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Immunochemical characterization of human TL-like (T48) and Ly 1-like (T72) glycoproteins using two-dimensional polyacrylamide gel electrophoresis

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SUMMARY

Xenoantisera, designated AT48 and AT72, were developed by immunizing rabbits with human thymus cell membrane and guinea-pigs with a T-cell glycoprotein purified from leukaemic T-cell membrane. Whereas AT48, after appropriate absorption, reacted exclusively with the majority of thymocytes (mainly cortical thymocytes) among normal lymphoid populations, AT72 reacted with virtually all of the thymus and T cells but not with B cells. Thymocytes, which were strongly reactive with AT72, existed in the thymic medulla, but cortical cells were also very weakly reactive with AT72. When cultured T-cell lines, all of which were derived from patients with T-cell-type acute lymphatic leukaemias, were tested for their reactivities with AT48 and AT72 by immunofluorescence, we found that AT48 stained certain T-cell lines, whereas AT72 stained all of the T-cell lines tested so far. Immunochemical data showed that AT48 precipitated a 48K molecular weight (mol. wt) glycoprotein from ^{125}I-labelled thymus cell surface glycoproteins, which appeared to be very weakly associated with a 12K mol. wt component. These 48K and 12K mol. wt components precipitated by AT48 showed almost identical isoelectric points (pI) to those of HLA heavy chain and β_2-microglobulin respectively. AT72, on the other hand, precipitated a 72K mol. wt glycoprotein from thymus and T cells as well as from leukaemic T cells. A less prominent 65K mol. wt glycoprotein was also precipitated by AT72 from thymus and T cells but not from leukaemic T cells. These two components showed almost identical pI ranging approximately from 4 to 7, and this marked charge heterogeneity observed was reduced by neuraminidase treatment, suggesting that it reflects the heterogeneity in sialylation of this molecular species. We concluded from these data that AT48 and AT72 used in this work could detect human homologues of mouse TL and Ly 1 antigens respectively.

INTRODUCTION

Human lymphocytes display distinctive functional and surface properties which enable us to classify them into T and B cells. For instance, human T cells are usually identified by their ability to form spontaneous rosettes with sheep erythrocytes (E), whereas B cells possess easily detectable immunoglobulins (Ig) on their cell surfaces (Lay *et al.*, 1971; Wilson & Nossal, 1971). Furthermore, human T cells express unique differentiation xenoantigens as defined by xenoantisera (Balch & Ades, 1979) or by monoclonal antibodies raised against human T cells (McMichael & Bastin, 1980).

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The immunochemical characterization of these antigenic markers clearly represents an important step in understanding the molecular basis of T-cell differentiation and the regulation of its function. In the present study, we have employed xenoantisera raised against human thymus cell membrane (Ishii *et al.*, 1980; Fujimoto *et al.*, 1981) or against a 72-kilodalton glycoprotein (T72) isolated from T-cell-type leukaemic cell membrane by sequential chromatographic procedures (Ishii *et al.*, 1981). Antigens isolated from radiolabelled cell surface glycoproteins of human lymphocytes by indirect immunoprecipitation with these reagents have been analysed by one- and two-dimensional polyacrylamide gel electrophoresis (1-D and 2-D PAGE), where the antigens can be separated from each other on the basis of their isoelectric point (pI) and/or molecular weight (mol. wt) characteristics (O'Farrell, 1975).

MATERIALS AND METHODS

Cell suspension. Cells obtained from thymuses and tonsils were washed three times with phosphate-buffered saline (PBS) and suspended in RPMI 1640 medium. Cell clumps were removed by squeezing them between glass slides and filtering through cotton gauze. Dead cells were removed by centrifugation on a Ficoll–Conray gradient ($\rho = 1{\cdot}078$) at 250 ***g*** for 20 min. Peripheral blood lymphocytes (PBL) and leukaemic cells separated from the blood on a Ficoll–Conray gradient were also suspended in RPMI 1640.

Separation of T and B cells. Tonsil lymphocytes were separated into T-cell and B-cell-enriched fractions by the E-rosette depletion technique described previously (Fujimoto *et al.*, 1981). Purity of those lymphocytes in each fraction was 80–90% for T cells and more than 98% for B cells.

