher MuLV and BALB:virus-2

| Virus | In vivo infectivity[a] MuLV p30 in serum at 5 months[b] | | | | Tumorigenicity Tumor at 10 months[b] | | |
|---|---|---|---|---|---|---|---|
| | XC pfu inoculated | | | | XC pfu inoculated | | |
| | $10^4$ | $10^3$ | $10^2$ | $10^1$ | $10^4$ | $10^3$ | $10^2$ |
| Rauscher MuLV | 10/10 | 10/10 | 10/10 | 7/10 | 9/10 | 7/7 | 3/7 |
| rec 25b-14 | 10/10 | 10/10 | 9/10 | 1/10 | 5/9 | 5/7 | 1/8 |
| rec 25c-3 | 0/10 | ND | ND | ND | 0/9 | ND | ND |
| rec 25d-22 | 7/10 | 0/10 | ND | ND | 0/10 | ND | ND |
| rec 25e-8 | 0/10 | ND | ND | ND | 0/10 | ND | ND |

[a] NIH/Swiss mice were injected at birth with 0.2 ml of the appropriate virus dilution
[b] Animals positive/total animals inoculated

nant viruses tested was at least 100-fold less tumorigenic.

## C. Discussion

The present studies demonstrate that the target cells for transformation by two clonal mouse leukemia virus strains, Rauscher and Moloney MuLV, contain markers that differentiate them readily within the lymphoid cell lineage. Our finding that Moloney MuLV caused tumors in NIH/Swiss mice containing T but not B cell markers (Reddy et al. 1980) confirms previous studies indicating that T cells are the in vivo target for transformation by this virus (Moloney 1960). Rauscher MuLV tumors of the same strain, tested either directly or as cell lines established in culture, invariably contained markers of B lymphoid cells. This in combination with their lack of Thy. 1 antigen, a well established marker of T lymphoid cells, provides the first demonstration of a B cell target for neoplastic transformation by a replication-competent MuLV (Reddy et al. 1980).

Among the most important biologic questions pertaining to replication-competent type-C viruses concerns the mechanism by which they cause neoplasia. Recombinants generated and characterized in this report should be useful in determining what regions of the viral genome are essential for oncogenicity. Our preliminary data indicate that recombinant viruses, which have a similar capacity to infect and replicate in mouse cells in tissue culture, show striking differences in their abilities to replicate and induce tumors in the animal. In collaborative studies with P. Arnstein (National Cancer Institute), it has been possible to show that each recombinant virus was capable of inducing tumors in (Nu/Nu) mice on an NIH/Swiss genetic background. Under the same conditions only the parental Rauscher MuLV and rec 25b–14 produced tumors in ($Nu^+$/Nu) heterozygous mice. These results indicate that the ability of the host to mount an immune response plays an important role in the ability of recombinant viruses to replicate in the mouse. Further studies will be necessary to dissect other host genetic factors that may also influence the expression of the viruses in vivo. Nonetheless, our studies to date argue for the importance of active virus replication in order for mouse type-C viruses to induce disease in their host.

Leukemia viruses might be postulated to induce transformation as a result of their site of integration or due to their coding for a specific transforming gene product from an already identified structural gene or an as yet unidentified transforming gene. Accumulating evidence indicates that there is no specificity with respect to the site of virus integration. This conclusion is derived both from analysis of the arrangement of added viral information in the cellular genome of individual virus-induced leukemias (Canaani and Aaronson 1979; Steffen and Weinberg 1978) and by DNA sequence analysis of cellular junctions of individual molecularly cloned integrated DNA proviruses (McClements et al. 1980; Shimotohno et al. 1980). Recently, Hayward (this volume) has obtained evidence that at least some cellular messages found in leukemia virus

transformants contain information of the viral large terminal redundancy (LTR) in the absence of detectable information derived from the rest of the viral genome. This has led to the hypothesis that a viral promoter in the LTR may be responsible for transformation by causing derepression of a cellular gene by means of "downstream" promotion. If this hypothesis were correct, the transformation of different target cells by Rauscher and Moloney MuLV would have to be explained on the basis of differences in susceptibility of target cells for virus infection.

The possibility that the leukemia virus mediates its transforming action on the host by means of one or more of its gene products must also be considered. To date, there is no direct evidence that the leukemia virus contains a discrete transforming gene that codes for a nonviral structural transforming gene product. However, continued genetic analysis will be necessary to completely exclude this possibility. It has been postulated that viral structural products, specifically the *env* gene product, gp70, may directly (McGrath and Weissman 1979) or indirectly (Lee and Ihle 1979) cause chronic blastogenesis of lymphoid cells. This chronic antigenic stimulation of cell division has been postulated to result in the eventual selection of a spontaneously transformed clone. Our findings argue that blastogenesis in response to different leukemia viruses would have to be very specific if this mechanism were to explain the reproducibly distinct lymphoid cell targets for transformation by different leukemia viruses. Studies are presently underway to generate recombinants between Rauscher and Moloney MuLV. Such recombinants may make it possible to map the region within the viral genome responsible for transformation of specific lymphoid cell populations and shed further light on the mechanisms by which these viruses induce malignancy.

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