VIRAL AND CELLULAR SURFACE ANTIGENS OF MURINE LEUKEMIAS AND MYELOMAS

SEROLOGICAL ANALYSIS BY IMMUNOELECTRON MICROSCOPY*

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(Received for publication 12 October 1971)

In studying the relation of the envelope antigens of oncorna viruses (1) to the antigens of cells they infect, it is necessary to consider: (*a*) infection, the penetration of virus into the cell, which may possibly leave residual antigens at the cell surface (2); (*b*) virus-directed synthesis of cell-surface components unrelated to the construction of virions (3, 4); and (*c*) virus assembly at the cell surface, during which the nucleocapsid becomes progressively enveloped by an outer membrane which is continuous with the plasma membrane of the cell (until maturation is complete) and which may or may not differ from it morphologically, according to the type of virus concerned (5–7).

In addition to problems relating to the constitution of the viral envelope, and to modifications of cell-surface composition resulting from infection, there arises the important question of whether the maturation of virus can take place at any region of the cell surface, or whether this is in any way governed by the remarkable regional differentiation which has recently been recognized as characteristic of cell surfaces.

We have already reported that H-2 alloantigen and GCSA[1] (a cell-surface murine leukemia virus (MuLV)-associated antigen) were not present on the envelope of MuLV from leukemia cells (E♂G2) induced by passage A Gross virus, and that budding appeared to take place preferentially at H-2$^-$GCSA$^-$ regions of the cell surface (4). In this communication we report an extension of these findings to other known antigens, cell-specified which contribute to the composition of the cell surface.

* The authors' work is supported in part by National Cancer Institute Grant CA 08748 and a grant from the John A. Hartford Foundation, Inc.

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[1] *Abbreviations used in this paper:* B6, C57BL/6; BALB, BALB/c; GCSA, Gross cell-surface antigen; GMuLV, Gross (wild-type) murine leukemia virus; GVEA, Gross viral envelope antigen; IEM, immunoelectron microscopy; MuLV, murine leukemia virus; SBMV, southern bean mosaic virus, χVEA, new myeloma virion antigen.

Materials and Methods

Serological Test Systems.—See Tables I and II. Medium 199 was used as the diluent and suspending medium.

Absorption of Antiserum.—

In vitro: The test serum was diluted to two doubling dilutions below the end point of the cytotoxic test, mixed with an equal volume of packed washed cells, incubated for 30 min at 4°C, and recovered by centrifugation in the cold.

In vivo: To remove anti-mouse heteroantibody, the rat MuLV typing serum (*W/Fu* × *BN*)F_1 *anti-W/Fu(C58NT)D* (10) was absorbed in vivo in 2-month-old C57BL/6 (B6) mice; 1 ml of undiluted antiserum was injected intraperitoneally and recovered 2½ hr later by cardiac puncture. The absorbed antiserum did not react with EL4 cells (GCSA$^-$) in immunoelectron microscopy (IEM) tests, confirming that absorption of heteroantibody was complete.

TABLE I

Test Systems for H-2, θ (8), Ly-A, and Ly-B (9) Alloantigens on Leukemia Cells

Leukemia (test cell)	Antiserum	Specificity
B6 transplanted leukemia E♂G2, induced by passage A Gross virus; (genotype of B6 mice is H-2^b:θ-C3H:Ly-A.2:Ly-B.2)	B6/H-2^{k}* anti-B6 leukemia EL_4	H-2^b(2, 6, 14, 22, 27, 28, 29, 33)
	A/θ-AKR* anti-A strain spontaneous leukemia ASL1	θ-C3H
	C3H/An anti-CE thymocytes	Ly-A.2
	(C3H/An × B6/Ly-B.1*)F_1 anti-B6 radiation leukemia ERLD	Ly-B.2
Transplanted AKR spontaneous leukemia K36 ascites cells; (genotype of AKR mice is H-2^k:θ-AKR:Ly-A.2:Ly-B.1)	AKR/H-2^{b}* anti-AKR leukemia K36	H-2^k(1, 3, 8, 11, 25, 32)
	C3H/An anti-AKR thymocytes	θ-AKR
	C3H/An anti-CE thymocytes	Ly-A.2
	B6/H-2^k anti-CE thymocytes	Ly-B.1

* Congenic strains.

Antigen Labeling.—5–10 × 10^6 washed viable test cells were incubated in the relevant antiserum (considerable excess antibody was always used). After two washings, the cells were incubated with *anti-mouse-γG/anti-southern bean mosaic virus* (*SBMV*) F(ab′)$_2$ or *anti-rat-γG/anti-ferritin* F(ab′)$_2$ hybrid antibody. The concentration of hybrid antibody was approximately 0.05 mg/ml with respect to *anti-γG*. (For detailed information on the hybrid antibody preparation see references 11, 12.) After two more washings, the cells were incubated with SBMV (0.05 ml of 10 mg/ml) or ferritin (0.05 ml of 0.05 mg/ml, six times crystallized) and then were finally washed twice. The incubations were carried out for 30 min at 0°C with occasional shaking, and washings were done in the cold.

Electron Microscopy.—The final pellets of viable cells were covered with 2 ml of buffered 2% glutaraldehyde for 20 min at 0°C. After fixation for 60–90 min in cold buffered 1% osmium tetroxide, they were kept overnight in cold 0.5% uranyl acetate, then dehydrated with ethanol and embedded in Epon. Thin sections were cut on a Sorvall MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) with a diamond knife, stained with 5% uranyl acetate in alcoholic solution and lead citrate, and examined with a Siemens Elmiskop 1A electron microscope (Siemens Corp., Electromedical Div., Iselin, N.J.).