Antisera. Rabbit anti-human thymus sera were prepared by immunizing the animals with crude cell membrane preparation of human thymocytes (Ishii *et al.*, 1980). The antiserum was absorbed extensively with human red cells, liver homogenate and tonsil lymphocytes. Since this absorbed antiserum precipitated a single glycoprotein with a molecular weight of 48K daltons (T48) from radiolabelled thymus cell glycoproteins (Ishii *et al.*, 1980; Fujimoto *et al.*, 1981), it is referred to as anti-T48 serum (AT48) in this work. Guinea-pig antiserum to the 72K mol. wt glycoprotein isolated from T-cell-type leukaemic cell membrane was also produced by the method described previously (Ishii *et al.*, 1981), absorbed with tonsil B cells and cultured Raji cells, and was used as anti-T72 serum (AT72) in this study. Rabbit anti-HLA (A, B, C) serum was kindly provided by Dr Katagiri, Asahikawa Medical School.

Immunofluorescence. Binding of antibody to antigens on cell surfaces was studied by indirect immunofluorescence as described previously (Ishii, Ueno & Kikuchi, 1974). $F(ab)_2$ fragments of FITC-labelled goat antibody to rabbit or guinea-pig IgG (Cappel Laboratories, Cochranville, Pennsylvania) were used as the second reagent. Each stain was applied to viable cells at 4°C for 30 min. The slides were examined under a Leitz Ortholux II microscope with an incident u.v. illuminator and the percentage of cells with surface fluorescence was counted.

Radioiodination. Freshly prepared lymphocytes were labelled with ^{125}I using lactoperoxidase (Sigma Chemical Co., St Louis, Missouri) according to the method previously described (Ishii *et al.*, 1980). The labelled cells were solubilized with 2% Lubrol-PX (Nakarai Chemicals, Kyoto, Japan) in PBS at 4°C for 1 hr. After centrifugation at 4,000 ***g*** for 15 min, the supernatant of cell lysate was collected, passed through a Millipore 0·45-μm membrane and applied to a Sepharose 4B column coupled with *Lens culinaris* haemagglutinin (LcH; Pharmacia Fine Chemicals, Uppsala, Sweden). The column was washed extensively with 0·1 M Tris-HCl buffer (pH 8·0) containing 0·2% Lubrol-PX and 0·5 M NaCl, and the bound material was eluted from the column with 4% methyl-D-mannoside in the same Tris buffer (Hayman & Crumpton, 1972). The bound material was dialysed against PBS containing 0·2% Lubrol-PX and subjected to radioimmunoprecipitation (RIP) experiments.

RIP. Aliquots of the labelled glycoprotein preparation (approximately 120,000 c.p.m.) were mixed with 10 μl of the antiserum or preimmune normal serum and were incubated at 4°C for 20 hr. Antigen–antibody complexes were precipitated by adding 100–200 μl of 10% *Staphylococcus aureus* Cowan I (SACI; The Enzyme Center Inc., Boston, Massachusetts) to the mixture (Cullen &

Schwarz, 1976). After 30 min at 37°C, the bacteria were washed three times with PBS containing 0·2% Lubrol-PX, and immune complexes bound to the bacteria were dissociated by heating them at 100°C for 4 min in 150 μl of 50 mM Tris–HCl (pH 7·0), containing 2% sodium dodecyl sulphate (SDS) and 5% 2-mercaptoethanol (2-ME) (SDS sample buffer), or in 50 μl of 9·5 M urea, containing 0·2% Nonidet P-40 (NP-40), 5% 2-ME and 6% Pharmalyte (pH 5–8, 2%; pH 3–10, 4%; Pharmacia Fine Chemicals, Uppsala, Sweden) (IEF sample buffer). Each sample solubilized in SDS or IEF sample buffer was applied to either one-dimensional SDS-PAGE or 2-D PAGE, as described below.

SDS-PAGE. SDS-PAGE (0·1% SDS and 10% polyacrylamide slab gel) was performed by Laemmli's method (Laemmli, 1970). The gel was electrophoresed at 25 mA per plate until a bromophenol blue marker reached the end of the gel. The gel was then stained with Coomassie brilliant blue for 20 hr, destained in 7% acetic acid solution containing 5% ethanol, and dehydrated using a gel slab dryer (Bio-Rad Laboratories, Richmond, California). Radioactivity in the gel slab was visualized by autoradiographic technique (O'Farrell, 1975). The protein standards used and their molecular weights were phosphorylase B (94K), bovine serum albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), trypsin inhibitor (20·1K) and alpha-lactalbumin (14·4K).

Two-dimensional PAGE. Two-dimensional PAGE was performed by a slight modification of O'Farrell's method (O'Farrell, 1975). We used isoelectric focusing (IEF) tube gels which contained 8 M urea, 0·2% NP-40 and 6% Pharmalyte (pH 5–8, 2%; pH 3–10, 4%), instead of 9·2 M urea, 2% NP-40 and 2% Ampholines. The first-dimension separations were carried out by IEF on 2 × 70 mm tube gels, and the second-dimension separations by SDS-PAGE on 10% slab gels.

