

Antigens Shared by Leukemic Blast Cell and Lymphoblastoid Cell Lines Detected by Lymphocyte-dependent Antibody¹

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SUMMARY

Lymphocyte-dependent antibodies (LDA's) directed against antigenic determinants present on lymphoblastoid cell lines as well as human leukemia blast cells were demonstrated in heterologous antisera obtained by immunizing rabbits with a membrane fraction from RPMI-4265 (a lymphoblastoid cell line derived from a patient with chronic myelogenous leukemia).

LDA was present at high titers against B-lymphoblastoid, myelomonocytic, and stem cell lines. The T-lymphoblastoid cell line MOLT-4, however, did not react. LDA was demonstrated against acute myelogenous as well as lymphoblastic leukemia cells. The reactivity was not directed against phytohemagglutinin-induced blastoid antigens, fetal antigens, or fetal calf serum. Absorptions with lymphoblastoid cell lines removed all LDA reactivity. Similar results were obtained by absorbing the rabbit antiserum with acute lymphoblastic and/or acute myelogenous leukemia cells.

These findings indicate the presence of cross-reactive antigens between lymphoblastoid cell lines and leukemia cells. Furthermore, cross-reactivity between acute lymphoblastic and acute myelogenous leukemia cells was demonstrated.

INTRODUCTION

Sera obtained from rabbits immunized with membrane components of LCL's³ contain specific antibodies directed against *HL-A* antigens (6, 9). On the other hand, Mann *et al.* (10) reported that antisera obtained against a cell fraction from RAJI cells (culture cell line derived from a patient with Burkitt's lymphoma) reacted against PBL's from patients with acute leukemia but not with remission lymphocytes or PBL's of normal donors. Thieme and

Columbe (15) reported on a rabbit serum raised against a cell membrane fraction from RPMI-4265 cells (culture cell line derived from a patient with chronic myelogenous leukemia) that was solubilized by papain digestion according to the procedure described by Mann *et al.* (10) and further purified by physicochemical means. This serum reacted with a variety of LCL's but not against PBL's from normal donors. The studies mentioned above characterized the serum reactivity by measuring cytotoxic CDA's.

The recognition of LDA in numerous heterologous sera as well as the exceptional sensitivity of the LDA assay prompted us to investigate further the serum raised in our laboratory by Thieme and Columbe (15), using this time an LDA assay system composed by ⁵¹Cr-labeled target cell (LCL, acute leukemia blasts, and PBL) rabbit antisera against RPMI-4625 as the source of antibodies and PBL obtained from adequately selected healthy donors as effector cells.

MATERIALS AND METHODS

Media

All work was carried out using MEM (Grand Island Biological Co., Grand Island, N. Y.) with 10% fetal calf serum (K. C. Biological Co., Lenexa, Kans.).

Preparation of Antigen

The procedure has been described amply by Thieme and Columbe (15). Briefly, a membrane preparation from RPMI-4265 was treated with papain in the presence of cysteine. The product of this reaction was centrifuged at 150,000 × *g* for 1 hr, and the supernatant was passed through a G-150 Sephadex column. Column fractions containing *HL-A* antigenic activity were pooled, concentrated by pressure dialysis, and subsequently subjected to disc-gel electrophoresis by the method described by Davis (5). Gels were sliced and eluted in buffer and were assayed. The fractions containing *HL-A* antigen activity were concentrated and used to immunize the rabbits.

Antisera Preparation

The method for preparation of rabbit anti-RPMI-4265 has been previously described (15). Briefly, rabbits were given 2 injections of a Sephadex G-150-purified fraction of papain-digested membrane material from RPMI-4265 with

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³ The abbreviations used are: LCL, lymphoblastoid cell lines; PBL, peripheral blood leukocyte; CDA, complement-dependent antibody; LDA, lymphocyte-dependent antibody; MEM, minimum essential medium; SRBC, sheep red blood cells; MEM-FCS; minimum essential medium with 10% fetal calf serum; TC, target cell; EC, effector cell; AML, acute myelogenous leukemia; MF, membrane fraction; AcLL, acute lymphoblastic leukemia; EBV, Epstein-Barr virus.

