Eur J Cancer Clin Oncol. Vol. 21, No. 6, pp. 687–700, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Antigens and Circulating Immune Complexes Related to the Primate Retroviral Glycoprotein SiSVgp70: Prevalence and Distribution in Human Sera*

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Abstract—*We have shown previously that antigens and also circulating immune complexes related to the primate retroviral envelope glycoprotein SiSVgp70 correlate with early mortality and survival of 56 patients with acute leukemias and chronic myelogenous leukemia in blast crisis. The prevalence and general distribution of these antigens and immune complexes in human sera was therefore of obvious interest. We now report an analysis of a total of 200 sera from 166 patients. Of these 113 sera were obtained from 84 patients with acute or chronic leukemias and 87 from 82 non-leukemic subjects, including laboratory workers and patients with non-leukemic neoplasias. Antigens and immune complexes were determined by enzyme-linked immunosorbent assays (ELISA). The anti-SiSVgp70 antiserum used predominantly recognized the protein moieties of the glycoproteins. The distribution of SiSVgp70-related antigens and immune complexes was similar among leukemic and non-leukemic sera. The prevalence of SiSVgp70-related antigens was 53% and of SiSVgp70-related immune complexes 49% in all sera. SiSVgp70-related antigens were detected in a somewhat higher proportion of non-leukemic (69%) than leukemic sera (40%), whereas SiSVgp70-related immune complexes and cross-reactive antibodies were more evenly distributed in leukemic and non-leukemic sera (in 46 and 51% of leukemic and 54 and 51% of non-leukemic sera). Presence of antigens correlated with presence of SiSVgp70-related immune complexes in 71% of all sera, but in 13% of all sera antigens were detectable only by determining SiSVgp70-related immune complexes. Total circulating immune complexes did not correlate with SiSVgp70-related immune complexes. The origin and pathophysiological role of the antigens are discussed.*

Accepted 1 October 1984.

*This work was supported by the Deutsche Forschungsgemeinschaft (SFB 51 and He 920/6-1).

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Abbreviations: AML, acute myeloblastic leukemia; AProL, acute promyelocytic leukemia; AMML, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; ALL, acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia; CLL, chronic lymphocytic leukemia; LS-L, lymphosarcoma-cell leukemia; CML, chronic myelogenous leukemia; CML-BC, chronic myelogenous leukemia in blast crisis; SiSV, simian sarcoma/simian sarcoma-associated virus; BaEV, endogenous baboon virus; GaLV, gibbon ape leukemia virus; MuLV, murine leukemia virus; CIC, circulating immune complexes; AG, antigen; AB, antibody; IC, immune complexes; SLE, systemic lupus erythematodes; RA, rheumatoid arthritis; RF, rheumatoid factor; AL, acute leukemia.

INTRODUCTION

RETROVIRUSES have been implicated in the etiology of various human neoplasias (leukemias, lymphomas, sarcomas, breast cancer) since viruses of this group have been found to cause corresponding malignancies in several animal species [1]. No etiological conclusion could be drawn for any human neoplasm, but retroviral structures, including reverse transcriptase, nucleic acids and proteins that cross-react serologically with retroviral structural proteins, have been isolated from human leukemic cells and sera (for review see [2]). Most retroviral structures in human tissues and sera resembled those of the primate retroviruses simian sarcoma virus/

simian sarcoma-associated virus (SiSV) and baboon endogenous virus (BaEV) (for review see [3]) and include proteins related to the p30 core proteins of SiSV and BaEV [4–6] and to the gp70 envelope glycoprotein of SiSV [7, 8]. Most recently retroviruses have been found in some human T cell leukemias and lymphomas that are unrelated to any known animal retroviruses [3].

Circulating immune complexes are being associated with an increasing number of neoplasias including human leukemias and lymphomas [9–16]. The nature and antigen-specificity of the immune complexes has not been elucidated in most instances. This lack of identification is partly due to the fact that most methods for the measurement of immune complexes do not allow the determination of their antigen-specificity [17–20]. One method for the determination of antigen-specific immune complexes has recently been established in our laboratory as an enzyme-linked immunosorbent assay (ELISA) and has been applied to the identification of retroviral immune complexes in various murine malignancies [21].

We have shown previously that SiSVgp70-related antigens and also immune complexes correlated with early mortality and shorter survival times of patients with acute leukemias and chronic myelogenous leukemia in blast crisis [7]). In the present paper we analyze the prevalence and distribution of these SiSVgp70-related antigens and immune complexes in a larger number of human leukemic and non-leukemic sera and discuss their origin and potential pathophysiological role.

MATERIALS AND METHODS

Compounds

Horseradish peroxidase grade VI RZ$\leqslant$3.0 (EC1.11.1.7), *O*-phenylene diamine-2HCl, Tween-20 and Thimerosal were purchased from Sigma, München. Trasylol was purchased from Bayer, Leverkusen. Mixed glycosidases used: α-mannosidase 94 units/mg, β-mannosidase 106 units/mg, α-glucosidase 3.0 units/mg, β-glucosidase 7.6 units/mg, α-galactosidase 40 units/mg, β-galactosidase 110 units/mg, α-L-fucosidase 60 units/mg, α-D-fucosidase, β-D-fucosidase, β-xylosidase 9.8 units/mg, α-*N*-acetylglucosaminidase 2.2 units/mg, β-*N*-acetylglucosaminidase 460 units/mg, α-*N*-acetylgalactosaminidase 30 units/mg, β-*N*-acetylgalactosaminidase 44 units/mg, α-L-arabinosidase, β-D-arabinosidase and sialidase were purchased from Miles, Frankfurt. Pronase R was from Calbiochem, Lahn (all in F.R.G.).

Technical supply

Microtiter plates M 129 were purchased from Dynatech (Plochingen, F.R.G.). For absorbance measurements of ELISA reactions, a Titertek multiscan from Flow (Bonn) or an SLT 210 ELISA reader from SEI/Kontron (Munich) with a 450 nm filter was used. For electrofocusing, a Multiphor from LKB (Munich) was used.

Cell lines and antigens

SiSV-71AP1 cells and SiSVgp70 were gifts from Dr R. Gallo, Bethesda, MD. HF cells were supplied by Dr F. Deinhardt, München. AKR-p30 was a gift from Dr J. Ihle, Frederick, MD. The viruses used and all other cell lines were grown in our own laboratory.

Antibodies

Antiserum against SiSVgp70 was prepared by injecting purified SiSVgp70 into a goat and was a gift from Dr R. Gallo, Bethesda, MD. IgG was purified as described [22].

Human sera

Leukemic sera were obtained from participating hospitals of the Süddeutsche Hämoblastosegruppe. Non-leukemic sera were from the Medizinische Poliklinik München. Sera from hospitals outside Munich were sent by express mail. In some instances, plasma was used instead of serum. All serum or plasma samples were treated with 5% trasylol on arrival or after separation from blood cells.

