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Characterization of HLA-DR antigens on leukaemic cells

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SUMMARY

A large series of leukaemias (1,512 cases) and leukaemic cell lines (40) have been tested for selective expression of a monomorphic HLR-DR determinant using a monoclonal antibody (DA2). Relatively mature myeloid leukaemias (APML, CGL) and erythroid leukemias are DR^-, in contrast to most (72% leukaemias of myeloid precursors (e.g. AML) which are DR^+. Non-T ALL are DR^+ but T (thymic) ALL are invariably DR^-. In contrast to the latter, some leukaemias with mature T cell phenotypes are DR^+. Leukaemias or lymphomas of B cells and B cell precursors (e.g. pre-B ALL) are invariably DR^+, whereas myeloma or plasma cell leukaemias are DR^-. This pattern of selective expression appears to closely parallel that seen in normal haemopoietic differentiation. Biochemical features of HLA-DR structures on leukaemic cells have been compared with the known features of B cell derived DR molecules and in one case of ALL compared with an autologous (EBV transformed) B cell line. Most leukemic cells showed the same general α and β two chain structure. However, B cell line and most chronic leukaemias showed the presence of an extra band of molecular weight 30,000 daltons (p30) with an intermediate electrophoretic mobility on SDS-PAGE between that of the α and β DR chains. In acute leukaemias and leukaemic cell lines (i.e. immature cells) p30 was not seen unless short labelling times were used. Two dimensional NEPHGE/SDS-PAGE under appropriate labelling conditions showed that the pattern of spots obtained from an ALL line (Nalm-6) and its autologous EBV transformed partner (B85) were similar though not identical. Pulse chase labelling of Nalm-6 and B85 showed that the turnover rate of p30 relative to DR α and β chains, differed in the two lines.

INTRODUCTION

A substantial proportion of acute lymphoblastic leukaemia and acute myeloblastic leukaemia express Ia-like or HLA-DR associated cell surface antigens as detected with either rabbit antisera (anti-p28/33) or monoclonal antibodies to monomorphic DR determinants (Schlossman *et al.*, 1976). The simplest interpretation of this observation is that DR positive leukaemias either derive from normal DR positive haemopoietic cells and/or are in apparent maturation arrest at

Abbreviations: SDS = sodium dodecyl sulphate; PAGE = polyacrylamide gel electrophoresis; NEPHGE = non-equilibrium pH gradient electrophoresis; FACS = fluorescence activated cell sorter; cALL = common acute lymphoblastic leukaemia; AML = acute myeloid leukaemia; AMML = acute myelomonocytic leukaemia; CLL = chronic lymphocytic leukaemia; NHL = non-Hodgkin lymphoma; PLL = prolymphocytic leukaemia; HCL = hairy cell leukaemia; AMonL = acute monocytic leukaemia; APML = acute promyelocytic leukaemia; CMML = chronic myelomonocytic leukaemia; CGL = chronic granulocytic leukaemia.

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developmental positions corresponding to normal haemopoietic cells that express DR (Greaves & Janossy, 1978). An alternative view is that DR expression on leukaemic cells is aberrant and reflects a distortion of normal gene expression. Support for the former view comes from the finding that normal myeloid precursors are DR positive (Winchester *et al.*, 1977; Janossy *et al.*, 1978; Koeffler *et al.*, 1979) as are terminal transferase positive lymphocyte precursors in normal bone marrow (Janossy *et al.*, 1979). These observations are of special interest since they may indicate that DR molecules play a role in regulatory interactions during early haemopoiesis (Greaves, 1978).

Acute leukaemias represent a convenient source for studying membrane antigens on cells arrested at early stages in the haemopoietic pathways. We have now determined more precisely the pattern of selective expression of DR antigens on leukaemic cells by examining more than 1,000 cases of human leukaemia using the anti-monomorphic DR monoclonal DA2. With few, if any, exceptions this pattern parallels the selectivity observed in normal haemopoietic differentiation. The biochemical features of DR antigens isolated from leukaemic cells has been compared with the known characteristics of these molecules when isolated from B cells or B lymphoblastoid cell lines; in one instance it has been possible to compare DR molecules isolated from a lymphoblastic leukaemia (of lymphoid precursor phenotype) with an autologous, EBV transformed, B cell partner.

