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Moloney Murine Leukemia Virus Variants with Distinct p30 Peptide Maps are Associated with Different Clinical Types of Leukemia*

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Abstract—*Mouse leukemias arising after neonatal inoculation of Moloney MuLV (M-MuLV) are of two main types. One is characterized by enlargement of spleen and lymph nodes, and has a normal diploid karyotype. Thymus enlargement is the most prominent feature of the second type, with or without spleen involvement. About half of the thymomas are trisomic with an extra chromosome* 15. *C-type viruses isolated from the two leukemia types differed with regard to their* p30 *peptide maps. The* p30s *isolated from the preleukemic tissues of a* 4-weeks-*old, neonatally MuLV-inoculated mouse were either of the 'thymic' or the 'splenic' type. These results suggest that the two virus types were already present in the original inoculum and had homed to different tissues. The peptide maps of the corresponding* gp70 *molecules showed no organ-related pattern: each virus isolate had a distinct* gp70 *profile.*

INTRODUCTION

NEONATALLY M-MuLV inoculated mice develop T-cell lymphomas, as a rule. In a previous study [1] we have found that 69% of the lesions involved the spleen and the lymph nodes; they all contained cells with a normal diploid karyotype. A smaller fraction (31%) developed thymomas with or without spleen involvement. The two thymomas examined contained cells with 41 chromosomes with an extra chromosome No. 15.

Depending on the primary tissue localization, the leukemias differed in their transplantation properties. Thymomas grew as subcutaneous tumours at the site of inoculation, while the transfer of cell suspensions from enlarged spleens led to splenomegaly with the proliferation of either donor or recipient type cells. Spleen cells grew only exceptionally (in one out of six cases) as solid tumors.

As one alternative explanation of these findings, we suggested that two different virus subtypes may be involved, with a predilection for different subsets of T cells and responsible for the 'splenic' and the 'thymic' forms of the leukemic syndrome. To explore this possibility, we have now compared the tryptic peptide maps of the viral p30 and gp70 components, isolated from leukemic and preleukemic spleen and thymus tissues.

MATERIALS AND METHODS

Four virus isolates were selected for detailed study. Newborn mice were inoculated intraperitoneally with 2.5×10^3 XC-PFU of M-MuLV (obtained from the Division of Cancer Cause and Prevention, National Cancer Institute). Virus Y36 was isolated from the trisomic thymus of a leukemic $(A \times C57Bl)F_1$ hybrid mouse. In the case of Y116, spleen

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enlargement was induced by the intravenous injection of bone marrow cells from a preleukemic M-MuLV inoculated (A × C57B1)F_1 hybrid mouse, into a 400 R irradiated syngeneic recipient. Cells of the enlarged spleen were serially transplanted and the Y116 virus was isolated from the third passage generation. Viruses 8104 and 8101 were isolated from the same one-month-old A/Sn mouse. In all cases, 24-hr culture supernatants from preleukemic or leukemic organs served as the virus source.

For tryptic peptide mapping, viruses were grown in roller cultures of infected NIH/3T3 or JLS-V9 cells. Culture fluids were concentrated in a hollow-fiber system and the viruses banded in sucrose gradients.

Viral proteins were separated by SDS–polyacrylamide gel electrophoresis, iodinated in the gel [2] and digested with trypsin [3]. To facilitate enzymatic cleavage of the glycoproteins, terminal sialic acid residues were removed by incubating the gel piece in 1 ml 0.05 N H_2SO_4 at 80°C for 90 min [4]. The labelled peptides were separated by chromatography on a Chromobead-(Technicon) cation exchange column and eluted with an exponential pH gradient (pH 2.5–5.0) [3].

RESULTS AND DISCUSSION

Clinical type, latency period and karyotype of leukemias

The chromosomal constitution of 21 M-MuLV induced leukemias in three genotypes is summarized in Table 1. As in previous observations [1], enlarged spleens contained only diploid cells while thymomas were more heterogeneous. Only 4 of 11 thymomas were trisomic, five contained only diploid cells and 2 were mixtures of diploid and trisomic cells. Regardless of the karyotype, the mean latency period of the thymic leukemias was approximately 50 days shorter than of the splenic leukemias. This confirms our earlier observation [1].

