A Non-virion Surface Antigen on Moloney Sarcoma Virus-transformed Non-producer and Producer Cells

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(Accepted 28 March 1980)

SUMMARY

Sera from STU mice bearing sarcomas induced by cells producing the Moloney sarcoma-helper-virus (M-MSV/MLV) complex were cytotoxic for these cells as well as for M-MSV non-producer and M-MLV producer cells. Analysis by polyacrylamide gel electrophoresis of 125I-labelled surface antigens immunoprecipitated with such sera revealed the virus envelope glycoprotein gp71 on the producer cells and an additional antigen of mol. wt 55 K on the M-MSV-transformed producer and non-producer cells. This antigen was not found on non-transformed M-MLV-producing cells and was neither related serologically to structural polypeptides of murine C-type viruses nor to components of embryonal STU mouse fibroblasts and foetal bovine serum.

INTRODUCTION

In a previous communication (Weiland et al. 1978) we reported on the non-producer tumour cell line Sac(-). These cells were derived from a secondary tumour which developed in a mouse of the STU inbred strain at the site of a regressed Moloney sarcoma virus (M-MSV)-induced tumour. Sac(-) cells grow progressively, killing their hosts within 3 to 7 weeks. Sarcoma virus genome could be rescued from the Sac(-) cells by infection with Moloney helper virus (M-MLV), resulting in the establishment of a cell line producing focus- and XC-plaque-forming virus [Sac(+) cells]. These Sac(+) producer cells induce tumours in STU mice which only regress occasionally.

Sac(-) cells failed to induce transplantation immunity and reacted only weakly in cellular cytotoxicity tests. However, they were sensitive to cytotoxic antibodies. These antibodies were found in sera of mice inoculated with cells producing the M-MSV/MLV complex [e.g. Sac(+)] but not in those from mice bearing Sac(−) cell-induced tumours or inoculated with cells producing M-MLV alone. From these observations it was assumed that M-MSV-transformed STU mouse cells express an antigen on the plasma membrane distinct from surface expressed structural polypeptides of mouse leukaemia virus identified earlier as gp85, gp71 and p12 (Hunsmann et al. 1976; Schwarz et al. 1976; Schneider & Hunsmann, 1978).

The present study identifies this antigen on M-MSV-transformed producer Sax(+) and non-producer Sac(−) cells by SDS-polyacrylamide gel electrophoresis of immunoprecipitated 125I-lactoperoxidase labelled cell surface polypeptides. This component, of 55 K

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mol. wt., is immunologically unrelated to M-MSV/MLV virion polypeptides and proteins of normal cells and foetal bovine serum.

METHODS

Cells. The non-producer Sac(−) cell line originates from a secondary tumour of an STU mouse. This tumour grew at the site where an M-MSV-induced sarcoma had regressed. Superinfection of these non-producer cells with Moloney helper virus derived from the syngeneic Bc cells (Weiland & Mussgay, 1976) generated the Sac(+) cell line producing both M-MSV and M-MLV. The cell lines were used between their 70th and 90th transfer generation. Details of their origin and virological state have been described by Weiland et al. (1978). An M-MLV-producing tissue culture cell line, Bc, was derived from an M-MSV-producing ascitic tumour of STU mice (Weiland & Mussgay, 1976). Friend leukaemia virus (FLV)-producing Eveline cells (FES) were grown in suspension (Seifert et al. 1975). Mouse embryo fibroblasts (MEF) were prepared from 14 to 17 day-old STU mouse embryos. All cells were grown in a modified Eagle’s medium supplemented with 10% inactivated (30 min, 56 °C) foetal calf serum (FCS; Gibco, Glasgow, U.K.).

Viruses. Moloney helper virus and sarcoma virus complex were harvested from culture supernatants of Bc and Sac(+) cells, respectively. For purification, virus was banded in a linear 10 to 50% sucrose gradient (Beckman SW27 rotor, 25 000 rev/min for 18 h).

Mice. STU inbred mice were initially obtained from Dr Werner Schäfer, Max-Planck-Institut für Virusforschung, Tübingen. They have already been used in previous studies (Weiland & Mussgay, 1976; Weiland et al. 1978).

