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Immunology

Human T cell surface antigens bearing a structural relationship to HLA antigens

(human TL antigen/HLA-like antigen)

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ABSTRACT **Three cell surface antigens that are structurally related to the human major histocompatibility antigens (called HLA antigens) have been characterized from the leukemic T cell line MOLT-4. One antigen is a glycoprotein of M_r 49,000 recognized by two monoclonal antibodies, OKT6 and NA1/34, and is associated with a M_r 12,000 subunit that crossreacts serologically with β_2-microglobulin but can be distinguished from it by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. A second antigen, defined by the monoclonal antibody OKT10, is a M_r 46,000 protein associated with a small subunit distinct from β_2-microglobulin. The OKT10 antigen is not restricted to T cells and is found on all T and B lymphoblastoid cell lines tested. The third protein is a β_2-microglobulin-associated glycoprotein of M_r 43,000 that is serologically distinct from the OKT6 (NA1/34), OKT10, and HLA antigens. It is found on some, but not all, T cell lines but is absent from any other hematopoeitic cell lines tested.**

The *HLA-A*, *HLA-B*, and *HLA-C* loci in the major histocompatibility complex (MHC) of man are located on chromosome 6 (1) and encode three major antigens, all glycoproteins of M_r 44,000. These are noncovalently associated with a peptide of M_r 12,000, β_2-microglobulin (β_2m). The HLA antigens are major membrane proteins that make up about 1% of membrane proteins on lymphoid cell lines and are expressed on virtually all cell types.

In the mouse, the analogous MHC-encoded antigens are called the H-2D, H-2K, and H-2L antigens (2). In addition, three other antigens, called TL (3), Qa-1 (4), and Qa-2 (5), have structures similar to the 44,000/12,000 subunit structure of the major histocompatibility antigens. The loci of these antigens are clustered and closely linked to the murine MHC at its distal end (6). TL and Qa can be distinguished from the major histocompatibility antigens by their cell distribution: TL is restricted to thymocytes (and some leukemic cells), whereas Qa-1 and Qa-2 are found on thymocytes, a variable number of peripheral T cells (60–95%), and some other lymphoid cells (6).

Recently, an "HLA-like" human T cell antigen (called HTA1) was recognized on thymocytes by the monoclonal antibody NA1/34 (7, 8). Its cell distribution and M_r were consistent with the characteristics expected of the human homologue of murine TL. The presence of β_2m-associated material on T cell leukemic lines, serologically distinct from the HLA antigens, has also been observed (8–10). Here we present evidence for the existence of three T cell antigens that are structurally related but serologically distinct from the classical HLA antigens.

MATERIALS AND METHODS

Monoclonal Antibodies. Monoclonal antibodies OKT6, OKT10 (11), and NA1/34 (8) were produced by immunization of CAF or BALB/c mice with human thymocytes. The monoclonal antibody W6/32 (obtained from C. Barnstable) was produced by using human tonsil lymphocytes as immunogen (12). The monoclonal antibody against β_2m, BBM.1 (obtained from P. Parham), was made in a mouse immunized with MOLT-4 cells (13).

Assays to Measure Antibody Bound to Cell Surface. ^{125}I-Labeled affinity-purified F(ab')$_2$ fragments of rabbit anti-mouse immunoglobulin (RαMIg) antibodies were used to detect binding of monoclonal antibodies to cell surfaces as described (13) except that 2×10^5 cpm of ^{125}I-RαMIg was used and cells were washed five times after addition of ^{125}I-RαMIg. Direct binding assays were performed by incubating 5×10^5 cells with iodinated monoclonal antibodies OKT6 and NA1/34 in phosphate-buffered saline, pH 7.2, for 1 hr on ice with 0.5% bovine serum albumin and 0.1% sodium azide. Cells were washed five times and bound radioactivity was measured. In inhibition experiments, cells were preincubated with unlabeled antibody for 1 hr on ice. Iodination of purified RαMIg and the immunoglobulin fractions of NA1/34 and OKT6 ascites fluids was as described (14). The specific activities of ^{125}I-RαMIg, ^{125}I-NA1/34 and ^{125}I-OKT6 were 18–24 $\times 10^6$ cpm/μg, 7.5×10^5 cpm/μg, and 4.5×10^5 cpm/μg, respectively. About 2×10^5 cpm were used per well.

Lymphocytes and Cell Lines. The T cell leukemic lines MOLT-4, TALL-1, 8402, JM, HPB-ALL, and HPB-MLT and the null cell lines NALM-1 and REH were obtained from J. Minowada; K562 and the T cell leukemic lines HSB and CEM were from H. Lazarus; U-937 was from K. Nilsson; and HL-60 was from R. Gallo. All lines were maintained in stationary bottle culture in RPMI 1640 medium with 8–10% fetal calf serum.