More than 100 virions and 100 cells of each sample were observed to determine whether antigens are present on or absent from the viral envelopes and cell surfaces. In order to avoid observing the same cells in different sections of one embedded sample, thin sections were obtained from eight blocks of each specimen.

TABLE II

Test Systems for GMuLV Antigens and PC Alloantigen on Myeloma Cells, Leukemia Cells, and Spleen Cells

Antiserum	Absorbed with	Tested on		
		MOPC-70A BALB myeloma cells	E♂G2 B6 leukemia cells	BALB spleen cells
		Known antigens identified		
(B6 × DBA/2)F_1 anti-MOPC-70A (standard anti-PC.1 typing serum) (15)	—	GCSA (23),* PC (15)	GCSA (23)	PC (15)
	BALB spleen	GCSA	GCSA	—
	E♂G2	PC	...	PC
	BALB spleen + E♂G2	—	...	—
	MOPC-70A	—	...	—
Serum from untreated, aged autommune NZB mice	—	See Results	GVEA‡ (4)	NT§
(W/Fu × Bn)F_1 anti-W/Fu (C58NT) D (standard MuLV [Gross] typing serum prepared in rats) (10)	In vivo in young B6 mice	See Results	GCSA (23), GVEA (4)	...

* GCSA, G (Gross) cell-surface antigen, previously designated G antigen (17).

‡ GVEA, G (Gross) viral envelope antigen (4).

§ NT, not tested.

RESULTS

H-2, θ, Ly-A, and Ly-B Antigens on the Two GCSA$^+$ Leukemias, E♂G2 (B6) and K36 (AKR).—These observations are summarized in Fig. 1.

E♂G2 (strain of origin: B6-genotype H-2^b, θ-C3H, Ly-A.2, Ly-B.2): H-2^b, θ-C3H, and Ly-A.2 antigens were detected on small discrete areas of the cell surface in some but not all sections (Figs. 2 and 3). Ly-B.2 was not demonstrable. A small proportion (approximately 1/8) of both budding and extracellular MuLV produced by E♂G2 cells were labeled by anti-θ-C3H antiserum. The label invariably appeared on only a small sector of the envelope; in no case was the entire circumference labeled. H-2^b, Ly-A.2, and Ly-B.2 antigens were not detected on virions at any stage of maturation (Fig. 1).

K36 (strain of origin: AKR-genotype H-2^k, θ-AKR, Ly-A.2, Ly-B.1): H-2^k and Ly-B.1 antigens were detected on small discrete areas of the cell surface in some but not all sections. θ-AKR and Ly-A.2 were not detectable. About one-fourth of the virions associated with K36, both incomplete (budding) as well as complete (extracellular), showed a restricted H-2^k sector on the viral envelope (Figs. 4 and 5). As the serum giving this reaction, *H-2^b* vs. *H-2^k* prepared in H-2 congenic mice, gave no reaction with E♂G2 cells (H-2^b) or its associated virions, this labeling of a sector on viral envelopes from K36 can confidently be ascribed to H-2^k. θ-AKR, Ly-A.2, and Ly-B.1 antigens were not detected on the viral envelope.

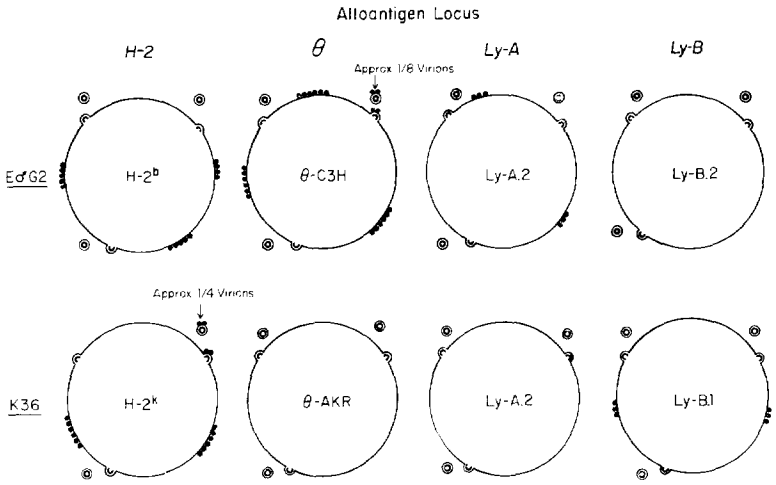


FIG. 1. Summary analysis of H-2, θ, Ly-A, and Ly-B antigens on the cell surface and the viral envelope of two Gross leukemias, E♂G2 and K36.

Once again, as in the case of θ-labeling of E♂G2 (above), the labeling produced by H-2 antiserum on K36 virions was restricted to small sectors of the viral envelope, and in no case was the entire circumference labeled (Figs. 4 and 5).

PC, GCSA, and GVEA on (a) MOPC-70A Myeloma Cells, (b) E♂G2 Gross Leukemia Cells, and (c) Normal BALB/c (BALB) Spleen Cells.—We shall be concerned here with known antigens of three classes: (*a*) the differentiation alloantigen (13, 14), PC (15), which is found on myeloma cells but not on leukemia cells, (*b*) the nonvirion MuLV-associated, cell-surface antigen, GCSA, and (*c*) the Gross viral envelope antigen, GVEA, which is found on Gross (wild-type) murine leukemia virus (GMuLV).

