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GLYCOPROTEINS OF 210,000 AND 130,000 M.W. ON ACTIVATED T CELLS: CELL DISTRIBUTION AND ANTIGENIC RELATION TO COMPONENTS ON RESTING CELLS AND T CELL LINES[1]

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A glycoprotein complex of 210,000 and 130,000 m.w., found on mitogen or alloantigen-stimulated human T cells and not on other hematopoietic cells, has been defined by a monoclonal antibody (Mab). The components of this complex are a subset of a larger family of proteins (210,000, 165,000 and 130,000 m.w.) defined by a second Mab. In a panel of hematopoietic cell lines and cell types, only activated T cells (including the cell line HUT-102) express the 210,000/130,000 complex and these cells also express the IL 2 receptor, a characteristic marker for activated T cells.

The 210,000/130,000 m.w. complex (reactive with the Mab TS2/7) is present on all long-term activated T cells, including both the OKT4 and OKT8 subsets. The 210,000 m.w. subunit is expressed only on activated T cells. Other lymphoid cells express either the 130,000 m.w. subunit alone (unactivated lymphocytes, thymocytes, HUT-78) or the 130,000 subunit together with a 165,000 subunit (MOLT-4, HSB, and other leukemic T cell lines). The 210,000/130,000 m.w., 165,000/130,000 m.w. and 130,000 m.w. complexes are antigenically related in that all share reactivity with the Mab A-1A5.

Among non-lymphoid hematopoietic cells and cell lines, none express the 210,000 m.w. chain; adherent cells (monocytes) and myeloid cell lines each express single proteins of 130,000 to 155,000 m.w. Granulocytes and red blood cells are negative and platelets express multiple bands (165,000 and 140,000 m.w.). Immunoperoxidase staining of tissue sections showed that a broad range of tissues and cell types had material cross-reactive with the lymphoid 130,000 m.w. protein. However, only a discrete subset of those tissues and cells including blood vessel walls, connective tissue, smooth muscle, kidney mesangial cells, and some non-cellular matrix tissue, had material cross-reactive with the 210,000 m.w. protein on activated T lymphocytes.

A monoclonal antibody (Mab)[3] (A-1A5) was used recently to define a novel glycoprotein complex of at least three proteins on the surface of mitogen or alloantigen-stimulated T cells (1). Two components of this complex (210,000 and 165,000 m.w.) appeared to be specific for activated T cells. The third protein (130,000 m.w.) was also found on unactivated peripheral blood lymphocytes. Immunoprecipitation with the Mab A-1A5 was also used to demonstrate that T lymphoblastoid cell lines express either the 130,000 m.w. protein or both the 165,000 and 130,000 m.w. proteins. In addition, B lymphoblastoid cell lines expressed only a weak 130,000 m.w. protein and the monocytoid cell line (U-937) expressed a single 145,000 m.w. protein, cross-reactive with the 130,000 m.w. protein.

The time course of appearance of several activation antigens has been studied on T cells (2). Aside from the 210,000 and 165,000 m.w. proteins co-precipitated by A-1A5, however, the only other proteins known to be relatively specific for activated T cells and not on other activated hematopoietic cell types (B cells, myeloid cells) are the IL 2 receptor (3, 4) and T200A in the mouse (5).

This report presents results obtained with an Mab (TS2/16) with specificity similar or identical to A-1A5 and a new Mab (TS2/7), which appears to react only with activation-specific subcomponents of the A-1A5 complex. The generation of the latter Mab provides a valuable tool for independent study of proteins specific for activated T cells separate from other components of the A-1A5 reactive complex.

MATERIALS AND METHODS

Antibodies and cell lines. The Mab A-1A5 (γ2b) (1) and B1.49.9 (γ2a) (4) were made against alloantigen-stimulated T cells, and OKT4 and OKT8 came from Ortho, Raritan, NJ. The negative control Mab, J-2A2 (γ2b), is directed against *Mycoplasma hyorhinis* (6), and P3 (γ1) is the antibody secreted by the myeloma line P3X63Ag8 (7). Long-term human CTL lines directed against HLA-A, B or HLA-DR were generated as described (8).

Hybridoma production. A human CTL line directed against HLA-DR was used to immunize BALB/c mice and mouse spleen cells were fused with the myeloma line NS1 as described (9). Mab that inhibit the cytolytic function of the anti-HLA-DR line were generated and these Mab (LFA-1, LFA-2, and LFA-3) have been described previously (9). Additional Mab that did not block cytolysis were selected because they immunoprecipitated unusual protein complexes with multiple polypeptide chains in the region of 80,000 to 210,000 m.w. Selection of clones and subclones of TS2/16 and TS2/7 was also by indirect binding assays with ^{125}I-labeled anti-mouse Ig.

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[3] Abbreviations: Mab, monoclonal antibody; SFI, specific fluorescence intensity; SaCI, *Staphylococcus aureus*, Cowan I strain.

Antibody preparation and characterization. Antibody subclass determination was by Ouchterlony double immunodiffusion, with anti-mouse subclass-specific antisera (Cappel Laboratories, Downingtown, PA). The antibodies TS2/7 (IgG1), TS2/16 (IgG1), A-1A5, and J-2A2 were purified by precipitation in 45% ammonium sulfate and S-300 column chromatography. Purified antibodies were labeled with ^{125}I, by using chloroglycoluril (IODOGEN, Pierce Chemical, Rockford, IL) (10).

Iodination and immunoprecipitation of cell surface proteins. Cells were labeled with ^{125}I by using chloroglycoluril, and then 1% Triton X-100 or Nonidet P-40 cell lysates were used for immunoprecipitation as described (1, 9). Immune complexes were adsorbed onto *Staphylococcus aureus* Cowan I strain (SaCI) and 100 μl of the Mab 187.1 (SaCI-binding anti-mouse κ-chain (11)] was included for mouse Mab that did not directly bind to SaCI. Immunoprecipitates were analyzed by $NaDodSO_4$/polyacrylamide gels (7 to 8%) and the protein standards myosin (200,000), phosphorylase b (92,500), and albumin (69,000) were used to estimate unknown m.w..

