JOURNAL OF VIROLOGY, Dec. 1990, p. 6176–6183
0022-538X/90/126176-08$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 64, No. 12

A Neutralizable Epitope Common to the Envelope Glycoproteins of Ecotropic, Polytropic, Xenotropic, and Amphotropic Murine Leukemia Viruses

LEONARD H. EVANS,[1]* RICHARD P. MORRISON,[2] FRANK G. MALIK,[1] JOHN PORTIS,[1] AND WILLIAM J. BRITT[3]

Laboratory of Persistent Viral Diseases[1] and Laboratory of Intracellular Parasites,[2] National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840, and Department of Pediatrics, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294[3]

Received 21 May 1990/Accepted 11 September 1990

An epitope common to all classes of murine leukemia viruses (MuLVs) was detected by reactivity of MuLVs with a rat monoclonal antibody (MAb) termed 83A25. The antibody is of the immunoglobulin G2a isotype and was derived after fusion of NS-1 myeloma cells with spleen cells from a Fischer rat immunized with a Friend polytropic MuLV. The antibody reacted with nearly all members of the ecotropic, polytropic, xenotropic, and amphotropic classes of MuLVs. Unreactive viruses were limited to the Friend ecotropic MuLV, Rauscher MuLV, and certain recombinant derivatives of Friend ecotropic MuLV. The presence of an epitope common to nearly all MuLVs facilitated a direct quantitative focal immunofluorescence assay for MuLVs, including the amphotropic MuLVs for which no direct assay had been previously available. Previously described MAbs which react with all classes of MuLVs have been limited to those which react with virion core or transmembrane proteins. In contrast, protein immunoblot and immunoprecipitation analyses established that the epitope reactive with MAb 83A25 resides in the envelope glycoproteins of the viruses. Structural comparisons of reactive and nonreactive Friend polytropic viruses localized the epitope near the carboxyl terminus of the glycoprotein. The epitope served as a target for neutralization of all classes of MuLV with MAb 83A25. The efficiency of neutralization varied with different MuLV isolates but did not correlate with MuLV interference groups.

Serological analyses have been an important tool for the identification and classification of murine leukemia viruses (MuLVs). In early studies, the reactivity of different antisera with MuLVs led to the detection of antigens restricted to particular types of MuLVs (e.g., exogenous ecotropic MuLVs) or of antigens which were shared among larger groups of MuLVs (e.g., exogenous ecotropic MuLVs and endogenous ecotropic and xenotropic MuLVs) (15, 18, 20, 23, 24, 49, 53). Antisera which were reactive with particular types of MuLVs frequently neutralized virus infectivity and were found to be directed predominantly toward antigens of the viral envelope proteins (18, 23, 36, 38, 52, 56), while antisera with broad specificity reacted predominantly with viral core proteins (15, 18, 20, 40, 49). With the advent of monoclonal antibody (MAb) technology, a much more precise antigenic characterization of MuLVs became possible such that different virus types, and even very closely related isolates of the same virus type, could be distinguished (5, 6, 30, 32, 37). Several studies from this and other laboratories have described a variety of MAbs which react with proteins of MuLVs (5, 6, 8, 30, 32, 34, 37, 43, 44). These include MAbs reactive with proteins of exogenous ecotropic MuLVs, of endogenous ecotropic, xenotropic, and polytropic MuLVs from inbred mice, and of endogenous ecotropic, xenotropic, and amphotropic viruses from feral mice. Group-specific MAbs, reactive with at least some members of all types of MuLVs, are directed at the viral core proteins (5, 6) or at the p15E protein of MuLVs (30, 34), whereas most type-specific MAbs, reactive with viruses of a particular type or with selected isolates within a type, are directed at the envelope glycoprotein (5, 6, 30, 32, 43, 44). In no instance has a MAb reactive with the envelope glycoproteins of all classes of MuLVs been reported. Indeed, no MAb, group specific or type specific, has been described which is reactive with the envelope glycoprotein of amphotropic MuLVs from feral mice. In this report, we describe the detection of an epitope which is nearly ubiquitous among MuLVs. The epitope was detected by using a rat MAb termed 83A25, and it resides near the carboxyl terminus of the envelope glycoprotein. MuLVs reactive with MAb 83A25 include all isolates of the ecotropic, xenotropic, polytropic, and amphotropic MuLV classes tested, except the Friend and Rauscher ecotropic MuLVs (F-MuLV and R-MuLV, respectively) and certain of their recombinant derivatives. MAb 83A25 exhibited neutralizing activity with all classes of MuLVs and was useful for histological detection of MuLV envelope glycoprotein expression, for immunoblotting and immunoprecipitation of MuLV envelope glycoproteins, and for direct quantitative assays of MuLVs.

MATERIALS AND METHODS

Viruses and cells. Numerous MuLVs were employed in these studies, including MuLVs of the ecotropic, xenotropic, and polytropic classes from inbred mouse strains as well as ecotropic, xenotropic, and amphotropic MuLVs from feral mice. The MuLVs and the references describing their origins are listed in Tables 1 and 2. All viruses except Moloney MuLV (M-MuLV) and the Friend virus complexes were propagated on *Mus dunni* cells (28). M-MuLV was propagated on SC-1 cells (21), and the Friend virus complexes were maintained as the originally infected FRE cells

* Corresponding author.

TABLE 1. Reactivity of MAb 83A25 with ecotropic, xenotropic, and amphotropic MuLVs

Virus	Membrane fluorescence	References
Ecotropic		
AKR 2A	+	9
WN1802N	+	10
CasBr-E	+	22
1504E	+	22
M-MuLV	+	12
F-MuLV[a]	−	5, 31, 39, 41, 45, 51
R-MuLV	−	46
Xenotropic		
AKR 6	+	9
NIH AT124	+	54
C58L1 xeno	+	4
Cas E no. 1	+	9
Balb IU-1	+	22
NFS-Th1	+	4
NZB-8882	+	47
NZB Cl. 35	+	29
Amphotropic		
1504A	+	22
4070A	+	22

[a] Nine strains of F-MuLV were tested and found to be unreactive with MAb 83A25.

