Assessment of Reactivities of Natural Antibodies to Endogenous RNA Tumor Virus Envelope Antigens and Virus-induced Cell Surface Antigens

M. G. Hanna, Jr., James N. Ihie, Barry L. Batzing, Raymond W. Tennant, and Cynthia K. Schenley

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

SUMMARY

The autogenous humoral immune response of mice to their endogenous leukemia virus (MuLV) has been examined with respect to the reactivities of natural antibodies to MuLV envelope antigens and virus-induced cell surface antigens. The natural reactivity of MuLV envelope antigens was evaluated by means of a radioimmune precipitation assay of intact and disrupted virus, as well as by virus neutralization tests. The specificity of natural antibody for MuLV envelope antigens was determined by immunoelectron microscopy and radioimmune precipitation. Antibody reactivity to virus-induced cell-surface antigens was evaluated by immunoelectron microscopy and a complement-dependent cytotoxicity test. The strains of mice selected for study were C57BL/6, C3H/Anf, and the C57BL/6 × C3H/Anf F1 hybrid. Although there were quantitative differences in the antibody levels among these various strains, the naturally recognized antigenic determinants of the virus were consistent, i.e., gp68, gp43, and p15. High levels of neutralizing antibody against the xenotropic BALB: virus-2 were detected in these various normal sera with the focus reduction assay; however, only marginal levels of neutralizing activity against Moloney leukemia virus were detected with the XC virus assay. No antacellular antibody could be detected in these normal sera with the complement-dependent cytotoxicity assay.

INTRODUCTION

The concept that mice are immunologically tolerant or hyporesponsive to their endogenous RNA tumor virus(es) is contradicted by published evidence. Early studies of naturally acquired immunity to endogenous MuLV3 (3, 4, 16) and others (2, 22), however, have recently demonstrated the widespread occurrence of natural antibody with specificity for VEA. The interaction between the natural immunities to VEA and to virus-induced cell-surface antigens and the significance of these reactivities in host control of viral expression and virus-mediated pathogenesis have not been resolved. With respect to the biological nature of the spectrum of natural antibody specific for MuLV-associated antigens, it would be important to determine whether virus-neutralizing as well as anticellular (cytotoxic) antibodies coexist in autogenous immune sera.

The purpose of this study was to correlate the reactivities of natural antibodies to VEA and virus-induced cell-surface antigens. Evaluations of the natural reactivity to VEA were made with a radioimmune precipitation assay of intact and disrupted virus and with virus neutralization tests. Antibody reactivity to virus-induced cell-surface antigens was evaluated by immunoelectron microscopy and complement-dependent serum cytotoxicity tests. The strains of mice selected for the study were C57BL/6, C3H/Anf, and the C57BL/6 × C3H/Anf F1 (hereafter called B6C3F1) hybrid. The B6C3F1, mice have a mean survival time of approximately 884 days and a low natural incidence of thymic lymphoma; a similar hybrid from a reciprocal mating has a much lower susceptibility to Gross virus as newborns than the parental strains (17). This resistance has been associated with the Rgy-1 locus, which is located proximal to or within the K-region of H-2, as is the Ir-1 gene, which determines immune responsiveness to certain antigens (18, 19). For this reason we feel that it is important that this strain combination be studied, since in this genetic combination the Ir-I and Rgy-1 genes may be identical and function in the immune control of the MuLV-associated antigens.

MATERIALS AND METHODS

Animals. Male B6C3F1 hybrids, C57BL/6 retired breeders from an inbred C57BL/6 colony, and C3H/Anf male retired breeders were used in these studies. The animals ranged in age from 4 weeks to 24 months. All mice were specific-pathogen free.

Test Sera. Blood was collected by orbital bleeding or
cardiac puncture and allowed to clot at room temperature, and serum was separated by centrifugation. Serum was aliquoted and frozen at −70° until use. Samples of serum were never thawed more than once.

