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Detection of the common acute lymphoblastic leukaemia antigen (CALLA) on B cells from human fetal tissues. A multiple phenotypic characterization

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SUMMARY

Fetal bone marrow liver and spleen of gestational age 15–20 weeks contain CALLA$^+$, HLA-DR$^+$ lymphoid cells. We show that a proportion of them expresses surface membrane immunoglobulins (SIg) as well as B cell differentiation antigens. A multiple phenotypic analysis reveals that CALLA$^+$ fetal B cells are: HLA-DR$^+$, SIg$^+$, FMC8$^+$, BA1$^+$, Y29.55$^+$ or Y29.55$^-$, B2$^+$, TdT$^-$. Tissue specific phenotypic differences concern the expression of B7 and HLA-DC on spleen but not on bone marrow B cells. This study indicates that the distribution of the CALL antigen across the B cell committed lineage is much wider in fetal than neonatal life since CALLA$^+$ B cells have not yet been detected in bone marrow and peripheral blood of normal infants and adults. In addition, the interpretation of our phenotypic data suggest that fetal bone marrow B cells are more immature than those present in the spleen, thus, further supporting the evidence that the bone marrow is the organ of B cell lymphopoiesis.

Keywords human fetal B cells CALLA composite phenotype

INTRODUCTION

The common acute lymphoblastic leukaemia (CALLA) antigen is a non-phosphorilated surface membrane glycoprotein of mol. wt 100,000 (Newman, Sutherland & Greaves, 1981), whose cellular distribution among normal and malignant hemopoietic tissues has been serologically characterized by the use of xenoantisera (Greaves *et al.*, 1980; Janossy & Greaves, 1978), and monoclonal antibodies (MoAb) such as J5 (Ritz *et al.*, 1980), and ViL-A1 (Knapp *et al.*, 1982). Thus, in haemoproliferative diseases CALLA is expressed in the majority of non-T, non-B lymphoblastic leukaemias and in some T-ALL (Greaves *et al.*, 1983), in Burkitt's lymphomas and some B-NHLs (Ritz *et al.*, 1981; Delia *et al.*, 1984). CALLA$^+$ haemopoietic cells are found in discrete amounts in fetal tissues such as liver and bone marrow (Hokland *et al.*, 1983), and in bone marrows from healthy children (Greaves *et al.*, 1980). Non-haemopoietic tissues can also express CALLA (Metzgar *et al.*, 1981). CALLA$^+$ leukaemic cells and their normal counterparts carry HLA-DR molecules, the nuclear enzyme terminal deoxynucleotidyl transferase (TdT) and are devoid of surface immunoglobulins though can exhibit cytoplasmic IgM (Janossy *et al.*, 1979).

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The finding that CALLA$^+$ leukaemias have rearranged immunoglobulin genes (Korsmeyer *et al.*, 1981), has unequivocally shown that they are committed B cell precursors. The observation that mature B cell neoplasias such as Burkitt's and non-Hodgkin lymphomas which are strongly positive for SmIg can be CALLA$^+$ suggests that this antigen has a wider distribution along the lymphoid maturation pathway than it was originally thought. Alternatively these B cell tumours could aberrantly express CALLA.

In this report we show that a proportion of B cells from fetal haemopoietic tissues are unexpectedly CALLA$^+$, this finding contrasts with what is observed in infants and adults where bone marrow B cells are CALLA$^-$, and may rule out the possibility that CALLA$^+$ B cell malignancies arise from an aberrant gene expression of this antigen.

MATERIALS AND METHODS

Preparation of cell suspensions from fetal tissues. Fetal tissues were obtained from cases undergoing abortions carried out at the Gynaecological Clinic of Milan University by dilatation and evacuation. The age of the fetuses was determined by the use of ultrasounds. Cell suspensions from livers and spleens were obtained by finely mincing the specimens with a scalpel and by pipetting the small clumps several times. Bone marrow cells were obtained by flushing with a syringe RPMI 1640 (GIBCO, UK) into the intramedullary cavities of femoral bones. All cell suspensions were subjected to Ficoll-Hypaque density gradient separations; the interphase cells were recovered, washed twice and used for the immunofluorescence (IF) staining.

