

THE USE OF ENVELOPE SPECIFIC MONOCLONAL
ANTIBODIES TO SEARCH FOR DIFFERENTIATION
ANTIGENS ENCODED BY ENDOGENOUS RETROVIRAL
SEQUENCES IN MURINE HEMATOPOIETIC CELLS

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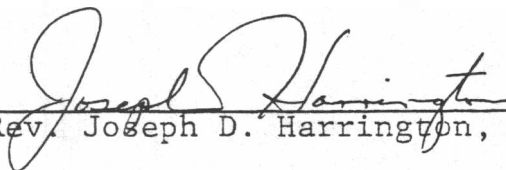
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ABSTRACT

Hematopoietic cells in the mouse were examined to detect retrovirus encoded differentiation antigens on their cell surfaces. Twenty-four established lymphoid, erythroid, and myeloid cell lines were studied. A panel of well defined envelope specific monoclonal antibodies were tested against each cell line in a ^{125}I -protein A cell binding radioimmunoassay to detect retroviral envelope antigens on the cell's plasma membrane. Results were analyzed to discern cell line specific expression of antigens as a basis for the search for differentiation antigens. A few cell lines were selected for further study. Two erythroid cell lines, 7727 and 7883, for example, were studied because of their singular reactivity with the broadly reactive 18-1 antibody. The bulk of this study attempted to determine if the gp70 reacting with the antibody 18-1 was encoded by the Moloney/SFFV pseudotype virus used to induce the cell lines or originated from an endogenous retrovirus. If the envelope antigens were encoded by endogenous retrovirus they would be good candidates for differentiation antigens. Membrane immunofluorescence, cytoplasmic fluorescence, and immunoprecipitation and PAGE analysis were used to determine that the gp70 did, in fact, originate from the inducing Moloney/SFFV pseudotype virus. The expression of this Moloney antigen was determined to be defective. In addition, several myeloid cell lines proved to be noteworthy. They expressed

antigens encoded by xenotropic endogenous virus in an unprecedented manner. These cell lines warrant further study since these antigens may be differentiation antigens. Continued use of the monoclonal antibody panel and the other cell lines utilized may also facilitate the search for these antigens in future research.

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INTRODUCTION

Monoclonal antibodies have proven recently to be a powerful tool in biological research. Their ability to detect a specific antigenic determinant makes them useful in immunology. One valuable function performed by these antibodies is to characterize the antigens expressed on the plasma membranes of a variety of cells.

Monoclonal antibodies have been successfully produced from hybrid cell lines resulting from the fusion of mouse myeloma cells and spleen cells. A series of these antibodies, developed at the National Institute of Health's Rocky Mountain Laboratories, react specifically with envelope glycoproteins from endogenous and exogenous murine retroviruses. This paper explores the use of these monoclonal antibodies to characterize the antigens expressed on the cell surfaces of twenty-four erythroid, lymphoid, and myeloid leukemia cell lines. Results of the serological tests employed were analyzed to learn two things about each cell line. First, a survey of the antigens expressed on each cell line quickly determined the types of virus present in a cell. The methods used resolved the viruses into three major categories: dualtropic (MCF), xenotropic, and ecotropic viruses. More importantly, the analysis discerned cell line specific patterns of antigen expression. Antigens peculiar to a cell line are noteworthy because they may be differentiation antigens, antigens connected

temporally and perhaps functionally with cellular differentiation.

Detection of these antigens is important because they may elucidate the differentiation process. Differentiation antigens serve as markers for specific stages of cellular differentiation. Their expression is controlled by regulatory elements operating during differentiation. The study of these antigens and the genes which encode them should provide information on genetic elements whose expression influences embryogenesis and differentiation in postnatal life.

All vertebrates, including man, contain within their genomes integrated retroviral sequences which are passed vertically like Mendelian genes from generation to generation. These sequences are rarely expressed as infectious viruses. In the mouse these retroviral sequences are scattered relatively heavily throughout the genome and may constitute as much as 0.3% of the entire genomic DNA. The expression of some of these viral sequences seems to be linked to cellular differentiation. It is not known whether these viral "differentiation antigens" play a role in these cellular processes or are merely transcribed because they are located near genes that do function in differentiation. The purpose of this study was to accumulate preliminary data on the cell lineage specificity of retroviral envelope gene products. The strategy involved was simple: A panel of envelope specific monoclonal antibodies was used to serologically and biochemically characterize any qualitative differences in the retroviral products expressed by a group of erythroid, myeloid, and

lymphocytic leukemia cell lines. Since it would be counter-productive for a differentiation antigen to be encoded by a movable genome originating from an infectious virus, it was necessary to determine whether selected cell lines expressed antigens arising from competent viruses. Thus, in searching for differentiation antigens, I attempted to identify a retroviral product which, while expressed on a single lineage of cells, was not related to an infectious virus.

LITERATURE REVIEW

Origin of Retroviral Antigens

A panel of monoclonal antibodies has been produced that reacts specifically with the antigens associated with murine retroviruses of endogenous and exogenous origin. Exogenous viral antigens appear on the mouse cell after viral infection. Because the retrovirus is a RNA virus, its genome must first be transcribed into a homologous DNA sequence via a viral polymerase, reverse transcriptase (1). As the infectious cycle continues, the viral DNA integrates into the host's genome and encodes the structural proteins required for the formation of progeny virus. Viral envelope proteins are expressed at the plasma membrane for subsequent viral assembly (2,3). These proteins hold the determinants that react with some of the monoclonal antibodies described herein.

