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Communication

Strong, Specific Anti-Human Leukemia Antisera Prepared with the Use of Purified Cell Membrane Antigen[1]

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ABSTRACT

Two rabbits immunized with 15 μg of a purified human thymus leukemia-associated antigen preparation and boosted once with the same amount of the antigen preparation yielded antisera that showed strong specificity for human leukemic T-cells without any prior absorptions. These antisera from the two rabbits showed a 50% killing of cells at antiserum dilutions of 5700- and 1600-fold, respectively, against JM, a leukemic T-cell line, and slightly weaker activity against MOLT-4, another leukemic T-cell line. These antisera, without any absorption, showed no or minimal reaction against two nonmalignant B-cell lines (RPMI 1788 and RPMI 8057), a leukemic non-T-, non-B-cell line (NALM-16), a leukemic pre-B-cell line (NALM-1), normal peripheral blood lymphocytes, and T-cells isolated from peripheral blood lymphocytes.

Antiserum 7557, which showed the higher antibody activity, was further studied by an absorption test using various human cell lines. The antiserum showed strong activity against all three leukemic T-cell lines tested, *i.e.*, CCRF-CEM, RPMI 8402, and CCCR-HSB-2, whereas it showed no significant activity against other cell lines which included two leukemic non-T-, non-B-cell lines (KM-3 and NALM-6), NALM-1 and RPMI 1788.

These are the first anti-human leukemia antisera, except for the monoclonal hybridoma antibodies, that showed good specificity for leukemia cells without prior absorption.

The present procedure of immunizing animals with a small amount of human thymus leukemia-associated antigen preparation isolated from cell membrane will also be useful for obtaining strong, specific antisera of other cell membrane antigens.

INTRODUCTION

HTL[4] antigens have been detected on T-type ALL cells and cell lines derived from them (*i.e.*, leukemic T-cell lines) as well as on normal thymocytes with the aid of extensively absorbed xenogeneic antisera raised against normal thymocytes, leukemic T-cell lines, or T-type ALL cells (3, 9, 10, 16, 18, 20, 22, 25). However, the antibody activity of these antisera is quite low because HTL antigens on the cell surface are not strongly immunogenic compared with other major cell components [*e.g.*, HLA(A,B,C) antigens], and the antisera obtained must be extensively absorbed with various tissues until they become operationally specific for HTL antigens. In the present study, we prepared rabbit anti-HTL antisera by injecting purified HTL antigen preparation which was isolated from cell membrane of MOLT-4 cells. These antisera have high antibody titers and showed strong specificity without prior absorption.

Recently, a few MH antibodies to HTL antigens were reported (6, 8, 13), and they appear to detect different antigen molecules. MH antibody has an advantage over conventional antiserum in that the former is monospecific and the latter is usually not. Conventional antiserum usually reacts with many different antigenic determinants of the same antigen molecule, whereas MH antibody reacts with one determinant only. However, some pitfalls may be associated with MH antibody because of this monospecificity (15). For instance, 2 different proteins having a common antigenic determinant but having important differences in other antigenic determinants would not be differentiated by a MH antibody directed to the common determinant, whereas a good conventional antiserum would recognize them as different. In addition, many MH antibodies do not bind complement and, consequently, are noncytolytic. In view of these facts, it will be of great value to use good conventional antisera in parallel with MH antibodies directed to the same antigen molecule.

MATERIALS AND METHODS

Human cell lines were cultured in RPMI 1640 supplemented with 5% heat-inactivated FCS. Peripheral blood lymphocytes were isolated from buffy coat specimens derived from healthy donors by centrifugation on a Ficoll-Paque gradient (25). T-cells were isolated from PBL as described by Julius *et al.* (4).

The amount of cell membrane protein was determined by a modified procedure of the method of Lowry *et al.* (7). In our modified procedure, DOC was added at 3% (final concentration), and the total volume of the reaction mixtures was decreased from a 1.1- to 1.3-ml range to 0.65 ml.

