Monoclonal Antibodies Recognizing Structural Components of Murine Retroviruses Including an FMR Antigen on Protein p12

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SUMMARY

Monoclonal antibodies were prepared from mice and rats immunized with Friend leukaemia virus and BALB/c xenotropic virus. By immunoprecipitation of 125I-labelled and [35S]methionine-labelled viruses and by protein blotting, ten antibodies were found to react with the viral components p12, p15, p30, gp70 and p15E/p12E. A dot-immunobinding assay was found to be a reliable method to type the antibody reactivity with different murine leukaemia viruses (MuLVs). When tested on a panel of ecotropic and xenotropic MuLVs the antibodies revealed the following antigenic specificities: ecotrop-specific on p15E/p12E; xenotrop-specific on p15E; group-specific on p30 and p15E; FM-specific on gp70; FR-specific on gp70 and p15. Of particular interest is a cytotoxic antibody recognizing an FMR determinant localized on p12.

INTRODUCTION

Monoclonal antibodies directed against the various components of retroviruses are potentially powerful tools for analysing the complex biology of this oncogenic class of viruses. Such antibodies are of obvious interest for genetic studies, for distinguishing antigenic and possibly biological variants, for identifying and manipulating virus-expressing cells and for studying immunological interactions between virus and host. To date, most monoclonal antibodies obtained are directed against the gp70 and p15E components (Nowinski et al., 1979; Lostrom et al., 1979; Niman & Elder, 1980; Chesebro et al., 1981; Portis et al., 1982; Cloyd et al., 1982). These have been successfully used to map antigenic and structural domains on the env gene products (O'Donnell & Nowinski, 1980; Nowinski et al., 1981; Niman & Elder, 1980; Pinter et al., 1982). However, at this time, only a few reports on gag-specific monoclonal antibodies have been published (Chesebro et al., 1981, 1983). A more recent development in retrovirology is to use this class of viruses as vectors of experimentally inserted genes (Mann et al., 1983). Monoclonal antibodies directed against the gag determinants situated between the 5' long terminal repeat and the inserted sequence should be valuable probes for vector expression.

The present work was undertaken to generate a set of well characterized monoclonal antibodies directed against FLV, i.e. the helper component of the Friend leukaemia virus complex, and against BALB/c xenotropic virus. We show that a recently introduced dot-immunobinding assay (Hawkes et al., 1982) is a reliable, fast and inexpensive method for antigenic typing. We have characterized ten monoclonal antibodies reacting with gp70, p30, p15, p12 and p15E/p12E. Of particular interest is an antibody recognizing an FMR antigen on protein p12.

METHODS

Immunization of animals. Male BALB/c mice and RAI/f rats, 10 to 14 weeks old, were primed with 200 μg of purified viral preparations (FLV or X-BALB), emulsified in complete Freund's adjuvant at day 1. The antigens were distributed into two hind footpads and two sites subcutaneously. The animals were boosted twice with 200 μg
antigen in incomplete Freund's adjuvant at day 7 and 14, respectively. A final injection of 400 μg antigen in phosphate-buffered saline was given intravenously or intraperitoneally at day 21. All the animals were test bled just before the third injection and also 4 days after the last boosting. Sera were tested for appropriate antibody titres by ELISA and/or immunofluorescence on cells infected with the appropriate virus.

Production of hybridomas. The procedure of Galfré et al. (1977) was used to produce hybridomas. The hybridization and the characterization of hybridomas were performed as previously described (Alkan et al., 1983), using the myeloma cell line SP2/0-Agl4 (Shulman et al., 1978). Positive cultures were determined by ELISA and/or immunofluorescence and cloned by limiting dilution. The heavy and light chain class of monoclonal antibodies were determined by the double-immunodiffusion method using class/subclass-specific rabbit antibodies from Bionetics (Kensington, Md., U.S.A.).

Large amounts of antibodies were produced in the peritoneal cavity of BALB/c mice using Pristan (Carl Roth, Karlsruhe, F.R.G.). The ascites fluids were tested in ELISA for the presence of specific antibodies as described by Voller et al. (1976).