Endogenous viral antigens arise in a somewhat different manner. Like the exogenous virus, the endogenous virus transcribes its RNA into a DNA sequence. The endogenous provirus that is incorporated into the cell's genome is not involved in producing polypeptides for an infectious virus; it is passed vertically from generation to generation like a normal gene (4). Transcription of this proviral sequence appears to fall under cellular regulation. While these proviral sequences are not typically involved in an infectious process, it is believed that they originated from some exogenous infection occurring millions

years ago (4). It has been estimated that such endogenous sequences comprise at least 0.01% (5) and as much as 0.3% of the murine genome (4). Since the endogenous provirus, unlike the exogenous viral genome, is intimately tied to the host's genome, it represents a more permanent fixture in the cell. Thus, in seeking differentiation antigens one most logically looks at antigens encoded by endogenous viruses because their permanence potentially allows them to become involved in crucial stages of cell development. Recent research indicates that many of the differentiation antigens in the mouse are encoded by endogenous proviruses (5,6).

Monoclonal Antibodies Specific for Retroviral Antigens

There are three general categories of murine retroviruses that are distinguished by the viruses' host ranges. Xenotropic viruses, for example, infect cells other than those of mice. They are necessarily endogenous since exogenous infection is not possible. Ecotropic viruses infect only mouse cells and may be endogenous or exogenous. Dualtropic (MCF) viruses, believed to be a recombinant form of the previous two, can infect mouse cells and those of other species. They may be of endogenous or exogenous origin (4).

Monoclonal antibodies have been developed that react specifically with antigens of viruses from each of these categories. Since each monoclonal antibody reacts specifically with one antigenic determinant of a complex antigen and arises from a unique clone, they are given identifying numbers. The specificity of the monoclonal antibodies used in this study are shown on

Table 1 (7-10).

Xenotropic viruses can be separated into four groups based on the antibodies with which they react. The four xenotropic virus serogroups have been defined by the reactivities of four prototypical viruses and are outlined on Table 2 (Portis, J., unpublished).

The panel of monoclonal antibodies represented on Table 1 and Table 2 was the basis for this study. By utilizing this panel, I was able to ascertain what types of viruses were present in each cell line. The rationale behind this diagnostic use of the panel will be discussed later. In addition, this panel may be used to quickly pinpoint cell line specific antigens, good candidates for differentiation antigens.

Precedence for the Study of Differentiation Antigens

The search for retrovirus encoded differentiation antigens is the crux of this paper. The precedence for such a study was established early in the 1970's by Elisabeth Stockert and her colleagues at the Sloan-Ketterling Institute for Cancer Research. They developed an antiserum, a relatively primitive serological tool, that would react specifically with antigens encoded by the Gross murine leukemia virus. Utilizing cytotoxicity tests, they found that only murine thymocytes expressed Gross antigens. Stockert turned to Howard Temin's work on Rous sarcoma virus of the previous few years to explain the expression of this antigen, which she called G_{IX}. Temin had proposed the germinal thoughts on retroviruses when he showed how viral RNA could be transcribed into DNA. Stockert reasoned that this DNA copy of the viral

Table 2

Xenotropic Virus Serogroups

GROUP	PROTOTYPE	MONOCLONAL ANTIBODY REACTIVITIES				
		18-1	24-6/24-9	603/609	613	615
1	Balb IU-1	+	+	+	-	+
2	NZBQ IU-3	+	+	+	+	+
3	NZB 1868	+	-	+	+	-
4	AKR-6	+	+	-	-	-

genome could be incorporated into the mouse's cell chromosomes (11). Eventually Stockert certified the proviral nature of the genes encoding the G_{IX} antigen (12).

Over the next ten years, a variety of similar antigens were discovered. The G_{ERLD} , G_{RADAI} , and G_{ASKL2} antigens exhibited expression on lymphocytes of spleen, bone marrow, and lymph node tissue in mice with radiation induced leukemia (12,13). In a similar way, the Friend erythroleukemia antigen (3) and Group 4 antigen from Table 2 (Britt, W., B. Chesebro and J. Portis, submitted for publication) were found expressly on murine erythroid cells.

Eventually researchers began to see that all of these antigens shared two traits. First, each is expressed on a specific cell type (14). Some, however, exhibit more restricted expression than others. The G_{IX} antigens, as an illustration, is found only on thymocytes. The second characteristic shared by these antigens is that they are encoded by sequences related to murine leukemia viruses of endogenous origin.

With these two traits in mind, researchers defined all such polypeptides as differentiation antigens. Their rationale was straightforward. They observed that the antigens showed a temporal connection with cellular differentiation. Since they appear on only specific cell types, they may be the cause or the product of differentiation from stem cell to committed cell. That these antigens may be linked to differentiation is supported by their relative permanence on a cell's membrane.

It is obvious that these antigens are connected with cellular differentiation. Their exact relation has, however, been

elusive. At least three correlations have been postulated.

The antigens might simply mark differentiation. The G_{IX} antigen provides the best illustration of this. Marco Pierotti and his colleagues have found that the G_{IX} antigen is encoded during the process of differentiation. The antigen serves no apparent purpose; rather it is a marker for T cell differentiation (14). Further studies have corroborated these findings by showing that the expression of G_{IX} is regulated by the genes controlling differentiation (15).