In order to study the possible representation of PC cell-surface antigen on virions produced by PC$^+$ cells (myeloma cells and their nonmalignant counterparts), we employed the standard PC.1 typing serum *(B6 × DBA/2)F$_1$ anti-*

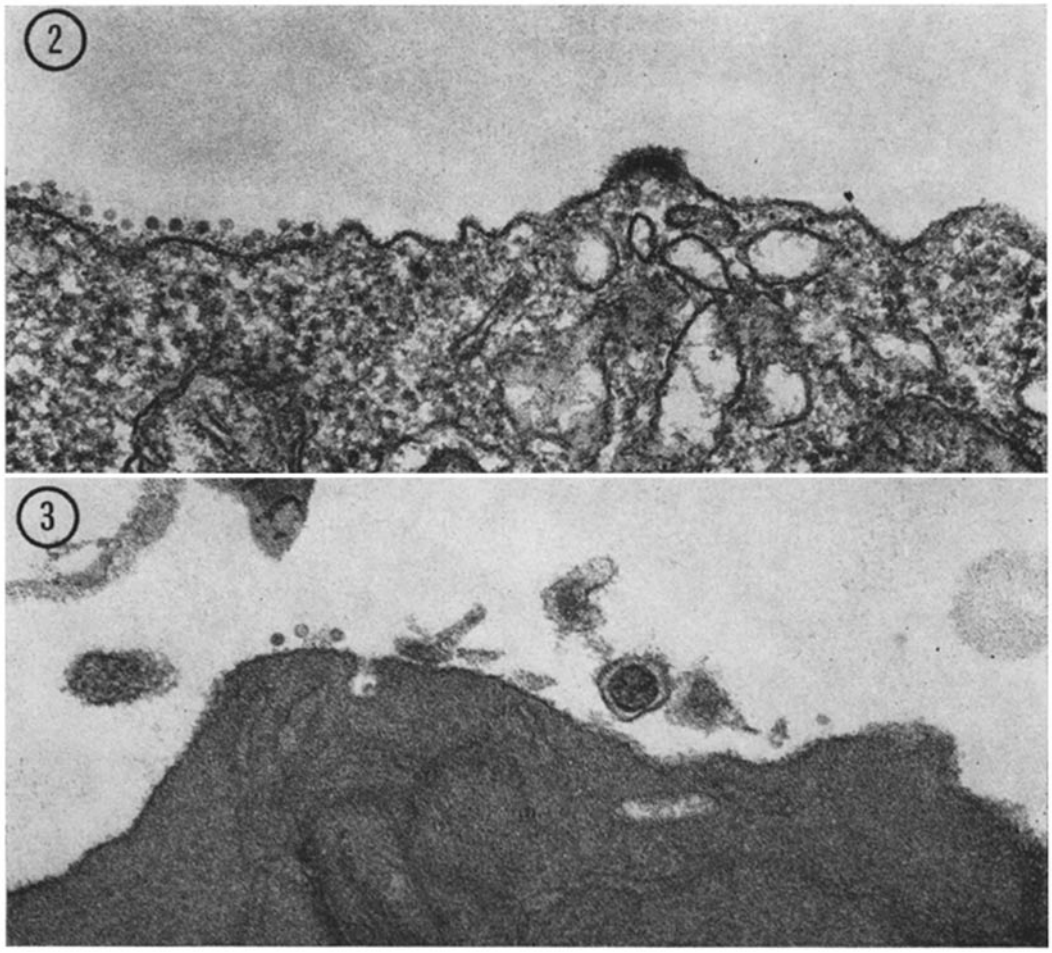


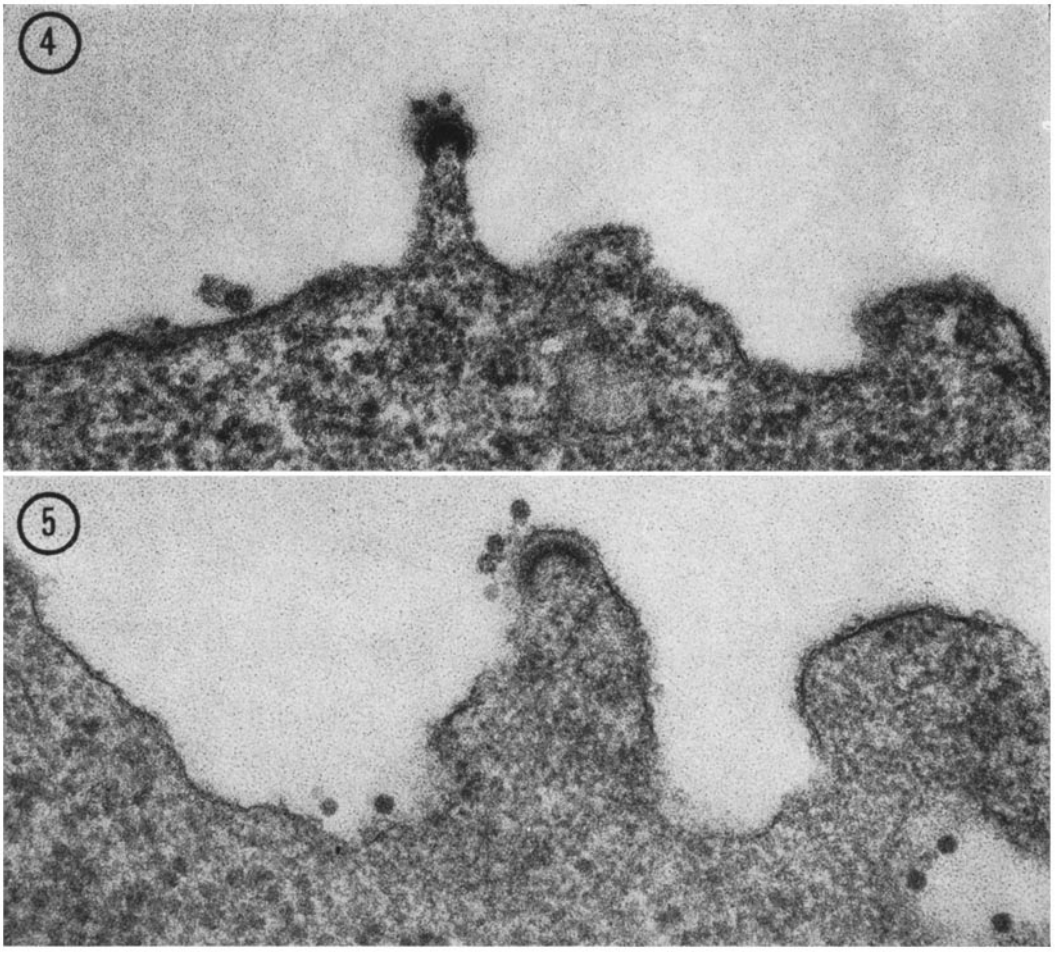
FIG. 2. E♂G2 cells were reacted with the serum *θ-ARK anti-θ-C3H* and labeled with SBMV. Small areas on the cell surface are labeled with SBMV but the viral envelope is not labeled at all. × 144,000.