(14). Other cell lines employed in these studies were Mv1Lu mink lung fibroblasts (ATTC CCL64) (26), the NS-1 nonsecretor clone, P3-NS-1-l-Ag4/1, of MOPC21 myeloma cells (27), SCL1 human squamous cell carcinoma cells (2), and HeLa human cervical carcinoma cells (19). Purified virions were prepared by sedimentation followed by isopycnic density gradient centrifugation.

Antibodies. 83A25 was one of several hybridoma cell lines obtained after immunization of a Fischer rat with a Triton X-100-disrupted, ether-extracted virion preparation of F-MCF 368-2T (11). Techniques for the immunization, fusion of cells, detection of antibody-producing hybridomas, and subsequent cloning of the hybridomas were done as described by Chesebro et al. (6). The isotype of the MAb released by 83A25 (MAb 83A25) was determined to be of the immunoglobulin G2a class by using a rat monoclonal typing kit (ICN ImmunoBiologicals). Unless otherwise indicated, the source of MAb 83A25 in all experiments was supernatant from saturated cultures of hybridoma cells cleared by centrifugation at 15,000 × *g* for 10 min. Other MuLV-reactive antibodies utilized in this study included MAb 48 (reactive with the envelope proteins of F-MuLV and R-MuLV) (6) and goat anti-gp70 serum, provided by J. Cole, Biological Carcinogenesis Branch, National Cancer Institute.

TABLE 2. Reactivity of MAb 83A25 with recombinant MuLVs

Recombinant virus	Membrane fluorescence	References
Akv-derived isolates[a]	+	4, 9, 10, 13, 25
M-MuLV-derived isolates[b]	+	12, 16
F-MuLV-derived isolates		
368-2T	+	11
368-2S	+	11
368-3T	+	11
368-5T	+	11
368-5S	+	11
368-6T	+	11
368-7T	−	11
368-7S	−	11
MCF-FrNx	−	1
F-MCF-1	−	55
F-MCF-1E	+	7
SFFVp	−	14
SFFVa	−	14

[a] The Akv-derived isolates examined were AKR L5, AKR L3 (10), AKR 247 (25), AKR 6AS, AKR 6AT (4), AKR 13, Akv-2-C34, Akv-1-C44-2 (9), M60P-T, M62P-S, M72P-S, M73P-S, M75P-T, M75P-S, M79P-T, and M81P-S (13). All isolates examined were positive.

[b] The M-MuLV-derived isolates examined were HIX (16), 383-1T, 383-1S, 383-2T, 383-2S, 383-4T, 383-4S, 383-5T, and 383-5-S (12). All isolates examined were positive.

Cell surface and cytoplasmic fluorescence assays. The reactivity of MAb 83A25 with various strains of MuLVs was determined by cell surface fluorescence of infected cells as previously described (6). In some cases, cells were examined for cytoplasmic fluorescence. In these instances the cells were grown on 13-mm cover slips to confluency. The cover slips were rinsed in 25 mM sodium phosphate–0.15 M sodium chloride (pH 7.2) (PBS) and then immersed in acetone prechilled to −20°C for 20 min and allowed to dry at ambient temperature. The cover slips were then rehydrated with PBS, incubated for 60 min at ambient temperature with undiluted hybridoma culture supernatants, washed, and incubated for 60 min with a 1:100 dilution of fluorescein isothiocyanate-conjugated goat anti-rat immunoglobulin G antiserum (Southern Biotechnology Associates, Inc.). The cover slips were washed two times for 10 min, counterstained with 0.2% Evans blue in water for 10 min, washed in PBS as described above, mounted under PBS:glycerol (70:30), and examined for fluorescence.

Focal immunofluorescence and virus neutralization assays for MuLVs. Quantitative assays of MuLVs were performed by using a focal immunofluorescence assay (FIA) as described previously (50). For neutralization assays, virus stocks were adjusted to approximately 2×10^2 focus-forming units per ml and then diluted 1:1 with fresh media or with serial dilutions of MAb 83A25 or MAb 48 and incubated for 30 min at 37°C. Virus titers of the MAb-treated and control samples were determined by using the FIA with MAb 83A25.

Immunoblotting and immunoprecipitation. Biosynthetic radiolabeling with [^{35}S]methionine, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoprecipitation, and immunoblotting procedures have all been previously described (3, 33). Immunoprecipitated proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 8% gels, while virion lysates to be analyzed by immunoblotting were electrophoresed in 12.5% gels. For immunoblots, detection of polypeptides immunoreactive with goat anti-gp70 serum was done by using a 1:500 dilution of the serum, while detection of polypeptides immunoreactive with MAb 83A25 was done by using a 1:5 dilution of culture supernatant and subsequent treatment with a 1:500 dilution of rabbit anti-rat immunoglobulin G (61-620-1; ICN Biomedical, Inc.).

RESULTS AND DISCUSSION

Detection of a common epitope among MuLVs. Cell lines infected with MuLVs exhibit virally coded cell surface antigens; thus, the reaction of an antibody with a particular MuLV can often be conveniently determined by the binding

or lack of binding of the antibody to infected cells. The binding of MAb 83A25 to MuLV-infected cells was determined by using a large array of MuLVs of different types derived from a variety of sources (Tables 1 and 2). Most MAbs we have studied are reactive with specific types of MuLVs, usually limited to certain isolates of a particular MuLV group (i.e., ecotropic, polytropic, or xenotropic MuLVs). In contrast, MAb 83A25 exhibited a remarkably broad specificity with MuLVs, reacting with almost all MuLVs tested, regardless of the class or source of the virus. This included most polytropic and all xenotropic MuLVs from inbred mouse strains that were tested, as well as all ecotropic MuLVs tested, except F- and R-MuLVs. Polytropic viruses which did not react with the antibody were limited to certain recombinant derivatives of F-MuLV. MAb 83A25 also exhibited strong reactivity to type C viruses from feral mice, including the wild mouse ecotropic MuLVs CasBr-E and 1504 E, the Cas E no. 1 xenotropic MuLV, and the amphotropic MuLVs 1504A and 4070A.

