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# Human Thy-1 antigen: cell surface expression on early T and B lymphocytes

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**Summary.** Human Thy-1 is present on the surface of only a small proportion of haemopoietic cells (thymus—0·2–10%; bone marrow—0·1–0·5%). We have analysed these Thy-1$^+$ cells in more detail using immunofluorescence on a wide range of human haemopoietic cell lines and fresh leukaemic blasts; both situations where rare cells can become clonally expanded. The data demonstrate that Thy-1 is confined to the early stages of T- and B-lymphocyte development, and is absent from all myeloid cells. The Thy-1$^+$ B cells represent late pre-B (c$\mu^+$)/early sIgM$^+$ B cells. The Thy-1$^+$ T cells are situated in the outer thymic cortex. Dual immunofluorescence analysis of FACS separated Thy-1$^+$ thymocytes shows them to have the antigenic phenotype and morphology of prothymocytes/early thymocytes (some overlap seen with CALLA, TdT, OKT6 and OKT1).

## INTRODUCTION

The Thy-1 antigen has provided a useful cell surface marker for thymocytes and mature T lymphocytes in mice (Raff, 1970), whilst in the rat the antigen is present on the pluripotent stem cell, myeloid and lymphoid progenitors and both immature T (thymocytes and early peripheral T cells) and immature B lymphocytes (Ritter, Gordon & Goldschneider, 1978; Goldschneider, Gordon & Morris, 1978; Crawford & Goldschneider, 1980; Hunt, 1979). However, little is known of the distribution of Thy-1 during human haemopoiesis, where very few cells appear to bear the antigen (McKenzie & Fabre, 1981; Ritter, Sauvage & Cotmore, 1981).

Recently we have shown that Thy-1 in the human thymus is predominantly associated with the epithelial microenvironment (Ritter *et al.*, 1981). Although the majority of thymocytes in man are Thy-1$^-$, we find a minority population (approximately 1%) that do bear the antigen. A similarly small (0·1–0·5%) population of Thy-1$^+$ cells is also found in normal human bone marrow. We present here an analysis of these rare Thy-1$^+$ cells. In addition, we have made a detailed study of a range of human haemopoietic cell lines and of fresh leukaemic cells (where rare cells can become clonally expanded and hence accessible to analysis) to determine at what stage during haemopoiesis in man Thy-1 is expressed on the cell surface.

## MATERIALS AND METHODS

*Normal haemopoietic tissues*

Foetal thymus, liver, bone marrow and blood were obtained from cases of spontaneous or induced abortion in the second trimester of pregnancy (kindly supplied by Dr S. Lawler, Royal Marsden Hospital, London). Paediatric thymus tissue was obtained from individuals aged 3–12 years undergoing open heart surgery (kindly supplied by the Institute of Child

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Health, London). Normal heparinized bone-marrow cell aspirates and blood were donated by healthy young adult volunteers.

Cell suspensions were prepared in Dulbecco's modified Eagle's minimal essential medium (DMEM) buffered with 20 mM Hepes and supplemented with 5% heat-inactivated foetal calf serum (FCS). Red cells were removed from blood and bone-marrow cell suspensions either by Ficoll–Isopaque density gradient centrifugation (also removes mature granulocytes) or by flash lysis (cell pellet is resuspended in small volume of distilled water for 10 seconds, followed by the addition of an appropriate volume of 10 × concentrated phosphate-buffered saline [PBS] to restore isotonicity). The cells were then washed and resuspended in DMEM.

*Fresh leukaemia cells and human haemopoietic cell lines*

Heparinized bone marrow and blood samples were obtained from untreated leukaemia patients, at presentation or relapse, who were attending hospitals throughout the U.K. Cell suspensions were prepared by Ficoll–Isopaque density gradient centrifugation; cells at the interphase were washed and resuspended in buffered DMEM containing 5% FCS.

A wide range of established leukaemic and normal EBV (Epstein Barr virus) transformed haemopoietic cell lines was maintained as previously described (Minowada *et al.*, 1978). Details of their origin and cell type are given in Table 2.