FIG. 3. E♂G2 cells were reacted with the serum *C3H/An anti-EC thymocytes* (*anti-Ly-A.2*) and labeled with SMBV but the viral envelope is not labeled at all. × 72,000.

BALB myeloma MOPC-70A (15). According to the cytotoxic test, the method by which the PC antigenic system was described, this antiserum detects only PC antigen, but IEM reveals the presence of additional, MuLV-associated antibodies that are not apparent by cytotoxicity. A summary analysis of the data is given in Fig. 6, which is largely self-explanatory.

PC antigen was seen to be represented on discrete areas of the surface of PC^+

spleen cells (Figs. 7 and 8), like all other cell-surface alloantigens so far studied (see above and reference 16). The reaction with E♂G2 leukemia cell surfaces shows that anti-GCSA is present in the PC typing serum (although not demon-



FIGS. 4 and 5. K36 cells were reacted with the serum *H-2^b anti-H-2^k* and labeled with SBMV. Small areas on both the cell surface and the envelope of budding viruses are labeled with SBMV. Fig. 4, × 72,000; Fig. 5, × 144,000.

strable by cytotoxicity) (Fig. 9). Furthermore, the antiserum contains antibody to a third antigen, tentatively named χVEA, which identifies a new virion antigen present on the myeloma virions but lacking from the leukemia cell virions both originally derived from passage A Gross virus (Figs. 9 and 10) and

SERUM:	ABSORBED:	REACTED WITH: MOPC-70A BALB myeloma cells (PC^+) ($GCSA^+$)	REACTED WITH: E♂G2 Gross leukemia cells (PC^-) ($GCSA^+$)	REACTED WITH: Cells from immunized BALB spleen (PC^+) ($GCSA^-$)	INTERPRETATION
(B6xDBA/2) F_1 anti-MOPC-70A myeloma cells	—	GCSA, PC, xVEA	GCSA	PC	Virions from MOPC-70A myeloma cells have an envelope antigen (xVEA) that is absent from passage A Gross MuLV.
	with normal BALB spleen cells	GCSA, xVEA	GCSA	(all negative)	xVEA is not PC.
	with E♂G2 Gross leukemia cells	PC, xVEA	(all negative)	PC	Confirms that xVEA is absent from passage A Gross MuLV.
	with both of the above	xVEA	(all negative)	(all negative)	xVEA occurs only on virions, not on the cell surface.
	with MOPC-70A myeloma cells	(all negative)	(all negative)	(all negative)	Control; all activities removed by absorption with MOPC-70A
from untreated aged NZB mice	—	(?) GVEA	(GVEA)	(all negative)	Aged NZB mice form anti-GVEA.
(W/Fu x BN) F_1 anti-W/Fu (C58NT) D (Standard rat MuLV Gross typing serum)	in vivo in B6 mice to remove natural heteroantibody	GCSA, GVEA	GCSA, GVEA (and G_{1X}?)	(all negative)	GVEA (as well as xVEA) is present on virions from MOPC-70A cells

FIG. 6. Summary analysis of GMuLV antigens and PC alloantigen on myeloma cells, leukemia cells, and spleen cells.