Thymus tissue was obtained as a by-product of cardiac surgery on infants (through the courtesy of A. Castenada). Thymocytes were obtained from the interface after Ficoll/Hypaque centrifugation. Spleen tissue was obtained from cadavers and viable splenocytes were separated by Ficoll/Hypaque centrifugation. T cells from peripheral blood were isolated and activated by phytohemagglutinin as described (15).

Isolation and Characterization of T-Cell Antigens. Iodo-Gen (Pierce) was used to label the external proteins of cells by incubating 10^7 cells for 15 min at 4°C, using 0.5–1 mCi of ^{125}I (1 Ci = 3.7×10^{10} becquerels) (16). Labeled cells were washed three times in phosphate-buffered saline or RPMI 1640 medium, then extracted for 45 min on ice with 1% Nonidet P-40 (NP-40, Particle Data Laboratories, Elmhurst, IL) in 10 mM Tris·HCl (pH 8.0)/10 mM iodoacetamide (Sigma)/freshly diluted 1 mM phenylmethylsulfonyl chloride (Sigma). After centrifugation at 100,000 $\times$ *g* for 1 hr, the supernatant was used for immunoprecipitation (17) or, after the addition of 5 mg of

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Abbreviations: β_2m, β_2-microglobulin; MHC, major histocompatibility complex; NP-40, detergent Nonidet P-40; NMS, normal mouse serum; rabbit anti-H, rabbit anti-HLA heavy chain; RαMIg, rabbit anti-mouse immunoglobulin; SaCI, *Staphylococcus aureus* Cowan I strain.

bovine serum albumin, for immunoaffinity chromatography. *Staphylococcus aureus* Cowan I strain (SaCI) was used as immunoadsorbant, and bound immune complexes were washed as described (17). Immunoaffinity columns were prepared by coupling the immunoglobulin fraction (40% saturated ammonium sulfate precipitate) of monoclonal ascites fluid to cyanogen bromide-activated Sepharose CL-4B (2–5 mg of protein per ml of packed beads) in phosphate-buffered saline, pH 7.5. All columns were preeluted with 0.5 M acetic acid and then washed with several column volumes of 0.1 M NaCl/10 mM Tris·HCl, pH 7.4/0.1% NP-40 (application buffer) prior to applying the detergent solubilized extract. The unbound material was reapplied to each column twice. Extracts were passed sequentially over a column of ovalbumin-Sepharose, a column of normal mouse IgG-Sepharose, and a column containing mouse immune complexes to remove cellular components binding nonspecifically. Columns were washed with 5 column volumes of the following buffers: (*i*) the application buffer (*ii*) the application buffer with 0.5 M LiCl instead of 0.1 M NaCl, (*iii*) the application buffer again, (*iv*) 10 mM Tris·HCl with 0.1% NP-40, and (*v*) 10 mM Tris·HCl. Bound proteins were eluted with one column volume of 0.5 M acetic acid, followed by 20 mM Tris·HCl, pH 7.4, and the acidic fractions were collected and lyophilized. Dried samples were dissolved in $NaDodSO_4$ sample buffer and analyzed on either 12% or gradient 7–15% polyacrylamide gels (18).

RESULTS

Reactivity of OKT6 and OKT10 with Hematopoeitic Cells and Cell Lines. The reactivities of the monoclonal antibodies OKT6 and OKT10 with normal hematopoeitic cells have been measured by fluorescence assays (11, 19). As a first step in obtaining a reproducible source of these antigens for biochemical analysis, a number of hematopoeitic cell lines of T, B, myeloid, and erythroid lineages were examined by indirect fluorescence microscopy and by indirect ^{125}I-RαMIg binding assay (20). OKT6 and OKT10 clearly had cell distributions distinct from each other and from W6/32, a monoclonal antibody that recognizes all HLA-A, -B, and -C antigens (Table 1). OKT10 was found on all T and B lymphoblastoid cell lines tested, and on essentially all thymocytes (11), but on only a small fraction (<10%) of peripheral blood lymphocytes or splenocytes. The expression of OKT6 was restricted to thymocytes and some T cell lines derived from leukemias. A direct comparison of NA1/34 and OKT6 was made on a panel of T cell lines and their reactivities were essentially indistinguishable, both qualitatively (Table 1) and with respect to the magnitude of their expression on T cell lines (20). The expression of OKT10 antigens (but not that of OKT6) was markedly increased on activation of peripheral T cells by phytohemagglutinin (see table 1 of ref. 20).

