Expression of Virus Structural Proteins on Murine Cell Surfaces in Association with the Production of Murine Leukaemia Virus

(Accepted 9 July 1976)

SUMMARY

We have used a quantitative radiolabelled antibody procedure to measure the amount of certain virus structural antigens on the surface of BALB/c RAG cells producing endogenous B-tropic type C virus. RAG cells expressed group specificities of MuLV p30 on their cell surface but did not express gp70 group specificities. However, type specificities of gp70 were expressed on BALB/c cell lines infected with Moloney leukaemia virus. The majority of p30 antigens detected on the RAG cell surface were removed by trypsin and their reappearance was prevented by cycloheximide, even in the presence of 'conditioned medium' containing MuLV. Passive adsorption of exogenous MuLV p30 to the surface of virus negative BALB/c fibroblasts reached a maximum of 20% of the protein detectable on virus producing RAG cells. These data support the hypothesis that much, but not all, of the surface p30 is expressed de novo on the cell membrane and not derived from passive adsorption of p30 released from shed virus or as a by-product of virus infection of a cell.

Recent work has suggested that certain of the type C virus structural proteins (p30, gp70 and p10) can be detected on the external cell surface of MuLV infected cells (Yoshiki, Mellors & Hardy, 1973; Grant et al. 1974; Yoshiki et al. 1974). The mode of origin of these virus proteins is unclear to date and could be derived from at least three possible events: (1) the insertion of these proteins in the membrane as a preliminary event to virus assembly on the cell membrane; (2) the adsorption of virus proteins to cell surfaces from ruptured viruses released in the culture milieu; (3) the placement of virus proteins on cell surfaces as a by-product of virus infection. We have employed a radioisotopic paired label assay for the study of virus associated surface antigens. We present here a quantitative estimation of p30 and gp70 on cell surfaces and evidence that the majority of cell surface p30 appears de novo in contrast to a smaller amount which is passively adsorbed.

RAG adenocarcinoma cells, a BALB/c derivative, which are resistant to 8-azaguanine (Klebe, Chen & Ruddle, 1970), were found to be positive for type C particles by electron microscopy and by particulate RNA-dependent DNA polymerase in the culture fluid (10 to 30 pmol deoxymonophosphates incorporated/h/10^6 cells). We found no evidence of type A or intracisternal B particles despite the history of the cell being derived from an adenocarcinoma.

DNA polymerase activity was assayed as described (Wu et al. 1972). Particle associated enzyme activity in RAG culture fluid was linear with time for one hour and with protein concentration up to a 100-fold concentrate of culture fluid. The activity was optimal at 1 mM-Mg^2+ (Mn^2+ would not substitute) using rAdT_{12-18} as a template and ^3H-thymidine triphosphate as a substrate. The host range of the RAG virus was determined by passing
Fig. 1. (a) Cell titration of RAG cells at a constant concentration of 125I-labelled IgG (20 μg/ml) from two antisera: (1) rat anti-Moloney sarcoma virus (MSV) from a Fischer rat with an MSV-derived tumour (Δ—Δ), (2) goat anti-Rauscher leukaemia virus (RLV) p30 (●—●). The sera were provided by Dr R. Wilsnack through the Office of Program Resources and Logistics, Viral Oncology Program, NCI. Monospecific antiserum was prepared by inoculating protein purified by agarose gel filtration (single band on polyacrylamide gels) into goats. The anti-p30 had a titre at least 2 logs higher for MuLV p30 than for MuLV gp70 in a competition radioimmunoassay (R. Wilsnack, personal communication). Cell surface antigen was detected by the paired label assay for cell surface antigens (Boone et al. 1971, 1973; O’Brien et al. 1976). Immune and normal IgG from the same species were prepared by ammonium sulphate precipitation and labelled catalytically with 125I and 123I. A mixture of equimolar amounts of the normal and immune IgG was adsorbed with 10^7 mosquito cells/mg IgG to remove non-specific binding protein. Cells were incubated with 20 μg immune and normal IgG in a final volume of 0.1 ml for 30 min at 25 °C, washed, and counted in a gamma spectrometer. The amount of 125I minus the 123I normalized according to the sp. act. of IgG indicated the nanograms of antibody bound on a per cell basis.

(b) Plot of data from (a) as a function of ng antibody bound/10^6 cells/ml against ng antibody bound at increasing cell concentration depicted in (a). Extrapolation of the linear portion of these curves provides an estimate of the number of antigenic determinants per cell (see text).

(c) Blocking of binding of goat anti-p30 to the RAG cell surface by purified Rauscher p30. Increasing amounts of Rauscher MuLV p30 purified by isoelectric focusing were added to 10 μg of labelled goat anti-p30 IgG in a paired label mixture in 0.1 ml PBS. After 4 h incubation at 4 °C, 0.1 ml inactivated foetal bovine serum and 1.5 x 10^6 RAG cells in 0.2 ml PBS were added and assayed for bound anti-p30 as in (a).