MATERIALS AND METHODS

Antisera and cells. Rabbit anti-HLA-DR serum was a gift from Dr M.J. Crumpton (ICRF) (Snary *et al.*, 1977). The monoclonal antibodies DA2, CA2.06 and TDR31.1 directed against HLA-DR monomorphic determinants were kindly provided by Drs W. Bodmer, M.J. Crumpton and T.A. de Kretzer (Brodsky *et al.*, 1979; Charron & McDevitt, 1979; DeKretzer *et al.*, 1982). Other monoclonal reagents used have been described elsewhere (Greaves *et al.*, 1981; Greaves, 1981).

Fresh leukaemic cells were obtained from many different hospitals throughout the country as part of an immunodiagnostic service (Greaves *et al.*, 1981b). Cell lines used in this study have been previously described (Minowada *et al.*, 1978) and include B85, Nalm-6, Nalm-1, KM3, Reh and U937.

Immunofluorescence and rosette formation. Indirect immunofluorescence was carried out as previously described using a fluorescein conjugated $F(ab')_2$ goat anti-mouse Ig second layer (Verbi *et al.*, 1982). Mouse and sheep rosette formation was assessed as previously described (Verbi *et al.*, 1982).

Immune complex formation and pulse chase labelling. Cells labelled with ^{35}S-methionine were lysed and immunoprecipitated as previously described (Newman, Sutherland & Greaves, 1981). Pulse chase labelling using ^{35}S-methionine and chasing with unlabelled L-methionine was carried out as described for HLA-AB antigens by Owen *et al.* (1980).

NEPHGE/SDS-PAGE analysis. Immunoprecipitates were run on 11 cm NEPHGE gels (2 mm diameter) as described by O'Farrell, Goodman & O'Farrell (1977), followed by separation in the second dimension by SDS-PAGE.

RESULTS

Selective expression of DR antigens in different types of leukaemia

One thousand, five hundred and twelve cases of acute and chronic leukaemia either at presentation (79%) or in relapse (21%) with high counts of leukaemic cells (> 70% blasts, or white blood cell counts of > 30,000) were examined for HLA-DR expression by indirect immunofluorescence using the monoclonal antibody DA2. Observations were made by fluorescence microscopy and, in parallel, by flow analysis (FACS-1). Table 1 summarizes these data.

In ALL, with one possible exception,* DR expression is restricted to non-T ALL in which more

* See footnote Table 1.

Table 1. Expression of HLA-DR in haemopoietic malignancies (tested using monoclonal DA2 anti-monomorphic HLA-DR determinant)

Leukaemia/subtype‡	DR positive (*n*)	DR negative (*n*)
Acute lymphoblastic leukaemia		
Common ALL	549	7
Null-ALL	87	1
T-ALL	0†	131
T-NHL*	0	21
Mature lymphoid leukaemias		
B cell lineage:		
B-CLL	54	0
B-PLL	13	0
B-ALL	14	0
B-NHL	23	0
B-HCL	6	0
B-myeloma/plasma cell leukaemia	0	10
T cell lineage:		
T-CLL	3	7
T-PLL	0	10
T-Sezary/mycosis fungoides	1	10
T-lymphoblastic lymphoma	2	2
Myeloid leukaemias		
AML	219	83
AMML	41	10
AMonL	12	1
APML	0	11
CMML	0	4
Ph^{1+} CGL		
CGL	3§	65
CGL blast crisis:		
'Lymphoid'	31	1
'Non-lymphoid'/'myeloid'	56	16
Erythroid	0	3
Erythroleukaemia	0	22
Megakaryoblastic leukaemia	0	7
Acute undifferentiated leukaemia	24	12

* childhood.

† One exceptional case not represented here had the phenotype E rosette$^+$, DR$^+$, cALL antigen$^+$, TdT$^+$ but was negative for other T cell antigens. The classification of this patient's cells is therefore difficult since it could be regarded as a DR$^+$ thymic ALL or a cALL expressing the E rosette receptor.

‡ Classified by standard haematological criteria. ALL and CGL blast crisis subtyped according to immunological markers as described in detail elsewhere (Greaves *et al.*, 1981c; Greaves, 1981).