It is, of course, possible that similar antigens may actually function in differentiation. One model of commitment and subsequent differentiation places import on the cell surface as a receiver and transmitter of molecular information. The stem cell is seen as a passive recipient of inductive signals in the form of hormones, inducing factors, and cellular messages. A cell receptor molecule responsible for a specific pattern of differentiation would be present on the cell surface in a uniform amount. Commitment and differentiation would result from changes in the inductive environment that affect these receptors (16). Thus, these antigens could function in differentiation if they appeared on the stem cell as such receptor molecules. The observations of Axelrad lend credence to this theory. He has shown that the gene for the Friend erythroleukemia antigen is linked to the Fv-2 locus in mice. The primary effect of this locus is to stimulate progenitor hemopoietic cell division. When this is coupled with the fact that the Friend erythroleukemia antigen is significantly amplified during hemopoietic regeneration, the link between the Friend erythroleukemia antigen and

differentiation becomes strengthened (3).

One final postulate attempts to tie the differentiation antigen to abnormal cell development. Recently some of these antigens have been indicted for playing a role in oncogenesis (15). Most of the work in this area has been done on lymphocytes. Researchers have focused their attention on lymphocytes with the gp70 phenotype: $G_{IX}^+ / G_{ERLD}^+ / G_{RADA1}^+ / G_{ASKL2}^+$. Because many different types of murine leukemia viruses produce these antigens, such a phenotype could represent a genotype that could easily recombine to create altered viral tropisms. Resultant viruses could have an increased capacity to infect lymphocytes. This could produce an amplified amount of the gp70 which, in turn, could cause a regulatory disturbance that would ultimately result in leukemia transformation (13).

Further research will be needed to fully understand the relationship between these antigens and cellular differentiation. It is sufficient to say that, from the outset, this study is fully warranted. Antigens that mark differentiation or that function in it, or some abnormal cellular development, may unlock the basis of cell differentiation.

MATERIALS AND METHODS

Mouse Cell Lines and Monoclonal Antibodies

All mouse cell lines and monoclonal antibodies were obtained from the Rocky Mountain Laboratories, Hamilton, Montana.

^{125}I -Protein A Cell Binding Assay for Antiviral Antibodies

An ^{125}I -protein A binding assay (IPA) was the fundamental means of detecting antibody binding to cell surface antigens. The ^{125}I -protein A was prepared from purified Staphylococcus protein A (Pharmacia) labeled with ^{125}I using chloramine T and the IPA was carried out as previously described by Bruce Chesebro (9). In simple terms, live cells were incubated with different monoclonal antibodies. The cells were washed and incubated, in turn, with amplifying antiserum. After a similar wash, the cells were incubated with ^{125}I -protein A, washed again, and then placed in borosilicate tubes to detect gamma radiation activity. The absolute ^{125}I activity of the cells was determined by measuring the gamma counts per one minute in a Gamma 4000 Counter (Beckman). Background radiation counts were subtracted from the absolute counts obtained. The relative amount of antibody binding to each cell line was determined by dividing this final gamma count by the count for the negative control: cells that had been incubated with amplifying antiserum and ^{125}I -protein A but not monoclonal antibody. By convention, if the tested cells produced a count per minute that was greater than four times the

count per minute of the negative control, then the cell exhibits a significant amount of binding of antibody to its antigens. In cases of borderline results (multiplicities of three to four), a membrane immunofluorescence was used to determine reactivity.

Membrane Immunofluorescence

In cases where the IPA gave borderline results, cell lines were tested again for antigen expression by using indirect membrane immunofluorescence as previously described by Bruce Chesebro (10). Briefly, live cells were incubated with monoclonal antibodies and subsequently washed. Antibody bound to the cell was detected by incubating the cells with goat antimouse immunoglobulin, washing them, and examining them for fluorescence under a Leitz Orthoplan incident light fluorescence microscope.

Cytoplasmic Immunofluorescence

Cytoplasmic immunofluorescence was utilized to detect antigens within the cytoplasm. Smears of cell line suspensions were made on glass slides by the brush technique. The smears were dried and fixed in acetone for 10 minutes and allowed to dry again. One hundred μ l of the tested monoclonal antibody was added to each and incubated for 30 minutes at 25° C. The slides were then washed in phosphate buffer solution (PBS) for 15 minutes. Twenty-five μ l of a mixture of fluoresceinated goat anti-mouse Ig absorbed twice with mouse liver powder, 1:10 goat serum, and 1:100 rhodamine dye, was added to each slide and incubated for 30 minutes at 25° C. The slides were washed again in PBS for 15 minutes, fixed in 1% formaldehyde in PBS, rinsed, and allowed to dry. Fluorescence, and thus antibody binding,

was observed under a Leitz Orthoplan microscope.

Immunoprecipitation and PAGE Analysis

These techniques were utilized to detect the presence of antigens amongst the cell-free lysates of the plasma membrane. Protocols have been described previously by Chesebro (10). In summary, live cells were surface labeled with ^{125}I using mild oxidizing conditions (Iodogen, Pierce Chemical Company). The cells were washed once and lysed in a Tris-saline EDTA buffer containing 0.5% NP-40. Lysates were incubated overnight at 4° C. with monoclonal antibodies and the immune complexes were recovered using Staphylococcus aureus Cowen strain as an immunosorbent. After washing, the complexes were dissociated with an eluting buffer containing SDS and 2-mercaptoethanol. The eluted protein were resolved in a 10% gel containing SDS. Gels were dried and autoradiography was performed using X-AR Kodak film and using a Lightning Plus intensifying screen (Dupont).