Anti-K-36 serum was prepared by immunizing C57BL/6 males with K-36 cells derived and passaged in AKR mice. The immunization regimen consisted of an initial s.c. injection of 2 × 10⁶ cells, followed by 4 i.p. injections of 2 × 10⁶ cells every 2 weeks. Mice were bled 10 to 14 days after the last immunization, and serum was prepared as described above.

**Immunoelectron Microscopy.** For electron microscopy, an indirect immunoferritin-labeling procedure was used, as described previously (8). Target cells included (a) E2G2 leukemia cells maintained by weekly i.p. transfer into male C57BL/6 mice, (b) an established AKR mouse embryo cell line that had spontaneously initiated replication of an RNA tumor virus, and (c) a cell line that was established from a BALB/c plasma cell tumor induced with mineral oil and that, upon continuous in vivo passage, spontaneously produced C-type virus.

**Radioimmune Precipitation Assays against Intact and Disrupted Virus.** [*3H]Leucine-labeled virus was prepared as previously described (13). For radioimmune precipitation of intact virus, the test serum (0.2 ml) was serially diluted 2-fold in TNE, 0.05 ml (6000 cpm) of labeled virus was added, and the mixture was incubated for 1 hr at 37°. Subsequently, an equal volume of 0.2 ml of a polyclonal anti-γ-globulin (Cappel rabbit anti-mouse γ-globulin) diluted 1:2 in TNE was added, and the mixture was incubated again at 37° for 1 hr and, finally, at 4° for 2 hr. The precipitates were collected by centrifugation at 1200 × g for 10 min, and the supernatant was removed for determination of radioactivity. The pellets were washed 3 times in a total of 3 ml TNE, resuspended in 0.4 ml TNE, and prepared for counting. All samples were counted in 10 ml Aquasol (New England Nuclear, Boston, Mass.) in a Nuclear-Chicago scintillation counter. Precipitation is expressed as the percentage of counts in the precipitate relative to the combined counts in the precipitate in the 1st supernatant. A number of parameters of the radioimmune precipitation assay were found to affect the results, as described in a previous publication (13).

Radioimmune precipitation assays against disrupted virus have been described in detail elsewhere (11). Intact [*3H]leucine-labeled AKR virus, purified, and recovered from isopyknic sucrose gradient, was disrupted in an equal volume of disruption buffer (1.2 ml KCl-1% Triton X-100-0.03 M β-mercaptoethanol-0.1 M Tris, pH 7.5). The virus was disrupted at 4° for 1 hr. Twenty-five to 50 μl of test serum were allowed to react with 2 × 10⁶ cpm of virus and subsequently were precipitated with polyclonal anti-γ-globulin (Cappel rabbit anti-mouse γ-globulin). The precipitates were washed 4 times in TNE and then sedimented through a cushion of 25% sucrose in TNE containing 0.5% deoxycholate. The precipitates were resuspended in TNE and pelleted at 1200 × g for 20 min. The pellets were carefully dried and resuspended in 0.05 ml of 1% sodium dodecyl sulfate-1% β-mercaptoethanol in 0.01 M sodium phosphate buffer, pH 7.4, and incubated at 60° for 1 hr and at 37° overnight to dissolve.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the precipitates was performed by the method of Weber and Osborn (27). Bromophenol blue was used as a reference standard to determine relative mobilities and was generally allowed to migrate 8 cm. Standard protein samples were used to calibrate the system. Radioactivity profiles of the gels were obtained by sectioning the gels in 1-mm sections with a Mickel gel slicer, dissolving individual sections in 0.2 ml of 30% hydrogen peroxide at 75° overnight, and determining radioactivity in a liquid scintillation counter after the addition of 10 ml of Aquasol.

**Complement-dependent Cytotoxicity Assays.** Cells used in these tests included E2G2 maintained by weekly transfer in C57BL/6 mice, K-36 cells maintained by weekly transfer in male AKR mice, an established virus-producing AKR cell line maintained in tissue culture, and an established non-virus-producing AKR cell line also maintained in tissue culture. All sera were stored at −70° in sterile tubes after Millipore filtration. Rabbit serum was used as a source of complement. Serum from a single rabbit that did not possess cytotoxic antibody to mouse cells was used throughout the study. Serum was stored in small aliquots so that thawing and refreezing were minimal.

