MEMBRANE ANTIGENS SPECIFIC FOR HUMAN LYMPHOID CELLS IN THE DIVIDING PHASE*

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The surface properties of cells vary during their division cycle as shown by change in electronegative charge (1) or binding affinity for plant agglutinins (2). Variation in structure or conformation of surface membranes can also be measured by the reaction of viable cells with specific antisera. In this way, it has been found that expression of the following surface markers change during cell division: immunoglobulin in human lymphoid cells (3); blood group H in HeLa cells (4); blood groups B and H in murine cells (5); and histocompatibility antigens in the murine cell lines YCAB (6), JLS-V9 (7), and P815Y (8). The significance of these events is not understood.

In this report, evidence is presented for antigenic differences between dividing and nondividing lymphoid cells. Heteroantisera have been prepared against human thymocytes or Burkitt's lymphoma EBI, both of which are mitotically active cells. After appropriate absorption these antisera are specific for a fraction of thymocytes and T lymphoblasts (TTBA: thymocyte-T blast antigen)[1] or B lymphoblasts and immunoglobulin (Ig)-positive lymphoblastoid cell lines (BBA: B-blast antigen).

Materials and Methods

Preparation of Antisera.—Rabbits were given two intravenous injections of 10^9 thymocytes (donor age: 7 yr) or Burkitt's lymphoma EBI at an interval of 14 days and bled out 7 days later. The serum was separated and the Ig fraction obtained by addition of ammonium sulfate (40% vol/vol). The precipitate was dissolved in a volume of phosphate-buffered saline (PBS), equal to that of the original serum, and dialyzed extensively against PBS. The Ig fraction was absorbed for 1 h at ambient temperature and 16 h at 3°C with an equal volume of kidney homogenate and packed red blood cells; anti-TTBA was absorbed further with the Ig-positive lymphoid cell line BEC-11 and with pooled peripheral blood lymphocytes from five healthy donors, while anti-BBA was absorbed with thymocytes followed by pooled peripheral blood lymphocytes.

Preparation of Cell Suspensions.—Peripheral blood lymphocytes were obtained from normal healthy adults. Venous blood was defibrinated by gentle agitation with sterile glass beads for

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[1] *Abbreviations used in this paper:* ALC, antihuman light chain; BBA, B-blast antigen; MEM, Eagle's minimal essential medium; NRS, normal rabbit serum; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; TTBA, thymocyte-T blast antigen.

10 min at room temperature. The erythrocytes were sedimented by incubating with 0.8 vol of 3.5% dextran (Pharmacia, Uppsala, Sweden) in 0.9% saline solution for 30 min at 37°C. The leukocyte-rich supernatant was removed and centrifuged at 150 *g* for 10 min. The cell pellet was washed twice with Eagle's minimal essential medium (MEM) before staining, cell culture, or absorption. Tonsils or thymus (collected in cold MEM immediately after removal) were teased in MEM to produce a cell suspension and the debris was allowed to settle by standing at 3°C for 10 min. Polymorphs were removed from tonsillar lymphocytes by incubation with carbonyl iron before staining or cell culture.

Lymphocyte Separation on Immunoabsorbent Columns.—Lymphocyte populations were enriched for non-Ig-bearing T cells or Ig-bearing B cells by fractionation on immunoabsorbent columns, as described in a previous report (9). Tonsillar lymphocytes were depleted of B cells by elution from columns of Degalan V-26 plastic beads (Degussa Wolfgang Au., Hanau am Main, Germany) (polymethylmeta acrylic plastic) (10) or Sephadex G-200 containing bound, purified rabbit antihuman light chain (ALC). The Ig-bearing B cells could be recovered from Sephadex columns by dextranase digestion (11).

Cell Culture.—Lymphocytes or thymocytes were cultured with phytohemagglutinin (PHA [Wellcome Research Laboratories, Beckenham, England] reagent grade, 1/100 dilution) in RPMI medium supplemented with 10% fetal calf serum at a cell density of 2×10^6/ml. Cell cultures were harvested at the plateau of the mitogenic response (72 h). The lymphoid cell lines BRI-7, BRI-8, BEC-11, and MICH were obtained from G. D. Searle and Co., High Wycombe, England, and all other cell lines were obtained from Dr. Elizabeth Arthur, Medical Research Council Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, Scotland.

The percentage blast transformation was determined by autoradiography. Lymphocyte cultures (at 72 h) were incubated with 1.0 μCi/ml of tritiated thymidine for a further 4 h at 37°C. The cells were washed extensively with cold MEM and autoradiographs were prepared from cytosmears. These were stained with May-Grünwald-Giemsa after development and examined under a light microscope.

Immunofluorescent Staining.—Surface antigens were detected by indirect immunofluorescent staining of viable cell suspensions at 0°–4°C. Cells (5×10^6; 100 μl) were incubated for 30 min with an equal volume of antiserum (anti-TTBA, anti-BBA, rabbit ALC, or normal rabbit serum [NRS]), washed three times with cold MEM, and incubated for 30 min with fluorescein-conjugated goat antirabbit Ig. After a further three washes in MEM, cells were examined with a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N. J.) under incident ultraviolet light. Blast cells were identified by the appearance of the same field viewed under phase contrast.