RESULTS

¹²⁵I-protein A Cell Binding Assay and Membrane Immunofluorescence

The initial stage of this study involved cataloging the antigens present on twenty-four mouse cell lines. Thirty-three monoclonal antibodies were tested against each cell line in an IPA. When this assay produced borderline results, reactivity was checked via a membrane immunofluorescence assay. Table 3 illustrates the results of these assays for a few significant cell lines and monoclonal antibodies. The cell lines were culled for study from the original list of twenty-four because some lucidly demonstrate the diagnostic value of the monoclonal antibody panel while others exhibit peculiar ensembles of antigens and, thus, are good candidates for differentiation antigen studies. The reactivity of all of the cell lines against all of the monoclonal antibodies has been relegated to the Appendix for clarity's sake.

Cell lines 2800, 2C, and 7229 are included on Table 3 to show how a panel of monoclonal antibodies can be used to quickly identify the viruses indigenous to each cell line. 2800, for example, reacted with antibodies specific for MCF and Friend exogenous ecotropic antigens (Table 1). Thus, it is apparent that the 2800 cell line carried a MCP and a Friend exogenous ecotropic virus. Cell line 2C reacted with antibodies specific for xenotropic, endogenous ecotropic, and Friend exogenous

ecotropic virus encoded antigens and therefore, evidently possesses these viral types. In addition, 2C's pattern of reaction with the monoclonal antibodies indicated that both a Group 1 and a Group 4 xenotropic virus was present. Cell line 7229, in a similar fashion, reacted only with antibodies specific for endogenous ecotropic envelope antigens and thus seemed to possess their viral counterpart alone.

Cell lines 7544, 7782, 7235, and 7301 are included in Table 3 because they exhibited unique expression of xenotropic endogenous envelope antigens. Each of the cell lines, because it expressed a cell line specific antigen contrary to that predicted for xenotropic viruses (Table 2) warrants further study.

Results of Serological Tests on Erythroid Cell Lines 7727 and 7883

After analyzing the results of the ^{125}I -protein A cell binding assay and the membrane immunofluorescence, two cell lines, 7727 and 7883, were chosen for further study because of their novel expression of an antigen that reacted only with antibody 18-1. 18-1 is a broadly reactive antibody. It reacts with MCF, xenotropic, and ecotropic determinants. In 7727 and 7883, 18-1 reacted with an antigen that apparently is not recognized by the usual antiviral antibodies. The reactivity of 18-1 could not be attributed to any MCF, xenotropic, or ecotropic virus in the cell line; none of these viruses' antigens were overly expressed on 7727 or 7883. This unique expression seemed worthy of study; the 18-1 reactivity could indicate the presence of a differentiation antigen.

7727 and 7883 are erythroid cell lines that have been

induced by a Moloney/SFFV psuedotype virus, a virion with a capsid comprised of Moloney encoded proteins that surround a nucleic acid core from the SFFV (spleen focus forming virus). The main line of attack on studying these lines sought to determine if 18-1 was reacting with a Moloney/SFFV antigen of exogenous origin or an antigen of endogenous origin. If 18-1 was reacting with a Moloney/SFFV antigen, the search for a differentiation antigen would end; the 18-1 reactive antigen would be accounted for solely as a by-product of exogenous infection by the input virus (Moloney/SFFV). A variety of tests was utilized to determine the nature of this antigen. The results of a membrane immunofluorescence, cytoplasmic fluorescence, and immunoprecipitation with PAGE analysis are shown on Table 4, Table 5; and Figure 1 respectively. Their relevance will be addressed in the Discussion.