Red blood cells present in the suspensions of K-36 and E2G2 were lysed before the cells were used. After centrifugation at 400 × g (4°) for 10 min, target cells were resuspended in Eagle’s basal medium with Earle’s salts and 10% fetal calf serum, counted in a hemocytometer with trypan blue as a viability indicator, and adjusted to 3 × 10⁷ live cells per ml in preparation for labeling with [*51Cr (as Na₂[*51CrO₄]. One ml of each target cell suspension was incubated for 45 min with 100 μCi [*51Cr in sterile 0.9% NaCl solution (New England Nuclear) at 37° in a 5% CO₂ and was shaken occasionally. Labeled cells were washed twice with 50 ml cold medium without serum, suspended in medium with serum, and recounted. A suspension of labeled cells at 1 × 10⁶ live cells per ml was used for all experimental conditions and standards.

Test sera were serially diluted in FPBS. Rabbit serum for C’ was diluted 1:10 in FPBS. Target cells (0.1 ml = 1 × 10⁶ cells) were incubated with 0.1 ml test serum and 0.1 ml C’ or the equivalent volume of FPBS at 37° with constant gentle agitation in glass tubes (10 x 75 mm) covered with Parafilm. After 45 min, 0.7 ml cold phosphate-buffered saline was added to each tube and the tubes were centrifuged for 10 min at 400 × g and 4°. One-half ml of supernatant was transferred to glass tubes (15 x 125 mm) and counted in a Packard Model 3002/model 574 Auto-Gamma spectrometer. Internal controls were (a) target cells for spontaneous [*51Cr release, (b) target cells with C’ but no serum, and (c) target cells with test serum but no C’. Three standards were used for each experiment or target cell: (a) dilutions of the Na₂[*51CrO₄ that was used for labeling, (b) total [*51Cr incorporation (1 ml labeled cells was centrifuged and both the pellet and supernatant were counted), and (c) maximal (100%) [*51Cr release (0.2 ml labeled cell was added to 0.8 ml distilled H₂O and frozen.

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(−70°) and thawed (hot water) 4 times. After centrifugation, 0.5 ml supernatant was counted. Standards, controls, and test conditions were run at least in duplicate.

All results were adjusted to the basis of cpm/10^6 cells, and calculations of percentage cytotoxicity were made by the methods of Johnson et al. (14).

Virus Neutralization Tests. The XC virus assay and neutralization test were performed in cultures of NIH strain Swiss mouse embryo cultures grown in Eagle’s minimal essential medium with 10% fetal calf serum. Moloney leukemia virus (originally obtained from Dr. Wallace P. Rowe, NIH, Bethesda, Md.) was grown in Swiss mouse embryo cultures.

Virus was assayed by the XC cell plaque technique (24). Secondary Swiss mouse embryo cells were treated with DEAE-dextran (25 μg/ml) for 1 hr and washed 3 times with medium before infection. The cells were incubated with virus for 2 hr, washed, given fresh medium, and incubated for 6 days. The infected cells were then exposed to UV (~ 1500 ergs/sq mm) and overlaid with XC cells. The cells were fixed after 4 days, and plaques or syncytia were counted.

Virus neutralization tests were performed by mixing dilutions of serum with ~100 syncytium-forming units of virus. After incubation for 1 hr at room temperature, the virus-serum mixture was inoculated into 2 or 3 cultures treated with DEAE-dextran. After incubation for 2 hr, the cultures were washed, given fresh medium, and incubated. The assay was completed as described above, and the percentage of neutralization was calculated by comparison to control plaque counts.