*Antibodies and pure Thy-1*

The $F(ab')_2$ preparation of a rabbit antiserum to rat-brain Thy-1 was used at 100 μg/ml to detect the human homologue of Thy-1, and was the gift of Dr R. J. Morris (Morris, Mancini & Pfeiffer, 1980). Approximately 25% of the anti-rat Thy-1 antibodies in this reagent recognize the human Thy-1 molecule (Kemshead *et al.*, 1982). Seven monoclonal antibodies to human Thy-1 (gifts of Dr J. W. Fabre, Dr S. Cotmore and Miss S. Crowhurst) gave the same staining profiles as those obtained with the xenoantiserum, although with some the immunofluorescence was weaker.

Monoclonal antibodies (ascites) against HLA-A, B, C (W6/32) and HLA-DR (DA2) monomorphic determinants were the gift of Dr W. Bodmer. Monoclonal antibodies (ascites) against human T lymphocyte-associated antigens (OKT1, 3, 4, 6, 8, 10 and 11A) were supplied by Drs P. Kung and G. Goldstein (Ortho Pharmaceutical Corp., Raritan, NJ) (Kung *et al.*, 1980; Greaves *et al.*, 1981a). Monoclonals NA134 (cortical thymocytes), J5 (ALL antigen), 8.11.13 (medullary thymocytes) and RFB1 (early lymphohaemopoietic cells) were the gifts of Drs A. McMichael, S. Schlossman, J. Fabre and M. Bodger, respectively (McMichael *et al.*, 1979; Ritz *et al.*, 1980; Dalchau & Fabre, 1981; Bodger *et al.*, 1981). Rabbit anti-TdT antibody was the gift of Professor Bollum. Rhodamine (TMRITC)-conjugated rabbit anti-human μ-chains and fluorescein (FITC)-conjugated goat anti-human Ig were purchased from Kallestad (Northampton, U.K.).

These antisera were followed in immunofluorescence by FITC- or TMRITC-conjugated $F(ab')_2$ sheep anti-rabbit $F(ab')_2$ (fluorescein/protein ration = 3·28) at 60 μg/ml (gift of Dr R. J. Morris) and FITC- or TMRITC-conjugated $F(ab')_2$ goat anti-mouse Ig as appropriate.

Purified rat-brain Thy-1 was the gift of Dr R. J. Morris (Morris *et al.*, 1980). Purified human-brain Thy-1 was the gift of Dr S. Cotmore (Cotmore, Crowhurst & Waterfield, 1981).

*Immunofluorescence*

Cell suspensions were stained by indirect immunofluorescence as in Ritter *et al.* (1979) using the above reagents. Dual immunofluorescence was carried out in four separate incubations using the appropriate FITC- and TMRITC-conjugated second-layer antibodies to distinguish the two first-layer antibodies (one rabbit, one mouse) used. Immunofluorescence was analysed using a Zeiss standard R.A. microscope equipped with epi-illumination optics. In addition, some samples were analysed using a fluorescence-activated cell sorter (FACS-1, Becton Dickinson).

Specificity of staining for Thy-1 was demonstrated by abrogation of FITC immunofluorescence on both cell suspensions and tissue sections by prior incubation (overnight at 4°) of the anti-Thy-1 antibody with a three-fold molar excess of either pure human or pure rat Thy-1 (Fig. 1). No immunofluorescence was seen when either the FITC $F(ab')_2$ sheep anti-rabbit $F(ab')_2$ or the FITC/TMRITC $F(ab')_2$ goat anti-mouse Ig (to follow monoclonals) antibodies were used in the absence of primary-layer antibodies, or when the mouse monoclonal reagents were replaced by a non-immune or an irrelevant immune ascites preparation.

*FACS separation of Thy-1$^+$ and Thy-1$^-$ cells*

Thymocytes were stained in cell suspension by indirect immunofluorescence for Thy-1 (see previous sections); for controls the first-layer antibody was preabsorbed

with pure human Thy-1. Experimental and control suspensions were analysed on the FACS, and windows were set to separate positive from negative cells (Fig. 1). Thy-1$^+$ thymocytes were enriched to approximately 80% and then processed further for dual immunofluorescence using mouse monoclonal first-layer antibody followed by F(ab')$_2$ rabbit anti-mouse F(ab')$_2$-TMRITC (Thy-1 visualized by FITC immunofluorescence).

