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Analysis of two monoclonal antibodies reactive with envelope proteins of murine retroviruses: One pan specific antibody and one specific for Moloney leukemia virus

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A B S T R A C T

Many monoclonal antibodies (MAbs) reactive with various proteins of murine leukemia viruses (MuLVs) have been developed. In this report two additional MAbs with differing and unusual specificities are described. MAb 573 is reactive with the envelope protein of all MuLVs tested including viruses in the ecotropic, xenotropic, polytropic and amphotropic classes. Notably, MAb 573 is one of only two reported MAbs that react with the envelope protein of amphotropic MuLVs. This MAb appears to recognize a conformational epitope within the envelope protein, as it reacts strongly with live virus and live infected cells, but does not react with formalin-fixed or alcohol-fixed infected cells or denatured viral envelope protein in immunoblots. In contrast, Mab 538 reacts only with an epitope unique to the envelope protein of the Moloney (Mo-) strain of MuLV, a prototypic ecotropic MuLV that is the basis for many retroviral tools used in molecular biology. MAb 538 can react with live cells and viruses, or detergent denatured or fixed envelope protein. The derivation of these antibodies as well as their characterization with regard to their isotype, range of reactivity with different MuLVs and utility in different immunological procedures are described in this study.

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1. Introduction

Monoclonal antibodies have proved to be invaluable for numerous investigations of MuLVs. During the course of previous studies many antibodies have been derived and characterized which have been used extensively (Chesebro et al., 1981, 1983; Cloyd et al., 1979, 1982; Evans et al., 1990; Portis et al., 1982; Robertson et al., 1991). Among these are antibodies to various core Gag-proteins as well as antibodies to the Env proteins of MuLVs. Some of the antibodies have been shown to react with the Env proteins of distinct classes of MuLVs such as xenotropic, polytropic, or ecotropic MuLVs; others with subclasses of MuLVs such as modified polytropic MuLVs (Lavignon et al., 1994) and still others that react very specifically with particular strains of MuLVs (Chesebro et al., 1981). MAbs that distinguish different types of MuLVs are useful to quantify particular MuLVs in complex virus mixtures (Evans and Britt, 1983; Sitbon et al., 1985). One of the antibodies that has been used extensively is MAb 83A25, a rat IgG2A antibody that recognizes an epitope on the carboxyl terminus of nearly all MuLV envelope SU proteins with the exception of MuLVs of the Friend (Fr-MuLV) and Rausher (R-MuLV) substrains (Evans et al., 1990). This antibody has been used to detect retrovirus infection of in vitro cell lines (Hartley et al., 2008; Yu et al., 2012), to detect the expression of endogenous viruses in mice (Young et al., 2012) to monitor virus production in gene therapy experiments (Donahue et al., 1992; Crooks and Kohn, 1993; Valsesia-Wittmann et al., 1996) and to study the role of the carboxyl-terminal region of SU in membrane fusion leading to infection (Burkhart et al., 2005).

Two additional MAbs that exhibit unique reactivity's with MuLVs are described in this study. One of the antibodies, MAb 573, reacts with all MuLVs tested and can be used to monitor retrovirus infection of cell lines and to reliably quantify MuLV stocks or identify MuLVs derived from infected animals. A second antibody, MAb 538, reacts specifically with Mo-MuLV, which is a prototypic MuLV that has been studied extensively and is the basis of numerous retroviral packaging cell lines (Miller, 1990), murine retroviral vectors (Soneoka et al., 1995; Valsesia-Wittmann et al., 1996; Miller, 2001) as well as commercial molecular biology products.

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2. Materials and methods

2.1. Derivation of hybridoma cell lines

Hybridoma 573 was generated after injection of an adult (C57B10 × A.BY)F_1 mouse by intravenous (i.v.) inoculation of 5×10^7 spleen cells from the grossly enlarged spleen of a (BALB/c × A/J)F_1 mouse infected previously with a Friend spleen focus-forming virus (SFFV) complex consisting of the replication-defective SFFV virus and the replication-competent Mo-MuLV. Thirteen days after immunization, spleen cells of the immunized mouse were removed, dissociated and fused to NS1 cells to generate hybridomas as described previously (Chesebro et al., 1981).

Hybridoma 538 was generated after injection of an adult (B10.AxA/WySn)F_1 mouse by i.v. inoculation with tissue culture medium containing the Mo-MuLV/SFFV complex. 75 days later this mouse received an i.v. inoculation of 3×10^7 spleen cells from the enlarged leukemic spleen of a (BALB/c × A/J)F, mouse infected previously with the Mo-MuLV/SFFV complex. 16 days after the booster inoculation, the spleen cells of the immunized mouse were fused to NS1 cells to generate hybridomas as described (Chesebro et al., 1981).