T cell activation. Phytohemagglutinin-P (PHA) (Difco, Detroit, MI) diluted 1/1000 was used to stimulate resting adherent cell-depleted peripheral blood T cells as described (1, 2). Alloantigen-stimulated peripheral blood T cells were obtained by co-culturing with irradiated B lymphoblastoid cell lines as described (1). For long-term culture, IL 2 (1 to 2 U/ml) was added two times per week (after the second week) and alloantigen was added one time per week. Culture supernatant from the T cell line JURKAT (12) was used as the source of IL 2.

After Ficoll-Hypaque separation, adherent cells (monocytes) were removed from the mononuclear cell fraction by adsorption onto plastic flasks (1 hr at 37°C), and then were eluted from the plastic after incubation at 0°C. After Ficoll separation, red blood cells were used without further preparation. Granulocytes were obtained after removal of red blood cells by two cycles of hypotonic lysis (45 sec in 0.2× PBS). Platelets were obtained from peripheral blood by centrifugation at 3000 × G in the presence of 5 mM EDTA after removal of mononuclear cells at 1000 × G. Thymocytes were isolated from human thymic tissue (removed during infant cardiac surgery) by passage through a wire screen and were filtered through cotton. T cell lines used in Table I originated from T lymphoblastic leukemias except for HUT-102 and HUT-78, which originated from patients with mycosis fungoides or Sezary syndrome (13). B lymphoblastoid lines are from Epstein Barr virus (EBV) transformation of normal B cells.

Source of human tissue. Specimens of human thymus, tonsil, lymph node, liver, kidney, lung, and skin were obtained from surgical procedures performed for diagnostic and therapeutic purposes at the Massachusetts General Hospital. Blocks of tissue were frozen in OCT compound (Ames Co., Division of Miles Laboratory, Elkhart, IN) and were stored at −70°C.

Immunoperoxidase procedure. Tissue sections were stained by the avidin-biotin complex method (14, 15). Acetone-fixed, 4-μm thick sections were incubated with optimal dilutions of the Mab TS2/7 and TS2/16 or the control Mab P3 for 30 min at room temperature. Subsequently, the sections were incubated with a biotinylated horse anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA) diluted 1/200 for 30 min and then with avidin-biotinylated peroxidase complexes (Vector Laboratories), diluted 1/120 for 45 min. Each incubation was followed by washes in three changes of PBS, pH 7.3. The sections were incubated in a solution of 0.1 M acetate buffer, pH 5.0, containing 3-amino-9-ethyl-carbazole (Aldrich Chemical Co., Milwaukee, WI), dimethylformamide, and hydrogen peroxide to develop a read reaction product. The reaction was terminated by washing with the acetate buffer. The sections were counterstained with Gill's triple strength hematoxylin (Lerner Laboratories, New Haven, CT). The sections were coverslipped with Gelvatol (Monsanto, St. Louis, MO).

RESULTS

Comparison of the protein complexes recognized by the Mab A-1A5, TS2/16, and TS2/7. The Mab A-1A5 has been used to define novel differences in high m.w. antigens on activated and resting T cells (1). New Mab (TS2/16 and TS2/7) have now been generated that can be used to support and extend the previous results. The Mab TS2/16 immunoprecipitated ^{125}I-labeled proteins of 210,000, 165,000, and 130,000 m.w. from a long-term anti-HLA-DR CTL line (Fig. 1, lane c). These proteins bore a striking resemblance to those recognized by the previously described Mab A-1A5 (1) (Fig. 1, lane a). The Mab

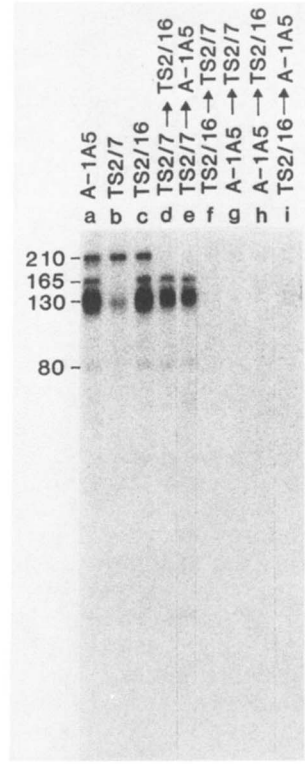

Figure 1. Comparison of proteins immunoprecipitated by TS2/7, TS2/16, and A-1A5. An ^{125}I-labeled extract from long-term CTL cells was immunoprecipitated with A-1A5, TS2/7, and TS2/16 (lanes a–c). For preclearing experiments (lanes d–i), the ^{125}I-labeled extract was preincubated with the indicated antibody and the immunoadsorbent SaCI two times to remove all reactivity completely with that antibody. Then incubation with a second antibody (*arrow*) was carried out to immunoprecipitate proteins still present in the extract.

TS2/7 (Fig. 1, lane b) immunoprecipitated a prominent protein band at 210,000 m.w., and a weaker protein band at 130,000 m.w., which appeared to have some relationship to those recognized by TS2/16 and A-1A5. When TS2/7-reactive proteins were removed from an ^{125}I-labeled extract by a pre-immunoprecipitation step, a second immunoprecipitation with TS2/16 (lane d) or A-1A5 (lane e) yielded only the 165,000 m.w. protein and a portion of the 130,000 m.w. protein. In the reciprocal experiments. TS2/16 (lane f) or A-1A5 (lane g) pre-immunoprecipitation removed all of the TS2/7 reactivity. Thus, TS2/7-reactive proteins are a subset of those reactive with A-1A5 and TS2/16. Pre-immunoprecipitation with A-1A5 removed all TS2/16 reactivity (lane h) and TS2/16 removed all A-1A5 reactivity (lane i). Thus, these two Mab are presumed to react with identical protein complexes. The same results were obtained with the use of CTL directed against HLA-DR (Fig. 1) or HLA-A, B (not shown). In contrast, immunoprecipitates from JY, which was used as a stimulator/feeder cell for CTL growth, showed no detectable bands with TS2/7 and only a weak 130,000 m.w. protein with TS2/16 (not shown).

