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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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Abstract

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.

Keywords

Monoclonal antibodies; Retroviruses; Env protein; Moloney MuLV; Amphotropic MuLV

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; Chesebro et al., 1983; Cloyd et al., 1979; Cloyd et al., 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

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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 MuLVs 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.

2. MATERIALS AND METHODS

2.1 Derivation of hybridoma cell lines

Hybridoma 573 was generated after injection of an adult (C57B10 $\times$ A.BY)F₁ mouse by intravenous (i.v.) inoculation of 5×10^7 spleen cells from the grossly enlarged spleen of a (BALB/c $\times$ A/J)F₁ 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.A $\times$ A/WySn)F₁ 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 $\times$ A/J)F₁ 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 IgM 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 III 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 of the MuLVs tested (Tables 1 and 2). In contrast, MAb 538 reacted only with Mo-MuLV infected cells (Table 1), and in one case with a recombinant polytropic MuLV (M-RV 14A). This recombinant virus was generated by substitution of a portion of the gene sequences encoding the SU protein of Mo-MuLV with analogous sequences of an endogenous mouse retrovirus (Figure 1) (Alamgir et al., 2005; Evans and Cloyd, 1985).

3.2. Identification of a region of the Mo-MuLV Env protein reactive with MAb 538

The observation that MAb 538 reacted with only one of the polytropic recombinant viruses in which a portion of the Mo-MuLV Env protein has been substituted with a polytropic Env suggests that this antibody reacts within a region of the Mo-MuLV Env protein that is retained in the recombinant polytropic MuLV, M-RV 14A. Sequence analyses of many Mo-MuLV recombinant viruses allowed a more precise definition of a region of the Mo-MuLV Env protein that contained a determinant of the epitope recognized by MAb 538 (Alamgir et al., 2005) (Fig. 1). The polytropic MuLV, M-RV 14A, in contrast to the non-reactive polytropic viruses, retained the entire proline-rich region (PRR) of the SU region of the Env protein and 1 additional carboxyl-terminal amino acid (Fig.1). Thus a determinant of MAb 538 reactivity appeared to reside in this region. The amino-terminal region of the Mo-MuLV PRR consists of a conserved region of 15 amino acids and a variable region of 31 amino acids (Fig. 1) (Wu et al., 2000). The conserved PRR region of Mo-MuLV is identical to a

Fr-MuLV (Oliff et al., 1980) that does not react with MAb 538, thus it is likely that the epitope for MAb 538 resides within a 32 amino-acid sequence (246-276) that encompasses the variable PRR region (Fig. 1).

3.3. Reactivity of MAb 573 with the Env proteins of MuLVs

The observation that MAb 573 is reactive with all MuLVs by cell surface fluorescence precluded the identification of the reactive MuLV protein by comparison of reactive and non-reactive MuLV as was done for MAb 538. Cells infected with MuLVs exhibit Env proteins as well as Gag proteins on their cell surface, thus it was not clear which of these proteins was the reactive target of MAb 573. This issue was resolved by examining the reactivity of MAb 573 with vaccinia viruses expressing different retroviral proteins. The results of these analyses indicated that MAb 573 reacted with vaccinia expressing the Env protein of Fr-MuLV but not with vaccinia expressing the core (gag) polyprotein of Fr-MuLV (Table 3). Furthermore, these antibodies did not react with the plaques of other vaccinia viruses expressing the Env protein of HIV (strain Ill B) or the influenza hemagglutinin (HA) protein. Thus, MAb 573 reacts with MuLV Env proteins.

3.4. Utility of the MAbs for flow cytometry and virus titrations

The reactivity of the MAbs to cell-surface SU proteins is apparent in flow cytometry analyses of infected cells (Fig. 2). Shown is a flow cytometry analysis of cells infected with the xenotropic MuLV Cas E no. 1, the amphotropic MuLV 4070 A, the ecotropic Fr-MuLV and the polytropic MuLV Fr98 using MAb 573 (Fig. 2A). Also shown is the analysis of cells infected with Mo-MuLV using MAb 538 (Fig. 2B). Furthermore both MAb 538 and 573 have been used for infectivity assays using the FIA that detects cell surface fluorescence of infected cells (Sitbon et al., 1985). Both MAbs gave similar results for Mo-MuLV (data not shown).