Immunoperoxidase staining. Cryostat sections of human thymuses were placed on glass slides and fixed in acetone for 1 min. The sections were dried and incubated with AT48 or AT72 for 30 min at room temperature. After washing three times with PBS, peroxidase-conjugated antibodies to rabbit or guinea-pig IgG (Cappel Laboratories, Cochranville, Pennsylvania) were placed on the tissue sections for 30 min at room temperature. The enzymatic reaction was developed with 0·05% 3,3′,5,5′-diaminobenzidine tetrahydrochloride and 0·01% hydrogen peroxide in 0·05 M Tris–HCl buffer (pH 7·6).

RESULTS

Tissue distribution of T48 and T72 antigens

The reactivity of AT48 and AT72 with various human lymphoid cell populations is shown in Table 1. Whereas AT48 reacted exclusively with the majority of human thymocytes among normal lymphoid cell populations tested, AT72 reacted with almost all thymus and T cells but not with B cells. AT72 serum reacted strongly with 15–20% of thymus cells and weakly with the remaining thymocytes. When the thymus tissue sections were stained with these antisera, we found that T48 was expressed on cortical thymocytes, whereas T72 was expressed largely on medullary thymocytes and to a much lesser extent on cortical cells (Fig. 1).

Several cultured T-cell lines, which were derived from patients with T-cell-type acute lymphatic leukaemias (T-ALL) and maintained in cultures with RPMI 1640 medium supplemented with 10% fetal calf serum, were then tested for their reactivity with AT48 and AT72 (Table 2). While AT48 reacted with three different T-cell lines (MOLT 4, P12/Ich, TALL-1), all of which had been shown to lack common ALL antigen (Koshiba, Minowada & Pressman, 1978), AT72 stained all the cultured T-cell lines thus far tested. Other non-T-cell lines including normal B-cell, Burkitt, non-T/non-B and myeloid cell lines were not stained with either AT48 or AT72 (data not shown).

To determine whether non-lymphoid tissues contain T48 and T72 antigens, AT48 and AT72 sera were absorbed with graded amounts of packed tissue homogenates of liver, kidney, lung and brain tissues, and were tested for residual reactivity on thymus cells by immunofluorescence. None of the non-lymphoid tissues tested could remove the reactivity of either AT48 or AT72 on the target cells (data not shown).

Immunochemical characterization of T48 and T72 glycoproteins

SDS-PAGE analysis performed on immunoprecipitates of AT48 with ^{125}I-labelled cell surface

Table 1. Reactivities of AT48 and AT72 with various normal lymphoid populations as defined by immunofluorescence

Cell type	AT48		AT72	
	Number of cases tested	Mean per cent fluorescence	Number of cases tested	Mean per cent fluorescence
Thymus	8	86*	2	99*
Tonsil	4	3	4	23
Blood†	2	2	3	72
Bone marrow	1	0	1	2
T-cell-rich				
Tonsil	2	2	2	82
Blood	2	2	2	94
B-cell-rich				
Tonsil	2	0	2	1
Blood	2	1	2	3

* Standard errors were less than 10%.

† Peripheral blood lymphocytes separated from the blood of normal donors by centrifugation on a Ficoll–Conray gradient.