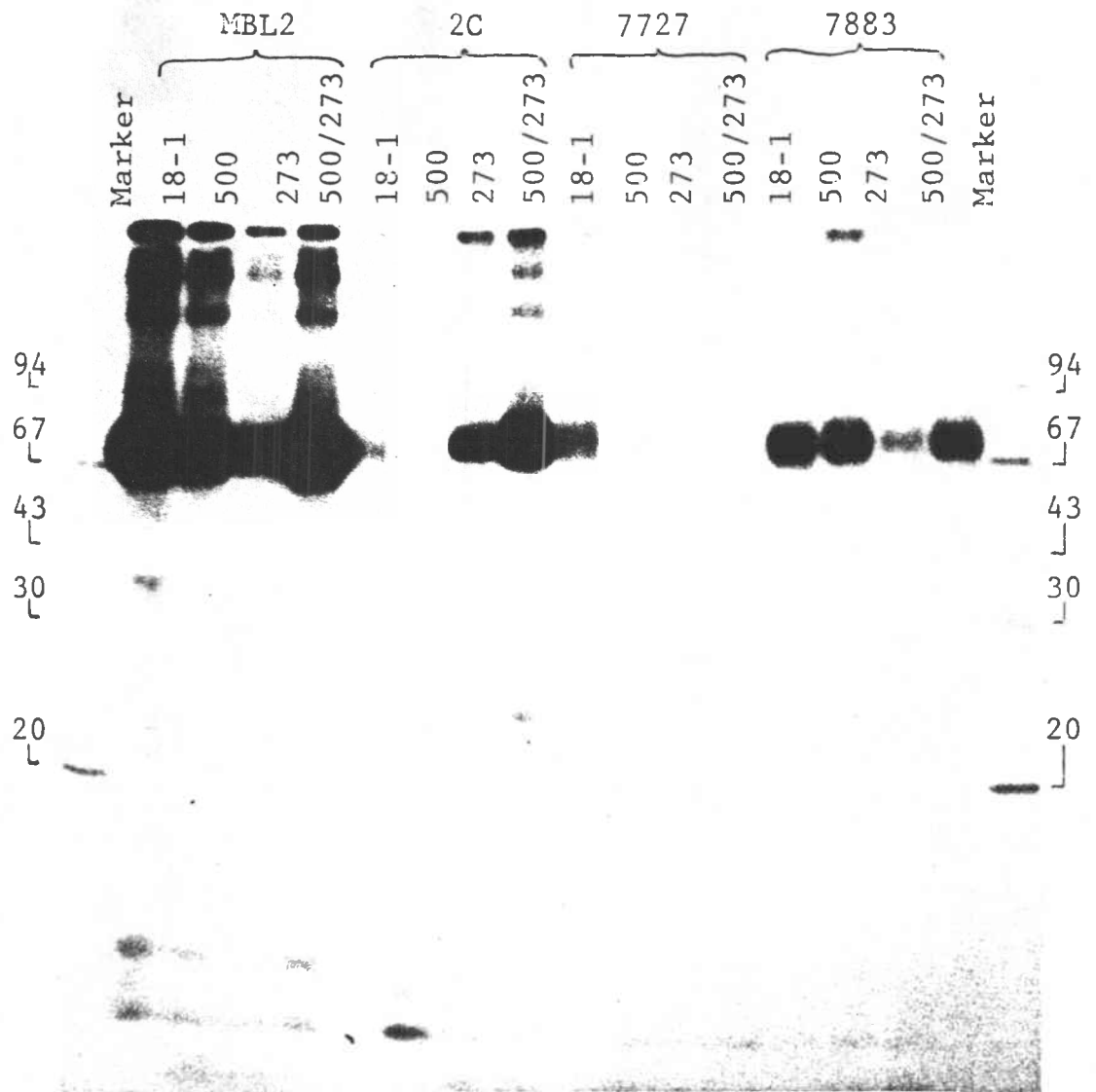


Figure 1. Autoradiogram showing the reactivity of anti-Moloney gp70 monoclonal antibodies (18-1, 500 and 273) with cell-free lysates of cell lines MBL2, 2C, 7727 and 7883. Lysates of ^{125}I labeled mouse cells were immunoprecipitated with the monoclonal antibodies. The precipitated immune complexes were resolved by SDS-PAGE and autoradiography of the dried gel was carried out on X-AR Kodak film using a Lightning Plus intensifying screen (Dupont). MBL2 served as a positive control; its expression of Moloney gp70 was well documented. 2C was included to illustrate the synergistic antigen binding created when antibodies 500 and 273 were mixed. Note that the 500/273 mixture precipitated a heavier band than the sum of the bands precipitated by 500 and 273 alone. 7727 and 7883 were the tested cell lines. The anti-Moloney gp70 monoclonal antibodies precipitated glycoproteins from the lysates of both cell lines. The numbers at the outer edges of the autoradiogram indicate the molecular weights of the reference ^{14}C labeled marker proteins in kilodaltons (Bio-rad).

Table 4

Membrane Immunofluorescence of Moloney
Specific Monoclonal Antibodies Against 7727, 7883, and MBL2

	MBL2	7727	7883
273	*+	-	-
500	*+	-	-

*Similar results have been described previously
(Morrison, R., unpublished).

Table 5

Cytoplasmic Fluorescence of Monoclonal
Antibodies Tested Against 7727,
7883, and MBL2 to Detect
Intracellular Moloney
Antigens

	MBL2	7727	7883
273	+	+	-
500	+	+	+

DISCUSSION

Viruses Indigenous to a Cell Line

By using the monoclonal antibody panel found in Table 1 in ^{125}I -protein A cell binding assays against murine cell lines, one can readily identify the types of viruses present in each cell line. Cell lines 2800, 2C, and 7229 provide an excellent example of this.

2800, for example, had antigens that reacted with antibodies 502, 514, 48, and 18-5. The reactivity with 502 and 514 showed, quite simply, that a dualtropic (MCF) virus was present in the cell. 18-5 reacts with endogenous and exogenous ecotropic viral envelope antigens. 18-5 could not have been reacting with an antigen of endogenous origin because no other endogenous specific antibody reacted with this cell line. The reactivity of 48 can account for 18-5's reaction. Both react with Friend exogenous ecotropic antigens. Thus, it seems 18-5 and 48 were binding to the same or different determinants on the same Friend ecotropic antigen encoded by indigenous Friend virus.

Cell line 2C showed positive reactions with 18-6, 24-6, 24-9, 603, 609, 18-1, 24-8, 24-13, 18-5, and 48. The reactions with 18-1, 24-6, 24-9, 603, 609, and 615 indicate that there was a Group 1 xenotropic virus present in 2C (Table 2). In the same vein, the reactivity of 18-6 indicates that there was also a Group 4 xenotropic virus present. 24-8 and 24-13 react specifically with endogenous ecotropic antigens. 2C thus showed expression of an envelope antigen of endogenous ecotropic origin. Antibodies of 48 and 18-5 also reacted. Their reactivity, as

explained for 2800, was due to the presence of Friend ecotropic virus. 18-1, the broadly reactive antibody, attached to these cells because they presented both endogenous and ecotropic and xenotropic determinants.

7229 possessed an interesting antigenic profile because it expressed only endogenous ecotropic antigens. It reacted only with 18-1, 24-8, and 18-5. 18-1 binds to polypeptides from xenotropic and endogenous ecotropic viruses. 18-5 reacts with endogenous and exogenous ecotropic viral antigens. Since 24-8 reacts solely with endogenous ecotropic viral antigens, one could logically assume that all three antibodies were binding to antigens of endogenous ecotropic virus. The absence of xenotropic and exogenous ecotropic specific reaction validates this assumption.