Coupling procedure of peroxidase to IgG

In essence, a modification of the procedure of Nakane and Kawaoi [23] and of Mesa-Tejada *et al.* [24] was followed, which involves blocking of the amino groups of the peroxidase with phenylisocyanate, introduction of aldehyde groups with Na-periodate and formation of a Schiff's base with the IgG by incubation of the IgG with the activated peroxidase.

ELISAS for recognition of antigens, immune complexes, and antibodies

Antigen [22, 25]. The microtiter plate was coated with 200 μl anti-SiSVgp70IgG per well (5 μg/ml) at 37°C for 2 hr in 50 mM carbonate buffer pH 9.6. After washing, the SiSVgp70 was applied to the plates in 1:4 dilution steps starting with 1 or 0.5 μg SiSVgp70/ml. Human sera were applied in 1:4 dilutions in duplicates or titrated in 1:2 dilution steps. Dilutions were carried out with D'PBS containing 5% non-immune goat serum, 0.1% Tween 20, 0.1 mM Thimerosal, and 1% Trasylol (= antigen buffer). A heat inactivation step (30 min, 56°C) was not included since its effect on the human antigen is still unclear. The

plates were incubated at 4°C overnight. The following day peroxidase-coupled anti-SiSVgp70 IgG was added and allowed to react at 37°C for 2 hr. After addition of substrate the extinction of the probe was determined at 450 nm for quantitation of the bound peroxidase-coupled antibody. Backgrounds were generally less than 0.01 A_{450}.

Immune complexes [21]. Monospecific antibody is coated onto the microtiter plate surface similar to the assays for antigen. Then serum containing the immune complexes in question is added to the antibody-coated microtiter plate. Both antigens and antigen-specific immune complexes will bind to the monospecific coating antibodies, provided a reactive site of the antigen is available for the antigen-antibody reaction. Since IgG molecules are connected with antigen within these immune complexes, peroxidase-coupled antibodies directed against the IgG molecules of the immune complexes are added. This step distinguishes immune complexes from antigens. The possible reactivity of anti-IgG antibodies with the coating IgG as tested by addition of anti-IgG antibodies to antigen is minimal under the conditions used.

In detail, anti-SiSVgp70 IgG (5 μg/ml) was coated onto the plate in 50 mM carbonate buffer, pH 9.6, at 37°C for 2 hr. After washing, human sera diluted 1:4 were applied in duplicates and allowed to react at 4°C overnight. The following day peroxidase-coupled anti-human IgG was added and incubated at 37°C for 2 hr. Substrate was added and the bound peroxidase-labeled IgG was measured at 450 nm.

Antibody. SiSVgp70 was coated onto the plate at a concentration of 50 μg/ml at 37°C for 2 hr in 50 mM carbonate buffer, pH 9.6. Anti-SiSVgp70 IgG was applied in 1:2 dilution steps starting with 5 μg/ml. Human sera were diluted 1:4 and applied in duplicates or in dilution steps of 1:2. Dilutions were carried out in antigen buffer. Incubation was at 4°C overnight. The following day peroxidase-coupled anti-goat IgG or anti-human IgG were added and incubated at 37°C for 2 hr. Substrate was added and the bound peroxidase-coupled IgG was measured at 450 nm.

Determination of total CIC

Total CIC were determined by a Clq binding ELISA. This assay measures the binding of CIC to Clq coated onto the surface of a microtiter plate via a color reaction at A_{450}. Since human Clq preparations frequently contain traces of IgG which non-specifically bind anti-human IgG molecules, goat-Clq was used for the Clq ELISA. Goat and human Clq preparations were found to give identical results as tested with sera of the high immune complex mouse strain NZB and of the low immune complex strain CBA. The purification of Clq was carried out according to Zubler and Lambert [24] by repeated precipitation of Clq with EDTA. Under these conditions Clq is one of the least soluble serum components. The purified Clq preparations were analyzed by SDS-gel electrophoresis. The human sera were diluted 1:100 in antigen buffer containing 1% BSA instead of goat serum. The background with uncoated microtiter plates was 0.03–0.05 A_{450}.

Antigen or antibody ELISA with sugar blocking

The plate was coated with anti-SiSVgp70 (5 μg/ml) for antigen tests or with SiSVgp70 (50 ng/ml) for antibody tests, in 50 mM carbonate buffer, pH 9.6, for 2 hr at 37°C. The following steps were carried out as described above, except that the antigen buffer contained 5% non-immune serum homologous to the peroxidase-coupled IgG and 0.1 M each of the following sugars: glucose, fructose, rhamnose, galactose, acetylglucosamine, maltose, lactose, arabinose and inositol.

Antigen ELISA after glycosidase treatment

The plate was coated with anti-SiSVgp70 (5 μg/ml) for 2 hr at 37°C. SiSVgp70 or human serum dilutions were applied to the plate and incubated at 4°C overnight. The following day the plates were washed and incubated with varying concentrations of mixed glycoxidases in 50 ml citrate buffer, pH 4.0, or 1% Triton X-100 and 100 μg/ml BSA at 37°C overnight. After removal of the glycosidase mixture, the peroxidase-coupled anti-SiSVgp70 IgG (5 μg/ml) was added and incubated at 37°C for 2 hr. After addition of substrate the extinction of the probe was determined at 450 nm for quantitation of the bound peroxidase-coupled antibody with a Flow Multiscan or a SEI/Kontron 210 ELISA-reader. The glycosidase treatment was controlled with SDS-PAGE of ^{125}I labeled SiSVgp70 [8].

Antibody ELISA after glycosidase treatment

The plate was coated with SiSVgp70 (50 ng/ml) for 2 hr at 37°C. The bound SiSVgp70 was treated with varying amounts of mixed glycosidases (0.1–1000 μg/ml) in 50 mM citrate buffer, pH 4.0, containing 0.1% Triton X-100 and 100 μg/ml BSA overnight at 37°C. After the glycosidase digest anti-SiSVgp70 (5 μg/ml) or human sera 1:4 diluted were added and incubated at 4°C overnight. Then peroxidase-coupled anti-goat or anti-human IgG were applied. The bound peroxidase-labeled antibody was measured by reading the color reaction at 450 nm.

Protease treatment

Three hundred and thirty three nanograms of SiSVgp70 (protein determination by the Lowry method) were incubated with 1 μg of pronase in 60 μl (= 5.55 μg SiSVgp70/ml) of 50 mM Tris-HCl, pH 7.5, for 1 hr at 37°C. The control was incubated with plain buffer. The reaction was stopped by diluting the solution with antigen buffer to a final concentration of 1 μg SiSVgp70/ml. Subsequently an ELISA was carried out as described above.

RESULTS

Specificity of the reactions with anti-SiSVgp70 antiserum

The specificity of the anti-SiSVgp70 antiserum was tested with various animal sera, unrelated retroviruses and retroviral proteins, extracts of uninfected cells and of cells infected with unrelated retroviruses, and normal human serum components (Table 1). In the assays with reagents other than retroviruses no reactivity, or reactivity only a little above background (approximately 5% of the homologous reaction with SiSVgp70), could be detected (Table 1). With MuLV whole virus about 20% interspecies reactivity is observed, as would be expected in reactions between SiSVgp70 and the envelope glycoprotein gp70 of MuLV [27, 28]. No cross-reaction has been observed with BaEV and core proteins of SiSV and MuLV. These data demonstrate that the antiserum recognizes predominantly type- or group-specific, and to some extent also interspecies-specific, determinants of the envelope glycoproteins of retroviruses of the SiSV-MuLV group.