Cell lines. The following virus-producing cell lines were used for immunofluorescence or virus preparation: ‘Eveline’ cells producing the helper component of the FLV complex (Seifert et al., 1975); CCL-64 mink lung cells producing spleen cell-derived BALB/c xenotropic virus (X-BALB) (Monckton & Moroni, 1980); NIH3T3 cells producing Moloney leukaemia virus (MLV) obtained from Dr P. Besmer; NIH3T3 cells producing spleen cell-derived AKR ecotropic (E-AKR) or xenotropic virus (X-AKR), 136.5 cells from C57BL/6 mice producing radiation leukaemia virus (RadLV) obtained from Dr M. Haas; mink lung 78918 cells producing NZB xenotropic virus (X-NZB) obtained from Dr J. Levy; JLSV-5 cells producing Rauscher leukaemia virus (RLV). Purified viruses to be used for immunization, iodination, immunoprecipitation, spotting and protein blotting were obtained by sucrose gradient centrifugation (Moroni, 1972).

Cell surface immunofluorescence. Indirect cell surface fluorescence was determined using virus-infected cells and the corresponding uninfected cells as controls. Cells (10⁶) were incubated with 50 μl of ascites fluid (diluted 10⁻² or 10⁻³) for 1 h on ice. As second antibody, fluorescein isothiocyanate-conjugated sheep anti-mouse Ig (Miles-Yeda, Rehovot, Israel) was used. The cells were examined for cell surface fluorescence using a Leitz microscope.

Dot-immunobinding assay (spotting). Purified viruses were disrupted in NET (50 mM-Tris–HCl pH 7-6, 1 mM-EDTA, 100 mM-NaCl) by repeated freezing, thawing and vortexing. Volumes of 1 μl containing 200 ng or 20 ng of viral proteins were dotted onto squares of a grid-marked nitrocellulose filter strip (type HA, pore size 0.45 μm; Millipore) using a microdispenser (Drummond Scientific, Ca., U.S.A.). Further steps were essentially as described by Hawkes et al. (1982).

Protein blotting. Purified FLV or X-BALB were dissolved in electrophoresis sample buffer and subjected to SDS-PAGE according to Laemmli (1970) and Maizel (1971). About 10 to 40 μg of viral protein was applied onto a 4 cm wide slot of a 12.5% SDS polyacrylamide gel. After electrophoresis separated proteins were blotted onto nitrocellulose filters as described by Towbin et al. (1979). Protein binding sites were blocked as for the dot-assay. Wet sheets were cut into 2 mm-wide strips and incubated with the first and second antibodies and stained as described by Hawkes et al. (1982).

Immunoprecipitation. Purified FLV or X-BALB was disrupted in NET/0-5% NP40 by repeatedly freezing, thawing and vortexing. Aliquots of about 50 μg of viral proteins were iodinated by the chloramine-T method (Greenwood et al., 1963). Alternatively, FLV was radiolabelled by [¹³⁵S]methionine incorporation using Eveline cells grown overnight in DMEM containing 1/10 the normal methionine concentration, 50 μCi/L [¹³⁵S]methionine (900 Ci/mmol, Amersham) per ml and 10% foetal calf serum. Labelled FLV was purified from the medium by sucrose density gradient centrifugation and dissociated with NET/0-5% NP40. Immediately prior to immunoprecipitation, the viral lysates were centrifuged at 100000 g for 30 min to remove aggregates and insoluble material.

Immunoprecipitations and analyses of the immunoprecipitates were carried out as described previously (Gambke & Deppert, 1981).

Antiserum. The following polyclonal antisera were used: rabbit anti-FLV (Hunsmann et al., 1974); rabbit anti-gp70 FLV (Hunsmann et al., 1974); rabbit anti-gp85 FLV (Schneider et al., 1980); rabbit anti-p65 RLV obtained from Dr R. C. Gallo; rabbit anti-X-BALB was prepared by immunization with spleen cell-derived BALB/c xenotropic virus grown in CCL-64 cells. The monoclonal antibody 1911FB2 reacting with a group-specific determinant of p15E was kindly provided by Dr R. C. Nowinski.