Antibodies. The MoAb used in this study are listed in Table 1 together with their specificity and references. Their binding to the cells was detected by IF using a fluorescein isothiocyanate (FITC) conjugated Fab_2 fragment of a goat anti-mouse. Fluorescein and rhodamine-labelled Fab_2 xenoantisera directed against surface immunoglobulins were the following: goat anti-Ig total, goat anti-IgM, goat anti-IgD, burro anti-κ and burro anti-λ, all obtained from Kallestad (Austin, Texas, USA). The nuclear enzyme TdT was detected by IF on methanol fixed cytocentrifuge preparations as previously described, Bollum (1979), using a rabbit anti-TdT (BRL, Rockville, Maryland, USA) followed by a rhodamine conjugated swine anti-rabbit (Nordic, The Netherlands). Normal mouse and normal rabbit sera were always included as negative controls for the murine MoAb antibodies and for the TdT determinations. All the antibodies were used at saturating concentrations, as previously determined. IF was evaluated by fluorescence microscope (Zeiss epi-illumination) and by fluorescence activated cell sorter (FACS IV, Becton-Dickinson, Mountain View, California, USA).

FACS separation and relabelling of SIg$^+$ cells. Cells from fetal tissues were sorted out by the FACS on the basis of their reactivity with the Fab_2 goat anti-human Ig. Prior to restaining with the relevant MoAb, the collected SIg$^+$ fraction was exposed to a diffuse laser light (wavelength 488 nm) in order to quench the fluorescence of the fluorochrome (this treatment, carried out at 4°C, had no

Table 1. List of MoAb used in this study

Antibody	Specificity	References
Vil-Al	GP 100 Kd CALL	Knapp *et al.* 1982
FMC8	B Subset + C-ALL	Brooks *et al.* 1982
OKB2	pan B	Mittler *et al.* 1983
OKB7	pan B	Mittler *et al.* 1983
BA1	pan B	Abramson *et al.* 1981
Y29.55	Early B + B Subset	Forster *et al.* 1982
DA2	HLA-DR monomorphic	Brodsky *et al.* 1979
TU22	HLA-DC monomorphic	Ziegler *et al.* 1981

effect on the cell viability). Quenching was checked by fluorescence microscopy and by FACS analysis. The cells were then relabelled by incubation with the specific monoclonal antibody or with irrelevant mouse Igs for the negative control, followed by another incubation with the fluorescein conjugated Fab_2 goat anti-mouse. The analysis was carried out with the FACS.

Dual immunofluorescence staining procedure. The simultaneous expression of SIg and anyone of the various cell-surface antigens was detected by the combination of fluorescein and rhodamine-labelled reagents.The staining sequence was the following: after incubation with the relevant MoAb (or normal mouse Ig for the negative control), the cells were washed twice in RPMI plus 2% FCS, and incubated with a rhodamine labelled Fab_2 goat anti-mouse. Following two more washes, the cells were incubated with the fluorescein labelled Fab_2 goat anti-human Ig. After two further washes, the double IF was evaluated by fluorescence microscopy. All incubations were carried out at 4°C for 20 min.

RESULTS

Composite phenotype of fetal tissue cell suspensions

Fifteen specimens (seven livers, five spleens, three bone marrows) were included in this study and the results are listed in Table 2. CALLA$^+$ cells, detected with Vil-A1, were consistently present in all specimens, with bone marrow containing a large number (> 20%), HLA-DR determinants were expressed in liver (~ 12%) spleen (~ 32%) and bone marrow (~ 68%) whereas the DC determinants were strongly detected in spleen and only occasionally in bone marrow; in this latter case the cells were dimly stained. B2, FMC8 and BA1 stained approximately a similar percentage of cells in the three tissues, with bone marrows containing a large number of positive cells, many of which of myeloid morphology (as judged by the phase contrast microscopic readings). Y29.55$^+$ cells were detected in all specimens and, similarly to the three previous markers, it also stained bone marrow myeloid cells. SIg$^+$ cells expressing the IgM and IgD isotypes were largely present in the spleen (> 26%) followed by the bone marrow (< 12%) and by the liver (< 6%). Spleen SIg$^+$ cells exhibited an extremely bright fluorescence in contrast to the dimly stained bone marrow and liver cells. κ and λ light chains were also detected and for each specimen the sum of their percentages never exceeded that of SIg; it actually tended to be lower.

