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AMINO ACID ANALYSIS OF A TUMOUR - ASSOCIATED ANTIGEN ISOLATED FROM THE TUMOUR TISSUE OF COWS WITH ENZOOTIC BOVINE LEUKOSIS

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A tumour-associated antigen (TAA) expressed in tumour cells of cattle with enzootic bovine leukosis (EBL) was isolated from the tumour tissue of cows serologically and haematologically positive for EBL and patho-anatomically and histologically positive for lymphosarkcomatosis of the prolymphocytic type. After homogenisation, ultrasound disintegration and ultrahighspeed centrifugation of the tumour tissue, the glycoproteins of the supernatant were extracted using perchloric acid and the TAA was purified by gel filtration and successive SDS-polyacrylamide electrophoresis. The molecular weight of the TAA was estimated to be 70.000 D. By aminoacid analysis the total aminoacid content of TAA was found to be 5,935 mmol/g. The proportion of particular aminoacid groups was as follows: 74,8% neutral amino acids, 14,4% acidic amino acids, and 10,8% basic ones. Compared with non-tumour fractions the tumour fractions contained elevated amounts of glycine, alanine, isoleucine, leucine, and lysine.

Key words: tumour-associated antigen, aminoacid analysis, enzootic bovine leukosis, bovine leukemia virus

INTRODUCTION

The molecular differences in the malignant cell, i. e. the antigenic differences and the altered biochemical outfit of the tumour cell compared with the normal cell, can be characterized immunologically and biochemically (Staab, 1984). On the basis of the biochemical characterization the tumour - associated antigens (TAAs), including histocompatibility antigens, differentiation antigens, embryonal antigens, and complex carbohydrates have been identified as molecules that are expressed in quantitatively and qualitatively different ways in transformed cells and their untransformed counterparts (Aida et al., 1994).

Enzootic bovine leukosis, the most common neoplasltic disease of cattle, is caused by the bovine leukemia virus (BLV). It is characterized by persistent lymphocytosis and development of B-cell lymphoma after a long latent period. Aida et al. (1992) analyzed TAAs, expressed in tumour cells of cattle with EBL, by

use of 13 monoclonal antibodies. The monoclonal antibody c143 recognized TAA expressed by the periferal blood monouclear cells of BLV-negative cattle as well as the tumour cells of cattle with EBL. This c143 TAA was characterised as 3 normal bovine glycoproteins with molecular weights of 32,000, 34,000 and 35,000 - 37,000 kDa, expressed in the B-cells mainly, differing from the antigens coded by the BLV (Aida et al., 1993.).

The origin and role of TAA in animals with EBL are so far not fully understood. TAA is expressed in the cytoplasm and the cell membrane of the tumour cells and is different from the antigens coded by the BLV. BLV does not express its gene products at a detectable level in freshly collected lymphocytes or tuour cells from the infected host, while it often expresses them when these cells are cultured in vitro. Thus, the expression of TAAs may play a key role in BLV-induced leukemogenesis and in the maintenance of the tumorous state (Aida et al., 1992b). Because the expression of this protein only occurs in the infected lymphocytes in vivo when the virus protein expression is blocked, it seems to be coded by genes of cell origin (Bicka et al., 1993).

The aim of this work was to isolate the tumour-associated antigen from the tumour tissue of EBL-positive cows, to perform aminoacid analysis of the isolated TAA and to compare the aminoacid composition of the isolated glycoprotein antigen with the aminoacid composition of some tumour-associated antigens occuring in other malignancies.

MATERIAL AND METHODS

Tumour and non tumour tissues. The tumour tissues used for preparing the basic biological material were obtained post mortem from cows with the lymphosarcomatosis of the prolymphocytic type detected by means of patho-anatomical and the histological examinations. Before or after slaughter the animals were examined for EBL serologically and haematologically.

The non-tumour tissues used for preparing the control biological material were obtained post mortem from BLV-serongative cows and cows shown to be EBL-negative by patho-anatomical examination.

Chemicals. The chemicals and the preparations used were supplied by Lachema, (n. p. Brno) and Medika, (n. p. Bratislava) at the purity degree p. a.

Isolation of the tumour-associated antigen

After washing the tumour tissue three times in buffered physiological solution (PBS, pH 7,2) the tumour tissue was homogenised in the biological homogenizator at 4^{0}C in the medium N2 TET (Grofova et al., 1981) with 1 ml of the antibiotics (PNC and STM) and 2 drops of potassium tylosine per 100 ml of mixture. The homogenised mixture was disintegrated by ultrasound at 15 amplitudes in 6 multiple intervals for 30 seconds at 4^{0}C and submitted to centrifugation at 4.000. g for 30 seconds at 4^{0}C. After removing the fat layer the supernatant was again centrifuged 2 times at 100 000. g for 1 hour at 4^{0}C. The clear supernatant was examined for the presence of BLV-antibodies by the immunodiffusion test and its protein concentration was determined. The non-

tumour tissues were processed in the same way, too. The protein concentration was determined by the method of Lowry et al. (1951) or BIOLA test (Lachema n. p., Brno). For the glycoprotein extraction 0.6 M perchloric acid was used. The resulting filtrate was dialysed against phosphate buffer solution (pH 7,2) for 24-72 hours. After centrifugation at 600. g, the protein concentration was determined in the supernatant

Purification of the tumour-associated antigen. The supernatant with the protein concentration adjusted to 20 mg/ml was used in the purification of the TAA by gel filtration through a colomn filled with Sephadex G-200 fine, which had been equilibrated with 0,01 mol phosphate buffer (pH 7,2). The separation passed at the flow rate of 5 ml/15 min. The optical absorbance was measured on the flow spectrophotometer. The possible presence of virus-specific antigens in the eluate fractions was detected with the imunodiffusion test and the protein concentration was determined.