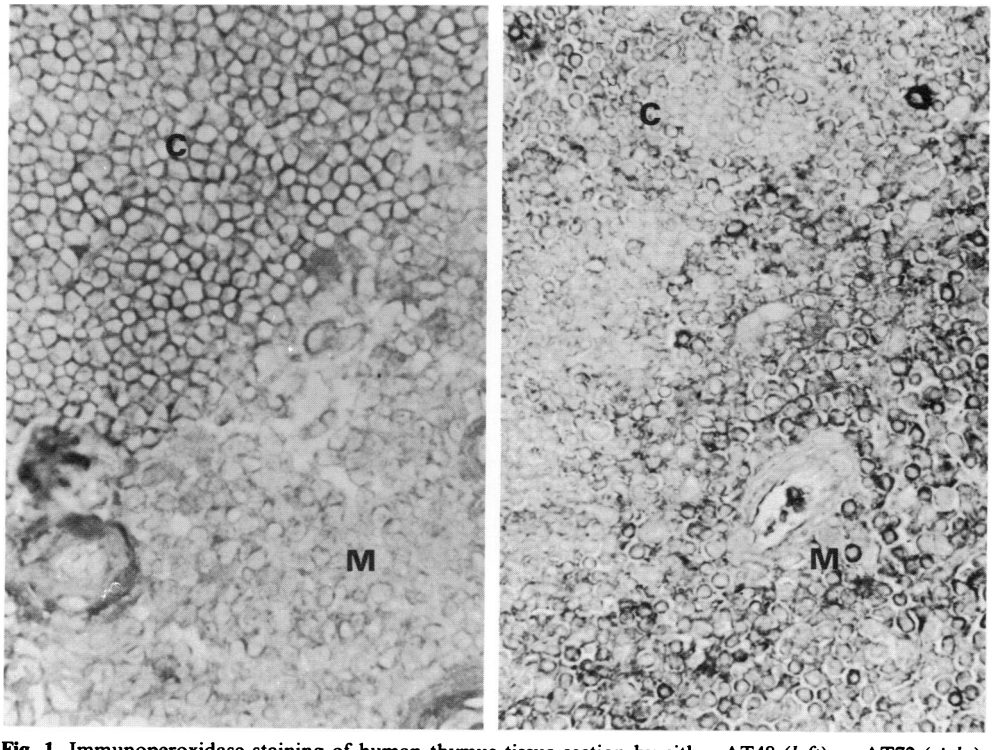

Fig. 1. Immunoperoxidase staining of human thymus tissue section by either AT48 (*left*) or AT72 (*right*). Whereas AT48 stains cortical thymocytes, AT72 reacts strongly with medullary thymocytes but weakly with cortical cells. C = cortex, M = medulla.

Table 2. Reactivities of AT48 and AT72 with various human leukaemic T-cell lines as defined by immunofluorescence

Cell line	AT48	AT72	Anti-cALL*
CCRF-CEM	−†	+	+
RPMI-8402	−	+	+
MOLT 4	+	+	−
JM	+	+	−
P12/Ich	+	+	−
HSB-2	−	+	−
Peer	−	+	−

* Common ALL antigen (cALL) defined by a xenoantiserum described by Koshiba *et al.* (1978).

† More than 90% fluorescence are positive. Less than 3% fluorescence are negative.

glycoproteins of thymus, T and B cells is shown in Fig. 2. AT48 precipitated a 48K mol. wt component from thymus cells but not from T and B cells. In addition, an extremely small peak with a molecular weight of around 12K was also observed with AT48, but with poor reproducibility (Fig. 3). These 48K and 12K mol. wt components seemed to be non-covalently associated, since almost identical patterns of their SDS-PAGE profiles were observed under both reducing and non-reduc-

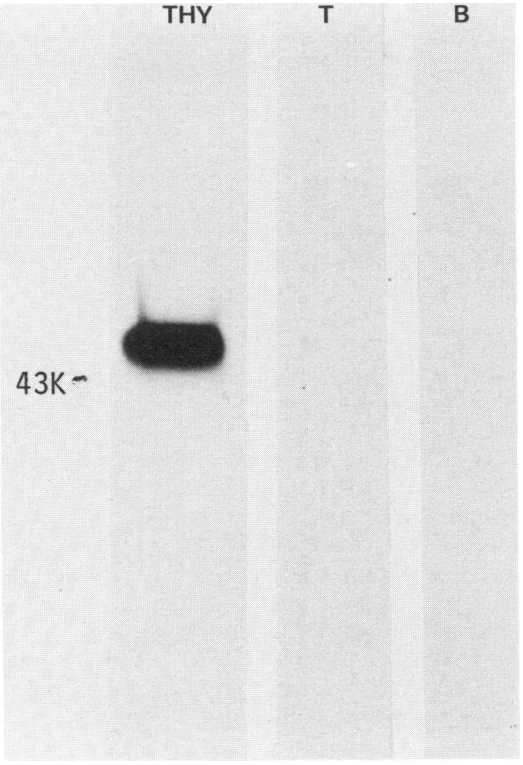

Fig. 2. SDS-PAGE analysis of immunoprecipitates of AT48 made with ^{125}I-labelled cell surface glycoproteins from human thymus (THY), tonsil T and B cells. AT48 clearly precipitates the 48K mol. wt component from thymus cells but not from T and B cells.