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1-month intervals and were bled 7 days after the 2nd injection. Sera were inactivated by heating at 56° for 45 min. The sera were stored at -20°.

Control Antisera

Normal rabbit sera obtained from 5 different rabbits were used for control puposes in LDA.

Rabbit Anti-human IgG (204). Rabbits received 1 mg of human IgG (G-myeloma protein) i.m. and s.c. in complete Freund's adjuvant. The rabbits were boosted by injection of 1 mg IgG i.m. and s.c. in incomplete Freund's adjuvant at 1, 2, 5, 6, 7, and 8 months and were finally bled 1 week after the last booster injection. Immunoelectrophoresis of the sera showed only 1 strong band in the IgG area.

Rabbit Anti-EL-4. Rabbits received i.v. 3 injections of 10⁸ EL-4 leukemia cells (chemically induced murine tumor) at weekly intervals and were bled 1 week after the last injection.

Rabbit Anti-SRBC. Rabbits were given i.v. injections of 1 ml of a 10% SRBC suspension and were boosted 3 times at weekly intervals with 1 ml of 20% SRBC suspension. The bleedings were performed 1 week after the last booster injection.

Target Cells

Eight lymphoblastoid cell lines established in culture were used. Some of the cells were derived from normal donors (UCLA-81, -91), while the rest were derived from patients with various types of leukemia (M. Jobin and J. L. Fahey, unpublished results). Peripheral blood mononuclear cells derived from 7 healthy donors were also used. T24 (4), a nonlymphoid culture cell line obtained from transitional cell carcinoma of the bladder, HT29M, derived from colon carcinoma (2), and FL, derived from amnion (7), were used in several occasions. These cells grow in monolayers and for use in a LDA assay the monolayers are trypsinized (0.25% trypsin) in order to obtain a cell suspension. The HL-A type and characteristics of the cells used in this study are shown in Table 1.

Labeling of TC

Cells (2 × 10⁷) contained in 1 ml of MEM-FCS were incubated with 400 μCi of ⁵¹Cr (Amersham/Searle Corp., Arlington Heights, Ill.) at 37° in a rocking platform (10 cycles/min) for 60 min in an atmosphere of 10% CO₂. At the end, the cells were washed twice, counted, and readjusted to a concentration of 25 to 50 × 10⁴/ml.

Effector cells

Peripheral blood mononuclear cells were obtained from heparinized blood by density flotation using a Ficoll (Ficoll 400, Pharmacia AB, Uppsala, Sweden) Hypaque (Hypaque sodium 50, Winthrop Laboratories, N. Y.) gradient (3). The separated cells were washed twice in MEM-FCS and adjusted to the appropriate concentration. Morphological

Table 1
Origin and characteristics of the various TC's used

LCL	Origin	Cell type	Synthesis of immunoglobulin	HL-A/ type
RPMI-4265	CML ^a	B-cell	IgG, K	2, 3, 7, 12
UCLA-81	Normal	B-cell	IgG	1, 9, 8, 12
UCLA-91	Normal	B-cell	IgG + IgM	9, 29, 50, 14
UCLA-102 ^b	CMML	Monocytoid		ND ^b
UCLA-109	Stem cell leukemia	Stem cell		ND
UCLA-173	CLL	B-cell	IgG	ND
UCLA-176 ^b	CMML	Monocytoid		ND
UCLA-62				ND
MOLT-4 ^c	AcLL	T-cell		ND
PBL				
A. D. ^d	Normal	Mononuclear cells		10, 29, 12
B. B.	Normal	Mononuclear cells		3, 9, 13
B. G. ^d	Normal	Mononuclear cells		2, 28, 5, 21
E. B.	Normal	Mononuclear cells		29, 30, 17, 18
J. Z. ^d	Normal	Mononuclear cells		1, 32, 8, 15
M. O.	Normal	Mononuclear cells		2, 10, 12, 14
T. T.	Normal	Mononuclear cells		9, 7, 12

^a CML, chronic myelogenous leukemia; ND, not done.