Antisera. Mouse anti-Sac(+) sera from three mice bearing small tumour nodules induced by Sac(+) cells were obtained 41 days after intramuscular (i.m.) inoculation of 1.8 x 10⁴ viable cells. Mouse anti-Bc sera from five mice were pooled 147 days after i.m. inoculation of 8 x 10⁵ viable Bc cells. Friend virus-specific antisera: antisera to Tween-ether (TE)-degraded FLV and purified FLV gp71 were prepared in goats, those against FLV p30, p125E, p12 and p10 in rabbits. Their origin and immunological reactivity have been described (Green et al. 1973; Schäfer et al. 1975; Schneider & Hunsmann, 1978). Feline leukaemia virus (FeLV) antiserum: this serum was raised in a goat immunized with FeLV/TE and obtained by F. de Noronha, Cornell University, Ithaca, N.Y. Rabbit antiserum to normal STU-MEF and FCS (Hunsmann et al. 1976) as well as normal sera of mice, goats and rabbits were included as controls.

Radioactive labelling of cells and viruses. Cell surface polypeptides were iodinated by a lactoperoxidase-catalysed reaction (Schneider & Hunsmann, 1978). Briefly, 5 x 10⁶ cells were detached with warm (37 °C) EDTA solution [0.2 g EDTA/litre of phosphate-buffered saline (PBS, pH 7.2)] and then suspended in PBS-F (PBS, 30 mm-phosphate) containing 1 mCi ¹²⁵I (NEN, Boston, U.S.A.) and 10 μg of lactoperoxidase (Boehringer, Mannheim, Germany). The reaction was started and sustained for 15 min at 30 °C by the addition of 0.3% H₂O₂ (Merck, Darmstadt, Germany). Virion surface polypeptides and total virion protein were iodinated with the lactoperoxidase and chloramine-T techniques, respectively (Schneider & Hunsmann, 1978). Cell surface glycoproteins were tritiated with ³H-borohydride after galactose oxidase treatment as described by Gähmberg (1976).

Sac(+) cells were grown to subconfluence in 250 cm² tissue culture flasks. Cultures were labelled for 7 h with ³H-amino acid mixture (100 μCi/ml) or ³H-glucosamine (60 μCi/ml) obtained from the Radiochemical Centre, Amersham, U.K. The culture media used for metabolic labelling contained 10% dialysed FCS. The concentration of unlabelled precursors was reduced to 20% of amino acid and glucose, respectively. Thereafter, viruses and cells were harvested as described above.
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Fig. 1. Cytotoxic activity of antisera to Sac(+) (△—△) and FLV gp71 (○—○) against various target cells. (a) Bc cells producing M-MLV; (b) Sac(+) cells producing M-MLV and M-MSV; (c) non-producer Sac(−) cells.

Immuno precipitation and PAGE. Labelled cells were washed five times in cold PBS, pH 7.2, and then disrupted with lysis buffer (10 mM-sodium phosphate, pH 7.2, 0.15 M-sodium chloride, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecylsulphate, 100 units/ml aprotinin (Sigma, München) as described by van Zaane et al. (1976). Insoluble material and nuclei were removed by ultracentrifugation (100000g for 30 min at 4 °C). Free 125I was removed by gel chromatography on Sephadex G-25 (Pharmacia, Uppsala, Sweden). Immunoprecipitates which formed after incubation (16 h, 4 °C) with 10 μl of antiserum or normal serum were adsorbed to 5 mg protein-A-Sepharose (Pharmacia) and washed four times by centrifugation (10000 g for 10 min at 4 °C) through 1 ml of lysis buffer containing 10% sucrose. Purified immunoprecipitates were dissolved in sample buffer and separated by SDS-PAGE on 9 to 16% acrylamide slab gels (Schneider & Hunsmann, 1978). 125I- or 3H-containing protein bands were visualized by autoradiography and fluorography (Bonner & Laskey, 1974), respectively.