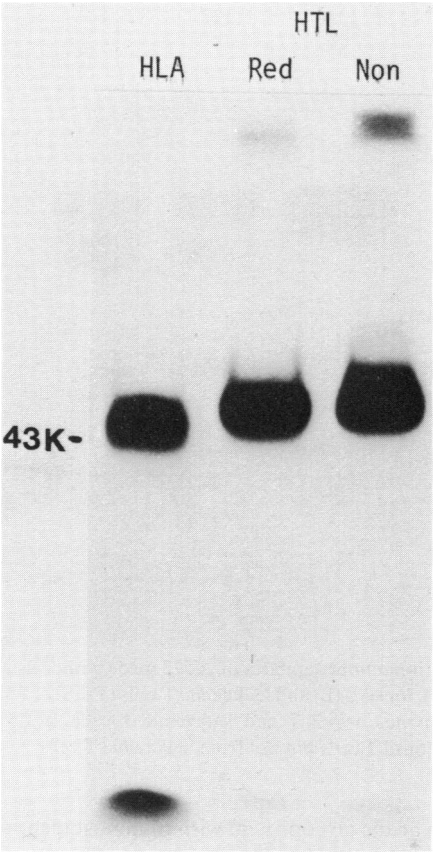

Fig. 3. SDS-PAGE analysis of immunoprecipitates of AT48 and anti-HLA made with ^{125}I-labelled thymus cell glycoproteins. Human TL-like (T48) antigen (HTL) precipitated by AT48 ran almost identically under both reducing (*Red*) and non-reducing (*Non*) conditions. Faint peak with 12K mol. wt component can be seen together with the prominent 48K mol. wt component in both reduced and non-reduced samples of HTL precipitated by AT48. HLA antigen, which was precipitated by anti-HLA and reduced by 2-mercaptoethanol, exhibits two components with molecular weights of 45K and 12K daltons, corresponding to HLA heavy chain and β_2-microglobulin.

ing conditions (Fig. 3). Sequential immunoprecipitation studies, however, have shown that anti-β_2-microglobulin (β_2m) serum could not remove more than 15% of the T48 antigen activity from ^{125}I-labelled thymus cell glycoproteins, whereas the same reagent could deplete more than 80% of HLA antigens from the same labelled glycoprotein preparation (Ishii *et al.*, 1980).

SDS-PAGE profiles of immunoprecipitates formed between AT72 and ^{125}I-labelled cell surface glycoproteins of thymus, T and B cells as well as from leukaemic T cells obtained from a patient with T-cell-type lymphosarcoma cell leukaemia are shown in Fig. 4. AT72 serum precipitated a 72K mol. wt component (T72) from thymus and T cells as well as from leukaemic T cells but not from B cells. The same antiserum precipitated a less prominent component with a molecular weight of 65K daltons, which could be observed with thymus and T cells but not with leukaemic T cells (Fig. 4). These two components were not the sulphydryl-linked subunit of each other, because they ran almost identically in SDS gels under both reducing and non-reducing conditions. The 72K mol. wt component precipitated from labelled leukaemic T-cell glycoproteins also showed almost identical electrophoretic mobility in SDS gels under both reducing and non-reducing conditions.

Two-dimensional PAGE analysis of antigens precipitated from labelled thymus cell glycoproteins by either AT48 or anti-HLA is presented in Fig. 5. The 48K mol. wt component precipitated by

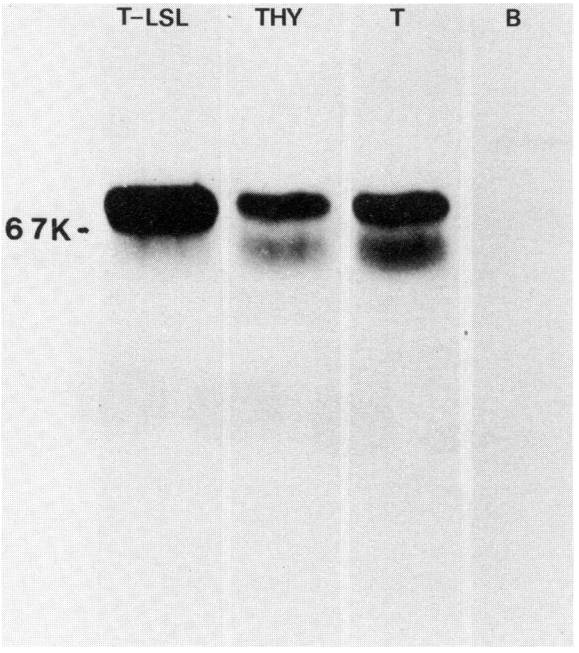

Fig. 4. SDS-PAGE analysis of immunoprecipitates of AT72 made with ^{125}I-labelled cell surface glycoproteins from thymus (THY), tonsil T (T), tonsil B (B) and leukaemic T cells (T-LSL). Whereas AT72 precipitates the 72K mol. wt glycoprotein from thymus, tonsil T and leukaemic T cells, it also precipitated the 65K mol. wt component from thymus and tonsil T cells but not from leukaemic T cells.

AT48 was recognized as an acidic glycoprotein with pI almost identical to that of the 45K mol. wt component (HLA heavy chain) precipitated by anti-HLA serum. While the 12K mol. wt component (β_2m), which was also precipitable with anti-HLA, was separated in the more basic side of pI than that of HLA heavy chains, the same component was barely seen in 2-D gels where the 48K glycoprotein precipitated by AT48 was separated.