RESULTS

Thymocytes and Lymphoblastoid Cell Lines.—After complete absorption, anti-TTBA antibody reacted with 1–3% peripheral blood lymphocytes and 8–15% tonsillar lymphocytes, while anti-BBA antibody reacted with <1% peripheral blood lymphocytes and 6–12% tonsillar lymphocytes (serum dilutions of 1:4 to 1:20). In contrast, anti-TTBA reacted with an appreciable number of thymocytes from donors of different ages (Table I) with maximum staining at dilutions <1:15. Anti-BBA was not reactive with thymocytes at all dilutions and all of the lymphoblastoid cell lines tested were negative for TTBA and positive for both surface Ig and BBA (Table II). It seemed unlikely that anti-BBA was directed against surface Ig determinants of the cell lines since the activity was unaltered after absorption with insoluble whole human serum. In further studies the antisera were used at a dilution of 1:10.

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TABLE I

Immunofluorescent Staining of Thymocytes

Donor	(Age)	Fluorescent cells		
		TTBA	BBA	Surface Ig
			%	
1	(17/36)	57	1	3
2	(18/36)	43	7	24
3	(20/36)	16	<1	2
4	(22/36)	32	<1	ND
5	(24/36)	35	10	5
6	(2 yr)	55	1	21
7	(4 yr)	4	<1	2
8	(8 yr)	4	<1	1
9	(10 yr)	9	<1	4

ND, not determined. There was no significant staining (<1%) with normal rabbit serum.

TABLE II

Immunofluorescent Staining of Lymphoblastoid Cell Lines

Cell line	Fluorescent cells		
	TTBA	BBA	Surface Ig
		%	
EBI	<1	55	50
BEC-11	<1	40	45
RAJI	<1	33	29
BRI-7	<1	60	55
BRI-8	<1	60	54
MICH	<1	63	63
YAK	<1	70	59
MON_1	<1	50	46
CLA4	<1	43	60
GOL	<1	38	51
DOU_1	<1	42	60
FLE-1	<1	19	88
GS-1	<1	16	57

Effect of PHA Stimulation.—Since thymocytes and lymphoid cell lines are mitotically active, it seemed possible that expression of these antigens was related to cell division. To test this proposal, PHA-treated cultures of thymocytes, tonsillar lymphocytes, or peripheral blood lymphocytes were stained at the peak of the mitogenic response (72 h; Tables III–V). In each case, the total number of stained cells with either antibody was markedly higher than in control cultures or cell samples before culture. The number of BBA-positive cells in thymocyte cultures was approximately equivalent to the number of Ig-positive cells (Table III) and a similar correlation was found with lymphocyte cultures (Tables IV and V). A majority of the stained cells had the morphological appearance of blasts on examination under phase contrast.

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TABLE III

Immunofluorescent Staining of PHA-Treated Cultures of Thymocytes

Donor	(Age)*	Fluorescent cells					
		Control cultures			PHA-treated cultures		
		TTBA	BBA	Surface Ig	TTBA	BBA	Surface Ig
				%			
4	(22/36)	14	2	ND	56	22	ND
8	(8 yr)	ND	ND	ND	34	1	1
9	(10 yr)	7	1	3	30	18	11

ND, not determined. There was no significant staining (<1%) with normal rabbit serum.
* Refer Table I.

TABLE IV

Immunofluorescent Staining of PHA-Treated Cultures of Peripheral Blood Lymphocytes

Donor	Fluorescent cells		
	TTBA	BBA	Surface Ig
		%	
1	40	24	15
2	48	11	6
3	30	26	13
4	36	18	11

The percentage blast transformation (>70%) was confirmed by autoradiography. There was no significant staining of blast cells with normal rabbit serum (<3%), and control cultures without PHA were negative (<1%) for TTBA and BBA.

The initial results obtained with thymocytes or the Ig-positive lymphoid cell lines (Tables I and II) suggested that these antisera might have specificity for dividing, T or B, lymphoid cells. Also, it has been shown in the human that both T and B lymphocytes can respond to the mitogenic stimulus of PHA (9). Therefore, purified populations of lymphocytes were prepared by column separation before mitogenic stimulation with PHA.

B Cell-Depleted or B Cell-Enriched Lymphocyte Cultures.—When tonsillar lymphocytes were depleted of B cells by elution from ALC-Degalan (Table VI) or ALC-Sephadex (Table VII) columns, there was a marked decrease in staining of the resultant blasts for surface Ig or BBA, and a corresponding increase in TTBA-positive cells as compared with control cultures. And in a reciprocal manner, the enriched B cell fraction (ALC-Sephadex digest; Table IV) contained a higher percentage of Ig-bearing, BBA-positive blasts. These results suggest that the antisera are indeed specific for T or B lymphoblasts. Further attempts were made to raise anti-TTBA using fetal thymocytes (four donors; 18–24 wk old) without success.