A similar schema of logic can be applied to any cell line. Therefore, with the simple tools of cell lines, monoclonal antibodies and radioimmunoassay can quickly identify the major types of virus indigenous to a cell.

Unique Xenotropic Antigens

Dr. John Portis (Rocky Mountain Laboratories, Hamilton, Montana, 59840) has discovered that xenotropic viruses can be divided into four serogroups (Table 2, p. 8). Four of the myeloid cell lines examined in this study exhibited unique expression of xenotropic viral antigens. Cell lines 7544 and 7235 reacted only with monoclonal antibody 24-9. None of Portis' four groups of viruses react singularly with 24-9. Indeed, the antigens on these cells was unique. The novelty of this antigen was heightened by the fact that, while these cells reacted with 24-9,

they did not react with 24-6. In the past, these two monoclonal antibodies have shown the exact same specificity.

Cell lines 7301 and 7782 showed a similar novel antigen expression. They reacted solely with antibody 609. This pattern of reactivity, again, falls outside those seen in the traditional xenotropic groups. 609 and 603, as well, have always shown parallel specificities. That 609 binds to the cell, while 603 does not, indicates that a unique antigen was present.

These four cell lines, because they expressed xenotropic antigens in a unique way, merit further study. The xenotropic antigens expressed appear to be novel glycoproteins and may be good candidates for differentiation antigens because their expression was limited to myeloid cell lines.

A Unique Moloney gp70

The bulk of this study focused on two particularly interesting cell lines: 7727 and 7883. The peculiar expression of 18-1 reactive antigens, mentioned earlier, offered an excellent departure point for the search for a differentiation antigen. After the initial screening of cell lines, it was difficult to account for the binding of 18-1 to the 7727 and 7883 cells. Other antibodies in the panel should have reacted with these cells because 18-1 is broadly reactive; it is specific for endogenous ecotropic and xenotropic antigens. If the 18-1 reactivity could not be explained as being a by-product of the Moloney/SSFV induction process, then the antigen may indeed be a good candidate for a differentiation antigen.

Several techniques were employed to determine if the

reactivity of 18-1 could be attributed to the presence of a Moloney/SFFV pseudotype encoded gp70.

First, Moloney specific antibodies 500 and 273 were tested against 7727 and 7883 in a membrane immunofluorescence assay (MF). MF was chosen because it is more sensitive than a cell binding radioimmunoassay (IPA). Results are shown on Table 4 (p. 20). 500 and 273, as expected, reacted with the positive control, MBL2, a lymphoid cell line induced by Moloney ecotropic virus. 500 and 273 did not bind to 7727 and 7883. These cell lines thus exhibited no overt expression of Moloney envelope antigens.

A logical conclusion of the MF results is that while Moloney viral antigens were not openly expressed on the cell surface, they might have been present intracellularly. In simple terms, transportation of the antigen to the cell surface may have been blocked. Cytoplasmic fluorescence was conducted with antibodies 500 and 273 against 7727 and 7883. MBL2 was the positive control. The results on Table 5 make it apparent that Moloney viral antigens were in fact present in the cells' cytoplasm.

Since the Moloney envelope antigen was present at all in these cell lines, its absence at the cell surface was unexpected. The plasma membrane was re-examined. Immunoprecipitation of the cell surfaces lysates with the Moloney specific antibodies seemed to be the most prudent course of investigation. It was hypothesized that the Moloney gp70 was present on the cell surface but its determinants were masked by some steric or chemical hindrance. In the immunoprecipitation protocol used, NP-40 lyses the cell's plasma membrane, freeing the surface antigens

from masking factors. Cell lines 7727, 7883, 2C, and MBL2 were immunoprecipitated with monoclonal antibodies 18-1, 500, 273, and a mixture of 500 and 273. The mixture was used because sometimes immunoprecipitation occurs only when two antigens effect a synergistic antibody-antigen binding (10). Results of the SDS-PAGE analysis of the immunoprecipitation appear on Figure 1.

Cell line 2C shows a synergistic phenomenon. The mixture of antibodies 500 and 273 precipitated more glycoprotein than the sum of the precipitates of 500 and 273 alone. In addition, it is important to note that 500 and 273 react with different determinants. The width of the bands precipitated by these two antibodies differed, indicating that the antibodies precipitated different determinants available in different amounts.

As expected for the positive control, MBL2, antibodies 18-1, 500, 273, and the mixture all precipitated a glycoprotein of 70K daltons. (All molecular weights were determined by log linear graph analysis of the autoradiogram using prepared molecular weight markers as a reference.) In 7727, antibodies 18-1, 500, and probably the 500 fraction of the 500/273 mixture precipitated proteins. It is interesting to note 500 precipitated a polypeptide but 273 did not. Usually these antibodies react with the same antigen. The reactivity seen in 7727 indicates that a Moloney viral antigen was present but its expression was defective in two ways. First, the gp70 was not overtly expressed at the cell surface; some factor inhibited its reactivity. That the Moloney envelope antigen could not be detected by IPA or MF assays illustrates its defective expression. Since the gp70 was found in the cytoplasm and among the cell surface lysates,

it seems that its determinants were masked or deleted in the intact plasma membrane. Second, and more specifically, the antigen's 273 reactive determinant was undetectable, suggesting that it was either blocked or deleted. In 7883, 18-1, 500, 273, and the mixture all precipitated glycoproteins. Thus, this cell line expressed Moloney viral antigen in a manner similar to 7727. In this case, however, the 273 reactive determinant was detectable.

