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STUDIES ON THE SPECIFICITY OF HUMAN ANTIBODIES REACTING WITH GP70 AND P15 ANTIGENS OF BABOON ENDOGENOUS (BaEV) AND GIBBON APE LEUKAEMIA (GaLV) VIRUSES

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Summary. — Antibodies reacting with gp70 and p15 antigens of baboon endogenous virus (BaEV) and gibbon ape leukaemia virus (GaLV) were detected by radioimmunoprecipitation (RIP) in blood plasma samples of patients with chronic granulocytic leukaemia, acute myeloid leukaemia and with potentially preleukaemic haematological disorders. Anti-gp70 antibodies were found more frequently than anti-p15 antibodies. Digestion of the carbohydrate part of gp70 antigens by glycosidase treatment abrogated the precipitation mediated by IgM antibodies, whereas that mediated by IgG antibodies was not markedly affected. Data suggest that antibodies detected in human plasma samples may have oncovirus specificity, but in considerable part of cases they can be of heterophil nature.

Key words: oncoviral gp70 and p15; radioimmunoprecipitation; glycosidase treatment; myeloid leukaemia; preleukaemic disorders

Introduction

Several laboratories have isolated viruses closely related to primate C-type viruses from cultured human cells (Gallagher and Gallo, 1975; Panem *et al.*, 1975; Nooter *et al.*, 1975; Kaplan *et al.*, 1977). Studies of the p30 and gp70 major oncovirus antigens suggested that they have been immunologically and functionally highly conserved during evolution and possess interspecies-specific antigenic determinants (Sherr *et al.*, 1974; Devare *et al.*, 1978; Fine *et al.*, 1980). It was suggested that humans might developed immune response possibly induced by human oncovirus strains. Immune reactivity to animal oncovirus glycoproteins was indeed demonstrated (Kurth *et al.*, 1977; Hirsch *et al.*, 1978: Thiry *et al.*, 1978). However, considerable controversy exists in the literature with respect to the specificity of antibodies to animal oncoviruses in human sera. Results of Barbacid *et al.* (1980) and Löwer *et al.* (1981) indicated that the carbohydrate moiety of the viral gp70 reacted

with the human sera. Therefore, these antibodies had to be directed against antigenic determinants specified by the host cell rather than against the virus-coded gene product. Moreover, Barbacid *et al.* (1980) have shown that oncoviruses grown in human cells cannot be recognized by natural human antibodies probably because man is tolerant to the corresponding human oligosaccharide side-chains in the virus glycoprotein provided by human host cells. Recently, Hehlmann *et al.* (1981) have succeeded in detection of antigens cross-reacting with gp70 of primate C-type viruses in sera of patients with leukaemia. The reaction was not due to the recognition of carbohydrate moieties as demonstrated by prior treatment with glycosidases. In the present study we show that human antibodies are capable of reacting with antigenic polypeptide determinants of primate oncoviral envelope antigens.

Materials and Methods

Cells, viruses and blood plasma samples. The NC37 and A204 cells were obtained through the Office of Program Resources and Logistics, Viral Oncology Program, National Cancer Institute. NC37 cells were grown in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS) (Gibco Bio-Cult. Ltd., Paisley, Scotland). A204 cells were grown in Dulbecco's modified Eagle's MEM with 10% FCS.

The baboon endogenous virus (BaEV) propagated in A204 cells (Lot. No. 990A) was from the Frederick Cancer Research Center (Frederick, Md., USA) and the gibbon ape leukaemia virus (GaLV) propagated in NC37 cells (Lot No. 18—76) was from the Pfizer Laboratory (Maywood, N. J., U.S.A.). Both viruses were provided in purified form through the Office of Resources and Logistics, N.C.I. (Bethesda, Md., U.S.A.).

Heparinized blood samples from patients with myeloid leukaemias, potentially preleukaemic haematological disorders and control persons were centrifuged and their plasma was decanted and stored at —20 °C until used. All plasma samples were absorbed with uninfected NC37 and A204 cells and FCS before RIP experiments. Cells destined for absorption were colleted and washed three times with phosphate buffered saline (PBS). FCS proteins were obtained by lyophilization of serum. All absorptions were carried out by incubating 0.8 ml of human plasma with either 10^7 cells or 10 mg of FCS proteins at 37 °C for 1 hr and at 4 °C for 4 hr.

Purification of gp70 and p15 antigens. A method for isolating pure envelopes from C-type RNA viruses (Van de Ven *et al.*, 1978) was combined with methods for preparation of envelope glycoproteins from Rauscher virus (Strand and August, 1973; Strand and August, 1976) and from endogenous primate retroviruses (Fine *et al.*,1980). Briefly, 200 μg of sodium dodecyl sulphate (SDS) was added to 10 ml of virus suspension containing 20 mg of GaLV in TNE buffer (10 mmol/l Tris-HCl, pH 7.4—100 mmol/l NaCl — 1 mmol/l EDTA). After incubation for 5 min at 4 °C, 2 vol of cold ether were added and the suspension was shaken for 10 min. The aqueous phase was centrifuged at 110,000 g for 90 min in a SW27 rotor (Beckman), and the supernatant was then dialysed against TNE buffer. Solubilized gp70 and p15 were isolated by chromatography on 1.5 × 6 cm columns of phosphocellulose (Whatman P-11) under conditions described by Strand and August (1973). The effluent was monitored continuously at 280 nm in a Beckman Model 25 spectrophotometer. Pools of fractions comprising gp70 and p15 peaks were concentrated by dextran. The pool containing p15 was checked for homogeneity by SDS-PAGE and dialysed against TNE buffer.