Multiphenotypic analysis of the SIg$^+$ cells

The data obtained from the study carried out on the FACS-enriched SIg$^+$ fractions are shown in Table 3. It can be seen that 100 per cent of the SIg$^+$ cells were HLA-DR$^+$, B2$^+$, BA1$^+$. The majority of bone marrow and liver B cells were CALLA$^+$; fewer cells expressed the CALL antigen in the spleen. FMC8 and Y29.55 stained 100% of bone marrow B cells; the former gave similar reactivity in the liver, the latter gave variable reactivity in the liver and spleen specimens ranging from 50 to 100%. The markers that gave strong differential binding were TU22 (anti-HLA-DC) and B7. Their reactivity was in fact restricted to the spleen, though they both displayed a weak reactivity with a small percentage of the bone marrow SIg$^+$ cells (see Fig. 1). Irrelevant mouse Ig, included to evaluate the non-specific cross-reactivity between the various antibodies gave consistently negative results.

Nuclear TdT versus other cell surface markers

Double staining for TdT and cell surface antigens was performed on bone marrow and liver specimens (see Table 4), but not on spleen since we had previously found that this latter contains less than 1/1,000 TdT$^+$ cells. TdT$^+$ mononuclear cells ranged between 5 and 13% in the bone marrow and 1 and 6% in liver. In the bone marrow 80% of the TdT$^+$ cells were CALLA$^+$ and in the liver 100% of the TdT$^+$ were CALLA$^+$. About 94% of the TdT$^+$ bone marrow cells were positive for FMC8; in liver only 68% of the TdT$^+$ cells expressed the FMC8 determinant. TdT$^+$ cells from bone marrows and livers were 100% positive for BA1. No TdT$^+$ bone marrow or liver cells were positive for Y29.55 or SIg.

Table 2. Surface phenotype of the fetal tissue cell suspensions

Specimen	CALLA	HLA-DR	HLA-DC	B2	B7	FMC8	BA1	Y29.55	SIg	SIgM	SIgD	κ	λ
Liver	4·7±2·26	12±5·71	nt	nt	nt	12·4±3·86	9·9±2·48	4·9±2·27	5·5±1·75	2·4±1·61	2·6±1·88	2·8±1·7	1·3±0·8
Spleen	10·4±3·64	32±7·45	27·4±7·53	30·6±10·5	32·6±9·23	38·2±5	34·6±9·07	21·8±7·39	28·4±9·7	25·2±4·19	26·3±8·5	20±4·6	14·3±3·2
Bone marrow	29·2±8·24	68·3±7·63	5·6±4·5	65·3±5·13	3·3±3·05	59·3±11·5	52±13·11	35±6·08	12·3±0·57	7·3±3·21	7·2±3·28	4·5±3·53	3·7±2·3

The data refer to the phenotypic analysis carried out on cell suspensions from seven livers, five spleens and three bone marrows; age range 15–19 weeks of gestation. The numbers in the table refer to the percentage of positive cells (after substraction of the background determined by a normal mouse serum) ± s.d.

nt = not tested.

Table 3. Phenotype of the FACS separated SIg^+ fractions

Specimen	CALLA	HLA-DR	HLA-DC	B2	B7	FMC8	BA1	Y29.55	NMS
Liver	75–100	100	nt	100	0	100	100	50–100	0
Spleen	25–58	100	90–100*	100	100	75–100	100	80–100	0
Bone marrow	65–100	100	10–20*	100	10	100	100	100	0

Cell sorting and phenotype of the SIg^+ cells was carried out as described in Materials and Methods. The purity of the sorted fractions was always >95%. At least three specimens of each tissue were tested and results are expressed in percentage of positive cells. NMS is an irrelevant mouse Ig, included to measure the non-specific binding of the reagents.

nt = not tested

* TU22 gave a strong binding with spleen B cells and a very weak one with a small proportion of bone marrow B cells, with average fluorescence intensities (as measured by the FACS) of 100 and >255, respectively.