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TABLE V

Immunofluorescent Staining of PHA-Treated Cultures of Tonsillar Lymphocytes

Donor	Fluorescent cells		
	TTBA	BBA	Surface Ig
		%	
1	50	45	37
2	45	64	53
3	57	32	21
4	61	38	32
5	41	57	33
6	43	58	33
7	47	35	25
8	55	36	24

The percentage blast transformation (>85%) was confirmed by autoradiography. Control cultures without PHA contained <10% stained cells for TTBA and BBA.

TABLE VI

Immunofluorescent Staining of B Cell-Depleted Cultures of Tonsillar Lymphocytes

	Fluorescent cells		
	TTBA	BBA	Surface Ig
		%	
ALC column effluent (T)*	68	15	6
NRS column effluent (T + B)*	46	51	38

* Immunoadsorbent columns of Degalan containing bound rabbit antihuman light chain (ALC) or normal rabbit Ig (NRS).

TABLE VII

Immunofluorescent Staining of B Cell-Depleted or B Cell-Enriched Cultures of Tonsillar Lymphocytes

	Fluorescent cells		
	TTBA	BBA	Surface Ig
		%	
Unfractionated cells (T + B)	24	20	30
ALC-Sephadex effluent* (T)	82	13	10
ALC-Sephadex digest (B)	10	51	56
NRS-Sephadex effluent* (T + B)	31	38	31

* Immunoadsorbent columns of Sephadex G-200, covalently linked to rabbit antihuman light chain (ALC) or normal rabbit Ig (NRS).

DISCUSSION

The present results indicate that the antigens TTBA and BBA are specific for T or B lymphoid cells in the dividing phase. What is not known is whether these membrane components are present at a discrete phase of division or throughout the cell cycle. Their temporal expression remains to be determined using synchronous cultures of human lymphoid cells.

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It is unlikely that the antisera are directed against isoantigens. Both antisera reacted with transformed lymphocytes from a panel of normal donors (Tables IV and V) and were consistently positive or consistently negative on all the lymphoblastoid cell lines tested (Table II). In contrast to normal peripheral blood, a considerable percentage (6–15%) of tonsillar lymphocytes reacted with either of the antisera. However, most surgical specimens were removed due to persistent infection and might therefore be expected to contain an increased number of "activated lymphocytes" (12).

It might be argued that BBA is a viral antigen since the antiserum was raised against an Epstein-Barr (EB) virus-positive lymphoma. However, BBA was present on PHA-induced blasts of all the normal donors tested. Also, a small percentage of BBA-positive cells was present in PHA-treated cultures of fetal thymocytes consistent with the number of Ig-positive cells (Table III). Since there is no evidence for vertical transmission of EB virus in the human, it would seem improbable that BBA is a viral antigen.

The TTBA antigen resembles the murine isoantigen "TL" (13) in that both determinants are present on thymocytes and lymphoblasts (TL+ murine leukemia cells) and absent on normal lymphocytes. However, it is not known whether TL is expressed on normal, PHA-induced lymphoblasts of some TL+ mouse strains. Similarly in the human, heteroantisera have been raised against membrane preparations of Burkitt's lymphoma, RAJI (14). The antisera react with acute leukemia cells but not with normal lymphocytes. It has not been determined whether these antisera react with normal, B lymphoblasts. If this is indeed the case, it follows that other "leukemia antigens" (15) might also be considered as "division membrane antigens." In a previous report (16) it was shown that sera of some patients (20%) with infectious mononucleosis contained cold IgM antibodies specific for B lymphoblasts and lymphoblastoid cell lines. This membrane antigen is similar to the present specificity, BBA. Both determinants are expressed on B lymphoblasts and absent on thymocytes, lymphocytes, and T lymphoblasts. Further work is necessary, using reciprocal blocking techniques, to determine whether the two antisera are directed against common membrane components.

Several questions remain to be answered. Firstly, we have not determined whether TTBA and BBA antigens are present on precursor cells in the bone marrow. Secondly, it is not known whether expression of TTBA and BBA require *de novo* synthesis or exposure of preexisting cryptic antigens. Finally, it remains to be shown whether certain antigens, considered to be specific for leukemia cells (15), are in fact membrane components common to both leukemia and normal lymphoid cells in the dividing phase.

SUMMARY

Heteroantisera were raised in rabbits against human thymocytes or Burkitt's lymphoma EBI cells. After suitable absorption, the sera were specific for thymocytes and T lymphoblasts, or B lymphoblasts and several lymphoblastoid cell

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lines. The specificity of the antisera was confirmed by preparing purified populations of T or B lymphocytes on immunoabsorbent columns, followed by mitogenic stimulation with phytohemagglutinin. The antisera did not react with normal resting lymphocytes.

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