In addition to 210,000, 165,000 and 130,000 m.w. proteins, A-1A5 and TS2/16 immunoprecipitated variable quantities of a 80,000 m.w. protein, usually weakly expressed (Fig. 1, lanes a, c, d, and e). The 80,000 m.w. protein was not detectable in TS2/7 immunoprecipitations (Fig. 1).

Competitive binding studies with TS2/16 and A-1A5 on long-term CTL lines showed that unlabeled A-1A5

blocked binding of ^{125}I-labeled TS2/16 (Fig. 2*A*) and unlabeled TS2/16 blocked ^{125}I-A-1A5 (Fig. 2*B*). Thus, TS2/16 and A-1A5 appear to react with similar or overlapping epitopes on the same molecular structure (s). In contrast, TS2/7 did not block ^{125}I-TS2/16 (Fig. 2*A* or ^{125}I-A-1A5 (Fig. 2*B*), and binding by ^{125}I-TS2/7 was not blocked by unlabeled A-1A5 or TS2/16 (Fig. 2*C*). Thus TS2/7 appears to react with an epitope distinct from the A-1A5 and TS2/16 epitope (Fig. 2), which is consistent with the TS2/7 epitope being on a subset of the proteins in the complex recognized by A-1A5 and TS2/16 (Fig. 1).

Distribution of A-1A5 and TS2/7 reactivity on lymphoid cells including activated T cells. Previous results have shown that among hematopoietic cells, the 210,000 m.w. protein in the A-1A5-reactive complex appears to be specific for mitogen or alloantigen-activated T cells (1). Because TS2/7 reactivity includes the 210,000 m.w. protein, it was hypothesized that TS2/7 may react with an activation-specific subset of the A-1A5-reactive complex. To test this hypothesis, a variety of lymphoid cells including activated T cells were analyzed for TS2/7 and A-1A5 reactivity.

Analysis of Mab binding by fluorescence-activated cell sorter (FACS) showed that A-1A5 was strongly present on all of the T cell lines examined (Fig. 3*A–C*, *H*) and weakly on the B cell line MAJA (Fig. 3*D*), as expected from previous results (1). In contrast, TS2/7 was present on HUT-102 (Fig. 3*H*) but was absent from the other cell lines (Fig. 3*A–D*). Further analysis of TS2/7 binding showed negligible reactivity with unstimulated peripheral blood T cells (Fig. 3*E*), weak binding to cells stimulated with alloantigen for 7 days (Fig. 3*F*), and more pronounced binding to a long-term culture carried for 4 wk (Fig. 3*G*). By comparison A-1A5 binding was strong on resting T cells (Fig. 3*E*) and was greater than TS2/7 binding on activated T cells (Fig. 3*F* and *G*). Significantly, the TS2/7 FACS profile (Fig. 3*G*) showed a single broad peak rather than distinct positive and negative peaks, indicating that TS2/7 may generally be found on all of the alloactivated cells, and is not limited to subpopulations. In this regard, long-term CTL lines that had been shown to contain ~50% OKT4-positive and ~50% OKT8-positive cells by FACS analysis (data not shown) at the same time still showed a single homogeneously positive peak for TS2/7 binding.

The results in Figure 3 were quantitated and extended to additional cell types (Table I). The Mab B1.49.9 directed against the IL 2 receptor (4) is included in Table I so that TS2/7 binding could be compared to a known marker for

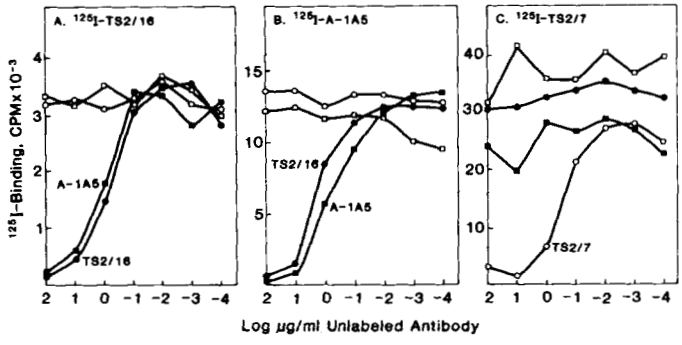

Figure 2. Competitive inhibition of binding by ^{125}I-TS2/16 (*A*), ^{125}I-A-1A5 (*B*), and ^{125}I-TS2/7 (*C*) to long-term CTL cells by unlabeled TS2/16 (●), A-1A5 (■), TS2/7 (○), and J-2A2 (□).

