Outer Membrane of Avian Myeloblastosis Virus

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Guinea pigs immunized intracerebrally with avian myeloblastosis virus (AMV) produced antiserum which reacted with intact virus particles in complement fixation. The antigen in question appeared to be located on the surface of the virion and could be distinguished from the type-specific virus envelope and the group-specific internal antigens of chicken leukosis-sarcoma viruses (ChiLSV). The material could be isolated by sequential treatments of AMV with bromelain, Tween 20, and freeze-thawing, and could be purified by differential centrifugation. Electron microscopy analysis indicated the presence of a component resembling the outer membrane of the particle. The antigenic determinant was designated virus membrane antigen (Vm). Further analyses revealed the presence of protein, lipid, and carbohydrate in a material having a molecular weight of about 6,000 as determined by sodium dodecyl sulfate gel electrophoresis. Serological studies suggested that the outer membranes of AMV and other ChiLSV are represented mainly by host cellular material.

Viruses belonging to the chicken leukosis-sarcoma complex (ChiLSV) possess two major classes of antigens which have been characterized immunologically and biochemically. The first of these are common to all ChiLSV and have, therefore, been designated as the group-specific (gs) antigens of the virus (4, 19). They are represented by at least four distinct polypeptides (5, 16), all of which appear to be situated in the interior of the particle (4, 7, 21).

An antigen on the surface of the virus presumably determines three distinct biological properties of ChiLSV, namely, the host range, the capacity to interfere with the infection by homologous but not heterologous strains, and the induction of neutralizing antibody (20, 33, 34). A component with these properties was isolated in highly purified form from avian myeloblastosis virus (AMV) and was shown to represent the surface projections of the particle (6, 29, 32).

ChiLSV are formed by budding at the cell surface; the outer cell membrane becomes the envelope of the virus (14). These agents, therefore, contain a considerable amount of host cellular material, principally that close to the surface membrane (for review, see 8). It is thus likely that the outer envelope of the virion consists of several antigenic determinants distinct from the strain-specific antigen. This

report describes the isolation and characterization of the outer membrane of AMV.

MATERIALS AND METHODS

Virus. The standard strain of AMV (BAI strain A) used in this work is a mixture of viruses of subgroups A and B (26). AMV consisting primarily of subgroup B virus (AMV-B) was obtained after repeated passage in C/A chickens (R. Ishizaki, unpublished data). Avian erythroblastosis virus (AEV), strain R, primarily of subgroup B, was also used. Both AMV and AEV were isolated from the plasma of leukemic chickens. Schmidt-Ruppin Rous sarcoma viruses of subgroups A (SR-RSV-A) and B (SR-RSV-B) and the Prague strain of subgroup C (PR-RSV-C) were kindly provided by T. Graf and R. E. Smith. An RSV pseudotype, RSV(AMV-B), was obtained by infection of RSV cells (18) with AMV-B.

Virus purification. Viruses derived from chicken plasma or tissue culture were purified by sedimentation velocity followed by sedimentation equilibrium as described previously (6). Only the peak fractions of the virus band ($\rho = 1.16 \text{ g/cm}^3$) were taken for study. Homogeneity of the virus preparations was also examined by electron microscopy. No evidence of contaminating proteins could be seen after analysis of the virus preparations for polypeptide content by sodium dodecyl sulfate (SDS) gel electrophoresis. Sucrose gradient profiles of the extracted RNA likewise revealed only virion-associated ribonucleic acids.

Virus particle estimation. Enumeration of virus particles (VP) by electron microscopy count was as previously described (30).

Antisera. Guinea pig sera were prepared by injecting 1-month-old animals intracerebrally with about 1 mg of virus or cellular material. A second inoculation was made intraperitoneally after 4 weeks. Animals

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were bled by cardiac puncture 10 days later, and the serum obtained was stored at -20°C .

Tumor-bearing pigeon serum for gs antigen detection was obtained as described previously (11).

Rabbit immune sera against the major AMV polypeptides isolated by gel filtration in guanidine hydrochloride (17) were also used.

Serological analyses. The micro-complement fixation (CF) test (11) and virus neutralization tests (20) have been described. When guinea pig serum was used in CF, a 1-h incubation at 37°C was used. Agar gel double diffusion studies were performed in 0.8, 1, and 2% agarose containing 0.15 M NaCl and 0.01 M phosphate buffer, pH 7.4.