Table 1. Specificity of the anti-SiSVgp70 antiserum

Antigens	% of maximal absorbance with anti-SiSVgp70
SiSVgp70	100
SiSVp30	0
N-MuLV virus (AKR)	21
MuLVp30	0
MuLVp12	0
M7 BaEV virus	0
M7 BaEV A204 cell extract	0
A204 cell extract	4
HF cell extract	7
N-C3H cell extract	5
Fetal calf serum	0
Newborn calf serum	0
Normal bovine serum	0
Normal cat serum	0
Normal rabbit serum	0
Normal goat serum	0
Normal chimpanzee serum	3
Normal mouse serum	3
Normal pig serum	0
Normal horse serum	6
Normal dog serum	0
Normal chicken serum	0
Normal human serum components:	
albumin	0
transferrin	0
IgG	0
IgM	0
IgA	0
IgD	0

The recognition of homologous and heterologous antigens or mammalian sera by the goat anti-SiSVgp70 IgG was measured by antigen ELISA. Background = less than 0.01 A_{450}.

To determine the amount of heterophilic reaction with the carbohydrate moieties of glycoproteins, the anti-SiSVgp70 antiserum was tested for reactivity with carbohydrate and protein moieties of SiSVgp70. The reactivity of anti-SiSVgp70 IgG was determined with varying concentrations of purified gp70-glycoprotein before and after treatment with a constant amount (100 μg/ml) of a glycosidase mixture. As shown in Fig. 1A, the reactivity with SiSVgp70 showed a reduction of reactivity of 5–18% after glycosidase treatment. In contrast, treatment of SiSVgp70 with pronase abolished all reactivity (Fig. 1A). The drop in sensitivity of glycosidase assay and untreated control is caused by the rigorous assay conditions (37°C overnight, pH 4.0) required for glycosidase treatment. For better comparison of different tests, maximal absorbance with a given antigen was designated 100%, representing A_{450} values ranging from 0.5 to 1.75.

Similarly, a constant amount of SiSVgp70 (5 μg/ml) submitted to treatment with varying concentrations of glycosidases showed a loss of antigenicity up to 20% at higher glycosidase concentrations (Fig. 1B). To determine further the amount of non-specific reactivity with carbohydrate moieties, various sugars (galactose, glucose, maltose, lactose, rhamnose, arabinose and inositol) were added to the test buffers of antigen ELISAs for purified SiSVgp70. Addition of 0.1 M concentrations of these sugars to the assays prior to the addition of SiSVgp70, however,

Table 2. Antigen ELISA with sugar blocking

	0.1M galactose	0.1M glucose	0.1M maltose	0.1M lactose	0.1M rhamnose	0.1M arabinose	0.1M inositol
SiSVgp70	97	90	104	95	112	106	110

The influence of various sugars on the binding of SiSVgp70 to anti-SiSVgp70 antibody was measured, expressed as percentage of absorbance. Antigen without sugar = 100%, corresponding to an A_{450} of 1.446. Background A_{450} = less than 0.01.

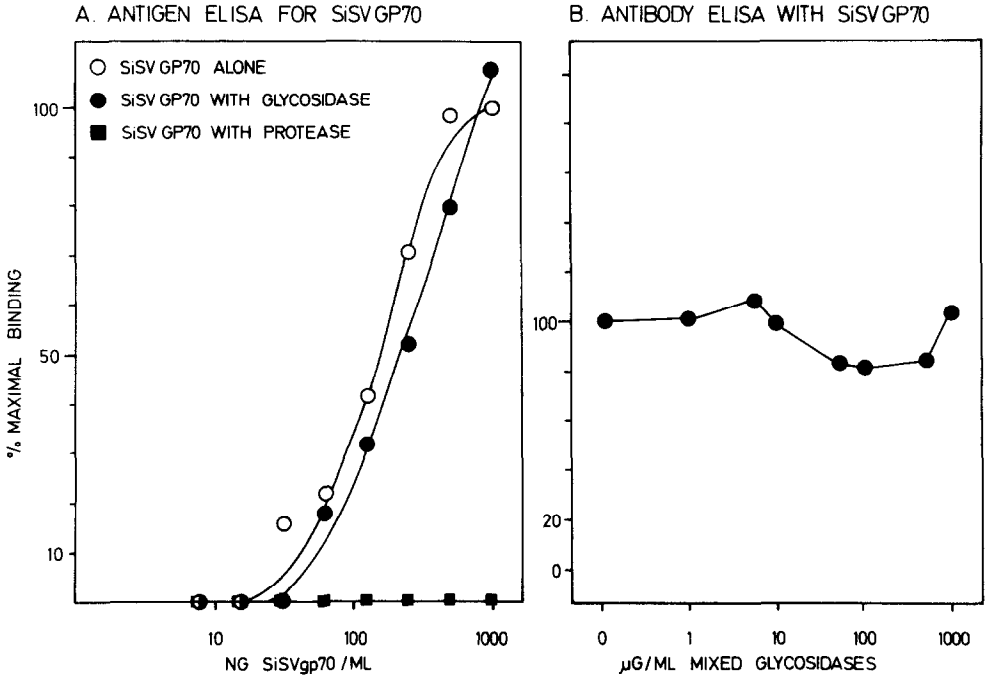

Fig. 1. (A) Binding of SiSVgp70 with (closed circles) and without (open circles) glycosidase treatment (constant amount, 100 µg/ml) to anti-SiSVgp70 antibodies. Reaction after protease treatment (squares). (B) Binding of anti-SiSVgp70 antibodies to SiSVgp70 (coating concentration 5 µg/ml) that had been subjected to treatment with increasing amounts of mixed glycosidases.

influenced the subsequent binding of SiSVgp70 to the antibody-coated plates only in the range of ±10% of SiSVgp70 alone (Table 2).

We conclude from these experiments that the anti-SiSVgp70 antiserum used in these studies recognizes preferentially the protein part, and only to a very low extent, if at all, the sugar moieties of the SiSVgp70 glycoprotein.

To exclude rheumatoid factor (RF) as a possible source of interference, all human sera were tested for RF prior to testing for cross-reacting antigens. Less than 3% of the sera were found positive for RF. Nevertheless, all sera were tested in the presence of 5% normal rabbit (or goat) serum to avoid interference by RF [6, 7].

An additional internal specificity control ruling out major non-specific IgG reactions is probably the fact that some human sera were positive for AG, but were entirely negative for IC and vice versa.

As a further specificity control human antigens were assayed with microtiter plates either coated with non-immune serum or uncoated. No reactivity was observed under these conditions.