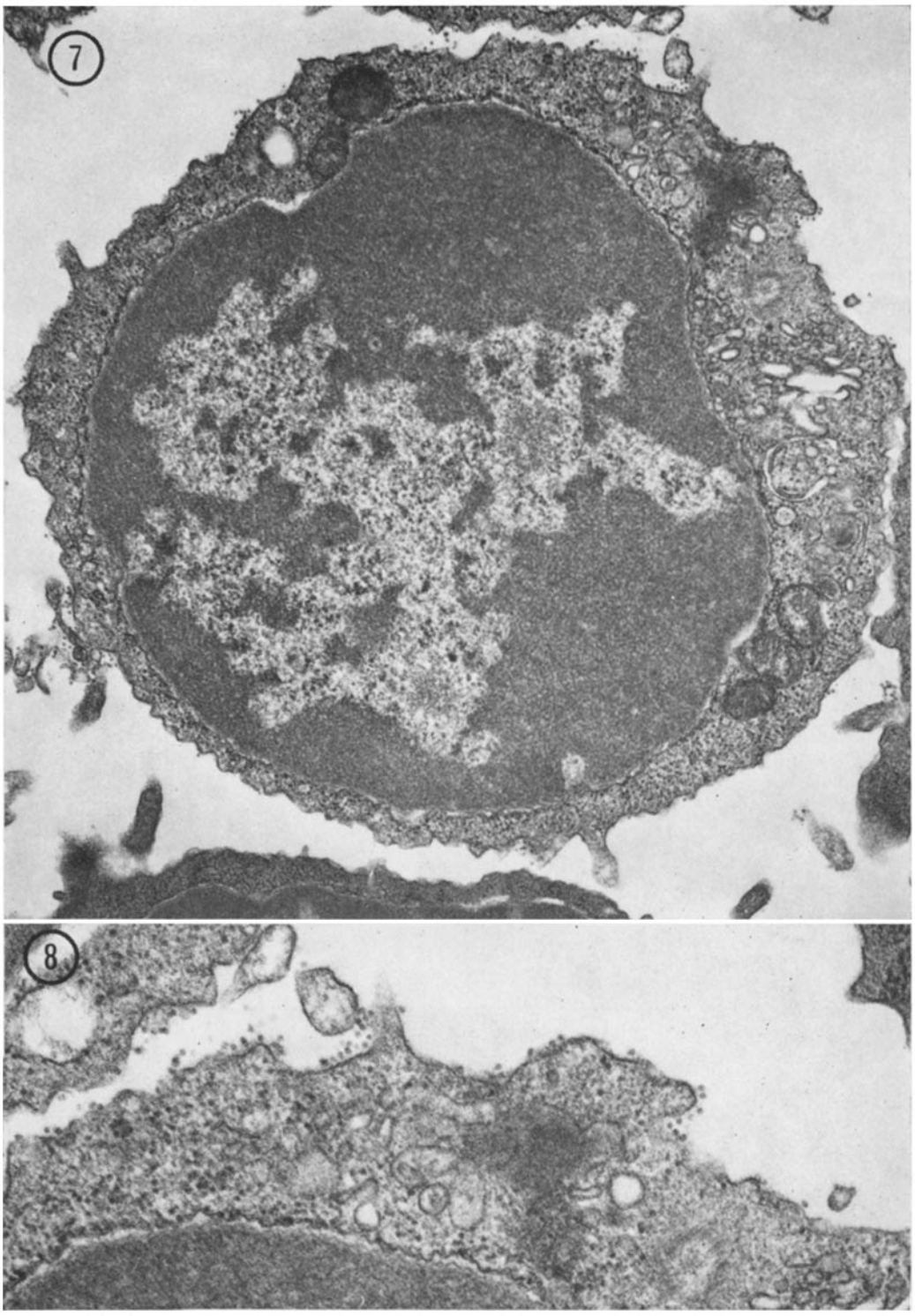


FIG. 7. BALB spleen cells (middle-size lymphocytes) were reacted with the standard PC.1 typing serum (*B6* × *DBA/2*) F_1 *anti-BALB myeloma MOPC-70A* and labeled with SBMV. Small sectors on the cell surface are labeled with SBMV. × 18,000.

FIG. 8. Higher magnification of one area in Fig. 7, showing several of the discrete SBMV-marked PC.1 sectors. × 30,000.

from wild-type Gross virus. The absorption tests illustrated in Figs. 6 and 11 confirm these conclusions.

The presence of χVEA on the myeloma virions precludes an absolute answer as to whether PC may be represented on the myeloma virions; this would require a demonstration that absorption with $\chi VEA^{+}PC^{-}$ cells (of which no examples are known so far) would remove all reaction with myeloma virions while leaving PC cell-surface reactivity intact.

The two other entries in Fig. 6 are included as controls. The serum of aged (autoimmune) mice of the NZB strain contains anti-GVEA antibody reactive with both the myeloma (Figs. 12 and 13) and the leukemia virions (Fig. 14), and so does the standard GMuLV typing serum produced by rats (Figs. 15 and 16). The latter, as expected, also gave cell-surface reactions referable to GCSA (and perhaps also to G_{IX} antigen, see reference 17). The reaction of autoimmune NZB mouse serum with the myeloma cell surface has not been identified.

DISCUSSION

Alloantigens.—In a previous study, neither $H\text{-}2^{b}$ alloantigen nor the MuLV-associated cell-surface antigen, GCSA, was present on the envelope of virions produced by cells bearing these antigens. This conclusion can now be extended to the differentiation alloantigens (13, 14), θ, Ly-A, and Ly-B. The occasional $H\text{-}2^{k}$ labeling of a small sector of some virions coming from $H\text{-}2^{k}$ leukemia cells is a fact to take note of, for it was not seen with $H\text{-}2^{b}$, and these two *H-2* alleles confer, respectively, sensitivity and resistance to leukemia induction by GMuLV (18–20). We noted earlier that the invariable absence of $H\text{-}2^{b}$ from virions produced by E♂G2 leukemia cells suggests that they are made preferentially at $H\text{-}2^{-}$ regions of the cell surface; evidently the same may not hold for $H\text{-}2^{b}$ and this may have something to do with the mediation of susceptibility of $H\text{-}2^{k}$ mice vs. $H\text{-}2^{b}$ mice.

The occasional θ-labeling of some virions produced by E♂G2 leukemia cells may similarly indicate that MuLV does not preferentially select either θ^{-} or θ^{+} regions of the cell surface for maturation sites; and in fact the fraction of virions showing a θ-labeled sector is roughly equivalent to the fraction of the E♂G2 cell surface which bears θ-antigen. In such circumstances it is not very surprising to find that such an alloantigen is occasionally and fortuitously associated with the surface of the virion. It seems highly unlikely that the respective alloantisera could neutralize any but an insignificant fraction of the infectivity of the virus population concerned.