^b UCLA-102 and -176 have lysozymes and migrate on a Boyden chamber in response to chemotactic factors (M. Jobin, B. Bonavida, and J. L. Fahey, submitted for publication).

^c Form spontaneous rosettes with SRBC's.

^d These cells were also incubated with 1% phytohemagglutinin (Burlington-Wellcome Co.) and, at 72 hr after blastic transformation (≥ 70%), were used in direct cytotoxicity assays and for absorption purposes.

examination revealed that cells obtained under these conditions contained 65 to 80% lymphocytes, 10 to 15% monocytes, and 10 to 20% polymorphonuclear leukocytes. The degree of contamination of our EC population with polymorphonuclear leukocytes depended on the time elapsing between blood drawing and Ficoll-Hypaque gradient separation.

LDA Assay

EC's in 0.1 ml containing from 2.5 × 10⁴ to 5 × 10⁵ cells (according to the EC to TC ratio) were added to 75 x 100-mm disposable test tubes containing 5 × 10⁸ to 5 × 10⁵ ⁵¹Cr-labeled target cells and 0.1 ml of the corresponding dilution of the antiserum. The tubes were incubated in a rocking platform for 4 hr (except when stated otherwise) at 37° in a 10% CO₂ atmosphere. Cold MEM was added to stop the reaction, and the tubes were centrifuged and counted in a γ scintillation counter. The degree of specific lysis was determined as follows:

$$\% \text{ specific lysis } (E - B/T - B) \times 100$$

where *E* represents counts released by TC's when incubated with EC and immune serum, *B* is background counts, rep-

Table 2
Nomenclature and titer against RPMI-4265 of the various types of absorbed sera

Nomenclature	Absorbing cells ^a	Titer ^b
R2-1		6
R2-1 (T)	Human RBC, liver, PBL	5
R2-1 (T, BM ^c , ^d 2 times)	Bone marrow, healthy donor (2 times)	3
R2-1 (T, PHA) ^d	PHA-blasts from PBL	5
R2-1 (T, AcLL 2 times)	AcLL-blasts (2 times)	1
R2-1 (T, AML 2 times)	AML-blasts (2 times)	1
R2-1 (T, LCL 2 times)	RPMI-4265 (2 times)	0
R2-1 (T, FCS)	Fetal calf serum	5
R2-1 (T, FT)	Fetal tissue	5

^a For all absorptions a ratio of 2 to 3:1 volume of undiluted sera per volume of packed cells was used. The suspension was incubated for 2 hr at 4° with intermittent shaking and then centrifuged at 500 × g for 10 min.

^b Serum titer, expressed as the reciprocal of the last 10-fold dilution giving ≥ 10% cytotoxicity above background values.

^c Bone marrow cells were obtained from healthy controls A. D. (HL-A-10, 29, 12) and J. Z. (HL-A-1, 32, 8, 15). RBC's were eliminated by hypotonic shock with distilled water.

^d BM, bone marrow; PHA, phytohemagglutinin; FCS, fetal calf serum; FT, fetal tissue.

It is worthwhile here to present some data on the assay conditions. Best results were obtained using a high number of target cells (25 to 50 × 10³), an EC:TC ratio of 4 to 10:1, and 4 hr of incubation time. Under these conditions the efficiency of the cytolytic reaction was considerably increased. Longer incubation periods were not advantageous because of high background values. A prozone effect was detected at 10⁻¹ serum dilution against most of the cells tested.