Table 1. Cell distribution of molecules recognized by W6/32, OKT6, NA1/34, and OKT10*

Cells or cell lines	W6/32	OKT10	OKT6	NA1/34
Thymocytes	≈80%†	≈95%‡	≈70%‡	65–80%†
T-cell lines				
MOLT-4, HPB-MLT	+	+	++	++
HPB-ALL, JM,	+	+	+	+
CEM	+	+	+/−	+/−
HSB, 8402, TALL-1	+	+	−	−
Peripheral T cells	100%	<5%‡	−	−
Splenocytes	100%	<10%		
B-cell lines (JY, SB)	+	+	−	−
Null cell lines (NALM-1, REH)	+	−	−	−
Promyelocytic line (HL60)	+	−	−	−
Histiocytic lymphoma (U937)	+	−	−	−
Erythroleukemic line (K562)	−	−	−	−

* Typing of cell lines was determined by binding of ^{125}I-RαMIg to cells pretreated with the respective monoclonal antibody. The percentage of reacting splenocytes was determined by indirect fluorescence microscopy.
† Values from ref. 21.
‡ Values from ref. 19.

OKT6 and NA1/34 Both Recognize a Heterodimer of M_r 49,000–12,000. The proteins recognized by OKT6 and NA1/34 were compared by immunoprecipitation from the T-cell line MOLT-4. OKT6 precipitated two bands of M_rs 49,000 and 12,000 (called p49,12) identical in size and amount to materials immunoprecipitated with NA1/34 (Fig. 1*A*). In order to determine whether the determinants recognized by OKT6 and NA1/34 were present on the same molecule, the technique of sequential immunoaffinity chromatography was used. An extract of ^{125}I-labeled MOLT-4 cells was passed over an OKT6-Sepharose column and the fractions passing through the column were then passed over a NA1/34-Sepharose column. The bound

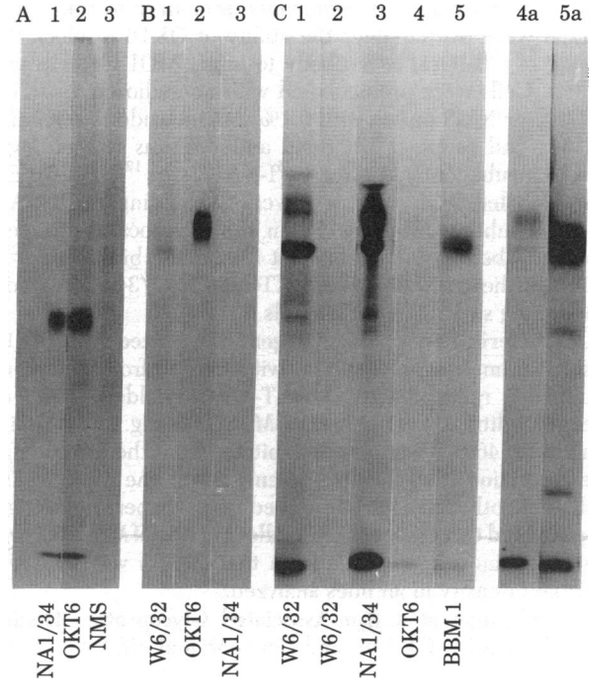

FIG. 1. Characterization of the OKT6 and NA1/34 antigens from ^{125}I-MOLT-4. (*A*) Parallel immunoprecipitation of aliquots of ^{125}I-MOLT-4 (12×10^6 initial cpm) with; lane 1, NA1/34 (14,600 cpm bound); lane 2, OKT6 (16,800 cpm bound); and lane 3, normal mouse serum (NMS) (1500 cpm bound). (*B* and *C*) Sequential chromatography of ^{125}I-MOLT-4 on W6/32, OKT6, and NA1/34 immunoaffinity columns. Lysates of ^{125}I-MOLT-4 [either 62×10^6 cpm (*B*) or 20×10^6 cpm (*C*)] were passed over three Sepharose columns conjugated with ovalbumin, normal mouse immunoglobulin, and mouse immune complexes. (*B*) Unbound material was passed in order over Sepharose columns conjugated with; lane 1, W6/32; lane 2, OKT6; and lane 3, NA1/34. Half of the bound radioactivity (lane 1, 9100 cpm; lane 2, 27,000 cpm; lane 3, 1850 cpm) was analyzed on a 7–15% acrylamide gel in which β_2m ran off. (*C*) ^{125}I-MOLT-4 was sequentially passed in the following order over Sepharose conjugated with: lane 1, W6/32; lane 2, W6/32 again; lane 3, NA1/34; lane 4, OKT6; and lane 5, BBM.1. Bound material (lane 1, 24,000 cpm; lane 2, 1100 cpm; lane 3, 20,000 cpm; lane 4, 1600 cpm; lane 5, 5000 cpm) was analyzed on a 7–15% acrylamide gel. Autoradiography was for 4 days (*A*), 48 hr (*B*), 40 hr (*C*, lanes 1–5), or 20 days (*C*, lanes 4a and 5a).

material was eluted with 0.5 M acetic acid, lyophilized, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The OKT6-Sepharose column bound 54,000 cpm, whereas only 3700 cpm was subsequently bound by the NA1/34-Sepharose column. As shown in Fig. 1*B*, an intense band of M_r 49,000 was bound to the OKT6-Sepharose, whereas no such band was recovered from the NA1/34-Sepharose.

