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Antibodies against Feline and Gibbon Ape Reverse Transcriptase on Surface of Spleen Cells in Human Myelogenous Leukemia

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Abstract—*We reported that antibody bound to blood leukocytes of patients in chronic myelogenous leukemia neutralize reverse transcriptase. The specificity of neutralization depended on the phase of the disease. Antibody bound to chronic phase leukemia cells neutralized Gibbon Ape leukemia virus reverse transcriptase, while that bound to blastic phase cells neutralized Feline leukemia virus reverse transcriptase. Spleen cells obtained from patients with chronic myelogenous leukemia have surface-bound immunoglobulins that recognize both reverse transcriptases. These data in the spleen suggest the existence of a progressive shift in cell population from a chronic phase type cell to a blastic phase type cell.*

INTRODUCTION

A CYTOPLASMIC reverse transcriptase (RT) has been described in some human leukemic cells [1, 2]. In a few cases enzymes have been partially purified and characterized as being immunologically related to two RT's of the primate virus group: Simian sarcoma virus (SiSV) and Gibbon Ape leukemia virus (GaLV) [3, 4]. Serum antibodies recognizing RT have thus far been demonstrated in only two leukemic patients, both of which had acute myelogenous leukemia (AML). In both cases the antibodies neutralized SiSV RT [5].

We have purified from the surface of human myelogenous leukemic cells, membrane-bound antibodies that were subsequently shown to preferentially neutralize different type-C viral RT's [6]. Antibodies purified from chronic myelogenous leukemic (CML) blood leukocytes isolated from patients in blast crisis (BC) neutralized Feline leukemia virus (FeLV) RT, while those from patients in the chronic phase of the disease neutralized GaLV RT. In addition, antibodies eluted from AML cells neutralized SiSV RT.

We report here that antibodies bound to CML spleen cells neutralize both FeLV and GaLV RT's unlike those found in blood leukocytes, where the specificity of RT neutralization depended on the stage of the disease.

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MATERIALS AND METHODS

Source of antibody and purification

Leukemic blood cells were obtained by leukophoresis of CML patients. Leukemic spleen cells before onset of BC were obtained by surgery as part of a clinical protocol. From 2 to 20 g of packed blood cells were washed three times with 10 vol. of cold RPMI 1640 to remove the serum. Spleen samples were minced on ice with razor blades and washed like the blood cell samples. The washed cells were suspended in 20 ml of RPMI 1640 per g of cells, containing gentamycin 50 μg/ml in absence of serum, and were incubated for 12 hr at 37°C in 5% CO_2. Cells were then removed by centrifugation at 800 ***g*** for 5 min and supernatants were made up with 50% ammonium sulfate and left overnight at 4°C. Ammonium sulfate precipitates recovered by centrifugation at 10,000 ***g*** were dialysed in 20 mM sodium phosphate, pH 8. IgG's were purified on a DEAE-Agarose column (10 mg of protein was applied on 1 ml of gel). Sample 11 (Table 1) was further purified on an affinity column of Sepharose-4B coupled to goat anti-human IgG. The affinity chromatography was performed in a buffer containing 0.05 M Tris–HCl, pH 7.4, 2% Triton

Table 1. *Neutralization of different viral* DNA *polymerases* (RT) *by antibody* (IgG) *preparations from different* CML-*cells*

Patient no.	Source of leukemic IgG's*	Amount (μg) of IgG necessary for 50% neutralization of RT from SiSV	$GaLV_{SF}$	$GaLV_{H}$	BaEV	MuLV	$FeLV_{FL74}$	$FeLV_{C}$	Ratio $GaLV_{SF}/FeLV_{C}$
1	CML blood chronic phase	5	2	2.2	> 10	> 10	NT	> 10	< 0.2
2	CML blood BC†	1.5	> 3	> 3	> 3	0.17	0.05	0.02	> 150
3	CML blood BC†	> 30	> 30	> 30	28	17	0.1	0.04	> 750
4	CML blood BC†	1.8	1.8	2	2.1	0.8	0.08	0.08	22
5	CML blood BC†	> 10	NT	NT	> 10	> 10	3.6	4	—
6	CML blood BC†	2.8	3.2	4	4	2.8	0.18	0.06	53
7	CML spleen†§	1.3	0.06	0.05	1	1.4	0.1	0.05	1.2
8	CML spleen†§	> 4	0.15	NT	> 4	2	0.05	0.05	3
9	CML spleen†§	> 10	3	2.8	> 10	> 10	2.7	0.9	3
10	CML spleen†§	> 10	7.5	8	> 10	> 10	1.2	0.7	10.7
11	CML spleen‡§	> 10	> 10	NT	> 10	8	1	0.4	> 25

*IgG purified from different individual cell samples obtained from different patients.
†Ammonium sulfate and DEAE-Agarose-purified IgG.
‡Ammonium sulfate, DEAE-Agarose and affinity-purified IgG.
§Obtained before onset of BC.

X-100, 0.1% Tween 80 and 10 mg/ml BSA. The unbound proteins were washed with 0.85% NaCl and the specifically bound IgG's were eluted with 0.2 M glycine pH 2.8.

Source of viruses utilized for the purification of reverse transcriptases

FeLV (Theilen strain) was grown in FL74 feline lymphoid cells and FeLV subgroup C was grown in canine thymus cells (FCf_2 Th), Rauscher-MuLV (R-MuLV) in JLSV/10 cells, $GaLV_{SF}$ (San Francisco strain) in NC37 cells, $GaLV_H$ (Hall's Island) in Gibbon lymphoid cells, BaEV in canine thymus cells (FCf_2Th) and SiSV in marmoset cells (71API). We used two FeLV viruses and GaLV viruses grown in different host cells to exclude a possible host cell effect as the cause of the positive data.