Immune cytotoxicity. Cytotoxic tests were carried out using non-toxic rabbit complement as described by Moroni et al. (1974).

RESULTS

Analysis by immunoprecipitation

Monoclonal antibodies selected for reactivity with FLV or X-BALB by ELISA testing were examined by immunoprecipitation to identify the recognized viral component(s). Fig. 1 shows
Monoclonal antibodies to murine retrovirus components

Fig. 1. Analysis of monoclonal antibodies by immunoprecipitation. 

Analyzing the patterns obtained with eight monoclonal antibodies using 

the patterns obtained with eight monoclonal antibodies using $^{125}$I-labelled FLV (Fig. 1a) or X-BALB (Fig. 1b) respectively. Analysis was carried out under non-reducing conditions where the gp70 and p15E/p12E molecules run as an 85000 mol. wt. (85K) complex, i.e. gp85 (Pinter & Fleissner, 1977). Proteins p15E/p12E, which migrated in our gel system as bands of 17K and 15K respectively, contain only two or one tyrosine residues, respectively (Koch et al., 1983) and hence were only poorly iodinated. We will first consider antibodies directed against FLV. Two monoclonal antibodies (174S3-38 and 174S18-12) exclusively reacted with gp70 and gp85 but not with p15E/p12E (Fig. 1, lanes 3, 4) and, therefore, are specific for gp70. In contrast, a rabbit anti-gp85 serum reacted also, as expected, with p15E/p12E (Fig. 1, lane 5). Antibody 9S41-8 precipitated gp85, p15E and p12E (Fig. 1, lane 6), and the same pattern was obtained with 9S47-26 (Fig. 2, lane 4). The minor amount of gp70 visible in the immunoprecipitate by 9S41-8 was probably due to partial dissociation of gp85 into gp70 and p15E/p12E during electrophoresis. This explanation is supported by the observation that a gp70 band was not consistently present in repeat experiments and appeared only after very long autoradiographic exposures of the gel which were necessary to visualize p15E and p12E. Therefore, antibodies 9S41-8 and 9S47-26 seemed to react with a determinant present on p15E/p12E. These conclusions could also be drawn from results shown in Fig. 2 (lanes 3, 4) where immunoprecipitation patterns from $^{35}$S-methionine-labelled FLV are presented. Two monoclonal antibodies (155 OK-1 and 155 OK-2) were found to react with p30 from iodinated FLV (Fig. 1, lanes, 8, 9) and $^{35}$S-methionine-labelled FLV (Fig. 2, lanes 5, 6). In our gel system gag protein p15 migrated very closely to envelope protein p12E, but it differed from p12E in that it could be strongly labelled with iodine (see Fig. 1, lane 7) but not with methionine (Shinnick et al., 1981; Fig. 2, lane 7). Applying these criteria, antibody 174S1-15 was specific for p15 since it precipitated an iodinated protein with a mol. wt. of 15000 (Fig. 1, lane 10), but no methionine-labelled polypeptide (Fig. 2, lane 7). Finally, antibody 173S25-9 reacted exclusively with p12 (Fig. 1, lane 11; Fig. 2, lane 8). Antibodies from hybridomas 11S3-10 and 176S34-24 were raised against X-BALB antigen, and accordingly analysed using $^{125}$I-labelled X-BALB. Whereas antibody 176S34-24 did not precipitate any labelled protein (data not shown), 11S3-10 recognized two polypeptides co-
Fig. 2. Immunoprecipitation of viral proteins from a [35S]methionine-labelled lysate of FLV virions using conventional antisera or hybridoma ascites fluids. Precipitates were analysed by SDS-PAGE under non-reducing conditions and labelled polypeptides were visualized by fluorography.

migrating with gp85 and p15E (Fig. 1, lane 13), indicating that the antigenic determinant recognized by this antibody resides also on p15E.