In five reciprocal experiments, passage of ^{125}I-MOLT-4 extracts first over NA1/34-Sepharose resulted in a 86–96% depletion in the amount of radioactivity subsequently bound by OKT6-Sepharose. Analysis of bound material by $NaDodSO_4$/polyacrylamide gel electrophoresis in a representative experiment yielded an intense band of M_r 49,000 from NA1/34-Sepharose in a 40-hr exposure, while a 20-day exposure revealed a faint band of M_r 49,000 from the OKT6-bound material (Fig. 1*C*). The small subunit of the OKT6 and NA1/34 antigen from MOLT-4 had a slightly slower mobility than β_2m, in agreement with previous results (8). Sequential immunoprecipitation also confirmed that OKT6 and NA1/34 can reciprocally and completely deplete the antigen recognized by the other. Both OKT6 and NA1/34 recognize determinants on the larger M_r 49,000 subunit and not its M_r 12,000 subunit, because the heavy chain can be precipitated in the absence of the light chain above 20°C (8) or after lectin affinity chromatography (20).

In order to see if the determinants recognized by OKT6 and NA1/34 were overlapping, the ability of OKT6 to block the binding of ^{125}I-NA1/34 antibody to intact MOLT-4 cells was studied. Cells were preincubated with nonradioactive OKT6, NA1/34, or NMS on ice with 0.1% sodium azide to minimize patching and capping. Unbound antibody was washed away prior to incubation with either ^{125}I-NA1/34 or ^{125}I-OKT6. Excess unlabeled OKT6 did not prevent the binding of ^{125}I-NA1/34 (<5% inhibition) (Table 2). In the reciprocal experiment excess unlabeled NA1/34 did not inhibit the binding of ^{125}I-OKT6, further indicating that OKT6 and NA1/34 occupy nonoverlapping sites on MOLT-4 cells.

Characterization of the Antigen Recognized by OKT10. Results of immunoprecipitation with OKT10 from detergent-solubilized, radioiodinated MOLT-4 cells yielded (under reducing conditions) a major band of M_r 46,000 (Fig. 2*B*). A similar band of M_r 46,000 was also precipitated from the OKT6-negative T-cell line HSB and its syngenic B-cell line SB (Fig. 2*B*). Unlike the other antigens described in this paper, this antigen failed to bind to lentil lectin. A smaller peptide of M_r 12,000 with a mobility indistinguishable from that of β_2m was seen with variable intensity in all lines analyzed.

Identification of a β_2m-Associated Glycoprotein Distinct from the OKT6, OKT10, and Conventional HLA-A, -B, and -C Antigens. Because β_2m is a known constituent of murine TL and both Qa-1 and Qa-2 molecules, it was important to know whether additional β_2m-associated proteins exist on human T cells. Sequential immunoaffinity columns or immunoprecipitations were used to deplete a radioiodinated MOLT-4 extract of molecules recognized by W6/32, OKT6, and OKT10 (Figs. 1*C* and 3). Then the unbound material was incubated with the monoclonal anti-β_2m antibody BBM.1. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis showed that treatment with W6/32, OKT6, and OKT10 was effective in depleting the respective antigens. However, after removal of these three antigens, bands of M_r 43,000 and 12,000 were then precipitated by BBM.1 (Fig. 1*C*, lane 5; Fig. 3*E*; also see ref. 20). The band of M_r 43,000 migrated slightly faster and was somewhat broader than the HLA-A, -B, -C band precipitated by the W6/32 antibody. In other experiments three sequential immunoprecipitations with each monoclonal antibody (W6/32, OKT6, and OKT10) also failed to deplete the molecule (p43,12) reacting finally with BBM.1.

W6/32 antibody was chosen as the reagent to deplete the HLA antigens because it recognizes all known HLA-A, -B, and -C allospecificities when they are associated with β_2m (12, 22) and in addition recognizes biosynthetic precursors of these antigens when they are associated with β_2m (23). It is extremely unlikely, therefore, that the protein of M_r 43,000 and 12,000 that is detected after exhaustive W6/32 precipitation is simply an HLA biosynthetic precursor. Furthermore, surface proteins were labeled by surface iodination of intact MOLT-4 cells for these experiments, again indicating that the β_2m-associated M_r 43,000 protein is not a biosynthetic precursor.