Absorption experiments. Absorption experiments were carried out to test for a possible inhibition of the immunoprecipitation of Sac(+) cell surface proteins by virus structural polypeptides. Before immunoprecipitation, 10 μl of mouse anti-Sac(+) serum were incubated for 2 h at 4 °C with increasing amounts of gradient-purified virus from Bc and Sac(+) cells, respectively. Viruses were degraded with lysis buffer as described above for surface labelled cells. Similar absorption experiments were performed with a lysate of Sac(−) cells instead of disrupted virus.
Fig. 2. PAGE analysis of immunoprecipitates from surface iodinated Sac(+), Sac(−), Bc and FES cells. (A, F, K, P) Goat anti-FLV gp71 serum; (B, G, L, Q) mouse anti-Bc serum; (C, H, M, R) mouse anti-Sac(+) serum; (D, I, N, S) normal goat serum; (E, J, O, T) normal mouse serum.
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Fig. 3. PAGE analysis of surface antigens on Sac(+) cells as precipitated with Sac(+) antiserum. Lactoperoxidase iodinated antigens separated under (A) non-reducing and (B) reducing conditions. Galactose oxidase "H-borohydride labelled antigens: (C) unprecipitated cellular lysate, immuno-precipitate of (D) normal and (E) anti-Sac(+) mouse serum.

Cytotoxicity test. The complement dependent humoral chromium release assay was employed as described earlier (Hunsmann et al. 1976; Schneider & Hunsmann, 1978).

RESULTS

Characterization of mouse anti-Sac(+) serum by the chromium release assay

To compare the specificity of mouse anti-Sac(+) serum with goat anti-FLV gp71 serum both sera were titrated against three lines of Moloney virus-infected target cells (Fig. 1). M-MLV-producing Bc cells were lysed about three times more effectively with FLV gp71 antiseraum (Fig. 1 a). In contrast, anti-Sac(+) serum reacts more strongly against Sac(+) cells producing M-MSV/MLV complex (Fig. 1 b). MSV-transformed non-producer Sac(−) cells, however, are destroyed only by anti-Sac(+) serum (Fig. 1 c).

Immunoprecipitation of surface polypeptides from Moloney- and Friend-MLV-infected cells

The results of the cytotoxic experiments suggested qualitative differences of the plasma membrane antigens of Bc, Sac(+) and Sac(−) cells. To identify these antigens we analysed immunoprecipitates of lactoperoxidase iodinated cell surface antigens in SDS–PAGE as shown in Fig. 2. In addition to the three Moloney virus-infected cells, FES cells producing FLV were included in this experiment for comparison. Clearly, both M-MSV-transformed tumour cells Sac(+) and Sac(−) expose a common antigen with an apparent mol. wt. of 55 000. This surface polypeptide was only precipitated by Sac(+) antiserum (lanes C and H).
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Fig. 4. PAGE analysis of surface antigens on Sac(+) cells precipitated with virus-specific antisera. (A) Goat anti-FeLV serum; (B) goat anti-FLV serum; (C) goat anti-FLV gp71 serum; (D) rabbit anti-FLV p15E serum; (E) rabbit anti-FLV p12 serum; (F) rabbit anti-FLV p30 serum; (G) rabbit anti-FLV p10 serum.

and was undetectable on non-transformed Bc and FES cells (lanes M and R). As a consequence of virus production Bc, Sac(+) and FES cells expressed gp71 on their surface. The virus glycoprotein was precipitated with goat anti-FLV gp71 (lanes A, K and P), anti-Bc (lanes B, L and Q) and anti-Sac(+) mouse sera (lanes C, M and R). Normal goat (lanes D, I, N and S) and mouse sera (lanes E, J, O and T) were non-reactive.

Under non-reducing conditions the 55 K polypeptide migrated to a position of 60 K in SDS-PAGE (Fig. 3, lane A). Three kinds of experiments failed to identify a sugar moiety on the 55 K component. It did not bind to concanavalin A, was not labelled by the galactose oxidase borohydride method on the cell surface (Fig. 3, lane E) and did not incorporate $^9$H-glucosamine metabolically (Fig. 6, lane F).