A HTL antigen preparation was obtained from cell membrane of MOLT-4 according to a slight modification of a procedure described previously (14).[5]

The HTL antigen preparation was isolated as follows. A suspension containing 1 × 10^8 MOLT-4 cells/ml was prepared in 10 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA, Trasylol (100 Kallikrein units/ml), 0.5 mM phenylmeth-

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[4] The abbreviations used are: HTL antigens, human thymus leukemia-associated antigens; ALL, acute lymphoblastic leukemia; MH antibody, monoclonal hybridoma antibody; RPMI 1640, Roswell Park Memorial Institute Medium 1640; FCS, fetal calf serum; PBL, peripheral blood lymphocytes; DOC, deoxycholic acid sodium salt; i.d., intradermally.

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ylsulfonyl fluoride, and 0.02% NaN_3. The cells were disrupted in a N_2 cell disruption bomb (Parr Instrument Co., Moline, Ill.) and subsequently further disrupted in a Stansted Model AO 612 cell disrupter equipped with a Model 716 disrupting valve (Stansted Fluid Power, Ltd., Stansted, Essex, England). The resultant cell homogenate was centrifuged at 2,000 × *g* for 20 min, and the supernatant was further centrifuged at 100,000 × *g* for 2 hr. The pellet representing the crude cell membrane was resuspended in 10 mM Tris-HCl, pH 7.8, containing 0.15 M NaCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, Trasylol (100 Kallikrein units/ml), and 0.02% NaN_3. To solubilize the cell membrane proteins, DOC (final concentration, 1%) and recrystallized iodoacetamide (final concentration, 5 mM) were added to the membrane suspension. The suspension was homogenized in a manual Dounce homogenizer and incubated for 1 hr with continuous shaking. The solubilized cell membrane proteins were subjected to affinity chromatography on a *Lens culinaris* lectin column, and the bound glycoproteins were eluted with 5% α-methyl-D-mannoside in 10 mM Tris-HCl buffer, pH 8.0, containing 0.5% DOC, 1 mM EDTA, and 0.02% NaN_3. The glycoproteins were fractionated on a AcA 34 (LKB-Produkter AB, Bromma, Sweden) column, and 2 HTL antigen peaks, one major and one minor, were detected. Substances in the fractions corresponding to the major antigen peak were pooled and passed through serially connected immunoadsorbent columns prepared with Sepharose-coupled $F(ab')_2$ fragments of anti-β_2-microglobulin, anti-B-cell, and anti-T-cell antibodies. The unbound substance was used for immunizing rabbits.

All the above operations for isolating HTL antigen preparation were carried out at 0–4°.

To obtain antisera, 2 New Zealand White rabbits were immunized i.d. at 20 different sites over the entire back of the animal with 15 μg of the membrane proteins in 0.5 ml of 10 mM Tris-HCl buffer, pH 8.0, containing 0.3% DOC (Tris-DOC buffer) mixed with an equal volume of Freund's complete adjuvant. A booster immunization was carried out on the 21st day after the first immunization by injecting s.c. at the edges of the erythema caused by the first immunization with 15 μg of the membrane proteins in 0.5 ml of Tris-DOC buffer mixed with an equal volume of Freund's incomplete adjuvant. The animals were bled once on the 7th day and bled out on the 14th day after the booster injection.

The antibody activity of the resultant antisera was tested by a complement-dependent cytotoxicity test using ^{51}Cr-labeled target cells (24) and by an absorption test (2).

RESULTS

Antisera obtained from 2 different rabbits (Rabbits 7556 and 7557) were tested, without prior absorption, against various cell lines and cells (Chart 1). Both antisera showed strong activity against 2 leukemic T-cell lines, JM and MOLT-4. Antiserum 7557 showed a 50% killing of cells against JM and MOLT-4 at the antiserum dilutions of 5700- and 4000-fold, respectively. Similarly, Antiserum 7556 killed 50% of JM and MOLT-4 cells at antiserum dilutions of 1600- and 1100-fold, respectively. Both antisera showed only minimal reactivity against 2 nonmalignant B-cell lines (RPMI 1788 and RPMI 8057), a leukemic non-T-, non-B-cell line (NALM-16), a leukemic pre-B-cell line (NALM-1), normal PBL, and T-cells isolated from PBL.