The protein of M_r 43,000 is similar to the large subunit of HLA-A, -B, -C antigens in that it is a glycoprotein that binds to lentil lectin (20) and is endogenously labeled with tritiated *N*-acetylglucosamine (data not shown). It can also be distinguished from the HLA-A, -B, and -C antigens by its cell distribution, being present on some, but not all, T cell lines (i.e., MOLT-4 but not HSB) and is not detectable on any of the four B cell lines tested (data not shown).

The Large and Small Subunits of the OKT6 Antigen but Not Those of the OKT10 Antigen Are Recognized by Antibodies Prepared Against the Large and Small Subunits of the HLA

Table 2. Direct binding and inhibition of binding of radiolabeled monoclonal antibodies OKT6 and NA1/34 to MOLT-4 cells

Pretreatment (5 μl)	^{125}I-Labeled monoclonal antibody*	cpm bound	% inhibition
NMS	NA1/34	12,773	—
NA1/34 ascites fluid	NA1/34	676	94.7
OKT6	NA1/34	11.561	5.1
NMS	OKT6	5,270	—
OKT6	OKT6	372	92.9
NA1/34	OKT6	5,548	(−5.3)

* About 2×10^5 cpm of labeled monoclonal antibody was added, and an average of 676 cpm and 751 cpm of ^{125}I-NA1/34 and ^{125}I-OKT6, respectively, were bound by a nonreactive cell line, K562 (which had been pretreated with NMS). The background binding to K562 is not subtracted. Results are the average of duplicates.

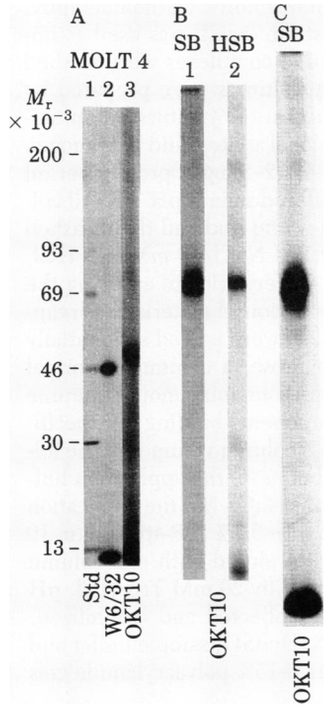

FIG. 2. Immunoprecipitation from lysates of ^{125}I-MOLT-4, ^{125}I-SB, and ^{125}I-HSB by OKT10. (*A*) Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis (7–15% gradient gel) of W6/32 and OKT10 immunoprecipitates of ^{125}I-MOLT-4. Lane 1, radioactive standards, M_rs indicated on the left; lane 2, W6/32, starting from 2×10^6 cpm; lane 3, OKT10, starting from 8×10^6 cpm. (*B*) Analysis of OKT10 immunoprecipitates from ^{125}I-SB (2.4×10^6 initial cpm) and ^{125}I-HSB (5×10^6 initial cpm) on a 12% acrylamide gel in which a 13,000 M_r standard had run off. (*C*) OKT10 immunoprecipitate from ^{125}I-SB (4.8×10^6 initial cpm). Autoradiographs were exposed for 8 days for *A*, 20 hr for SB in *B*, 5 days for HSB in B, and 4 days for SB in *C*.