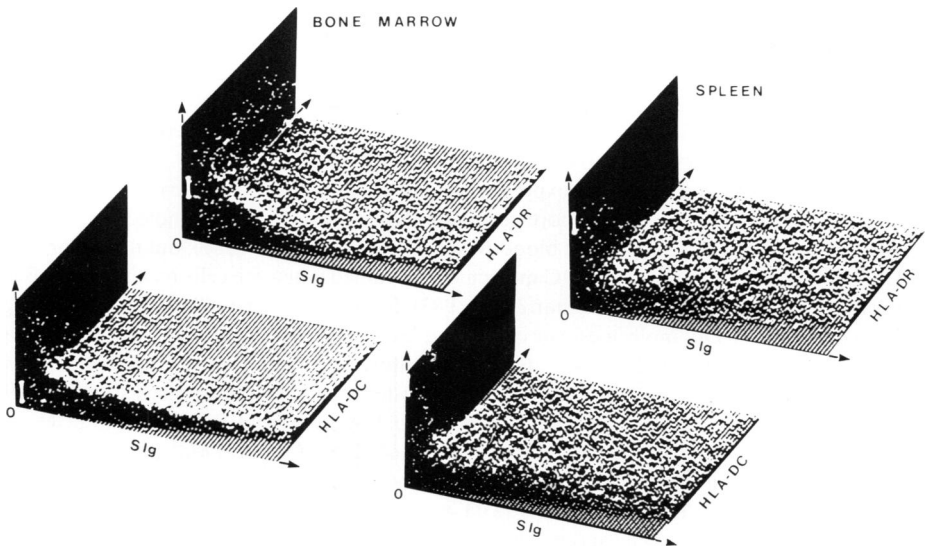

Fig. 1. Simultaneous detection of SIg, HLA-DR and DC by FACS analysis and tridimensional display. SIg and DR or DC determinants were detected with the use of fluorescein and phycoerythrin-labelled reagents, respectively. The staining sequence was the following: the cells were first incubated with DA2 or TU22 MoAb, then with a biotynilated horse anti-mouse (1:50) (Vector Labs, California) followed by an incubation with avidin conjugated phycoerythrin (Becton-Dickinson, California) and finally with a Fab_2 goat anti-human Ig-FITC. The double immunofluorescence was evaluated by the FACS equipped with a 600 LP and 520 LP plus 540 SP interference filters (Ditric Optics, Massachusetts, USA) for the detection of the red and green fluorescencies, respectively. Excitation wavelength: 501 nm. Negative controls were used to determine the non-specific cross-reactivity of the reagents (which was consistently low) and to set up the instrument. In this particular experiment positivities for HLA-DR, HLA-DC and SIg were 40, 39 and 41%, respectively; for the spleen and bone marrow 46, 1 and 14%, respectively.

DISCUSSION

Lymphocytes bearing surface Ig can be detected, during lymphopoiesis, from 9 weeks gestation onwards, Asma *et al.* (1977) and from 10 to 12 weeks can synthesize IgM stimulation, Gitlin & Biasucci (1969). Though we have not investigated this latter point, our data confirm the presence, in fetuses of 15–20 weeks gestation age, of SIg^+ cells, which, in addition, have B cell lineage, multiple phenotypic markers. Morphologically, these cells appear as small lymphocytes in bone marrow and liver and medium size lymphocytes in spleen (see Fig. 2). Our data are also in agreement with previous reports on the presence of $CALLA^+$ lymphoid cells in fetal tissues (Hokland *et al.*, 1983). By extending the study on the nature of such $CALLA^+$ fetal cells we found that a percentage of them bear surface immunoglobulins. Cell separations and dual IF studies have unequivocally shown that the SIg^+ cells are $HLA\text{-}DR^+$, $BA1^+$, $B2^+$ and that a percentage of them is $CALLA^+$, $FMC8^+$, $Y29.55^+$, $B7^+$; none of the SIg^+ cells is TdT^+.