Infectious properties of the viruses reisolated from the leukemic cells

As in our previous study, all viruses were NB-tropic and gave large XC-plaques regardless of whether isolated from leukemic tissues or from the thymus or spleen of preleukemic mice. Four viruses were selected for more detailed studies. Y36 and 8104, the viruses derived from the leukemic and the preleukemic thymus, respectively, contained both xenotropic and ecotropic activity (Table 2). The xenotropic activity of the reisolated virus harvests could be eliminated by one passage though mink lung cells, suggesting that no dual-tropic viruses were present. The spleen derived Y116 was different from the three other viruses, and also from the original M-MuLV, since it failed to induce the Moloney virus-determined cell surface antigen (MCSA) in JLS-V9 cells in spite of its ability to replicate. Infected JLS-V9 cultures thus became XC-plaque positive but remained MCSA negative.

Tryptic peptide mapping

Figure 1 shows the tryptic peptide maps of the p30 proteins from different viruses. The presence or absence of the 7 major identifiable peptides (or peptide complexes) is summarized in Table 3. Virus from the preleukemic thymus (8104) and from the trisomic thy-

Table 1. Karotype and latency of thymic and splenic leukemia forms

Genotype	No of leukemic mice tested	Latency in days	Enlarged organ	Chromosome number* 40	41	40/41
(A × C57B1)F_1	5	140, 152, 152, 183, 207	spleen	5	—	—
(A × C57B1)F_1	5	100, 143, 147 183, 193	thymus	3	2	—
(A × C57L)F_1	2	136, 349	spleen	2	—	—
(A × C57L)F_1	1	90	thymus	—	1	—
CBA	3	147, 261, 281	spleen	3	—	—
CBA	5	132, 135, 146, 176, 199	thymus	2	1	2

Mean ± S.E. 199 ± 0.84. 10 spleens. 150 ± 0.55. 11 thymuses, $0.02 > P > 0.01$.
*Chromosome analysis was done from 20 metaphase plates according to a slightly modified method of Wang and Fedoroff [9] and C-banding according to Arrighi and Hus [10].

Table 2. Infectious properties of reisolated virus harvests

Virus		Ecotropic virus			Xenotropic virus
		XC-plaque		MCSA-induction on JLS-V9 cells‡	Focus induction S^+L^- mink cells§
Designation	Source*	CBA	C57Bl†		
Y36	leukemic thymus	4.0	3.8	+++	1.0
Y116	leukemic spleen	4.3	3.7	–	–
8104	one-month-old thymus	1.3	1.4	++	1.0
8101	one-month-old spleen	1.6	1.7	++	–

*All mice were neonatally M-MuLV inoculated.
†Mouse embryo fibroblasts. CBA: Fv-1^n, C57Bl: Fv-1^b. The XC-plaque test was carried out according to Rowe *et al.* [11]. Log_{10} virus titers.
‡The Moloney cell surface antigen (MCSA) was detected in indirect membrane immunofluorescence with mouse antisera produced by 3–6 inoculations of syngeneic Moloney lymphoma cells [12]. More than 30% of cells positive at 2 weeks +++ or 3 weeks ++ after infection. – Cultures negative 4 weeks after infection.
§Mink S^+L^- cells were infected with undiluted culture media and observed for foci at day 8 and, after one subculture, at day 16 [13]. Log_{10} virus titers.

moma (Y36) had identical p30 maps. The viruses from the preleukemic (8101) and leukemic (Y116) spleen, while identical among themselves with regard to their p30 tryptic maps, differed from the 'thymus type' p30 by the presence of an extra major peptide (H) and the absence of a minor peptide (B). Regions A and E showed further differences.

These results suggest that viruses localized in different organs may differ with regard to their p30 composition. The ability of the viruses to induce MCSA did not correlate with any identifiable p30 pattern.

Figure 1 also shows the tryptic map of the original M-MuLV p30. Seven major peptides were discernible here as well. Peptide H was absent while peptide B was present; this resembled the p30 protein of the virus recovered from the thymus, rather than the spleen-associated virus. However, regions A and E were similar to the corresponding regions of the spleen-derived viruses. These findings are in line with the assumption that the original M-MuLV is a mixture of the two (or more) viruses.