LDA Reactivity of R2-1(T) against LCL's. Eight LCL's derived from normal donors and patients suffering from acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myelomonocytic leukemia, and stem cells leukemia were used as TC's in assays designated to evaluate LDA reactivity of R2-1 sera (Table 3). High-titer LDA's were demonstrable against all cell lines (irrespective of their origin), tested with the exception of MOLT-4, a cell line with T-cell characteristics, *i.e.* forms spontaneous rosettes with SRBC (12). In all the experiments, a 4-hr incubation and an EC:TC ratio of 4:1 were used. A total of 4 rabbit anti-MF's (RPMI-4265) were tested against MOLT-4, and in each case no reactivity was detected.

LDA Reactivity of R2-1 against PBL's. PBL's obtained from healthy donors including B. G. and M. O. (HL-A2), A. D. (HL-A12), B. B. (HL-A3), and T. T. (HL-A7,-12) were used as TC's in experiments aimed at defining the presence of LDA against human histocompatibility antigens. These tests were performed 3 times with each of the TC's. The presence of LDA against HL-A2 antigens was demonstrated by the serum reactivity against B. G. and M. O., while it did not react against PBL carrying either HL-A3-7, or, -12. It became clear, however, that absorption of R2-1 with B. G. or M. O. removed totally the anti-HL-A2 reactivity while retaining high titer of reactivity against the LCL's (Table 4).

LDA Reactivity against Human Leukemia Blasts. The presence of LDA reactivity against human leukemia cells

representing the counts released by the target cells when incubated with EC and normal serum, and *T* is total releasable counts, representing the counts released by incubating the TC with 1 ml of distilled water.

One of the main questions revolving around the LDA assay relates to the criteria used to determine whether or not an antiserum has positive LDA reactivity and to determine the appropriate method for calculating maximum releasable counts and background. As far as maximum releasable counts, we have chosen to use the counts released by hypotonic lysis with distilled water. On numerous occasions we have observed that the counts released in this manner increase with incubation time, thus better mimicking the experimental conditions. For background we have used the counts released by the TC's in the presence of EC's and normal or unrelated immune rabbit serum. The values are lower than those obtained by incubating the TC's with MEM-FCS or antiserum in the absence of EC's. Although the explanation for this is still unclear, it probably has to do with cell density in the culture conditions.

A positive LDA activity in a serum was recorded when the counts of ⁵¹Cr released by the TC's in the presence of that serum was 10% above background, with a *p* value of ≤ 0.01. The LDA titer of a serum was defined by the last dilution that would have a positive reactivity as defined above.

Mitogenic Stimulation

PBL's (20 × 10⁶) (1 × 10⁶/ml) were cultured in a 250-ml Falcon tube with 1% phytohemagglutinin (Burroughs Wellcome Co., Burlingame, Calif.) for 72 hr in a 10% CO₂ atmosphere. The harvested cells were washed 2 times with MEM-FCS and used when ≥ 70% were blasts.

Absorption of Immune Sera

The procedure used consisted in mixing the antiserum with packed cells in a ratio of 2 to 3:1 volume of antiserum per volume of packed cells. This mixture was incubated for 2 hr at 4° with intermittent shaking every 10 min. Finally, the suspension was centrifuged at 500 × g, and the serum obtained was properly labeled and stored at -20°. The cells to be used for absorption were washed 3 times in MEM before mixing with the antiserum. Table 2 summarizes the types and origins of the various cell preparations used for absorption as well as the nomenclature and titers of the absorbed sera that will be used in this paper.

RESULTS

LDA Reactivity in Rabbit Antisera to MF (RPMI-4265). Antisera obtained from 5 different rabbits at various times after immunization were tested against ⁵¹Cr-labeled RPMI-4265 TC's. LDA reactivity was demonstrated in each of the sera tested. The titer, however, varied among the various sera (10⁻³ to 10⁻⁶). These observations indicated that LDA was generated in each of the rabbits immunized with MF (RPMI-4265). The reactivity of R2-1 against LCL (the serum to be described in this paper) therefore does not represent a unique feature of this serum.