**Analysis by protein blotting**

Towbin *et al.* (1979) designed a method for the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose filters and subsequent detection of immobilized proteins by serological procedures. In this way, distinct antigens may be visualized if a specific antibody is available. Likewise, distinct proteins immobilized on nitrocellulose sheets can be used to detect their respective antibodies. This method requires that the antigenic conformation recognized by the antibody is not unsuitably altered during SDS-PAGE. We applied this blotting technique to the analysis of our monoclonal antibodies and asked whether (i) the results obtained from immunoprecipitation described above could be confirmed, (ii) the antigenic determinants could still be recognized following SDS denaturation, and (iii) in particular, whether the protein specificity of antibody 176S34-24, which did not immunoprecipitate, could be determined by this means.

We chose non-reducing conditions in order to avoid dissociation of gp85. Representative results are shown in Fig. 3(a, b). Antibodies 9S41-8 and 9S47-26 stained p12E (in a very few blotting analyses also p15E, data not shown), but never gp85. The corresponding antigenic determinant possibly was not accessible for the antibody after binding of gp85 to the filter. That gp85 was actually transferred to the filter was visible in the staining pattern obtained with both conventional anti-gp85 serum and monoclonal antibodies 174S3-38 and 174S18-12. It is noteworthy that the blotting procedure allowed ready identification of both the gp69 and gp71 components (Strand & August, 1973) which are difficult to identify in ordinary systems. Antibody 174S1-15 which precipitated p15 (Fig. 1, lane 10) also recognized a 15K protein on the blot. The anti-p30 antibodies (155 OK-1 and 155 OK-2) were also positive using the blotting
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Fig. 3. Analysis of monoclonal antibodies by protein blotting. Purified FLV (a) or X-BALB (b) were subjected to SDS-PAGE under non-reducing conditions. Separated proteins were electrophoretically transferred from the polyacrylamide gel to a nitrocellulose filter. Filter strips were incubated with conventional antiserum or hybridoma ascites fluids and the corresponding antigen(s) were visualized as described in Methods.

Table 1. Properties of ten anti-MuLV monoclonal antibodies

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>ImmunoPrecipitation of 125I-labelled virus</th>
<th>[35S]Methionine labelled virus</th>
<th>Protein specificity</th>
<th>Ig class</th>
<th>Cytotoxicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>9S41-8</td>
<td>p12E, p15E, gp85</td>
<td>p12E, p15E, gp85</td>
<td>p12E, (p15E)</td>
<td>IgG2a/K</td>
<td>+</td>
</tr>
<tr>
<td>9S47-26</td>
<td>p12E, p15E, gp85</td>
<td>p12E, p15E, gp85</td>
<td>p12E, (p15E)</td>
<td>IgG2a/K</td>
<td>+</td>
</tr>
<tr>
<td>174S3-38</td>
<td>gp80, gp70</td>
<td>gp85, gp69/71</td>
<td>gp85, gp69/71</td>
<td>IgG2a/K</td>
<td>+</td>
</tr>
<tr>
<td>174S18-12</td>
<td>gp85, gp70</td>
<td>gp85, gp69/71</td>
<td>gp85, gp69/71</td>
<td>IgG1/K</td>
<td>+</td>
</tr>
<tr>
<td>173S25-9</td>
<td>p12</td>
<td>p12</td>
<td>p12</td>
<td>IgG2a/K</td>
<td>+</td>
</tr>
<tr>
<td>174S1-12</td>
<td>p15</td>
<td>p15</td>
<td>p15</td>
<td>IgG2/K</td>
<td>-</td>
</tr>
<tr>
<td>11S3-10</td>
<td>p15E, gp85</td>
<td>p15E</td>
<td>p15E</td>
<td>IgG2/K</td>
<td>-</td>
</tr>
<tr>
<td>176S34-24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>IgM/K</td>
<td>-</td>
</tr>
<tr>
<td>155 OK-1†</td>
<td>p30</td>
<td>p30</td>
<td>p30</td>
<td>IgG1/K</td>
<td>-</td>
</tr>
<tr>
<td>155 OK-2†</td>
<td>p30</td>
<td>p30</td>
<td>p30</td>
<td>IgM/K</td>
<td>-</td>
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</table>