## RESULTS

### Normal haemopoietic tissues

Table 1 summarizes the distribution of Thy-1$^+$ cells in normal haemopoietic tissues, assessed by indirect immunofluorescence. Adult bone marrow and blood, and foetal liver contain between 0·1 and 0·5% Thy-1$^+$ cells; in foetal bone marrow and blood the percentage is slightly higher (up to 2·0%). Although many samples of paediatric and foetal thymus contain equally low percentages of Thy-1$^+$ cells, higher values are found in early foetal (11 weeks, 10%) and neonatal (4 months, 6%) thymuses. FACS analysis gave similar results for bone marrow and blood, but gave consistently higher values for thymus samples (e.g. FACS 7% *vs.* microscopy 1%) indicating the presence of an additional population of Thy-1$^+$ thymocytes whose immunofluorescence intensity is below the detection threshold of the microscope.

**Table 1.** Distribution of Thy-1 on cells from normal human haemopoietic tissues

| Tissue | | % Thy-1$^+$ cells* |
|---|---|---|
| Bone marrow: | foetal | 1·4– 2·0 |
| | adult | <0·1– 0·5 |
| Thymus: | foetal | 0·2–10·0‡ |
| | childhood | 0·2– 6·0‡ |
| Liver: | foetal | <0·1– 0·2 |
| Blood: | foetal | 1·5† |
| | adult | 0·2– 1·0 |

*Range of % Thy-1$^+$ cells is based on a minimum of six separate experiments.

†Single determination only.

‡Higher values are found in early foetal and early (neonatal) paediatric thymus.

Cells were analysed by indirect immunofluorescence using F(ab')$_2$ rabbit anti-rat pure Thy-1 antibody followed by FITC-F(ab')$_2$ sheep anti-rabbit F(ab')$_2$. For controls the rabbit antibody was pre-absorbed with pure human Thy-1.

**Table 2.** Distribution of cell surface Thy-1 antigen in human haemopoietic cell lines

| B-lymphoid lineage | | | T-lymphoid lineage | | | Non-lymphoid | | |
|---|---|---|---|---|---|---|---|---|
| Cell line | Cell type | Presence of Thy-1 | Cell line | Cell type | Presence of Thy-1 | Cell line | Cell type | Presence of Thy-1 |
| Reh†[1]‡ | pre-B | – | HPB MLT[5] | early T | + (>90) | KG1[6] | myeloid | – |
| Ru3[1] | pre-B | – | RPMI8402[5] | early T | + (>90) | HL60[7] | myeloid | – |
| Nalm 6[1] | pre-B | – | CCRFHSB2.1[5] | early T | + (30) | | | |
| Nalm 1[2] | pre-B | + (23)* | CCRFHSB2.2[5] | early T | – | K562[2] | erythroid | – |
| | | | DND 41[5] | early T | – | | | |
| B85[3] | B | + (24) | JM[5] | early T | – | | | |
| Bristol 7[3] | B | + (4) | Peer[5] | early T | – | | | |
| Bristol 8[3] | B | + (7) | GH1[5] | early T | – | | | |
| Riva[4] | B | – | HPB ALL[5] | early T | – | | | |
| Hill[3] | B | – | Molt 3[5] | early T | – | | | |
| | | | Molt 4[5] | early T | – | | | |

*Percentage positive, based on at least four separate determinations. Cells were analysed by indirect immunofluorescence using F(ab')$_2$ rabbit anti-rat pure Thy-1 antibody followed by FITC-F (ab')$_2$ sheep anti-rabbit F(ab')$_2$. For controls the rabbit antibody was pre-absorbed with pure human Thy-1.

†c$\mu^-$, but shows $\mu$ gene rearrangement (Ford, Mulgaard, Gould & Greaves, submitted for publication).

‡Origin of cell lines: 1, cALL; 2, Ph' CML-BC; 3, Normal EBV$^+$ B cell; 4, B lymphoma; 5, T-ALL/NHL; 6, AML; 7, APML.

### Analysis of cell lines

Cell lines representing lymphoid, myeloid and erythroid lineages were studied. Thy-1$^+$ cells were found only in pre-B-, early B- and early T-cell lines (Table 2; Figs 1a, b). Dual immunofluorescence analysis of these Thy-1$^+$ cell lines (Table 3) showed the phenotype of the Thy-1$^+$ B cells to be: HLA-DR$^+$, ALL$^{+\text{ or }-}$, TdT$^{+\text{ or }-}$, c$\mu^{+\text{ or }-}$, sIgM$^{+\text{ or }-}$, sIgG$^-$ and that of the Thy-1$^+$ T cells to be: ALL$^+$, TdT$^{+\text{ or }-}$, HLA-DR$^-$, OKT1$^+$, 3$^-$, 4$^-$, 6$^-$, 8$^-$, 11$^{+\text{ or }-}$.