§ These three cases had modest proportions of blast cells, 25–30%, with increased percentages of immature myeloid cells and were considered to be in the accelerated (= 'early' blast crisis) phase of leukaemic evolution.

than 95% of cases are positive. These cases include a proportion of (~25%) of pre-B ALL (Greaves *et al.*, 1979). ALL and non-Hodgkin lymphoma (in children) with thymic phenotypes were DR negative.

In contrast to leukaemias and lymphomas of thymic phenotype, six out of 35 cases of mature T cell malignancy were DR positive (Table 1). Five of these were tested with monoclonal antibodies reactive with T subsets, and all five had an $OKT4^+/OKT8^-$ 'helper/inducer' phenotype.

All mature B lymphocyte leukaemias and lymphomas were DR positive but myelomas and plasma cell leukaemias were DR negative.

Amongst the leukaemias involving immature myeloid cells (AML, AMML, CGL in 'myeloid' blast crisis) most but not all cases were DR^+ (72%). In contrast, megakaryoblastic leukaemia (seven cases), erythroleukaemia (22 cases) and leukaemia of relatively more mature granulocytic cells (APML, CGL) were DR^- in all but three cases (Table 1).

A similar spectrum of DR expression was observed in leukaemic cell lines. All 13 lines derived from T ALL/NHL were DR^- whereas all lines derived from non-T ALL or B cells malignancies (excluding myeloma) were DR^+ (data not shown).

Isolation of HLA-DR antigens on acute leukaemias and leukaemic cell lines

Two cases of common acute lymphoblastic leukaemia (cALL), three cases of acute myelomonocytic leukaemia (AMML), two cases of acute myeloid leukaemia (AML) and one case of null ALL were

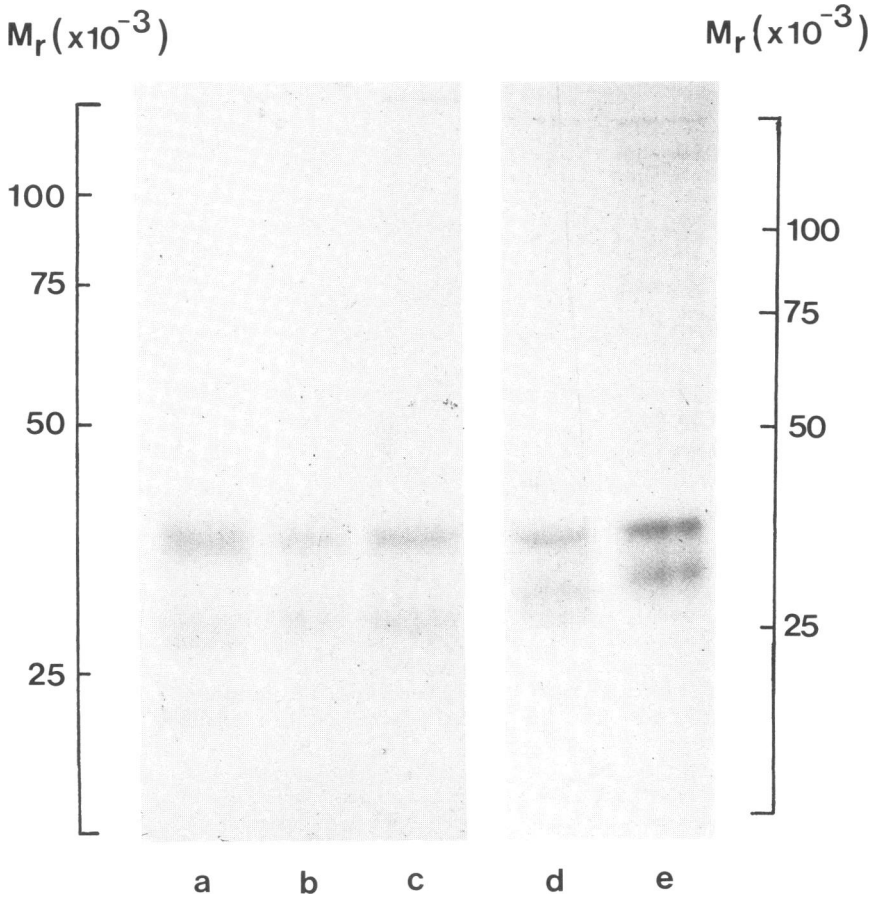

Fig. 1. Fluorogram of ^{35}S-methionine labelled cell lysates immunoprecipitated with rabbit anti-DR serum and run on 10% SDS-PAGE gels under reducing conditions. (a) KM3; (b) Reh; (c) NALM-1; (d) AMML; (e) U937.

examined for the presence of HLA-DR antigens by ID SDS-PAGE. After immunoprecipitation of ^{35}S-methionine labelled extracts all the above leukaemias showed the presence of α and β chains only (Fig. 1). A trace of an intermediate band was seen in one case of cALL, however.