The focus reduction assay for virus neutralization was performed with normal rat kidney cells grown in Dulbecco’s modified Eagle’s medium containing 10% calf serum, in plastic Petri dishes as previously described (1, 2). Viruses used in the tests were BALB:virus-1 and BALB:virus-2, biologically distinguishable viruses of BALB/c mouse cells, which were propagated in normal rat kidney cells (1). A rat type C virus induced from normal rat kidney cells was also used. Test sera were heat inactivated at 56° for 30 min and filtered through 0.45-μm Millipore filters before use. Neutralization tests were performed by a focus reduction method. Approximately 100 focus-forming units of each helper-virus pseudotype of KiMSV were incubated with serially diluted test serum samples for 30 min at 37°. The surviving virus fraction was assayed on normal rat kidney cells treated with Polybrene (2 μg/ml). The number of KiMSV foci was scored at 7 days. Neutralizing antibody titer is presented as the reciprocal of the highest serum dilution that reduced the number of KiMSV foci by 67% or more.

RESULTS

Specificity of Antibody Reactivity as Determined by Immunoelectron Microscopy. Serum from B6C3F1 mice between 5 weeks and 2 years of age was determined by indirect immunoferritin labeling to contain antibody specific for VEA. When E×G2, K-36 leukemia cells, or an established AKR mouse embryo cell line, which chronically replicates C-type MuLV, were used as target cells, specific antibody was associated with the entire envelope of the majority of budding and free C-type virus particles (Fig. 1a). Only limited areas of the leukemia cell surface were labeled in occasional target cells. No detectable labeling of VEA or virus-induced cell-surface antigens was present when target cells were treated with ferritin conjugate alone. Pooled serum from normal male C3H/Anf breeders showed a labeling pattern similar to the B6C3F1; against these target cells, i.e., consistent labeling of budding and free C-type viruses and restricted labeling of the cell surface in occasional cells (Fig. 1b).

Pooled serum from normal female breeders retired from our C7BL/6 colony also was assayed by immunoelectron microscopy. In these tests, antibodies with specificity for virus-induced cell-surface antigens and VEA were revealed. When E×G2 target cells were used, extended regions of the cell surface were labeled, in addition to the entire envelopes of free and budding C-type virus particles (Fig. 1c). The surface reactivity was qualitatively similar to but quantitatively less than that of C57BL/6 anti-K-36 hyperimmune test serum (Fig. 1d).

Radioimmune Precipitation Titer against Intact Virus. Chart 1 shows a comparison of the levels of antibody specific for VEA in sera of parents and hybrids of similar ages (8 to 12 months) with sera of young adult C7BL/6 males immunized to MuLV-replicating K-36 cells. All test sera precipitated approximately 90% of the virus at lower dilutions, but a weak overall titer was associated with the C3H/Anf parent strain. The C57BL/6 strain definitely showed a higher antibody titer than its C3H/Anf counterpart; in addition, it can also be boosted for VEA antibody by immunization with MuLV-producing K-36 cells. The highest titer was achieved with the B6C3F1, serum, which had a 50% precipitation end point that reached 1:5020.

Antibody Reactivity for Specific Viral Antigens. Sera of the various mouse strains were examined by radioimmune precipitation and gel electrophoresis of the disrupted virus to determine the consistency of the recognition of viral antigenic determinants of the natural antibody. In tests of
B6C3F1 serum, the precipitated viral proteins migrated during electrophoresis with mobilities (relative to bromophenol blue) corresponding to molecular weights of approximately 68,000, 43,000, and 17,000, based on the standard curve shown in Chart 2a. The profile for C57BL/6 serum is qualitatively similar to that for B6C3F1 serum but shows additional virus components with molecular weights of 19,000 and 15,000. The reactivity of C3H mice is also identical to that of C57BL/6 mice with respect to the antigens precipitated but differs quantitatively with respect to the ratio of M.W. 68,000 to M.W. 17,000 components. Although the radioimmune precipitation assay against intact virus indicates that the titer of C57BL/6 anti-K-36 serum is significantly higher than that of the control C57BL/6 serum, gel electrophoresis of an immune precipitate results in a profile almost identical to that of the control serum. An additional minor reactivity against a polypeptide with a molecular weight around 30,000 is found with the C57BL/6 anti-K-36 serum. This reactivity has been detected in several tests with C57BL/6 anti-K-36 serum.