In normal lymphoid differentiation BA1 and FMC8 determinants appear at an early stage of maturation and are retained down to the level of circulating B cells, LeBien *et al.* (1981). The reactivity of these two markers with $HLA\text{-}DR^+/SIg^+$ fetal cells is therefore not unexpected. B2 shows a similar reactivity in that it stains 100% of fetal B cells. We also found that a large proportion of SIg^- fetal bone marrow cells (FACS separated) are $BA1^+$, $FMC8^+$, $B2^+$ (D. Delia, personal observation) some of which with a myeloid morphology. The remaining probably representing the B cell precursors. In adult bone marrow B2 labels approximately the same percentage of cells (60–70%) (Mittler *et al.*, 1983) as in fetal bone marrow.

OKB7 reacts in normal peripheral blood with a percentage of B cells and with about 10% of bone marrow cells (Mittler *et al.*, 1983). In fetal tissues it has exhibited a strong reactivity with spleen B cells though, occasionally it has bound (weakly) to bone marrow B cells.

The OKB7 differential tissue reactivity has been paralleled by that of TU22 (the anti-HLA-DC) which, on the whole reflect distinct B cell maturation stages.

Serological studies on the cellular expression of MHC class II antigens by the haemopoietic system have shown that DC have a more restricted distribution than DR molecules; HLA-DC molecules are found in adult peripheral blood B cells (Navarrete *et al.*, 1981), but not on precursor cells. During normal B-cell ontogeny DC molecules are gained when the cells reach the pre-B level of maturation ($Cyt.IgM^+$, SIg^-) (Newman *et al.*, 1983) though leukaemic cell studies would suggest that the acquisition of DC molecules can occur at a later stage, since some chronic lymphocytic leukaemias of B cell type are $HLA\text{-}DC^-$ (Guy & Heyningen, 1983). Not all B cells reacted with Y29.55, as evident from Table 3, thus suggesting the existence of at least two B cell subsets; Y29.55 recognizes the majority of cells from secondary lymphoid organs, from B cell malignancies and a minority of peripheral blood B lymphocytes (Forster *et al.*, 1982; Gudat *et al.*, 1981). In agreement with a previous report (Bonati *et al.*, 1983) we found nuclear TdT^+ cells in liver and bone marrow and only rarely in spleen. The expression of TdT is, in normal B cell maturation, confined to the $CALLA^+$, $HLA\text{-}DR^+$, SIg^- cells and it is lost when the cells acquire cytoplasmic IgM (Gathings, Lawton & Cooper, 1977); circulating and sessile B cells are TdT^-. Our results show that none of the SIg^+, $Y29.55^+$ cells is TdT^+. Given the fact that a percentage of these SIg^+ cells is also $CALLA^+$, it follows that all the $SIg^+/CALLA^+$ cells are TdT^-. Taken together our data show that during fetal life B cells retain the CALL antigen and display many of the phenotypic characteristics of mature B cells, moreover the lack of or very weak reactivity of B7 and TU22 with bone marrow B cells, as opposed to their strong binding with spleen B cells is suggestive of two distinct discrete stages of maturation of which the bone marrow B cells reflect the early stage.

Finally, the existance of $CALLA^+$ normal B cells indicates that the $CALLA^+$ subgroup of B non-Hodgkin's lymphomas do not express this molecule aberrantly: more likely, these cells arise from normal B cell counterparts that exist in extremely (so far undetected) rare numbers in lymphoid organs of infants and adults but in large numbers in fetal life.