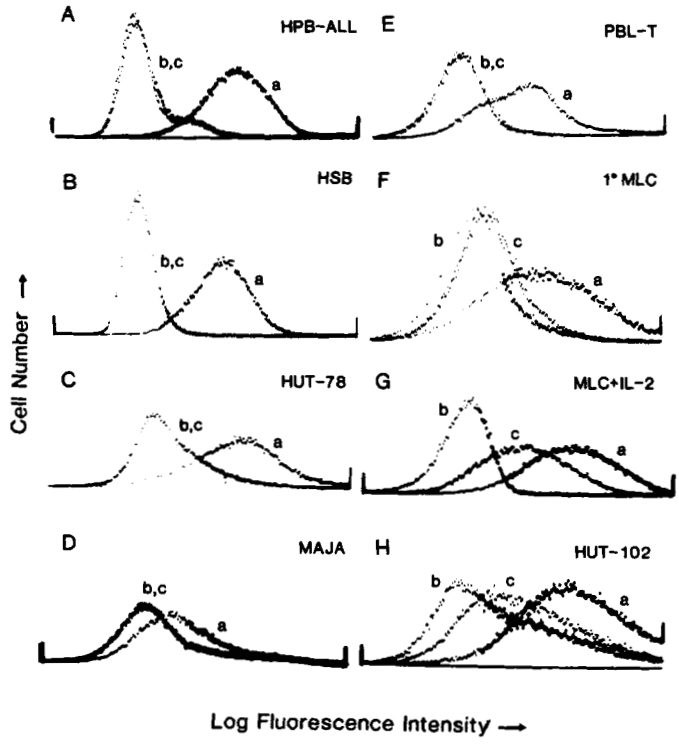

Figure 3. Flow cytometric analysis of A-1A5 and TS2/7 binding to various lymphoid cells. Analyses of cells labeled with A-1A5 (a), the negative control P3 (b), and TS2/7 (c) were carried out by using a FACS II (Becton Dickinson). FITC-labeled goat anti-mouse Ig heavy and light chains (Cappel) was used as the fluorescent second antibody, and 50,000 cells were counted for each picture. Results with A-1A5 (IgG2b) and TS2/16 (IgG1, not shown) were similar, indicating that the second antibody had comparable reactivity for these two antibody subclasses. Thus, results with TS2/7 (IgG1) can be compared directly to A-1A5 results.

activated T cells. The TS2/7 Mab did not bind significantly to any of the B cell lines listed nor to any of the T cell lines, except HUT-102. Also TS2/7 showed minimal binding to thymocytes and resting peripheral blood T cells. A low percentage of cells (10 to 22%) appeared to be positive for TS2/7 binding after 4-day PHA stimulation or alloantigen stimulation (7-day 1°MLC). These cells were only weakly positive, as reflected in the specific fluorescence intensity (SFI, proportional to antigen sites per cell), which remained at less than 0.1 units. In longer term cultures, TS2/7 binding was greatly increased (see also, Fig. 3*G*) as reflected by SFI (0.57) and percentage of cells (73%). Notably, all cell cultures with significant TS2/7 binding (including HUT-102) had the properties of activated T cells insofar as they expressed the IL 2 receptor, as measured by B1.49.9 binding. TS2/7 binding, however, was greatly amplified in long-term culture compared to short-term activated T cells, and conversely, IL 2 receptor expression was diminished in long-term culture and highest in short-term culture.

Binding of A-1A5 was elevated on short-term activated T cells and twofold increased in SFI on the long-term activated population, relative to unactivated T cells. Also, A-1A5 binding was generally about sixfold higher on T cell lines when compared to B cell lines. Binding by A-1A5 was always greater than TS2/7 binding (Table I), consistent with the concept that TS2/7 reacts with a subset of the proteins recognized by A-1A5. TS2/7 and A-1A5 immunoprecipitations from a variety of lymphiod cell sources were carried out to test further the hypothesis that TS2/7 reacts with activation-specific components

TABLE I

FACS analysis of binding of A-1A5, TS2/7, and B1.49.9 to lymphoid cells

Cell Type	Cells[a]	Mab Binding					
		A-1A5		TS2/7		B1.49.9	
		SFI[b]	%+[c]	SFI	%+	SFI	%+
T lines	MOLT-4 (1)	3.6	73	0	0	0	0
	HSB (3)	2.8	95	0.01	0.7	0	1.0
	CEM (1)	2.2	79	0	0	0	0
	JURKAT (1)	2.2	92	0	0.6	0	0.4
	HPB-ALL (2)	3.4	89	0.04	0.5	0.06	0
	JM (1)	1.9	97	0	0	0	0
	SKW-3 (2)	2.5	95	0.03	2.3	0	0
	HPB-MLT (1)	2.0	96	0.04	0	0	0
	HUT-78 (2)	4.8	72	0	1.6	0	0.4
	HUT-102 (3)	4.7	72	0.89	32	26.2	83
B lines	JY (1)	0.32	39	0	0	0	2
	HOM-2 (1)	0.79	67	0	0	0	1
	BJAB (1)	0.97	30	0	0	0	1
	MAJA (2)	0.37	45	0	0.1	0.02	5
	IDF (1)	0.77	57	0	0	0	0
T cells and activated T cells	Thymocytes (1)	0.11	25	0.01	5.4	0.08	16
	PBL-T (3)	0.78	77	0.02	5.4	0.01	5.6
	4-day PHA (2)	0.84	70	0.03	10.1	3.8	75
	1° MLC (3)	1.10	71	0.09	22	0.92	54
	4 wk MLC (1)	1.60	93	0.57	73	0.14	42

[a] Number in parentheses after cell name is number of replicate experiments used to derive mean data values.

[b] SFI is a linear parameter and was calculated from the channel number corresponding to peak log fluorescence intensity relative to negative control fluorescence intensity as described (16). Negative control antibodies were P3 (IgG1) and J-2A2 (IgG2b). In the unusual cases of an asymmetrical fluorescent peak, the channel number corresponding to median fluorescence intensity was used to calculate SFI.