Table 3
LDA reactivity of R2-1 and R2-1(T) against LCL's

Antisera	TC's ^a							
	RPMI-4265	UCLA-81	UCLA-91	UCLA-176	UCLA-62	UCLA-109	UCLA-173	MOLT-4
R2-1	6	6	6	4	5	4	3	0
R2-1(T)	5	5	5	3	4	ND ^b	ND	0

^a The conditions of the assays were: EC:TC = 4:1; 2.5×10^4 TC's; 4 hr-incubation. Background values, 20 to 30%. The numbers represent the serum titer, which is expressed as the reciprocal of the last 10-fold dilution giving $\geq 10\%$ cytotoxicity over background values.

^b ND, not done.

Table 4
LDA^a reactivity of R2-1 and R2-1(T) against PBL's

Antisera	TC's ^b						
	B. G.	E. B.	M. O.	B. B.	T. T.	A. D.	J. Z.
R2-1	4 ^c	0	4	0	0	0	0
R2-1(T)	0	0	0	0	0	0	0

^a EC:TC = 4:1; 2.5×10^4 TC's; background values, 25 to 30%.

^b *HL-A* phenotype B. G. (2, 28, 5, 21); E. B. (29, 30, 17, 18); M. O. (2, 10, 12, 14); B. B. (3, 9, 13); T. T. (9, 7, 12); A. D. (10, 29, 12); J. Z. (1, 32, 8, 15).

^c Serum titer, expressed as the reciprocal of the last 10-fold dilution giving $\geq 10\%$ cytotoxicity over background values.

obtained from patients with AML or AcLL is demonstrated in Table 5. LDA reactivity was present against 3 AML and 2 AcLL blast cells tested. These antibodies were present in high titer (10^{-3} to 10^{-5}).

Specificity of the LDA Assay. To evaluate specificity at the antisera level, 8 different sera obtained from normal as well as immunized rabbits were tested for LDA against ⁵¹Cr-labeled RPMI-4265. Only R2-1(T) showed LDA reactivity.

To evaluate specificity at TC level, R2-1(T) was tested against 3 different target cells: T24, HT29, and FL (non-lymphoid cell lines growing in monolayers). In these experiments (performed at least twice with each TC), no LDA reactivity was encountered against any of the TC's tested, while positive control sera show reactivity. Furthermore, absorption of R2-1(T) with T24 or FL did not decrease the titer of reactivity against LCL or leukemia blast cells.

Antigenic Specificity of LDA Demonstrated in R2-1(T) serum: Reactivity against blastoid antigens. The presence of LDA against LCL raised the question as to whether these antibodies might be directed against blastoid antigens. R2-1(T) serum was tested against blastoid cells obtained by incubating peripheral blood lymphocytes with phytohemagglutinin for 72 hr. The serum, which was tested 2 times against 3 different blast cells, showed no LDA reactivity. R2-1(T) failed to react with blastic lymphocytes obtained from B. G., a cell with which the serum reacted before absorptions. Furthermore, absorptions with these blastoid cells failed to remove the anti-LCL and antileukemic reactivity.

Absorptions. To rule out that the anti-LCL and antileukemia reactivities present in R2-1 are directed against histocompatibility antigens, the serum was sequentially

Table 5
LDA reactivity of R2-1(T) against human leukemia blasts

Serum dilution	TC's ^a				
	AcLL-F	AcLL-J	AML-HL	AML-Mu	AML-Ca
10^{-3}	65.3 ± 6.7^b	45.2 ± 6.9	50.0 ± 6.9	56.4 ± 5.6	38.6 ± 5.2
10^{-4}	46.8 ± 5.3	7.3 ± 2.0	47.2 ± 3.8	42.3 ± 4.2	6.1 ± 2.1

^a The conditions of the assays were: EC:TC = 4:1; 2.5×10^4 TC's; 4 hr-incubation. Background value, 25 to 35%.