A possible source of confusion in detection reactions for immune complexes may be non-specific reactivities for normal rabbit IgG preparations. Therefore different normal rabbit IgGs have to be compared and/or assayed for preimmune IgG reactivities prior to testing.

Antigens and immune complexes related to SiSVgp70 in human sera

Two hundred sera from 166 patients were assayed for SiSVgp70-related antigens and immune complexes using a solid phase enzyme immunoassay (ELISA).

Antigens. Antigens were detected in leukemic sera as well as in sera from non-leukemic patients and from laboratory personnel. In total, antigens were present in 97 out of 185 sera tested, representing 52.4%. All reactions above background level of assay (0.01 A_{450} or 0.1 ng SiSVgp70 equivalent) were designated positive. No obvious differences were present among different groups of sera, though the sera of laboratory personnel tend to show somewhat higher reactivities. Whereas antigens cross-reacting with SiSVgp70 could be found in 40 out of 102 sera tested from patients with leukemia (39.2%) [i.e. in 24 out of 53 sera from patients with acute leukemias (45.3%), and in 16 out of 49 sera from patients with chronic leukemias (32.7%)], these antigens were present in 58 out of 84 non-leukemic sera tested (69%), including 16 out of 21 sera from laboratory personnel (76%). The results grouped according to diagnoses are presented in Fig. 2A. The values for SiSVgp70-related antigen are given in ng SiSVgp70 equivalents/ml serum as determined from a standard concentration curve with purified SiSVgp70 which was included in each set of

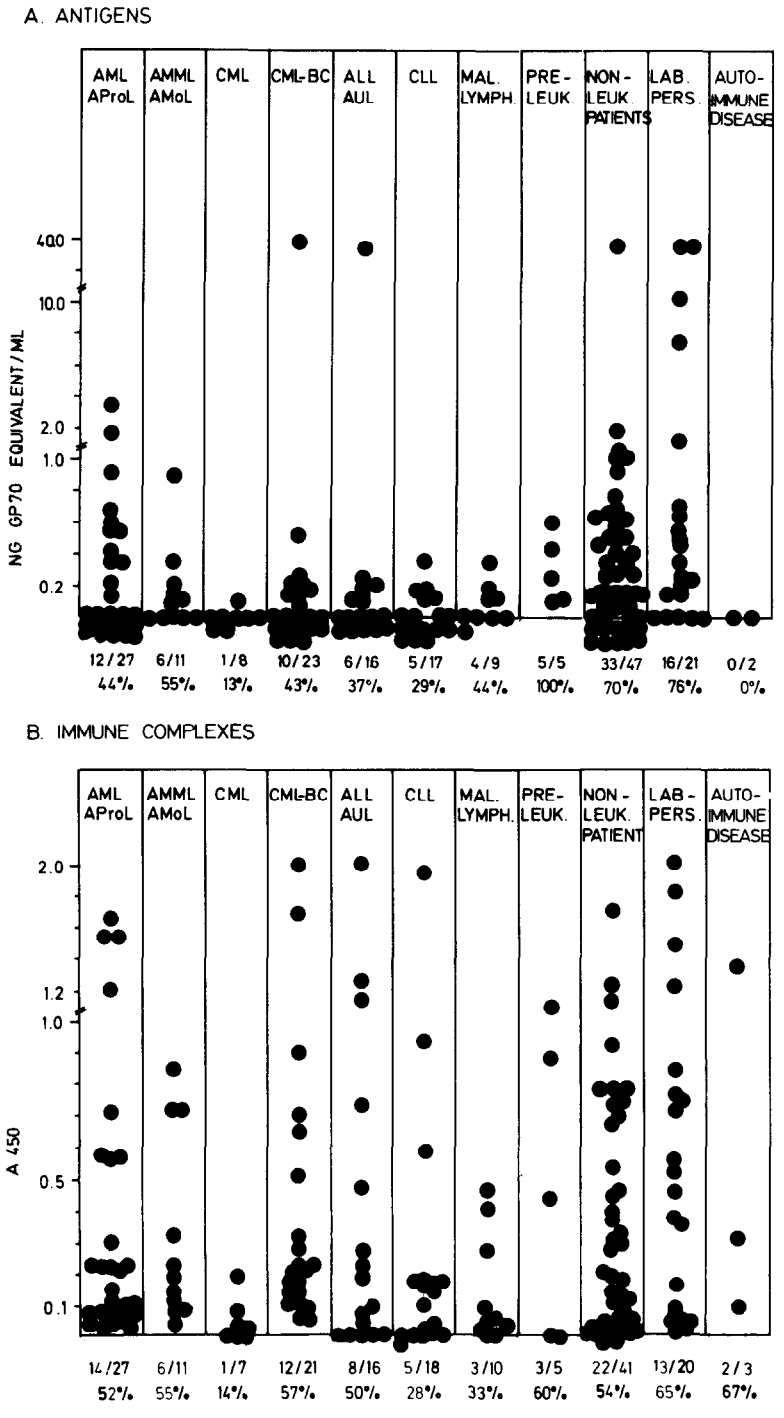

Fig. 2. (A) Antigens in human sera related to SiSVgp70. Cut-off line is the background of the reaction (0.01 A_{450} *corresponding to less than* 0.1 ng *SiSVgp70 equivalent*/ml). *(B) Circulating immune complexes in human sera related to SiSVgp70. Arbitrary cut-off line representing the background of the assay plus two times the standard deviation is* 0.174 A_{450}. *The numbers under the figures indicate the number of positive sera vs the number of sera tested.*

ELISAs. The numbers of sera positive for SiSVgp70-related antigens vs numbers of sera tested were: 12/27 for AML and AProL, 6/11 for AMML and AMoL, 1/8 for CML, 10/23 for CML-BC, 6/16 for ALL and AUL, 5/17 for CLL, 4/9 for malignant lymphomas, 5/5 for preleukemic conditions conditions (polycythemia vera, essential thrombocythemia), 33/47 for non-leukemic patients, 16/21 for laboratory personnel and 0/2 for autoimmune diseases (SLE, RA). The lowest number of positive sera was observed for the chronic leukemias and autoimmune diseases, the highest for sera of the laboratory personnel.

Assays for antigen only, however, may give

incomplete results since antigens might be complexed to antibodies and thus escape recognition. We therefore decided to assay the same sera also for SiSVgp70-specific immune complexes.

Immune complexes. The ELISA technique was also used to detect antigen-specific immune complexes. The test system was worked out and standardized with MuLVgp70 and monoclonal anti-MuLVgp70 antibodies [21]. In tests with human sera all reactions that gave absorptions above 0.174 A_{450} (= background of the assay plus two times the standard deviation) were designated positive. SiSVgp70-related immune complexes were present in leukemic as well as in non-leukemic sera in similar proportions. In total, immune complexes were detected in 88 out of 179 sera tested, representing 49.2%. Their prevalence in leukemic sera was 46%, in non-leukemic sera 54.4%. The results grouped according to diagnoses are presented in Fig. 2B.

