IMMUNIZATION OF MICE WITH SYNGENEIC MOLONEY LYMPHOMA CELLS INDUCES SEPARATE ANTIBODIES AGAINST VIRION ENVELOPE GLYCOPROTEIN AND VIRUS-INDUCED CELL SURFACE ANTIGENS*

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Immunization of mice with syngeneic Moloney lymphoma cells induces virus-neutralizing antibodies. The same sera also react, as a rule, with the surface of Moloney, Friend, and Rauscher virus-induced neoplastic cells. Operationally, the latter type of reactivity is usually designated as anti-Friend-Moloney-Rauscher or anti-MCSA1 (Moloney virus-induced cell surface antigen) antibody (1). Since the virally induced lymphoma cells continue to produce virus, as a rule, it was difficult to decide whether membrane-inserted virion proteins or virally induced cellular antigen(s) serve as the target of the latter reaction. Under certain conditions, virus neutralizing activity could be absorbed without affecting anti-MCSA cytotoxicity, supporting the hypothesis of nonidentity (2). These experiments were performed using a Moloney lymphoma (YAC) and an L-cell subline (A9). Both cells reacted with mouse sera immunized against syngeneic Moloney lymphoma cells but while absorption with L cells removed both virus-neutralizing activity and reactivity against L cells, the reactivity against the YAC cells was not affected. YAC cells removed the reactivity against both. We concluded therefore that at least two antibody specificities were present, one reacting with the virus envelope, and another with the surface of the lymphoma cells. L cells carry the former antigen only, whereas YAC cells contain both.

Monospecific antisera directed against purified C viral proteins were found to react against membrane-associated virion proteins, regularly expressed on the surface of virus-producing cells (3–5). The question of whether any of these could be identical with MCSA was approached by comparing sublines of the YAC lymphoma differing in their MCSA expression. Immunoresistant sublines,

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Abbreviations used in this paper: BSS, balanced salt solution; FITC, fluorescein isothiocyanate; FV, Friend virus; IR, immunoresistant; MCSA, Moloney cell-surface antigen; anti-ML sera, mouse antisera to Moloney lymphoma cells; MLV, Moloney leukemia virus; RLV, Rauscher leukemia virus; TRITC, tetramethylrhodamin isothiocyanate; YAC, Moloney lymphoma cells.
low or deficient in MCSA were derived by immunoselection, consisting of serial
cytotoxic exposure to mouse antisera, combined with passage in preimmunized
mice (6). The original and the MCSA-deficient lines reacted in a virtually
identical fashion with a spectrum of antivirion protein sera, directed against
gp71, p30, p15, p15(E), p12, and p10 (7). This suggested that MCSA was
distinct from these virion proteins.

We have recently obtained direct biochemical evidence showing that MCSA
was distinct from H-2 and from the virion proteins gp71, p30, p15(E), p12,
and p10 (8).

The present paper deals with the question of whether there is any relationship
(including steric association) between MCSA and viral envelope proteins on
the cell surface, as judged by antibody-induced redistribution and capping. We
have also approached the same problem by reciprocal inhibition tests with
complete or disrupted virus or purified viral proteins compared to YAC and L
cells.

Materials and Methods

Cells. The YAC and YA7C lymphomas were induced by inoculating Moloney leukemia virus
(MLV) to newborn A and (A × C57B1)F1 mice, respectively (9). The characteristics of YAC cell-
surface antigens and virus production have been described in a series of publications (6, 10, 11).
Both tumors were maintained in the ascites form in syngeneic mice.

The L-cell subline used in the present experiments was a clonal derivative (clone 3C) of
A9HT, a highly malignant A9 variant (12). Like other L-cell sublines, A9HT produces a C-type
virus that shows a certain resemblance to endogenous mouse viruses. It is N-tropic (10, 11, 13)
and lacks leukemogenic activity (14, 15). In the XC test, it induces distinct syncytia rather than
plaques (16). The A9HT cells were grown in Eagle's Minimum Essential Medium with 5% fetal
calf serum.

Viruses. Two different Rauscher leukemia virus (RLV) batches and one MLV batch was
obtained through the Office of Resources and Logistics, Virus Cancer Program, National Cancer
Institute, Bethesda, Md. Batch 448 contained concentrated culture fluid from RLV-infected
JLS-V9 cells (RLV/JLS), purified through sucrose gradient centrifugation. The protein content
was 3.93 mg/ml (17).

