ISOLATION AND IMMUNOLOGIC CHARACTERIZATION OF A HUMAN, B-LYMPHOCYTE-SPECIFIC, CELL SURFACE ANTIGEN*

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Lymphocytes bear many types of surface molecules, some of which are restricted to subpopulations of lymphocytes and may be necessary for their specialized immune functions. Examples of such antigens include: the Ia antigens; cell surface immunoglobulin; the Fc receptor, complement (C) receptors, and Ly-4 all present predominantly on B lymphocytes and Thy (theta) antigen; and sheep erythrocyte receptor, and the Ly-1, 2, and 3 antigens all present on T cells. H antigens (H-2 or HL-A) and β_2-microglobulin are found on all populations on lymphocytes. Because a non-H-2, B-lymphocyte-specific antigen had been serologically defined on the murine lymphoblast line L1210 (reference 1 and footnote 1) and because a similar human, non-HL-A antigen could be present on the human B-lymphoblast line IM-1, from which the isolation of the HL-A antigen was in progress, attention has been paid to the "contaminant" molecules of the HL-A antigen preparations.[2]

Several cell surface antigens have been identified, one of which (p23,30) is similar to Ia antigens which have been described on mouse B lymphocytes. The purification of that antigen, its distribution on distinct subsets of human lymphocytes, and the properties of heteroantisera to p23,30 in lymphocyte functional assays are described in this and the accompanying paper (3).

Materials and Methods

Alloantisera. Alloantisera BC (anti-HL-A3), DAL (anti-HL-A27), and WMS (anti-W28) were gifts from Dr. D. Bernard Amos, Duke University, Durham, N. C. BEL (anti-HL-A27) was the gift of Dr. Thomas Fuller, Harvard Medical School, Boston, Mass. Several alloantisera which were

[1] Humphreys, R. E., A. Ahmed, R. E. Budd, K. W. Sell, and W. E. Vannier. 1976. Identification of a B lymphocyte-specific alloantigen on the L1210 lymphoblast cell line. Manuscript submitted for publication.

[2] This work was presented at the Sixth International Histocompatibility Workshop, 1975 at Aarhus, Denmark (2).

obtained from multiparous women of an Amish population, were found to histotype for an HL-A-linked alloantigen present only on B lymphocytes (4, 5). These sera are listed in Table II.

Heteroantisera. Antisera to the p23,30 complex were raised by injection of 25 μg of the antigen (material identical to that in gel C of Fig. 1) in 0.01 M Tris buffer, pH 8.0, homogenized with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) into the foot pads of a rabbit. One subcutaneous booster injection at 6 wk was made with 25 μg of soluble antigen in saline. Antisera raised from four rabbits were not significantly different in cytotoxic titers or in precipitin patterns. Antiserum to p12 (β_2-microglobulin) was prepared by R. Robb, Harvard University, Cambridge, Mass., by injection of 100 μg of purified β_2-microglobulin (the gift of Dr. M. D. Poulik, Wayne State University, Detroit, Mich.) in saline solution and Freund's complete adjuvant into the foot pads of a rabbit. Booster injections with 100-μg portions of saline-soluble antigen were given at two 6-wk intervals, after which the animal was exsanguinated.

Heat-Aggregated IgG. Heat-aggregated IgG was prepared by heating a 2 ml/ml solution of human IgG (Worthington Biochemical Corp., Freehold, N. J.) in phosphate-buffered saline at 63°C for 30 min. Large aggregates were removed by centrifugation at 3,000 g for 20 min.

Separation of B, T, and Null Lymphocytes from Peripheral Blood. Highly purified populations of T, B, and Null cells were isolated by methods which have been described in detail previously (6, 7). In brief, human mononuclear cells were obtained from Ficoll-Hypaque separated peripheral blood (8). These cells were washed in medium 199 containing 5% fetal calf serum and 2.5 mM EDTA, and then passed through a Sephadex G-200 antihuman Fab column. The effluent cell population (nonretained) contained <2% cells bearing surface Ig and 70–85% of these cells formed spontaneous rosettes with sheep erythrocytes (RBC's) (E rosettes) (7). This population was defined as the T plus Null population. In contrast, the lymphocytes which bound to the immunoabsorbent column and which were subsequently recovered by elution with soluble immunoglobulin (10 mg/ml) were >97% surface immunoglobulin positive and >98% E-rosette negative. These cells represent the B-cell population. The nonimmunoglobulin-bearing population (T plus Null) was depleted of T cells by the formation and subsequent removal by Ficoll-Hypaque sedimentation of E-rosetting cells (7). The nonrosetted cells which were recovered at the interface were immunoglobulin negative and E-rosette negative. This population was depleted of EAC-rosetting cells by Ficoll-Hypaque sedimentation, leaving at the interface a population of EAC-negative Null cells. Highly purified T cells were isolated from the T plus Null preparation by the formation and subsequent depletion of EAC-rosetting cells. The cells recovered after this separation were immunoglobulin negative but more than 92% formed spontaneous E rosettes (7).

Separation of B Lymphocytes by Adherence in a Microtiter Plate. Microtiter plates were coated with trinitrobenzene sulfonate (TNP), washed, and antisera to TNP was added. After 30 min of incubation at 37°C, the plates were washed and human peripheral blood lymphocytes, obtained by Ficoll-Hypaque gradient centrifugation (8), were added to each well of a second microtiter plate. Cytotoxic antisera (5 μl) were added to each well and incubated for 30 min at 37°C. After washing these cells, 5 μl of normal rabbit serum was added as a source of C and the plates were incubated for 1 h at room temperature. A trypan blue solution containing 1.4% EDTA was added and cell death was scored by light microscopy. This procedure was used for results presented in Tables I and II.