Two-dimensional PAGE analysis was also performed on immunoprecipitates of AT72 made with labelled cell surface glycoproteins of normal and leukaemic T cells (Fig. 6). The 72K mol. wt glycoprotein precipitated by AT72 from both normal and leukaemic T cells was identified as an acidic glycoprotein, which exhibited marked electric charge heterogeneity with pI ranging approximately from 4 to 7. The 65K mol. wt component, which was also precipitable by AT72 from normal T cells, also showed marked charge heterogeneity with a wide pI range comparable to that of T72. When the leukaemic T-cell glycoproteins were incubated with *Vibrio cholerae* neuraminidase (100 units for 120,000 c.p.m.; Calbiochem–Behring Corp., San Diego, California) in 0·1 M acetate buffer (pH 6·0) at 37°C for 2 hr (Shackelford & Strominger, 1980) and analysed by 2-D PAGE, we found that the marked charge heterogeneity observed with native T72 molecules was reduced, and they moved to the more basic side of the gel with pI around 6–7 (Fig. 7).

DISCUSSION

Human thymus and T-cell antigens have been characterized using xenoantisera (Balch & Ades, 1979) or monoclonal antibodies raised against those lymphocytes (McMichael & Bastin, 1980). These studies have shown that human thymus and T cells have unique differentiation antigens, which enable us to distinguish human T-cell subsets with distinct functional properties. The present study has also demonstrated two different kinds of antigens expressed on human thymus and T cells, which, as described below, are essentially similar to mouse TL and Ly 1 antigens.