^b Percentage of specific lysis \pm S.D.

absorbed with PBL, human RBC (AB type), and human liver. The PBL used were a mixture from 3 donors, comprising within them all *HL-A* specificities present in RPMI-4265 cells, i.e., *HL-A2*, -3, -7, -12. The serum so obtained, R2-1(T) devoid of antihistocompatibility and species specific antibodies, retained high LDA titer against LCL's and leukemic cells (Table 6). Serum R2-1(T) was studied by complement-dependent cytotoxicity against a panel of 105 PBL's and showed no reactivity against these cells (J. Zigelboim and A. Durantez, unpublished results), further substantiating the fact that this serum is devoid of antibodies against histocompatibility or species-specific antigens. To further evaluate the specificity of R2-1(T), the serum was absorbed with fetal tissue,³ fetal calf serum,⁴ and non-LCL's. None of the absorptions had a significant effect on LDA titer. Absorption with bone marrow 2 times reduced moderately the LDA titer. Absorption with LCL's (RPMI-4265 or UCLA-81) totally removed anti-LCL and antileukemic LDA. Absorptions (2 times) with AML or AcLL had identical results. No selectivity was demonstrable within absorption experiments, indicating that the serum was directed against common antigens present in the LCL, AML, and AcLL blasts.

DISCUSSION

Immunization of rabbits with a purified membrane component from a LCL (RPMI-4265) induced LDA's directed against antigenic specificities shared by several

³Tissue from 12-week-old fetus obtained from therapeutical abortion was lyophilized and used as such for absorption. A serum:tissue ratio of 2:1 was used during absorption.

⁴Lyophilized FCS was used for absorption. The serum was subsequently centrifuged at 5000 rpm using a PR-j centrifuge.

LCL's and human leukemia cells. LDA with specificity for histocompatibility antigens (*HL-A2*) was also demonstrated.

The type of preparation used as the immunogenic stimuli may play a fundamental role in determining the nature of the immune response generated. In this regard, Shulman *et al.* (13) were unable to obtain anti-*HL-A* sera when immunizing rabbits with whole lymphoid cells. By contrast, Einstein *et al.* (6) succeeded using membrane components. It can be inferred from this that the immunization of rabbits with the purified membrane preparation facilitated the production of antibodies directed against antigenic determinants shared by leukemic cells and LCL's. Such antibody response might have been aborted if whole cells with numerous and multiple types of antigenic determinants were used as the immunogenic material.

Thieme and Columbe (15) reported on the presence of cytotoxic CDA's in rabbit antisera obtained using a similar protocol. These antibodies reacted also with antigens present in various LCL's and failed to react with PBL's. On the contrary, using a LDA assay, we were able to demonstrate antibodies directed against normal PBL's carrying *HL-A2* antigens. These findings illustrate the importance of using several serological assays when attempting to characterize the reactivity of a serum. It is not possible to determine from the data available whether LDA and CDA reactivities are mediated by the same antibody molecules or not. The presence of LDA in the absence of CDA in certain sera (16) indicates that LDA is indeed a distinctive antibody type from a biological standpoint.

Our results confirm the findings of Mann *et al.* (10) and indicate that antileukemic sera can be obtained with other cell lines as well, *i.e.*, RPMI-4265.

The presence of LDA reactivity against LCL's is particularly interesting because tissue culture lines derived from normal donors share antigens with leukemic cells. RPMI-4265 is a cell line derived from a patient with chronic myelogenous leukemia. It possesses, however, characteristics of B-cells (synthesizes immunoglobulin), indicating that the cell line probably derived from 1 of the pa-

tients' B-lymphocytes and not from 1 of the myeloid leukemia cells (M. Jobin, personal communication). It was thus expected that R2-1(T) would react with cell lines of B-cell origin. The demonstrated reactivity against cell lines with characteristics of myelomonocytes and stem cells indicates the presence of common or cross-reactive antigenic specificities between these cell lines. The nature of the antigen(s) remains to be defined.