3.5 Utility of the MAbs for immunoblotting procedures

MAbs 573 and 538 are both of the IgM class. IgM antibodies are frequently reactive with conformational antigens that may not be suitable for immunoblotting procedures. While the Env determinants of the epitope reactive with MAb 573 are not closely defined, a major determinant of the MAb 538 epitope resides in a relatively short sequence corresponding to the variable portion of the PPR region of the SU protein and might correspond to a non-conformational epitope. Both MAbs were tested for their ability of detect MuLV SU proteins by immunoblotting. MAb 573 was unable to detect the SU proteins of several MuLVs which are reactive with the antibody when expressed on live infected cells (data not shown). In contrast, by immunoblotting, MAb 538 specifically detected the SU protein of Mo-MuLV but not of the closely related ecotropic Fr-MuLV nor of the Mo-MuLV-derived polytropic MuLV A383-5S (Fig. 3). The ability to specifically detect the Mo-MuLV SU protein may prove useful in experiments in which Mo-MuLV is present in mixed virus infections.

3.6 Virus neutralization

We have reported a number of monoclonal antibodies that are capable of neutralization of murine retroviruses including MAb 83A25 that is reactive with most MuLVs but not with the Friend or Rauscher ecotropic MuLV strains (Evans and Britt, 1983). We found that tissue culture supernatant containing MAb 573 was capable of neutralizing Fr-MuLV as well as Mo-MuLV (Fig. 4A). Tissue culture supernatant containing MAb 538 neutralized Mo-MuLV but not Fr-MuLV further illustrating the specificity of this antibody by this technique (Fig 4B).

4. DISCUSSION

The MAbs described here should prove useful for a number of applications. MAb 538 specifically reacts with Mo-MuLV which has been the prototypic MuLV for the development of retroviral vectors for gene therapy as well as for the study of MuLV structure and function. MAb 538 has been used in studies describing production of Mo-MuLV in the RAW264.7 cell line, the most commonly used macrophage mouse cell line in medical research (Hartley et al., 2008) and also in studies describing a stepwise mechanism of lymphocytic leukemia induction by Mo-MuLV (Lavignon and Evans, 1996). MAb 538 is useful for the quantification of Mo-MuLV in mixtures of MuLVs (Lavignon and Evans, 1996; Hartley et al., 2008) as well as determining the infectious titer of purified Mo-MuLV stocks. Furthermore, the identification of the PRR region of the Mo-MuLV Env protein as the region which contains determinants of reactivity to MAb 538 and the ability to detect the protein by immunoblotting procedures may facilitate structural studies of the Mo-MuLV Env protein.

Most of the MAbs developed in this laboratory have exhibited neutralizing activity only after the addition of serum complement (Chesebro et al., 1981). It is noted that both MAbs 538 and 573 exhibited significant neutralization of viruses without the addition of complement or without concentration of the MAbs from the tissue culture supernatant. The ability to neutralize a wide variety of MuLVs expands the usefulness of these antibodies.

MAb 573 is useful for the titration of all purified retroviral stocks tested including members of the ecotropic, xenotropic, amphotropic and polytropic MuLV classes. The MAb does not react with denatured Env protein and very likely corresponds to a conformational epitope. The observation that it reacts with all MuLVs tested suggests that it reacts with a broadly conserved tertiary or quaternary structure of MuLV Env proteins. The precise identification of such a conserved structure would be of great interest. In contrast to MAb 83A25, which does not react with Fr- or R-MuLVs (Evans et al., 1990), MAb 573 reacts with the env proteins of all MuLVs tested and facilitates routine screening of cells from tissues or tissue culture cells for infection by MuLVs. Among the MAbs described previously that are reactive with MuLV env proteins, only MAb 83A25 is reactive with the env protein of amphotropic MuLVs (Evans et al., 1990). As noted, MAb 573 also reacts with amphotropic MuLVs but with an intensity of cell surface fluorescence higher than with MAb 83A25 (Fig 5). Amphotropic MuLVs are capable of infecting human cells and have been utilized in a number of clinical gene therapy applications (Hasegawa et al., 1993; Purcell et al., 1996; Ram et al., 1997; Kang and Malech, 2012). The ability to monitor the replication of viruses expressing the env proteins of amphotropic MuLVs is of utmost importance in such applications and should be greatly facilitated using MAb 573.