### Analysis of fresh leukaemic samples

Approximately 400 leukaemic samples were analysed (Table 4). All myeloid, erythroid and megakaryoblastic leukaemias were consistently Thy-1$^-$. Approximately 5% of cALL (common acute lymphocytic leukaemia) and null-ALL samples, two out of three CML (chronic myelocytic leukaemia) in pre-B lymphoid blast crisis and one out of eight T lymphomas were Thy-1$^+$. All other classes of lymphoid leukaemia were Thy-1$^-$. In some Thy-1$^+$ patients, essentially 100% of the leukaemic blasts were Thy-1$^+$, whilst in others only a subpopulation (25–50% of blasts) bore the antigen. Thy-1$^+$ patients were studied further by immunofluorescence for the presence of additional cell surface and intracellular markers (Table 5). Three of the eight Thy-1$^+$ cALL samples were tested for cytoplasmic $\mu$ (c$\mu$). One of these was c$\mu^+$ (patient no. 12) and therefore of pre-B phenotype. Patient no. 5

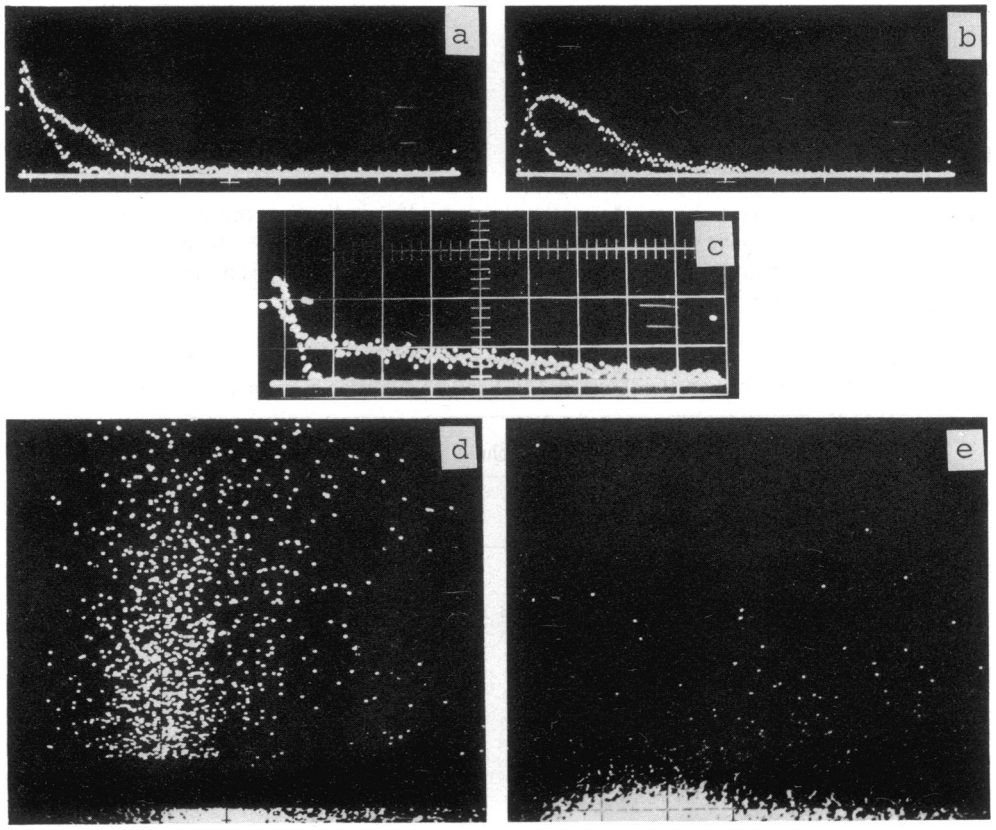


**Figure 1.** FACS analysis of Thy-1$^+$ immunofluorescence on human haemopoietic cell lines, leukaemia and normal thymocytes. (a) B 85; (b) HPB MLT; (c) pre-B ALL (patient no. 11, Table 5); (d) paediatric thymocytes with windows set for sorting; (e) negative control for (d).

Cells were stained in indirect immunofluorescence using F(ab')$_2$ rabbit anti-rat pure Thy-1, followed by F(ab')$_2$ sheep anti-rabbit F(ab')$_2$ FITC. For controls—lower line in (a), (b), (c) and (e)—the first-layer antibody was preabsorbed with pure human Thy-1.