^{51}Cr-Release Cytotoxic Assays. The ^{51}Cr-release microtiter plate cytotoxic assay of antisera and the cytotoxic inhibition assay of antigen was based on established techniques (9–12). One unit of antigen activity was that amount required to reduce the percentage chromium release from 90 to 50% with a given, diluted antiserum under the conditions of this assay.

Antibody Absorptions by Platelets. Washed platelets (blood groups O, Rh$^+$) were suspended at 10^{10} platelets/ml and diluted 1:2 serially in dextrose-gelatin-veronal buffer (DGV).[3] Aliquots of platelets (25 μl) were incubated for 1 h at 4°C with 25-μl aliquots of *(a)* 1:50 diluted anti-p23,30 or *(b)* 1:25 diluted anti-β_2 microglobulin serum in microtiter plates following a previously described absorption technique (13). After absorption, the plates were centrifuged and 5-μl aliquots of the

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supernate were assayed for the presence of cytotoxic antibodies against peripheral blood lymphocytes (PBL's).

Internal Labeling and Double Antibody Precipitation of Antigens. [35S]methionine was prepared by hydrolysis of *Escherichia coli* which had been grown on a sulfate-limited minimal medium and fed [35S]sulfate (14). Lymphoblast cells (2×10^6/ml) were grown overnight in a limited medium composed of four parts methionine-free RPMI 1640 medium and one part spent culture medium, and 1-5 mCi 35S-labeled *E. coli* hydrolysate. These internally labeled cells were washed in 0.01 M Tris buffer, pH 8.0, containing 0.14 M NaCl, solubilized in 0.15% Nonidet P-40 detergent (Shell Chemical Corp., New York) in the same buffer, and centrifuged at 100,000 g for 1 h. Aliquots of the supernatant solution (500 μl) were incubated for 4 h with a rabbit heteroantisera, followed by an overnight incubation with goat antirabbit IgG serum. The precipitates were washed and dissolved in sodium dodecyl sulfate (SDS) gel loading buffer without reduction. Aliquots of the precipitates were electrophoresed. The gels were fixed, sliced longitudinally, and dried. Autoradiograms of the gels were scanned on a Joyce-Loebl densitometer (Joyce, Loebl & Co., Inc., Burlington, Mass.).

Antibody-Binding Assay. An assay for the blocking of binding to lymphoblasts of a radioiodinated (second) antibody by previously incubating lymphoblasts with serial dilutions of an unlabeled (first) antiserum has been adapted to microtiter plates. IM-1 cells (2×10^3 in 5 μl of DGV) (Grand Island Biological Co., Grand Island, N. Y.) were incubated at 4°C with 5 μl of blocking unlabeled antiserum, serially diluted in DVG for 1 h. 5 μl of a second, radioiodinated IgG fraction of antibody was added for a second 1 h incubation at 4°C. The IgG fraction had been prepared by Na_2SO_4 precipitation and dialysis (15) and was radioiodinated by the chloramine-T method (16). The cells were washed three times with 100-μl aliquots of DGV and the cells were transferred from the well to a 15 x 125 mm plastic tube. The radioiodine bound to the cells in this tube was counted in a gamma counter. Results are plotted as counts of radioiodine on labeled antibody bound to the cells as a function of the dilution of unlabeled blocking antiserum.

Mixed Lymphocyte Culture Assay. Standard one-way mixed lymphocyte cultures (MLC) were established by the method of Hartzman et al. in flat-bottomed microtiter plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) using triplicate wells, each containing 0.2×10^6 responding cells and 0.2×10^6 stimulating cells which had been pretreated with 50 μg/ml of mitomycin-C for 30 min at 37°C and then washed three times (17). All cultures were established in "final medium" (Media 199 containing 1% penicillin-streptomycin, 200 mM L-glutamine, 25 mM HEPES buffer, and 0.5% $NaHCO_3$; Microbiological Associates, Bethesda, Md.) supplemented with 20% heat-inactivated human AB serum. In addition, in some cultures 0.1 ml of normal rabbit serum (NRS) or anti-p23,30 were added in final dilution of 1/20 and 1/100. After 5 days the cells were pulsed with 0.2 μCi of [3H]thymidine (sp act 1.9 Ci/mmol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. J.) and harvested 16 h later with a MASH II apparatus (Microbiological Associates). Radioactivity was measured in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), and the results of triplicate wells expressed as counts per minute ± standard error of the mean.

Results

Preparation of the p23,30 Antigen. This polypeptide complex was separated from HL-A antigen at a late stage of preparation. The purification of papain-solubilized HL-A antigen from the cell line RPMI 4265 has already been described (18). Briefly, in the present study membranes of the IM-1 cell line were digested with dithiothreitol (DTT)-preactivated papain under nonreducing conditions.[4] After centrifugation at 100,000 g for 1 h, the supernatant solution was passed onto a DEAE-cellulose column in 0.01 M Tris-HCl buffer, pH 8.0, and HL-A antigen-containing material was eluted with 0.3 M NaCl in the same buffer. The eluant was concentrated and applied to a Bio-Gel A-15m gel filtration column (Bio-Rad Laboratories, Richmond, Calif.) in 0.01 M Tris-HCl buffer,

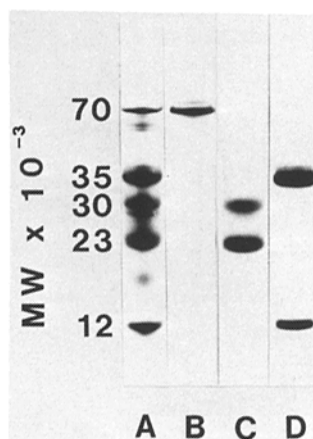[4] Humphreys, R. E. Manuscript in preparation.