A similar analysis was performed on the major viral envelope glycoprotein gp70, after removal of the terminal sialic acid residues by sulphuric acid [4, 5]. The gp70 peptide maps are shown in Fig. 2. Table 4 summarizes the presence or absence of the identifiable peptides. The pattern was more complex than for p30. Each profile differed with regard to two or more peptides. Unlike p30, there were no discernible 'spleen' or 'thymus' associated patterns in this case. Our results are in line with previously demonstrated high genetic polymorphism of gp70 [6]. It is likely that this polymorphism, whether a result of mutations or recombinations, provides the virus population with rapid escape mechanisms across immunological barriers and/or extension of its host range at the level of virus–receptor interaction. The reason for the great variability of gp70 in our experiments may be due to the presence of two or more different viruses in the original M-MuLV stock and/or

Table 3. Presence or absence of identifiable peptides after tryptic digestion of p30

Virus source	A	B	C	E	F	G	H
8104 Preleukemic thymus	double	+	double	+	+	+	–
Y36	double	+	+	+	+	+	–
M-MuLV	+	+	+	+	+	+	–
Y116 Leukemic spleen	+	–	+	+	+	+	+
8101 Preleukemic spleen	+	–	+	+	+	+	+

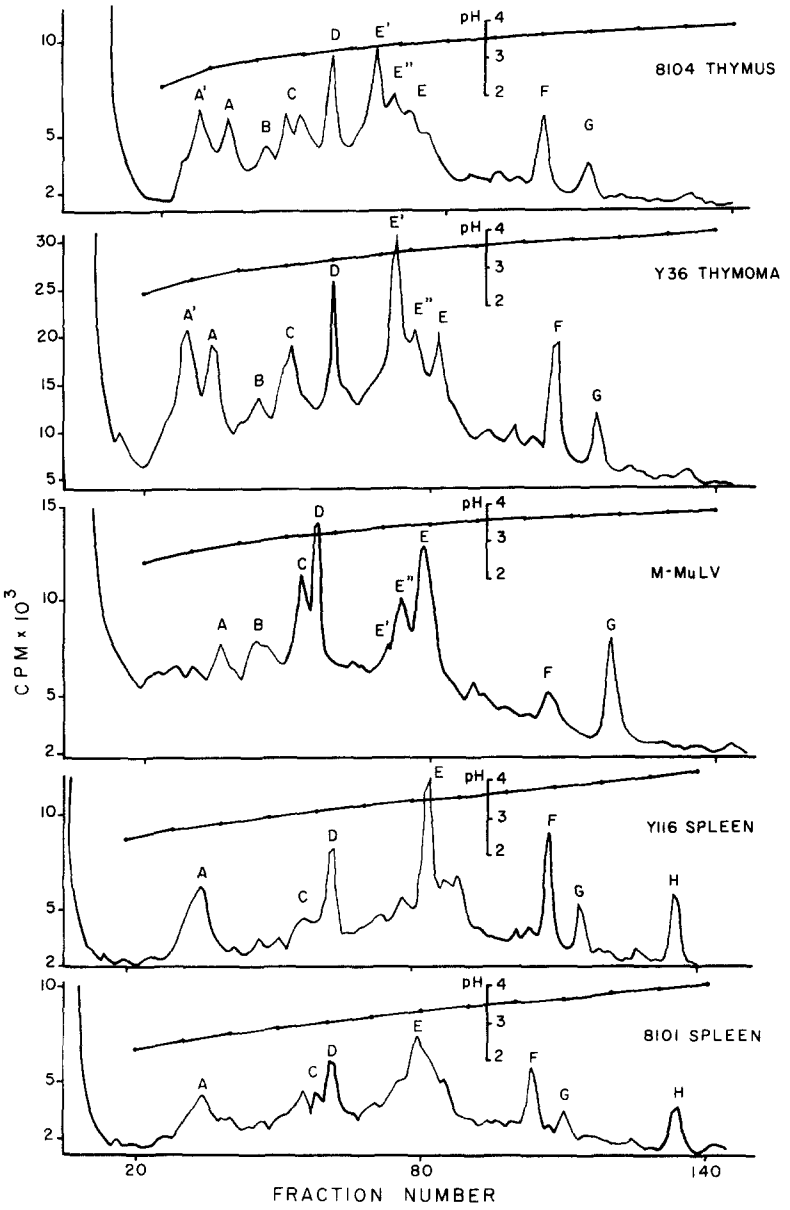

Fig. 1. Cation exchange chromatography of ^{125}I*-labelled tryptic peptides of* p30 *from virus preparations as indicated.*

Table 4. Presence or absence of identifiable peptides after tryptic digestion of gp70

Virus source	A	B	C	D	E	F	G	H	I	K	L	M	N	O
8104 Preleukemic thymus	+	+	+	double	−	+	+	+	+	+	+	+	−	+
Y36 thymoma	+	+	?	single	+	double	+	+	+	+	+	+	+	+
M-MuLV	+	+	+	double	+	+	+	+	−	+	+	−	+	+
Y116 Leukemic spleen	+	+	+	double	+	+	+	+	−	+	+	+	−	−
8101 Preleukemic spleen	+	+	+	single	+	+	+	+	+	+	−	−	+	−