The other batch (RLP-QQ) consisted of viremic plasma of RLV-inoculated BALB/c mice, and
was designated RLV/BALB plasma. In the focus induction test on S × L - cells (18) it contained
10-3 focus-inducing units/ml. MLV was concentrated and purified on sucrose gradient from the
culture fluid of NIH 3T3 cells (batch 599-18-3, designated MLV/NIH) to a concentration of 1.16
mg protein/ml.

MLV was also collected from roller cultures of YAC lymphoma cells, the medium was
concentrated and banded in sucrose gradients (MLV/YAC). Protein concentration was 0.78 mg/ml.

Antigens. Purified Friend virus (FV), gp71, and p15(E) were the generous gift of Dr. W.
Schäfer, Max-Planck-Institut für Virusforschung, Tübingen, Germany. RLV p12 was kindly
provided by Dr. J. R. Stephenson, National Cancer Institute. RLV p30 was obtained through the
Office of Resources and Logistics, Virus Cancer Program, National Cancer Institute.

Antisera. Anti-H-2* serum was produced by immunizing ASW mice with pooled spleen,
kidney, and liver suspensions from A mice, inoculated subcutaneously at biweekly intervals
during 14-24 wk. The mice were bled 7-10 days after the last injection. In the microcytotoxicity
test, the serum was used at a 1:6,000 dilution against A lymph node cells (titer 1:24,000).

Mouse antisera to YAC (designated anti-ML sera) were produced in (A × C57B1)F1, or (A ×
C57L)F1 mice by three to six injections of 6,000 rads irradiated YAC or YAC cells. Sera were
collected 1 wk after the last injection.

The rabbit and goat anti-FV gp71 and rabbit anti-FV p15(E) sera were the generous gift of Dr.
W. Schäfer. The goat antisera to purified RLV proteins were kindly provided by Dr. B. Hampar,
National Cancer Institute, (anti-p30) by Dr. M. Strand, Albert Einstein College of Medicine,
Bronx, N.Y.; (anti-p15 and anti-Gross p12) by Dr. J. R. Stephenson National Cancer Institute, Bethesda, Md. (anti-p12). None of the goat or rabbit antisera reacted with a series of control mouse cells, including normal A lymphocytes and the cell lines, NIH/3T3, BALB/3T3, and JLS-V9 (7).

Cytotoxicity-Inhibition Assay. A microassay (7) was used in which 2 μl of serial antiserum dilutions in balanced salt solution (BSS) with 1% gelatin (BSS/gel) were injected under paraffin oil into rings of microtrays (Møller-Coats A/S, Moss, Norway) with an automatic Hamilton syringe. For the inhibition assay, antigenic material was diluted in antiserum at the final dilution which still gave 60-90% killing. 6 to 10 three-to fivefold dilution steps were tested in at least two repeat experiments. 10⁸ target cells in 1 μl were added to each droplet and the plates were incubated for 20 min at 37°C. Subsequently, 1 μl of rabbit complement was added at a dilution of 1:10 for A9HT, 1:20 for YAC, and 1:40 for lymph node cells. Plates were further incubated for 45 min. Thereafter 0.5 μl trypan blue was added to each drop and the results were read microscopically. The ASW anti-A serum was tested against normal A lymph node cells. All other sera were tested against the YAC lymphoma.

Membrane Immunofluorescence and Capping Experiments. gp71 and p15(E) were detected on the cell membrane using a 1:15 diluted rabbit anti-FVgp71 and a 1:2 diluted rabbit anti-FVp15(E) antisera, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit Ig antibodies (Hyland Laboratories, Los Angeles, Calif.), diluted 1:20. MCSA and H-2d were detected by exposure to the corresponding mouse antiserum, diluted 1:2, followed by tetramethylrodamine isothiocyanate (TRITC)-conjugated goat anti-mouse Ig (Hyland Laboratories) diluted 1:4.

Redistribution and capping were induced by incubating stained cells at 37°C. Aliquots were taken every 5 min, fixed with glycerol: BSS 1:1 and the proportion of cap-positive cells was determined in relation to the total number of membrane fluorescence-positive cells. Membrane fluorescence-positive cells with less than half of their circumference stained were registered as capped.