In Vitro Virus Neutralization Tests. We next attempted to determine whether the natural antibody reactivity to viral envelope antigens would neutralize a variety of MuLV in tests in vitro. The results of extensive tests against AKR virus, Moloney virus, BALB: virus-1, and BALB: virus-2, utilizing both the XC plaque assay and a focus cocultivation assay, are shown in Tables 1 and 2. A series of XC neutralization against AKR virus proved highly variable with respect to the virus control. The number of plaques for virus control ranged from approximately 70 to 200. Although there was a suggestion of weak neutralization, the test results were not significant. Moloney virus assays with the XC neutralization tests were more reproducible and, as seen in Table 1, a low level of neutralization was achieved at low serum dilutions. Similar results were achieved in the focus assay when the sera were tested against BALB: virus-1. However, a high level of neutralization was achieved when all sera were tested against the X-tropic BALB: virus-2. The levels of neutralization of C57BL/6, C57BL/6 anti-K-36, C3H/Anf, and B6C3F1 test sera against the BALB: virus-2 are proportional to their radioimmune precipitation titers.

Complement-dependent Serum Cytotoxicity Test. As shown in Table 3, complement-dependent serum cytotoxicity as measured by 51Cr release was achieved with C57BL/6 serum.
Table 3
Complement-dependent serum cytotoxicity assay
Results are shown as percentage cytotoxicity, given as the mean ± S.E. for 3 to 5 tests or as the mean for 2 tests.

<table>
<thead>
<tr>
<th>Test serum</th>
<th>Dilution</th>
<th>K-36</th>
<th>E-G2</th>
<th>AKR high-passaged</th>
<th>AKR low-passaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit C' alone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>11.3</td>
<td>2.1</td>
<td>2.7</td>
<td>0.7</td>
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<tr>
<td></td>
<td>1:8</td>
<td>61.4</td>
<td>43.2</td>
<td>35.5</td>
<td>21.7</td>
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<tr>
<td></td>
<td>1:16</td>
<td>51.4</td>
<td>41.7</td>
<td>54.6</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>1:32</td>
<td>52.3</td>
<td>33.1</td>
<td>63.6</td>
<td>31.1</td>
</tr>
<tr>
<td></td>
<td>1:64</td>
<td>46.9</td>
<td>24.9</td>
<td>51.9</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>1:128</td>
<td>28.4</td>
<td>10.8</td>
<td>39.7</td>
<td>9.6</td>
</tr>
<tr>
<td>C57BL/6 female retired breeder + C'</td>
<td>1:8</td>
<td>5.8</td>
<td>11.2</td>
<td>4.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>7.1</td>
<td>13.3</td>
<td>2.2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>1:32</td>
<td>7.2</td>
<td>14.8</td>
<td>3.2</td>
<td>0.3</td>
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<td>C3H/Anf male retired breeder + C'</td>
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<td>1.2</td>
<td>6.3</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
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<td>4.3</td>
<td>14.0</td>
<td>2.2</td>
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<tr>
<td></td>
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<td>5.2</td>
<td>12.3</td>
<td>2.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>1:32</td>
<td>5.6</td>
<td>8.8</td>
<td>2.9</td>
<td>5.3</td>
</tr>
<tr>
<td>B6C3F, male, 1.5 hr. + C'</td>
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<td>0.8</td>
<td>8.1</td>
<td>0.25</td>
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<tr>
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<tr>
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<td>11.1</td>
<td>0.5</td>
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</tbody>
</table>

anti-K-36 test sera. The reactivity against the AKR non-virus-producing control cell line is considered primarily a result of antibody reactivity against H-2K sites. The enhanced cytotoxicity with this serum against the K-36 and AKR high-passaged virus-producing cell lines is a result of combined reactivity of antibody with H-2K sites as well as virus-induced cell-surface antigens. Proportionally, the anti-virus-induced cell-surface antigen reactivity of this test serum would be expressed by the reactivity against E@G2 cells.