*Axes.* (a)–(c): Horizontal, immunofluorescence intensity; vertical, cell number. (d) & (e): Horizontal, cell size; vertical, immunofluorescence intensity.

**Table 3.** Detailed analysis of Thy-1$^+$ human haemopoietic cell lines

| Cell line | Antigenic markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALL | TdT | HLA-DR | cμ | sIg | Eros/ OKT11A | OKT 1 | 3 | 4 | 6 | 8 |
| Nalm 1 | + | + | + | + | − | − | − | − | − | − | − |
| B85 | − | − | + | ± | ±*† | − | − | − | − | − | − |
| Bristol 7/8 | − | − | + | + | − | − | − | − | − | − | − |
| RPMI 8402 | + | + | − | − | − | − | + | − | − | − | − |
| HPB MLT | + | ±* | − | − | − | ±* | + | − | − | − | − |

+ Essentially all cells are positive.
± Population consists of some positive, some negative cells.
− Essentially all cells are negative.
* Thy-1 present on both positive and negative cell populations.
† sIgM$^+$, sIgG$^-$.
Cells were analysed by single and dual indirect immunofluorescence to detect the simultaneous presence of Thy-1 and other antigenic markers.

**Table 4.** Distribution of cell surface Thy-1 antigen in human leukaemia

| Lymphoid | | | Non-lymphoid | | |
|---|---|---|---|---|---|
| Type | Number Thy-1$^+$ | Total screened | Type | Number Thy-1$^+$ | Total screened |
| cALL | 7 | 152 | AML | — | 62 |
| Null ALL | 3 | 29 | CML | — | 9 |
| pre-B ALL | 2 | 4 | CML in myeloid blast crisis | — | 24 |
| B-ALL | — | 2 | AMML | — | 25 |
| T-ALL | — | 35 | APML | — | 2 |
| B-CLL | — | 30 | CMML | — | 3 |
| T-CLL | — | 5 | Erythroleukaemia | — | 4 |
| B lymphoma | — | 13 | Megakaryoblastic leukaemia | — | 4 |
| T lymphoma | 1 | 11 | | | |
| CML in lymphoid blast crisis: | | | | | |
| cALL | — | 9 | | | |
| pre-B ALL | 2 | 4 | | | |
| null | 1 | 1 | | | |

Fresh leukaemic samples were analysed by indirect immunofluorescence using F(ab′)$_2$ rabbit anti-rat pure Thy-1 antibody followed by FITC-F(ab′)$_2$ sheep anti-rabbit F(ab′)$_2$. For controls the rabbit antibody was pre-absorbed with pure human Thy-1.

**Table 5.** Detailed analysis of Thy-1$^+$ leukaemic samples

| Patient no. | Diagnosis | Antigenic markers on leukaemic blasts | | | | | |
|---|---|---|---|---|---|---|---|
| | | ALL | TdT | HLA-DR | cμ | sIg | Eros/OKT11A |
| 1 a* | null ALL | − | + | + | − | − | − |
| 2 c | null ALL | − | + | ±† | − | − | − |
| 3 c | null ALL | − | + | + | − | − | − |
| 4 a | cALL | + | + | + | nt | − | − |
| 5 a | cALL/pre-T‡ | + | − | + | nt | − | − |
| 6 c | cALL | + | + | + | − | − | − |
| 7 c | cALL | + | + | + | nt | − | − |
| 8 c | cALL | + | + | − | − | − | nt |
| 9 c | cALL | + | + | + | nt | − | − |
| 10 c | cALL | + | + | + | nt | − | − |
| 11 a | pre-B ALL | − | − | + | + | − | − |
| 12 c | pre-B ALL | ±† | + | + | + | − | − |
| 13 b | CML pre-B BC | + | + | + | + | − | − |
| 14 c | CML pre-B BC | + | + | + | + | − | − |
| 15 c | T lymphoma | − | − | − | nt | − | +§ |
| 16 a | CML-null ALL BC | − | − | + | − | − | − |

* (a) >90% blasts = Thy-1$^+$; (b) 50–90% blasts = Thy-1$^+$; (c) 20–50% blasts = Thy-1$^+$.

† Not all blasts were positive for the given marker. Thy-1 was present only on the positive blast population.

‡ Mediastinal mass.