4.1. CONCLUSIONS

This report describes two new MAbs to MuLV Env proteins that exhibit unique properties and provide valuable reagents to facilitate research in a number of areas. Notably, MAb 573 provides a means of detecting infectious MuLVs in substrates used for vaccine development, in clinical gene therapy applications, in mouse strains and tissues and in murine cell cultures maintained in the laboratory. In this regard, myeloma cells used to generate MAbs as well as the hybridoma cells maintained in various laboratories produce infectious MuLVs (Deo et al., 1994).

MAb 538 reacts with the PRR region of the Env protein of Mo-MuLV and is useful for immunoblotting experiments and assays of Mo-MuLV in complex virus mixtures. MAb 538 is the first reported MAb that reacts specifically with Mo-MuLV, a virus that has been utilized extensively in studies of murine retrovirology.

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Reference List

- Alamgir AS, Owens N, Lavignon M, Malik F, Evans LH. Precise identification of endogenous proviruses of NFS/N mice participating in recombination with moloney ecotropic murine leukemia virus (MuLV) to generate polytropic MuLVs. *J Virol.* 2005; 79:4664–4671. [PubMed: 15795252]
- Burkhardt MD, D'Agostino P, Kayman SC, Pinter A. Involvement of the C-terminal disulfide-bonded loop of murine leukemia virus SU protein in a postbinding step critical for viral entry. *J.Virol.* 2005; 79:7868–7876. [PubMed: 15919941]
- Butchi NB, Du M, Peterson KE. Interactions between TLR7 and TLR9 agonists and receptors regulate innate immune responses by astrocytes and microglia. *Glia.* 2010; 58:650–664. [PubMed: 19998480]
- Chattopadhyay SK, Lander MR, Gupta S, Rands E, Lowy DR. Origin of mink cytopathic focus-forming (MCF) viruses: comparison with ecotropic and xenotropic murine leukemia virus genomes. *Virology.* 1981; 113:465–483. [PubMed: 6267794]
- Chesebro B, Britt W, Evans L, Wehrly K, Nishio J, Cloyd M. Characterization of monoclonal antibodies reactive with murine leukemia viruses: use in analysis of strains of friend MCF and Friend ecotropic murine leukemia virus. *Virology.* 1983; 127:134–148. [PubMed: 6305011]
- Chesebro B, Wehrly K. Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J.Virol.* 1988; 62:3779–3788. [PubMed: 3047430]
- Chesebro B, Wehrly K, Cloyd M, Britt W, Portis J, Collins J, Nishio J. Characterization of mouse monoclonal antibodies specific for Friend murine leukemia virus-induced erythroleukemia cells: friend-specific and FMR-specific antigens. *Virology.* 1981; 112:131–144. [PubMed: 6787798]
- Cloyd MW, Chesebro B, Portis JL, Weir M. MCF-specific murine monoclonal antibodies made against AKR-247 MCF virus recognize a unique determinant associated with the gp70-p-15(E) complex. *J.Virol.* 1982; 41:1112–1117. [PubMed: 6178839]
- Cloyd MW, Hartley JW, Rowe WP. Cell-surface antigens associated with recombinant mink cell focus-inducing murine leukemia viruses. *J.Exp.Med.* 1979; 149:702–712. [PubMed: 85682]
- Cloyd MW, Hartley JW, Rowe WP. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. *J.Exp.Med.* 1980; 151:542–552. [PubMed: 6244357]
- Crooks GM, Kohn DB. Growth factors increase amphotropic retrovirus binding to human CD34+ bone marrow progenitor cells. *Blood.* 1993; 82:3290–3297. [PubMed: 7694678]
- Deo Y, Ghebremariam H, Cloyd M. Detection and characterization of murine ecotropic recombinant virus in myeloma and hybridoma cells. *Hybridoma.* 1994; 13:69–76. [PubMed: 8200661]
- Donahue RE, Kessler SW, Bodine D, McDonagh K, Dunbar C, Goodman S, Agricola B, Byrne E, Raffeld M, Moen R. Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. *J Exp Med.* 1992; 176:1125–1135. [PubMed: 1383375]
- Earl PL, Moss B, Morrison RP, Wehrly K, Nishio J, Chesebro B. T-lymphocyte priming and protection against Friend leukemia by vaccinia-retrovirus env gene recombinant. *Science.* 1986; 234:728–731. [PubMed: 3490689]
- Evans LH, Britt WJ. An immunological focus assay for murine leukemia viruses on viable attached cell lines. *Virology.* 1983; 129:212–217. [PubMed: 6310879]
- Evans LH, Cloyd MW. Generation of mink cell focus-forming viruses by Friend murine leukemia virus: recombination with specific endogenous proviral sequences. *J.Virol.* 1984; 49:772–781. [PubMed: 6422051]
- Evans LH, Cloyd MW. Friend and Moloney murine leukemia viruses specifically recombine with different endogenous retroviral sequences to generate mink cell focus-forming viruses. *Proc.Natl.Acad.Sci.U.S.A.* 1985; 82:459–463. [PubMed: 2578666]