[c] Percent of cells binding specific Mab (SP) to the right of the negative control (C) was calculated from $\frac{\text{SP-C}}{\text{Total} - \text{C}} \times 100$ for 10,000 to 50,000 total cells counted. This number is an underestimation of the "percent positive" because a 100% weakly positive population with a homogeneous symmetrical peak overlaps with the negative control to give "false-negative" cells.

of the A-1A5 complex. When the activation-specific 210,000 m.w. protein was present within the A-1A5 complex on long-term CTL (Fig. 4*A*, lane d) or HUT-102 (Fig. 4*B*, lane c), TS2/7 reactivity was also present (210,000 and 130,000 m.w.) (Fig. 4*A*, lane b; Fig. 4*B*, lane b). When the 210,000 m.w. protein was absent, however, as in unactivated peripheral blood lymphocytes (Fig. 4*A*, lane c) or T cell lines (Fig. 4*C*, lanes a, c, e, and g), then TS2/7 reactivity was absent (Fig. 4*A*, lane a; Fig. 4*C*, lanes b, d, f, and h). On unactivated peripheral blood T cells (Fig. 4*A*, lane c), HUT-78 (not shown), or thymocytes (not shown), A-1A5 reacted with only a single protein (130,000 m.w.), but on the other T cell lines (Fig. 4*C* and *D*), a 160,000 to 165,000 m.w. protein was co-precipitated with the 130,000 m.w. protein. Figure 4*D* is included because it illustrates a more distinct gel separation of the 160,000 and 130,000 proteins than seen in Figure 4*C*. The A-1A5 but not the TS2/7 immunoprecipitations from the T cell lines in Figure 4*B*, *C*, and *D* also showed variable expression of an 80,000 m.w. protein, as noted in Figure 1.

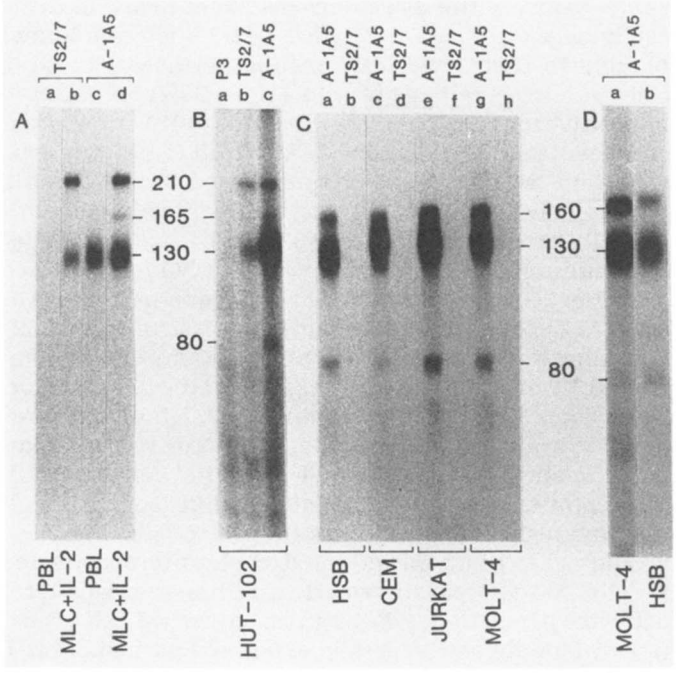

Figure 4. ^{125}I-labeled proteins immunoprecipitated by A-1A5 and TS2/7 from T cells and T lymphoblastoid cell lines. *A*, ^{125}I-labeled extracts from unstimulated peripheral blood lymphocytes (PBL) and alloantigen-stimulated T cells (MLC + IL 2, cultured for 3 wk including 1 wk with exogenous IL 2) were immunoprecipitated with A-1A5 and TS2/7. *B–D*, immunoprecipitations from various T lymphoblastoid cell lines are shown. *D*, included to show better resolution of the two protein bands immunoprecipitated from MOLT-4 and HSB as shown in *C*.

Reactivity of TS2/7 and A-1A5 on non-lymphoid hematopoietic cells. In addition to lymphoid cells, cells and cell products in the myeloid lineage were analyzed for A-1A5 and TS2/7 reactivity. FACS analyses similar to those shown in Figure 3 are summarized in Table II. Reactivity

TABLE II

Binding of TS2/7 and A-1A5 to non-lymphoid cells and cell lines

Cell Population	Mab Binding[a]			
	A-1A5		TS2/7	
	SFI	%+	SFI	%+
Adherent cells	2.4	85	0.01	1.6
Granulocytes	0	0	0	0
Platelets	0.23	44	0	0
Red blood cells	0	0	0	0
K-562	4.2	79	0	0
ML-1	4.9	98	0	0
HL-60	3.5	90	0	0
U-937	6.2	91	0	0

[a] Binding and FACS II analysis was carried out as described for Figure 4 and Table I.

with TS2/7 was not expressed by granulocytes, platelets, red blood cells, and the four myeloid cell lines listed. TS2/7 was also generally unreactive with adherent cells, although sometimes variable weak binding was observed, perhaps due to Fc receptor binding. In contrast to results with TS2/7, A-1A5 was prominent on K-562 (an erythroid precursor), U-937 and ML-1 (both in the monocytoid lineage), and HL-60 (in the granulocyte and monocyte lineage) and weakly reactive with platelets. Granulocytes and red blood cells were negative for A-1A5 and thus are the only hematopoietic cells so far known not to express this reactivity. Interestingly, induction of HL-60 with tetradecanoyl phorbol acetate at 5 ng/ml for 3 days caused a loss of A-1A5 reactivity (results now shown), perhaps consistent with HL-60 maturation in the granulocyte pathway.

Analysis by immunoprecipiation confirmed that TS2/7 was absent from the four myeloid cell lines (Fig. 5*A*) and platelets and adherent cells (Fig. 5*B*). The single band immunoprecipitated by A-1A5 from ML-1, HL-60, and U-937 (Fig. 5*A*) was significantly elevated in m.w. (150,000 to 160,000) compared to the single band (130,000) seen from K-562 (Fig. 5*A*, lane j) or PBL-T (Fig. 4*A*, lane c). The single A-1A5-reactive band immunoprecipitated from peripheral blood monocytes was also slightly higher in m.w. (~140,000) (Fig. 5*B*, lane d). Among materials in the myeloid lineage, platelets appeared to be unique in that two distinct bands (165,000 and 140,000 m.w.) were co-precipitated by A-1A5. In this regard, platelets showed similarity to the T cell lines in Figure 4*C* and *D*. Weak 80,000 m.w. proteins were associated with A-1A5 immunoprecipitations from adherent cells, U-937, HL-60, and ML-1, but were only seen upon longer autoradiographic exposure.