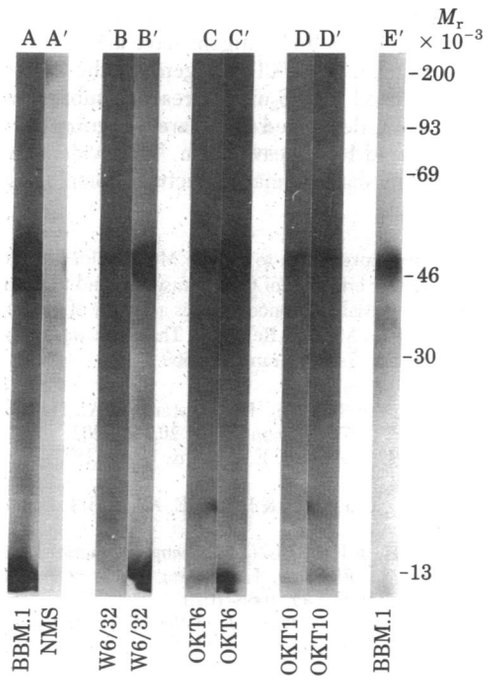

FIG. 3. Sequential immunoprecipitation from ^{125}I-MOLT-4 (8×10^6 initial cpm) lysate by W6/32, OKT6, and OKT10 before and after depletion of β_2m-associated material. Lysate was precleared twice with immune complexes formed between NMS and RαMIg, then divided in half. One half was allowed to react twice sequentially with BBM.1 and the second half, with NMS and RαMIg. Each half was then allowed to react sequentially in the order W6/32, OKT6, OKT10. Precipitates from the half previously treated with BBM.1 are displayed on 12% acrylamide gel under unprimed letters with radioactivity precipitated in parenthesis: (*A*) BBM.1 (6000 cpm); (*B*) W6/32 (1700 cpm); (*C*) OKT6 (1000 cpm); (*D*) OKT10 (2500 cpm). Primed letters indicate precipitates from the half not pretreated with BBM.1: (*A′*) NMS–RαMIg (700 cpm); (*B′*) W6/32 (5200 cpm); (*C′*) OKT6 (6800 cpm); (*D′*) OKT10 (2200 cpm). This half was allowed to react once again with W6/32, OKT6, and OKT10 and then with BBM.1 (*E′*) (1200 cpm). Autoradiography was for 6 days.

Antigens. Immunoprecipitates of OKT10 and OKT6 from MOLT-4 brought down small peptides of M_r 12,000 that migrated either with or slightly slower than β_2m. Because the murine TL and Qa molecules are associated with β_2m, experiments were conducted to see if the small peptides precipitated by OKT6 and OKT10 reacted with anti-β_2m antibody. The effect of depletion of β_2m-associated material on subsequent precipitation with OKT6, OKT10 and, as a control, W6/32, was examined. A radioiodinated MOLT-4 extract was divided into halves; one half was allowed to react twice sequentially with an anti-β_2m monoclonal antibody, while the second half was allowed to react with a control immune couplex, NMS–RαMIg (Fig. 3 *A* and *A′*). Precipitation with OKT6, OKT10 and W6/32 was carried out sequentially on each half and immunoprecipitates were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Pretreatment with BBM.1 was successful in depleting all HLA-A, -B, and -C antigens precipitated by W6/32 (Fig. 3*B*, compare to Fig. 3*B′*). Significantly, it also dramatically decreased, but did not completely eliminate, the antigen precipitated with OKT6 (Fig. 3 *C* and *C′*). In three experiments depletion of the β_2m-associated material reduced the radioactivity to an average of 26% of the level bound in the control OKT6 precipitation. Unlike OKT6, precipitation with OKT10 was unaffected by prior removal of β_2m-associated material (Fig. 3 *D* and *D′*).

The M_r 12,000 subunit of the OKT6 antigen was more directly demonstrated to have β_2m determinants by reprecipitating it with a goat antiserum to β_2m from the NaDodSO$_4$-eluted material of an OKT6 immunoprecipitate. W6/32, OKT6, OKT10, and BBM.1 (after preclearing; see Table 3) immunoprecipitates were eluted from SaCI cells by boiling in 1% NaDodSO$_4$. They were then made up to 1% NP-40/0.2% NaDodSO$_4$ and allowed to react with goat anti-β_2m. Comparable amounts of radioactivity were precipitated from material eluted from W6/32, OKT6, and BBM.1 immunoprecipitates, whereas no radioactivity above control values could be precipitated from material eluted from OKT10 immunoprecipitates (Table 3).

Table 3. Immunoprecipitation of the heavy and light chains of the HLA antigens and the three T cell antigens by rabbit anti-H serum and by goat anti-β_2m serum

First immunoprecipitation	Second immunoprecipitation	cpm added	cpm pptd	% pptd
W6/32	Normal rabbit serum	2680	302	11
	Goat anti-β_2m	2350	704	30
	Rabbit anti-H	3203	1210	38
OKT6	Normal rabbit serum	2595	330	13
	Goat anti-β_2m	2550	751	29
	Rabbit anti-H	3103	1098	35
OKT10	Normal rabbit serum	2180	385	18
	Goat anti-β_2m	2380	277	12
	Rabbit anti-H	2715	379	14
BBM.1*	Normal rabbit serum	1730	197	11
	Goat anti-β_2m	1730	770	44
	Rabbit anti-H	1780	482	27

Initial immunoprecipitations were from ^{125}I-MOLT-4 lysate treated sequentially with W6/32, OKT6, OKT10, and finally BBM.1. Immunoprecipitates were washed five times (17), material was eluted with 1% NaDodSO$_4$, made up to 1% NP-40 and 0.2% NaDodSO$_4$, and divided into three aliquots for the second immunoprecipitation. The second series of immunoprecipitates were washed once before bound radioactivity was measured.
* After clearing with W6/32, OKT6, and OKT10.

