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Characterization of monoclonal antibodies recognizing neurotropic Friend murine leukemia virus

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Abstract

We isolated a replication-competent, neurotropic retrovirus (FrC6 virus) and its molecular clone A8 from the NB-tropic Friend murine leukemia virus (FLV) complex. For detection and characterization of the FrC6 and A8 viruses, monoclonal antibodies (MAbs) against the FLV complex were established. Thirty MAbs, each of which reacted with the FLV-producing cell line, were tested for potential neutralizing activities; only two MAbs inhibited the proliferation of the A8 virus. These two MAbs were ineffective or had very weak neutralizing activities toward the non-neurotropic FLV strain clone 57 virus. Further characterization of MAbs by immunoprecipitation revealed that 4 MAbs recognized the envelope protein of the A8 virus. Two of these 4 MAbs recognized the surface glycoprotein gp70, requiring the conformational epitope of the virus for this recognition, while the other two MAbs, which were reactive with the transmembrane protein p15E, were conformation-independent. Both of the MAbs against gp70 distinguished neuropathogenic and non-neuropathogenic viruses to some extent, through neutralizing activity or binding activity detected by immunoprecipitation, whereas the two MAbs against p15E reacted with the viruses in a similar manner. Furthermore, one of the MAbs distinguished the viral antigen in the wall of the vacuolation that composes the spongiotic lesion induced by FrC6 viral infection of the brain.

Keywords: Friend murine leukemia virus; Monoclonal antibody; Neurotropism; Conformation-dependent recognition

The Friend virus complex, composed of the replication-competent Friend murine leukemia virus (FLV) and the replication-defective spleen focus-forming virus

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(SFFV), induces rapid erythroleukemia in mice (Weiss et al., 1984). FLV without SFFV is also capable of inducing leukemia after injection into newborn mice. However, FLV-induced leukemia is slower in onset than leukemia induced by the FLV complex (Steeves et al., 1971). Kai and Furuta (1984) isolated the neurotropic virus strain PVC211 from the ecotropic FLV complex (Odaka, 1973). PVC211 induced hind leg paralysis in rats. We also isolated a replication-competent neurotropic retrovirus (FrC6 virus) from the ecotropic FLV complex after several passages through newborn rat brain in vivo and adapted it to the rat glial cell line C6 in vitro. This FrC6 virus proliferated in the central nervous system (CNS) of rats in vivo as well as in the C6 cells in vitro (Takase-Yoden and Watanabe, 1994). The FrC6 virus also induced hind leg paralysis after injection into newborn rats. Neuropathological changes in infected rats were characterized by spongiotic lesions in the thalamus, brain cortex and brainstem (see Fig. 3c). We obtained the molecular clone A8 from the FrC6 virus. The biological activities of the A8 virus both in vitro and in vivo were identical to those of the FrC6 virus (manuscript in preparation).

The envelope protein of murine leukemia virus (MuLV) is a major determinant of the viral host range (Pinter and Honnen, 1983; Heard and Danos, 1991) and is composed of two polypeptides: gp70 surface glycoprotein (SU) and p15E transmembrane protein (TM) (Pinter and Fleissner, 1979; Chesebro et al., 1983). These proteins are encoded by the *env* gene and are processed from a common precursor, Pr80. For detection and characterization of the FrC6 and A8 viruses, we established anti-FLV monoclonal antibodies (MAbs) that recognized the envelope proteins of the FLV. The biological activity of these MAbs was characterized by the use of two FLV clones with different tissue tropisms: the neurotropic A8 virus and the non-neurotropic FLV clone 57 virus (Oliff et al., 1980). The 57 virus was used as a non-neuropathogenic, erythroleukemogenic wild-type virus because its biological activity has been studied extensively (Masuda et al., 1992) and its nucleotide sequence is available (Koch et al., 1983).