FIG. 1. SDS-polyacrylamide gels run in Tris-bicine buffer. The materials used were from the experiment of Fig. 2. (A) HL-A antigen prepared with DTT-preactivated papain contaminated with other cell surface antigens (the material applied to the gel filtration column, Fig. 2). (B) The 70,000 dalton glycoprotein. (C) The complex of 30,000 and 23,000 dalton subunits. (D) HL-A antigen with polypeptides at 35,000 and 12,000 daltons.

pH 8.0. Fractions of column effluent containing HL-A antigens were applied to a second DEAE-cellulose column in 0.01 M Tris-HCl buffer, pH 8.0, and eluted with a linear gradient of NaCl between 0 and 0.2 M, in the same buffer. The HL-A antigen-containing peak was pooled and concentrated. An SDS polyacrylamide gel electrophoresis pattern of this material is shown in tube A of Fig. 1. This material was passed onto a 1.5 × 110 cm Sephadex G-150 gel filtration column in 0.01 M Tris-HCl buffer, pH 8.0. The absorbance profile at 280 nm of fractions of this column is shown in Fig. 2, as is the profile of HL-A cytotoxic inhibitory units. SDS gels of material from the three largest peaks are shown in gels B, C, and D, respectively (Fig. 1).

Molecular Weight and Subunit Composition. The G-150 column was calibrated with the dimethylsuberimidate dimer of bovine serum albumin, ovalbumin, lysozyme, and [^{14}C]glycine. Peak B material, p70, eluted at an apparent mol wt of 70–80,000 daltons. Peak C material, which eluted from the Sephadex column at an apparent size of 50–70,000 daltons, showed in SDS gels two bands at 23,000 and 30,000 daltons. For convenience, this complex of peptides will be referred to as p23,30.

Antisera to p23,30. Antisera to p23,30 was prepared in four rabbits. Precipitant arcs in Ouchterlony gels between these sera and p23,30 did not show any cross-reaction with purified HL-A antigen (gel D, Fig. 1); with p70 (gel B, Fig. 1); with β_2-microglobulin (gift of Dr. Poulik); with orosomucoid (gift of Dr. Jeanloz, Harvard Medical School); or with p13.5, a B-cell-specific protein which is distinct from β_2-microglobulin. No cross-reaction with antisera to various immunoglobulins (IgG, M, A, D, and E) (Meloy Laboratories Inc., Springfield, Va. and Cappel Laboratories, Inc., Downingtown, Pa.) or to haptoglobin (Meloy Laboratories Inc.) was found. Cytotoxic assays of these four sera against PBL's from several individuals and IM-1 lymphoblasts gave similar patterns with each serum. However, lytic patterns of these four sera against purified T-, B-, and
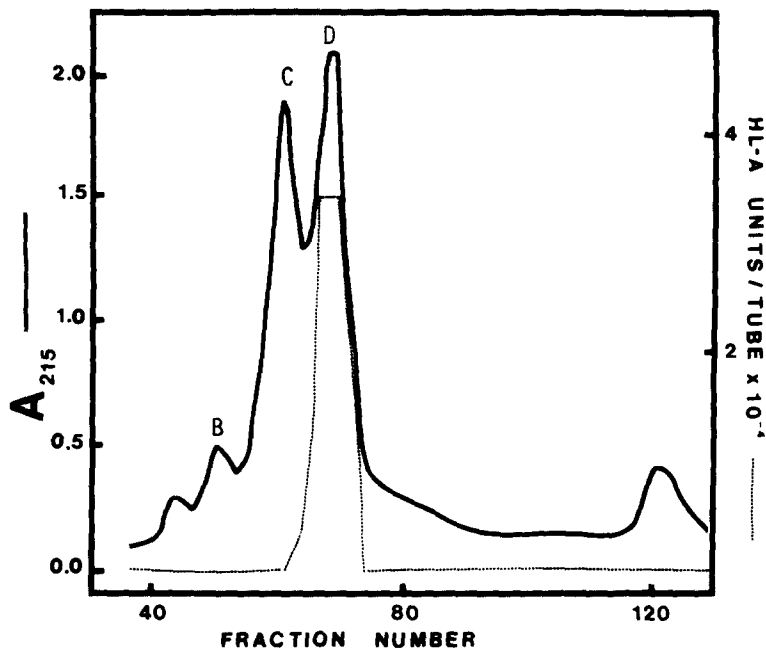
FIG. 2. Sephadex G-150 gel filtration of the antigen preparation shown in gel A of Fig. 1. The 70,000 dalton dimer (B), the 23,000 and 30,000 daltons complex (C), and the HL-A antigen (D) are resolved. Cytotoxic inhibitory units determined with anti-HL-A3 (BC) human alloantiserum are shown. MW, molecular weight.