Specificity of the immunoprecipitation of the 55 K component

Experiments were performed to test the specificity of the 55 K antigen precipitation and to obtain information about its derivation. The 55 K antigen could not be precipitated with anti-Sac(+) serum from lactoperoxidase iodinated normal embryonal STU fibroblasts. Absorption of this serum with a 20 times excess of a lysate of embryonal fibroblasts (MEF) did not interfere with the precipitation of gp71 and 55 K antigen from iodinated Sac(+) cells. Furthermore, rabbit anti-MEF sera and anti-FCS did not detect a component of 55 K on Sac(+) cells.

To test whether the 55 K antigen was related to a structural polypeptide of murine type-C viruses, we tried to precipitate the 55 K of Sac(+) cells with complex antisera against FeLV and FLV as well as those specific for FLV gp71, p15E, p12, p30 and p10. Only gp71 was
detectable (Fig. 4) with antisera reacting with MLV envelope components. We then attempted to block the precipitation of the 55 K antigen by anti-Sac(+) serum with detergent-disrupted virus released from Bc and Sac(+) cells; 220 μg Bc virus lysate suppressed the precipitation of gp71 from Sac(+) cells completely, while the precipitation of 55 K was affected only slightly (Fig. 5, lane A). In contrast, 350 μg of Sac(+) virus abolished the reaction of both gp71 and 55 K (Fig. 5, lane D). As expected, unlabelled Sac(−) extract only abolished precipitation of 55 K (Fig. 5, lane G).

Precipitation of radioactively labelled Sac(+) virus should help to decide whether the 55 K antigen is a virion polypeptide or a co-purified cellular contaminant in the Sac(+) virus preparation. A ³H-amino acid- (Fig. 6, lane A) and ³H-glucosamine- (Fig. 6, lane B) labelled M-MSV/MLV complex was detergent disrupted, precipitated with anti-Sac(+) serum and analysed on PAGE (Fig. 6, lanes C and D). Immunoprecipitates of lysates of Sac(+) cells were run on the same gel (Fig. 6, lanes E and F). In the ³H-amino acid labelled virus only gp71, p30 and two components in the p15 region were detected, while three additional polypeptides were precipitated from lysate of ³H-amino acid labelled Sac(+) cells (Fig. 6, lane E). One migrated to the position of about 100 K and the other two to 60 and 55 K, respectively. Neither component incorporated ³H-glucosamine (Fig. 6, lane F). As in metabolically labelled Sac(+) virus, the 55 K antigen was undetectable after immunoprecipitation of lactoperoxidase iodinated intact virus (not shown). However, 55 K could be immunoprecipitated from ¹²⁵I-labelled total protein of the same virus preparation (Fig. 6, lane H).
Fig. 6. Identification of 55 K antigen in labelled M-MSV/MLV and Sac(+) cells. PAGE analysis of \(^{3}H\)-labelled M-MSV/MLV (C, D) and Sac(+) cellular polypeptides (E, F) immunoprecipitated with mouse anti-Sac(+) serum. (A) \(^{3}H\)-amino acid and (B) \(^{3}H\)-glucosamine labelled, unprecipitated M-MSV/MLV. Immunoprecipitated virus polypeptides: (C) amino acid label, (D) glucosamine label. Immunoprecipitated cellular polypeptides: (E) amino acid label, (F) glucosamine label, (G) amino acid label immunoprecipitated with normal mouse serum. Chloramine T iodinated M-MSV/MLV was immunoprecipitated with (H) mouse anti-Sac(+) and (I) normal mouse serum.

**DISCUSSION**

The existence of a surface antigen on a Moloney sarcoma virus-transformed non-producer cell line Sac(−) has been suggested by Weiland et al. (1978). In this paper we have characterized a polypeptide of mol. wt. 55000, found on the surface of Sac(−) cells and on its rescued producer derivative [Sac(+) cells], using sera from mice bearing Sac(+) cell-induced tumours in a humoral chromium release test and PAGE analysis of immunoprecipitates of lysates from surface iodinated M-MSV sarcoma cells.