For double staining and cocapping experiments, cells were exposed to the first antibody for 30 min at 37°C to induce cap formation, and subsequently transferred to BSS/gel containing 10⁻² M sodium azide and chilled to 4°C to avoid further redistribution. Thereafter the cells were stained for the second antigen with the opposite conjugate, fixed, and examined for red and green fluorescence in a Leitz Ortholux microscope (E. Leitz, Wetzlar, West Germany) with a Ploem-type "Opakilluminator" system as described (19). For FITC fluorescence, 470 nm blue excitation light was used, with 4 mm BG38 + 1.5 mm BG12 + AL470 (Schott, Mainz, West Germany) as primary filters, 495 nm interference plate and K510 barrier filter. For TRITC fluorescence, 546 nm green excitation light was used, with a 4-mm BG38 + 2 mm BG36 + AL546 as primary filters, 580 nm interference plate, and K590 barrier filter. Single cells were photographed for green and red fluorescence differentially while the field between the exposures was shifted to observe the distribution pattern of both antigens.

Results

Expression of Viral Structural Proteins on YAC and A9HT Cells. YAC and A9HT cells were tested for their cytotoxic sensitivity to antisera directed against purified viral components (Table I). Both cell lines reacted with all sera. With the exception of anti-FVp15(E) and anti-Gross p12, A9HT cells reacted more weakly than did YAC cells with the antiviron protein reagents. Also, in accordance with previous results (2), A9HT cells reacted more weakly with the mouse anti-ML serum.

Analysis of the Mouse Anti-ML Serum by Inhibition Tests

Inhibition with Virus. Four different preparations were used; two RLV and two MLV. As expected, the cytotoxicity of goat anti-FVgp71 serum was inhibited completely by all four virus preparations (Table II and Fig. 1, upper part), although at varying efficiency, 0.73 ng RLV/JLS, 4.0 ng MLV/NIH, and
DISTINCT ANTIBODIES TO MOLONEY CELL SURFACE ANTIGEN

TABLE I
Expression of Viral Structural Proteins on YAC and A9HT Cells

| Antiserum         | Target cells |         |         |
|-------------------|--------------|---------|---------|
|                   | YAC          | A9HT    |         |
| Goat anti-FVgp71  | 8,700*       | 2,900   |         |
| Goat anti-RLVp30  | 25           | 10      |         |
| Rabbit anti-FVp15(E) | 17          | 27      |         |
| Goat anti-RLVp15  | 40           | <10†    |         |
| Goat anti-Grossp12| <10‡         | 35      |         |
| Goat anti-RLVp12  | 25           | 10      |         |
| Mouse anti-ML     | 250          | 62      |         |

* Reciprocal of antiserum dilution which gave 0.5 cytotoxic index.
† Cytotoxic index 0.40 at 1:10 dilution of antiserum.

TABLE II
Inhibition of Cytotoxic Activity with Various Virus Preparations

| Antiserum* | Target cell | RLV/JLS | RLV/BALB plasma | MLV/NIH | MLV/YAC |
|------------|-------------|---------|-----------------|---------|---------|
|            | Inhibition  |         |                 |         |         |
|            | Intact†     |         |                 |         |         |
|            | Disrupted   |         |                 |         |         |
| Mouse anti-ML | YAC       | None    | 30%             | 200    | None    |
|            | Complete    | 0.9     | Complete        | 90.5   | Complete|
|            |             | 15.3    | Complete        | 725    | Complete|
|            | ALt         | None    | >31.3           | >2,000 | None    |

* Dilution: anti-ML, 1:160; anti-FVgp71, 1:5,000; anti-RLVp30, 1:10; anti-A, 1:5,000.
† Figures correspond to the maximum amount of protein tested in cases where there was no or only partial inhibition, or the minimum amount which gave complete (100%) inhibition.
§ FIU, focus-inducing units on S+L- cells.
| A lymph node cells.

123.0 ng MLV/YAC was needed for 50% inhibition. The goat anti-RLVp30 antiserum was also inhibited completely. None of the virus preparations inhibited anti-H-2b cytotoxicity. With the mouse anti-ML serum, different results were obtained, depending on the target cell. RLV/JLS and MLV/YAC did not inhibit cytotoxicity against YAC cells while RLV/BALB plasma and MLV/NIH were partially inhibitory in doses above 1 μg. All virus preparations inhibited cytotoxicity against A9HT cells (Fig. 1, lower part) This is in line with our previous finding that the mouse antiserum reacts with viral envelope antigen on A9 cells while YAC cells have additional antigens, not expressed on the viral envelope.