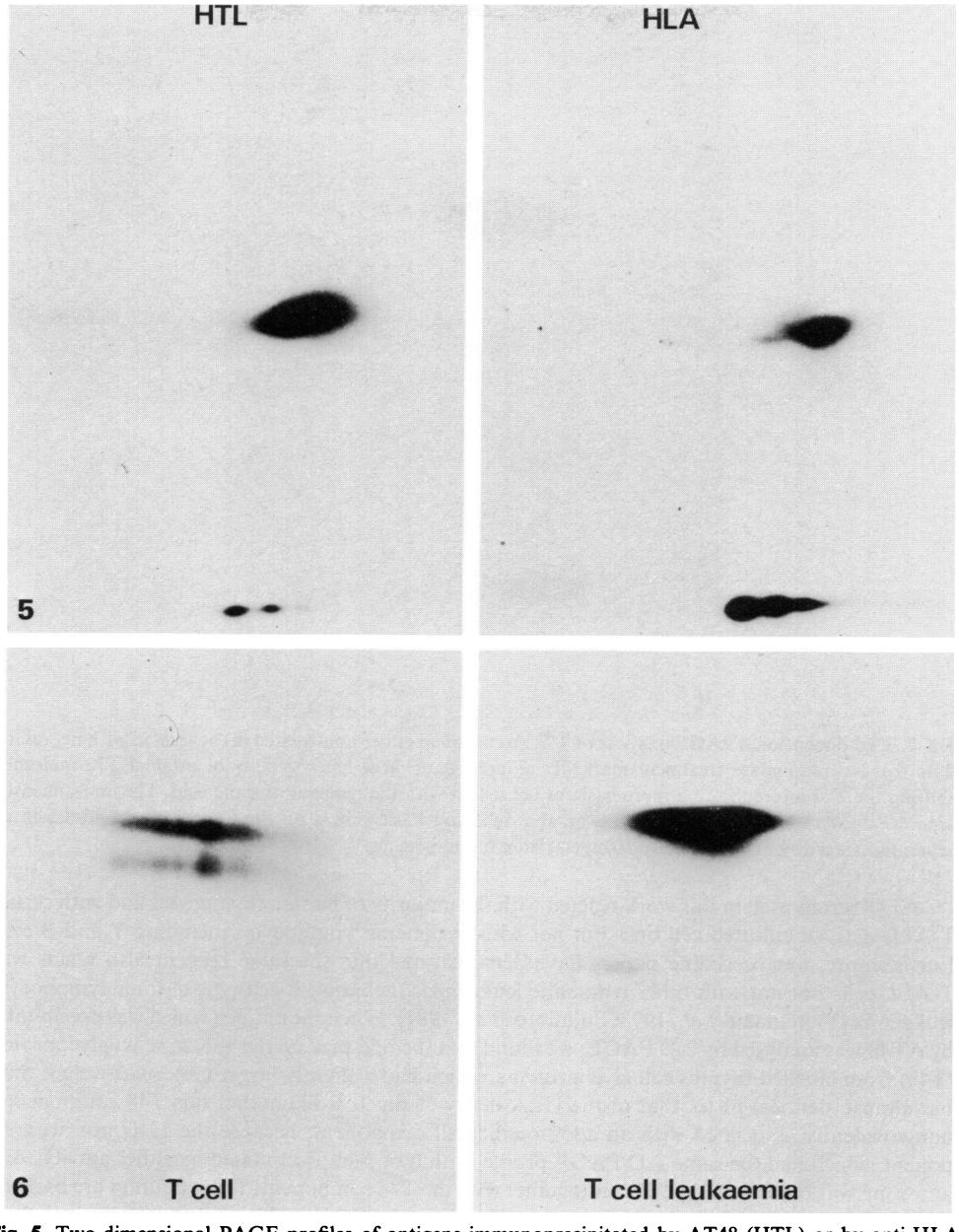

Fig. 5. Two-dimensional PAGE profiles of antigens immunoprecipitated by AT48 (HTL) or by anti-HLA (HLA). HLA antigen consists of 45K and 12K mol. wt components, whereas HTL is composed of 48K and 12K mol. wt components. The 12K mol. wt component is precipitated largely by anti-HLA and to a much lesser extent by AT48 from ^{125}I-labelled thymus cell glycoproteins. The first-dimension separations were by IEF (acidic side is on the right and basic side is on the left). The second-dimension separations were by SDS-PAGE on 10% gels (from top to bottom).

Fig. 6. Two-dimensional PAGE profiles of antigens precipitated by AT72 from tonsil T (*left*) and leukaemic T-cell glycoproteins (*right*). Whereas AT72 precipitates the 72K mol. wt glycoprotein from both normal and leukaemic T cells, the 65K mol. wt component is observed only with normal T cells. Note almost identical pI between T72 and T65 glycoproteins, ranging approximately from 4 to 7. The first-dimension separations were by IEF (acidic side is on the right and basic side is on the left). The second-dimension separations were by SDS-PAGE on 10% gels (from top to bottom).

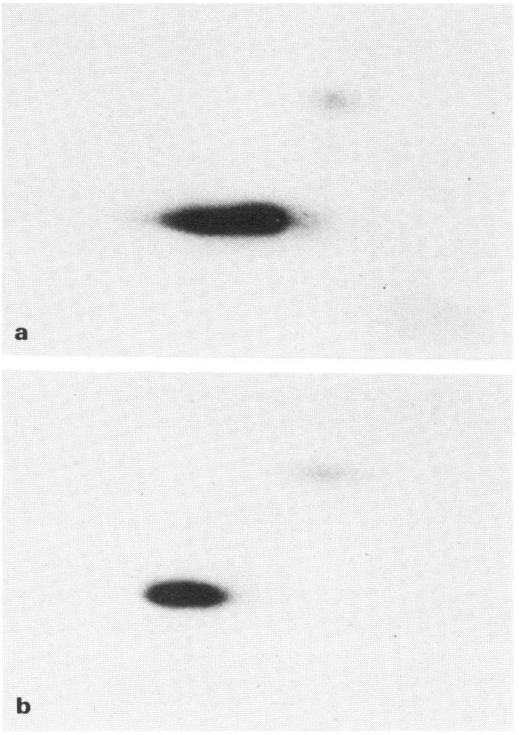

Fig. 7. Two-dimensional PAGE analysis of T72 glycoprotein either non-treated (a) or treated with neuraminidase (b). Neuraminidase treatment markedly reduced the charge heterogeneity of original T72 molecules, shifting the T72 molecules to a more basic pI consistent with the removal of sialic acid. The first-dimension separations were by IEF (acidic side is on the right and basic side is on the left). The second-dimension separations were by SDS-PAGE on 10% gels (from top to bottom).

AT48 serum used in this work reacted with the majority of human thymocytes and with certain T-ALL-derived cultured cell lines but not with peripheral lymphocytes including T and B cells. Furthermore, our preceding papers have demonstrated that the same reagent also reacts with T-ALL cells but not with other lymphatic leukaemias including T-cell-type chronic lymphocytic leukaemia (Yamanaka *et al.*, 1978; Fujimoto *et al.*, 1981). When the antigen which was precipitated by AT48 was analysed by 2-D PAGE, we found that it could pick up the 48K mol. wt glycoprotein (T48) from labelled thymus cell glycoproteins, which had a slightly larger molecular weight than, but almost identical pI to, that of the HLA heavy chain. It is likely that this T48 component is non-covalently associated with an additional small component, because the 12K mol. wt component, which had the same 2-D PAGE profile as that of β_2m precipitated by either anti-HLA or anti-β_2m, was barely seen in 2-D gels together with the T48 component. These findings are basically comparable to the data reported for mouse TL antigens (Katz, 1977), and thus suggest that the antigen defined by our AT48 is homologous to mouse TL antigens. The reason why AT48 could not precipitate so much of the 12K mol. wt component as observed with anti-HLA is not clear, but it is possible that this small component bound non-covalently to the T48 glycoprotein is easily dissociated during immunoprecipitation under the experimental conditions employed in this study (Ziegler & Milstein, 1979).