The cell lines Reh, KM3, Nalm-6, all of which were originally derived from cALLs, Nalm-1 which originated from a chronic myeloid leukaemia in lymphoid blast crisis, and U937 which was established from a histiocytic lymphoma, all showed only HLA-DR α and β chains on SDS-PAGE (Fig. 1).

HLA-DR antigens on chronic lymphocytic cells

Seven cases of CLL were examined. Four cases showed the presence of an intermediate band (p30) on one dimensional SDS-PAGE which gave a pattern of spots on 2D NEPHGE/SDS-PAGE similar although simpler than that reported by other authors for B cells (Charron & McDevitt, 1980). Two cases showed faint p30 bands and in one case this was completely absent even after prolonged gel exposure. This was seen in 2D gels as the presence or absence of the basic or invariant spot (Fig. 2).

If CLL cells were examined for mouse rosetting ability or SIg, generally the samples with heavily labelled invariant spot had a high percentage of SIg^+ cells and how a low percentage of $M_R{}^+$ cells. The case with no invariant spot had 84% $M_R{}^+$ cells with only 4% SIg^+ cells. Mouse rosetting ability and SIg are often taken as a relative measure for immature and more mature B cells respectively and although the sample is too small for any conclusions to be drawn, there does appear to be a possible correlation with the expression (or detection) of p30 and the maturation status of the cell.

HLA-DR antigens on Nalm-6 and B85 autologous cell lines

The autologous pair of cell lines Nalm-6 and B85 have the advantage of representing a HLA-DR^+ cell arrested early in the haemopoietic differentiation pathway (Nalm-6) and the more mature

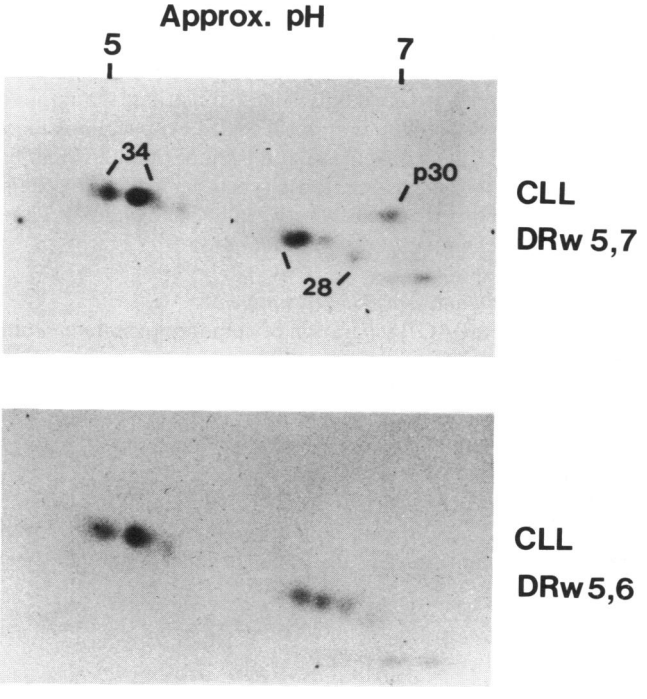

Fig. 2. Fluorogram of a two dimensional NEPHGE/SDS-PAGE separation of immunoprecipitates from CLL cells. (Top) CLL sample showing basic p30 polypeptide spot. (Bottom) CLL sample without p30 spot. Gels were run under reducing conditions after immunoprecipitation with rabbit anti-DR.