- Evans LH, Malik FG. Class II polytropic murine leukemia viruses (MuLVs) of AKR/J mice: possible role in the generation of class I oncogenic polytropic MuLVs. *J. Virol.* 1987; 61:1882–1892. [PubMed: 3033319]
- Evans LH, Morrison RP, Malik FG, Portis J, Britt WJ. A neutralizable epitope common to the envelope glycoproteins of ecotropic, polytropic, xenotropic, and amphotropic murine leukemia viruses. *J. Virol.* 1990; 64:6176–6183. [PubMed: 1700832]
- Fischinger PJ, Nomura S, Bolognesi DP. A novel murine oncornavirus with dual eco- and xenotropic properties. *Proc. Natl. Acad. Sci. U.S.A.* 1975; 72:5150–5155. [PubMed: 1061100]
- Hartley JW, Evans LH, Green KY, Naghashfar Z, Macias AR, Zerfas PM, Ward JM. Expression of infectious murine leukemia viruses by RAW264.7 cells, a potential complication for studies with a widely used mouse macrophage cell line. *Retrovirology.* 2008; 5:1. [PubMed: 18177500]
- Hartley JW, Rowe WP. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. *J. Virol.* 1976; 19:19–25. [PubMed: 59816]
- Hartley JW, Wolford NK, Old LJ, Rowe WP. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 1977; 74:789–792. [PubMed: 191826]
- Hasegawa Y, Emi N, Shimokata K, Abe A, Kawabe T, Hasegawa T, Kirioka T, Saito H. Gene transfer of herpes simplex virus type I thymidine kinase gene as a drug sensitivity gene into human lung cancer cell lines using retroviral vectors. *Am J Respir Cell Mol Biol.* 1993; 8:655–661. [PubMed: 8391827]
- Henderson IC, Lieber MM, Todaro GJ. Mink cell line Mv 1 Lu (CCL 64). Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. *Virology.* 1974; 60:282–287. [PubMed: 4366800]
- Kang EM, Malech HL. Gene therapy for chronic granulomatous disease. *Methods Enzymol.* 2012; 507:125–154. [PubMed: 22365772]
- Lander MR, Chattopadhyay SK. A Mus dunni cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ectropic, amphotropic, xenotropic, and mink cell focus-forming viruses. *J. Virol.* 1984; 52:695–698. [PubMed: 6092693]
- Lavignon M, Evans L. A multistep process of leukemogenesis in Moloney murine leukemia virus-infected mice that is modulated by retroviral pseudotyping and interference. *J. Virol.* 1996; 70:3852–3862. [PubMed: 8648721]
- Lavignon M, Walker JL, Perryman SM, Malik FG, Khan AS, Theodore TS, Evans LH. Characterization of epitopes defining two major subclasses of polytropic murine leukemia viruses (MuLVs) which are differentially expressed in mice infected with different ecotropic MuLVs. *J. Virol.* 1994; 68:5194–5203. [PubMed: 7518532]
- Levy JA, Kazan P, Varnier O, Kleiman H. Murine xenotropic type C viruses I. Distribution and further characterization of the virus in NZB mice. *J. Virol.* 1975; 16:844–853. [PubMed: 170419]
- Mathieu-Mahul D, Heard JM, Fichelson S, Gisselbrecht S, Sola B, Larsen CJ. Viral expression in two myelomonocytic cell lines obtained from long-term bone marrow culture infected with the Friend polycythemia-inducing virus (FV-P). *Virology.* 1982; 119:59–67. [PubMed: 7072154]
- Miller AD. Retrovirus packaging cells. *Hum. Gene Ther.* 1990; 1:5–14. [PubMed: 2081186]
- Miller AD. Production of retroviral vectors. *Curr. Protoc. Hum. Genet.* 2001 Chapter 12, Unit.
- Miyazawa M, Nishio J, Chesebro B. Protection against Friend retrovirus-induced leukemia by recombinant vaccinia viruses expressing the gag gene. *J. Virol.* 1992; 66:4497–4507. [PubMed: 1534853]
- Oliff AI, Hager GL, Chang EH, Scolnick EM, Chan HW, Lowy DR. Transfection of molecularly cloned Friend murine leukemia virus DNA yields a highly leukemogenic helper-independent type C virus. *J. Virol.* 1980; 33:475–486. [PubMed: 6245244]
- Ostertag W, Pragnell IB. Changes in genome composition of the Friend virus complex in erythroleukemia cells during the course of differentiation induced by dimethyl sulfoxide. *Proc. Natl. Acad. Sci. U.S.A.* 1978; 75:3278–3282. [PubMed: 277925]
- Portis JL, McAtee FJ, Cloyd MW. Monoclonal antibodies to xenotropic and MCF murine leukemia viruses derived during the graft-versus-host reaction. *Virology.* 1982; 118:181–190. [PubMed: 6281969]