Purification of reverse transcriptase and assay conditions

RT was purified from 1000-fold concentrated viruses. After disruption of viruses in a buffer containing 0.6 M KCl and 0.5% Triton X-100, RT was purified by successive chromatography on DEAE-cellulose, phosphocellulose, poly(G)-Agarose and hydroxyapatite. Details of these methods are described elsewhere [7]. Assays for enzymatic activity were done in 0.05 M Tris–HCl, pH 7.4, 140 μM [^{3}H]-dTTP (3.6 Ci/m mole), 130 μM dATP, 50 μg/ml dT_{12-18} poly rA, 0.5 mM $MnCl_2$, 5.0 mM DTT and 190 mM NaCl at 30°C for 30 min. Assays were stopped by addition of cold TCA and carrier calf thymus DNA.

Neutralization tests of enzymatic activity were done as follows: enzyme and antibody were preincubated at 4°C for 4 hr in preincubation buffer (0.05 M Tris-HCl, pH 7.4, 190 mM NaCl, 1% Triton X-100, 10 mg/ml BSA and 0.005% Tween 80) and assayed as described above. The 100% activity was obtained in absence of antibody and varied between 40,000 and 70,000 cpm (20–40 pmole) of [^{3}H]-dTMP incorporated into acid-insoluble material. Comparable amounts of enzyme units were used for each polymerase.

RESULTS

Membrane-bound antibody was purified from blood and spleen cell samples from ten different CML patients. The antibodies released from the membrane as described in Materials and Methods were precipitated with 50% ammonium sulfate purified on a DEAE-Agarose column and tested for their neutralizing activity of RT's. Table 1 compared the amount of each IgG preparation necessary to neutralize RT from seven different sources. The blood sample IgG (no. 1), obtained from a patient in chronic phase CML, specifically neutralized GaLV RT's, while samples from patients 2–6 in CML BC showed a good specificity in neutralization of FeLV enzymes and almost as well the GaLV enzymes: there was a less than 3-fold difference in the amount of IgG necessary to neutralize the two types of RT's. Figure 1 shows the neutralization curves obtained with spleen-eluted IgG from a patient in reactions with different RT's. Complete inhibition of the FeLV enzymes was reached with as little as 0.5 μg of IgG. The BaEV and SiSV enzymes were not neutralized, while there was a significant neutralization of GaLV and MuLV enzymes. MuLV RT is immunologically closely related to FeLV RT [8, 9]: this may explain the 50% neutralization noticed. A cross-reactivity of this type does not exist between GaLV RT and FeLV RT [8, 9], so that the striking neutralization of the GaLV RT's (e.g. 70%) was probably due to a second population of antibodies. The IgG from spleen no. 10 (Table 1) was more specific than that from other spleens (nos. 7–9) in that it neutralized FeLV RT's much more than GaLV RT's. This might indicate that the process of blastic transformation was more advanced in the case of the donor of spleen no. 10. This last sample was further purified on an affinity column of goat anti-human IgG to verify that further purification did not significantly modify the results (no. 11).

DISCUSSION

Antibody eluted from blood leukocytes of CML patients in chronic phase specifically

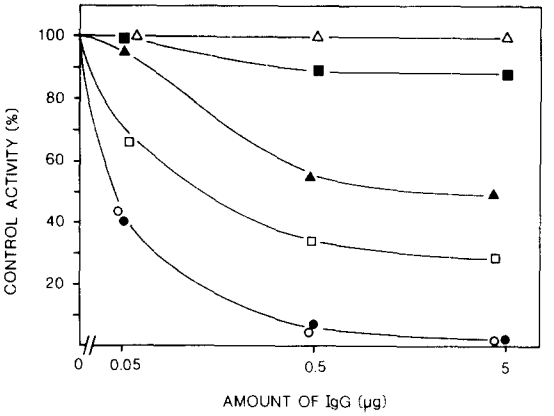

Fig. 1. Specificity of neutralization of viral RT by different amounts (μg) of IgG purified from spleen cells of CML patient no. 8 (Table 1). 100% corresponded to the activity of different enzymes in absence of antibody. Enzymes tested for neutralization were: (●) FeLV RT FL74, (○) $FeLV_CRT$, (□) $GaLV_{SF}RT$, (▲) MuLV RT, (■) SiSV RT and (△) BaEV RT.

neutralized GaLV RT, as opposed to antibody eluted from blood leukocytes of patients in blast crisis which neutralized FeLV RT. Antibody eluted from spleen cells of CML patients neutralized FeLV RT but also neutralized GaLV RT with comparable amount of IgG's. These spleen-eluted antibodies can be associated with spleen B lymphocytes reactive against different leukemic cell-associated antigens, but they can also reflect the existence in the spleen of two different populations of leukemic cells bearing different membrane-bound antibodies. We have previously shown that the specificity of antibody found on CML blood leukocytes was related to the phase of the disease [6]. Chronic phase blood cells were found to bear associated surface antibodies against GaLV RT and blastic cell antibodies against FeLV RT. The presence of an antibody response against both of these populations of cells in CML spleen raises the hypothesis of a dual event in the process of leukemogenesis in CML patients. A long chronic phase associated with a GaLV RT-like antigen on cell membranes could be followed by a blastic transformation associated with the appearance of a FeLV RT-like antigen on cell membranes. The existence on the cell surface of those types of virus-related proteins does not imply the existence of a viral infection since it can be due to the appearance of cross-reactive proteins with no relationship to viruses.

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