Turning to the general topic of cell-surface constitution, it is interesting that according to IEM the phenotype of the two leukemias, with respect to their surface differentiation alloantigens (13, 14), was θ^{+}Ly-A^{+}Ly-B^{-} (E♂G2) and θ^{-}Ly-A^{-}Ly-B^{+} (K36) (Fig. 1). This accords with serological absorption tests (personal observations). Clearly the plasma membrane components recognized

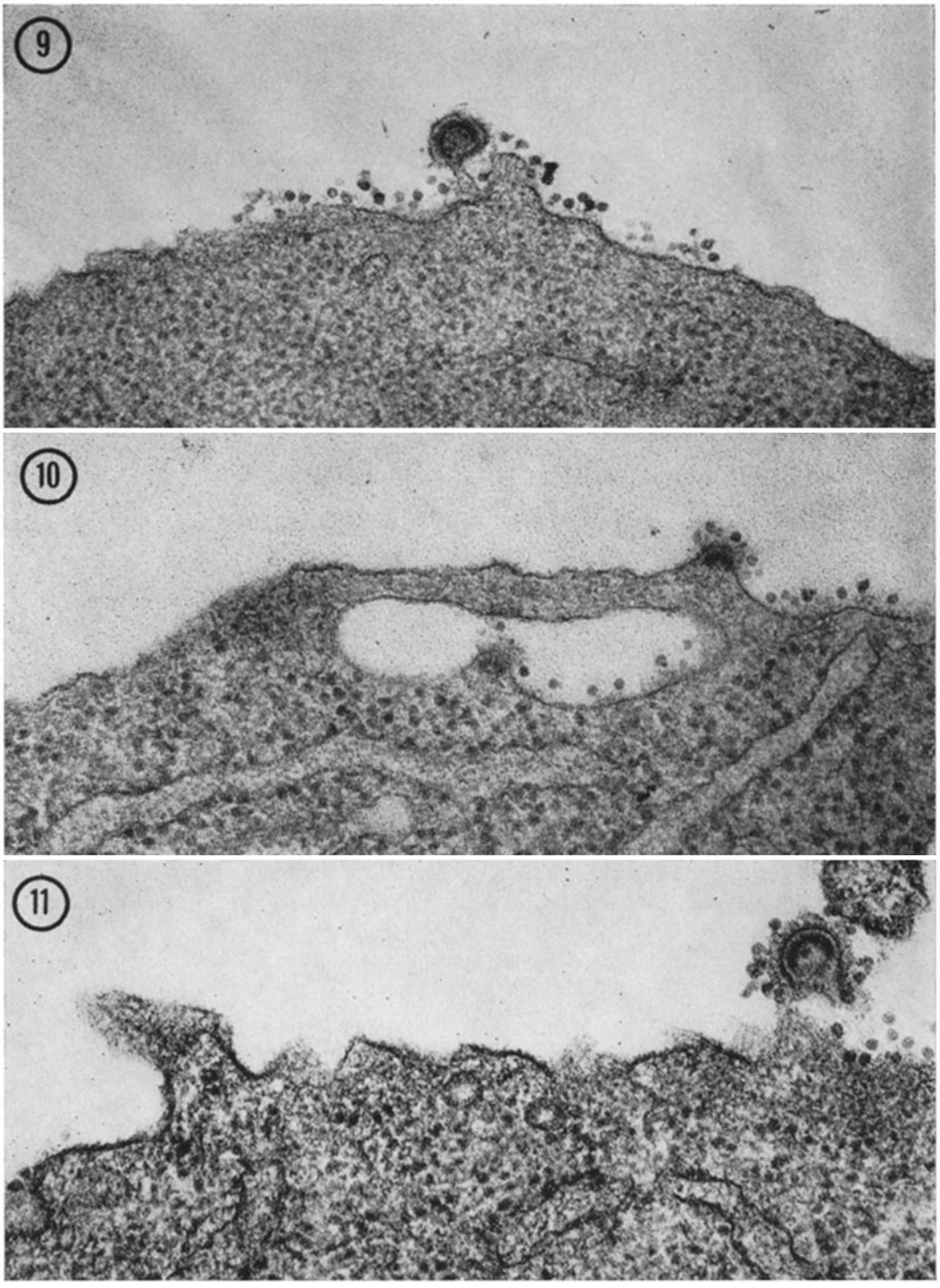


FIG. 9. E♂G2 cells were reacted with the standard PC.1 typing serum (*B6* × *DBA/2*) F_1 *anti-BALB myeloma MOPC-70A* and labeled with SBMV. One large sector on the cell surface is labeled with SBMV, showing $GCSA^+$, but the envelope of budding virus in the middle of $GCSA^+$ area is not labeled at all. × 144,000.

FIG. 10. BALB myeloma MOPC-70A cells were reacted with the standard PC.1 typing serum (*B6* × *DBA/2*) F_1 *anti-MOPC-70A* and labeled with SBMV. Both the discrete areas on the cell surface and the entire envelope of budding viruses are labeled with SBMV. × 144,000.

as θ-antigen or as antigens of the Ly series are not essential to cell viability, for in fact they are limited to certain cell types only. Their loss from malignant cells therefore may be inconsequential, which may not be the case with the *H-2* locus, whose products are found on possibly all cells and may constitute essential elements of the plasma membrane. (Loss of the antigens of one *H-2* haplotype from malignant cells occurs readily under circumstances of immunoselection, but losses of entire H-2 complements have not been found; one explanation of this is that the products of the *H-2* locus itself are essential to cell viability [see references 13 and 21]). The fact that both leukemias retain at least one of the markers, θ, Ly-A, and Ly-B, identifies them as derivatives of the T lymphocyte (22). It seems highly likely therefore that the selective loss of these antigens from the two leukemias (assuming the losses to be absolute) has produced phenotypes that have no counterpart in the nonmalignant precursor population. Whether the loss is due to exclusion of the coding genes, as is probably the case with the *H-2* haplotype losses mentioned above, or whether it is epigenetic, is unknown.