§ Cells were OKT 3$^-$, 4$^-$, 6$^-$ and 8$^-$.

Cells were analysed by indirect immunofluorescence. Where <90% of blasts were Thy-1$^+$, overlap with other antigenic markers was confirmed by dual immunofluorescence using fluorescein and rhodamine labels for Thy-1 and the second antigenic marker respectively. BC = blast crisis.

combined a cALL phenotype with a mediastinal mass and was probably early T in type. Of the four null-ALL patients tested for cμ, one (no. 11) was pre-B in type (cμ$^+$) (Fig. 1c). The Thy-1$^+$ T lymphoma was OKT3$^-$, 4$^-$, 6$^-$, 8$^-$, 11A$^+$.

### Additional analysis of Thy-1$^+$ thymocytes by dual immunofluorescence

Dual immunofluorescence was performed either on whole thymocyte suspensions or on the Thy-1$^+$ fraction after separation on the FACS (Figs 1d, e). The overlap of cells bearing a second marker (TdT, CALLA, RFB1, 8.11.13, and OKT1, 3, 4, 6, 8, 10, 11A) with the Thy-1$^+$ thymocyte population is presented in Figs 2 and 3. RFB1 and OKT10 were present on all Thy-1$^+$ thymus cells, whilst 8.11.13 showed no overlap. The remaining marker-defined populations showed varying degrees of partial overlap.

## DISCUSSION

A small but consistent number of Thy-1$^+$ haemopoietic cells can be detected by immunofluorescence in normal human tissues (McKenzie & Fabre, 1981; Ritter *et al.*, 1981). In order to analyse these rare cells in greater detail we studied the clonally expanded populations of fresh leukaemias and haemopoietic cell lines, and FACS-enriched Thy-1$^+$ populations of normal cells.

Only a small proportion (approximately 3%) of lymphoid leukaemias were Thy-1$^+$, as might be expected from the low frequency of Thy-1$^+$ cells in normal tissues (Table 4). Thy-1 was not detected on any non-lymphoid leukaemic cells. The majority of Thy-1$^+$ leukaemias were of the 'lymphoid progenitor' null and cALL types, at least some of which had a cμ$^+$ pre-B phenotype (Table 5), whilst others (cμ$^-$) may have rearranged μ genes and thus be of an earlier pre-B

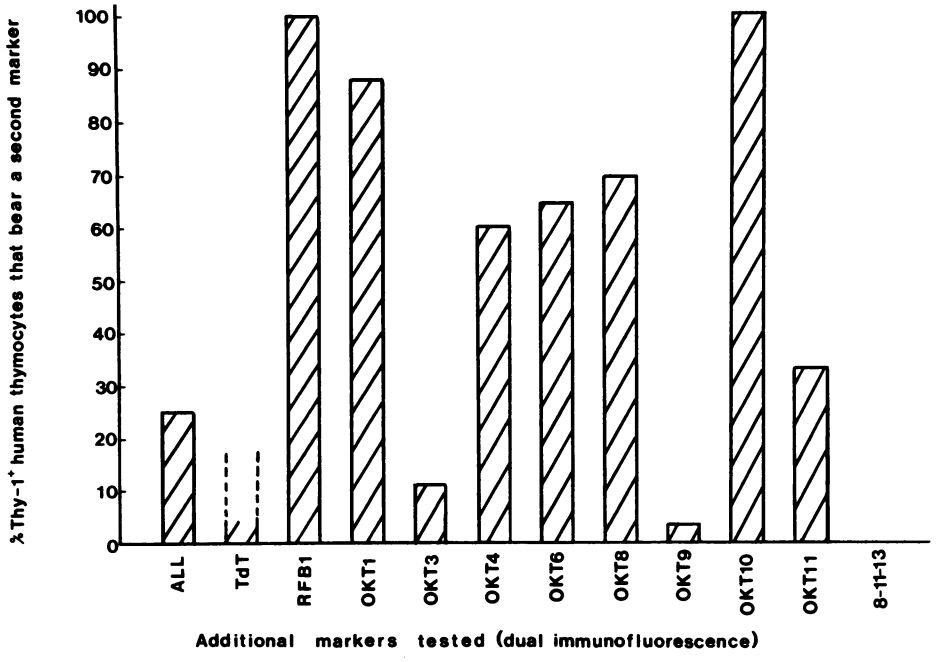


**Figure 2.** Human thymocytes were stained in indirect immunofluorescence and FACS sorted for Thy-1 (FITC), then tested for the presence of a second antigen using TMRITC immunofluorescence. Data are expressed as the % of Thy-1$^+$ cells that were also positive for the second marker.