Polyacrylamide gel electrophoresis. SDS-containing polyacrylamide gels were prepared and used as reported earlier (5). Staining procedures with Coomassie blue for protein, periodic acid Schiff (PAS) for carbohydrates, and sudan black-B for lipids were also previously described (5).

Radioactive virus. AMV from myeloblasts in tissue culture (24) labeled with ^{14}C -glucosamine (New England Nuclear Corp., Boston, Mass.; $2\ \mu\text{Ci/ml}$) and ^3H -amino acids (New England Nuclear Corp., Boston, Mass.; $10\ \mu\text{Ci/ml}$) was prepared as described earlier (6).

Electron microscopy. Preparation of support films and specimens and examination of specimens with the electron microscope were carried out as described in other work (25).

Isolation of virus outer membrane. Approximately 1.1 mg of AMV per ml in 0.01 M Tris-hydrochloride, 0.02 M NaCl, and 0.001 M EDTA, pH 7.2 (TSE) was added to an equal volume of 20.8 mg of bromelain per ml (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M Tris-hydrochloride, 0.001 M EDTA, pH 7.3, and 0.05 M dithiothreitol and incubated for 60 min at 37°C . Iodoacetamide (0.5 M) was added to stop enzyme activity. The sample was then centrifuged at 36,000 rpm for 30 min at 4°C in a Spinco rotor 40 (Beckman Instruments, Palo Alto, Calif.), and the pellet was resuspended in TSE. After a similar additional centrifugation, the pellet was resuspended in 1 ml of TSE and freeze-thawed 30 times by using dry ice-alcohol and 37°C water baths alternatively. Tween 20 was then added to the suspension at a final concentration of 1.0%, and the material was incubated at 37°C for 30 min. After three cycles of centrifugation as described above, the final pellet was resuspended in 0.5 ml of TSE and layered on a 10 to 70% sucrose gradient (in TSE) and centrifuged for 120 min at 50,000 rpm in a Spinco SW50 rotor at 4°C . The virus membranes could be recovered as a band in the low-density region of the gradient. Material presumably representing other virion substructures such as cores and ribonucleoprotein (7) could be seen in the denser region of the gradient.

Isolation of normal host antigens from chicken chorioallantoic membranes. The chorioallantoic membranes (CAM) obtained from 14-day-old C/E chicken embryos which were negative for gs antigen and avian leukosis viruses were washed in saline and freeze-thawed three times in distilled water. The material was then further disrupted in a VirTis (model

45) homogenizer and centrifuged at 2,000 rpm for 10 min. The supernatants were used to prepare the host membrane antigen, and the procedure employed was similar to that used for the virus membranes with the exception that the bromelain and Tween 20 treatments were omitted. Cell membranes, like the virus membranes, were present in the low-density region of the sucrose gradient.

RESULTS

Reactivity of AMV in CF with guinea pig serum. Guinea pigs immunized with purified AMV produced an antiserum which reacted to a high extent in CF with the intact virus particles. The strong CF activity could be correlated directly with the optical density of the virus-containing band (Fig. 1). In contrast, only barely detectable CF activity was obtained with antisera directed against the internal gs antigens of the virus. These two types of antisera, therefore, seem to react with different components of the virion.

When the same fractions (Table 1) were treated with ether to disrupt the virus, the CF activity with the guinea pig serum disappeared almost completely. In contrast, CF activity with anti-gs serum increased markedly, consistent with the notion that the gs antigens are internal components of the virion. With the exception of Tween 20, treatment with various detergents and lipid solvents resulted in the same finding.

FIG. 1. Detection of AMV in a sucrose gradient by optical density and complement fixation. Purified AMV was layered onto a 20 to 60% sucrose gradient and centrifuged for 4 h at 23,000 rpm in a Spinco SW25 rotor at 4°C . Fractions were taken from the bottom of the tube and analyzed for optical density at $260\ \text{m}\mu$ (—○—), antigen titer in CF with guinea pig serum (○), and antigen titer in CF with pigeon serum (gs) (●).