- Pragnell IB, McNab A, Harrison PR, Osterag W. Are spleen focus-forming virus sequences related to xenotropic viruses and expressed specifically in normal erythroid cells? *Nature*. 1978; 272:456–458. [PubMed: 204875]
- Purcell DF, Brosius CM, Vanin EF, Buckler CE, Nienhuis AW, Martin MA. An array of murine leukemia virus-related elements is transmitted and expressed in a primate recipient of retroviral gene transfer. *J Virol*. 1996; 70:887–897. [PubMed: 8551628]
- Ram Z, Culver KW, Oshiro EM, Viola JJ, DeVroom HL, Otto E, Long Z, Chiang Y, McGarrity GJ, Muul LM, Katz D, Blaese RM, Oldfield EH. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat Med*. 1997; 3:1354–1361. [PubMed: 9396605]
- Rauscher FJ. A virus-induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia. *J.Natl.Cancer Inst*. 1962; 29:515–543. [PubMed: 14490596]
- Rein A, Schultz A. Different recombinant murine leukemia viruses use different cell surface receptors. *Virology*. 1984; 136:144–152. [PubMed: 6330990]
- Robertson MN, Miyazawa M, Mori S, Caughey B, Evans LH, Hayes SF, Chesebro B. Production of monoclonal antibodies reactive with a denatured form of the Friend murine leukemia virus gp70 envelope protein: use in a focal infectivity assay, immunohistochemical studies, electron microscopy and western blotting. *J.Virol.Methods*. 1991; 34:255–271. [PubMed: 1744218]
- Shoemaker C, Goff S, Gilboa E, Paskind M, Mitra SW, Baltimore D. Structure of a cloned circular Moloney murine leukemia virus DNA molecule containing an inverted segment: implications for retrovirus integration. *Proc.Natl.Acad.Sci.U.S.A*. 1980; 77:3932–3936. [PubMed: 6449003]
- Sitbon M, Nishio J, Wehrly K, Lodmell D, Chesebro B. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. *Virology*. 1985; 141:110–118. [PubMed: 2983493]
- Sitbon M, Sola B, Evans L, Nishio J, Hayes SF, Nathanson K, Garon CF, Chesebro B. Hemolytic anemia and erythroleukemia, two distinct pathogenic effects of Friend MuLV: mapping of the effects to different regions of the viral genome. *Cell*. 1986; 47:851–859. [PubMed: 3465451]
- Soneoka Y, Cannon PM, Ramsdale EE, Griffiths JC, Romano G, Kingsman SM, Kingsman AJ. A transient three-plasmid expression system for the production of high titer retroviral vectors. *Nucleic Acids Res*. 1995; 23:628–633. [PubMed: 7899083]
- Todaro GJ, Arnstein P, Parks WP, Lennette EH, Huebner RJ. A type-C virus in human rhabdomyosarcoma cells after inoculation into NIH Swiss mice treated with antithymocyte serum. *Proc.Natl.Acad.Sci.U.S.A*. 1973; 70:859–862. [PubMed: 4123693]
- Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, Malathi K, Magi-Galluzzi C, Tubbs RR, Ganem D, Silverman RH, Derisi JL. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS.Pathog*. 2006; 2:e25. [PubMed: 16609730]
- Valsesia-Wittmann S, Morling FJ, Nilson BH, Takeuchi Y, Russell SJ, Cosset FL. Improvement of retroviral retargeting by using amino acid spacers between an additional binding domain and the N terminus of Moloney murine leukemia virus SU. *J Virol*. 1996; 70:2059–2064. 1996 Mar;70(3). [PubMed: 8627737]
- Wu BW, Lu J, Gallaher TK, Anderson WF, Cannon PM. Identification of regions in the Moloney murine leukemia virus SU protein that tolerate the insertion of an integrin-binding peptide. *Virology*. 2000; 269:7–17. [PubMed: 10725193]
- Young GR, Eksmond U, Salcedo R, Alexopoulou L, Stoye JP, Kassiotis G. Resurrection of endogenous retroviruses in antibody-deficient mice. *Nature*. 2012; 491:774–778. [PubMed: 23103862]
- Yu P, Lubben W, Slomka H, Gebler J, Konert M, Cai C, Neubrandt L, Prazeres da CO, Paul S, Dehnert S, Dohne K, Thanisch M, Storsberg S, Wiegand L, Kaufmann A, Nain M, Quintanilla-Martinez L, Bettio S, Schnierle B, Kolesnikova L, Becker S, Schnare M, Bauer S. Nucleic acid-sensing Toll-like receptors are essential for the control of endogenous retrovirus viremia and ERV-induced tumors. *Immunity*. 2012; 37:867–879. [PubMed: 23142781]