The gp70 fractions dialysed against Con-A buffer containing 0.05 mol NaCl, 1 mmol/l $MgCl_2$, 1 mmol/l mercaptoethanol and 2 mmol/l Tris-HCl (pH 7.4), were further purified by affinity chromatography on 0.9 × 10 cm Con-A-Sepharose column. The column was washed extensively with Con-A buffer and the absorbed glycoproteins were eluted with 0.1 mmol/l α-methylmannoside. Fractions containing gp70 were pooled, concentrated by dextran and dialysed against TNE buffer. Molecular weights for gp70 and p15 were determined by SDS-PAGE (Segrest *et al.*, 1971). Gels were stained for protein with Coomassie brilliant blue and for carbohydrate by the PAS

procedure (Glossmann and Neville, 1971). Protein quantitation was performed according to Lowry *et al.* (1951).

Purification of BaEV gp70 and p15 was essentially similar as for GaLV antigens except that the glycoprotein was further purified on lens culinaris Sepharose column and eluted with 1 mol/l α-methylmannoside (Fine *et al.*, 1980).

Glycosidase treatment of viral gp 70. The procedure was carried out as described by Ohno *et al.* (1979) was followed. 40 ug of a glyxosidase mixture (Miles, Frankfurt, FRG) was added to 400 μg of BaEV or GaLV gp70 in 50 mmol/l sodium citrate buffer, pH 4.0. The efficacy of the glycosidase treatment was controlled by SDS-PAGE of glycoproteins with and without glycosidase treatment on parallel gels. The glycosidase-treated antigens were free of carbohydrate.

Radioimmunoprecipitation (RIP) assay. Purified viral antigens were labelled to high specific activity with ^{125}I by the chloramine-T method (Greenwod *et al.*, 1963). The experimental conditions described by Kurth *et al.* (1977) were utilized throughout the present study. The carrier and washing buffer contained 2 mmol/l phenylmethyl-sulphonyl fluoride. All solutions were centrifuged just before assay to remove possible aggregates. 10 μg of virus antigen, 70 μg of carrier buffer and 20 μg of test plasma at twofold dilutions (started from 1 : 5) were incubated for 3 hr at 37 °C in siliconized tubes. Then heavy chain specific anti-human IgM or anti-human IgG serum (Hyland, Costa Mesa, Ca., U.S.A.) was added in 20-fold excess over the test plasma and incubation was terminated after 18 hr at 4 °C.s Centrifugation of the immune complex (1000 g, 15 min.) was followed by two washings. Radioactivity of sediments was assessed in a gamma scintillation counter.

Results

Frequency of antiviral IgM and IgG

Frequency of IgM and IgG antibodies reacting with native gp70 and p15 antigens of BaEV and GaLV is shown in Table 1. Plasma samples were collected from patiens with chronic granulocytic leukaemia (CGL), acute myeloid leukaemia (AML) and potentially preleukaemic haematological disorders and from normal donors. In patiens with potentially haematological disorders the diagnosis was idiopathic refractory sidoroblastic anaemia (IRSA) or pancytopenia. In patiens with IRSA the presence of ringed sideroblasts in the bone marrow was regarded as an early indicator of imminent leukaemic transformation. In those with pancytopenia, the polymorphonuclear leukocytes showed pseudo-Pelger anomaly or nuclear hyposegmentation, the platelets were unusually large and bizarre, appearing pseudopodies and

Table 1. Frequency of human antibodies reacting with gp70 and p15 antigens of BaEV and GaLV

Plasma source	Antibody to							
	BaEV				GaLV			
	gp70		p15		gp70		p15	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
CGL	12/86*	35/86	3/86	11/86	11/86	42/86	2/86	21/86
AML	2/34	11/34	1/34	8/34	5/34	13/34	3/34	10/34
Preleukaemia	3/22	10/22	2/22	7/22	3/22	11/22	1/22	8/22
Control	6/102	28/102	0/102	3/102	1/102	4/102	0/102	0/102

* = positive plasma samples / number of plasma samples tested

Table 2. Frequency of human antibodies reacting with native and glycosidase-treated gp70 antigens

Plasma source	Antibody to							
	BaEV gp70				GaLV gp70			
	native		treated		native		treated	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
CGL	12/86*	35/86	0/86	28/86	11/86	42/86	0/86	38/86
AML	2/34	11/34	0/34	10/34	5/34	13/34	0/34	13/34
Preleukaemia	3/22	10/22	0/22	10/22	3/22	11/22	0/22	10/22
Control	6/102	28/102	0/102	4/102	1/102	4/102	0/102	0/102

* positive plasma samples/number of plasma samples tested

hypergranularity. Their bone marrow was hypercellular; the megakaryocytes increased in number and many of them being small with few or no nuclear lobulations and intense cytoplasmic granularity. The erythropoiesis was megaloblastoid, and intermediate macronormoblasts were increased. IgM reactivities were less frequently observed, than IgG reactivities. As for the antibodies reacting with BaEV antigens, plasma samples from patients with myeloid leukaemias and potentially preleukaemic disorders did not show significant differences as compared to samples from control persons. In contrast, the antibodies reacting with GaLV antigens could be detected more frequently in patients with myeloid leukaemias and potentially preleukaemic disorders, than in healthy humans. Finally, antibodies reacting with p15 antigens were less frequently observed, than antibodies to gp70 antigens.