Reactivity of TS2/7 and TS2/16 on human tissue sections. The results of staining frozen sections of human tissue with TS2/7 and TS2/16 by the avidin-biotin complex immunoperoxidase method (14, 15) are presented in Table III and Figure 6. Sections stained with P3 (control Mab) showed no staining. No TS2/7 staining of thymocytes or lymphocytes (Fig. 6*A*) was seen. Blood vessel walls (but not endothelial cells), fibroblasts, smooth muscle, and connective tissue stained with TS2/7 in all or most organs. The kidney showed TS2/7 staining of some of the mesangial cells and the mesangial matrix (Fig. 6*C*). Liver sinusoids and lung alveolar lining cells also were positive, whereas most of the other tissue areas listed in Table III were TS2/7-negative. In contrast to the very limited distribution for TS2/7, TS2/16 had a much wider distribution of staining, including all areas stained by TS2/7. TS2/16 stained most T cells, germinal center B cells (Fig. 6*B*), and thymocytes. It also stained a variety of other cells (Fig. 6*D*), such as smooth muscle, epidermis, bile duct epithelium, glandular epithelium, chondrocytes, peripheral nerves, and endothelial cells. In addition to cells, both TS2/7 and TS2/16 stained extracellular material in connective tissue and kidney mesangial matrix. TS2/16 but not TS2/7 stained kidney tubular basement membranes.

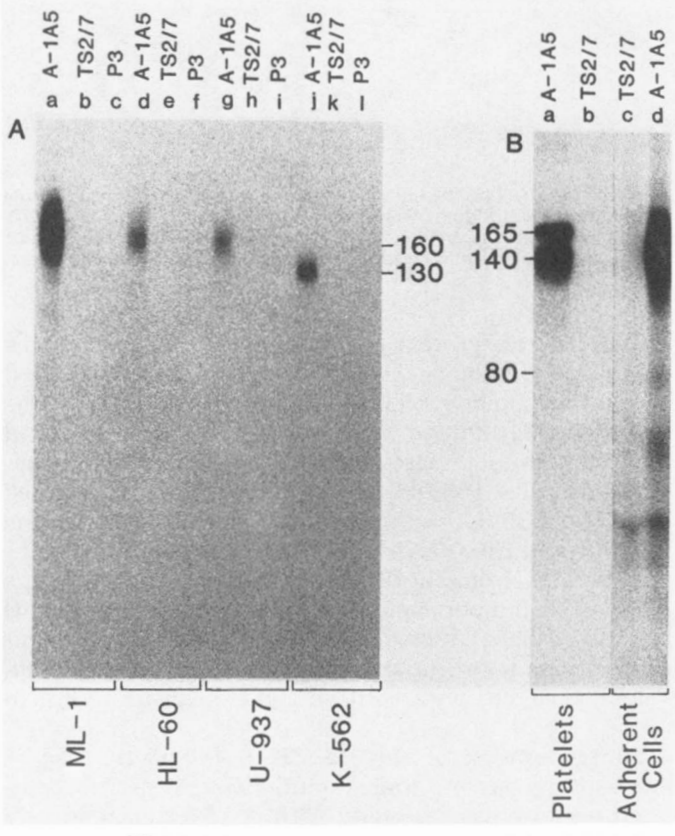

Figure 5. ^{125}I-labeled proteins immunoprecipitated by A-1A5 from non-lymphoid hematopoietic cells. *A*, the monocytoid cell lines ML-1 and U-937, the promyelocytic cell lines HL-60, and the erythroleukemic cell line K-562 were immunoprecipitated with TS2/7, A-1A5, and the control antibody P3. *B*, extracts from platelets and adherent cells (monocytes, greater than 90% OKM1-positive) were immunoprecipitated with A-1A5 and TS2/7.

DISCUSSION

An Mab (TS2/7) has been produced that defines a novel protein structure found on activated T cells. Unactivated

TABLE III

Immunoperoxidase staining of tissue sections

Tissue		TS2/7	TS2/16
Thymus	Cortical and medullary thymocytes	–	+
	Blood vessels	+	+
Lymph node	Germinal center cells	–	+ (most)
	T cells	–	+ (most)
	Blood vessels	+	+
	Connective tissue	+	+
Liver	Sinusoids	+	+
	Lymphocytes	–	+
	Bile ducts	–	+
	Liver cell membranes	weak	weak
Kidney	Glomeruli: epithelial cells	–	+
	endothelial cells	–	+
	mesangial cells	+ (some)	+
	mesangial matrix	+	+
	Tubules: basement membrane	–	+
	distal and collecting duct epithelium	–	+
	others	–	–
	Interstitial stromal cells	+(?)	+(?)
Lung	Bronchus: epithelium	–	+
	glands	–	+
	cartilage	–	+
	smooth muscle	+	+
	Blood vessels	+	+
	Peripheral nerve	–	+
	Elastic tissue	–	–
	Alveolar lining cells	+	+
	Lymphocytes	–	+
Skin	Epidermis, basal layer	–	++
	Epidermis, spinous layer	–	+
	Blood vessels	+	+
	Peripheral nerves	–	+
Skin	Smooth muscle	+	+
	Eccrine gland	–	+
	Fibroblasts	+	+