INHIBITION WITH PURIFIED VIRAL PROTEINS FVgp71, RLVp30, FVp15(E), AND RLVp12. Further evidence was obtained to confirm that the mouse anti-ML serum reacted with viral envelope components on A9HT cells when we found that purified FVgp71 inhibits cytotoxicity completely (Fig. 2). No such inhibition was obtained with YAC target cells (Fig. 2 and Table III).

None of the three virion proteins RLVp30, FVp15(E), and RLVp12 showed any inhibition of the mouse anti-ML serum against YAC. The anti-FVp15(E), anti-RLVp15, and anti-Gross p12 antisera were completely inhibited by
Fig. 1. Inhibition of the cytotoxic activity of goat anti-FVgp71 (upper) and mouse anti-ML (lower) sera by MLV/NIH virus. Target cells: YAC ○ and A9HT △.

FVp15(E) antigen (Table III), indicating that all three detect the same antigen.

INHIBITION TESTS WITH DISRUPTED VIRUS. Three virus preparations were disrupted by 10 cycles of freeze-thawing. Increase of the p30 activity was taken as the measure of disruption, assayed by the inhibition of the anti-p30 serum. For RLV/JLS 30-fold increase, for MLV/NIH 10-fold, and for MLV/YAC 3-fold increase was obtained (Fig. 3). The increase seems to relate to the state of the virus preparation before treatment. For example, untreated RLV/JLS contained less p30 than gp71 and showed the largest increase of p30 activity among the virus preparations tested. MLV/YAC contained more p30 than gp71 and freeze-thawing led to a threefold increase only.

Disrupted virus particles still failed to inhibit the cytotoxicity of the anti-ML serum against YAC cells (Fig. 3). This argues against the possibility that MCSA is located within the virus particle.

Membrane Immunofluorescence Reactivity of YAC Cells

KINETICS OF CAP FORMATION. Fig. 4 shows the frequency of capped YAC cells as a function of time after exposure to four antisera. A high proportion of
DISTINCT ANTIBODIES TO MOLONEY CELL SURFACE ANTIGEN

1526

FIG. 2. Inhibition of the cytotoxic activity of the goat anti-FVgp71 (upper) and mouse anti-ML (lower) serum by purified viral proteins. Target cells: YAC O and A9HT Δ.

TABLE III

Cross-Inhibition of Antisera with Virion Proteins

| Antiserum* | FVgp71 | RLVp30 | FVpl5(E) | RLVp12 |
|------------|--------|--------|----------|--------|
|            | Inhibition ng | Inhibition ng | Inhibition ng | Inhibition ng |
| Mouse anti-ML | 10-30% | 900-3,600 | None | 360 | None | 2,800 | None | 80 |
| Goat anti-FVgp71 | Complete | 1 | None | 360 | None | 2,800 | None | 80 |
| Goat anti-RLVp30 | None | 2,600 | Complete | 350 | None | 80 |
| Rabbit anti-FVpl5(E) | None | 2,600 | None | 360 | Complete | 350 | None | 80 |
| Goat anti-RLVp15 | Complete | 500 | Complete | 40 |
| Goat anti-Grossp12 | Complete | 175 | Complete | 40 |
| Goat anti-RLVp12 | None | 2,600 | None | 360 | None | 2,800 | Complete | 40 |

* Dilution: anti-ML 1:160 (anti-YAC), 1:10 (anti-YAC); anti-FVgp71 1:5,000; all others 1:10. Target cell: YAC.
† Figures denote the maximum amount of protein tested in cases of partial or no inhibition, or the minimum amount which gave complete (100%) inhibition.

The cells capped after exposure to anti-FVgp71, anti-FVpl5(E), and anti-\(\text{H-2}^a\) sera, (66, 53, and 59% respectively), plateauing after 25-30 min at 37°C. The caps were restricted to one pole of the cell and appeared as one continuous brilliant spot of fluorescence. In contrast, only about 15% of the cells capped with anti-ML serum after 30 min incubation. The pattern was also different,
redistribution rather than capping, with numerous small spots and dots, appearing over one-half of the cell circumference (Fig. 5). This is in agreement with our previous observation (20) showing a very poor MCSA capping, compared to that of H-2. It is of interest that the capping of the virion proteins resembles the pattern of H-2 and differs clearly from the redistribution of MCSA.