Null-lymphocyte populations showed that one rabbit had absolutely no C-dependent lysis of T cells at 1:1 dilution, while a 1:2,000 titer against B cells was obtained. The studies reported here are with one antiserum. The other three rabbit antisera reacted with T cells to a lysis titer of 1:4–1:16 while showing a 1:2,000 titer against B lymphocytes.

Lysis of Purified Cell Populations with Anti-p23,30 Serum and with HL-A Typing Sera and Anti-β₂-Microglobulin. Cytotoxic titration of various cell populations with anti-p23,30 is shown in Fig. 3. 28% of RH-PBL's were lysed to an end point of 1:2,000, in a plateau pattern indicating that a subpopulation of PBL's was lysed. Study of the lysis of separated T and B PBL's of 46 other individuals showed that only B lymphocytes were lysed at high titer. The lysis of purified lymphocyte subpopulations from one individual was examined in detail (Fig. 3). Complete lysis of B cells and no lysis of T lymphocytes was observed. In contrast, only 15–20% of the purified Null-cell population was lysed. Lysis of the Null-cell population also occurred in plateau fashion, suggesting that only a subpopulation of Null cells expressed p23,30. Further experiments showed that anti-p23,30 serum lysed that portion of Null cells which bear the EAC receptor. Removal of the subpopulation of Null cells which formed EAC rosettes eliminated reactivity with anti-p23,30 serum, while a repeated E-rosetting procedure (which removed contaminating T cells) did not reduce the percentage of Null cells lysed.

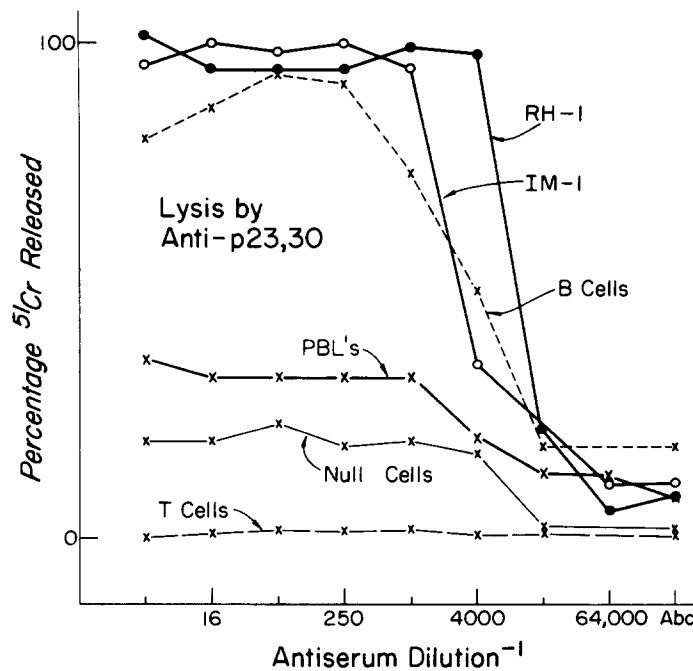The RH-1 and IM-1 lymphoblast lines were completely lysed by anti-p23,30

FIG. 3.   Lysis of peripheral blood lymphocytes and purified T, B, and Null cells (from RH) and of two lymphoblast lines (RH-1 and IM-1) with anti-p23,30 serum.

serum to a titer of 1:2,000 (Fig. 3). Several continuous B- and T-cultured lymphoblast lines were examined (Table I). All four B-cell lines were lysed at high titer. No reaction was observed with two of the T-cell lines. Some reaction was seen with the third T-cell line (HSB) (19) but only at a high antibody concentration. In contrast to the lytic pattern observed with the anti-p23,30 serum, all subpopulations of lymphocytes (T, B, and Null) were lysed by a first *HL-A* locus antiserum (BC, anti-HL-A3), by a second *HL-A* locus antiserum (DAL, anti-HL-A27), and by rabbit anti-β_2-microglobulin serum. Fig. 4 illustrates the lytic pattern with anti-β_2-microglobulin serum. The patterns with BC and DAL sera were similar with 50% lytic end points of 1:125 and 1:32, respectively.

Absence of p23,30 from Platelets.   Under conditions at which 4×10^8 platelets/ml absorbed 50% of the cytotoxic activity of anti-β_2 microglobulin serum (1:25 dilution), 100×10^8 platelets had no effect in absorbing anti-p23,30 serum (1:100 dilution). Under conditions of this assay p23,30 antigen could not be detected on human platelets.

Cytotoxic Inhibitory Assay with Anti-p23,30 Serum.   A cytotoxic inhibitory assay using a 1:800 dilution of anti-p23,30 was used to follow the presence of p23,30 antigen in a preparation of cell surface antigens which were solubilized from IM-1 cells by cysteine-activated papain. The absorbance tracing, and cytotoxic inhibitory units of p23,30 and of three HL-A antigen specificities in fractions of a Sephadex G-150 column are presented in Fig. 5. SDS gels of fractions of these columns (Fig. 6) showed bands at 23,000 and 30,000 daltons