Highlights

- We describe two monoclonal antibodies to murine leukemia virus (MuLV) env proteins.
- One of the antibodies reacts with all MuLVs tested including amphotropic MuLVs.
- Another antibody reacts with the proline-rich region of the Moloney MuLV SU protein.
- The Moloney MuLV-specific antibody is useful for immunoblotting procedures.
- Both antibodies are capable of neutralizing virion infectivity.

Figure 1.

Recombinant polytropic MuLV SU proteins and their reactivity to MAb 538. The bar diagrams depict the amino acid sequences of several polytropic MuLV SU proteins derived by recombination with Mo-MuLV (Alamgir, Owens et al., 2005). Open (white) regions of the bars represent Mo-MuLV-derived regions while closed (black) regions represent regions derived from endogenous polytropic MuLV sequences. Numbers above the diagrams indicate the positions of amino acids numbered from the beginning of the processed SU protein. The sequence at the bottom of the figure depicts the proline rich region (PRR) of the Mo-MuLV SU protein with conserved and variable regions indicated. The reactivity of each of the SU proteins to MAb 538 is indicated.

Figure 2.

Detection of cell surface expression of MuLV SU proteins by MAb 573 and MAb 538 by flow cytometry. (A). 3T3 cells chronically infected with the xenotropic MuLV, CasE no.1, the amphotropic MuLV, 4070A, the ecotropic MuLV, Fr-MuLV or the polytropic MuLV, M-RV 7A, were treated with MAb 573 and a secondary FITC goat Ab directed at mouse Ig (white) or with the secondary FITC antibody alone (grey) and analyzed by FACS. (B). 3T3 cells chronically infected with Mo-MuLV were treated with MAb 538 and the secondary goat FITC Ab (white) or with the secondary antibody alone (grey).

Figure 3.

Detection of the Mo-MuLV SU protein by immunoblotting using MAb 538. Virions released from cells producing the Mo-MuLV-derived polytropic recombinant virus MCF 383-5S, Mo-MuLV or Fr-MuLV were purified by isopycnic gradient centrifugation, and equivalent levels of virion protein were electrophoresed on 10% polyacrylamide gels, transferred to Immobilon-FL PVDF membranes (Millipore) and subsequently developed using MAb 538 and goat anti-mouse DyLight 680 (Thermo Scientific).

Figure 4.