The effect of glycosidase treatment on the reactivity of human plasma samples

Precipitation of native and enzymatically digested, carbohydrate-free gp70 by the same human plasma samples was compared in Table 2. It can be seen that digestion of the carbohydrate part of the antigen molecules resulted in the abrogation of IgM-mediated precipitation. On the contrary, precipitation mediated by IgG antibodies was not significantly influenced by the elimination of the carbohydrate part of envelope glycoproteins. However, a great decrease in the number of positively reacting plasma samples from normal donors after glycosidase treatment could be observed.

The average precipitating activities of plasma samples containing IgG antibodies reacting with oncoviral antigens

Average precipitating activities are summarized in Table 3. Control serum samples gave a weak reaction.

The highest titres were observed in sera of patients with potentially preleukaemic haematological disorders. A striking difference in the antibody

Table 3. Average RIP of iodinated oncovirus antigens by IgG

Plasma source	Precipitated antigen (in ng*)					
	BaEV			GaLV		
	gp70		p15	gp70		p15
	native	treated		native	treated	
CGL	6.3 ± 1.6	3.8 ± 0.9	4.1 ± 0.2	6.8 ± 1.4	3.1 ± 0.7	4.5 ± 0.3
AML blastosis	2.2 ± 0.5	1.1 ± 0.4	2.3 ± 0.4	3.1 ± 1.1	2.2 ± 0.5	1.9 ± 0.3
AML remission	7.8 ± 1.8	5.7 ± 1.1	5.8 ± 0.3	8.4 ± 2.5	6.1 ± 0.4	4.9 ± 0.4
Preleukaemia	16.2 ± 3.0	11.9 ± 1.8	9.3 ± 1.4	14.8 ± 2.2	8.2 ± 0.9	8.6 ± 0.8
Control	3.1 ± 0.7	1.4 ± 0.3	1.1 ± 0.3	3.1 ± 0.5	—	—

* viral antigens precipitated with 10 μl of plasma diluted 1 : 10

titres could be detected in the case of patients with AML depending on the stage of the disease. In patients with untreated AML or being in relapse, the average titre of antibodies was very low, whereas AML patients being in remission had the same values as patients with CGL. Titres depended on the quality of antigens, too. The highest titres were observed in the case of native gp70-s. Titres found in the case of glycosidase-treated gp70 and p15 were lower.

Discussion

Antibodies precipitating purified antigens of BaEV and GaLV could be detected in human blood plasmas by RIP. We were able to show that viral glycoproteins of simian oncoviruses grown in human cells were precipitated by plasma of leukaemic and preleukaemic patients. Recent findings indicate that the oncovirus genome may influence or control the glycosylation of the viral protein regardless of the cell type in which the viruses had been grown (Kemp *et al.*, 1979; Rich-Rosmer *et al.*, 1980). The method used by us for isolation of purified viral envelopes results in an envelope fraction containing gp70, p15 (E) and a larger amount of the *gag* gene product p15 (Van de Ven *et al.*, 1978). Thus, antibodies reacting with the p15 component prepared from BaEV and GaLV may be directed against p15(E) and core p15 as well. The data suggest that IgM antibodies to native gp70 antigens must be directed against carbohydrate determinants, i.e. they may have a Forssman-like character. In other cases, the virus-specific nature of antibodies reacting with carbohydrate-free gp70 and p15 polypeptides cannot be excluded. This assumption is supported by the recovery of endogenous retroviral sequences in human DNA (Martin *et al.*, 1981) and by the detection of antigens related to structural polypeptides of primate C-type viruses in human cells (Suni *et al.*, 1981; Derks *et al.*, 1982). The presence of an antigen cross-reacting with carbohydrate-free gp70 of simian sarcoma virus in human leukaemic sera (Hehlmann *et al.*, 1981) seems to strengthen the existence

of antibodies specific for simian C-type viruses in humans. Our results indicate that the percentage and titre of antibody-positive plasma samples may be quite different in the various groups of patients. The biological significance of antioncoviral antibodies is not clear at present, but one has to keep in mind that such antibodies may possess both virus neutralizing (Bendinelli *et al.*, 1980) and cytotoxic (Portetelle *et al.*, 1980) activities. RIP studies based on the competitive effect of monoclonal antibodies produced to envelope antigenic determinants of BaEV and GaLV might be useful in the evaluation of the specificity of natural human antibodies.

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