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DETECTION BY RADIOIMMUNOASSAY OF ANTIGENS RELATED TO THE P30 POLYPEPTIDES OF PRIMATE TYPE C ONCOVIRUSES IN PERIPHERAL LEUKOCYTES FROM PATIENTS WITH CHRONIC MYELOGENOUS LEUKAEMIA

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Summary. — Leukocytes from patients with chronic myelogenous leukaemia were examined for antigens related to the p30 polypeptides of baboon endogenous virus (BaEV) and gibbon ape leukaemia virus (GaLV). Samples from patients with the quiescent phase of the disease proved to be negative or contained p30 of BaEV as the only viral antigen. In cases of blastoid crisis or acceleration, an antigen related to p30 of GaLV could be detected. In 5 of 6 patients, acceleration or blastoid crisis was preceded by expression of GaLV p30.

Key words: p30 oncoviral polypeptide; radioimmunoassay; chronic myelogenous leukaemia

Introduction

Oncoviruses are aetiologically involved in naturally occurring leukaemias in a number of vertebrate species (Kaplan, 1978). The widespread distribution of these viruses among vertebrates has led to intensive efforts to demonstrate their presence in man. A major approach has been the application of highly sensitive radioimmunological techniques to the search for the presence of viral antigens in cells or body fluids in humans at potentially high risk to virus exposure or disease. However, reports on the presence or absence of viral structural proteins in human cells have been rather contradictory. The detection of p30 of primate type C oncoviruses by radioimmunoassay (RIA) in myelogenous leukaemia has ranged from 5 out of 7 (Sherr and Todaro, 1974) to 0 out of 12 cases (Spiegelman, 1976). It has been clear that validating the specificity of competitive RIA, particularly in extracts, is very difficult. Incubation damage by proteolysis of labelled antigen may result in diminished radioactivity and apparent competition (Charman and Gilden, 1978). In a study reporting positive RIA reactions in human tissues, a total of 20 hr incubation at 37 °C in the absence of protease inhibitors was employed (Strnad and August, 1974). On the other hand, it has to kept in mind that EDTA in the generally used buffers removes Ca^{2+}

Table 1. p30 proteins of BaEV and GaLV in extracts of leukocytes from patients with the quiescent phase of CML

Patient No.	Age (years)	Leukocytes per μl blood	Leukocytes blasts %	ng competitor per mg of total protein BaEV*
1	51	15 900	3	1.11
2	16	30 000	2	0.84
3	44	22 500	0	2.42
4	60	48 000	0	0
5	45	22 800	0	0.55
6	35	24 000	0	0.82
7	35	19 100	0	0.14
8	51	13 200	0	2.18
9	69	10 200	1	8.15
10	79	27 000	2	0.92
11	64	10 400	0	0
12	79	13 000	0	0
13	74	25 700	0	0.19
14	66	24 200	0	0.63

* The results with GaLV were invariably negative.

and Mg^{2+} which in turn are advantageous for the electrostatic forces involved in antigen-antibody binding (Kurth *et al.*, 1979). Recently, we have succeeded in demonstrating antigens related to the p30 polypeptide of primate type C viruses by immunofluorescence in leukocytes from patients suffering from chronic myelogenous leukaemia (Váczi and Tóth 1980). In the present work we attempted to confirm the presence of oncoviral antigens in leukaemic leukocytes by competitive RIA.

Materials and Methods

Purification of leukocytes. Boyle's method as modified by us was used. Erythrocytes were removed by lysis in 0.83% ammonium chloride solution. For details see Tóth *et al.* (1977).

Preparation of cell extracts. One ml of phosphate buffered saline (PBS) pH 7.2 containing Ca^{2+} and Mg^{2+} and supplemented with ovalbumin (0.5 mg/ml), 0.5% Nonidet P-40 and 2 mM phenylmethyl-sulfonyl fluoride (PMSF) was added to 10^8 leukocytes. The cells were disintegrated by five ultrasonic treatments (1.5 mA, 10 sec). The treated samples were then incubated at 37 °C for 15 min and finally centrifuged at 18,000 × g for 15 min at 4 °C. The supernatants were used as competing antigens in RIA. Proteins were quantitated by the method of Lowry *et al.* (1951).

Purification of p30 polypeptides from primate oncoviruses. The M7 strain of baboon endogenous virus (BaEV) and the gibbon ape leukaemia virus (GaLV) were provided by the Pfizer Laboratories (Maywood, NJ). The BaEV and GaLV p30 proteins were purified by phosphocellulose column chromatography as described by Strand and August (1973). Viral polypeptides were radioactively labelled with ^{125}I by the chloramine method of Greenwood *et al.* (1963).

Immune sera. Goat immune sera to BaEV p30 and GaLV p30 were provided by R. Wilsmack (Huntingdon Research Center, Baltimore, MD). Precipitating rabbit anti-goat immune serum was from the Human Institute (Budapest, Hungary).