Neutralization of MuLVs by MAbs 573 and 538. 0.5 ml of virus stocks of Mo-MuLV or Fr-MuLV adjusted to ~6000 focus-forming units/ml were mixed with 0.5 ml dilutions of hybridoma supernatant tissue culture medium containing MAb 573 (A) (16.7 ug IgM/ml) or MAb 538 (15 ug IgM/ml) (B), and incubated for 30 minutes at 37°C. The treated MuLV stocks were subsequently assessed for infectivity on NIH 3T3 cells using a focal immunofluorescence assay (Sitbon et al., 1985).

Figure 5.

Detection of cell-surface env protein on amphotropic 1504A-infected cells. The reactivity of Mus dunni cells chronically infected with the amphotropic MuLV 1504A with MAb 83A25 or MAb 573 was compared by indirect membrane immunofluorescence assays using trypsinized virus-infected cells (Chesebro et al., 1981).

TABLE 1

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

		MAb		Reference
Class	Strain	538	573	
Ecotropic				
	F-MuLV ^a	–	+	
	R-MuLV	–	+	(Rauscher, 1962)
	AKR 2A	–	+	(Cloyd et al., 1979)
	M-MuLV 8.2	+	+	(Shoemaker et al., 1980)
	M-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 IU-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)

^aNine strains of F-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.

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

TABLE 2

Reactivity of MAbs 538 and 573 with recombinant polytropic MuLVs.

Recombinant virus	MAb		Reference
	538	573	
AKv-derived isolates ^a	–	+	
F-MuLV-derived isolates			
368-2S	–	+	(Evans and Cloyd, 1984)
368-2T	–	+	(Evans and Cloyd, 1984)
368-3T	–	+	(Evans and Cloyd, 1984)
368-5S	–	+	(Evans and Cloyd, 1984)
368-5T	–	+	(Evans and Cloyd, 1984)
368-6T	–	+	(Evans and Cloyd, 1984)
368-7S	–	+	(Evans and Cloyd, 1984)
368-7T	–	+	(Evans and Cloyd, 1984)
F-MCF-1T	–	+	(Evans and Cloyd, 1984)
F-MCF-1E	–	+	(Evans and Cloyd, 1984)
M-MuLV-derived isolates ^b	–	+	
M-RV 14A	+	+	(Alamgir et al., 2005)

^a AKv-derived isolates examined were AKR-L3, AKR-L5 (Cloyd et al., 1980), AKR 247 (Hartley et al., 1977), AKR-6AS, AKR-6AT (Chattopadhyay et al., 1981), AKR-13, Akv-2-C34, Akv-1-C44-2 (Cloyd et al., 1979), M60P-T, M62P-S, M72P-S, M73P-S, M75P-T, M79P-T and M81P-S (Evans and Malik, 1987).

^b M-MuLV-derived isolates examined included HIX (Fischinger et al., 1975), 383-1S, 383-1T, 383-2S, 383-2T, 383-4S, 383-4T, 383-5S, and 383-5T (Evans and Cloyd, 1985) as well as twenty-four additional isolates that were sequenced in an earlier study (Alamgir et al., 2005). One of the additional recombinants, M-RV 14A, was reactive with Mab 538 (Figure 1).

TABLE 3

Reactivity of MAb 573 with recombinant vaccinia viruses expressing viral protein.

MAb	Recombinant vaccinia viruses ^a				Reference
	F-MuLV env	F-MuLV gag	HIV (NIB) env	Influenza HA	
573	+	–	–	–	
34	–	+	–	–	(Chesebro et al., 1981)
48	+	–	–	–	(Chesebro et al., 1981)
901	–	–	+	–	(Chesebro and Wehrly, 1988)

^aVaccinia virus plaques were made in CV-1 cells, and 48 h later live cell monolayers with plaques were exposed for 30 min. to monoclonal antibodies. After appropriate washing, antibodies were detected by immunofluorescence using FITC-conjugated goat anti-mouse immunoglobulin serum as previously described (Earl et al., 1986) except that cells were not fixed prior to indirect immunofluorescence assay because MAbs 573 failed to react specifically with plaques after methanol fixation. MAbs 34, 48 and 901 that react with the MuLV matrix (MA) protein, the F-MuLV env protein and the HIV env protein, respectively, were used as controls.