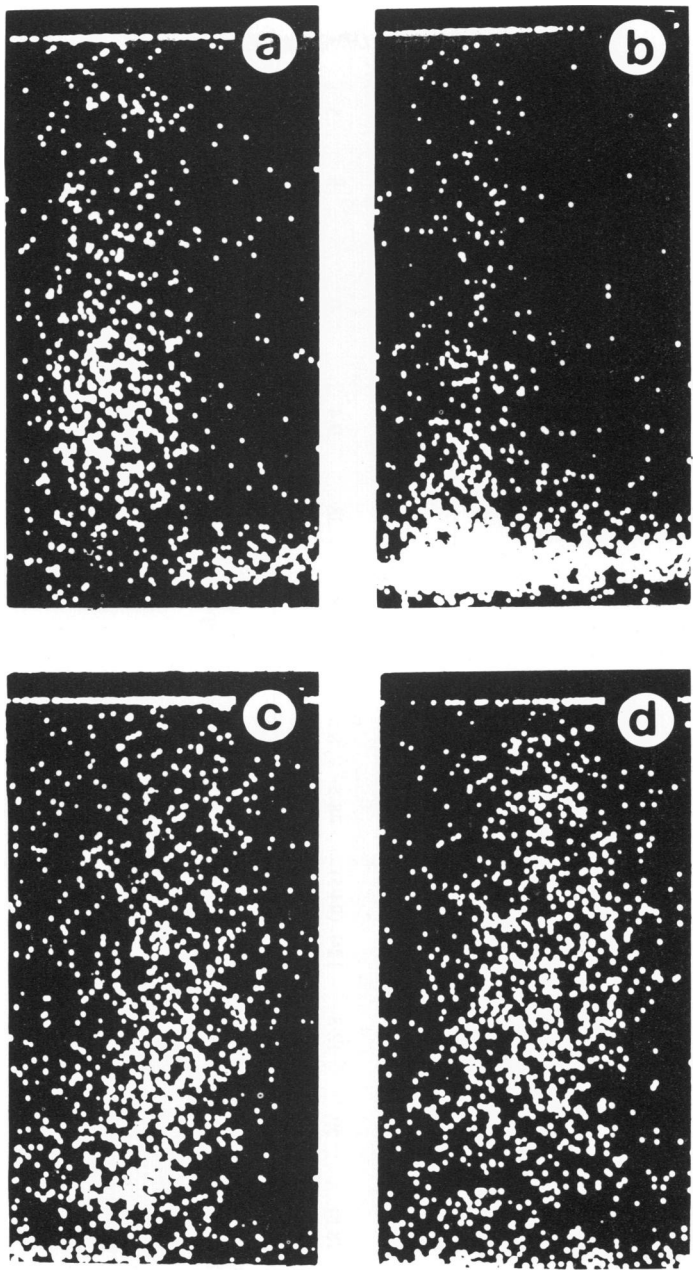

Fig. 2. HLA-DR and SIg on CALLA$^+$ enriched fractions. Bone marrow and spleen Vil-A1$^+$ mononuclear cells sorted out with the FACS were restained and re-analysed as described in Materials and Methods. FACS dot plots of CALLA$^+$ bone marrow (top panel) and spleen (bottom panel) cells counterstained for HLA-DR (a), (c) and SIg (b) (d). It can be seen that the majority of CALLA$^+$ cells is HLA-DR$^+$ in both tissues; however, only a small proportion of them is SIg$^+$ in the bone marrow in contrast to the spleen where they are all SIg$^+$. It can be seen that the bone marrow B cells are smaller than those of the spleen. Horizontal axis: light scattering (cell size). Vertical axis: fluorescence intensity.

Table 4. Correlation between TdT and cell-surface markers in fetal bone marrow and liver

Specimen	CALLA$^+$	TdT$^+$	TdT$^+$/CALLA$^+$	FMC8$^+$	TdT$^+$	TdT$^+$/FMC8$^+$	BA1$^+$	TdT$^+$	TdT$^+$/BA1$^+$	Y29.55$^+$	TdT$^+$	TdT$^+$/Y29.55$^+$	SmIg$^+$	TdT$^+$	TdT$^+$/SmIg$^+$
Bone Marrow	45·5	10·3	8·1 (78·6)	50	12·8	12·1 (94·5)	48·5	8·8	8·8 (100)	28	9·4	0	5	5	0
Liver	9	2·3	2·3 (100)	16·2	2·2	1·5 (68)	9·2	1·4	1·4 (100)	3·6	1·4	0	4	1·3	0

The study was done on three bone marrows and three livers. The numbers, expressing percentage of positive cells were obtained from the average of three separate labellings. Numbers in parenthesis indicate the percentage of TdT$^+$ cells that were also positive for the relevant surface marker. Fetal spleens were not included as they contained less than 1/1,000 TdT$^+$ cells which were SmIg negative.