The anemia and polycythemia strains of spleen focus-forming virus (SFFV) were tested for membrane fluorescence with MAb 83A25 and were found to be negative (Table 2). Since SFFV-infected cells have been reported to exhibit low levels of cell surface envelope protein (48), we also examined SFFV-infected cells for reactivity with MAb 83A25 by cytoplasmic fluorescence. Under conditions in which cells infected with an amphotropic virus (1504A) were easily identified by cytoplasmic fluorescence, assays of SFFV-infected cells were negative (data not shown), suggesting that the gene products of this virus do not contain the appropriate epitope.

The finding that MAb 83A25 reacts with nearly all MuLVs indicated the presence of a highly conserved epitope among MuLVs. Yet the presence of this epitope is not required for efficient viral replication, as evidenced by the lack of reactivity of the MAb with F-MuLV or R-MuLV, both of which are fully replication-competent viruses. The lack of reactivity of MAb 83A25 with F-MuLV was observed with all strains tested and may reflect the common origin of these strains from a single original isolate (17). However, R-MuLV, which exhibits very similar biological and structural properties to F-MuLV, is an independent isolate (46), suggesting that F-MuLV and R-MuLV may have originated from a common mouse genomic sequence which does not encode an epitope reactive with MAb 83A25.

Utility of MAb 83A25 in quantitative assays of MuLVs. The observation that the epitope reactive with MAb 83A25 was detectable on the surface of nearly all MuLV-infected cells suggested that it may a useful target for a broadly applicable quantitative assay of MuLVs. Figure 1 illustrates the detection of focal infections of ecotropic, amphotropic, polytropic, and xenotropic MuLVs with different cell lines. The foci in Fig. 1 were developed on live monolayers and could also be developed on fixed cells (not shown). In the former instance, viruses can be biologically cloned by subculturing foci of infected cells directly from the assay (50). Focal infections were apparent for all reactive viruses and could be enumerated on most of the permissive cell lines. In the case of the amphotropic MuLVs, a direct assay has not been previously available. Titrations of the amphotropic virus 1504A on *M. dunni* cells and on mink cells are shown in Fig. 2. The assay was dose responsive and exhibited one-hit kinetics with either cell line, although the *M. dunni* cells were more susceptible to infection than were the mink cells. Mink cell cultures from the assay which were infected at dilutions of virus beyond the detection of fluorescent foci ($>6.4 \times 10^4$) did not show evidence of infection upon subculturing or after cocultivation with *M. dunni* cells. Interestingly, infection of human cell lines (SCL1 or HeLa cells) did not result in discernible cell surface fluorescence in the assay. However, when the infected human cells were subcultured (1:10) for 1 week and subsequently seeded as infectious centers with *M. dunni* cells (10^3 human cells with 5×10^4 *M. dunni* cells), foci of infected *M. dunni* cells could be detected and endpoints of infection of the human cells by amphotropic virus could be determined. Endpoint dilutions of amphotropic MuLV infection of SCL1 and HeLa cells determined by cocultivation with *M. dunni* cells were $10^{1.4}$ and $10^{4.9}$, respectively. Thus, large differences in the susceptibility to infection by the amphotropic virus exist between the human cell lines as well as among cell lines from heterologous species.

Other fluorescence applications of MAb 83A25 include flow cytometry procedures and the detection of MuLV expression in histological sections (data not shown). In these applications, the MAb was used in conjunction with a fluorescein isothiocyanate-conjugated polyclonal anti-rat immunoglobulin serum adsorbed with mouse immunoglobulin.

MAb 83A25 reacts with MuLV envelope glycoproteins. Several different virus-encoded proteins have been detected by MAbs on the surface of MuLV-infected cells (5, 6, 8). These include *gag*-encoded as well as *env*-encoded proteins. The MuLV envelope glycoprotein was identified by immunoblotting to be the protein containing the epitope reactive with MAb 83A25 (Fig. 3). Virtually identical results were obtained by using immunoprecipitation procedures (data not shown). Immunoblots of purified virus preparations from representatives of each of the virus classes were done in parallel by using either MAb 83A25 or a broadly reactive anti-gp70 serum. Of the viruses tested, all reacted except F-MuLV 57, which is in agreement with the membrane fluorescence data presented earlier. It is noteworthy that MAb 83A25 is the first reported MAb that is reactive with the envelope glycoproteins of amphotropic MuLVs. Two proteins in the F-MCF-E virion preparation were detected by using the polyclonal anti-gp70 serum on the immunoblot in Fig. 3, whereas MAb 83A25 reacted with only the larger protein. The F-MCF-E used for the immunoblot was a virion preparation containing both F-MCF-1E (a polytropic isolate subsequently cloned from the F-MCF-E stock) and F-MCF-1. It is likely that the smaller protein which was unreactive with MAb 83A25 corresponds to the envelope glycoprotein of F-MCF-1. Variation in the intensity of the glycoprotein band among the different viruses was observed with both the antiserum and the MAb. The virion preparations employed in the immunoblots and radioimmune precipitations were normalized with respect to total protein; however, the level of the envelope glycoproteins can vary widely in different virus preparations. Thus, it is not clear if the different intensities of the bands in Fig. 3 are due to different levels of the glycoproteins in the various preparations or, alternatively, to variations in the efficiency of detection of the glycoproteins. Considering that MAb 83A25 detected denatured envelope glycoproteins (Fig. 3), it is likely that it is reactive with a contiguous rather than a conformational epitope.