Several theories can account for such defective expression of antigens at the cell surface. A defect, for example, in the viral genome could produce an incomplete polypeptide. This change in the primary structure of the protein would, in turn, alter secondary and tertiary organization of the antigen resulting in a determinant with weak affinity for the antibody, especially when it is placed in the restricting constraints of the plasma membrane.

A second possibility is that there could have been a fault in the processing of the antigen. The antigen may not have been fully glycosylated before reaching the cell surface. The resultant alteration of the antigen's structure would affect its ability to react with antibody.

Finally, the nature of the plasma membrane itself could affect the antigen's expression. Some chemical or steric phenomenon intricately tied to the membrane's physiology could block the antigen's expression at the cell surface. This hypothesis seems the most reasonable. The first two possibilities are unlikely in light of the evidence present. Both of the explanations would involve a change in the apparent molecular weight

of the protein molecule. By log linear graph analysis the proteins precipitated were determined to be 70K daltons, the same weight as those in the control, MBL2.

Conclusion

Thus, a unique gp70 was expressed on the erythroid lines 7727 and 7883. Their reactivity with 18-1, which was enigmatic before, has now been resolved. 18-1 reacted with an antigen arising from the envelope of the inducing Moloney/SSFV pseudotype virus. Since the gp70 comes from an exogenous source, it is no longer considered to be a good candidate for a differentiation antigen. Nevertheless, its faulty expression is noteworthy, and further study of it is warranted. A two-dimensional oligopeptide mapping of the antigen is, for instance, in order here. By such a map, one could compare the structure of this gp70 with that of similar antigens in cell lines like MBL2. This comparison could divulge a structural basis for the faulty expression.

The general thesis of this paper, the search for the all-important differentiation antigen, should not be abandoned either. As mentioned earlier, the unique xenotropic viral antigens of the four myeloid cell lines should be investigated further. The strategy involved in the study of these cell lines should, of course, focus on determining if the antigens expressed are of endogenous origin and thus are good candidates for differentiation antigens. Membrane immunofluorescence and cytoplasmic fluorescence assays could be done to verify the results of the IPA. The membrane immunofluorescence would provide a more

sensitive method of detecting antigens expressed at the cell surface. Cytoplasmic fluorescence would be useful in deciding whether the unique expression of antigens at the cell surface was due to blockage or deletion of determinants at the plasma membrane. If this was the case, one would find that the intracellular antigens reacted with the more complete complement of antibodies as predicted by Table 2. If the MF and cytoplasmic fluorescence corroborated the results of the IPA, then one can rule out the possibility that an exogenous genome encoded the antigens because exogenous viruses do not react in the pattern ascribed to these myeloid cell lines. Thus, it would be likely that these antigens were encoded by endogenous retroviral sequences and are therefore good candidates for differentiation antigens.

The next step in studying these cells would be to isolate the antigen, inject it into a mouse, and select cells from the spleen that produce antibody against the original gp70. By fusing the spleen cells with mouse myeloma cells, monoclonal antibodies against the gp70 could be produced. These antibodies could be used to examine different myeloid cells in normal mice to determine whether the antigen appears during a specific stage of differentiation or embryogenesis. To prevent confusing results, the mice used should be free from endogenous ecotropic sequences in their genomes. NFS, an inbred strain of mouse, is an example; it contains only endogenous xenotropic sequences within its genome. If the antigen was found to be associated with cellular differentiation, DNA probes could be produced for the genes encoding it. Ultimately, data should arise that would explain the presence of gp70, its genetic origin, and its function in

differentiation.

In a more general sense, the panel of monoclonal antibodies, itself, can continue to serve as a basis for research. Several cell lines found in the Appendix could also prove to be worthy candidates for future studies. The stage has been set; the preliminary data compiled in this paper should fuel the continued search for differentiation antigens.

APPENDIX

This appendix represents a compilation of all results of all IPA and MF assays. IPA results are indicated by numbers representing the multiplicities of the counts/minute of the test mixture over the counts/minute of the negative control. A multiplicity of four or greater indicates significant binding of the monoclonal antibody to antigens on the cell's surface. The results of the MF assays, which were used to verify the results of the IPA, are represented as minus or plus signs in the upper corners of boxes for which the assay was run.

Lymphoid Cell Lines

	MBL2	7303
601	4 ⁻	2
602	4 ⁻	2
604	5 ⁻	2
605	5 ⁻	2
607	4 ⁻	1
608	4 ⁻	1
612	3	2
617	4	2
618	3	2
20-1	4 ⁻	2
502	5	1 ⁻
514	3	1 ⁻
18-4	3	2
18-6	4 ⁻	2 ⁺
18-7	2	2
19-1	3	2

Monoclonal Antibodies

	MBL2	7303
24-6	6 ⁻	5 ⁺
24-7	3	1
603	3	2
609	5	3
610	3 ⁻	2
613	4 ⁻	2 ⁻
614	5 ⁻	1
615	9	12
616	3 ⁻	2
518	2	1
24-9	9 ⁻	6 ⁺
24-10	5	5
18-1	7 ⁺	8 ⁺
24-8	6	3 ⁻
24-13	4	2
48	3 ⁻	4 ⁺
18-5	7 ⁻	6 ⁺