In addition, immunoprecipitation was carried out with rabbit anti-H serum (an antiserum prepared against the separated, denatured heavy chain of highly purified HLA-B7) (23, 24). The heavy chains of the W6/32, OKT6, and the β_2m-associated M_r 43,000 protein were immunoprecipitated by rabbit anti-H, but the heavy chain of the OKT10 antigen was not (Table 3).

DISCUSSION

This study describes the identification of three different human T cell antigens that are structurally similar to the HLA-A, -B, and -C antigens. One, first detected with monoclonal antibody NA1/34 (7) is recognized by OKT6 on MOLT-4 cells [also immunoprecipitated from HPB-MLT (20), JM, and HPB-ALL cells]. It was found to be a glycoprotein of M_r 49,000 in association with a subunit of M_r 12,000. The small subunit was reactive with a monoclonal anti-β_2m antibody but when from MOLT-4 cells had a slower mobility in NaDodSO$_4$/polyacrylamide gel electrophoresis. The second, precipitated by antibody OKT10 from T and B cell lines, consisted of protein of M_r 46,000 and a small subunit of M_r 12,000 that was not reactive with the anti-β_2m antibodies. The third protein, a glycoprotein of M_r 43,000, was precipitated by the anti-β_2m antibody after MOLT-4 lysate had been depleted of all HLA-A, -B, -C, and OKT6 antigens. All of these T cell proteins were readily radioiodinated at the surface of MOLT-4 cells and were isolated independently of one another by sequential immunoaffinity chromatography. The presence of a β_2m-associated protein of M_r 43,000 on T leukemic lines has previously been noted (8–10).

The cell distribution of OKT6 reactivity was essentially identical to that of another monoclonal antibody, NA1/34 (7, 11, 21)

with respect to their expression on both thymocytes and T cell leukemia lines. Sequential immunoaffinity chromatography and immunoprecipitation established that OKT6 and NA1/34 recognized the same protein. The antigenic determinants recognized by OKT6 and NA1/34 were nonoverlapping, however, because the two antibodies did not compete with each other for binding to MOLT-4 cells.

The small subunit (M_r 12,000) of the OKT6 antigen from the T cell leukemic line MOLT-4 was distinguished from β_2m by a slightly slower mobility in $NaDodSO_4$/polyacrylamide gel electrophoresis, in agreement with the findings of Ziegler and Milstein (8), who used the NA1/34 antibody. This difference in gel mobility may be the result of a modified β_2m, a second related gene product, or a β_2m polymorphism as has been recently noted in the mouse (25). Comparative structural studies will be necessary to resolve this question. Two types of analysis were used to see if this small subunit was recognized by anti-β_2m antibodies. First, the depletion of β_2m-associated material from MOLT-4 by a monoclonal anti-β_2m antibody resulted in a dramatic reduction of the amount of antigen subsequently precipitated by OKT6. Second, goat anti-β_2m precipitated the separated denatured small subunit of the OKT6 antigen. This discrepancy with an earlier report (8) could be due to the use of a different anti-β_2m serum. In contrast, the small subunit of the OKT10 antigen was nonreactive with either of these anti-β_2m antibodies. Thus, the four types of "p43–49,12" surface antigens on MOLT-4 cells may be associated with three different small subunits. (*i*) β_2m associated with the M_r 44,000 glycoproteins (HLA-A, -B, and -C antigens) or with a M_r 43,000 glycoprotein; (*ii*) a small subunit of the OKT6 antigen that crossreacts with anti-β_2m antibodies but can be distinguished from β_2m by $NaDodSO_4$/polyacrylamide gel electrophoresis; and (*iii*) the small subunit of the OKT10 antigen, which is not distinguished from β_2m by $NaDodSO_4$ gel electrophoresis and does *not* crossreact with anti-β_2m antibodies. Further experiments are needed to confirm these conclusions regarding the nature of the small subunits, but it seems clear that there are four types of molecules of the p43–49,12 structure on some human T cell lines.