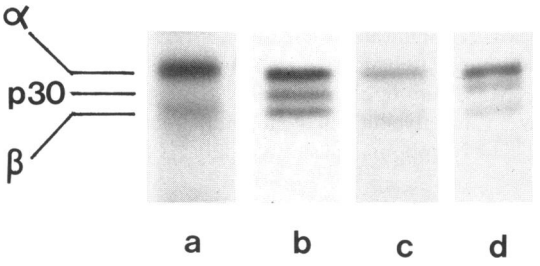

Fig. 3. Immunoprecipitation of DR antigens from autologous cell lines B85 and Nalm-6 with rabbit antiserum. (a) ^{125}I-lactoperoxidase labelled B85; (b) B85 cells labelled with ^{35}S-methionine for 6 hr; (c) Nalm-6 cells labelled with ^{35}S-methionine for 6 hr; (d) Nalm-6 cells labelled with ^{35}S-methionine for 1 hr.

HLA-DR$^+$ B cell line (B85). If these two cell lines are labelled under standard conditions of 6 or 16 hr, B85 shows a band at 30K which is not detectable in the line Nalm-6 (Fig. 3).

If B85 cells are labelled by ^{125}I-lactoperoxidase then only two chains are seen (Fig. 3). If Nalm-6 cells are labelled briefly, for 1 hr with ^{35}S-methionine, then p30 becomes visible in immunoprecipitates. Increasing the labelling time leads to a progressive decrease in p30, as seen in SDS-PAGE of immunoprecipitates. Thus p30 is indeed present in Nalm-6 cells but requires brief labelling times in order to be observed satisfactorily. This phenomenon can be further examined by pulse chase labelling.

Pulse chase labelling of Nalm-6 and B85

Fig. 5 shows the results of pulse chase labelling experiments on B85 and Nalm-6 cells. It can be seen that in B cell line B85, α and β chains as well as p30 are labelled very rapidly (Fig. 4). After longer times (e.g. 8 hr) both α chains and p30 are almost chased out simultaneously. In Nalm-6, p30 is present and begins to appear strongly at 20 min chase. At 5 hr it is either chased out or chased into the higher molecular weight position at which the α chain runs. The rabbit antiserum used possibly reacts with both α and β chains and therefore it is difficult to quantitate the amounts of each chain as there may be non-stoichiometric synthesis of individual chains. Application of more material to the gel or longer exposure times did not change the relationship of the individual bands.

There is no obvious precursor–product relationship between p30 and α chains and the rate of turnover between these chains differs significantly between B85 and Nalm-6. Thus it is unlikely that p30 is a precursor of the DR α chain.

Two dimensional separation of Nalm-6 and B85 DR antigens

Two dimensional NEPHGE/SDS-PAGE separation of immunoprecipitates from B85 and Nalm-6 show a highly complex yet essentially similar pattern. The set of 28K spots described by Charron & McDevitt (1980) as being characteristic for a given DR specificity show identical distributions (Fig. 5). The basic, p30 spot can be seen in an identical position in each cell line and thus is probably the same component. In the B cell line B85, higher molecular weight spots stretching between the basic (p30) spot and the α set of spots can be seen. This series has been previously observed in DR patterns from various B cell lines and may represent α spot precursors and/or processed forms of the basic spot (De Kretzer *et al.*, 1982). Nalm-6, by contrast, shows only a faint pattern of spots in this region. This may be a reflection of different turnover rates of p30 or other components between the two cell lines.

DISCUSSION

This study documents the selective expression of an HLA-DR monomorphic determinant on different types of leukaemic cells. A comparison with the known pattern of expression on normal