Individual groups of patients showed somewhat different percentages for the presence of SiSVgp70-related immune complexes (IC). Again the lowest ratio of presence to absence of IC was observed in the chronic leukemias, the highest ratio in laboratory workers. The numbers of sera positive for SiSVgp70-related IC vs number of sera tested were: 14/27 for AML and AProL, 6/11 for AMML and AMoL, 1/7 for CML, 12/21 for CML-BC, 8/16 for ALL and AUL, 5/18 for CLL, 3/10 for malignant lymphomas, 3/5 for preleukemic states (polycythemia vera, essential thrombocythemia), 22/41 for non-leukemic patients, 13/20 for laboratory and 2/3 for autoimmune diseases.

Determination of total circulating immune complexes (CIC)

As a control for the determination of SiSVgp70-specific immune complexes a selected number of leukemic and non-leukemic human sera which were either negative or positive for SiSVgp70-related immune complexes were analyzed for total circulating immune complexes (CIC) by Clq binding on the basis of a Clq binding ELISA (Fig. 3). As can be seen by comparison of Fig. 3 with Fig. 2B, and also from Figs 4 and 5, a clear difference exists between the patterns for total CIC and for SiSVgp70-related immune complexes in acute leukemic and non-leukemic sera. Whereas high levels of SiSVgp70-related immune complexes are found in both leukemic and non-leukemic sera, high levels of total CIC are only found in leukemic sera. About 50% of acute leukemia sera contain CIC levels above the levels of CIC observed in non-leukemic sera. In contrast, total CIC levels in sera from chronic leukemias in blast crisis do not differ significantly from those in non-leukemic sera (Fig. 3). These data indicate that SiSVgp70-related immune complexes represent only a minor part of total CIC in human sera and are not proportional to the amounts of total CIC.

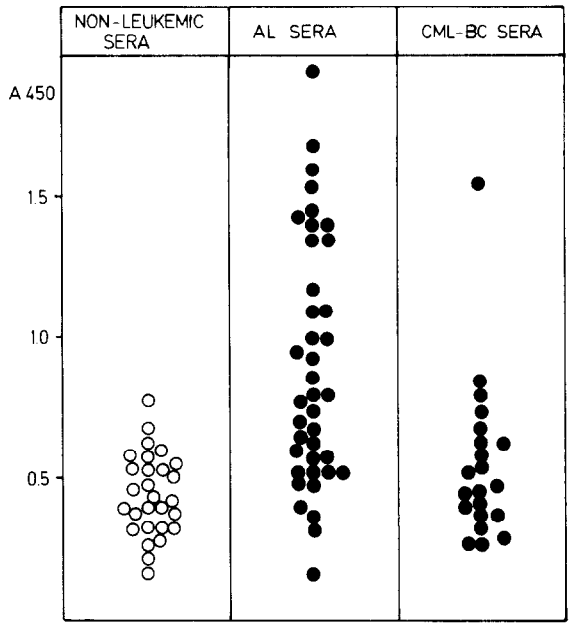

Fig. 3. Total circulating immune complexes in human leukemic and non-leukemic sera as determined by Clq binding ELISA. The mean of the A_{450} values of the 28 normal-donor sera was 0.450 (0.140 S.D.). 0.450 plus two times the standard deviation (= 0.73) was taken as 95% confidence limit for the assay.

Correlation of SiSVgp70-related antigens, immune complexes and antibodies in individual sera

In order to correlate SiSVgp70-related antigens with SiSVgp70-related immune complexes in individual sera the results were plotted for each individual serum in the order of increasing amounts of antigens. For correlation free antibodies reactive with SiSVgp70 as determined by antibody ELISA were also included. As in the immune complex ELISAs all antibody ELISAs with A_{450} absorptions of 0.175 or more were designated positive. Cross-reacting antibodies were detected in 96 out of 188 sera, representing 51.1%, and were equally present in leukemic (51%) and non-leukemic sera (51.2%). A marked difference, however, of antibody positively between sera from non-leukemic patients (33.3%) and from laboratory personnel (71.4%) was noted.

Figures 4 and 5 depict the correlation analysis of 159 sera (time course analyses of leukemic sera not included) from patients with acute leukemias, chronic leukemias, and no leukemia including sera from laboratory personnel, preleukemias,