TABLE I

Lysis of B- and T-cell-Cultured Lymphoblasts by Anti-p23,30
Serum

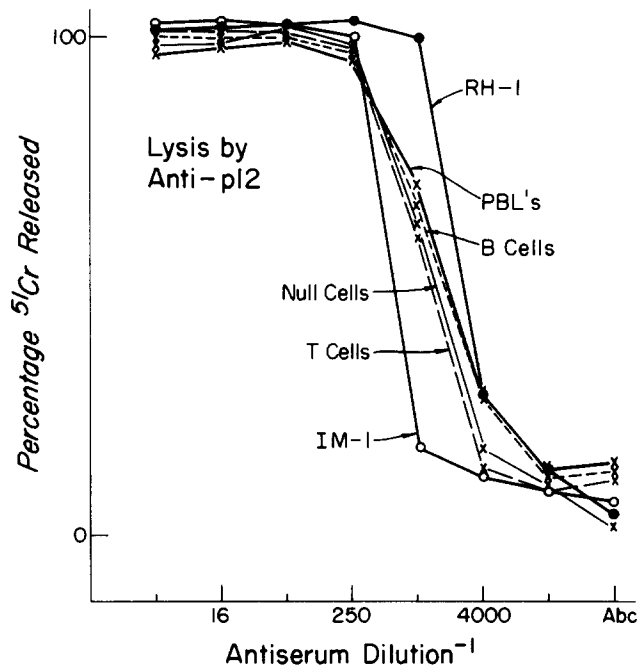| Cell line	B- or T-cell character	Lysis	Dilution^{-1}
		%	
SB	B	100	500
HSB	T	60	20
8392	B	100	500
8402	T	0	10
PA3	B	100	500
CEM	T	0	10
4265	B	100	500

FIG. 4. Lysis of PBL's; purified T, B, and Null cells; and two lymphoblast lines (RH-1 and IM-1) by rabbit anti-β_2-microglobulin serum.

corresponding to the occurrence of anti-p23,30 cytotoxic inhibitory activity (i.e., fractions 53–63). Likewise, the fractions which contained the 35,000 and 12,000 dalton bands of HL-A antigen corresponded to those which had HL-A cytotoxic inhibitory activity (i.e., fractions 60–70). These patterns are similar to those of Fig. 1 in which the p23,30 complex eluted from the gel filtration column at a higher molecular weight than the HL-A antigen complex of a glycopeptide and β_2-microglobulin.

Size of the Detergent-Solubilized Antigen which is Precipitated by Anti-p23,30. Cells labeled with [^{35}S]methionine were solubilized in Nonidet P-40
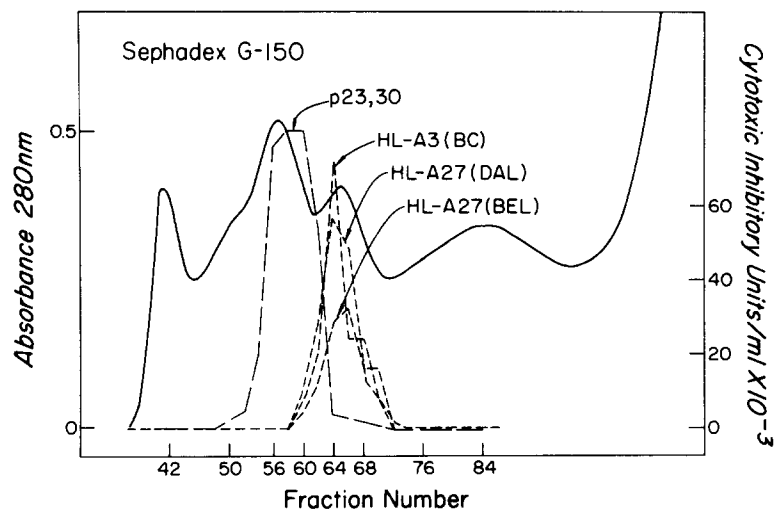
FIG. 5. Sephadex G-150 gel filtration of papain-released cell surface proteins from the IM-1 lymphoblast cell membranes.
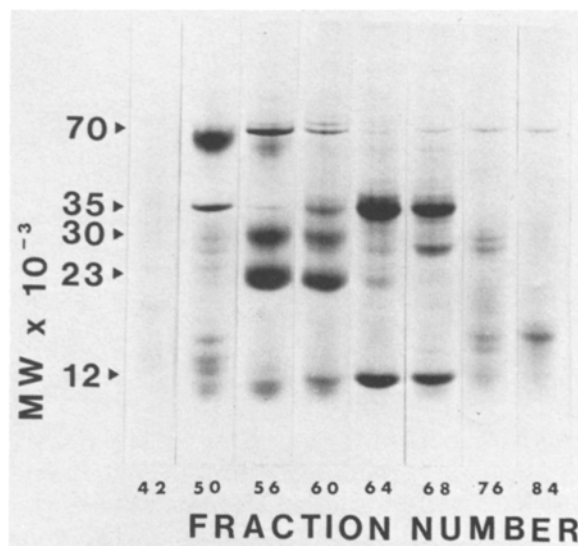

FIG. 6. Polyacrylamide gels (SDS in Tris-bicine) of the Sephadex G-150 gel filtration column of Fig. 5. The p23,30 antigen complex is in fractions 56 and 60. HL-A glycopeptide and β_2-microglobulin are most clearly seen in fractions 64 and 68 corresponding to the peak of HL-A cytotoxic inhibitory activity. MW, molecular weight.