Competitive radioimmunoassay. The dilution of antiserum added was that which precipitated approximately 50% of the labelled antigen, and the competitive inhibition of binding of the ^{125}I-labelled antigen was measured. At first 200 μl of leukocyte extract and 20 μl of anti-p30 serum were incubated at 37 °C for 1 hr. Then 10 μl of ^{125}I-labelled antigen were added and

Table 2. Detection of p30 proteins of BaEV and GaLV in leukocytes from patients with the blastoid or accelerated phase of CML

Patient No.	Age (years)	Phase of CML	Leukocytes per μl blood	Leukocytes blasts %	ng competitor per mg of total protein BaEV	ng competitor per mg of total protein GaLG
1	48	Blastoid	21 700	15	0.48	1.26
2	41	Blastoid	121 500	15	0.36	1.88
3	49	Blastoid	73 000	20	0.74	0.48
4	61	Blastoid	186 000	74	0.85	1.09
5	24	Blastoid	40 000	35	1.03	0.85
6	47	Accelerated	32 600	3	1.97	1.12
7	68	Accelerated	41 000	5	0.72	1.34
8	44	Accelerated	35 000	0	1.89	0.85
9	45	Accelerated	41 000	0	0.56	0.24
10	57	Accelerated	38 000	3	4.41	0.41

the mixture was incubated at 37 °C for 3 hr. Thereafter 15 μl of rabbit anti-goat serum was added to the samples which were subsequently incubated for 1 hr at 37 °C and then for 18 hr at 4 °C. After termination of incubation, the immune complexes were sedimented (200 × g, 15 min) and washed twice in 0.5 ml of buffer at 4 hr intervals at 4 °C to allow for reaggregation of small, soluble immune complexes. Radioactivity of sediments resuspended in buffer was assessed in a gamma scintillation counter. The same buffer as used for preparation of cell extracts was employed as diluent and for washing.

Results

Data for 14 untreated patients with chronic myelogenous leukaemia (CML) are presented in Table 1. These patients were easily kept in balance by chemotherapy, i.e. they were in the quiescent phase of CML. Leukocytes from 11 of the 14 patients contained an antigen related to the p30 polypeptide of BaEV. Their antigen content was expressed in ng of competitor per mg of total protein. In leukocytes of the remaining 3 patients p30 antigen specific of BaEV could not be detected. All the samples proved to be negative for p30 of GaLV.

Table 2 presents the results of detection of p30 proteins in leukocytes from 10 patients with the accelerated phase (development of resistance to chemotherapy) or blastoid crisis of CML. All the extracts tested by competitive RIA proved to be positive for the presence of both BaEV- and GaLV-specific p30.

Six CML patients showed changes in the course of their illness during the four-year period of observation. The results obtained at three intervals for each patient are given in Table 3. The first date corresponds to that of the first blood sampling, the second to the first change observed in the p30 antigen spectrum and the third to the onset of the change in clinical picture. In the first two patients the quiescent phase CML turned into blastoid crisis. The remaining four patients developed resistance to chemotherapy (accelerated phase). In these patients the antigen spectrum changed (appearance

Table 3. Changes in the clinical picture and in the p30 antigen spectrum of patients with CML

Patient No.	Age (years)	Phase of CML	Months	Leukocytes per µl blood	Leukocytes blasts %	ng antigen per mg of total protein BaEV	ng antigen per mg of total protein GaLV
1	61	Quiescent	0	32 000	1	0.72	0
		Quiescent	3	42 500	0	0.61	0.41
		Blastoid	6	90 000	45	0.85	1.09
2	24	Quiescent	0	36 000	2	1.31	0
		Accelerated	2	56 000	9	0.83	0.41
		Blastoid	3	50 000	30	1.03	0.36
3	47	Quiescent	0	37 000	0	0.12	0
		Quiescent	1	26 200	4	0.41	0.36
		Accelerated	3	32 600	13	1.98	1.12
4	68	Quiescent	0	16 300	0	0.21	0
		Quiescent	6	17 800	0	0.71	0.34
		Accelerated	10	41 000	5	0.72	1.34
5	44	Quiescent	0	28 000	0	1.42	0
		Quiescent	3	28 500	0	0.66	1.19
		Accelerated	10	35 000	1	1.89	0.85
6	45	Quiescent	0	11 800	0	0.15	0
		Quiescent	5	21 600	0	0.11	0.66
		Accelerated	8	56 600	2	1.92	0.31

of GaLV-specific p30) 2—7 months before the acceleration or blastoid crisis with the exception of patient No. 2.

The presence of BaEV-specific p30 was demonstrated in six of 40 control persons. No control samples contained a competitor related to p30 of GaLV.

Discussion

The present results suggest that purified viral proteins provide a means to test for possible correlations of viral protein concentration to analogous human diseases. The presence of a potent protease inhibitor (PMSF) and Ca^{2+} and Mg^{2+} seems to be necessary in all buffers. It appears that the expression of p30 polypeptide of BaEV is not unique to malignant tissue, because a related antigen could also be detected in samples from control persons. However, the frequency of the expression of BaEV-specific p30 in control samples was very much lower as compared to that in samples from CML patients. Immunological studies of the p30 antigen of endogenous primate type C viruses found in different Old World monkey tissues have suggested that this major antigenic determinant has not undergone extensive evolutionary divergence (Sherr *et al.*, 1974). The present results showed that the BaEV-specific p30 antigen detected in human cells is also closely related to those found in tissues of other primates. This assumption is supported by the presence of antigen-antibody complexes related to the BaEV in humans with acute lymphoblastic leukaemia (Stass *et al.*, 1980). Whether the proteins of BaEV and GaLV reflect the occurrence of infectious virus spread from man to man or from animal to man, or of endogenous viruses, has to be

determined. DNA sequences related to BaEV have been detected in both normal and leukaemic human tissues (Wong-Staal *et al.*, 1976), whereas GaLV-related DNA sequences could not be found in normal cells (Campbell *et al.*, 1978). The reason for this discrepancy is not clear, but it is possible that the extent of nucleic acid homology for GaLV is too small to be detected in nucleic acid hybridisation experiments, and that the expression of GaLV-specific gene product observed in CML patients occurred during the leukaemogenic process. The present results may be of interest mainly from prognostical point of view. Ultimate proof of this model should include the complete purification and characterization of these putative viral proteins.

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