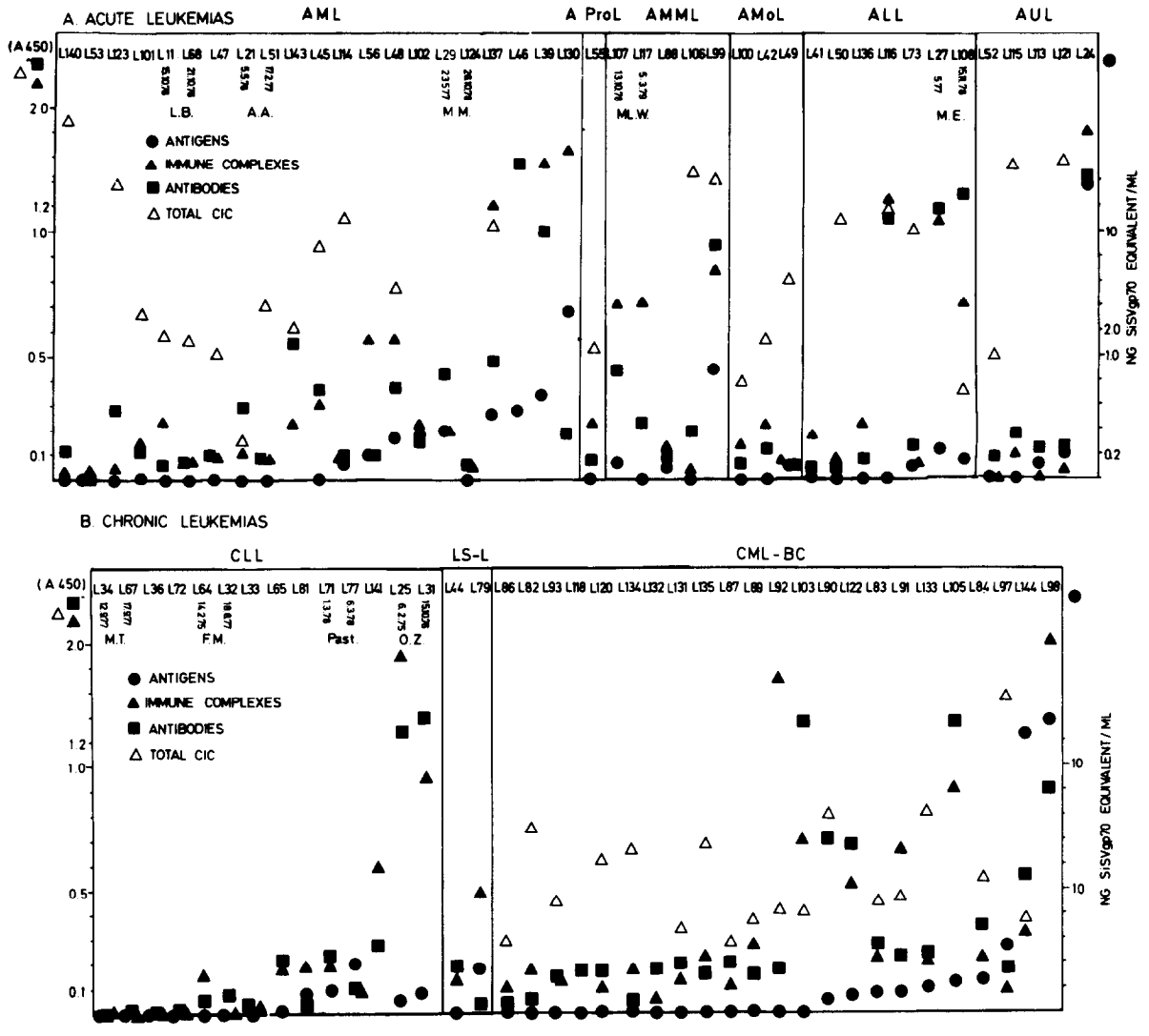

Fig. 4. SiSVgp70-related antigens, circulating immune complexes and antibodies and total circulating immune complexes in individual sera from patients (A) with acute leukemias and (B) with chronic leukemias.

autoimmune diseases, and malignant lymphomas. A good, although not complete correlation exists between the presence of SiSVgp70-related antigens and immune complexes. This correlation is most obvious in sera from acute myeloblastic leukemias (81%) (Fig. 4A), chronic myelogenous leukemias in blast crisis (76%) (Fig. 4B), and laboratory workers (95%) (Fig. 5B). Altogether, presence (or absence) of SiSVgp70-related antigens correlates with presence (or absence) of SiSVgp70-related immune complexes in 123 sera (71%). In 50 sera (29%), however, presence of antigens and immune complexes does not correlate. In 22 of these sera (about 13% of all evaluable sera) the presence of SiSVgp70-related antigens could only be recognized by the determination of SiSVgp70-related immune complexes.

Cross-reacting antibodies were found in most sera that were positive for antigen and for immune complexes. In some sera, cross-reacting antibodies were detected in the absence of SiSVgp70-related antigens or immune complexes, suggesting elimination of the antigen in the presence of continued immunity (L123, L106, L132, N107). Some sera that were antigen-positive did not show evidence of any immune response and were antibody- and immune complex-negative (N87, N47, N44, PV5). In some sera SiSVgp70-related antigens and free cross-reacting antibodies were found, but no evidence for the formation of immune complexes (L113, L121, N39, N96, PV4).

Total CIC are included for selected sera and demonstrate that no obvious correlation exists between total CIC and SiSVgp70-related IC.

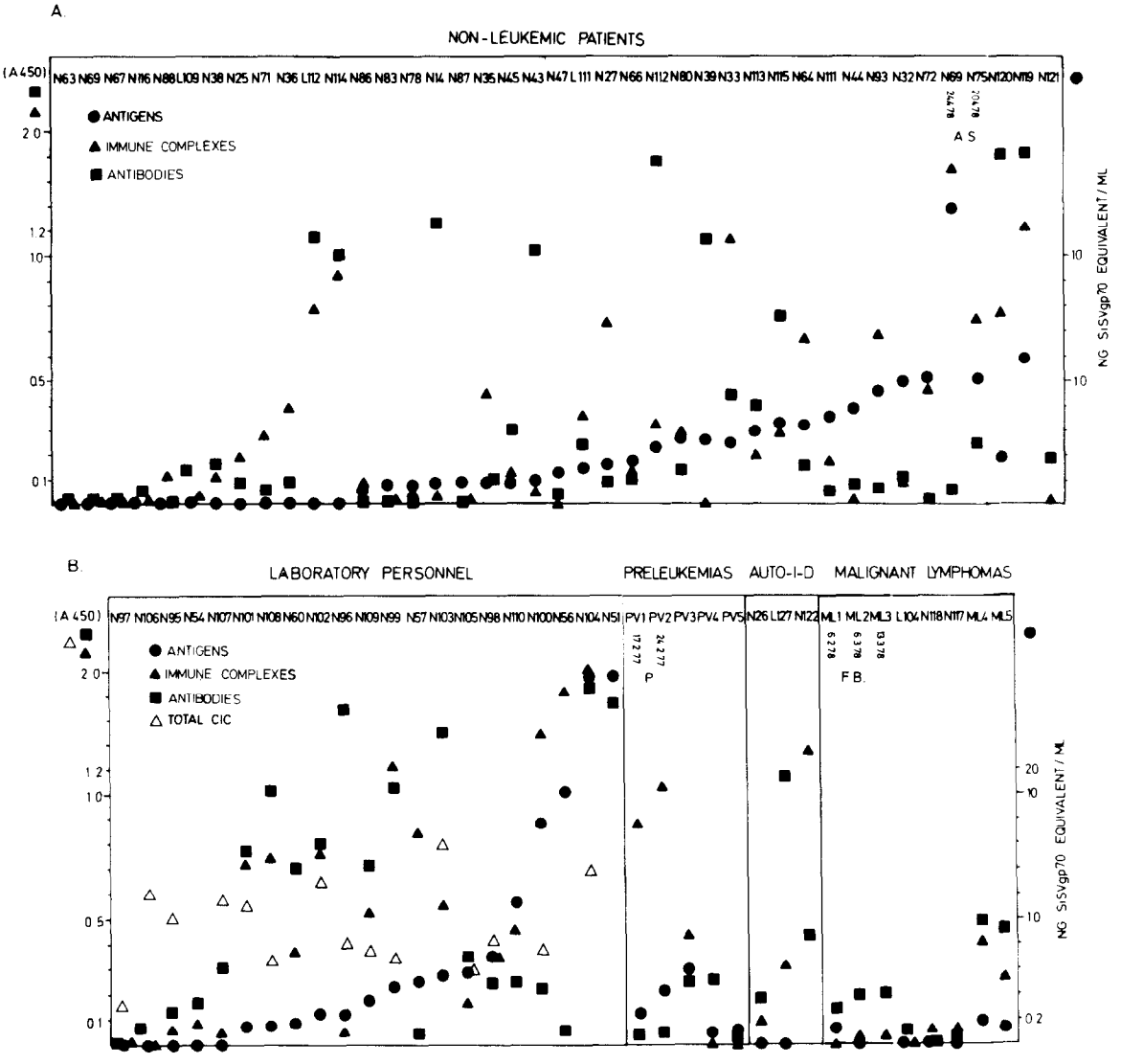

Fig. 5. SiSVgp70-related antigens, circulating immune complexes and antibodies and total circulating immune complexes in individual non-leukemic sera (A) from non-leukemic patients and (B) from laboratory personnel and from patients with preleukemias (polycythemia vera = PV 1-3; essential thrombocythemia = PV 4 and PV 5), autoimmune diseases (= auto.-I.-D.; lupus erythematodes = N26, L127; rheumatoid arthritis = N122) and malignant lymphomas (L104 = Hodgkin's disease; N117 = multiple myeloma; N118, ML 1-3, ML 4, 5 = non-Hodgkin's lymphomas).