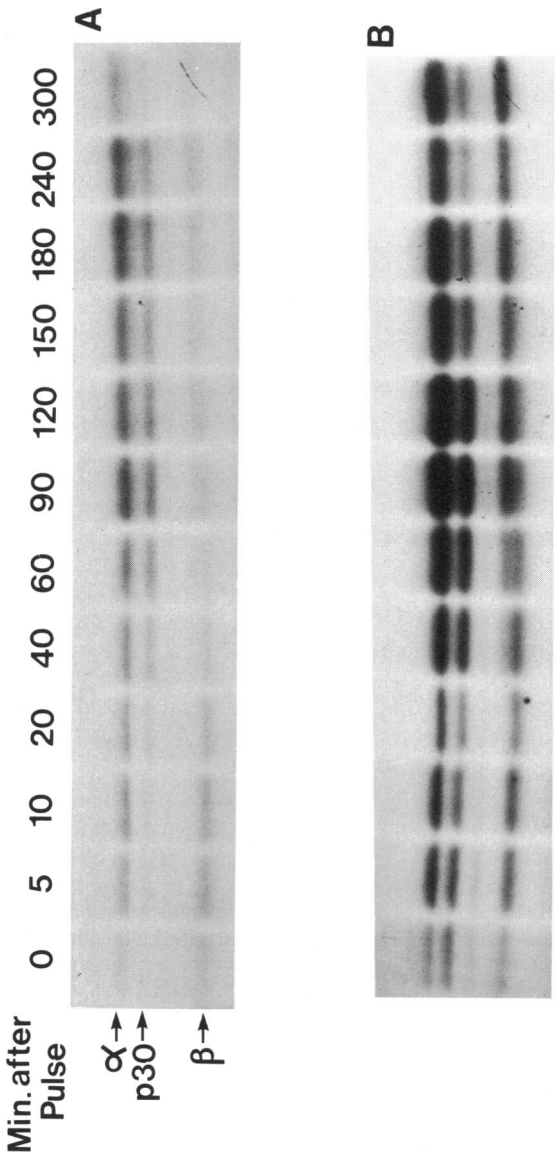

Fig. 4. Pulse chase labelling of B85 and Nalm-6 cells with ^{35}S-methionine. (a) Nalm-6 cells showing appearance of p30 after 20 min which virtually chased out after 5 hr; (b) B85 cells showing early appearance of p30 and chase of β chain into higher molecular weight at 60 min.

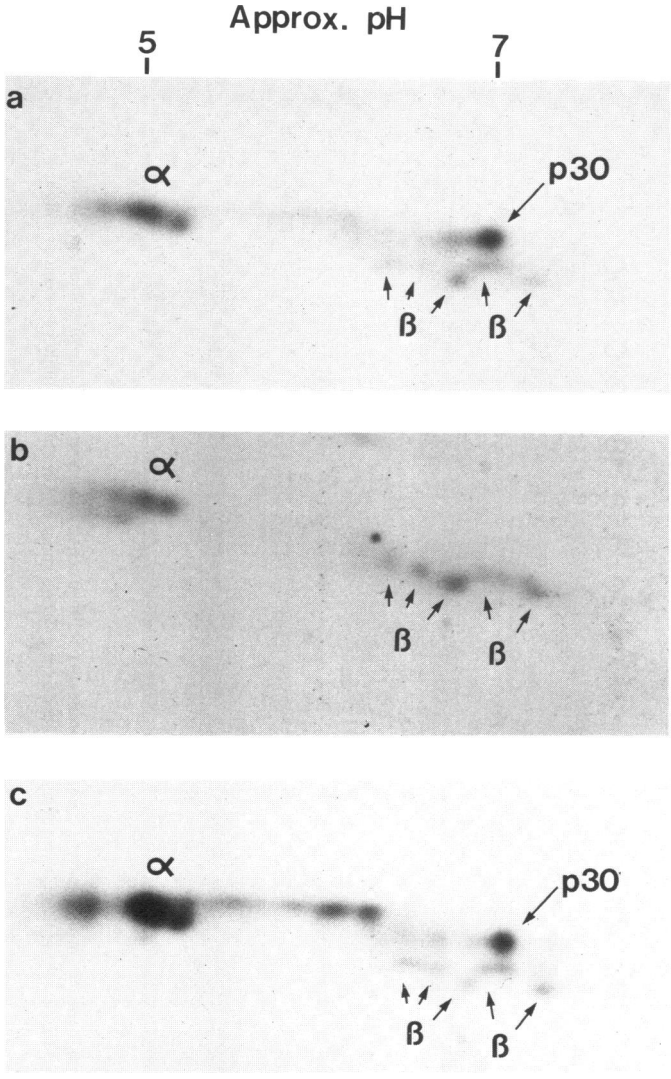

Fig. 5. Two dimensional NEPHGE/SDS-PAGE pattern of polypeptides precipitated from ^{35}S-methionine labelled B85 and Nalm-6 cells with rabbit anti-DR. (a) Nalm-6 cells labelled for 1 hr showing clear presence of p30 as well as α and β groups of spots. (b) Nalm-6 cells labelled for 6 hr showing absence of p30 basic spot. No basic spot is visible even after prolonged exposures. (c) B85 cells labelled for 6 hr showing basic p30 spot, α spots, two possible sets of β chains and an intermediate series of spots stretching between p30 and the α chain group.