**Double staining and cocapping.** These experiments were carried out to see if the different antigens represent distinct molecular entities on the surface of YAC cells and whether or not their antibody-induced redistribution is independent. YAC cells were stained for gp71, allowed to cap, and transferred to sodium azide-containing medium at 4°C. Subsequently, the cells were stained for MCSA. Capping of gp71 did not affect the distribution of MCSA, as shown by the double-staining procedure. Fig. 6 shows complete gp71 capping (green) with virtually continuous MCSA ring fluorescence.

Redistribution of gp71 (green) followed by staining for H-2a (red) showed no cocapping of H-2a (Fig. 7). Fig. 8 shows capping of p15(E) on YAC cells with no subsequent cocapping of MCSA (Fig. 8). These experiments confirm that MCSA is distinct from gp71 and p15(E).
Mouse anti-ML sera neutralize MLV (1, 2), presumably due to their reactivity against gp71 (21, 22). To assess this reactivity, YAC cells were first incubated with mouse anti-ML serum at 37°C for 1 h. They were subsequently transferred to sodium azide-containing medium at 4°C, and stained for either gp71 or p15(E). Finally, the cells were stained with the red anti-mouse conjugate and fixed. As shown in Fig. 9 and 10, the anti-MLV serum induced redistribution and capping of gp71. This was visualized by creating a sandwich with the green anti-gp71 reagent at conditions which did not allow capping of the latter. Since the maximum frequency of cells with MCSA caps was always low (Fig. 4), most of the cells that capped gp71 did not redistribute their MCSA. When stained with the red conjugate, the latter appeared as a complete ring. Fig. 5 shows that a similar procedure did not affect p15(E). The latter antigen remained dispersed over the total cell circumference and neither redistributed nor capped after exposure to the anti-ML antiserum, not even on the few cells that capped their MCSA. Incubation of YAC cells for 1 h at 37°C in BSS/gel before staining for gp71 or p15(E) at 4°C showed a diffuse distribution of the antigens, confirming that incubation at 37°C does not induce redistribution by itself.

This procedure has thus confirmed that the mouse anti-ML serum also reacts with gp71. However, when assayed by membrane immunofluorescence or cytotoxicity against YAC cells, the anti-gp71 reactivity is usually overshadowed by the MCSA reaction.
FIG. 5. Pre-incubation of YAC with anti-ML antiserum does not induce capping of pl5E. Cells were incubated with anti-ML for 1 h at 37°C, transferred to a sodium azide-containing medium at 4°C, and stained for pl5 (green), and with anti-mouse immunoglobulin conjugate (red). MCSA is redistributed into several spots and dots, spread over one half of the cell circumference. Magnification is 540 times.

FIG. 6. Capping of gp71 (green) does not induce cocapping of MCSA (red). YAC-IR cells were stained for gp71 and incubated at 37°C for 30 min. Subsequently, they were transferred to sodium azide-containing medium at 4°C and stained for MCSA. Magnification is 540 times.

FIG. 7. Capping of gp71 (green) does not induce cocapping of H-2a (red). YAC-IR cells were stained for gp71 as in Fig. 6 and then transferred to a sodium azide-containing medium at 4°C and stained for H-2a. Magnification is 540 times.

FIG. 8. Capping of pl5 (green) does not induce cocapping of MCSA (red). YAC cells were stained for pl5E and incubated 30 min at 37°C. Subsequently, they were transferred to a sodium azide-containing medium at 4°C and stained for MCSA. Magnification is 540 times.

FIG. 9. Pre-incubation of YAC cells with anti-ML antiserum induces capping of gp71 (green), while MCSA remains distributed (red). Cells were treated as in Fig. 5, but gp71 was visualized instead of pl5E. Magnification is 700 times.

FIG. 10. Same as Fig. 6 but gp71 is redistributed into aggregates which precede cap formation. Magnification is 700 times.

Discussion

MCSA could first be distinguished from virion protein antigens in immunoresistant (IR) lines of the YAC Moloney lymphoma. The IR lines were derived by repeated immunoselection, alternating between in vitro cytotoxic treatment.
with anti-ML sera in the presence of complement, and passage through preimmunized mice. They are resistant to mouse anti-ML sera, while their sensitivity to the cytotoxic effect of antisera against gp71, p30, p15, p15(E), p12, and p10 virion antigens is unchanged (7). NP40 solubilization of YAC cells allowed the physical separation of MCSA from the virion proteins and from H-2 (8). In a similar manner to H-2a and gp71, solubilized MCSA bound to concanavalin-A columns and could be eluted with α-methyl-mannoside. The eluted material could be separated by further fractionation on Sephadex G-200 into distinct MCSA, gp71, and H-2 peaks. MCSA was identified by inhibition of cytotoxicity of the mouse anti-ML serum against YAC lymphoma cells. It appeared as an approximately 110,000 mol wt species.