No significant levels of complement-dependent serum cytotoxicity were detected by this assay utilizing C57BL/6 retired breeder serum. This was somewhat surprising, since this serum did show some level of cell surface reactivity by immunoelectron microscopy. While it is inconclusive, the level of reactivity detected by immunoelectron microscopy may be suggested in the cytotoxicity test against E@G2 where a slight increase (10 to 12%) over the control level was achieved. No other normal serum showed such a significant increase over the control level. In some respects this is surprising, since numerous labeled virus particles were detected on the test cells by immunoelectron microscopy. One must conclude that the virus buds rapidly, not allowing time for complement-dependent damage to the cell membrane.

DISCUSSION
It has been clearly shown that, within the strain combinations used in this study, various levels of natural antibody with specificity for endogenous MuLV envelope antigens are present, although there appear to be genetically determined quantitative differences in antibody levels. The specificity of the antibody has been shown by immunoelectron microscopy and radioimmune precipitation. Furthermore, although there are quantitative differences in antibody levels among these various strains, the naturally recognized antigenic determinants of the virus are a consistent aspect of autogenous immunity. The 68,000-dalton component has previously been shown to be a glycoprotein (11) and appears to correspond to gp71, the main constituent of the MuLV surface, as described by Moenning et al. (21) and by Strand and August (26). Similarly, the 43,000-dalton component is also a glycoprotein (11) but appears to be a minor component of the virion surface. The 17,000-dalton component is not glycosylated and has been shown by Ihle et al. (12) to correspond to the p15 virion envelope protein described by Schäfer et al. (25). The consistency among strains of the recognition of natural antigenic determinants of virus envelope is also borne out by the quantitative difference between the levels of intact virus-precipitating antibody in C57BL/6 normal and C57BL/6 anti-K-36 hyperimmune sera and by the similarity of the specific VEA recognized by these 2 sera. It is also interesting to note from these results that antibody with specificity for VEA generated in what would be considered N-, B-, or NB-tropic systems reacts comparably with N- or X-tropic viruses, with respect to both specific immunological recognition and biological function.

It is also important to keep in mind that, in these studies,
normal sera were compared with 1 allogeneic hyperimmune serum. Past immunological studies characterizing virus-associated antigens have primarily used hyperimmune systems or allogeneic or xenogeneic test sera (5, 6). Under those conditions, not only are the virus-associated antigenic determinants measured, but so also is the immunological competence of the hyperimmunized recipients. We feel that studies of the specificity and biological activity of autogenous immune sera with respect to natural antigenic determinants are more valuable for defining the immunological functions required for immune control of viral carcinogenesis, as well as for determining the essential immunological recognition of and reactivities to vaccine preparations to be used for therapeutic purposes (28).

We attempted in this study to determine whether virus-neutralizing and antacellular (cytotoxic) antibodies coexist in autogenous immune serum. On the basis of immunoelectron microscopy, there is variation among strains in natural recognition of virus-induced cell-surface antigens. Since all of the sera tested recognize similar VEA components, the presence of dissimilar VEA and virus-induced cell-surface antigens might be concluded from the present results. In contrast, the results of Kennel et al. (15) suggest that the virus-induced cell-surface antigen is a glycoprotein and has an approximate molecular weight of 70,000 daltons and appears to be identical to the virus envelope antigen gp71. However, the possibility exists that the various heterologous hyperimmune test sera used in the studies by Kennel et al. (15) recognize different antigenic determinants on the same molecule, and the availability of these sites varies, depending upon whether the antigen is a virion knob or a part of the cellular membrane. Another important point is that the presence of p30, the major internal viral protein, has recently been detected on the cell surface of cultured cells infected or transformed by C-type viruses (9, 29). While a question remains as to whether this is consistent and of biological significance (9), the autogenous immune sera would be negative by virtue of its inability to recognize p30 as a natural antigenic determinant, as demonstrated by the present results.