The immunofluorescence studies presented earlier suggested that the SFFV does not encode a protein containing the epitope reactive with MAb 83A25. To further test the reactivity of SFFV-encoded proteins with MAb 83A25, extracts of metabolically radiolabeled infected cells were subjected to immunoprecipitation procedures by using MAb

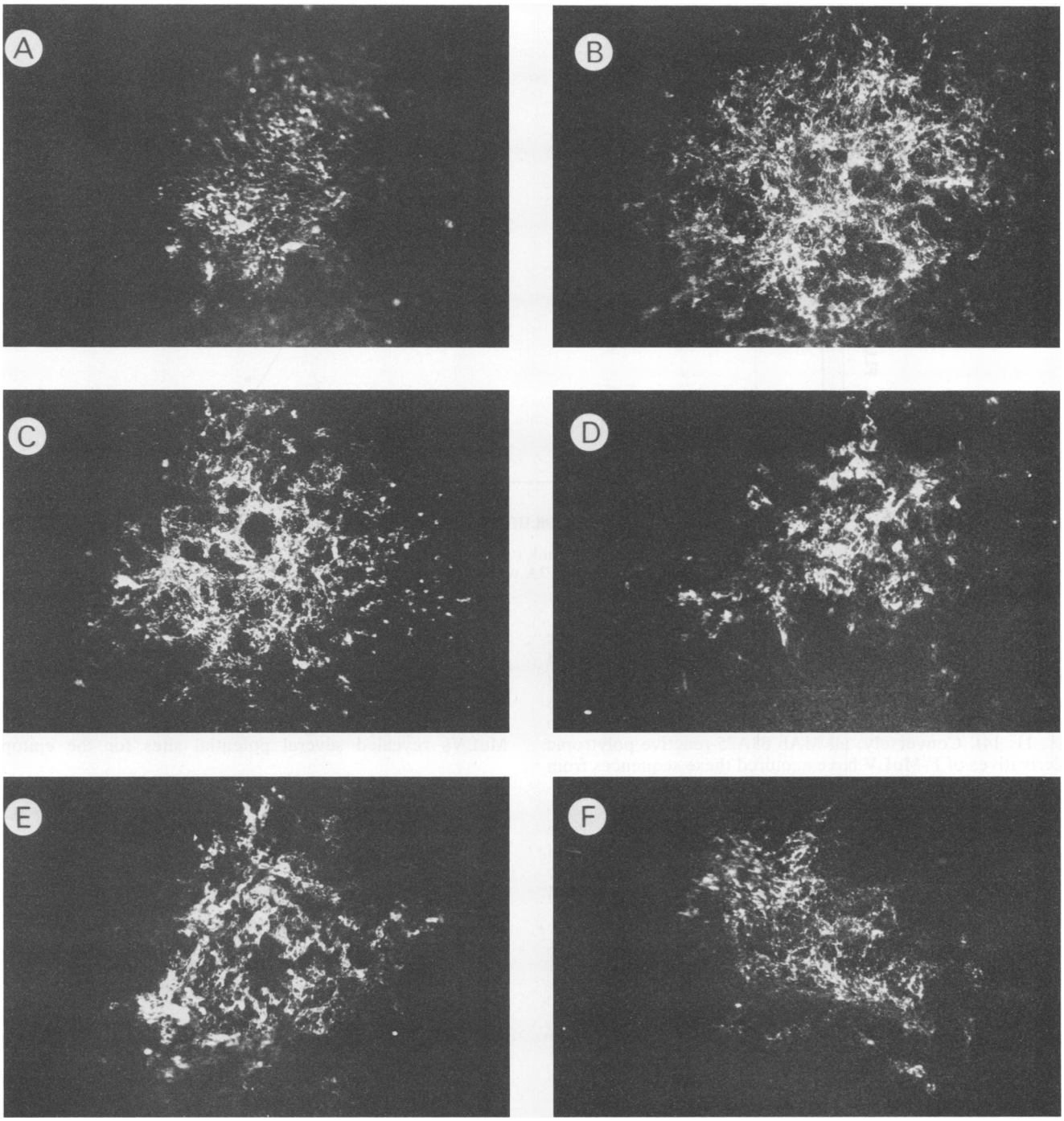

FIG. 1. Fluorescence detection of ecotropic, polytropic, xenotropic, and amphotropic MuLV infections on live monolayers with MAb 83A25. Following infection of cell cultures by MuLVs, the cells were allowed to grow to confluency and were assayed for focal infections by the FIA with MAb 83A25. (A) Ecotropic MuLV AKR 2A on *M. dunni* cells; (B) polytropic MuLV M72P-S on *M. dunni* cells; (C) polytropic MuLV M73P-S on *M. dunni* cells; (D) polytropic MuLV M73P-S on Mv1Lu mink lung fibroblasts; (E) xenotropic MuLV Balb IU-1 on Mv1Lu mink lung fibroblasts; (F) amphotropic MuLV 1504A on Mv1Lu mink lung fibroblasts. Magnification, ×25 before enlargement.

83A25 or polyclonal antiserum to the envelope glycoprotein. The antiserum readily precipitated the envelope glycoprotein-related products from SFFV-infected cells (gp55) and the glycoprotein precursor from amphotropic MuLV 1504A-infected cells, whereas MAb 83A25 precipitated proteins from 1504A-infected cells but failed to precipitate proteins from SFFV-infected cells (data not shown). These results further indicated that SFFV does not encode an epitope reactive with MAb 83A25.

The epitope reactive with MAb 83A25 is located near the carboxyl terminus of envelope glycoproteins. The lack of reactivity of MAb 83A25 with F-MuLV and certain polytropic MuLVs allowed us to deduce the probable location of the reactive epitope on the glycoproteins. As noted earlier,