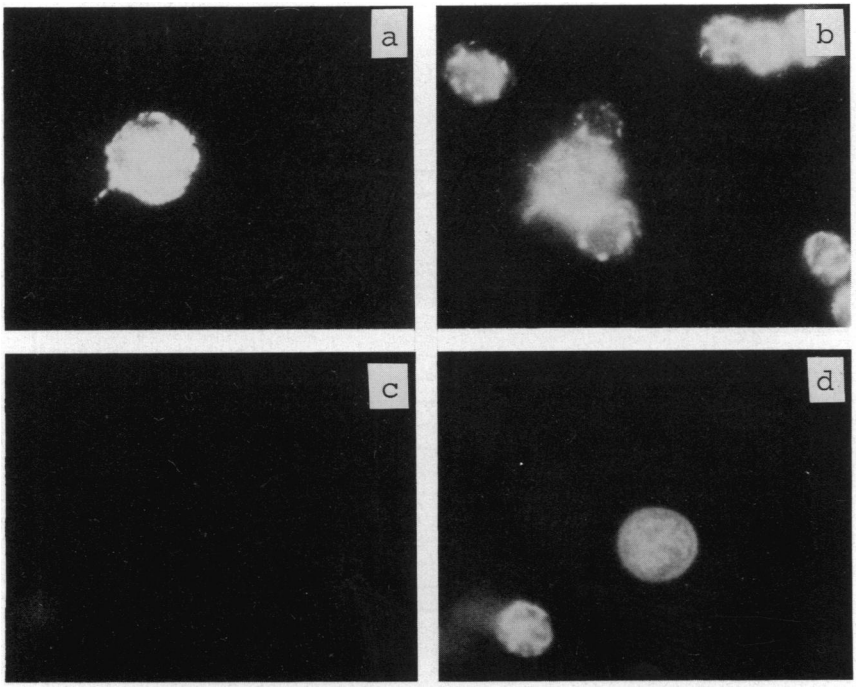


**Figure 3.** Human thymocytes were stained in indirect immunofluorescence and FACS sorted for Thy-1 (FITC), followed by TMRITC immunofluorescence for intracellular TdT. (a) Thy-1$^+$ prothymocyte. (b) The same cell is TdT$^+$. (c) Thy-1$^-$ prothymocyte. (d) The same cell is TdT$^+$. Magnification ×917.

cell type (Korsmeyer *et al.*, 1981). In addition, one patient (no. 5) had a mediastinal mass, suggestive of a T-cell malignancy (Greaves *et al.*, 1981b). Thy-1$^+$ null and pre-B phenotypes were also seen in some lymphoid blast crises of CML. The most mature Thy-1$^+$ malignancy was a T lymphoma (no. 15), although additional testing showed this to be of an early thymocyte type (OKT 11$^+$; OKT 3, 4, 6 and 8$^-$). The data from our haemopoietic cell line studies were concordant with the leukaemia data. All myeloid cells were Thy-1$^-$. Those lymphoid lines that were Thy-1$^+$ were of early thymocyte (RPMI 8402 and HPB MLT), pre-B (Nalm 1) or pre-B/sIgM$^+$ early B (B 85) type. The human T cell-specific cell surface molecule recently described by Saji & Tanigaki (1982) is therefore unlikely to be Thy-1.