(NP-40). After high speed centrifugation, the supernatant solution was treated with rabbit anti-p23,30 serum or with a rabbit anti-β_2-microglobulin serum followed by goat antirabbit IgG serum ("sandwich precipitation") and the precipitates were subjected to SDS gel electrophoresis. The anti-p12 (β_2-microglobulin) serum precipitated two peptides of 44,000 and 12,000 daltons. The anti-p23,30
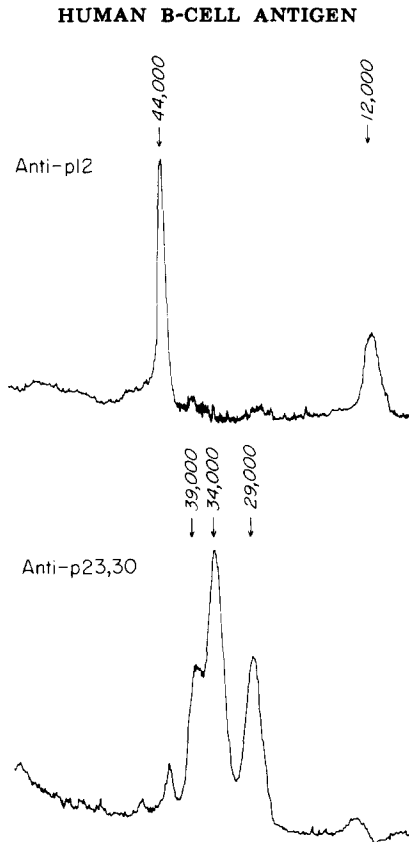
FIG. 7. [^{35}S]methionine internally labeled and NP-40-solubilized membranes were copre-cipitated with anti-p23,30 and anti-p12 (β_2-microglobulin), respectively. Densitometer scans of autoradiograms of SDS-polyacrylamide gels of these precipitates show three peaks of detergent-solubilized antigen bound to anti-p23,30 and two peaks bound to the anti-β_2-microglobulin antiserum (the HL-A antigen glycopeptide and β_2-microglobulin).

serum precipitated three polypeptides of 39,000, 34,000, and 29,000 daltons (Fig. 7).

Precipitation with Anti-HL-A Typing Sera. Similar antibody precipitates with histotyping alloantisera were made from NP-40-solubilized, [^{35}S]methionine-labeled cells. Each pattern showed a dominant labeled material at 44,000 daltons (the glycoprotein of HL-A antigen) (Fig. 8). The β_2-microglobulin component of HL-A antigen at 12,000 daltons is best seen in the DAL (anti-HL-A27) pattern. In each pattern, materials in the 30,000–40,000 dalton range were also precipitated. These materials may correspond to the materials precipitated by anti-p23,30 serum (Fig. 7). BEL antiserum also precipitated another larger material in the molecular weight range of p70 (cf. Fig. 1).

Relationship of HL-A and p23,30 Antigens on the Cell Surface. The relationship of p23,30 and the glycoprotein chain of HL-A antigen on the surface of IM-1 lymphoblasts may be judged to some extent by competitive blocking experiments with antisera against these antigens. Anti-p23,30 serum gave a 50% inhibition end point of 1:64 in blocking the subsequent binding of the radioiodinated gamma globulin fraction of anti-p23,30 serum (Fig. 9). Under

BC
HL-A3

MWS
W-28

DAL
HL-A27

BEL
HL-A27
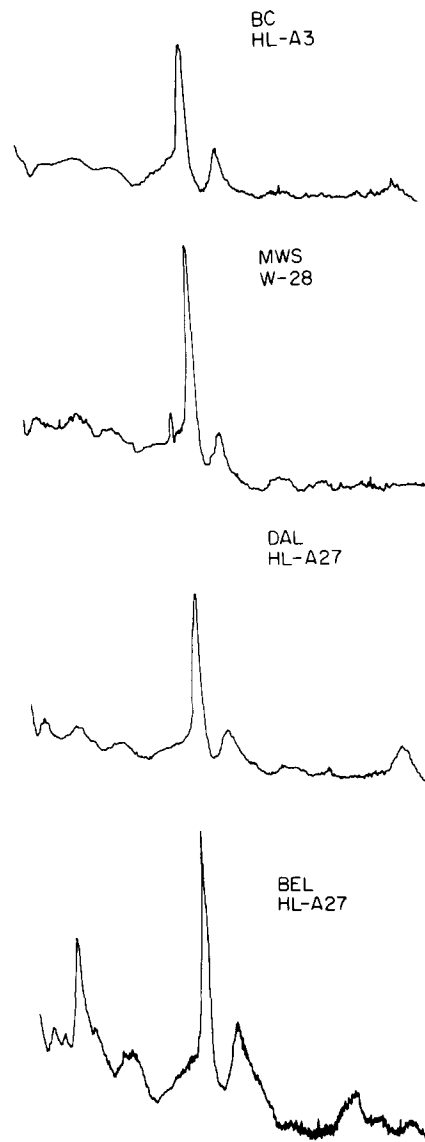
FIG. 8. Double antibody coprecipitates of HL-A and other alloantigens of the RH-1 cell line, formed with histotyping alloantisera. Note the high molecular weight alloantigen recognized by BEL antiserum.

identical conditions anti-p35 serum (raised against the papain-solubilized glyco-protein chain of HL-A antigen) gave a parallel inhibition curve with a 50% blocking titer of 1:16. Minimal blocking was seen with anti-p12 serum (directed against β_2-microglobulin). No blocking was seen with anti-p70 serum or with heat-aggregated human IgG.