Our previous work has suggested that the 72K mol. wt component (T72) precipitated by AT72 from labelled cell surface glycoproteins of thymus and T cells and of leukaemic T cells represents a human homologue of Ly 1 antigens in the murine system (Ishii *et al.*, 1981). Thus AT72 reacted with virtually all thymus and peripheral T cells. In the thymus, medullary thymocytes possessed a much higher amount of the T72 antigen than did cortical thymocytes. Furthermore, functional studies have shown that T cells highly susceptible to the cytotoxic effect of AT72 contain a subpopulation of

T cells that can help immunoglobulin synthesis of B cells driven by pokeweed mitogen, whereas suppressor T cells induced by concanavalin A which reside in those cells are rather resistant to its cytotoxic effect. Two-dimensional PAGE analysis of this T72 glycoprotein clearly demonstrated that T72 is a glycoprotein composed of a single polypeptide, rather than sulphydryl-linked subunits, showing marked charge heterogeneity with pI between 4 and 7. When the T72 glycoprotein was treated with neuraminidase, the marked charge heterogeneity observed with the native T72 molecules was reduced and they were shifted to more basic pI (6–7) consistent with the removal of sialic acid, suggesting that the charge heterogeneity of T72 molecules is attributed largely to that in sialylation of this molecular species.

In addition to T72, AT72 precipitated a less prominent component with a molecular weight of 65K daltons (T65) from thymus and T cells. These two components appear to be similarly glycosylated, because they could bind to LcH and showed almost identical pI in 2-D gels. We do not know at present the exact relationship between T72 and T65 molecules, but our preliminary experiments suggest that T65 might be the precursor of T72, because the T65 molecule was clearly precipitated by AT72 from leukaemic T cells when they were internally labelled by radiolabelled amino acids (unpublished observation).

It is of interest to compare the antigens defined by AT48 and AT72 to those detected by recently developed monoclonal antibodies. In our preceding paper (Ishii *et al.*, 1981), it has been fully described that AT72 recognizes the same molecular species as that detected by monoclonal anti-Leu-1 antibody. AT48, on the other hand, appears to detect a similar antigen to HTA-1 (McMichael *et al.*, 1979) or OKT6 (Reinherz *et al.*, 1980), both of which have been found on human cortical thymocytes but not on medullary thymocytes and peripheral T cells using monoclonal antibodies (Bradstock *et al.*, 1980; Bhan *et al.*, 1980). However, the precise molecular comparison of T48 to those antigens defined by monoclonals should await further chemical studies using sequential co-precipitations with those reagents.

Based on surface antigenic characteristics including T48, T72 and common ALL (cALL) antigens (Koshiba *et al.*, 1978), leukaemic T-cell lines, all of which were derived from T-ALL patients, can be divided into three different categories: $T48^+T72^+cALL^-$ (MOLT 4, JM, P12/Ich), $T48^-T72^+cALL^-$ (HSB-2, Peer) and $T48^-T72^+cALL^+$ (CCRF-CEM, RPMI-8402). The first category ($T48^+T72^+cALL^-$) can readily be compared with the phenotype of cortical thymocytes, thus suggesting their cortical thymocyte origin. The second category ($T48^-T72^+cALL^-$) can also be compared to the phenotype of medullary thymocytes. In fact, one of the representative T-cell lines in this category (Peer) has been suggested to be of medullary thymocyte origin (Ravid *et al.*, 1980). However, HSB-2, the other T-cell line with $T48^-T72^+cALL^-$ phenotype, has been shown to possess high terminal deoxynucleotidyl transferase (TdT) activity, which is expressed little or not at all in medullary thymocytes (Minowada, 1978). This cell line might originate from cells intermediate between cortical and medullary thymocytes, if the former cells can differentiate into the latter cells. The third category is characterized by the expression of cALL and T72 and repression of T48. The T-cell lines included in this category also express high TdT activity (Minowada, 1978). It might be possible that these T-cell lines originate from a minor thymocyte population described by Bradstock *et al.* (1980), who demonstrated the presence of immature thymocytes comprising 1–3% of human thymus cells that expressed human T-cell xenoantigens as well as high TdT activity but lacked TL-like (HTA-1) antigen.

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