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Detection of Group and Interspecies Reactivities in Mammalian C-Type Virus p30 Proteins and Corresponding Human Antigens *

H. Schetters, V. Erfle, and R. Hehlmann

A. Introduction

We previously reported the presence of RNA tumor virus related antigens in human leukemic sera. The antigens were detected by the ELISA method in 30%–40% of patients with acute leukemia [1, 2]. Since these antigens from human sera that cross-react with primate C-type viral p30 protein had been observed with the ELISA method and since retroviral group and interspecies reactivities had been previously characterized mostly by competition radioimmunoassay, we examined the applicability and reliability of the ELISA method for the detection of group and interspecific determinants in p30 proteins of mammalian C-type retroviruses [4].

The specificity of the observed reaction was further demonstrated by competition ELISA, by preabsorption of the coating antibody with homologous and heterologous antigen, and by ELISA with $F(ab')_2$ fragments of the IgG molecules [5].

After exclusion of rheumatoid factor (RF) interference, the immunoaffinity purified human antigen was characterized by SDS polyacrylamide gel electrophoretic analysis, enzymatic digest, and peptide maps. The antigen found in human leukemic sera is a protein distinct from common serum proteins.

B. Results

I. Specificity of the Test System

To determine the specificity of the ELISA technique five different approaches were chosen: specificity of the antisera used, competition ELISA, preabsorption test, ELISA with $F(ab')_2$ fragments of IgG, and interspecies reactivities detected by the ELISA technique.

1. Specificity of the Antisera

For the search for human antigens, antisera against three different RNA tumor viral p30s (MuLV p30, SiSV p30, and BaEV p30) were used. To establish their specificity for the homologous p30, they were tested with purified p30 proteins, disrupted whole viruses, and virus-producing cell lysates. It was shown that the antisera recognized the homologous antigen very well, while the heterologous antigens bound only to a very limited extent [3].

2. Competition ELISA

In this assay the antiserum was incubated with increasing amounts of homologous or heterologous viral p30, virus, extracts of virus-producing cells, or human sera. The remaining binding activity to the homologous p30 protein was measured. The human sera competed the binding partially in the anti SiSV p30 system while homologous viral protein blocked the antibody activity completely (Fig. 1).

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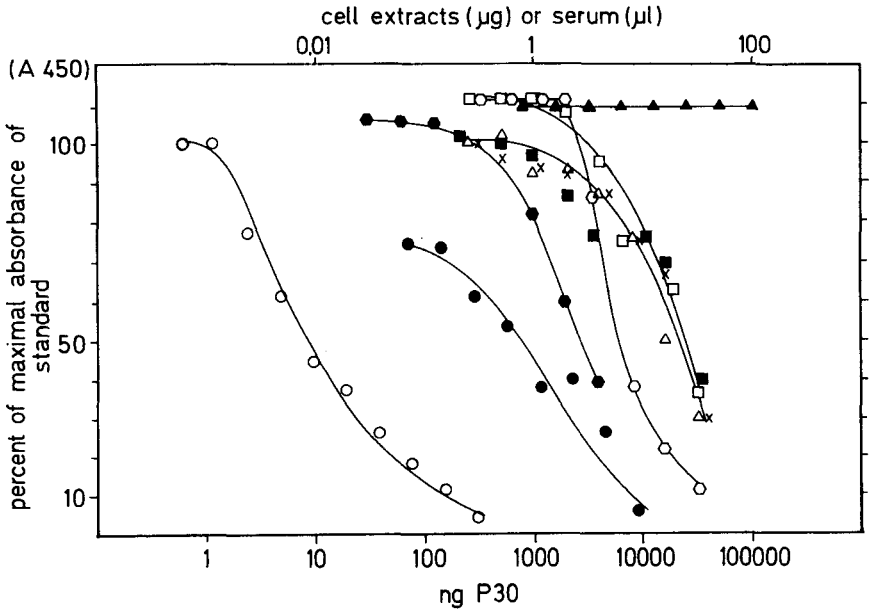

Fig. 1. Competition of human sera for SiSV p30 measured by the competition ELISA. The plate was coated with SiSV p30 (30 ng/ml) and the antiserum was preabsorbed with increasing amounts of antigen or human serum. The competing antigens were: ○ SiSV p30; ● SiSV-71AP1 cell extract; ■ L175, ALL; □ L153, CML-BC; × L148, CML; ⬡ L183, ALL; ⬬ L40, AProl; △ Mult.Myel.; ▲ FCS, HSA, MuLV inf. C3H cell extract, BaEV inf. A204 cell extract

3. *ELISA After Preabsorption of Coating Antibody with Homologous p30 Protein*

Identical results to the C-ELISA assay were achieved, when the coating antibody for an antigen test was preabsorbed with the homologous p30 protein. Corresponding to the increasing amount of absorbent the binding of the homologous p30 and of the human sera tested decreased, thus confirming the specificity of the test system.