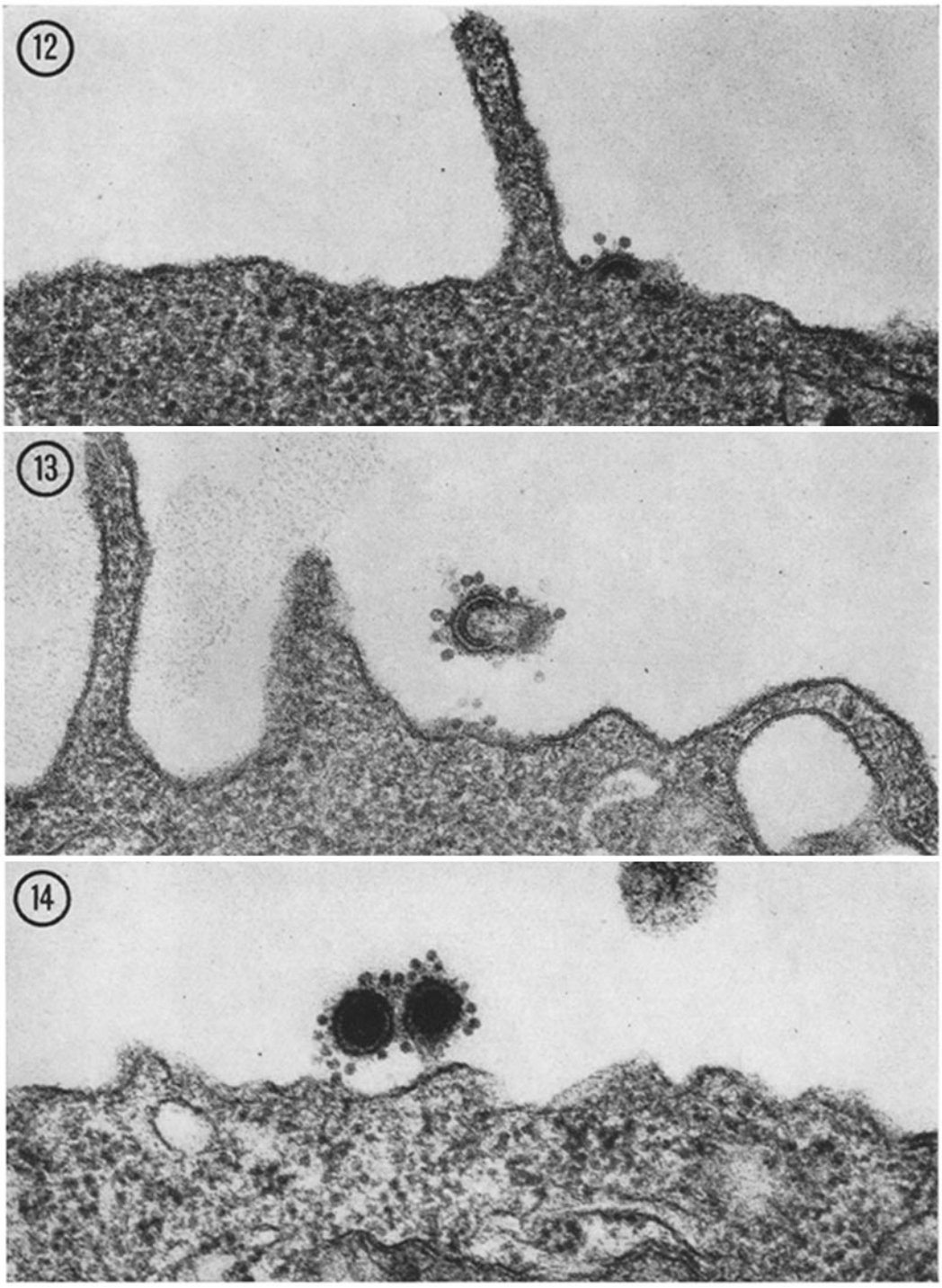
MuLV-Related Antigens.—The finding of a new VEA on virions produced by myeloma MOPC-70A was an unexpected outcome of testing for PC.1 antigen. The PC typing serum is monospecific for PC.1 antigen in cytotoxic tests, but IEM shows it contains *anti-GCSA* (cell-surface antigen specified by GMuLV) and also antibody to the new VEA antigen which denotes the virus produced by MOPC-70A as an MuLV subtype vis-à-vis passage A Gross MuLV.

The identification of the MOPC-70A–myeloma virus as a distinct subtype may help explain the usual phenotype of these cells, which is $GCSA^+G_{IX}^-$ (23). (In normal mice, G_{IX} is a thymocyte-differentiation alloantigen whose expression is dependent on two cellular genes, one of them at the G_{IX} locus in linkage group IX; if MuLV is productively expressed, however, the rule is that G_{IX} antigen will be expressed, regardless of the inherited cellular genotype. Thus $MuLV^-$ to $MuLV^+$ conversion of G_{IX}^- cells is generally accompanied by G_{IX}^- to G_{IX}^+ conversion.) The MOPC-70A virus may now perhaps be classed with the Friend, Moloney, and Rauscher variants, all of which induce the $GCSA^+$ but not the G_{IX}^+ (23) phenotype. The G_{IX}^- to G_{IX}^+ conversion may therefore be a particular function of wild-type GMuLV as represented in AKR and C58 mice and the derivative passage A Gross virus.