* Tested on FLV-producing Eveline cells, except 176S34-24, which was tested on CCL-64 cells producing X-BALB: NT, not tested; -, negative in this test; +, positive in this test.
† Rat origin.

technique (data not shown). However, monoclonal 173S25-9 directed against p12 (Fig. 1, lane 11; Fig. 2, lane 8) failed to react with the filter-bound antigen (data not shown).

Antibodies 11S3-10 and 176S34-24 were tested by blotting using X-BALB-derived proteins (Fig. 3b). Anti-p15E antibody 11S3-10 did not bind to denatured p15E (data not shown), while 176S34-24, which failed to immunoprecipitate, stained a single band on the blot co-migrating with p15E.

The results obtained by immunoprecipitation and blotting with our ten monoclonal antibodies are summarized in Table 1 together with their class specificity and cytotoxic activity.
Fig. 4. Analysis of monoclonal antibodies by a dot-immunobinding assay (spotting). Purified ecotropic or xenotropic MuLVs were disrupted and spotted in different amounts (200 ng and 20 ng in a volume of 1 µl) onto each square of a nitrocellulose filter strip. Strips were incubated with hybridoma ascites fluids diluted from $10^{-4}$ to $10^{-9}$. Spotted antigens were visualized as described in Methods. Controls: (a, b) monoclonal antibody 1911FB2 (reacting with p15E) at dilutions of $10^{-3}$ or $10^{-6}$, respectively; (c) no first antibody was added.

Cross-reactivity patterns of monoclonal antibodies with various murine leukaemia viruses (MuLVs): comparison of spotting and immunofluorescence

Hawkes et al. (1982) have recently developed a dot-immunobinding assay (spotting) which is a highly sensitive and easy procedure for the analysis of antibodies reacting with antigen
Monoclonal antibodies to murine retrovirus components

Table 2. Reactivity of monoclonal antibodies with different MuLVs

<table>
<thead>
<tr>
<th>Hybridoma specificity</th>
<th>Protein</th>
<th>FLV</th>
<th>RLV</th>
<th>MLV</th>
<th>AKR</th>
<th>RadLV</th>
<th>AKR</th>
<th>BALB</th>
<th>NZB</th>
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</thead>
<tbody>
<tr>
<td>9S41-8</td>
<td>p12E/p15E</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+†</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>9S47-26</td>
<td>p12E/p15E</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+†</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>174S3-38</td>
<td>gp70</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>174S18-12</td>
<td>gp70</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+†</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>173S25-9</td>
<td>gp70</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>174S1-15</td>
<td>p15</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
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<td>-/-</td>
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<tr>
<td>173S25-9</td>
<td>p15</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
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<tr>
<td>173S25-9</td>
<td>p15</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
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<tr>
<td>174S18-12</td>
<td>gp70</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
<td>155 OK-1</td>
<td>p30</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
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</tr>
<tr>
<td>155 OK-2</td>
<td>p30</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
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<tr>
<td>11S3-10</td>
<td>p15E</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
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<td>-/-</td>
</tr>
<tr>
<td>176S34-24</td>
<td>p15E</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>-/-</td>
<td>-/-</td>
<td>-/-</td>
<td>+/+</td>
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</tr>
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</table>

* (+), weak reaction only; +, positive in this test; -, negative in this test; NT, not tested.
† 50% positive cells.

immobilized on nitrocellulose filters. We were interested to see whether this test would also be suitable for typing the antibody specificity against a battery of viruses. Results scored as positive in this dot-assay had to meet the following criteria: (i) a positive reaction on different batches of virus preparations, and (ii) a decrease in colour intensity of the spots at high antibody dilutions; spots of the same low colour intensity at all antibody dilutions were considered as ‘background’.

