Key words: Marek's disease/tumour-associated antigen/MSB-1 cells

Characterization of an Antigen Associated with the Marek's Disease Lymphoblastoid Cell Line MSB-1

(Accepted 1 February 1982)

SUMMARY

A Marek's disease lymphoblastoid cell line (MSB-1) has been analysed by immunoprecipitation for expression of tumour-associated antigen, Marek's disease virus (MDV)-specific antigens and antigens specific to avian leukosis–sarcoma viruses. Rabbit antisera raised against two independently derived cell lines after extensive absorption with normal chick cells reacted with a polypeptide of mol. wt. 40000 (40K) in extracts of MSB-1 cells. The 40K polypeptide was not present in myeloblasts or in chick embryo fibroblasts (CEF) infected with MDV and did not react with antiserum raised against normal chicken thymus antigens. The possibility that the 40K polypeptide is a tumour-associated antigen is discussed. Seven MDV-specific antigens were noted in infected CEF (mol. wt. 110K, 100K, 80K, 70K, 50K, 35K and 32K) but none of these was detected in MSB-1 cells. The avian leukosis–sarcoma group-specific antigen P27gag and its precursor Pr76gag were not found in MSB-1 cells, confirming that expression of mature gag protein is not required for transformation by MDV. However, two polypeptides of unknown origin and function (mol. wt. 180K and 110K) were precipitated from MSB-1 cells with a rabbit anti-Rous sarcoma (Schmidt–Rupin, subgroup D) antiserum.

Marek's disease is a herpesvirus-induced lymphoma of chickens characterized by transformation of T cells. Previous studies have shown that the majority of the cells of several lymphoblastoid cell lines established from Marek's disease lymphomas express a cell surface tumour-associated antigen which is distinct from virus-specified antigens found in lytically infected non-lymphoid cells (Powell et al., 1974; Witter et al., 1975). In vivo studies showed that cells bearing the tumour-associated antigen appear in the spleen early after infection, increase in number as the disease progresses and comprise 10 to 30% of the cells of gross lymphomas. These results, together with the finding of reduced numbers of cells bearing the antigen in infected genetically resistant birds, have provided good evidence that this antigen is a marker for transformation. It is currently believed that tumour-associated antigen plays an important role in immunity by stimulating a cell-mediated immune response against malignantly transformed cells (Powell & Rowell, 1977). Molecular characterization of the tumour-associated antigen is essential in order to identify the genes that code for the antigen and for the eventual development of vaccines using molecular cloning or synthetic antigen.

The main objective of this study was to characterize electrophoretically the antigen that reacts with rabbit antisera that have been used extensively in studies on the role of tumour-associated antigen in pathogenesis and immunity (Witter et al., 1975; Murthy & Calnek, 1978; Powell & Rowell, 1977). Cell extracts were also examined for the presence of MDV-specific antigens and avian leukosis–sarcoma antigens in view of the controversial role of retrovirus expression in Marek's disease oncogenesis (Peters et al., 1973; Calnek & Payne, 1976).

The MSB-1 cell line (obtained originally from Dr S. Kato, Osaka University, Japan) was labelled metabolically with L-[35S]methionine (1000 Ci/mmol) at 400 μCi/ml for 4 h at 37 °C.
in methionine-free Eagle's medium. The cell line which had been subcultured extensively did not express MDV-specific antigens as shown by immunofluorescence and only 1 cell out of $10^7$ cells scored as infective centres. A non-producer cell line of myeloblasts transformed by avian myeloblastosis virus (AVM, MAV 2 obtained from Dr C. Moscovici, Veterans' Administration Hospital, Gainesville, Fla., U.S.A.) was similarly labelled and served as controls. Immunoprecipitation was carried out as described by Oppermann et al. (1979). Cells were washed after labelling, lysed in buffer (10 mM-tris pH 7.2, 100 mM-NaCl, 1 mM-EDTA, 1% Nonidet P40, 1 mg/ml bovine serum albumin), centrifuged at 80,000 g for 1 h and the supernatant collected. Rabbit antisera were added to aliquots of the solubilized antigen using 10 µl per 100 µl of antigen and were incubated at 4 °C for 1 h. Immune complexes were removed using formalin-fixed Staphylococcus aureus as described by Kessler (1976).

Antiser to Marek's disease tumour-associated antigen were prepared in rabbits by hyperimmunization with lymphoblastoid cell lines and were absorbed with normal chicken tissues until specific for transformed cell lines. The antisera used in this study were raised against MSB-1 and CUCL 1 cell lines respectively (Calnek et al., 1978). These antisera reacted strongly in immunofluorescence tests with cell surface antigens in 80 to 100% of the cells of several independently derived Marek's disease cell lines, but failed to react with normal thymus cells or with cells from avian leukosis lymphomas and avian leukosis cell lines (Murthy et al., 1979). Antiserum against avian leukosis-sarcoma antigens was obtained from rabbits bearing fibrosarcomas induced by the Schmidt–Rupin (subgroup D) strain of Rous sarcoma virus (RSV). This antiserum, generously given to us by Dr H. Oppermann (Department of Immunology and Microbiology, University of California, San Francisco, Ca., U.S.A.), contained antibodies to virion structural proteins as well as to transformation proteins (Oppermann et al., 1979). Convalescent Marek's disease serum was a pool of sera from three adult Rhode Island Red chickens that had survived infection with MDV. Antiserum against chicken thymus antigens was prepared by immunizing rabbits with chicken thymus cells and absorbing the serum with chicken liver homogenates, erythrocytes, bursa cells and insolubilized chicken IgG (Payne et al., 1974) until it reacted only with thymus cells in immunofluorescence tests.