(d) Cell titration of RAG (Δ—Δ), Basc-1 (●—●) and LSTRA (●—●), with goat anti-RLV gp70. Increasing cell concentrations were tested for immune IgG binding as in (a).
tissue culture filtrates (0.45 μm) of log phase RAG cells on to test cells for 6 h in the presence of 2 μg/ml polybrene. After 2 to 4 weeks, the test cells were tested for virus production by assay for particle associated reverse transcriptase in the culture fluid. The host range of the virus was B-tropic with respect to the Fv-z locus since it infected the murine cell lines 3T3-BALB, SC-1, and Basc-2 (a BALB/c adult fibroblast line), but not 3T3-NIH, VA-2 (human), FCO 121 (cat), SIRC (rabbit) or MS-1 (Chinese hamster). A B-tropic virus has been previously reported to occur in BALB/c mice tumours and in aged BALB/c mice (Peters, Spahn & Rabstein, 1973; Peters et al. 1973).

Titrations of RAG cells at constant immune serum concentration (20 μg/ml) were performed with a broadly reactive rat anti-MSV serum and a monospecific goat anti-Rauscher MuLV p30 using the paired label assay (Fig. 1a; Boone, Irving & Rubenstein, 1971; Boone, Gordin & Kawakami, 1973; O'Brien et al. 1976). The RAG cell surface clearly bound large quantities of both sera relative to normal serum binding and relative to virus negative Basc-2 cells (adult BALB/c fibroblasts). For a given incubation volume, the amount of antibody bound per cell is inversely proportional to the total amount of antibody bound in regions of antigen excess (Fig. 1b; Boone et al. 1971, 1973). By extrapolation to the region of low cell concentration (antibody excess), it is possible to estimate the maximum amount of IgG which can bind to the cell surface at the indicated antiseraum concentration. For the rat anti-MSV, 39 ng/10^6 cells represents (39 x 10^{-6} x 6.023 x 10^{23})/(1.5 x 10^{6} x 10^{9}) = 1.6 x 10^5 bound antibody molecules per cell (Boone et al. 1971). For goat anti-MuLV p30, 26 ng/10^6 cells represent 1.0 x 10^5 bound antibody molecules per cell. The number of antigenic sites per cell is, therefore, the same as or up to twice these numbers depending upon whether the IgG binds mono- or bivalently to the cell surface.

Since MuLV p30 is a core protein, we considered that the detection of p30 on the RAG cell surface could be the result of goat antibodies against small amounts of virus surface proteins which have contaminated the inoculating p30 antigen. In order to investigate this question, the ability of purified Rauscher MuLV p30 to interfere with the binding of goat anti-p30 was examined (Fig. 1c). Purified Rauscher p30 was added to a paired label mixture of goat anti-p30 and normal goat sera at increasing concentrations for 4 h at 4 °C. The mixture was tested for binding to RAG cells. After an initial rise in binding, probably due to adsorption of p30 to the cell surface (see below), increasing amounts of p30 effectively block the binding of 60 to 70 % of the measured sites. The failure to block the binding to the remaining sites may be because the p30 used to raise the antiserum differed in source and purification from that used to compete for antiseraum binding.

Between 150 and 200 ng of RLV p30 was required for complete adsorption of 10 μg anti-p30. From the cell titration (Fig. 1b) it is possible to estimate the amount of specific antibodies/mg IgG by dividing the maximum antibody bound to RAG cells by the μg of IgG in the reaction mixture. Thus the immune serum contains 200 ng binding antibody/20 μg input IgG. With the requirement of divalent antibody binding for adsorption, the 10 μg IgG used in the adsorption (Fig. 1c) would require 100 ng x 2 x 30000/150000 = 67 ng for equivalence of antigen to binding site concentration. Empirically, a threefold excess was required for complete adsorption.