4. *Preabsorption Test*

The specificity of the test was further analyzed by using $F(ab')_2$ fragments of the anti SiSV p30 and anti BaEV p30 IgG molecules. The binding activity of the fragments was decreased to some extent for the homologous antigens and for the human antigens compared to undigested IgG molecules. But a specific binding of homologous antigen and human antigen remained while sera of healthy donors and animal sera showed no binding activity (Fig. 2).

5. *Interspecies Reactivities Detected by the ELISA Technique*

For the determination of the recognition of interspecific reactivities by the ELISA method, four groups of C-type retroviruses (MuLV, FeLV, SiSV/GaLV, and BaEV/RD114) were assayed for group-specific and for interspecific reactivities of their p30 proteins. We found that the ELISA can detect group-specific as well as interspecies

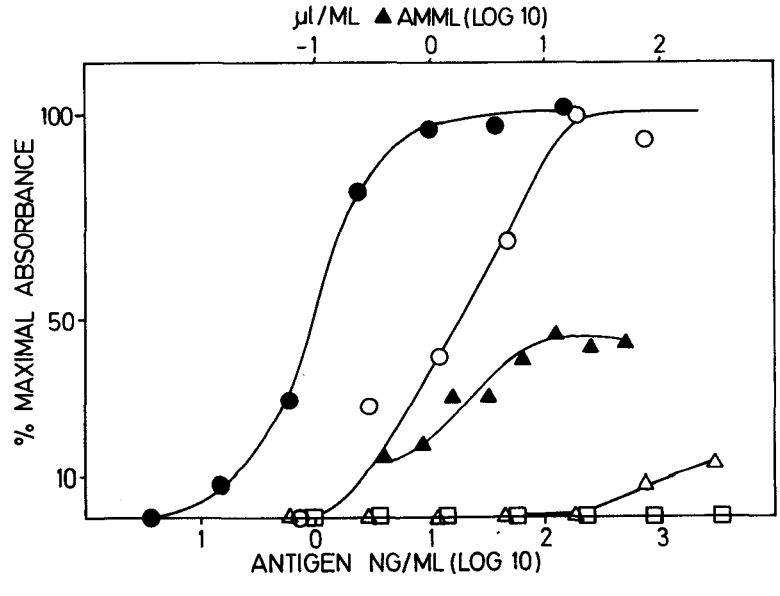

Fig. 2. Binding of homologous and heterologous antigen and one human leukemic serum to $F(ab')_2$ fragments of anti-SiSV p30 IgG detected by the ELISA technique. Antigens used were: ● SiSV p30; ○ SiSV virus; □ BaEV virus; △ MuLV virus; ▲ L117, AMML serum

determinants sensitively and reproducibly in purified p30 proteins, disrupted viruses, and cell extracts if an anti p30 multispecies antiserum is used [4]. When monospecific antisera against MuLV p30, SiSV p30, or BaEV p30 were used only specific reactivities were detected reproducibly, whereas the detectability of interspecies determinants depended on the antisera used. Comparing native and SDS denatured p30 proteins the detectability of the denatured MuLV p30 was better than that of the native MuLV p30, suggesting that some of the reactive determinants are localized inside the protein molecule and are freed by the denaturation process (Table 1).

II. Characterization of the Purified Human Antigen

For the characterization of the human antigens (a) the nature of the antigen (b) the molecular weight, and (c) peptide maps were determined.

1. The Nature of the Human Antigen

To determine the nature of the human antigen from a selection of human sera (Table 2) that were isolated by immunoaffinity chromatography, they were subjected to digests with five different enzymes (RNAase A, DNAase I, lipases, mixed

Table 1. Recognition of group and interspecies determinants in purified p30 proteins, disrupted viruses, and cell extracts with antisera against native p30 proteins

Antisera	Anti-MuLV p30	Anti-SiSV p30	Anti-BaEV p30	Anti-p30 interspecies
Antigens				
EV-purified p30				
MuLV	1.2[a]	>2,500	>2,500	1.1
SiSV	>214	0.55	214	1.7
BaEV	>3,400	>3,400	4.4	3.6
FeLV	2,400	>2,500	>2,500	NT
SDS gel purified p30				
MuLV	1.3	340	1,900	4.1
SiSV	>2,100	<0.38	>2,100	44
BaEV	>1,300	600	2.8	2.1
FeLV	>170	>170	>170	0.8
GaLV	>1,000	10.0	>1,000	44
RD114	90	>450	450	1.7
Viruses				
MuLV	3.2	2,300	>22,000	<0.8
SiSV	3,800	<2.0	>32,000	8.5
BaEV	>23,000	>6,000	2.7	<0.85
FeLV	>18,000	>18,000	>18,000	<0.66
GaLV	>32,000	3.2	>32,000	10.0
RD114	>48,000	>48,000	6,700	<3.5
Cells				
A7573	>100,000	>100,000	>100,000	>100,000
CCL88	>96,000	>96,000	>96,000	>96,000
MuLV-Balb/c spleen	115	1,600	>108,000	1,800
SiSV-71AP1	6,000	43	>96,000	1,800
M7BaEV-A204	>100,000	40,000	900	950
FeLV-A7573	>100,000	25,000	>100,000	1,100
GaLV-CCL88	>100,000	1,700	>100,000	22,000