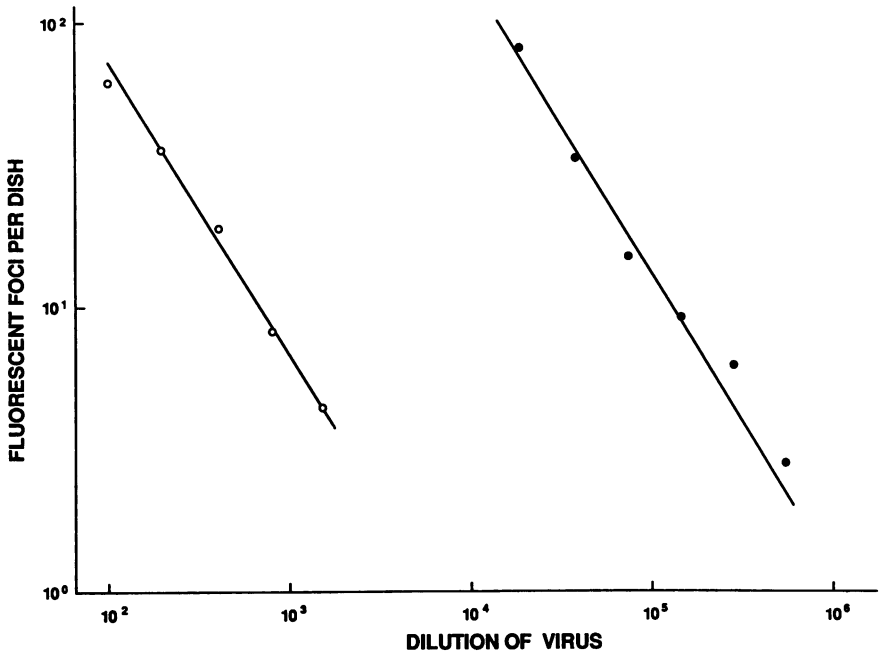

FIG. 2. Titration of amphotropic MuLV 1504A infection of mink (○) and *M. dunni* (●) cells. Cells were infected with twofold serial dilutions of amphotropic MuLV 1504A and assayed by using the FIA with MAb 83A25.

the unreactive polytropic MuLVs were limited to those derived by recombination with F-MuLVs. Comparisons of the genomic structures of these derivatives revealed that each of them has retained the F-MuLV gene sequences encoding the carboxyl terminus of the envelope glycoprotein (1, 11, 14). Conversely, all MAb 83A25-reactive polytropic derivatives of F-MuLV have acquired these sequences from endogenous polytropic virus-like sequences (11). Furthermore, the replication-defective SFFVs, which are also recombinant derivatives of F-MuLVs, exhibit *env* gene deletions which include the region encoding the carboxyl terminus of the envelope glycoprotein (14, 57). A schematic representation of the genomic structures of reactive and nonreactive F-MuLV recombinant derivatives is presented in Fig. 4. It is quite likely from these comparisons that the epitope reactive with MAb 83A25 is encoded by sequences within the 3′ one-fourth of the envelope glycoprotein-coding region. Comparisons of published amino acid sequences of MuLVs revealed several potential sites for the epitope

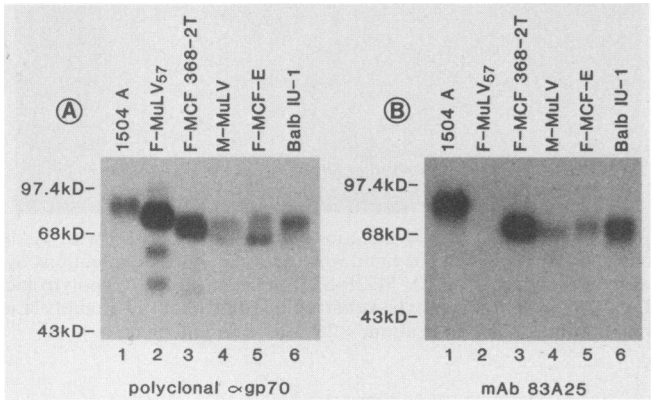

FIG. 3. Protein blot immunoassay of virion proteins with MAb 83A25. Virion preparations were dissociated, electrophoresed in 12.5% sodium dodecyl sulfate-polyacrylamide gels, and electrophoretically transferred to nitrocellulose paper. Parallel blots were reacted with goat anti-gp70 serum (A) or with MAb 83A25 (B), and bound antibody was detected as described in Materials and Methods.

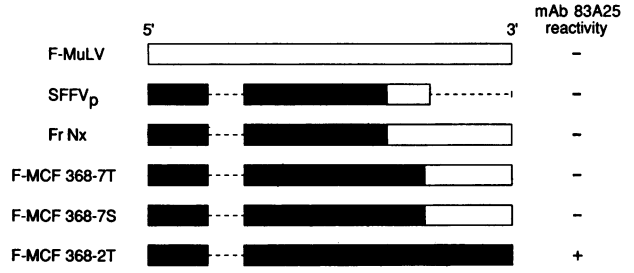

FIG. 4. Ecotropic and polytropic regions of the envelope glycoproteins of recombinant derivatives of F-MuLV. Sequences encoding the envelope glycoproteins of F-MuLV, of MAb 83A25-reactive and -unreactive F-MuLV-derived polytropic MuLVs, and of the Lilly-Steeves strain of SFFV are depicted by bar diagrams. Open (white) regions of the diagrams correspond to sequences derived from the ecotropic F-MuLV parent. Solid (black) regions correspond to sequences derived from endogenous polytropic virus-like sequences. Areas of the bar diagrams which are interrupted by dashed lines correspond to sequences which are deleted from the polytropic and SFFV envelope genes, compared with the ecotropic F-MuLV sequences. The indicated deletions have been confirmed by nucleotide sequence analysis in the case of $SFFV_p$ (57) and FrNx (1) and are assumed to be present in the remaining polytropic isolates. Additional small deletions which minimally affect the colinearity between F-MuLV and its derivatives are not indicated. Five additional F-MCF isolates not included in the figure (F-MCFs 368-2S, 368-3T, 368-5T, 368-5S, and 368-6T) have derived their entire gp70 coding sequences from endogenous polytropic virus-like sequences similar to F-MCF 368-2T and were all reactive with MAb 83A25.