Since gp70 is a major surface component of the MuLV virion, and therefore a likely contaminant in the p30 immunogen, we examined the surface of RAG cells with a goat anti-Rauscher MuLV gp70 (Fig. 1d) using a cell titration. The amount of anti-gp70 bound to RAG cells and to Basc-1 cells (a BALB/c adult skin fibroblast which expresses endogenous type C virus at high levels) was very low. The possibility that the group-specific region(s) of cell membrane bound gp70 were relatively inaccessible to antibody, while the type-specific
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Fig. 2. (a) Passive adsorption of MuLV p30 to cell surface. 1.2 x 10⁶ Basc-2 (virus negative adult BALB/c fibroblasts) were incubated with MuLV-p30 for 2 h at 37 °C. The cells were washed 3 times and tested with goat anti-p30 as in Fig. 1(a). 1.2 x 10⁶ RAG cells bound 31 ng/10⁶ cells under the same reaction conditions. (b) The effect of cycloheximide and supernatant oncornavirus on the appearance of p30 on RAG surface following trypsin removal. RAG cells were scraped with a rubber policeman from near confluent 150 mm tissue culture dishes (viability = 68 to 73 %), washed twice and resuspended at 4 x 10⁶ cells in 2 ml phosphate buffered saline containing 0.625 mg/ml trypsin for 20 min. The reaction was terminated by the addition of ovamucoid trypsin inhibitor (2.5 mg). The cell clumps caused by trypsin treatment were dispersed by addition of 100 μg DNase for 5 min following the trypsin treatment. Cells were washed twice, and dispensed into 4 ml nutrient medium (RPMI-1640 + 10 % foetal bovine serum (●—●, △—△), or in ‘conditioned’ medium (○—○, Δ—Δ), which was a 24 h supernatant fluid of a log phase RAG cell culture (contained 15 to 20 pmol reverse transcriptase activity units/ml culture fluid). The triangles represent cultures supplemented with 100 μg/ml cycloheximide. Circles are controls with no antibiotics. At the indicated period the cells were washed once and assayed for p30 on their surface by the paired label assay. Square (□) indicates RAG cells not treated with trypsin. Cycloheximide treatment failed to affect antibody binding to RAG cells in control experiments.
determinants were accessible, has been suggested (Obata et al. 1975; Tung et al. 1975). Since we would not expect to detect type specificities of BALB/c endogenous viruses with goat anti-Rauscher (of the FMR group) gp70, we examined a BALB/c lymphoma line, LSTRA, which was infected with Moloney leukaemia virus for surface gp70 (Fig. 1d). Any ‘type’-specific surface components of gp70 shared by Rauscher and Moloney virus would be detected on the LSTRA cell surface. A considerable amount of antibody of the goat anti-RLV gp70 bound to the surface of LSTRA (0.24 x 10^6 molecules/cell as a maximum) and to other cell lines infected with Moloney leukaemia virus (S. J. O’Brien, unpublished data). These results are consistent with the proposition that some gp70 ‘type’ specificities are accessible to antibody on cells making the glycoprotein, but intraspecies group specificities are not. Nevertheless, whether gp70 type specificities are expressed on RAG cell surfaces or not, the antigen detected by the goat anti-RLV p30 is clearly not gp70 because monospecific anti-gp70 prepared from the same virus strain (Rauscher) as the anti-p30 has a negligible binding activity.

We have mentioned that the mechanism by which p30 arises at the cell surface might be through passive adsorption of the protein from viruses in the culture fluid. Virus negative Basc-2 cells were incubated with increasing amounts of purified exogenous MuLV p30 for 2 h and tested for surface-associated p30 (Fig. 2a). MuLV p30 increasingly binds to the surface of the virus negative cells resulting in specific binding of anti-RLV p30 to the cell surface in agreement with the report of Grant et al. (1974). The amount of p30 bound, however, reaches a plateau corresponding to 4 to 5 ng anti-p30 binding 1 x 10^6 cells. This value is less than 20% of the amount of p30 detected on the RAG cell surface.

A second experimental approach to this question involved monitoring p30 reappearance on the cell surface following its removal with trypsin (Fig. 2b). Cells treated with sufficient trypsin to remove p30 were incubated in nutrient medium for up to 5 h in the presence of cycloheximide. Cells were similarly treated with trypsin and incubated in ‘conditioned’ medium which was taken from a log phase RAG cell culture after 27 h of virus production. If the p30 on the surface were derived from adsorption of supernatant p30, or as a by-product of virus infection, then cycloheximide would not be expected to block the post-trypsin appearance of p30 on the cell surface. However, if the p30 is synthesized by the cell and inserted on the cell’s membrane, then cessation of protein synthesis by cycloheximide would block the reappearance of the antigen. Fig. 2(b) demonstrates that antigen reappearance was effectively blocked by cycloheximide both in fresh or ‘conditioned’ (virus containing) medium. The reappearance of p30 on the RAG cell surface is complete in approx. 4 h.

Our results are compatible with the hypothesis that a considerable amount of MuLV-p30 is found on the cell surface, and that although some of it can be explained by passive absorption of culture fluid p30, the majority of it cannot. The simplest interpretation is that it is placed on the surface rapidly from within, after it is synthesized. An alternative, but less likely possibility, is that trypsin treatment also removes a receptor for exogenous p30 adsorption, the synthesis of which is also blocked by cycloheximide. This explanation, however, fails to account for the low binding of exogenous p30 to virus negative Basc-2 cells (Fig. 2a). Furthermore, the re-growth of a receptor might be detected by differential kinetics of reappearance of cell surface associated MuLV-p30 in the presence and absence of exogenous virus. The results in Fig. 2(b) failed to reveal any differences in antigen growth which were affected by exogenous virus.

The expression of cell surface p30 on m3SR and BALB/3T3 cells which produce no detectable MuLV (Grant et al. 1974) further suggests that p30 may arise on the cell surface.
in a process other than virus infection or passive adsorption. The relationship of cell surface p30 and gp70 to virus assembly and to immune recognition of cells expressing these antigens is at present unclear and requires further examination.

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**REFERENCES**


*(Received 31 March 1976)*