For generation of MAbs, mice were immunized with the persistent FLV complex-producing mouse cell line FBα, established from spleen cells of FLV complex-infected Balb/c mice. Antibody-producing hybridoma cells were screened by testing their supernatant culture fluids in an indirect immunofluorescence assay against FBα cells. Immunoglobulin isotypes of isolated MAbs were determined using the Mouse Monoclonal Sub-isotyping Kit (American Qualex International Inc.) in accordance with the manufacturer's protocol. To characterize these MAbs, we performed radio immunoprecipitation, as previously described by Kubo et al. (1993) with minor modifications (see caption of Fig. 1). Reactivity of each MAb to denatured proteins was examined by treating the lysate of cells infected with A8 virus with 4 M urea and 0.5 M 2-mercaptoethanol before immunoprecipitation. The neutralization assay was performed as previously described (Takase-Yoden and Watanabe, 1994) with the following modification: MAbs were purified from ascites with MAb Trap II (Pharmacia) in accordance with the manufacturer's protocol. Serial dilutions of each MAb were incubated with the A8 virus or the 57 virus at 2×10^2 XC-PFU (plaque-forming units) titer for 30 min at 4°C before

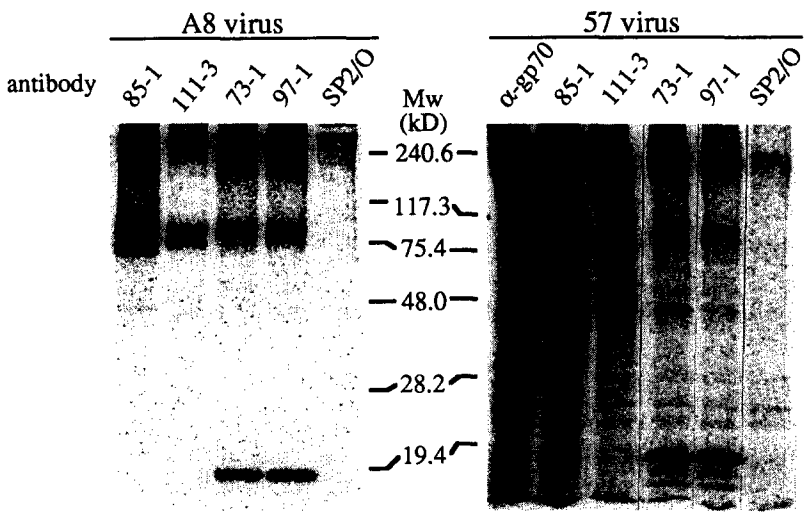

Fig. 1. Radio immunoprecipitation analysis of MAbs. Lysates prepared from NIH 3T3 cells infected with A8 virus or clone 57 virus were immunoprecipitated with a panel of MAbs, anti-Rauscher murine leukemia virus gp70 goat serum (α-gp70, kindly provided by the Division of Cancer Cause and Prevention, NCI), or ascites of mice inoculated with mouse myeloma cell line SP2/O (SP2/O). Molecular weight markers are shown in the center. NIH 3T3 cells were infected with A8 or 57 virus at an m.o.i. of 1. Three days later, the medium was replaced with DMEM containing 50 μCi [^{35}S]methionine (EXPRE^{35}S^{35}S-Protein Labeling Mix, NEN). Cells were pulse-labeled for 2 h and lysed with radio immunoprecipitation assay buffer (1% Triton X-100, 1% sodium dodecylcholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris/Cl, 10 mM EDTA; pH 7.4) containing 0.5 mM phenylmethylsulfonyl fluoride. Lysates were incubated with each MAb overnight at 4°C. Immune complexes, precipitated by the addition of protein G Sepharose 4FF (Pharmacia), were analyzed by 15% SDS–PAGE. Precipitates were visualized by use of Bio-imaging analyzer BAS-2000 (Fuji).

administration into NIH 3T3 cell cultures. Viral production was measured by recording reverse transcriptase activity in the supernatant medium of inoculated cell cultures and by the XC focus-forming assay.