The results of analysis of immunoprecipitates by electrophoresis in 8% acrylamide gels (Fig. 1) show the following. (i) Both anti-CUCL1 and anti-MSB-1 antisera reacted with a 40K polypeptide present in extracts of MSB-1 cells (b, lanes 3 and 4 respectively) but lacking in myeloblasts (a, lanes 3 and 4). The failure to precipitate this polypeptide from extracts of MSB-1 cells with normal rabbit serum (b, lane 1), with anti-RSV tumour antiserum (b, lane 5) and with anti-thymus serum (c, lane 2) suggests that the 40K polypeptide is tumour-associated. (ii) Several antigens, presumably thymus-specific, were precipitated from extracts of MSB-1 cells by anti-thymus serum (c, lane 2). Of these, a 55K polypeptide was prominent and appears to be a major thymus antigen. None of the thymus-specific antigens had the same electrophoretic mobility as the 40K tumour-associated antigen. (iii) At least three antigens that are normally synthesized during productive infection by leukosis–sarcoma viruses were identified in myeloblasts (a, lane 5) but not in MSB-1 cells (b, lane 5). Of these antigens, the mature group antigen P27$^{ gag}$ and its precursor Pr76$^{ gag}$ were the most obvious; p12 and p19 were not resolved in this gel and migrated with the dye band. We conclude from this result that MSB-1 cells do not express antigens associated with productive infection of exogenous leukosis–sarcoma viruses. However, it appears that an antigen of approximately the same mobility as the polymerase-gag precursor polyprotein (Pr180$^{ poly/gag}$) which was present in myeloblasts was also expressed in MSB-1 cells (lane 5 in a and b). In addition, another polypeptide of approx. mol. wt. 110K was also synthesized in MSB-1 cells. The identity or function of this antigen is not clear at present.
To determine whether MDV-specific antigens are synthesized in MSB-1 cells, a variation of the immunoprecipitation test was used because chicken immunoglobulin does not bind to *S. aureus*. Labelled MSB-1 cells or MDV-infected CEF were lysed in buffer (150 mM-NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM-tris pH 7.2, phenylmethylsulphonyl fluoride), centrifuged at 80,000 g for 1 h and the soluble fraction incubated with convalescent Marek's disease serum (10 µl per 100 µl antigen) for 4 h at 4 °C. Excess (200 µg IgG) of rabbit anti-chick IgG (Miles Laboratories) was added and, after incubation for 1 h at 4 °C, immune complexes were isolated with *S. aureus* and analysed electrophoretically (Fig. 2). At least seven polypeptides with mol. wt. 110K, 100K, 80K, 70K, 50K, 35K and 32K were isolated. The 35K and 32K proteins were very faint in the autoradiograms and cannot be seen clearly in Fig. 2. None of these seven antigens was present in MSB-1 cells (Fig. 1b, lane 2).
Moreover, anti-MSB-1 and anti-CUCL 1 antisera failed to precipitate any antigen in extracts of MDV-infected CEF (Fig. 2, lane 4).

This study has demonstrated that heterologous antisera raised against two independently established Marek's disease cell lines reacted with a 40K polypeptide in extracts of MSB-1 cells. This antigen appears to be neither a thymus tissue antigen nor a virus-coded antigen (Fig. 1 and 2) but is probably tumour-associated. The MSB-1 cell line used here had been
extensively subcultured, and although each cell contained approx. 15 copies of virus DNA, transcription of virus DNA was almost negligible (Ross et al., 1981). It is therefore possible that the tumour-associated antigen is host-coded. In view of recent reports on the presence of host-coded components in the T antigen of simian virus 40 (Lane & Hoeffler, 1980) and in EBNA in Epstein–Barr virus-transformed lymphocytes (Klein, 1981), it would not be surprising if Marek’s disease tumour-associated antigen also contained a cellular component. At present it is not known whether the 40K polypeptide is a virus-modified histocompatibility antigen or a derepressed host antigen unrelated to histocompatibility antigens but similar in size to these antigens (Ziegler & Pink, 1976). We cannot exclude either, the possibility that the 40K polypeptide is a virus-coded protein that is short-lived and undetectable in lytically infected CEF but which accumulates in abortively infected lymphocytes. Conclusive evidence on the nature and origin of the tumour-associated antigen must await the production of monoclonal antibodies and in vitro translation of its mRNA.

Our results are in accord with the failure of others to detect expression of avian leukemia–sarcoma group-specific antigen in MSB-1 cells using the COFAL test (Nazerian et al., 1978). However, at least two antigens were precipitated from extracts of these cells in this study with RSV tumour antiserum. One was similar in mol. wt. to Pr180\textsuperscript{pol-gag} and the other mol. wt. 110K was also present but in smaller amounts in myeloblasts and in uninfected cells (results not shown). It is of interest that synthesis of abnormal gag-related products by endogenous virus and by replication-defective leukemia viruses has been reported (Hayman et al., 1977).

Part of this work was carried out at the University of California, San Francisco during the tenure of a Research Training Fellowship awarded by the International Agency for Research on Cancer. I am grateful to Professor J. M. Bishop and H. Varmus for advice, Dr H. Oppermann for help with the immunoprecipitation tests and to Professor B. Calnek for the generous gift of antisera to MSB-1 and CUCL I cell lines. I am grateful to Dr P. M. Biggs and Dr H. Williams-Smith for critical reading of the manuscript.

Houghton Poultry Research Station
Houghton, Huntingdon, Cambs. PE17 2DA, U.K.

L. J. N. Ross

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*(Received 28 October 1981)*