2.2. Detection and of MuLV-reactive antibodies and identification of antibody classes

Identification of wells containing MuLV-reactive hybridoma MAbs was accomplished by indirect membrane immunofluorescence assays using trypsinized virus-infected cells (Chesebro et al., 1981). Both antibodies (MAbs 538 and 573) were found to be of the lgM class by agar immunodiffusion using antisera reactive with individual mouse immunoglobulin isotypes (Chesebro et al., 1981).

2.3. Determination of viral strain and viral protein specificity

The reactivities of the MAbs to different strains of MuLVs were determined using assays of cell-surface fluorescence of M. dunni cells (Lander and Chattopadhyay, 1984) infected with a variety of individual murine retroviruses (Chesebro et al., 1981). The MuLVs used in these studies are described in the citations included in Tables 1 and 2.

The identity of viral proteins recognized by mAb 538 was deduced by comparisons of reactive and non-reactive MuLV of known structure. To determine which viral proteins were recognized by MAb 573, supernatant fluids were tested by membrane immunofluorescence against plaques induced by various recombinant vaccinia viruses in CV-1 monkey cells including vaccinia virus expressing the Fr-MuLV Gag protein (Miyazawa et al., 1992), the Fr-MuLV Env protein (Earl et al., 1986), the HIV (strain lll B) or the influenza hemagglutinin protein (Chesebro and Wehrly, 1988).

2.4. Flow cytometry and infectivity assays

Analysis of cell-surface fluorescence by flow cytometry was performed as described previously (Butchi et al., 2010). Virus titers were assessed by a focal immunofluorescence assay (FIA) (Sitbon et al., 1985).

3. Results

3.1. Reactivities of MAbs 573 and 538 with MuLVs

The infected cells used for identification of the reactive MAbs included cells infected with Fr-MuLV as well as cells infected with Mo-MuLV. It was noted in the initial assays that hybridoma 538 released a MAb (MAb 538) that was reactive with Mo-MuLV-infected cells, but not with Fr-MuLV-infected cells, indicating that these two closely related ecotropic MuLVs could be distinguished by this MAb. In contrast, hybridoma 573 released a MAb (MAb 573) that reacted with both Fr- and Mo-MuLV-infected cells indicating a broader reactivity.

The reactivities of the MAbs were subsequently tested against an extensive array of MuLVs that included many members of the ecotropic, amphotropic, xenotropic and polytropic classes. MAb 573 was found to react with all the MuLVs tested (Tables 1 and 2). In contrast, MAb 538 reacted only with Mo-MuLV infected

Table 1
Reactivity of MAbs 538, 573 with ecotropic, amphotropic and xenotropic MuLVs.

Class	Strain	MAb		Reference
		538	573	
Ecotropic	Fr-MuLV[a]	–	+	
	R-MuLV	–	+	Rauscher (1962)
	AKR 2A	–	+	Cloyd et al. (1979)
	Mo-MuLV 8.2	+	+	Shoemaker et al. (1980)
	Mo-MuLV 1387	+	+	Evans and Cloyd (1984)
	WN1802N	–	+	Cloyd et al. (1980)
	CasBr-E	–	+	Hartley and Rowe (1976)
	1504E	–	+	Hartley and Rowe (1976)
Xenotropic[b]	AKR 6	–	+	Cloyd et al. (1979)
	NIH AT124	–	+	Todaro et al. (1973)
	Balb lU-1	–	+	Hartley and Rowe (1976)
	C58L1 xeno	–	+	Chattopadhyay et al. (1981)
	Cas E no. 1	–	+	Cloyd et al. (1979)
	NFS-Th1	–	+	Chattopadhyay et al. (1981)
	XMRV VP62	–	+	Urisman et al. (2006)
	NZB-8882	–	+	Rein and Schultz (1984)
	NZB Cl. 35	–	+	Levy et al. (1975)
Amphotropic[b]	1504A	–	+	Hartley and Rowe (1976)
	4070A	–	+	Hartley and Rowe (1976)

[a] Nine strains of Fr-MuLV (Chesebro et al., 1983; Mathieu-Mahul et al., 1982; Oliff et al., 1980; Ostertag and Pragnell, 1978; Pragnell et al., 1978; Sitbon et al., 1986) were tested and found to be reactive with MAb 573 but not with Mab 538.

[b] Xenotropic and amphotropic MuLVs were tested on both M. dunni- (Lander and Chattopadhyay, 1984) and Mv1Lu mink lung fibroblasts (ATCC CCL64) (Henderson et al., 1974).