In similar experiments, unlabeled anti-p12 serum inhibited the subsequent binding of a radioiodinated gamma globulin fraction of anti-p12 serum with a 50% inhibition end point at a titer of about 1:64. Anti-p23,30 and anti-p35 sera
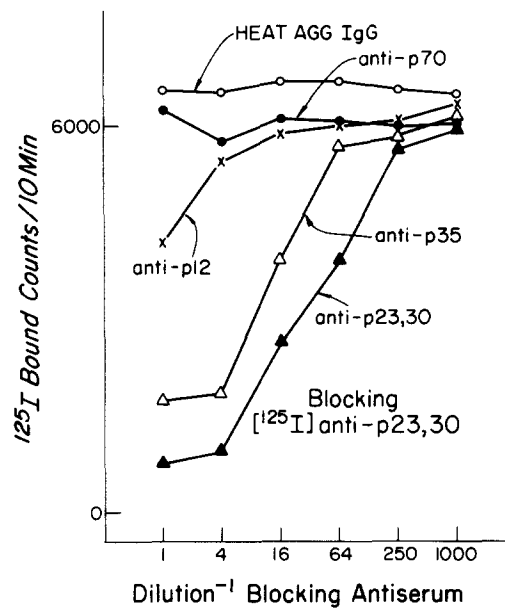
FIG. 9. The binding of radioiodinated anti-p23,30 to IM-1 cells is blocked by previously incubating the cells with anti-p35 (the glycopeptide of HL-A antigen) and partly by preincubation of the cells with anti-p12 (β_2-microglobulin). Preincubation with heat-aggregated (AGG) IgG or anti-p70 had no effect. The heat-aggregated IgG was serially diluted from a 2 mg/ml solution.

blocked the binding of anti-p12 serum with titer of about 1:4. Minimal blocking was seen with anti-p70 serum and no blocking was seen with heat-aggregated IgG. These experiments suggest the possibility that the p35,12 complex (HL-A antigen) and p23,30 complex may be close to each other on the cell surface.

Inhibition of Amish Antisera by p23,30 Antigen. Several Amish alloantisera have been described which recognize a non-HL-A, B-cell-specific human alloantigen (6). Several HL-A homozygous cell lines have been established in continuous cell culture from Amish individuals and have been found to bear antigens recognized by some of these sera. Purified p23,30 antigen from the IM-1 cell line (material shown in gel C of Fig. 1) and p23,30 containing portions of papain-solubilized antigens from the cell lines RPMI-4265 and JY were tested as inhibitors of cytolysis of the Amish cell lines by the Amish alloantisera (Table II). The following points may be noted: *(a)* JY antigen inhibited all of the antisera when the target cells were homologous (JY or PY cells); 6 out of 10 antisera were inhibited when the target cells were the other two Amish cell lines (KL and SL). The four remaining antisera (177, 359, 590, and MAB) were inhibited only when the target cells (JY and PY) and the p23,30 antigen (JY) were homologous. *(b)* Sera 35 and 289 were similar and were inhibited by antigen from IM-1 cells but not by antigen from 4265 cells. By contrast the reverse was true from serum 76. *(c)* Serum RMB was inhibited by all three of the p23,30 antigens. It must recognize a common determinant on these antigens. These observations are consistent with the possibility either that the three p23,30 antigens from IM-1, RPM1 4265, or JY cells are alloantigen alleles at a

TABLE II

Inhibition by p23,30 Antigens of Amish Cell Lines by Amish Antisera

Amish cell line	Cell source of p23,30 added	Amish antiserum									
		35	76	124	177	192	289	359	590	MAB	RMB
KL	IM-1	+	−	−	−	−	+	−	−	−	+
	RPMI 4265	−	+	−	−	−	−	−	−	−	+
	JY	+	+	+	−	+	+	−	−	−	+
SL	IM-1	+	−	−	−	−	−	−	−	−	+
	RPMI 4265	−	+	−	−	−	−	−	−	−	+
	JY	+	+	+	−	+	+	−	−	−	+
PY	IM-1	+	−	−	−	−	+	−	−	−	+
	RPMI 4265	+	+	−	−	−	−	−	−	−	+
	JY	+	+	+	+	+	+	+	+	+	+
JY	IM-1	+	−	−	−	−	+	−	−	−	+
	RPMI 4265	−	+	−	−	−	−	−	−	−	+
	JY	+	+	+	+	+	+	+	+	+	+

The antisera were obtained from multiparous women of the Indiana Amish community and define non-HL-A antigens represented on B lymphocytes (5, 6). The B-cell lines were established from four individuals of the same community by transformation with Epstein-Barr virus and were labeled with [^{51}Cr]sodium chromate. The antigens (100 μg/ml) were used as inhibitors of cytolysis at a 1:5 dilution in a chromium-release assay. +, inhibition of 40% or greater and was most often in the range of 60-70%. −, inhibition of 20% or less and was most often in the range of 5-15%.

TABLE III

Inhibition of Mixed Lymphocyte Cultures by Anti-p23,30

	Mixed cultures*	Media control‡	Normal rabbit serum‡		Rabbit anti-p23,30‡	
			1/20 dilution	1/100 dilution	1/20 dilution	1/100 dilution
Exp. 1	XX$_m$	548 ± 36	1,334 ± 81	855 ± 574	561 ± 59	819 ± 68
	XY$_m$	73,084 ± 4,371	73,557 ± 9,987	82,887 ± 18,330	4,590 ± 1,962	12,370 ± 2,422
Exp. 2	XX$_m$	601 ± 36	549 ± 196	4,761 ± 902	1,542 ± 591	4,892 ± 666
	XY$_m$	88,675 ± 4,693	79,815 ± 11,743	73,365 ± 9,361	5,926 ± 620	3,612 ± 110

* One-way mixed cultures were established using responding lymphocytes from individual X and stimulating cells from an allogeneic individual Y. The subscript m implies mitomycin-treated populations.