We obtained 30 hybridomas that produced MAbs reactive with the FLV complex-producing cell line FBα and/or the FrC6 virus-producing cell line FrC6 (Takase-Yoden and Watanabe, 1994). These MAbs were tested for potential neutralizing activity against proliferation of the A8 and 57 viruses. Only two of the MAbs, MAb 25 and MAb 85-1, exhibited neutralizing activity. Interestingly, these two MAbs partially distinguished the A8 virus and non-neurotropic FLV strain clone 57 virus by their neutralizing activities. MAb 25, classified as an IgM, did not inhibit proliferation of the 57 virus, and 2.5 μg/ml of purified MAb 85-1 was required to exert a neutralizing effect on the 57 virus, whereas 0.1 μg/ml of this MAb was sufficient to inhibit growth of the A8 virus (Table 1). The fact that only a small percentage of the isolated MAbs exhibited neutralizing activity is consistent with previous reports. McAtee and Portis (1985) reported that virus neutralization capacity was detected in only two of the 22 tested *env*-specific MAbs, and

Table 1
Biological activity of anti-FLV MAbs

Group	MAb	IgG class	Virus specificity [a]	Protein specificity [a]	Reactivity to denatured protein [b]	Neutralization titer [c]	
						A8	57
I	85-1	IgG2b	A8, 57	gp70	−	4000	160
	111-3	IgG1	A8	gp70	−	< 160	< 160
II	73-1	IgG1	A8, 57	p15E	+	< 160	< 160
	97-1	IgG1	A8, 57	p15E	+	< 160	< 160

[a] Specificity of MAbs determined by immunoprecipitation (see Fig. 1).
[b] Reactivity of MAbs to urea-treated lysate (see Fig. 2).
[c] Data shown are reciprocals of purified antibody dilutions (0.4 mg/ml) that yielded a 50% reduction in XC-PFU titer.

Nowinski et al. (1981) detected this capacity in only one of the 9 MAbs tested. These findings indicate a rather low population of neutralizing antibody-producing cells in animals infected by a viable virus or immunized with a whole virus instead of a fragmented viral protein or peptide (Pinter et al., 1986).

Further characterization by immunoprecipitation of MAbs classified as IgG revealed that 4 MAbs recognized the envelope protein of the A8 virus (Fig. 1). These 4 MAbs were classified into two groups according to the specificity of their protein recognition (Table 1). Group I MAbs (85-1 and 111-3) reacted with the SU protein gp70, and group II MAbs (73-1 and 97-1) recognized the TM protein p15E. All of these MAbs precipitated from the precursor protein Pr80 (Fig. 1), and did not react with mock-infected cells (data not shown). As shown in Fig. 1, MAb 111-3 distinguished the two FLV clones.

These results indicate that some of the gp70 epitopes of the two virus clones were different. This conclusion was supported by analysis and comparison of the nucleotide sequences of clones A8 and 57. We found 24 points of discrepancy in the putative amino acid sequences of the two clones in the gp70 region, whereas only one difference was revealed in the p15E region, which was located at the carboxyl terminal (manuscript in preparation). In previous reports, group-specific MAbs, reactive with several members of each type of MuLV, have been directed to viral core proteins (Chesebro et al., 1981, 1983) or to TM protein of MuLVs (Lostrom et al., 1979; Nowinski et al., 1979), whereas most type-specific MAbs have been directed to SU protein (Lostrom et al., 1979; Chesebro et al., 1981, 1983; Portis et al., 1982; Portis and McAtee, 1983; McAtee and Portis, 1985). Our results confirm these reports. As shown in Fig. 1, MAb 111-3, which distinguished the A8 virus from the 57 virus, precipitated SU protein, and MAb reacted with TM protein precipitated both viral clones in a similar manner.