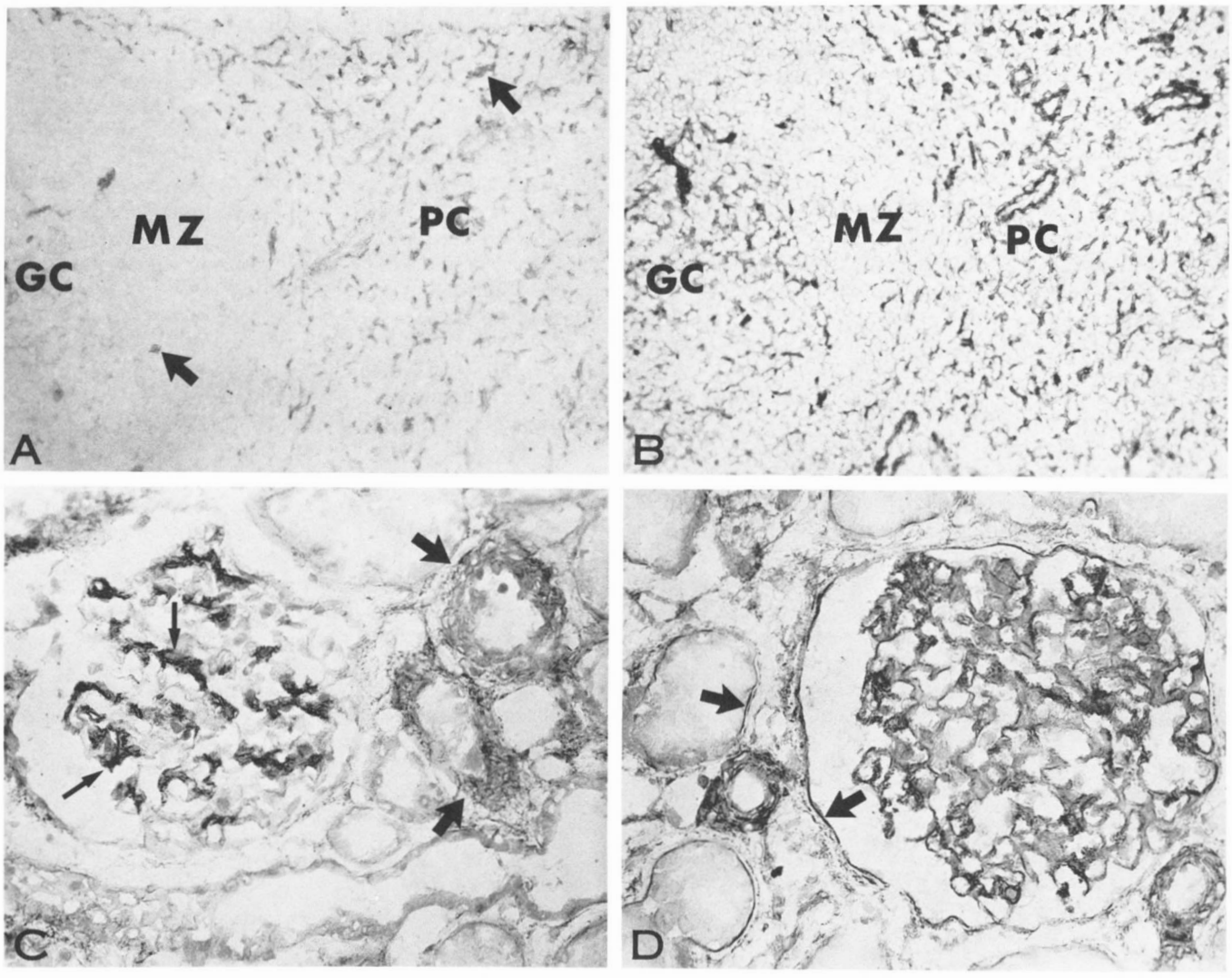

Figure 6. Human lymph node was stained with TS2/7 (*A*) or TS2/16 (*B*). The secondary follicle, including the mantle zone (MZ) and the germinal center (GC), and the paracortical area (PC) and blood vessels (arrows) are indicated. Human kidney was stained with TS2/7 (*C*) and TS2/16 (*D*). Staining is indicated (*C*) in mesangial cells and matrix (*thin arrow*), blood vessels (*thick arrow*), and (*D*) in the basement membrane of Bowman's capsule and of tubules (*arrows*). Staining was by immunoperoxidase with hematoxylin counterstain (see *Materials and Methods*) and magnification is ×256 (*A* and *B*) and ×400 (*C* and *D*).

T cells, T cell lines (except HUT-102), and all other hematopoietic cell types tested failed to express TS2/7 reactivity. The protein complex (210,000 and 130,000 m.w.) immunoprecipitated by TS2/7 is a subset of the family of proteins (210,000, 165,000, and 130,000 m.w.) immunoprecipitated by A-1A5 from activated T cells. Results from cell surface binding assays confirmed that cells positive for TS2/7 are a subset of the broader range of cells positive for A-1A5 (or TS2/16) both for hematopoietic cells and other tissue (Tables I to III). Thus, the availability of TS2/7 now provides an important complement to A-1A5 for specifically analyzing the 210,000/130,000 T activation complex alone. By using this tool, an absolute positive correlation was demonstrated between cells bearing the 210,000 m.w. antigen and cells binding to TS2/7. These results include TS2/7 binding to HUT-102 and the immunoprecipitation of 210,000 (and 130,000) m.w. proteins from HUT-102. In the previous study (1), HUT-102 radiolabeling had been weak and the activation-related proteins had not been seen by immunoprecipitation.

From the results in Figure 1, it is hypothesized that the A-1A5-reactive protein complex may be composed of a TS2/7-reactive dimer (210,000 and 130,000 m.w.) as well as another dimer (165,000 and 130,000 m.w.). In this regard, the 210,000 m.w. protein has always been found to be co-immunoprecipitated with the 130,000 m.w. protein. Also, the 165,000 m.w. protein is co-precipitated with the 130,000 m.w. protein, independent of the 210,000 and 130,000 m.w. proteins. The former complex is separable from the 210,000/130,000 m.w. proteins both by immunoprecipitation (after preclearing; Fig. 1) and by cell distribution (Fig. 4). The hypothesized subunit associations for these two dimers are probably non-covalent, because they are known not to be disulfide linked (1).