TABLE 1. *Effect of detergents on CF activity^a*

Treatment	CF titer obtained with AMV from			
	Chicken plasma (1.4×10^{13} VP/ml)		Myeloblast cell tissue culture (1.6×10^{12} VP/ml)	
	Guinea pig serum	Pigeon serum (gs)	Guinea pig serum	Pigeon serum (gs)
None	2 ¹⁰⁰	2 ⁵	2 ⁷	2 ²
Triton X-100 (0.2%) ..	2 ³	2 ¹⁹	2 ³	2 ¹³
Nonidet P40 (0.2%) ..	2 ³	2 ¹⁸	2 ³	2 ⁹
Tween 20 (0.2%)	2 ¹⁰	2 ²²	2 ⁵	2 ¹⁰
Tween 20 + ether	2 ¹	2 ²²	2 ¹	2 ¹⁴
Ether	2 ²	2 ²²	2 ¹	2 ¹⁵

^a The virus suspension was mixed with Triton X-100, Nonidet P40, and Tween 20, each at a final concentration of 0.2%. For ether extraction, 4 vol of ethyl ether were mixed with the virus. After 10 min at room temperature, these treated samples were reacted in CF with 4 U of each antiserum (determined in preliminary checkerboard tests with appropriate antigens) in twofold dilution steps.

^b Numbers represent the reciprocal of the virus dilution giving a positive reaction with the antibody.

These preliminary observations suggested that the guinea pig serum reacts with a component on the virus surface which is rendered serologically inactive by detergent treatment.

Treatment of the virus with various enzymes indicated that the antigenicity was not strongly affected by lipolytic enzymes or by neuraminidase. The antigenicity was sensitive to treatment with proteolytic enzymes at high concentrations (Table 2).

The experiments presented thus far strongly suggest that antigenic activity is directly associated with the virus particles. However, it could not be excluded that the material might represent a contaminant of the preparations which possessed similar physical properties to those of the virus. Virus preparations were therefore subjected to further purification steps and analyzed for specific CF activity. Additional purification by sedimentation velocity in both continuous and discontinuous gradients indicated that the peak of CF activity coincided with the bulk of the virus material. No noticeable changes in specific CF activity resulted from further purification attempts. Furthermore, highly purified virus preparations completely absorbed the CF activity from immune serum prepared against virus membrane preparations (see below). Since examination of the preparations in the electron microscope did not reveal the presence of contaminating material, the CF

activity appears to be intimately associated with the virus.

Removal of AMV surface projections with bromelin. It was initially of interest to determine whether the antigenicity was related to the type-specific envelope (Ve) antigen of the virus (32). This could best be approached by enzymatic removal of the type-specific virion surface projections as described initially by Rifkin and Compans (29). Electron microscopy observation of the particles after treatment with freshly prepared bromelin indicated that intact spherical particles remained, devoid of the surface projections (Fig. 2). This could be seen most clearly in the insets where phosphotungstic acid was used to stain the particles. Uranyl acetate was employed to best illustrate the conformation of the particles since staining with phototungstic acid often results in distortion and blebbing (10).

Analysis of the treated virion preparations by gel electrophoresis showed that most of the PAS staining material (carbohydrate) in the region of the major glycoprotein was removed by the bromelin (Fig. 3). In contrast, the PAS staining material at the bottom of the gel was not removed by the enzyme (see below). Similarly,

TABLE 2. *Effect of enzymes on CF activity^a*

Treatment	Expt no.	CF titer ^b	
		Intact virus	Bromelin-treated virus
None	1	2 ⁷	2 ⁶
	2	2 ⁶	2 ⁶
Trypsin			
1 mg/ml	1	2 ⁵	2 ⁴
2.5 mg/ml	2	2 ³ (pt) ^c	2 ² (pt)
Pronase			
5 mg/ml	1	ACE ^d	ACE
3.3 mg/ml	2	2 ¹ (pt)	2 ¹ (pt)
Phospholipase-C			
1 mg/ml	1	2 ⁷	2 ⁵
2.5 mg/ml	2	2 ⁶	2 ⁶
Neuraminidase			
250 µg/ml	1	2 ⁵	2 ⁴
400 µg/ml	2	2 ⁴	2 ⁴

^a The various enzymes were added to intact and bromelin-treated virus and incubated at 37 C for 60 min. After the treatment, the mixtures were tested in CF as described in Table 1.

^b Expressed as reciprocal of the virus dilution giving a positive reaction with the antibody.

^c (pt), After enzyme treatment, the mixture of virus and enzyme was centrifuged at 36,000 rpm for 40 min in a Spinco rotor 40 and resuspended in TSE before testing.

^d ACE, Anticomplementary effect was observed.