Purified ecotropic and xenotropic viruses were disrupted, applied in two different amounts (200 ng and 20 ng protein) onto nitrocellulose filters and reacted with antibodies present in ascites fluids at dilutions of $10^{-4}$ to $10^{-9}$. The results presented in Fig. 4 demonstrate that our monoclonal antibodies could be classified into six groups: (i) two antibodies (9S41-8 and 9S47-26, both directed against p15E/p12E) recognized all ecotropic MuLVs tested but not xenotropic ones and, therefore, seemed to be ecotrop-specific; (ii) a single hybridoma, 173S25-9 (anti-p12) appeared to have the Friend–Moloney–Rauscher (FMR) pattern of specificity as it reacted exclusively with these antigens; (iii) hybridomas 174S1-15 (anti-p15) and 174S3-38 (anti-gp70) detected determinants expressed by FLV and RLV (FR pattern) but not by other MuLVs; (iv) a single monoclonal antibody, 174S18-12, recognizing gp70, was restricted to the Friend and Moloney (FM) type specificity; (v) both anti-p30 antibodies 155 OK-1 and 155 OK-2 reacted with all ecotropic and xenotropic viruses tested so far (data not shown) and thus are group-specific. Hybridoma 11S3-10 (anti-p15E) most likely also belonged to this broad-reacting group since it recognized all viruses tested, but it should be noted that it reacted only weakly with E-AKR virus (Fig. 4); (vi) antibody 176S34-24 (anti-p15E) appeared to detect xenotrop-specific determinants only; the weak staining of FLV seen in Fig. 4 was not obtained with different FLV preparations and therefore was considered as non-specific.

We wished to see whether these typing data obtained by the spotting method could be confirmed by conventional immunofluorescence microscopy. Monoclonal antibodies were tested for membrane fluorescence on a battery of infected and uninfected cell lines. A result was considered as positive and specific if the infected cell line was stained at an ascites dilution of $10^{-3}$ and if the uninfected cells were negative at a dilution of $10^{-2}$. The results are summarized in Table 2, together with the spotting data. As can be seen, the results with 7/10 antibodies fully agreed, demonstrating the value of this new method. Three antibodies (174S1-15, 11S3-10 and 176S34-24) did not stain the cell surface of unfixed infected cells but reacted in the dot-assay. We have not attempted to test these antibodies on fixed cells.

Immune cytotoxicity

Monoclonal antibodies, binding to cell surface antigens and belonging to a complement-fixing class of Ig, may lyse target cells in the presence of complement. We first tested those
antibodies for cytotoxicity that reacted with the cell surface in immunofluorescence microscopy (Table 1) and which belonged to the IgM or IgG2 classes. Fig. 5 shows that antibodies 9S47-26, 9S41-8 (p12E/p15E-specific), and 173S25-9 (p12-specific) had high cytotoxic titres, giving 50% dead cells at dilutions of $10^{-5}$ to $10^{-6}$. Antibody 155 OK-2 (anti-p30), although weakly reactive in cell surface fluorescence, was unable to lyse cells in the presence of complement (data not shown). We also tested the anti-p15 antibody 174S1-15 for cytotoxicity as polyclonal (Hunsmann et al., 1974) and monoclonal (Chesebro et al., 1981) antibodies have localized p15 on the cell surface of infected cells. Our antibody, although belonging to the complement-fixing class IgG2, was not cytotoxic, in accordance with the negative membrane fluorescence result. It appears that the corresponding epitope belongs to a non-exposed domain of p15.