Monoclonal Antibodies

Erythroid Cell Lines

Monoclonal Antibodies

	2800	2C	7C	D1B	401	7847	7909	7727	7883	AA60	AW28
601	1	5 ⁻	1	4	5	2	1	1	2	5 ⁻	6 ⁻
602	1	6 ⁻	1	3	5	2	2	1	2	6 ⁻	4
604	1	6 ⁻	1	3	4	1	2	1	2	7 ⁻	5
605	1	5 ⁻	1	2	12	1	2	1	1	3	3
607	1	4 ⁻	1	3	15	1	1	1	2	2	3
608	1	4 ⁻	3	3	17	1	4	1	1	4	3
612	1	4 ⁻	2	4	2	1	2	2	2	4	5
617	1	5 ⁻	2	3	3	2	2	1	2	6 ⁻	4 ⁻
618	4 ⁺	6 ⁻	1	5	15 ⁺	3	2	1	2	7 ⁻	6
20-1	1	5 ⁻	2	3	3	2	2	1	2	6	6 ⁻
502	14 ⁺	4 ⁻	2	7	14 ⁺	1 ⁺	2 ⁻	2 ⁻	2 ⁻	3	2
514	7 ⁺	6 ⁻	2	7	18	2 ⁺	2 ⁻	2 ⁻	2 ⁻	5 ⁻	2 ⁻
18-4	1	6	1	3	1	1	2	1	2	5	5
18-6	1	7	2 ⁻	3	2 ⁻	1 ⁻	2 ⁻	1 ⁻	2 ⁻	7	4 ⁻
18-7	1	3	2	3	2	2	1	2	2	4	2
19-1	2	3	1	3	3	1	1	1	2	4	3
24-6	1	9	2	4	3 ⁻	3 ⁻	4	3 ⁻	3 ⁻	9 ⁻	4 ⁻
24-7	1	5	1	4	2	1	2	1	1	3	4
603	1	8	1 ⁻	3 ⁻	1 ⁻	2 ⁻	4 ⁺	2 ⁻	2 ⁻	4 ⁻	4 ⁻
609	1	5	1	5	4	2	3	1	3	5	4
610	1	4	1	3	3	1	2	1	2	2	3
613	1	3	1	3	2	1	1	1	2	3	2 ⁻
614	1	4	1	3	2	1	2	1	2	5	4
615	2	3 ⁺	6 ⁻	15 ⁻	8 ⁻	8 ⁻	8	7 ⁻	7 ⁻	11 ⁻	8
616	1	4	1	3	1	1	2	1	1	4	3
518	1	4	1	2	2	1	2	1	2	3	3
24-9	2	11	3	6 ⁻	3	5 ⁻	4 ⁺	4 ⁺	3 ⁺	14	14 ⁻
24-10	4	7	2	4	4	2	4	3	3	6	4
18-1	2	20	8 ⁺	18	6	6 ⁻	6 ⁻	12 ⁺	21 ⁺	13	9 ⁺
24-8	1	9	2 ⁻	8	2 ⁻	3 ⁻	3 ⁻	2 ⁻	5 ⁺	8 ⁻	6 ⁺
24-13	1	5	1	3	1	2	1	1	1	5	3
48	11 ⁺	36 ⁺	6 ⁺	19 ⁺	2 ⁻	1	1	1 ⁻	2	4 ⁻	16 ⁺
18-5	11 ⁺	33	5 ⁻	12	11 ⁺	3	3	2 ⁻	3	8 ⁻	8 ⁺

Myeloid Cell Lines

	5402	7320	7557	7542	7544	7229	7301	7782	7235
601	2	6	3	5	3	3	2	2	5
602	2	5	2	4	2	2	1	2	2
604	2	9 ⁻	2	5	3	2	1	1	4
605	1	3	2	3	2	1	1	1	1
607	1	4	2	4	2	1	1	1	3
608	1	6	2	4	2	1	1	1	2
612	2	6	3	6	3	2	1	2	4
617	2	5	1	4	4	2	1	3	3
618	2	6 ⁻	3	4	3	3	1	2	3
20-1	2	11 ⁻	3	6	4	4	1	2	6
502	1	2 ⁻	2	3	2	1	2	1	2
514	1	5 ⁻	2	4	2	2	2	3	3
18-4	2	7 ⁻	1	3	2	3	1	2	3
18-6	2	8 ⁻	1	5	2	3	1	2	3
18-7	2	4	1	3	2	2	1	1	2
19-1	2	6	2	4	2	2	1	2	3
24-6	2	9 ⁻	3	5 ⁻	2	2	2	2	2
24-7	2	2	1	1	2	2	1	1	3
603	1	2 ⁻	1	2	3	1	1	3	2
609	2	6	2	5	1	5	8	10	1
610	2	3	1	3	1	2	1	1	2
613	2	2	2	3	2	2	1	2	2
614	1	10 ⁻	1	3	2	2	1	1	1
615	6	9 ⁻	3	11	8	3	4	4	3
616	2	6	2	3	2	2	1	2	4
518	2	5	2	3	2	2	1	2	2
24-9	6 ⁺	15 ⁺	3	9	7	4	3	5	11
24-10	9	17	3	16	7	5	1	5	10
18-1	5	17 ⁺	2	9	6	5	3	4	7
24-8	2	14 ⁻	2	7	4	4	3	3	6
24-13	1	7	2	3	2	5	2	1	1
48	15	31 ⁺	10	13	7	4	2	9	5
18-5	11	21 ⁺	6	10	7	7	2	9	10

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