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REFERENCES

ABRAMSON, C.S., KERSEY, J.G. & LEBIEN T.W. (1981) Monoclonal antibody (BA-1) reactive with cells of human B lymphocytes lineage. *J. Immunol.* **126,** 83.

ASMA, G.E.M., PICHLER, W., SCHUIT, H.R.E., KNAPP, W. & HIJMANS W. (1977) The development of lymphocytes with T- or B-membrane determinants in the human foetus. *Clin. exp. Immunol.* **29,** 278.

BOLLUM, F. (1979) Terminal deoxynucleotidyl transferase as a hematopoietic cell marker. *Blood,* **54,** 1203.

BONATI, A., CASOLI, C., STARCICH, B. & BUSCAGLIA, M. (1983) Terminal deoxynucleotidyl transferase in human fetuses. *Scand. J. Hemat.* **31,** 447.

BRODSKY, F.M., PARHAM, P., BARNASTABLE, C.J., CRUMPTON, M.J. & BODMER, W.F. (1979) Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* **47,** 3.

BROOKS, D.A., BRADLEY, J. & ZOLA, H. (1982) A differentiation antigen expressed selectively by a proportion of human blood cells: Detection with a monoclonal antibody. *Pathology,* **14,** 5.

DELIA, D., COSTA, A., GIARDINI, R., DEL BINO, G., SILVESTRINI, R. & ZOLA, H. (1984) Surface phenotype and cell kinetics in leukaemias and lymphomas. In *Leucocyte Typing* (ed. by A. Bernard, L. Boumsell, J. Daussett, C. Milstein & S.F. Schlossman) pp. 579. Springer Verlag, Berlin.

FORSTER, H.K., GUDAT, F.G., GIRARD M.F. ALBRECHT, R., SCHMIDT, J., LUDWIG, C. & OBRECHT, J.P. (1982) Monoclonal antibody against a membrane antigen characterizing leukemic human B-lymphocytes. *Cancer Res.* **42,** 1927.

GATHINGS, W.E., LAWTON, A.R. & COOPER, M.D. (1977) Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. *Eur. J. Immunol.* **7,** 804.

GITLIN, D. & BIASUCCI, A. (1969) Development of yG, yA, yM, $B_{1C}/B_{1A}C'1$ esterase inhibitor, caerutoplasmin, transferin, hemopexin, haptoglobin, fibrinogen, plasminogen, antitrypsin, orosomucoid, B lipoprotein, a_2 macroglobulin and pre-albumin in the human conceptus. *J. clin. Invest.* **48,** 1433.

GREAVES, M., DELIA, D., JANOSSY, G., RAPSON, N., CHESSELLS, J., WOODS, M. & PRENTICE, G. (1980) Acute lymphoblastic leukaemia associated antigen. IV. Expression on non-leukaemic 'lymphoid' cells. *Leuk. Res.* **4,** 1, 15.

GREAVES, M.F., HARIRI, G., NEWMAN, R.A., SUTHERLAND, D.R., RITTER, M.A. & RITZ, J. (1983) Selective expression of the common acute lymphoblastic leukemia (gp100) antigen on immature lymphoid cells and their malignant counterparts. *Blood,* **61,** 4, 628.

GUDAT, F.G., FORSTER, H.F., GIRARD, M.F., ALBRECHT, R., LUDWIG, C. & OBRECHT, J.P. (1981) Recognition of leukemic B lymphoma cells by monoclonal anti-Y29/55. In *Leukemic Markers* (ed. by W. Knapp) p 109. Academic Press, New York.

GUY, K. & HEYNINGEN, V. (1983) An ordered sequence of expression of human MHC class II antigens during B cell maturation? *Immunol. Tod.* **4,** 186.