Aminoacid analysis of the tumour-associated antigen

1 gramme ofthe sample was hydrolysed in 150 ml of 6N HCl in the laboratory drier at 105^{0}C for 22 hours. The cooled hydrolysate was filtered, washed in 6N HCl and dilated with distiluted water. After evaporation to as small volume in a rotating evaporator, neutralising and repeating the evaporation the sample was diluted with the dosing buffer solution (pH 2,2) to an extent depending on the protein concentration. The adjusted sample (100 μl) was submitted to aminoacid analysis based on the principle of separation chromatography in the presence of the ninhydrin agent (12 ml/hour) on a colomn (35 cm) filled with the ionex ostion LGAN B in Na+ cycle and the buffer solutions I, II and III at a flow rate of 14 ml/hour. The colour intensity was detected by the photocell and the individual amino acids registered by the digital integrator IC-26. The amounts of individual amino acids in the samples were calculated in relation to three standards recorded before eluting the samples.

RESULTS

Isolation of the tumour-associated antigen.

The protein concentration in the soluble fractions of the ultracentrifuged tumour and no-tumour homogenates was measured before and after precipitation with percipitation with perchloric acid. After precipitation the protein concentration ranged from 20 to 57 mg/ml according to the type of examined tissue.

To exclude the presence of virus-specific antigens in the soluble fractions of tumour and non-tumour tissues, the immunodiffusion test was performed. No precipitation lines between the tumour homogenate fractions and the BLV-positive serum were observed. Soluble fractions prepared in this way served as the basic material for the next purification steps.

Purification of the tumour-associated antigen

1) Purification by means of the chromatographic methods.

The fraction resulting from the extraction with perchloric acid was submitted to gel filtration on the column. The chromatogram from the soluble fraction of the tumour homogenate showed five fractions.

2) Purification with SDS-polyacrylamide electrophoresis

The fractions containing the tumour-associated antigen resulting from the perchloric acid extraction and the succeeding gel filtration were submitted to SDS-PAGE. The molecular weight of the antigen was estimated using the calibration curve prepared by means of the protein standards (bovine serum albumin - m. w. 66 500, ovoalbumin - m.w. 44 500, trypsin - m.w. 24 000). The molecular weight of the isolated antigen was about 70.000.

Aminoanalysis of the tumour-associated antigen

The total amino acid content in the tumour fraction was 5.935 mmol/g. Neutral amino acids made up 74.8%, acid amino acids 14.4%, and basic ones 10.8% of the total amount.

The total amino acid content in the non-tumour fraction was 3.995 mmol/g. Neutral amino acids acconated for 15.4%, acid amino acids 8.7%, and basic ones 75.9%.

Regarding particular amino acids, the tumour fractions were compared with the non-tumour fractions and elevated amounts of (in mmol/g): glycine (0.223:0.063), alanine (0.074:0.00), isoleucine (0.144:0.075), leucine (0,205:0.090), and lysine (4.695:3. 196) were found.

DISCUSSION

The results of the aminoacid analysis of the TAA isolated from the tumour tissues of the BLV-positive cattle showed the highest proportion of neutral amino acids (74.8%). Substantial elevation of glycine, alanine, leucine, isoleucine, and lysine was observed.

These differences in the quantitative composition of particular aminoacid groupd show that the purified preparations are not homogenous but they express intramolecular heterogeneity.

In contrast with our results, aminoacid analysis of the tumour-associated surface antigen (TASA) expressed by turkey cells transformed with the avian leukosis virus (strain Mc31) (Vesselinova 1995) showed a substantially elevated content of the basic amino acids and glycine, too. TASA with the molecular weight of 14 000 D was purified by immunoadsorption, SDS-PAGE and immunoblotting. According to the author, the extremely high glycine content may be due to the fact that generally the membrane proteins contain a high portion of glycine and the isolated TASA is a membrane-bound protein. Staab (1984) investigated the aminoacid composition of carcinoembryonic antigen (CEA) preparations originating from various research workplaces and from the National Institute for Biological Standards, England. The deviations in the aminoacid composition were insignificant. All of them contained a high portion of the acid amino acids (asparagine and glutamic acid) and the hydroxyamino acids (serine and

threonine), while the proportion of the basic amino acids (lysine, arginine, histidine, cysteine and methionine was lower.

According to Masiar et al. 1983, malignancy can be regarded not only as a local phenomenon but also as a disorder of the metabolic activity and of the physiological functions of the whole organism. After aminoacid analysis of human sera this author found that all kinds of malignant bone tumours were accompanied by a decrease of serum alanine and cysteine and by an increase of branched chain amino acids. Thus malignant tumour growth imitated starving. The tumour energetic demands increased with the malignant cell number. After the liver glycogen reserve is exhausted, alanine is converted into glucose in the glucoso-alanine cycle in liver, so it represents not only a source for protein synthesis but also an energetic source.

In relation to the biochemical processes in organisms with malignant disease it could be worth following up the connection between the proportion of some serum amino acids and the proportion of these amino acids in the tumour-associated antigens isolated from the respective tumour tissue.

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ANALIZA AMINOKISELINA TUMORSKIH ANTIGENA IZOLOVANIH IZ TUMORSKOG TKIVA KOD KRAVA SA ENZOOTSKOM BOVINOM LEUKOZOM

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SADRŽAJ

U radu je analiziran aminokiselinski sastav tumorskih antigena izolovanih iz tumorskog tkiva kod krava obolelih od leukoze. Ukupna koncentracija aminokiselina u tumorskim antigenima iznosila je 5,975 mmol/g a molekulska masa antigena bila je 70 000Dlt. Utvrđeno je prisustvo 7,8% neutralnih, 14,4% kiselih i 10,8% baznih aminokiselina.