haemopoietic cells (Greaves & Janossy, 1978; Greaves *et al.*, 1981a; Winchester & Kunkel, 1979) suggests that in qualitative terms, at least, DR expression in leukaemia reflects a remarkable conservation or fidelity of phenotype, i.e. the DR$^+$ leukaemias originate in a DR$^+$ cell type and/or are in apparent maturation arrest in a 'developmental position' and proliferative state corresponding to DR$^+$ normal cells. Thus immature myeloid cells in AML are frequently DR$^+$ as are normal myeloblasts and CFU-GM (Janossy *et al.*, 1978; Koeffler *et al.*, 1979; Winchester & Kunkel, 1979). Similarly, granulocytic cells in Ph^1 positive CGL, like normal granulocytes Winchester *et al.*, 1977), are DR$^-$ but clonal evolution leads to the dominance of DR$^+$ myeloblasts in maturation arrest in blast crisis or acute leukaemia develops.

Thymic leukaemias are DR$^-$ but some mature T cell leukaemias are DR$^+$ paralleling normal

thymocytes and 'activated' mature T cells (Winchester & Kunkel, 1979) respectively. TdT^+ common ALL are DR^+ in common with the same putative $cALL^+/TdT^+$ lymphocyte progenitor population in normal bone marrow. Pre-B ALL (Greaves *et al.*, 1979) and mature B cell leukaemias and lymphomas also show 'conservation' of DR expression since their normal counterparts are DR^+ (Winchester & Kunkel, 1979). In contrast, plasma cell tumours (myeloma, plasma cell leukaemia) are DR^-, thus mimicking the loss of DR expression which occurs during normal maturation of B cells to plasma cells (Winchester & Kunkel, 1979). This conservation of normal gene expression appears to be a constant feature of leukaemias (Greaves & Janossy, 1978; Greaves *et al.*, 1981a) but is particularly striking in this instance since such large numbers of leukaemias were tested for a gene product which appears to have a strict pattern of control in normal haemopoiesis and probably serves an important role in regulatory interactions of cells.

The general structure of DR molecules isolated from leukaemic cells was very similar to that previously reported for B cells and B cell lines (Snary *et al.*, 1977; Charron & McDevitt, 1979, 1980; Springer *et al.*, 1977; Humphreys *et al.*, 1976). Acute leukaemias and leukaemic cell lines labelled metabolically with ^{35}S-methionine for long periods (6–18 hr) generally give two bands when examined by SDS-PAGE. These bands have molecular weights of 34,000 and 28,000 and are usually designated α and β chains respectively. HLA-DR^+ cALLs, null ALLs, AML and AMML all give this characteristic two band pattern with identical molecular weight for α and β chains respectively. When brief labelling times (30–60 min) were used, a third band (p30) becomes visible in extracts from ^{35}S-methionine labelled leukaemic cells.

The results reported here suggest that p30 is indeed present in acute leukaemias but cell labelling time is important for its visualization in immune precipitates. It is not obviously clear why short labelling times lead to better visualization of p30. Without antibodies directed against each individual chain, a full understanding is impossible.

The apparent correlation between maturation status and presence of p30 is most likely indirect; the presence of p30 in immunoprecipitates is more likely a reflection of metabolic activity than maturation *per se*. However, when cells are labelled for long periods, B cells and most CLLs show a strong p30 band whereas in most acute leukaemias (lymphoid and myeloid) this band is absent or weak.