We next studied Thy-1$^+$ normal thymocytes after FACS enrichment and dual immunofluorescence for additional cell surface molecules (Figs 2, 3). All Thy-1$^+$ thymocytes were RFB1$^+$ and OKT10$^+$, but 8.11.13$^-$. Since the RFB1 antigen is present on all cortical thymocytes (Bodger *et al.*, 1982) whilst 8.11.13, which detects a differential determinant on certain human leucocyte-common molecules, is restricted to medullary thymocytes (Dalchau & Fabre, 1981), the data indicate that Thy-1$^+$ thymocytes form part of the cortical thymocyte population. This fits well with earlier observations on tissue sections where all Thy-1$^+$ cells, both epithelial and lymphoid, were located within the thymus cortex (Ritter *et al.*, 1981; McKenzie & Fabre, 1981). Our additional double-marker studies show that some Thy-1$^+$ thymocytes also carry CALLA, a cell surface molecule on human lymphoid progenitor cells (Greaves *et al.*, 1980; Greaves *et al.*, 1982), thus suggesting that the thymocytes that bear Thy-1 are amongst the most immature in the thymus. This is further supported by the fact that some Thy-1$^+$ thymocytes are large TdT$^+$ prothymocyte-like cells (Figs. 3a, b). However, since not all TdT$^+$ prothymocytes bear Thy-1 (Figs 3c, d) and not all the CALLA$^+$ cells are Thy-1$^+$, the earliest prothymocytes may be CALLA$^+$, TdT$^+$, Thy-1$^-$ giving rise to later prothymocytes that also carry Thy-1. These Thy-1$^+$ prothymocytes may then give rise to the Thy-1$^+$ smaller thymocytes which bear the various OKT-defined T lymphocyte markers (Fig. 2). A simplified scheme of lymphocyte development within the human thymus cortex is presented in Fig. 4, although clearly the data are also consistent with the presence of several separate T cell-differentiation pathways.

If the Thy-1$^+$ prothymocytes are not the earliest

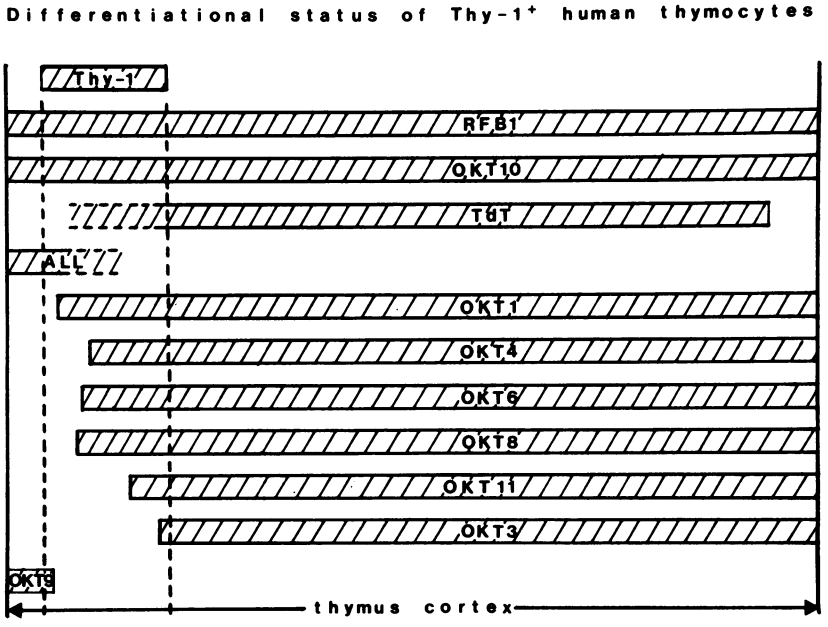


**Figure 4.** Simplified scheme of lymphocyte differentiation within the human thymus cortex. Differentiation proceeds from left (subcapsular cortex) to right (cortico-medullary junction).

cells in the human thymus, then it is unlikely that any of the Thy-1$^+$ cells in normal bone marrow or foetal liver (Table 1) are themselves T lymphoid progenitors destined for migration to the thymus. Instead, they are probably all pre-B cells, as suggested by the leukaemia and cell-line data. However, since less than 1% are Thy-1$^+$, the Thy-1 antigen must be present during only part of the human pre-B cell stage of differentiation. Further work was undertaken to identify this stage more precisely. Dual immunofluorescence studies on the B85 cell line showed Thy-1 to be present on c$\mu^+$ and some sIgM$^+$ cells, although the majority (70%) of sIgM$^+$ cells were Thy-1$^-$. The simplest interpretation of this data is that Thy-1 is present on the surface of fairly late pre-B cells, and is lost soon after their maturation to sIgM$^+$ B cells.

Thus the Thy-1 antigen is present during two phases of human haemopoietic development, late pre-T and late pre-B. The distribution of Thy-1 in man therefore differs considerably from both that in the mouse (Raff, 1970) and that in the rat (Ritter *et al.*, 1978)—differences that can also be seen in the haemopoietic microenvironment (Ritter & Morris, 1980; Ritter *et al.*, 1981). The relationship between haemopoietic cell surface and microenvironmental Thy-1 and its functional significance remain to be established.

## ACKNOWLEDGMENTS

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