Characteristics of the SiSVgp70-related antigens and antibodies

SiSVgp70-related human antigens were submitted to the same treatment with glycosidases as described for SiSVgp70. The reactivity of the SiSVgp70-related cross-reacting antigens with the anti-SiSVgp70 antiserum was reduced by only 5-18% by prior treatment with the glycosidase mixture (Fig. 6). Again it is evident that preferentially the protein moieties of the cross-reacting human antigens are recognized by the SiSVgp70 antiserum. In addition, on the basis of serological cross-reactivity, the human SiSVgp70-related antigens are not detectably related to any of the cellular, retroviral or normal serum components summarized in Table 1 except to MuLV and SiSVgp70.

Also, the specificity of the SiSVgp70-reactive human antibodies was controlled by prior glycosidase treatment of the coating SiSVgp70. No loss of reactivity of the antibodies with SiSVgp70 was observed after glycosidase treatment (Fig. 7). On the contrary, an up to 80% increase of binding was found with increasing amounts of mixed glycosidases. This is probably due to additional determinants that become available for reactivity after glycosidase treatment. We conclude that the human cross-reactive antibodies described here preferentially recognize the protein and not the carbohydrate part of SiSVgp70.

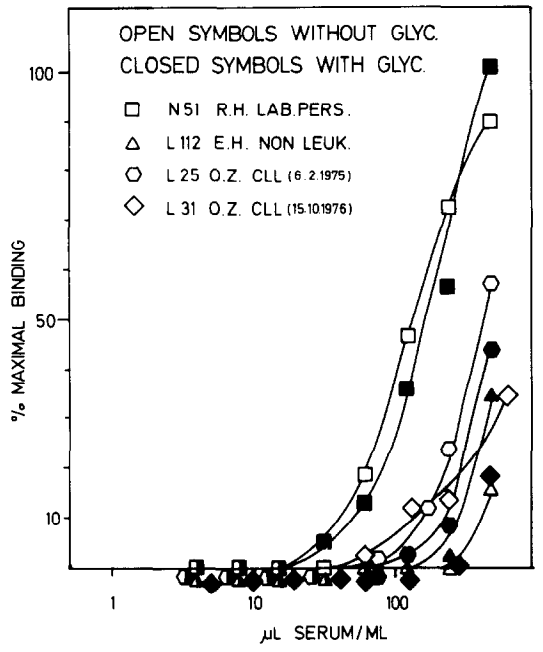

Fig. 6. Binding of SiSVgp70-related human antigens that had been treated with glycosidase (100 µg/ml) *to anti-SiSVgp70 IgG.* 100% = A_{450} 0.665.

DISCUSSION

In this paper we report the detection and distribution of antigens and immune complexes related to SiSVgp70 in human sera. In contrast to SiSVgp70-related human proteins which were almost exclusively found in leukemic sera [6], SiSVgp70-related antigens are present in leukemic and non-leukemic sera to similar extents. The prevalence of SiSVgp70-related antigens and immune complexes in human sera is about 50% (range 29–76% for antigens and 28–65% for immune complexes). The antigens and immune complexes seem to be ubiquitous and are distributed rather evenly amongst all groups of sera tested. Exceptions may be laboratory workers, who had the highest fraction, and patients with chronic leukemias (CML in chronic phase and CLL), who had the lowest fraction of positives. The low fraction of positives among CML-sera (chronic phase) may not be significant since sera of only two patients were examined.

Previous attempts by others to detect this kind of antigen may have failed because of too stringent assay conditions of competition RIAs. The ELISA [25] as a binding assay of high sensitivity permits a broader recognition of cross-reactivity than that allowed by the competition RIA.

The presence of SiSV-related antigens in man is in agreement with the earlier isolation of SiSV/GaLV-related retroviruses from human cell cultures (see [3] for review) and with the previous detection of SiSVgp70-reactive antibodies [29, 30]. These antibodies, however, were found to react predominantly with the sugar moieties of SiSVgp70 and therefore were regarded as heterophilic antibodies [31, 32]. The detection of SiSVgp70-related antigens as reported here does not appear to be mediated by a heterophilic reaction with sugar moieties, since the antiserum used predominantly reacts with the protein parts of SiSVgp70 [8] and of the SiSVgp70-related human antigens.

The presence of SiSVgp70-related immune complexes indicates that SiSVgp70-related antigens elicit an antibody response and are complexed with natural antibodies to immune complexes. This assumption was further confirmed by the demonstration of SiSVgp70-reactive free antibodies in human sera. The SiSVgp70-reactive antibodies described here differ from the antibodies mentioned above [29–32] in that they are directed predominantly against the protein moieties of SiSVgp70. In the present experiments carbohydrate moieties were removed from the coating SiSVgp70 of antibody ELISAs by prior treatment with a mixture of glycosidases (as monitored by polyacrylamide gelelectrophoresis), and the antibody determinations were controlled by ELISAs with glycosidase-treated SiSVgp70. Subtractions of non-specific reactivities (as determined with preimmune IgG) from total SiSVgp70-related IC, which would be required for quantitative determinations of SiSVgp70-related IC, have not been included here. Studies with preimmune sera, however, do not show significant qualitative differences between total and corrected SiSVgp70-related IC determinations [Kreeb *et al.*, in preparation].

For a complete evaluation of the SiSVgp70-related AG status the determination of SiSVgp70-related AB and IC in addition to AG is necessary. In 13% of all evaluable sera SiSVgp70-related AG were only detectable by determining SiSVgp70-related IC. Determination of IC therefore avoided false negative results in about 20% of all AG- or IC-positive sera. In 12 sera (7% of the evaluable sera) only SiSVgp70-related IgG antibodies indicated presence of, or previous contact with, SiSVgp70-related AG. Persisting IgG-mediated immunity in the absence of antigen is a feature of many previous antigenic contacts of infections. Conversely, in a few patients, SiSVgp70-related AG appears not to elicit an immune response (AG positive, AB and IC negative).

The detection of antigens resembling structures of primate C-type viruses in human sera is support for the presence of related agents in man. It is, however, a matter of speculation whether the SiSVgp70-related structures described here are of