‡ Cultures were established for 6 days at 37°C in a 96% CO$_2$-5% air atmosphere in the presence of either media, NRS, or varying dilutions of anti-p23,30. [^{3}H]thymidine incorporation was measured at day 6.

single locus or that the p23,30 antigen in each case arises from at least two genetic loci, or both.

Inhibition of MLC by Anti-p23,30. Several of the above findings were consistent with the view that p23,30 antigen is the human analog of murine Ia antigens: the molecular weight of the detergent product, the cellular distribution of the antigen, and inhibition of Amish sera which recognize an HL-A-linked B-cell alloantigen. Since murine anti-Ia serum is known to inhibit MLC reactivity it was therefore important to determine the effect of anti-p23,30 on human MLC assays. Standard one-way MLC's were established in the presence of either anti-p23,30 serum, NRS, or media controls. The presence of anti-p23,30 serum during the 5-day culture period markedly reduced and in some instances totally eliminated MLC reactivity (Table III). In contrast, NRS had no signifi-

cant effect. In other experiments we attempted to see whether the inhibition of MLC by anti-p23,30 was observed after precoating either responding or stimulating cells. Thus, stimulating or responding cells were pretreated with anti-p23,30 or NRS, washed, and then placed in mixed cultures. Under these conditions no significant reduction in MLC was seen. Whether the lack of inhibition under these pretreatment conditions is secondary to the rapid turnover of cell membranes during culture or alternatively whether sufficient anti-p23,30 was washed free from the cell surface before culture has not been resolved. The same phenomena has been observed with murine anti-Ia sera.

Discussion

p23,30 is a unique, B-lymphocyte-specific cell surface protein complex. It is immunochemically different from previously identified membrane antigens; including immunoglobulins, HL-A antigen, and β_2-microglobulin. The antigen is restricted to B lymphocytes and to a subpopulation of Null lymphocytes which bear a receptor for C. The antigen is not found on T lymphocytes, C-receptor-negative Null lymphocytes, or platelets. The distribution on nucleated cells has been followed by cytotoxic and immunofluorescence assays at both low and high titers of antiserum. The lack of reactivity of this unabsorbed heteroantiserum with cell populations not bearing p23,30 antigen has been striking and underlines the value of this approach in the study of membrane antigens, i.e., the preparation of antisera against purified proteins.

Because many structural and functional characteristics of this antigen are very similar to those of the murine Ia antigen, one may view this antigen complex to be the human counterpart of the murine Ia antigen. The antigen complex p23,30 resembles murine Ia antigens in four ways: (a) chemical structure, (b) tissue distribution, (c) linkage to the major histocompatibility complex, and (d) biological function as judged by effects of antisera to p23,30 in various systems. The detergent-solubilized form of the papain-derived p23,30 antigen was identified in SDS-polyacrylamide gels of double antibody-precipitated, radiolabeled antigen. This native antigen resembles the structure of mouse Ia antigens which have been reported by others (20–22), although three components were resolved in the human antigen. Several human histotyping sera precipitated molecules of weights similar to the native, detergent-solubilized precursor of p23,30. These sera presumbly recognized alloantigenic determinants on the p23,30 antigen. Cytotoxic Amish alloantisera which recognize an HL-A-linked B-cell alloantigen were inhibited by p23,30 antigen. The genes for the p23,30 antigen appear to be linked to the major histocompatibility complex, as are genes for Ia antigens. It should be noted that antibodies with similar specificity to p23,30 antibody have been described in pregnancy sera (23, 24). These antisera also inhibit the MLC reaction and react predominantly with human B cells but not T cells. In contrast to anti-p23,30 antibody, however, these antibodies seem to react with antigenic structures on lymphocyte membranes of mol wt 11,000 daltons (β_2-microglobulin) and 50–45,000 daltons (23). Whether the p23,30 membrane antigen complex, which is present on all B cells, is the common determinant recognized by these B-cell alloantisera remains to be defined. Others have also reported on the existence of Ia-like human B-cell antigens (25, 26). Finally, it is of interest that both the B-cell-specific alloanti-

sera and anti-p23,30 serum in addition recognize antigens on non-T-cell leuke-mias (27, 28).

Summary

In addition to HL-A antigens, another cell surface protein complex has been obtained from membranes of the human B-lymphoblast cell line IM-1. This complex which was solubilized with papain, consisted of polypeptides of 23,000 and 30,000 daltons (p23,30). Rabbit antisera to this material precipitated from [^{35}S]methionine-labeled detergent-solubilized cells, three proteins of 39,000, 34,000, and 29,000 daltons. These antisera were specifically cytotoxic for B lymphocytes of peripheral blood, for B-lymphoblast cell lines, and for EAC rosette receptor-positive surface Ig-negative (Null) lymphocytes. The p23,30 complex was not present on T lymphocytes, EAC rosette receptor-negative Null lymphocytes, or platelets. In addition, the p23,30 complex from several cell lines inhibited alloantisera from multiparous Amish women which had been shown to recognize non-HL-A, B-lymphocyte antigens. Some other properties of the anti-p23,30 sera antisera were described.

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