LDA specificities do not seem to be directed against EBV membrane antigens because some of the cell lines, including RPMI-4265, have no demonstrable EBV surface antigens. Thieme and Columbe (15) showed that sera containing antibodies against EBV-membrane antigens did not react by complement dependent cytotoxicity assay with RPMI-4265 target cells, but they were strongly reactive against lymphocytes from patients with infectious mononucleosis.

The demonstration of an antibody response in patients receiving multiple injections of autologous lymphoid cells (8) as well as a cellular proliferative response when lymphocytes from healthy donors were cultured *in vitro* with their autochthonous LCL's (1) suggests the development of neoantigens on these cells. Thieme and Columbe (15) indicated in their study that lymphocytes of normal volunteers, placed in tissue culture conditions, will acquire these antigenic specificities after a period of 3 to 6 weeks, substantiating the concept that these antigens are indeed neoantigens. These neoantigens may result from: (a) differentiation or expression of embryonic antigens (1); (b) expression of new histocompatibility antigens (14); (c) viral-induced antigens; or (d) new membrane antigenic specificities in mutant clones.

The lack of reactivity of R2-1 against MOLT-4 indicates that these antigenic determinants are not present on all LCL's, especially T-cell lines. We have since tested our serum once against a cell line with similar characteristics (HSB) and have found identical results.

LDA reactivity against human leukemic cells was clearly established. The ability of LCL's to absorb completely the antibodies reactive with leukemic blasts and *vice versa* indicates that cross-reactive or identical antigenic determinants are present on each of the cell lines mentioned. Why the *in vitro* establishment of a cell line in culture and leukemic transformation *in vivo* are associated with the appearance of cross-reactive or identical cell surface antigens is an intriguing question that is currently being investigated in our laboratories.

This study also revealed the presence of cross-reactive antigens between AML and AcLL cells. Such findings are in agreement with observations reported before that sera raised in rabbits contained cytotoxic CDA's to acute leukemia antigens (10). Both tests using LDA and CDA techniques detected common antigen(s) on leukemic cells. The AML- and AcLL-specific antibodies obtained by immunizing monkeys (11), however, were not identified with the rabbit antisera.

The ability of normal human bone marrow to reduce LDA titer was of particular interest because it suggests the probable presence of leukemia antigens on normal bone marrow cells. It is possible that immature cells from the myeloid or erythroid series carry similar antigens as those

Table 6
Effect of absorptions on LDA^a reactivity against LCL's

Antisera	RPMI-4265		UCLA 81	
	% specific lysis ^b	Titer ^c	% specific lysis	Titer
R2-1 (T)	42.3 ± 5.2	5	58.1 ± 7.0	5
R2-1 (T, 2 BM) ^d	30.6 ± 4.2	3	40.5 ± 3.3	3
R2-1 (T, AcLL 2 times)	40.4 ± 6.1	1	61.7 ± 3.6	1
R2-1 (T, AML 2 times)	26.3 ± 7.2	1	38.6 ± 5.5	1
R2-1 (T, LCL 2 times)	NS		NS	

^a EC:TC = 4:1; 4-hour incubation time; serum dilution, 10⁻¹. For titer, we used the same method as described in Table 4.

^b Specific lysis, mean of triplicates ± S.D., obtained with antisera diluted to 10⁻¹.

^c Titer, reciprocal of last 10-fold dilution giving 10% ⁵¹Cr release over background values.

^d BM, bone marrow; NS, not significant.

expressed on leukemic cells and LCL's that are lost during differentiation and maturation.

From a more practical standpoint, the presence on LCL's of antigenic specificities shared by leukemic blasts makes them a useful resource for producing specific antibodies to leukemic antigens. These antibodies will be of primary importance in attempts directed towards the isolation and characterization of the leukemia antigens. These cells also represent a standard source of TC's that can be used to detect antileukemic antibodies in patients with acute leukemia. Furthermore, the presence on these cells of leukemic antigens makes them a useful resource for specific immunotherapy of acute leukemia. Such an approach has been initiated in several centers, but the results are still unknown.

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