The electrophoretic mobility of this novel antigen in a 10 % acrylamide SDS–PAGE corresponds to a mol. wt. of 55000 under reducing and 60000 under non-reducing conditions. The latter finding may indicate that a smaller polypeptide of about 5 K mol. wt. is disulphide linked to the 55 K moiety under non-reducing conditions. Interestingly, a 60 K and a 100 K component are found in addition to 55 K after metabolic protein labelling. Since 100 K and 60 K are not found after surface iodination they may represent intracellular precursor polypeptides for the 55 K antigen. It is remarkable that this surface component does not seem to be glycosylated.

By immunoprecipitation, the 55 K antigen was found neither on normal cells nor on non-transformed, non-oncogenic cells replicating M-MLV or FLV in the absence of M-MSV. In addition, it could not be precipitated with rabbit anti-STU-MEF or rabbit anti-FCS sera.

There are indications that this 55 K surface antigen is unrelated to MLV structural
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polypeptides. Complex antisera against FeLV and FLV as well as FLV-component specific antisera failed to precipitate the 55 K antigen. Gradient-purified M-MLV did not block anti-55 K antibodies of the anti-Sac(+) serum, whereas the M-MSV/MLV complex harvested from Sac(+) cells did (Fig. 5). In this virus preparation the antigen was demonstrable only after iodination of proteins to high specific activity (Fig. 6). Thus, the antigen is either a virion polypeptide of M-MSV or a non-virion component co-purified with, or integrated into, virus particles budding from Sac(+) cells. Even though the results presented are not conclusive we favour the latter explanation since short time ³H-amino acid labelled virus from Sac(+) cells did not contain a 55 K polypeptide nor could such a component be enriched from labelled virus by immunoprecipitation with Sac(+) antiserum.

Antigens associated with MSV transformation have been described before. Aoki et al. (1973, 1974) detected, by immuno-electron microscopy, and antigen on rat and mouse cells non-productively transformed by M-MSV and the Kirsten strain of MSV. Earlier investigations (Strouk et al. 1972; Stephenson & Aaronson, 1972), however, could not detect new surface antigens on MSV-transformed cells. More recently, the Moloney cell surface antigen, a glycoprotein of mol. wt. 110,000 (Siegert et al. 1977), has been described on M-MLV-induced YAC lymphoma cells (Klein et al. 1966). Even though it cross-reacts with other protein species of mol. wt. 52,000, 92,000 and 180,000 to 190,000 (Troy et al. 1977) its relationship to our 55 K antigen remains to be determined.

The 55 K protein described here does not appear to be analogous to the pp60 src of avian sarcoma viruses (Brugge & Erikson, 1977) which has not been detected on the cell surface (Rohrschneider, 1979). Furthermore, the 55 K protein is non-phosphorylated (unpublished observation) and does not display a protein kinase activity under conditions in which pp60 src does (Rübsamen et al. 1979; H. Rübsamen, unpublished data). Finally, in vitro translation of the M-MSV genome gave no indications of a 55 K product (Philipson et al. 1978).

Recently, transformation-associated 50 to 60 K polypeptides have been identified by immunoprecipitation with anti-SV40 tumour sera in a number of laboratories (Lane & Crawford, 1979; Linzer & Levine, 1979; Melero et al. 1979). It appears likely that these antigens are of cellular origin rather than virus coded. DeLeo et al. (1979) described a transformation-related 53 K antigen precipitable with sera from mice bearing methylcholanthrene-induced tumours. It was found in murine cells transformed spontaneously in culture, in chemically induced sarcomas and leukaemias as well as in SV40- and murine sarcoma virus-transformed cells.

Experiments are under way to see whether the 55 K protein described here belongs to this new family of transformation-associated polypeptides. If so, this report would be the first indication of their presence on the cell surface.

The technical assistance of Annegret Schultz and Andrea Schenk is gratefully acknowledged. We thank Professor Werner Schäfer for his gift of FLV component specific antisera, Dr Helga Rübsamen for the protein kinase assay with the 55 K antigen and Professor Gernot Walter for help with the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft.

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