HOKLAND, P., ROSENTHAL, P., GRIFFIN, J.D., NADLER, L.M., DALEY, J., HOKLAND, M., SCHLOSSMAN, S.F. & RITZ, J. (1983) Purification and characterization of fetal hematopoietic cells that express the common acute lymphoblastic leukemia antigen (CALLA). *J. exp. Med.* **157,** 114.

JANOSSY, G. & GREAVES, M.F. (1978) Diagnostic use of an antiserum made against acute lymphoid leukaemia associated antigen. *Bibliotheca Haemat.* **45,** 156.

JANOSSY, G., BOLLUM, F.J., BRADSTOCK, K.F., MCMICHAEL, A., RAPSON, N. & GREAVES, M.F. (1979) Terminal transferase-positive human bone marrow cells exhibit the antigenic phenotype of common acute lymphoblastic leukemia. *J. Immunol.* **123,** 4, 1525.

KNAPP, W., MAJDIC, O., BETTELHEIM, P. & LISZKA, K. (1982) VIL-A1 a monoclonal antibody reactive with common acute lymphatic leukemia cells. *Leuk. Res.* **6,** 2, 137.

KORSMEYER, S.J., HIETER, P.A., RAVETCH, J.V., POPLACK, D.G., LEDER, P. & WALDMAN, T. (1981) Patterns of immunoglobulin gene arrangement in human lymphocytic leukemias. In *Leukemia Markers* (ed. by W. Knapp) p. 85. Academic Press, New York.

LEBIEN, T.W., BRASHEM, C.J., ABRAMSON, C.S., FRENZEL, E.M., BOLLUM, F.J. & KERSEY, J.H. (1981) Studies of human lymphopoiesis with monoclonal antibodies BA1 and BA2. In *Leukemia Markers* (ed. by W. Knapp) p. 81 Academic Press, New York.

METZGAR, R.S., BOROWITZ, M.J., JONES, N.H. & DOWELL, B.L. (1981) Distribution of common acute lymphoblastic leukemia antigen in non hematopoietic tissues. *J. exp. Med.* **154,** 1249.

MITTLER, R.S., TALLE, M.A., CARPENTER, K., RAO, P.E. & GOLDSTEIN, G. (1983) Generation and characterization of monoclonal antibodies reactive with human B lymphocytes. *J. Immunol.* **131,** 1754.

NAVARRETE, C., FAINBOIM, L, FESTENSTEIN, H., LISTER, A., AMESS, J. & GREAVES, M. (1981) Selective

expression of HLA-DR and HLA-MB antigens on acute leukemic cells. In *Leukemia Markers* (ed. by W. Knapp) p. 65 Academic Press, New York.

NEWMAN, R.A., SUTHERLAND, R. & GREAVES, M.F. (1981) The biochemical characterization of a cell surface antigen associated with acute lymphoblastic leukemia and lymphocyte precursors. *J. Immunol.* **126,** 5, 2024.

NEWMAN, R.A., DELIA, D., GREAVES, M.F., NAVARRETE, C., FAINBOIM, L. & FESTENSTEIN, H. (1983) Differential expression of HLA-DR and DR-linked determinants on human leukemias and lymphoid cells. *Eur. J. Immunol.* **13,** 172.

RITZ, J., PESANDO, J.M., NOTIS-MCCONARTY, J., LAZARUS, H. & SCHLOSSMAN, S.F. (1980) A monoclonal antibody to human acute lymphoblastic leukaemia antigen. *Nature*, **283.** 583.

RITZ, J., NADLER, L.M., BAHN, A.K., NOTIS-MCCONARTY, J., PESANDO, J.M. & SCHLOSSMAN, S.F. (1981) Expression of common acute lymphoblastic leukemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. *Blood*, **58,** 3, 648.

ZIEGLER, A., UCHANSKA- ZIEGLER, B., ROSENFELDER, G., BRAUN, D.G. & WERNET, P. (1981) Heterogeneity of established human hemopoietic cell lines: Surface antigens detected by monoclonal antibodies and glycosphingolipid patterns. In *Leukemia Markers* (ed. by W. Knapp) p. 317. Academic Press, New York.