The generation of the Mab TS2/16 and the use of competition binding and immunoprecipitation to demonstrate its cross-reactivity with A-1A5 independently confirms and supports results obtained with A-1A5. A summary of the distribution of the components of the A-1A5-TS2/7 antigen family as related to T cell maturation and cell line origin is shown in Figure 7. As indicated, immature thymic T cells mature to PBL-Ts while bearing

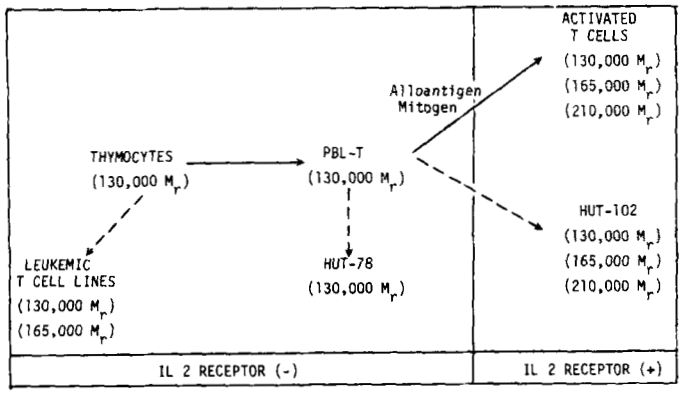

Figure 7. Expression of A-1A5-reactive antigens correlated with T cell maturation and T cell line origin.

only low amounts of the 130,000 m.w. protein. Then upon activation, the 165,000 and 210,000 m.w. proteins are acquired, and thus associated with the A-1A5-reactive 130,000 m.w. protein. In this regard, recent evidence suggests that the 165,000 m.w. protein may arise from a gene on a different chromosome than the gene for the A-1A5 antigenic determinant (17). The 165,000 m.w. protein appears to be less specific for T cell activation than the 210,000 m.w. protein in that it also appears on leukemic cell lines originating from thymic T cell precursors. Such cell lines are thus phenotypically different from both resting PBL-T cells and thymocytes, as well as activated mitogen or alloantigen-stimulated PBL-Ts.

The T cell line HUT-102, derived from a patient with Sezary syndrome (13), has phenotypic similarities to mitogen or alloantigen-stimulated T cells. The presence of human T leukemia virus in HUT-102 (18) is associated with the expression of the IL 2 receptor (3, 19, 20) and both the secretion and utilization of IL 2 (19). On the other hand, the T cell line HUT-78, derived from a patient with mycosis fungoides (13), appears not to use IL 2 (3) or express the IL 2 receptor, and also has only the 130,000 m.w. protein characteristic of unactivated cells. Although the appearance of the 210,000 protein correlates with the presence of IL 2 receptor on T cells, the latter structure (50,000 to 55,000 m.w. (3, 4)) is clearly unrelated biochemically.

On non-lymphoid hematopoietic cells, TS2/7 reactivity was totally absent and A-1A5 generally reacted with only a single protein of 130,000 to 160,000 m.w. This variability in size is hypothesized to be due to glycosylation differences, causing an increased size above the 130,000 m.w. seen on lymphoid cells. In this regard, the A-1A5-reactive protein from HL-60 (150,000) was larger than that from K-562 (130,000), which may be analogous to the 4F2 antigen (21) on HL-60 (91,000) compared to K-562 (85,000). The size difference in the latter antigen is known to be due to glycosylation. Among all non-lymphoid hematopoietic cells tested, the only source expressing multiple bands was platelets (165,000 and 140,000 m.w.). It is not yet clear if this fact relates to the level of maturation or activation of platelets compared to other myeloid components. The variable appearance of an 80,000 m.w. protein in A-1A5 but not TS2/7 immunoprecipitations from a variety of sources suggests that this may be either a precursor or a proteolytic cleavage product that bears the A-1A5 epitopes. In this regard, the 80,000 m.w. protein might not be a glycoprotein because it has not been co-precipitated with the other proteins (210,000, 165,000 and 130,000) after ricin or lentil lectin enrichment for glycoproteins (results not shown).

On non-hematopoietic tissues, the presence of a subset of tissues with simultaneous expression of both TS2/7 and TS2/16 epitopes suggests that the protein structures on those tissues are related to those found on T cells and are not merely unrelated cross-reactive proteins.

All of the components of the A-1A5-reactive complex, including the TS2/7-reactive subcomplex (210,000 and 130,000 m.w.) have been shown to be unrelated to the human T200 antigen (1). Also, the TS2/7-reactive antigen complex is different than the human LFA-1 antigen (9, 22, 23), which is a heterodimer of 177,000 and 95,000 m.w. and is preent on unactivated and B lymphocytes. The A-1A5-reactive proteins also appear to be unrelated to T305 (24), another T cell activation-associated protein. The latter protein of 160,000 m.w. is not reported to have multiple associated proteins, and has a more limited tissue distribution than the A-1A5-reactive determinant.

At present, a major significance of the discovery of new T cell activation proteins, and the generation of an Mab (TS2/7) against one of them, lies in the specificity among hematopoietic cells for activated T cells. The majority of other antigens [such as the transferrin receptor, 4F2, or HLA-DR (2)] known to appear on activated T cells also are found on other rapidly growing hematopoietic cell types. The IL 2 receptor appears to be relatively specific for activated T cells, but the level of that antigen appears to diminish at about the time that TS2/7-reactive antigen starts to become prominent (Table I). Thus, the latter antigen may provide a unique capability as a marker for long-term activated T cells.

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