FIG. 2. Electron micrographs of bromelin-treated AMV and isolated membranes. AMV subjected to bromelin (10.4 mg/ml) treatment was examined in the electron microscope by 2% uranyl acetate staining (25). In comparison with the nontreated sample (B), bromelin treatment removed outer knobs from the virus particles (A). Arrows point to knobs on perimeter of some particles. The same results as seen with A and B were obtained when particles were prefixed in 15% glutaraldehyde before 2% uranyl acetate staining or when 1% sodium-phototungstic acid (see insets, arrows) was used as negative stain. (C) AMV membranes obtained with the procedure described in text after the bromelin treatment and stained with 2% uranyl acetate. A heterogeneous array of membranous structures varying from 100 to 200 nm in diameter can be seen. This is consistent with the size expected from the AMV particles of A and B whose diameter is about 140 nm. Magnification scale bar, 0.1 μ m. A and B, $\times$ 130,000; C, $\times$ 81,000.

FIG. 3. SDS gel electrophoresis of bromelin-treated AMV. AMV preparations treated with bromelin as described in Materials and Methods were analyzed by SDS gel electrophoresis. From right to left: untreated AMV stained with Coomassie blue; untreated AMV stained with PAS; bromelin-treated AMV stained with Coomassie blue; bromelin-treated AMV stained with PAS. Arrow indicates position of major glycoprotein. Migration is from top to bottom.

with the exception of some of the higher-molecular-weight minor polypeptides, the major virion proteins were not appreciably affected by the enzyme treatment. This suggests that some of the minor protein bands, like the glycoproteins, are associated with the virion surface. Some of these could represent chicken serum proteins adhering to the particles. As shown in a separate study (7), some high-molecular-weight polypeptides are associated with the internal core and ribonucleoprotein constituents of AMV. The latter minor polypeptides may represent the various nucleic acid-specific enzyme activities associated with oncornaviruses (for review, see 31).

In addition to their conformation, the treated particles retained their buoyant density (1.16 g/cm³) after bromelin treatment (Fig. 4). As indicated by the gels (Fig. 3), most of the protein radioactivity remained associated with the virus band, whereas a significant portion of the carbohydrate radioactivity was removed and found at the top of the gradient (Fig. 4B).

These findings are consistent with the notion that bromelin treatment is effective in removal of the type-specific AMV glycoproteins, without noticeable disruption of the particles (29). Sero-

logically, the virus preparations retained most of their CF activity with guinea pig serum (Table 2), indicating that this antigen is distinct from the Ve antigen of ChiLSV. An attempt was therefore made to isolate and characterize this material in more detail.

Isolation and characterization of the outer membrane of the virion. The procedure described in Materials and Methods for isolation of virus membranes made use of two basic treatments: (i) removal of the type-specific surface projections of the virus by exposure to bromelin, and (ii) rupturing the bromelin treated "naked" particles by physical means followed by mild detergent treatment to remove materials adhering to the membrane.

Examination of the material after centrifugation on a 10 to 70% sucrose density gradient revealed a somewhat broad band in the low-density (1.06 g/cm³) region of the gradient which coincided with the peak of the CF activity with guinea pig serum (Fig. 5). Examination of this material with the electron microscope (Fig. 2C) revealed particles bearing a strong resemblance to membranous structures which are apparently devoid of surface projections. Although there was considerable heterogeneity

FIG. 4. Treatment of labeled AMV with bromelin. Purified AMV labeled with ^3H -amino acids (●) and ^{14}C -glucosamine (○) was treated with 10.4 mg of bromelin per ml (B), and the control was untreated (A). The material was then layered onto a 20 to 70% sucrose density gradient and centrifuged for 4 h at 50,000 rpm in a Spinco SW50 rotor at 4°C. Fractions were taken from the bottom and analyzed for radioactivity.

(diameters varied from 100 to 200 nm), their size was consistent with that of the AMV particle whose diameter was about 140 nm (Fig. 2A).

Analysis of this material by electrophoresis in SDS acrylamide gels indicated the presence of a component staining with Coomassie blue which migrated more rapidly than any of the virion polypeptides (Fig. 6). However, it was necessary to apply a relatively large amount of material (obtained from about 10^{13} particles representing 5 mg of protein) to the gel before any detectable staining could be visualized with Coomassie blue. Therefore, this material may represent a minor component not previously described as a virion constituent. Alternatively, the component may be a breakdown product after proteolytic digestion of one of the major polypeptides by the enzyme bromelin (see below). A likely possibility would be that the peptide (about 6,000 daltons) represents that portion of the spike glycoprotein (37,000 daltons) which is embedded in the virus membrane.