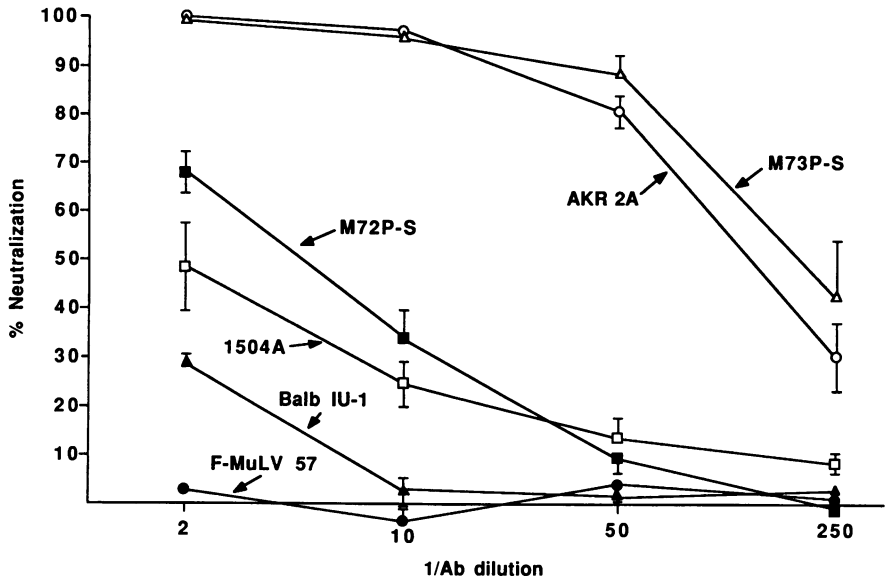

FIG. 5. Neutralization of MuLV infectivity by MAb 83A25. Stocks of ecotropic (AKR 2A), Class I polytropic (M73P-S), Class II polytropic (M72P-S), xenotropic (Balb IU-1), and amphotropic (1504A) MuLVs were incubated with dilutions of hybridoma 83A25 tissue culture medium for 30 min. The treated MuLV stocks were subsequently assayed in triplicate for infectivity on *M. dunni* cells (AKR 2A, M73P-S, M72P-S, and 1504A) or Mv1Lu mink lung fibroblasts (Balb IU-1) by using the FIA. The limit bars indicate the standard error for that determination. For the sake of clarity, overlapping standard error bars are shown in only one direction.

distributed within this region. Thus, further studies will be necessary to more precisely define the immunoreactive domain.

Virus neutralization by MAb 83A25. Strong determinants of the host range of MuLVs reside in the amino-terminal half of the viral *env* gene and likely correspond to receptor-binding regions. From our results described above, it would appear that the epitope reactive with MAb 83A25 lies outside of the receptor-binding domain. Nevertheless, the MAb was found to neutralize MuLV infectivity (Fig. 5). Most anti-MuLV MAbs tested in this laboratory have exhibited neutralizing activity against MuLVs only when facilitated by guinea pig complement (6). In contrast, MAb 83A25 exhibited significant neutralization of reactive MuLVs in the absence of added complement and without the use of more-concentrated MAb preparations such as ascites fluid or affinity-purified antibody. In control experiments to test for nonspecific inhibition, unreactive MAbs failed to influence the infectivity (not shown). F-MuLV, shown above to be unreactive by immunofluorescence, immunoprecipitation, and immunoblotting, was not neutralized by MAb 83A25, further indicating the specificity of neutralization.

The neutralization titers varied greatly with different reactive MuLVs, ranging from 50% neutralization titers of approximately 1:100 with the ecotropic virus AKR 2A and the polytropic MuLV M73P-S to titers of less than 1 with the xenotropic virus Balb IU-1, using unconcentrated tissue culture supernatant from the hybridoma culture. The efficiency of neutralization did not correlate with virus interference groups, in that the neutralization titer for the polytropic MuLV M73P-S was substantially higher (50-fold) than for the polytropic MuLV M72P-S. Both of these viruses were derived from AKR/J mice and have 5′ *env* gene sequences that are indistinguishable by T_1-oligonucleotide fingerprinting (13). However, M73P-S, which exhibits neutralization properties nearly identical to those of the ecotropic virus from AKR/J mice (Fig. 5; AKR 2A), has derived the carboxyl one-third of the glycoprotein from the ecotropic MuLV parent, whereas the allelic region of M72P-S is derived from endogenous polytropic-like retroviral sequences. Thus, our results suggest that the efficiency of neutralization correlates with the identity of the carboxyl region of the envelope glycoprotein rather than the interference properties which reflect the receptor-binding region in the amino-terminal region of the protein. In this regard, a neutralizing MAb reactive with the carboxyl end of the gp70 from the ecotropic MuLV of AKR mice has been described (35, 42). It was suggested that additional determinants which influence binding of gp70 to cellular receptors may reside in the carboxyl region of the molecule.

The finding of such a broadly distributed neutralizable epitope of MuLV envelope glycoproteins is surprising, particularly in the case of the amphotropic MuLVs. Although antigenic cross-reactivity is found between the major structural protein (p30) and the transmembrane protein (p15E) of amphotropic and other MuLVs, the lack of cross-neutralization by antibodies directed at the envelope glycoproteins of other MuLVs has been an important criterion distinguishing the amphotropic MuLVs (22).

LITERATURE CITED

1. **Adachi, A., K. Sakai, N. Kitamura, S. Nakanishi, O. Niwa, M. Matsuyama, and A. Ishimoto.** 1984. Characterization of the *env* gene and long terminal repeat of molecularly cloned Friend mink cell focus-inducing virus DNA. J. Virol. **50**:813–821.
2. **Boukamp, P., W. Tilgen, R. T. Dzarlieva, D. Breitkreutz, D. Haag, R. K. Riehl, A. Bohnert, and N. E. Fusenig.** 1982. Phenotypic and genotypic characteristics of a cell line from a squamous cell carcinoma of human skin. JNCI **68**:415–427.
3. **Britt, W. J., J. K. Collins, and B. Chesebro.** 1981. New cell surface gp70 related to Friend mink cell focus-inducing virus is expressed on Friend virus-induced erythroleukemic spleen cells after elimination of Friend virus gp70 in Rfv-$3^{r/s}$ mice. J. Exp. Med. **154**:868–882.
4. **Chattopadhyay, S. K., M. R. Lander, S. Gupta, E. Rands, and D. R. Lowy.** 1981. Origin of mink cytopathic focus-forming (MCF) viruses: comparison with ecotropic and xenotropic murine leukemia virus genomes. Virology **113**:465–483.