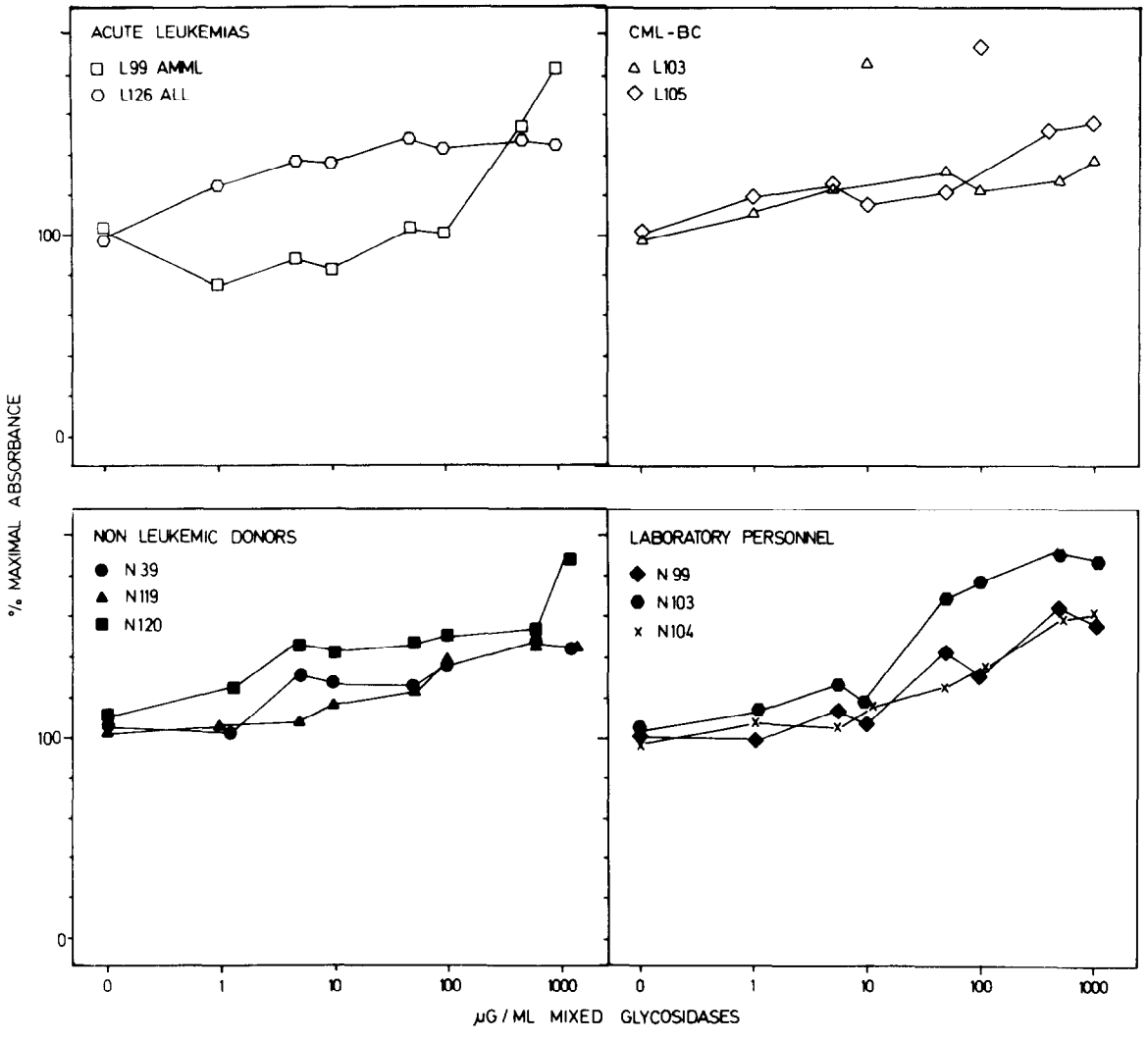

Fig. 7. Binding of human antibodies to SiSVgp70 (5 µg/ml coating concentration) that had been subjected to treatment with increasing amounts of glycosidases. The glycosidase digest was monitored by gelelectrophoretic analysis of ^{125}I-labeled SiSVgp70 which was treated with glycosidases in a parallel assay. Maximal A_{450} binding values were for: L103 = 0.970; L99 = 0.693; L105 = 0.997; L126 = 1.214; N39 = 0.850; N119 = 0.805; N120 = 0.486; N99 = 0.678; N103 = 0.590; N104 = 0.677.

retroviral origin. SiSV-related nucleic acids, reverse transcriptase, structural proteins and complete viral particles have all been reported in human leukemic and embryonic tissues (for review see [2]. Further, endogenous retroviral sequences have been detected in the human genome [33–35] and characterized by molecular cloning [36]. Therefore a partial derivation of the SiSVgp70-related antigens from cellular sequences had to be taken into account. More detailed studies on sequence homologies of human DNA to SiSV envelope sequences are necessary to verify a possible endogenous origin of SiSVgp70-related antigens. On the other side, the high percentage of SiSVgp70-related antigen- and antibody-positive sera among laboratory workers who were exposed to SiSV and related viruses (GaLV, MuLV, also BaEV) may be an indication for a horizontal transmission and an exogenous origin of the antigens.

The pathophysiological role of SiSVgp70-related antigens in man remains uncertain. In mice expression of the envelope glycoprotein gp70 of murine leukemia viruses (MuLVgp70) is not correlated with development of leukemia *per se* [37]. However, MuLVgp70 appears to play a key role in leukemogenesis by certain murine leukemia viruses [38, 39] after recombination events have occurred between envelope (gp70) genes of ecotropic and xenotropic murine C-type retroviruses [39, 40]. In order to decide on recombination events of human SiSVgp70-related proteins in leukemic sera as compared to non-leukemic sera their structure and coding sequences have to be characterized.

Also a receptor-based model which has been

proposed for murine leukemogenesis [41-43] might explain the correlation of SiSVgp70-related human antigens with prognosis of acute forms of leukemias. This model proposes a mitogenic effect of retroviruses on target cells via cell surface receptors for retroviral envelope glycoproteins with the consequence that the target cells may produce blastogenic factors (lymphokines) which induce proliferation of another cell population [42-44, 37]. If human white blood cells (e.g. lymphocytes) possess similar receptors, binding of SiSVgp70-related proteins to these receptors might result in mitogenic stimulation with production of lymphokines and thereby modulate the course of the disease. The correlation of SiSVgp70-related antigens with prognosis in acute leukemias and chronic myelogenous leukemia in blast crisis [7] suggests that further characterization of these antigens and analysis of their genomic structure should be interesting and might shed some light on mechanisms underlying human leukemogenesis and on factors determining the course of the disease.

Acknowledgements—The expert technical assistance of E. Jatho, L. Ireland and S. Schulte-Overberg is acknowledged. We thank Dr R. Brack for reading the manuscript and U. Böck for drawing the graphs. This work was conducted, in part, as study of the Süddeutsche Hämoblasttosegruppe (SHG). Participating institutions: Abt. Innere Medizin II der Med. Fakultät, Aachen (U. Essers); Onkolog. Abt. des Kantonsspitals, Basel (W. Weber, P. Obrecht); Onkolog. Zentrum der Städt. Krankenanstalten, Mannheim (M. Mayer, W. Queißer); Med. Klinik III. Klinikum Großhadern der Universität, München (W. Wilmanns); Zentrum Innere Medizin der Universität, Ulm (D. Hoelzer, H. Heimpel), Zentrum Innere Medizin der Universität, Gießen (H. Pralle), Med. Klinik der Universität, Tübingen (K. Wilms), Zentrum für Innere Medizin der Universität, Frankfurt (F. Walther, P. S. Mitrou), Med. Klinik der Universität, Mainz (J. Fischer), Med. Klinik der Universität, Heidelberg (P. Drings), Med. Poliklinik der Universität, München (R. Hehlmann).

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