Analyses were also made for the presence of material other than protein by differential staining procedures. The band staining with Coomassie blue (protein) could also be stained with amido black (protein) but not with PAS (carbohydrate) or sudan black-B (lipid) (Fig. 7). However, there was a considerable amount of material which migrated slightly more slowly than this component which stained both with PAS and sudan black-B (Fig. 7) and may have represented glycolipid. This material corresponded to the PAS staining component present in the intact and bromelin-treated virus preparations (see Fig. 3). Prior precipitation of the virus in acetone or alcohol removed this material completely, substantiating the notion it is partly lipid in nature. Previous studies (6) have shown that the same component constitutes about 40% of the virus material which can be labeled with glucosamine. The precise composition of total membrane fraction, specifically with regard to lipid, must be determined since the staining techniques are not quantitative and may not be totally reliable. Preliminary results using direct chemical analyses indicate that the membrane fraction is rich in carbohydrate. The protein-to-carbohydrate ratio is approximately 0.7, which is about 80 times lower than that in the intact virion (about 60).

Serological analyses. Immune sera prepared in guinea pigs to (i) intact AMV (standard and

FIG. 5. Sedimentation of the virus membrane fraction in sucrose gradient. Intact purified virus (○) and the virus membrane preparation (●) were layered onto a 10 to 70% sucrose gradient and centrifuged for 12 h at 50,000 rpm in a Spinco SW50 rotor at 4°C. Fractions were collected from the bottom and analyzed for CF activity and density (■).

FIG. 6. Migration of membrane components in polyacrylamide gel. The major gs polypeptides and glycoproteins of intact virions (A) are indicated. The material staining with Coomassie blue is seen migrating more rapidly than the virion polypeptide with the highest electrophoretic mobility (B). Migration is from left to right. Molecular weights of indicated peaks determined by gel filtration in GuHCl (16, 17) are as follows: gs 1, 15,000; gs 2, 12,000; gs 3, 19,000; and gs 4, 27,000.

AMV-B), (ii) bromelin-treated AMV, (iii) virus membrane isolated without prior bromelin treatment, (iv) virus membrane, and (v) normal CAM were reacted with intact virus and nor-

mal cell CAM in CF tests (Table 3). Throughout these studies, CAM were used rather than chick embryo fibroblast cell membrane preparations since the latter produced a strong anti-complementary effect in the CF tests. The titers of the immune sera prepared with virus materials were eightfold higher when tested with virus than with CAM. Reciprocally, titers to intact virus with serum prepared against normal CAM were eightfold lower than when homologous CAM was used. These results suggest that the AMV membrane is not identical serologically to normal CAM.

The various sera were also tested against the major gs antigens of AMV after isolation and purification by gel filtration in 6 M guanidine hydrochloride (17). A component with no gs reactivity, having a molecular weight of about 10,000 and originally described by Fleissner (16), was also tested. At antigen concentrations where homologous sera prepared in rabbits demonstrated a strong CF reactivity (1:1,024), no titer could be demonstrated by using guinea pig sera. The 10,000-dalton material which contained no gs activity likewise tested negative with guinea pig sera.

These sera were also examined in the immunodiffusion test by using 0.25 mg of each of the virus gs antigens per ml (3, 5) (gs 1 to gs 4, see Fig. 6). Whereas rabbit sera demonstrated a clear precipitin line with the homologous antigens, no reaction was observed when guinea pig sera were used.

Analysis of the virus membrane material with guinea pig serum and with rabbit sera prepared against the major group and type-specific antigens of the virus gave the expected results. No reactivity was obtained except with antisera prepared in guinea pigs to intact virus or virus membranes. Guinea pig sera obtained after immunization with standard AMV or AMV-B were also tested for their capacity to neutralize viruses of a homologous subgroup such as RSV(AMV-B). Only a very slight neutralization activity could be detected. Sera prepared by immunization with bromelin-treated virus or virus membranes gave negative results in neutralization tests.

The isolated membranes, therefore, do not appear to contain either the known type- or group-specific antigens of the virus, but rather an antigenic determinant(s) distinct from these which will be designated virus membrane (Vm) antigen.