5. **Chesebro, B., W. Britt, L. Evans, K. Wehrly, J. Nishio, and M. Cloyd.** 1983. Characterization of monoclonal antibodies reactive with murine leukemia viruses: use in analysis of strains of Friend MCF and Friend ecotropic murine leukemia virus. Virology **127**:134–148.
6. **Chesebro, B., K. Wehrly, M. Cloyd, W. Britt, J. Portis, J. Collins, and J. Nishio.** 1981. Characterization of mouse monoclonal antibodies specific for Friend murine leukemia virus-induced erythroleukemia cells: Friend-specific and FMR-specific antigens. Virology **112**:131–144.
7. **Chesebro, B., K. Wehrly, J. Nishio, and L. Evans.** 1984. Leukemia induction by a new strain of Friend mink cell focus-inducing virus: synergistic effect of Friend ecotropic murine leukemia virus. J. Virol. **51**:63–70.
8. **Cloyd, M. W., B. Chesebro, J. L. Portis, and M. Weir.** 1982. MCF-specific murine monoclonal antibodies made against AKR-247 MCF virus recognize a unique determinant associated with the gp70-p15(E) complex. J. Virol. **41**:1112–1117.
9. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1979. Cell surface antigens associated with recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **149**:702–712.
10. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **151**:542–552.
11. **Evans, L. H., and M. W. Cloyd.** 1984. Generation of mink cell focus-forming viruses by Friend murine leukemia virus: recombination with specific endogenous proviral sequences. J. Virol. **49**:772–781.
12. **Evans, L. H., and M. W. Cloyd.** 1985. Friend and Moloney murine leukemia viruses specifically recombine with different endogenous retroviral sequences to generate mink cell focus-forming viruses. Proc. Natl. Acad. Sci. USA **82**:459–463.
13. **Evans, L. H., and F. C. Malik.** 1987. Class II polytropic murine leukemia viruses (MuLVs) of AKR/J mice: possible role in the generation of class I oncogenic polytropic MuLVs. J. Virol. **61**:1882–1892.
14. **Evans, L. H., M. Nunn, P. H. Duesberg, D. H. Troxler, and E. M. Scolnick.** 1980. RNAs of defective and nondefective components of Friend anemia and polycythemia virus strains identified and compared. Cold Spring Harbor Symp. Quant. Biol. **44**:823–835.
15. **Fink, M. A., L. R. Sibal, N. A. Wivel, C. A. Cowles, and T. E. O'Conner.** 1969. Some characteristics of an isolated group antigen common to most strains of murine leukemia virus. Virology **37**:387–394.
16. **Fischinger, P. J., S. Nomura, and D. P. Bolognesi.** 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. Proc. Natl. Acad. Sci. USA **72**:5150–5155.
17. **Friend, C.** 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J. Exp. Med. **105**:307–318.
18. **Geering, G., L. J. Old, and E. A. Boyse.** 1966. Antigens of leukemias induced by naturally occurring murine leukemia virus: their relation to the antigens of Gross virus and other murine leukemia viruses. J. Exp. Med. **124**:753–772.
19. **Gey, G. O., W. D. Coffman, and M. T. Kubicek.** 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. **12**:264–265.
20. **Gregoriades, A., and L. J. Old.** 1969. Isolation and some characteristics of a group specific antigen of the murine leukemia viruses. Virology **37**:189–202.
21. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology **65**:128–134.
22. **Hartley, J. W., and W. P. Rowe.** 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. J. Virol. **19**:19–25.
23. **Hartley, J. W., W. P. Rowe, W. I. Capps, and R. J. Huebner.** 1965. Complement fixation and tissue culture assays for mouse leukemia viruses. Proc. Natl. Acad. Sci. USA **53**:931–938.
24. **Hartley, J. W., W. P. Rowe, W. I. Capps, and R. J. Huebner.** 1969. Isolation of naturally occurring viruses of the murine leukemia group in tissue culture. J. Virol. **3**:126–132.
25. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia viruses associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. USA **74**:789–792.
26. **Henderson, I. C., M. M. Lieber, and G. J. Todaro.** 1984. Mink cell line Mv1Lu (CCL-64). Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology **60**:282–287.
27. **Kohler, G., and C. Milstein.** 1976. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur. J. Immunol. **6**:511–519.
28. **Lander, M. R., and S. K. Chattopadhyay.** 1984. A *Mus dunni* cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ecotropic, amphotropic, xenotropic, and mink cell focus-forming viruses. J. Virol. **52**:695–698.
29. **Levy, J. A., P. Kazan, O. Varnier, and H. Kleiman.** 1975. Murine xenotropic type C viruses. I. Distribution and further characterization of the virus in NZB mice. J. Virol. **16**:844–853.
30. **Lostrom, M. E., M. R. Stone, M. Tam, W. N. Burnette, A. Pinter, and R. C. Nowinski.** 1979. Monoclonal antibodies against murine leukemia viruses: identification of six antigenic determinants on the p15(E) and gp70 envelope proteins. Virology **98**:336–350.
31. **Mathieu-Mahul, D., J. M. Heard, S. Fichelson, S. Gisselbrecht, B. Sola, and C. J. Larsen.** 1982. Viral expression in two myelomonocytic cell lines obtained from long-term bone marrow culture infected with the Friend polycythemia-inducing virus (FV-P). Virology **119**:59–67.
32. **McAtee, F. J., and J. L. Portis.** 1985. Monoclonal antibodies specific for wild mouse neurotropic retrovirus: detection of comparable levels of virus replication in mouse strains susceptible and resistant to paralytic disease. J. Virol. **56**:1018–1022.
33. **Morrison, R. P., K. Lyng, and H. D. Caldwell.** 1989. Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57-kD protein. J. Exp. Med. **169**:663–675.
34. **Nowinski, R. C., M. E. Lostrom, M. R. Tam, M. R. Stone, and W. N. Burnette.** 1979. The isolation of hybrid cell lines producing monoclonal antibodies against the p15(E) protein of ecotropic murine leukemia viruses. Virology **93**:111–126.
35. **Nowinski, R. C., R. Pickering, P. V. O'Donnell, A. Pinter, and U. Hammerling.** 1981. Selective neutralization of ecotropic murine leukemia virus by monoclonal antibodies: location of a site on the gp70 protein associated with ecotropism. Virology **111**:84–92.
36. **Obata, Y., H. Ikeda, E. Stockert, and E. A. Boyse.** 1975. Relation of G_{IX} antigen of thymocytes to envelope glycoprotein of murine leukemia virus. J. Exp. Med. **141**:188–197.
37. **Obata, Y., E. Stockert, A. B. DeLeo, P. V. O'Donnell, H. W. Snyder, and L. J. Old.** 1981. $G_{(ERLD)}$: a cell surface antigen of the mouse related to xenotropic MuLV defined by naturally occurring antibody and monoclonal antibody. J. Exp. Med. **154**:659–675.
38. **O'Donnell, P. V., and E. Stockert.** 1976. Induction of G_{IX} antigen and Gross cell surface antigen after infection by ecotropic and xenotropic murine leukemia viruses in vitro. J. Virol. **20**:545–554.
39. **Oliff, A. I., G. L. Hager, E. H. Chang, E. M. Scolnick, H. W. Chan, and D. R. Lowy.** 1980. Transfection of molecularly cloned Friend murine leukemia virus DNA yields a highly leukemogenic helper-independent type C virus. J. Virol. **33**:475–486.
40. **Oroszlan, S., C. L. Fisher, T. B. Stanley, and R. V. Gilden.** 1970. Proteins of the murine C-type RNA viruses. I. Isolation of a group-specific antigen by isoelectric focusing. J. Gen. Virol. **8**:1–10.
41. **Ostertag, W., and I. B. Pragnell.** 1978. 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. USA **75**:3278–3282.
42. **Pinter, A., W. J. Honnen, J.-S. Tung, P. V. O'Donnell, and U. Hammerling.** 1982. Structural domains of endogenous murine leukemia virus gp70s containing specific antigenic determinants defined by monoclonal antibodies. Virology **116**:499–516.

