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Purification of a Reverse Transcriptase-like Protein from the Plasma of a Patient with Chronic Myelogenous Leukemia and Production of Monoclonal Antibodies

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A. Introduction

Some human leukemic cells have been shown to contain a cytoplasmic reverse transcriptase (RT) [1]. The RT from cells of acute myelogenous leukemia (AML) patients has been shown to be specifically neutralized by IgG from hyperimmune sera prepared against RT of simian sarcoma virus (SiSV) and of gibbon ape leukemia virus (GaLV) [2]. An RT-like polymerase was also isolated from other neoplastic tissues, including the spleen of a child with myelofibrosis [3] and some melanoma tissue [4]. We have previously reported the presence of surface immunoglobulin on cells of patients with chronic myelogenous leukemia (CML) in blast crisis (BC) that specifically neutralized RT from feline leukemia virus (FeLV), and of surface IgG on AML and on some normal blood cells that preferentially reacted with RT from SiSV and GaLV [5, 6]. These membrane-bound IgGs were recovered by spontaneous release in the medium after overnight incubation. Since we found it difficult to identify a RT-like antigen on the surface of fresh leukemic cells, we looked at the plasma of a CML-BC patient for the presence of a RT-like antigen.

B. Results

I. Purification of a Protein with Chromatographic Properties of RT

A large quantity of plasma was obtained by repeated leukophoresis from a patient in CML-BC. Forty milliliters of it was supplemented with Triton X100 (0.5%), DTT (0.1 m*M*), NaCl (0.3 *M*), Tris HCl (0.05 *M*) pH 7.9, and PMSF (0.2 m*M*) and chromatographed on a DEAE-Agarose column to remove the nucleic acids. After a threefold dilution, the flow through was incubated for 10 min with 50 μ*M* polyguanilic acid [poly (G)] and 0.5 m*M* $MnCl_2$ and applied again to DEAE-Agarose following a procedure described by Sarangadharan et al. [7]. The 0.3-*M* eluate which did not possess enzymatic activity was iodinated and precipitated with different hyperimmune sera raised against type C virus proteins. A rabbit anti-Rauscher murine leukemia virus (R-MuLV) RT antiserum and an anti-FeLV p15 antiserum precipitated preferentially a protein of 74 kilodaltons. This protein was precipitated neither by a normal rabbit serum, nor by rabbit sera raised against R-MuLV gp70, R-MuLV p30, Baboon endogenous virus (BaEV) RT, and SiSV RT. In addition, goat antisera raised against SiSV gp70 or against human albumin did not precipitate the protein. The antiserum raised against R-MuLV RT appears very cross-reactive in enzyme neutralization assays; it neutralizes both R-MuLV RT and BaEV RT almost equally well. The antiserum against FeLV p15 appears to recognize p15E.

II. Production of Monoclonal Antibodies Against the 74K Protein

Balb/c mice were immunized subcutaneously with the crude preparation of RT-like antigen. Four weeks later, the spleen cells of the immunized mice were fused with SP/2 drug marked, non-immunoglobulin secreting myeloma cells [8].

Hybrid clones obtained were tested in a solid-phase radioimmunoassay for production of antibody recognizing the immunizing preparation. The iodine-labeled preparation of immunizing antigen was precipitated with the positive monoclonal antibodies. Two-dimensional electrophoresis of the immune precipitates showed that they precipitated the same 74K protein as the anti-R-MuLV RT and the anti-FeLV p15 antisera.

III. Further Purification of the RT-Like Protein

Taking advantage of the specificity of the monoclonal antibodies, an affinity column was prepared to further purify the antigen. From 30 mg crude RT-like antigen described above, 1.4 mg purified protein was eluted from the affinity column. The purified protein was labeled with ^{125}iodine and shown to be a clean preparation of a 74K protein by SDS polyacrylamide gel electrophoresis. It was also precipitable up to 100% by the rabbit anti-FeLV p15 antiserum and up to 80% by the anti-R-MuLV RT antiserum. The monoclonal antibody used to prepare the column also precipitated 100% of the labeled antigen. A monoclonal antibody against P15E (9) precipitated the protein up to 60% but with a low titer. The precipitation of the antigen could be competed by virus cores (R-MuLV, GaLV) and by purified FeLV RT (the only one tested in competition) but not by pure R-MuLV p30. The purified antigen still bound to poly (G) in the presence of $MnCl_2$ and when introduced in a RT assay competed with the active enzyme for the template primer dT_{12-18}-poly rA.

C. Conclusion

We purified a 74K protein with chromatographic properties like RT but no enzymatic activity from the plasma of a patient with CML-BC. After labeling with ^{125}iodine the protein was precipitable by a broadly cross-reactive antiserum raised against R-MuLV RT and by a rabbit antiserum raised against FeLV p15. Monoclonal antibodies were developed that recognized specifically the 74K protein. They allowed us to purify the protein further. We found that the purified protein still had affinity for poly (G) and could compete for the template primer with active enzymes in an RT assay. When labeled the pure protein could also be precipitated by monoclonal antibodies. We are currently investigating whether this protein is expressed on the surface of cells from CML patients in BC, for which we have previously demonstrated the presence of immunoglobulin with anti-RT activity.

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