Distribution and origin of Vm. The guinea pig immune sera were further examined as to their reactivity with viruses of other subgroups. Purified preparations of SR-RSV-A, SR-RSV-B, and PR-RSV-C all tested positive and

FIG. 7. Staining of membrane components for lipid and carbohydrate. AMV membranes were solubilized with SDS, electrophoresed as in Fig. 3, and stained as described in Materials and Methods. From left to right: Coomassie blue (protein); amido black (protein); periodic acid Schiff (carbohydrate); and sudan black-B (lipid). Migration is from top to bottom.

to a similar degree as did AMV. However, the reactions with these tissue culture-grown viruses could all be removed after absorption of the sera with normal CAM membrane. However, the absorbed serum still contained a high reactivity with AMV and AEV both derived from the plasmas of leukemic chickens. When

the sera were absorbed with chick red blood cells obtained from leukosis-free, gs-negative C/E roosters, the reactivity with AMV and AEV was removed.

These results indicate that viruses derived from hematopoietic cells (AMV and AEV) contain VM determinants distinct from those pres-

TABLE 3. *Antibody titers of guinea pigs immunized with virus and normal CAM material^a*

Guinea pig no.	Immunized with	CF titer against	
		AMV	CAM
1	Intact virion of AMV-B	32	8
2	Intact virion of AMV-B	256	32
3	Intact virion of AMV-B	128	16
4	Intact virion of AMV-B	1,024	128
5	Intact virion of AMV-B	32	4
6	Intact virion of AMV-B	256	32
14	Bromelin-treated AMV	256	32
16	Non-bromelin-treated AMV membrane	64	8
17	Bromelin-treated AMV membrane	256	16
20	CAM	4	32

^a Antigen titers for the purified virus and CAM were first determined with homologous antisera. In each case, 4 CF units of antigen were used to test for reactivity against the various antisera. The CF test was performed as described in Table 1.

ent in fibroblast-grown viruses (SR-RSV-A, SR-RSV-B, PR-RSV-C) and that these determinants seem to be related to those present in the host cell of origin.

DISCUSSION

The antiserum produced in guinea pigs after inoculation of intact AMV is directed mainly against a surface component of the virion (Vm) which is distinct from the strain-specific virus envelope antigen (Ve). The antigenic determinant is common to ChiLSV of at least three distinct subgroups, but its specificity is probably determined by the host cell from which the virus is derived. Although it is well known that ChiLSV contain host components which can be detected serologically (3, 5), such material has not been investigated in detail. The guinea pig serum which reacts specifically and in high titer with this material in CF presents an opportunity to study some of its properties.

The guinea pig serum used in this study is apparently unique in that little or no antibody activity is present against other virus antigenic components. Antisera prepared in rabbits against intact virus possess both neutralizing activity and antibodies to gs antigens (3). Those prepared in chickens likewise demonstrate neutralizing activity (3, 6). Apparently, either the immunological system of the guinea pig or the route of inoculation used prevented antibody formation to all possible antigenic determinants. It is of interest that guinea pig antiserum prepared against the 31,000-dalton polypeptide

of mammalian oncornaviruses reacts only with one of the two major determinants (27).

The antigen detected in this study is apparently not virus specific since it can be found also in uninfected cells. The initial studies have suggested that hematopoietic cells differ from fibroblastic cells in terms of the VM determinants incorporated in the virions replicating in the two cell types. A similar situation exists for another host cellular component which is incorporated in the virion, the ATPase activity associated with AMV. As shown by others (2, 13), AMV grown in myeloblasts possesses ATPase activity, whereas AMV grown in fibroblasts does not. This probably results from the observation that in contrast to fibroblasts, myeloblasts contain large amounts of ATPase on their surface membrane, some of which becomes incorporated in the particles during the budding process. AMV derived from myeloblasts also possesses Forsmann antigen (15), but since guinea pigs cannot produce antibody against Forsmann (12), the CF activity is apparently distinct from this widespread antigen.

The exact nature of the antigenic activity or the chemical composition of the virus outer membrane has not been determined. Certainly detailed analyses such as those performed on parainfluenza viruses (22, 23) and on ChiLSV particles (9, 28) must be applied to the isolated membrane. Preliminary findings indicate the presence of lipid, carbohydrate, and protein and indicate that the observed antigenicity is to some extent dependent on the protein moiety. Studies to separate these components and to determine their characteristics are planned.

The present work indicates that in addition to the virus-specific Ve antigens, the Vm antigens, which are apparently cell specific, represent highly reactive determinants in serological tests with appropriate antisera. Since the guinea pig serum used may not be detecting all possible antigenic determinants, it cannot be presently excluded that other virus-specific antigens are present on the virion surface. Virion antigens having group-specific properties and apparently residing on the outer virus envelope have been described for murine leukemia viruses (1). Other approaches are being developed to search for such antigens on the surface of ChiLSV.

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