Cell distribution and other properties of these antigens suggest that they may include the human homologues of murine TL and Qa antigens. Considerable data suggest that the OKT6 antigen is the human homologue of murine TL. The OKT6 antigen has a heavy chain of M_r 49,000 [compared to a value of 48,000 reported for TL (26)], and is associated with a subunit of M_r 12,000 recognized by anti-β_2m antibody. Its distribution on thymocytes and T cell leukemias but not peripheral T cells is identical to that found for murine TL. In addition, OKT6 antigen has recently been found to be expressed on Langerhans cells in the skin (27). The relationship of the OKT10 target antigen and the β_2m-associated protein of M_r 43,000 to the Qa-1 and Qa-2 antigens is unclear. The OKT10 antigen is similar to the Qa antigens in its expression on bone marrow cells, myeloid precursors, and mitogen-stimulated T cells (7, 28). However, the OKT10 antigen is found on 10% or less of human peripheral lymphocytes or splenocytes (Table 1), whereas the different murine anti-Qa antisera all react with 30–70% of peripheral mouse T cells (7). It is possible that the Qa alloantisera are polyspecific and recognize more than one class of molecule, but similar observations have been made with monoclonal antibodies to Qa antigens (29). In addition, the two structurally defined Qa antigens are associated with β_2m, whereas the OKT10 antigen appears not to be. The similarities of the TL and Qa antigens in size, their affinity for β_2m, and the close genetic linkage of their genes to those encoding the major histocompatibility antigens have led to the hypothesis that genes for TL, Qa, and the major histocompatibility antigens have arisen by gene duplication (3). In man, the OKT6 antigen and the protein of M_r 43,000 are associated with β_2m-crossreactive subunit and both of their $NaDodSO_4$-denatured chains are recognized by an antiserum to denatured HLA heavy chain. This evidence suggests that both antigens share primary structural homology with the HLA antigens.

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1. Jongsma, A., van Someran, H., Westerveld, A., Hagemeijer, A. & Pearson, P. (1973) *Humangenetik* **20**, 195–202.
2. Hansen, T. H., Cullen, S. E. & Sachs, D. H. (1977) *J. Exp. Med.* **145**, 438–442.
3. Vitetta, E. S., Uhr, J. W. & Boyse, E. A. (1978) *J. Immunol.* **114**, 252–258.
4. Stanton, T. H. & Hood, L. (1980) *Immunogenetics* **11**, 309–314.
5. Michaelson, J., Flaherty, L., Vitetta, E. S. & Poulik, M. D. (1977) *J. Exp. Med.* **145**, 1066–1070.
6. Flaherty, L. (1981) in *The Role of the Major Histocompatibility Complex in Immunobiology*, ed., Dorf, M. (Garland, New York), pp. 33–55.
7. McMichael, A. J., Pilch, J., Galfré, G., Mason, D. Y., Fabre, J. W. & Milstein, C. (1979) *Eur. J. Immunol.* **9**, 205–210.
8. Ziegler, A. & Milstein, C. (1979) *Nature (London)* **279**, 243–244.
9. Tada, N., Tanigaki, N. & Pressman, D. (1978) *J. Immunol.* **120**, 513–519.
10. Tanigaki, N., Tokuyama, H., Fukunishi, T., Minowada, J. & Pressman, D. (1979) *J. Immunol.* **123**, 2906–2914.
11. Reinherz, E. L., Kung, P. C., Goldstein, G. & Schlossman, S. F. (1979) *J. Immunol.* **123**, 1312–1317.
12. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfré, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14**, 9–20.
13. Brodsky, F., Bodmer, W. F. & Parham, P. (1979) *Eur. J. Immunol.* **9**, 536–545.
14. Sonada, S. & Schlamowitz, M. (1970) *Immunochemistry* **7**, 885–898.
15. Judd, W., Poodry, C. A., Broder, S., Friedman, S. & Strominger, J. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6805–6809.
16. Markwell, M. A. L. & Fox, C. F. (1978) *Biochemistry* **17**, 4807–4817.
17. Shackelford, D. A. & Strominger, J. L. (1980) *J. Exp. Med.* **151**, 144–164.
18. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
19. Reinherz, E. L., Kung, P. C., Goldstein, G., Levey, R. & Schlossman, S. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1588–1592.
20. Cotner, T., Hemler, M. & Strominger, J. L. (1981) *Int. J. Immunopharmacol.*, in press.
21. Janossy, G., Lidman, N., Selby, W. S., Thomas, J. A., Granger, S., Kung, P. C. & Goldstein, G. (1980) *Nature (London)* **288**, 81–84.
22. Parham, P., Barnstable, C. J. & Bodmer, W. F. (1979) *J. Immunol.* **123**, 342–349.
23. Krangel, M. S., Orr, H. T. & Strominger, J. L. (1979) *Cell* **18**, 979–991.
24. Ploegh, H. L., Cannon, L. E. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2273–2277.
25. Michaelson, J., Rothenberg, E. & Boyse, E. A. (1980) *Immunogenetics* **11**, 93–95.
26. Rothenberg, E. & Boyse, E. A. (1980) *J. Exp. Med.* **150**, 777–791.
27. Fithian, E., Kung, P., Goldstein, G., Rubenfeld, M., Fenoglio, C. & Edelson, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2541–2544.
28. Kincaide, P. W., Flaherty, L., Lee, G., Watanabe, T. & Michaelson, J. (1980) *J. Immunol.* **124**, 2879–2885.
29. Hammerling, G. J., Hammerling, U. & Flaherty, L. (1979) *J. Exp. Med.* **150**, 108–116.