The quantitative levels of natural antibody reactivity to virus-induced cell-surface antigens are below the point of detection in a complement-dependent cytotoxicity assay. Nevertheless, there was a significant level of antibody specific for VEA in all strains tested. While it must be concluded from the immunoelectron microscopy studies that there is reactivity of antibody to budding viruses, the lifetime of the virus as a budding particle on the cell surface must be short, since antibody-complement reactions with budding particles were not capable of damaging the cell surface to an extent that would elicit chromium release.

It is clear that there are high levels of neutralizing antibody, as tested against the xenotropic BALB: virus-2 in the focus-reduction assay. However, there is a marked discrepancy between the biological activity of autogenous immune sera to VEA indicated by the focus-reduction assay of BALB: virus-2 and that indicated by a similar assay of BALB: virus-1 or by XC tests of the Moloney leukemia virus. In a separate communication (16), we have demonstrated that virus neutralization of BALB: virus-2 is a function of the 19 S fraction of normal B6C3F1 serum, and that no neutralization occurs with antibodies of the 7 S fraction. Also, we demonstrated by immunoprecipitation of disrupted virus that the 19 S antibody class precipitates all 3 of the natural antigenic determinants (gp68, gp43, and p15), whereas the 7 S class precipitates only the p15 antigen. Thus it must be concluded that for neutralization of BALB: virus-2 by autogenous immune sera there is a requirement for 19 S antibody and a requirement of reactivity to at least the glycoprotein (gp68 and/or gp43) antigenic determinants of the virus envelope.

It is difficult to reconcile the sensitivity of BALB: virus-2 to neutralization with the lack of sensitivity of the Moloney leukemia virus and BALB: virus-1. There are several possible explanations for this: (a) the sensitivities of the assays, (b) the confirmation of the viral antigenic determinants on the viruses, allowing for radioimmune precipitation with autogenous antibody but not conducive to neutralization, or (c) a marked difference in the number of infectious particles to viral particles in the various viral preparations. We tend to favor the latter factor as one explanation for the capacity of autogenous immune sera to neutralize BALB: virus-2.

Since BALB: virus-2 has been shown to be an endogenous C-type virus of mice but has not yet been shown to be infectious or to have any contribution to MuLV pathogenicity in mice (2), it should be difficult to invoke autogenous immunity to VEA, or more specifically the recognition of VEA by humoral antibody, as a beneficial immune component with respect to spontaneous viral carcinogenesis on the basis of the results presented here. It still remains to be determined, however, whether virus neutralization is a primary requisite for immunological control of virus burden and virus-mediated carcinogenesis. Nevertheless, natural recognition of viral antigenic determinants of endogenous leukemia virus has been demonstrated here to be a general phenomenon, while natural antigenic recognition of virus-induced cell surface antigens appears to be a more selective or restricted autogenous immune function.

ACKNOWLEDGMENTS

We wish to thank Dr. Stuart A. Aaronson for his general assistance in performing the virus-neutralization tests.

REFERENCES

Fig. 1. In a, is an immunoelectron microscopic demonstration of specificity of humoral antibodies from normal B6C3F1 mice. E.G2 leukemia cells were incubated with serum and labeled with ferritin-conjugated rabbit anti-mouse γ-globulin. A selected region of an E.G2 leukemia cell with an entire envelope of budding C type virus is labeled. × 70,000. b, immunoelectron microscopy using B6C3F1 serum reacted with BALB/c plasma cell tumor cell. An entire envelope of free virus that apparently has just completed budding from the cell is labeled. × 83,000. c, E.G2 leukemia cells reacted with serum from female C57BL/6 retired breeders and labeled with ferritin-conjugated rabbit anti-mouse γ-globulin. Extended regions of the cell surface and the entire envelope of a budding C type particle are labeled. × 130,000.
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