SUMMARY

Immunoelectron microscopy (IEM) of mouse cells which were productively infected with murine leukemia virus (MuLV) yielded the following conclusions:

FIG. 11. BALB myeloma MOPC-70A cells were reacted with the standard PC.1 typing serum (*B6* × *DBA/2*) F_1 *anti-MOPC-70A* previously absorbed with E♂G2 cells, and labeled with SBMV. The cell surface is nearly negative, but the entire envelope of budding virus remains labeled with SBMV, showing GVEA of MOPC-70A virions different from GVEA of E♂G2 virions. × 144,000.



FIGS. 12 and 13. BALB myeloma MOPC-70A cells were reacted with the serum of aged (autoimmune) NZB mice and labeled with SBMV. The entire envelope of budding viruses at the initial stage (Fig. 12) and at the advanced stage (Fig. 13) and minute areas on the cell surface are labeled with SBMV. × 144,000.

FIG. 14. E♂G2 cells were reacted with the serum of aged (autoimmune) NZB mice and labeled with SBMV. The entire envelope of both complete and budding viruses is labeled with SBMV but the cell surface is not labeled at all. × 144,000.

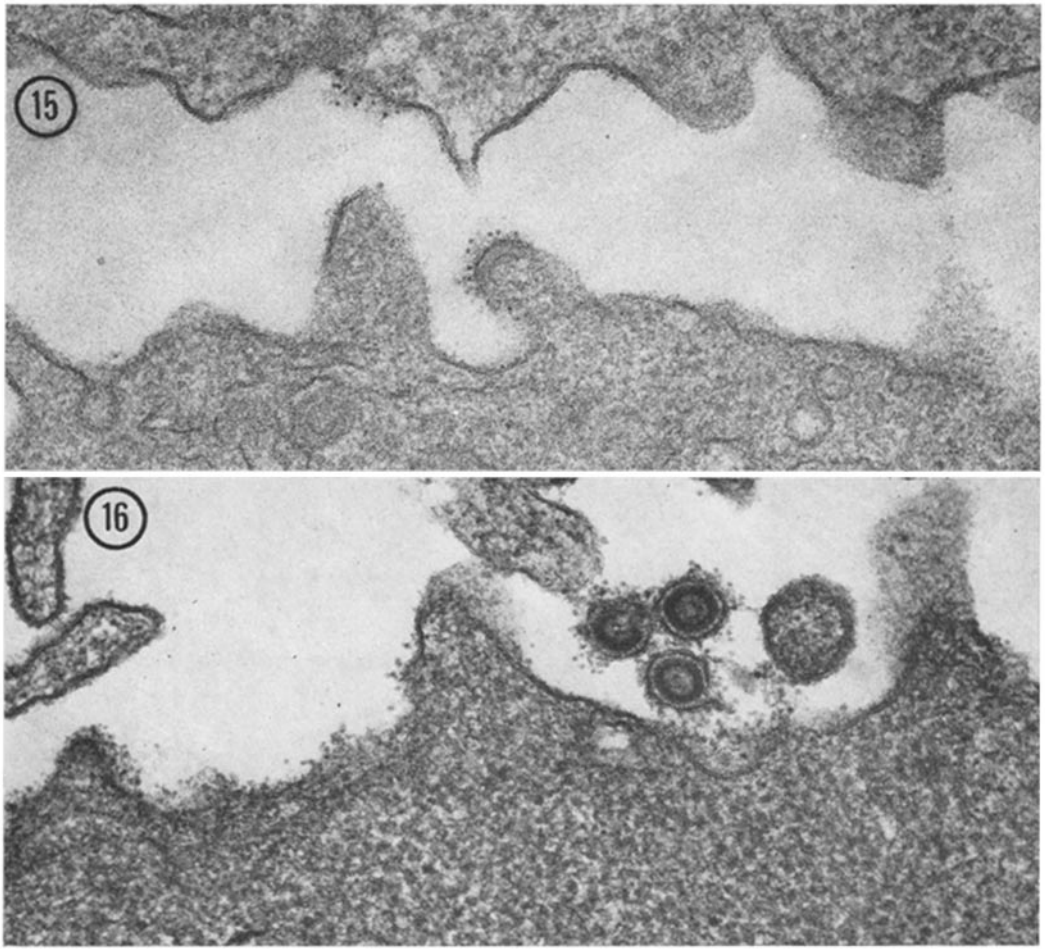


FIG. 15. BALB myeloma MOPC-70A cells were reacted with the standard GMuLV typing rat serum (*W/Fu* × *BN*) F_1 *anti-W/Fu* (*C58NT*)*D* and labeled with ferritin. The entire envelope of budding virus and small sectors on the cell surface are labeled with ferritin. × 144,000.

FIG. 16. E♂G2 cells were reacted with the standard GMuLV typing rat serum (*W/Fu* × *BN*) F_1 *anti-W/Fu* (*C58NT*)*D* and labeled with ferritin. The entire envelope of virions and small sectors on the cell surface are labeled with ferritin. × 144,000.

With two exceptions, the alloantigens H-2, θ, Ly-A, and Ly-B were not present on complete or incomplete virions produced by cells bearing these antigens. The exceptions were H-2^k (but not H-2^b) and θ; both appeared on only a minority of virions and never on more than a small part of the circumference.

The incidental discovery of an additional envelope antigen on virions produced by a BALB/c mouse myeloma but lacking from passage A Gross virions distinguishes these two viruses as MuLV subtypes; it also illustrates that IEM can be applied as a primary tool for antigenic analysis, as well as for its usual purpose of finding out where antigens are situated.

We are indebted to Dr. H. A. Wood for supply of SBMV; Doctors L. J. Old, E. A. Boyse, and E. de Harven for valuable advice and discussion; and Mr. M. P. Lardis, Miss S. Ohno, and P. A. Johnson for excellent technical assistance.

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