It has been unclear whether conformational epitopes exist in the envelope proteins of MuLV. Therefore, we examined the reactivity of our MAbs against denatured proteins. Immunoprecipitation of urea-treated lysate revealed that group I MAbs (85-1 and 111-3) failed to react with the denatured proteins, whereas group II MAbs (73-1 and 97-1) recognized denatured Pr80 and p15E as well as the corresponding untreated proteins (Fig. 2). This indicates that MAb 85-1

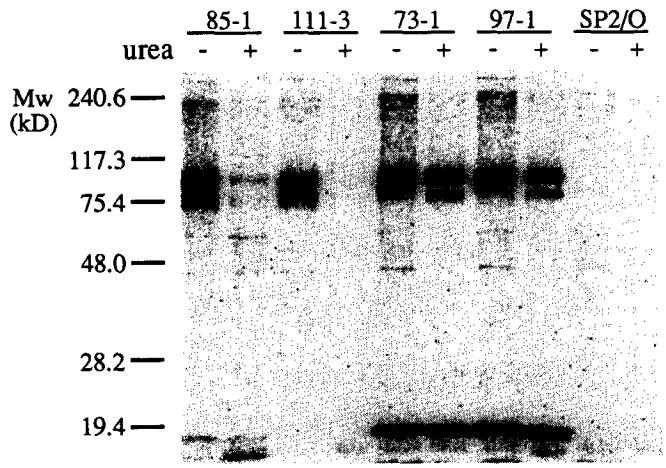

Fig. 2. MAb recognizing conformational epitope. Cell lysate infected with A8 virus was treated (+) or not treated (−) with urea and 2-mercaptoethanol, and immunoprecipitated with a panel of MAbs. Molecular weight markers are indicated on the left.

and 111-3 recognized the conformational epitope(s) of the FLV gp70. It follows that in the present study, MAbs that distinguished between the two FLV clones through neutralizing or binding activity recognized conformational epitopes, whereas MAbs that reacted with both clones also recognized denatured proteins.

It has also been unclear whether conformation of viral protein is required for recognition of MuLV by neutralizing MAbs generated by the immunization of mice with whole viral particles or untreated viral protein without fragmentation. For simian immunodeficiency virus (Javaherian et al., 1994) and murine coronavirus (Kubo et al., 1993), it has been reported that a conformational epitope is required for recognition by neutralizing MAbs. The present study demonstrates that a neutralizing antibody recognized a conformational epitope of the FLV gp70 (Table 1). Furthermore, in addition to the rather low quantity of neutralizing antibody-producing cells in the spleen cell population, a conformational change in the viral envelope protein, possibly due to a point mutation, might allow a retrovirus persistent infectivity in vivo by natural infection because the main neutralizing antibody raised in vivo might be conformation-dependent. If neutralizing antibodies were to recognize the peptide of fragmented viral protein, point mutations occurring at the only restricted point would rescue the potent infectivity of mutant virus in vivo. Conformational change can occur after mutation at any other position.

Immunohistochemical methods showed that all of the MAbs reactive with the envelope protein could detect the viral antigen in the brains of rats infected at birth with the FrC6 virus. MAb 85-1 was the most efficient MAb for the detection of the viral antigen, especially in materials embedded in paraffin (Fig. 3). The viral antigen was found in glial-like cells (Fig. 3b) as well as in the vascular wall (Fig.