DISCUSSION

We have characterized monoclonal antibodies directed against all major structural components of murine retroviruses with the exception of p10. As other workers have similarly failed in isolating an antibody of this specificity, it may be that immune recognition of p10 occurs less readily under the conditions used. Of particular interest is antibody 173S25-9 reacting with p12 and defining a cell surface FMR antigen on this protein. The FMR antigen has been initially described by the cytotoxic action of sera from leukaemic mice reacting with cells infected with FLV, MLV and RLV, respectively, but not with cells infected with Gross leukaemia virus (Old et al., 1963; Old & Boyse, 1965). This antigen, initially thought to be a non-virion antigen, was subsequently detected in disrupted but not in intact viral preparation (Friedman et al., 1974). Absorption experiments using purified viral proteins localized the FMR antigen on p15 (Strand & August, 1975). This was confirmed by the isolation of a monoclonal anti-p15 antibody with FMR reactivity (Chesebro et al., 1981). A similar antigenic specificity was also found to reside on gp70 as shown by polyclonal (Nowinski et al., 1978) and monoclonal (Chesebro et al., 1981) antibodies. That the 'FMR antigen' is an antigenic complex rather than as initially thought a unique distinct structure is further exemplified by our novel finding that a membrane-located FMR specificity can also reside on p12. We observed the FMR specificity pattern both by the spotting test and by immunofluorescence. As FMR leukaemias are classical models in tumour immunology our new antibody may become a useful tool in this field.

Three more antibodies have been obtained reacting with gag proteins. Two recognized p30 and belonged to the IgM and IgG1 classes, respectively. Although both reacted weakly with the cell membrane in fluorescence microscopy, none of them was cytotoxic. It may be that the
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antigenic epitope is exposed in a configuration which does not allow sufficient antibody and hence complement binding to occur in order to allow cell lysis. As p30 carries mainly group-specific antigenic determinants, it is not surprising that the two antibodies reacted with all viruses tested. The anti-p15 antibody described here (174S1-15) could only be detected when virus was labelled with $^{125}$I, but not with $^{35}$S-methionine, as p15 does not contain this amino acid (Shinnick et al., 1981). Since the antigenic determinant was not destroyed by SDS, the p15 component could also be visualized by protein blotting. The antigenic site recognized by this antibody is not exposed at the membrane and could not be revealed by serology on the intact membrane. This is in contrast to another monoclonal anti-p15 antibody (Chesebro et al., 1981) which reacts with a cell membrane-exposed FMR epitope as discussed above, while our anti-p15 is FR-specific.

The different specificities of the antibodies reacting with gp70 are of interest. 174S3-38 reacted with FLV and RLV but not with MLV, and 174S18-12 reacted with FLV and MLV but not with RLV. While a monoclonal anti-gp70 antibody with FR specificity has been described before (Chesebro et al., 1981), an FM-specific one, to our knowledge, has not.

Four antibodies were obtained reacting with p15E. 176S34-24 and 11S3-10, both raised against BALB/c xenotropic viruses, were unable to distinguish between the BALB/c, AKR and NZB xenotropic viruses. The former was xenotrop-specific, while the latter recognized in addition the ecotropic viruses. Portis et al. (1982) have obtained different monoclonal antibodies reacting with xenotropic viruses by fusing spleen cells from animals undergoing a graft-versus-host reaction. In contrast to the xenotropic virus-specific anti-p15E (176S34-24), two anti-p15E/p12E antibodies recognizing only ecotropic viruses were obtained (9S41-8 and 9S47-26). These antibodies may react in part with a cluster of six amino acids at the C-terminal end of p15E which differs in ecotropic FLV and Friend spleen focus-forming virus (SSFV). In SSFV this sequence may be derived from an endogenous xenotropic virus (Amanuma et al., 1983; Clark & Mak, 1983; Wolff et al., 1983).

That several techniques must be used in parallel for characterizing monoclonal antibodies is exemplified by our anti-p12 (173S25-9) and p15E (176S34-24) hybridomas. The latter did not react in immunoprecipitation, perhaps due to lack of sufficient affinity, but could be used in protein blotting. In contrast, anti-p12 was negative in protein blotting, as the antigenic site was lost after SDS-gel electrophoresis, but was active in immunoprecipitation.

We wish to emphasize how simple, rapid and inexpensive the dot-immunobinding assay is in comparison with the more laborious procedures such as immunofluorescence, radioimmunoassay or ELISA. It is a good method to choose for typing a new virus or identifying the specificity of a new monoclonal antibody.

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