The present study reaffirms the conclusion that MCSA is an entity, distinct from surface-associated virion proteins. While H-2 and the virion proteins underwent rapid, high-frequency redistribution and capping, on ligand contact, MCSA showed only a very limited redistribution and virtually no capping. Furthermore, MCSA did not ccope with the virion proteins gp71 or p15(E). H-2 did not ccope with any of the virion proteins either; results which are at variance with those of Schrader et al. (23).

Mouse anti-ML sera contain virus-neutralizing activity (1, 2), attributed to antibodies against gp71 (21, 22). Our cytotoxicity and fluorescence tests confirmed that the anti-ML serum reacts with at least two different sites on the surface of the YAC cell, identified as gp71 and MCSA, respectively. The serum could induce the redistribution and capping of surface-associated gp71 molecules, confirmed by a subsequent second labeling with heterologous monospecific anti-gp71 serum. Under ordinary test conditions, the stronger and much less cappable anti-MCSA reaction predominates, and gp71 capping remains masked. Since gp71 was clearly capped by the mouse serum, this experiment also shows that the lack of MCSA capping cannot be ascribed to the serum source and must be therefore due to a relatively firmer membrane anchorage of the antigen.

Cytotoxicity by the anti-ML serum against YAC target cells was only partially inhibited or not at all inhibited by purified gp71 (or virus). This is in line with the fact that YAC cells carry both gp71 and MCSA. A9HT cells express gp71 but lack MCSA (2). As expected, their reaction with the anti-ML serum could be completely inhibited by gp71 (or by virus).

Our findings that the mouse anti-ML sera contain antibodies against both virion (anti-gp71) and nonvirion cell-surface components (anti-MCSA) on a Moloney virus-induced lymphoma cell are at variance with those of Friedman et al. (24) on the Friend system. They found that the cytotoxic effect of mouse antisera against FV-infected spleen cells could be completely inhibited by FV. Similar findings were reported by Hogg (25) using sera of MSV regressor mice and an established culture line of a Moloney sarcoma as target cells.

The mouse anti-ML serum did not contain antibodies to p15(E), p30, or p12 at a level detectable by the methods used; cytotoxicity and membrane immunofluorescence. In addition, the membrane redistribution of p15(E) was not influenced by the mouse anti-ML serum, unlike gp71 which was capped.

Several experimental findings emphasize the in vivo importance of MCSA. First, there was a close parallelism between in vivo rejectability and in vitro
MCSA expression when a large number of Moloney lymphomas were compared (9). Second, in a group of backcross mice segregating for low and high anti-MCSA reactivity, only those with antibodies to MCSA showed protection against lymphoma transplants (26). On the contrary, the presence of anti-viral antibodies did not show any correlation to the ability of tumor rejection. Third, immunoselection of YAC invariably results in sublines with decreased MCSA content (6) but leaves viral protein antigens unchanged (7).

**Summary**

Immunization of mice with heavily irradiated syngeneic Moloney lymphoma cells evokes antibodies against the major viral envelope antigen, gp71, and the Moloney virus-induced cell surface antigen (MCSA). A9HT cells, an L-cell subline, react with the antibodies against the viral envelope antigen only; this reaction can be completely inhibited by virus or purified gp71. Reactivity to Moloney lymphoma cells (YAC) was only partially inhibited (maximum 30%) or not at all. This can be attributed to the reaction of the YAC cells with antibodies directed against MCSA, a nonvirion cell surface component according to both biological and biochemical evidence.

Antibody-induced capping of gp71 or p15(E) did not change the membrane distribution of MCSA or H-2, indicating that these antigens represent distinct entities on the cell surface. MCSA showed only minimal capping and thereby differed in behavior from both H-2 and virion antigens. gp71 could be capped by the mouse antiserum as revealed by subsequent staining with monospecific anti-gp71 antiserum. Under ordinary test conditions this reactivity is overshadowed by the reaction against MCSA. The lack of MCSA capping reflects a difference in anchorage of this antigen.

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