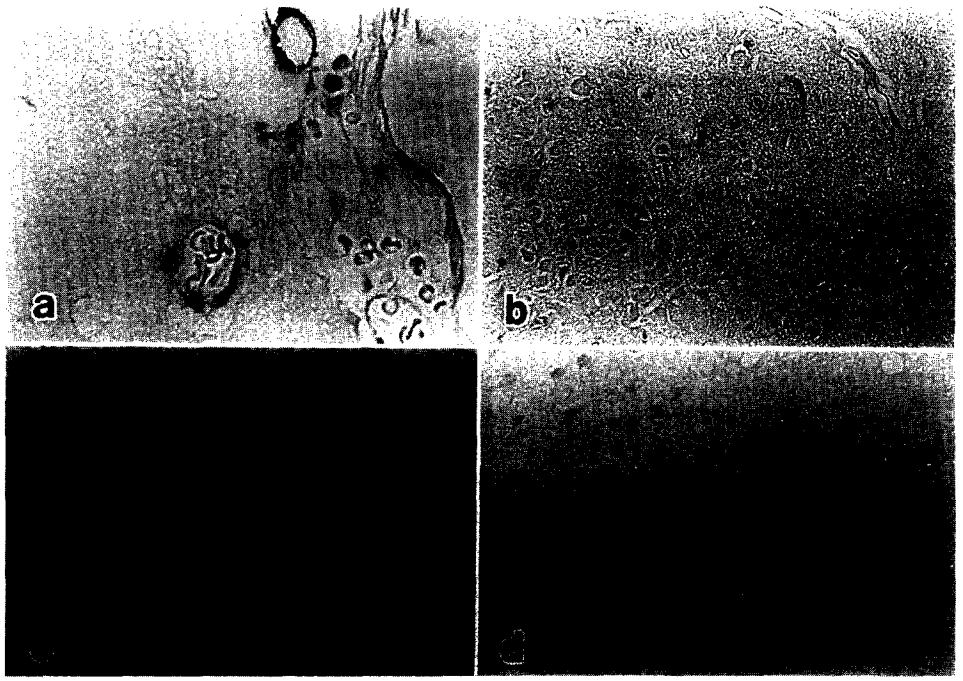

Fig. 3. Immunohistochemical detection of FrC6 viral antigen. Viral antigen was detected in vascular wall (a), glial-like cells (b), and wall of vacuolation composing spongiotic lesion (c, arrow head). d: control. Magnification: a, ×600; b, ×150; c and d, ×300. Rat infected with FrC6 virus was perfused with 4% paraformaldehyde (Merck) in 0.12 M phosphate buffer of pH 7.3 via the left ventricle of the heart after deep anesthesia with Nembutal. Brain was embedded in paraffin, and sections were prepared. Glass slides were dipped in phosphate-buffered saline (PBS, pH 7.2) containing 0.1% fetal calf serum followed by application of blocking sera containing PBS, 45% normal horse serum (Hatzelton), and 5% normal swine serum (Dako). FLV antigen was detected by application of 10 μg/ml purified MAb 85-1 followed by biotinylated sheep anti-mouse IgG (Amersham) and then by avidin–peroxidase complex (Wako). Between each step, glass slides were washed with PBS. For peroxidase reaction, 0.1 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (DOTITE) in 0.1 M Tris buffer (pH 7.6) was used. For the control, normal mouse serum was applied instead of MAb 85-1.

3a). Interestingly, MAb 85-1 detected the viral antigen in the walls of vacuoles that compose the characteristic spongiotic lesions induced by FrC6 virus infection (Fig. 3c). This indicates that spongiotic changes are produced by direct infection of the cell by the virus, leading to degeneration and vacuolation. The molecularly cloned infectious virus of PVC 211 was shown to infect endothelial cells of the CNS both in vitro and in vivo (Hoffman et al., 1992). The neurovirulent MLV Cas-Br-E strain has been detected in neurons, oligodendrocytes, and endothelial cells of the CNS (Morey et al., 1990; Nagra et al., 1992). The FrC6 virus antigen in the CNS localized in different cell lineages than other neurotropic MLVs.

We are now conducting experiments in order to determine which amino acid replacement is responsible for constructing the conformation of gp70 for antibody

recognition, using a chimera virus derived from the A8 and 57 viral genes. These analyses should clarify the mechanism(s) of A8 virus infection of neuronal cells.

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