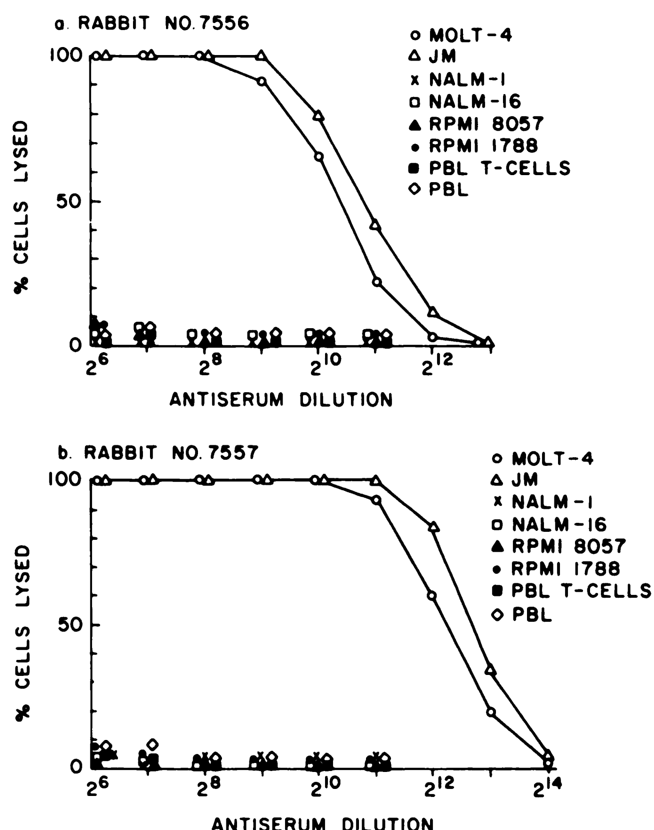

Chart 1. Activity and specificity of rabbit antisera prepared by injecting a purified HTL antigen preparation isolated from MOLT-4 cell membrane. The antisera were prepared in 2 rabbits (Rabbits 7556 and 7557) as described in "Materials and Methods." The antisera were tested in serial dilutions by a complement-dependent cytotoxicity test using the ^{51}Cr-labeled target cells indicated.

Antiserum 7557 was further studied by means of an absorption test. In this test, we used the IgG of the antiserum which was obtained by ammonium sulfate precipitation at 45% saturation. Nine μg of the IgG dissolved in 100 μl of RPMI 1640 containing 10% heat-inactivated FCS was incubated with 3 × 10^7 cells of each of the various cell lines indicated in Chart 2. The remaining antibody activity in each of the absorbed IgG preparations was tested against ^{51}Cr-labeled MOLT-4 cells after the IgG was serially diluted with RPMI 1640 containing 10% FCS. Significantly decreased antibody activity was observed only for those IgG preparations which had been absorbed with each of the 3 leukemic T-cell lines (CCRF-CEM, RPMI 8402, and CCRF-HSB-2). No significant decrease of the antibody activity was observed for any of those IgG preparations which had been absorbed with leukemic non-T-, non-B-cell line (KM-3 or NALM-6), leukemic pre-B-cell line (NALM-1), or nonmalignant B-cell line (RPMI 1788). Thus, the results of the absorption test (Chart 2) completely agree with those of a complement-dependent cytotoxicity test (Chart 1).