EF, electrofocusing; NT, not tested

[a] Numbers are nanograms of proteins to reach 20% of maximal binding

Table 2. Crossreacting antigens in human leukemic serum

Sera/follow-up sera of same patient	Diagnosis	Crossreaction with	
		SiSV p30	BaEV p30
		in ng equivalents/ml	
L22/L23/L107/L117	AMML	>600/>600/>600/>600	>1840/>1840
L99	AMML	30	16
L80/L96/L119	AMML	80/9.6/0	>1840/78/9.6
L88	AMML	14	170
L39	AML	>600	>1840
L29/L124	AML	72/>600	0
L130	AML	>600	>1840
L102	AML	8.5	1040
L150/L151	AML	12.4/13.2	NT
L101	AML	0	8
L40/L66/L69	AProL	6.4/11/0	NT
L73	ALL	14	0
L183	ALL	70	NT
L175	ALL	60	NT
L74/L75/L149/L158	CML	14/8.4/18/6.4/10	0
L159	CML-BC	8.8	NT
L163	CML-BC	8.8	NT
L83	CML-BC	15	>1840
L98	CML-BC	>600	>1840
L103	CML-BC	0	4.8
L82	CML-BC	6.8	18.4
L105	CML-BC	5.6	6.8
L153	CML-BC	19.4	NT

AMML, acute myelomonocytic leukemia; AML, acute myeloblastic leukemia; AProl, acute promyelocytic leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; BC, blast crisis; NT, not tested

glycosidases, or protease). From these five enzymes only the protease degraded the antigen as could be judged by SDS polyacrylamide gel electrophoretic analysis and by measuring remaining binding activity in the ELISA.

2. Determination of the Molecular Weight

On SDS polyacrylamide gel the antigen showed mainly one band. From its position, compared to BSA, it has an approximate molecular weight of 70,000 daltons.

3. Correlation of the Human Antigen to SiSV p30 and BaEV p30 by Peptide Mapping

The relatedness of the human antigen to the primate viral p30 proteins was determined by peptide maps. The peptide maps of the antigens isolated from two different patients were compared to each other, to SiSV p30 or BaEV p30, and to common human serum components like human serum albumin, transferrin, fibrinogen, plasmin, immunoglobulins G and M, and α-fetoprotein. The peptide patterns of the human antigens are identical and revealed homologies to SiSV p30 or BaEV p30 of 50%–60% of the number of p30 peptides. To the serum components the peptide homology reached up to 25% what is within the background range of specificity.

C. Discussion

It was possible to recognize specifically antigens in human sera with the ELISA

method that crossreact with primate C-type viral proteins. The ELISA method was previously shown to recognize specifically the homologous antigen and also to detect interspecies reactivity, which is crucial for the recognition of human crossreacting antigens. To show that these reactions, despite of broad reactivity, remain specific for the viral p30 proteins further supporting tests were undertaken. C-ELISA and ELISA with preabsorbed antibody or $F(ab')_2$ fragments of IgG all showed that the reaction was specific, excluding interfering reactions like rheumatoid factor or artifactual binding to other determinants of the IgG molecule rather than to the antigen binding site.

The antigen seen in human sera with antiserum against primate C-type viral p30 are proteins with a molecular weight of 70,000 daltons. From other publications, however, it is known that incomplete processing may lead to an accumulation of precursor proteins in the cell, virus-coded proteins like reverse transcriptase or *onc* proteins should be found to be present in this molecule. Another possibility would be that this protein is a fusion protein of virus-related p30 protein and cellular protein, as has been shown for some transformation proteins that were first detected in the avian leukemia virus system with anti p30 protein. In the first case it should be possible to detect other viral proteins in this molecule and in the second case transformation specific reaction like protein kinase activity must be looked for. The first possibility is favored by Jacquemin (this volume), who found in a leukemic patient a protein with a molecular weight of 74,000 daltons, reactive with anti MuLV reverse transcriptase that besides the reverse transcriptase determinants also contains feline leukemia virus p15 determinants. That we can find fusion proteins might be a sign for the presence of RNA tumor viruses, even when infectious viruses cannot be isolated.

Acknowledgments

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