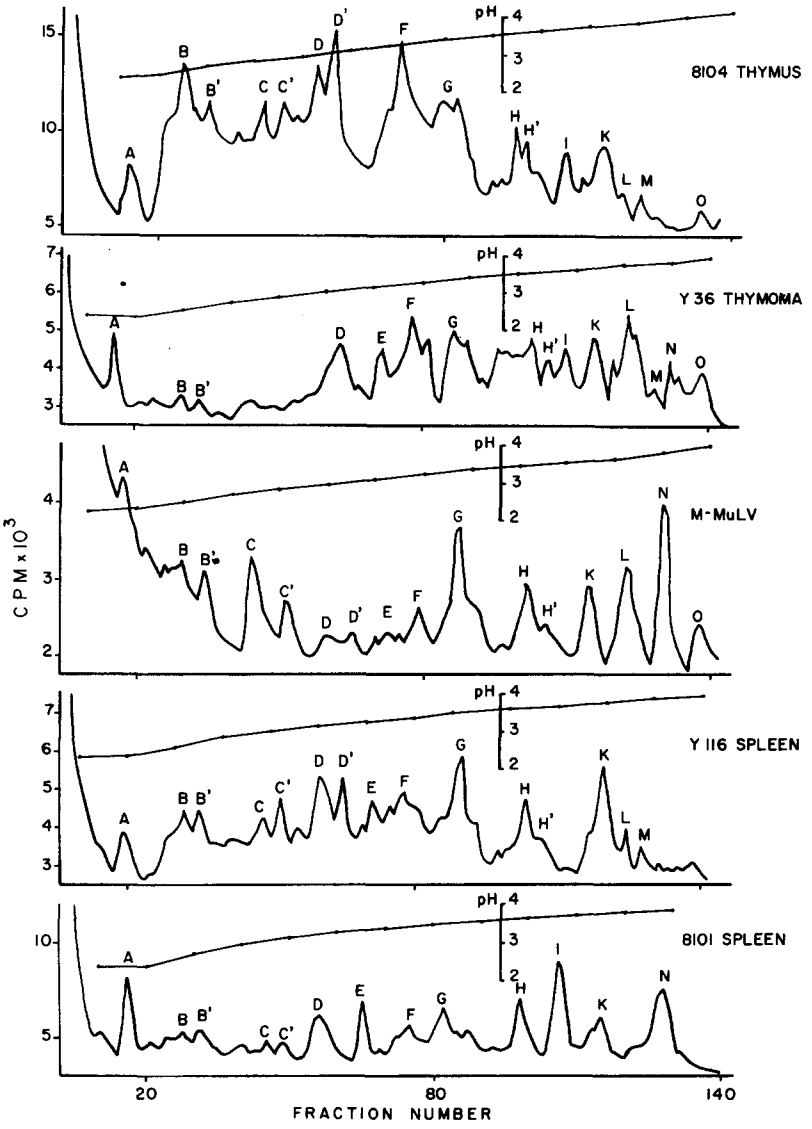

Fig. 2. Cation exchange chromatography of ^{125}I*-labelled tryptic peptides of* gp70 *molecules from virus preparations as indicated.*

the recombination of M-MuLV with endogenous mouse viruses.

In contrast to gp70, the primary structure of the M-MuLV gag gene p30 products is highly conserved [7]. Since the p30 must function within a limited structural framework, high mutability may be a disadvantage. Small changes in, e.g., one out of twelve peptides may occur in p30 as well; however, Gautsch *et al.* [8] have found a functional correlation between the p30 peptide map and the Fv-1 tropism.

Our findings indicate that the peptide map of p30 may differ, depending on the organ localization of the virus. Moreover, we have found that organ-associated similarity of viral isolates applies both to the leukemic and the preleukemic tissues. This decreases the likelihood that leukemogenesis-associated recombination is responsible for the generation of the different viral subtypes. It is more likely that the original M-MuLV homogenate was heterogeneous and contained virus particles that can infect and transform different subtypes of T-cells. Such a mixture would be overlooked on peptide mapping if the ratio of splenotropic and thymotropic virus was low. The homing of the viruses to different tissues *in vivo* would separate them. Experiments with cloned M-MuLV are in progress.

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