DISCUSSION

It was previously shown by several groups of investigators that established human leukemic cell lines retain many cell membrane markers including leukemia-associated antigens found on the noncultured, fresh leukemia cells (1, 3, 5, 6, 8, 10, 13, 14, 21, 23). On the basis of this fact, we used mainly

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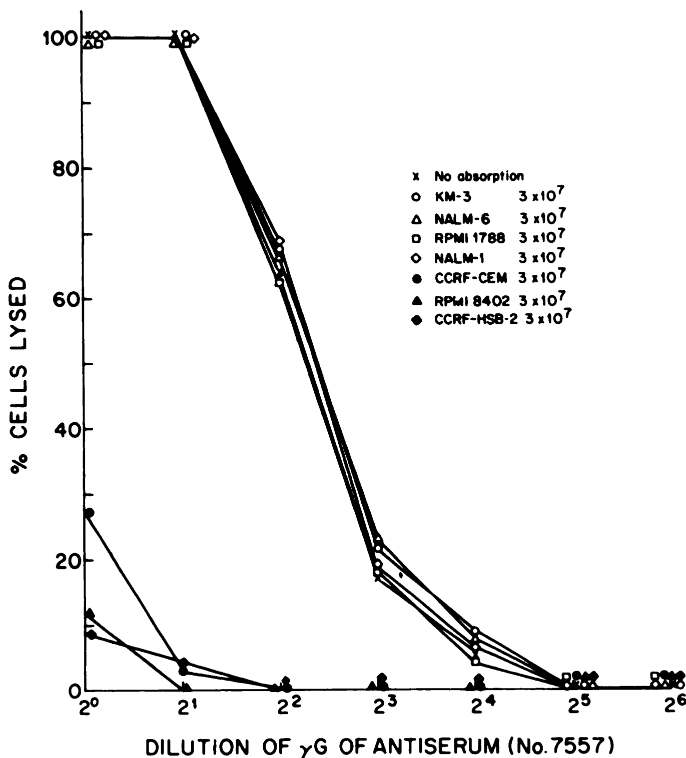

Chart 2. Absorption of the IgG (γG) of anti-HTL Antiserum 7557 with various human cell lines. Portions of the IgG (9 μg in 100 μl) of the antiserum were individually incubated for 30 min in ice water with 3×10^7 cells of each of the various cell lines indicated. As a control, the IgG was treated in the same manner but in the absence of cells. Each of the resultant absorbed and control IgG preparations was tested in serial dilutions by a complement-dependent cytotoxicity test against ^{51}Cr-labeled MOLT-4 cells. The 3 cell lines (CCRF-CEM, RPMI 8402, and CCRF-HSB-2) which show significant inhibition activity against the IgG of Antiserum 7557 are all leukemic T-cell lines.

established cell lines in the present study. All of 5 leukemic T-cell lines used in the present study, *i.e.*, MOLT-4, JM, CCRF-CEM, RPMI 8402, and CCRF-HSB-2, were derived from the peripheral blood of patients with T-type ALL (Ref. 17; reviewed in Ref. 10) and were previously shown to express HTL antigens on the cell surface (3, 6, 8, 10, 13, 20, 25). NALM-6, NALM-16, and KM-3 were derived from the peripheral blood of patients with non-T-, non-B-type ALL, whereas NALM-1 was derived from the peripheral blood of a patient with chronic myelocytic leukemia in lymphoid blastic crisis (Ref. 11; reviewed in Refs. 10 and 12). The specificity of the present anti-HTL antisera is in good agreement with that of previously reported anti-HTL antisera which were prepared by immunizing rabbits with leukemic T-cell line and normal thymocytes followed by extensive absorptions (10, 20, 25).[6] Furthermore, the antibody titers of the present antisera are much higher than those of the previously reported conventional antisera.

The present antisera will be very useful for diagnostic purposes because of their high antibody titers. In addition, relatively large quantities of antibodies can be obtained from the present antisera and will permit us to prepare immunoadsorbents which will be very useful for isolating HTL antigens from leukemia cells as well as from body fluids of leukemia patients by a simple procedure.

[6] S. Negoro, K. Yoshizaki, E. S. Henderson, and B. K. Seon. New antigenic determinant common to human thymus-leukemia-associated antigens and common acute lymphoblastic leukemia antigen, accepted for publication, Clin. Exp. Immunol.

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