43. **Portis, J. L., and F. J. McAtee.** 1983. Monoclonal antibodies derived during graft-versus-host reaction. II. Antibodies detect unique determinations common to many MCF viruses. Virology **126**:96–105.
44. **Portis, J. L., F. H. McAtee, and M. W. Cloyd.** 1982. Monoclonal antibodies to xenotropic and MCF murine leukemia viruses derived during the graft versus host reaction. Virology **118**:181–190.
45. **Pragnell, I. B., A. McNab, P. R. Harrison, and W. Ostertag.** 1978. Are spleen focus-forming virus sequences related to xenotropic viruses and expressed specifically in normal erythroid cells? Nature (London) **272**:456–458.
46. **Rauscher, F. J.** 1962. A virus-induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia. J. Natl. Cancer Inst. **29**:515–543.
47. **Rein, A., and A. Schultz.** 1984. Different recombinant murine leukemia viruses use different cell surface receptors. Virology **136**:144–152.
48. **Ruta, M., and D. Kabat.** 1980. Plasma membrane glycoproteins encoded by cloned Rauscher and Friend spleen focus-forming viruses. J. Virol. **35**:844–853.
49. **Schafer, W., F. A. Anderer, H. Bauer, and L. Pister.** 1969. Studies on mouse leukemia viruses. I. Isolation and characterization of a group-specific antigen. Virology **38**:387–394.
50. **Sitbon, M., J. Nishio, K. Wehrly, D. Lodmell, and B. Chesebro.** 1985. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range class in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. Virology **141**:110–118.
51. **Sitbon, M., B. Sola, L. Evans, J. Nishio, S. F. Hayes, K. Nathanson, C. F. Garon, and B. Chesebro.** 1986. Hemolytic anemia and erythroleukemia, two distinct pathogenic effects of Friend MuLV: mapping of the effects to different regions of the viral genome. Cell **47**:851–859.
52. **Stockert, E., A. B. DeLeo, P. V. O'Donnell, Y. Obata, and L. J. Old.** 1979. $G_{(AKSL2)}$: a new cell surface antigen of the mouse related to the dualtropic mink cell focus-inducing class of murine leukemia virus detected by naturally occurring antibody. J. Exp. Med. **149**:200–215.
53. **Stockert, E., L. J. Old, and E. A. Boyse.** 1971. The G_{IX} system. A cell surface allo-antigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. J. Exp. Med. **133**:1334–1355.
54. **Todaro, G. J., P. Arnstein, W. P. Parks, E. H. Lennette, and R. J. Huebner.** 1973. A type C virus in human rhabdomyosarcoma cells after inoculation into NIH Swiss mice treated with anti-thymocyte serum. Proc. Natl. Acad. Sci. USA **70**:859–862.
55. **Troxler, D. H., E. Yuan, D. Linemeyer, S. Ruscetti, and E. M. Scolnick.** 1978. Helper-independent mink cell focus-inducing strains of Friend murine type-C virus: potential relationship to the origin of replication-defective spleen focus-forming virus. J. Exp. Med. **148**:639–653.
56. **Tung, J. S., E. S. Vitetta, E. Fleissner, and E. A. Boyse.** 1975. Biochemical evidence linking the G_{IX} thymocyte surface antigen to the gp69/71 envelope glycoprotein of murine leukemia virus. J. Exp. Med. **141**:198–205.
57. **Wolff, L., E. Scolnick, and S. Ruscetti.** 1983. Envelope gene of the Friend spleen focus-forming virus: deletion and insertions in 3′ gp70/p15E-encoding region have resulted in unique features in the primary structure of its protein product. Proc. Natl. Acad. Sci. USA **80**:4718–4722.