The question arises therefore whether p30 is an obligatory component of the α–β DR chain complex or simply an unrelated component with a high affinity for the hydrophobic domains of α and β chains. Some interesting observations by McMillan *et al.* (1981) have recently shown that the association of the basic spot with α and β Ia chains in mouse immunoprecipitates varies with the haplotype used and thus probably p30–β chain interaction is important. De Kretzer *et al.* (1982) have suggested from observations on several B cell lines, using 2D pulse chase analysis, that p30 may be processed via a series of steps to a higher molecular weight form with a molecular weight similar to that of the α chain. Whether this form reaches the cell surface is not known. It is also not known whether p30 is a product of the HLA-D locus or some related locus; it is tempting to speculate that the turnover of p30 is independent of DR α and β chains and that a differential expression between these two sets of products reflect the metabolic turnover of a cell in either an immature (e.g. acute luekaemic cells) or mature (e.g. B cells, CLLs) haematological state. These observations support the contention that, qualitatively, normal gene expression and cell surface antigen are usually preserved in leukaemia (Greaves & Janossy, 1978; Winchester *et al.*, 1977).

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REFERENCES

BRODSKY, F.M., PARHAM, P., BARNSTABLE, C.J., CRUMPTON, M.J. & BODMER, W.F. (1979) Hybrid myeloma monoclonal antibodies against MHC products. *Immunol. Rev.* **47**, 3.

CHARRON, D. & MCDEVITT, H.O. (1979) Analysis of HLA-D region-associated molecules with monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* **76**, 6567.

CHARRON, D.J. & MCDEVITT, H.O. (1980) Characterisation of HLA-D-region antigens by two-dimensional electrophoresis. Molecular genotyping. *J. exp. Med.* **152,** 18s.

DE KRETZER, T.A., CRUMPTON, M.J., BODMER, J.G. & BODMER, W.F. (1982) Demonstration of two distinct light chains in HLA-DR associated antigens by two-dimensional gel electrophoresis. *Eur. J. Immunol.* **12,** 214.

GREAVES, M.F. (1978) Cell surface structures, differentiation and malignancy in the haemopoietic system. In *Cell–Cell Recognition* (ed. by A.S.G. Curtis) pp. 429. Cambridge University Press, Cambridge.

GREAVES, M.F. (1981) Monoclonal antibodies as probes for leukaemic heterogeneity and haemopoietic differentiation. In *Leukemia Markers* (ed. by W. Knapp) pp. 19. Academic Press, New York.

GREAVES, M.F., DELIA, D., ROBINSON, J., SUTHERLAND, R. & NEWMAN, R. (1981a) Exploitation of monoclonal antibodies: A 'who's who' of haemopoietic malignancy. *Blood Cells*, **7,** 257.

GREAVES, M.F. & JANOSSY, G. (1978) Patterns of gene expression and the cellular origins of human leukaemias. *Biochem. Biophys. Acta.* **516,** 193.

GREAVES, M.F., JANOSSY, G., PETO, J. & KAY, H. (1981b) Immunologically defined subclasses of acute lymphoblastic leukaemia in children: their relationship to presentation features and prognosis. *Br. J. Haematol.* **48,** 179.

GREAVES, M.F., RAO, J., HARIRI, G., VERBI, W., CATOVSKY, D., KUNG, P. & GOLDSTEIN, G. (1981c) Phenotypic heterogeneity and cellular origins of T cell malignancies. *Leuk. Res.* **5,** 281.

GREAVES, M.F., VERBI, W., VOGLER, L., COOPER, M., ELLIS, R., GANESHAGURU, K., HOFFBRAND, V., JANOSSY, G. & BOLLUM, F.J. (1979) Antigenic and enzymatic phenotypes of the pre-B subclass of acute lymphoblastic leukaemia. *Leuk. Res.* **3,** 353.

HUMPHREYS, R.E., MCCUNE, J.M., CHESS, L., HERMAN, H.C., MALENKA, D.J., MANN, D.L., PARHAM, P., SCHLOSSMAN, S.F. & STROMINGER, J.L. (1976) Isolation and immunologic characterisation of a human B-lymphocyte-specific cell surface antigen. *J. exp. Med.* **144,** 98.

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

JANOSSY, G., FRANCIS, G.E., CAPELLARO, D., GOLDSTONE, A.H. & GREAVES, M.F. (1978) Cell sorter analysis of leukaemia-associated antigens on human myeloid precursors. *Nature*, **276,** 176.

KOEFFLER, H.P., NISKANEN, E., CLINE, M., BILLING, R. & GOLDE, D. (1979) Human myeloid precursors forming colonies in diffusion chambers express the Ia-like antigen. *Blood*, **54,** 1188.

MCMILLAN, M., FRELINGER, J.A., JONES, P.P., MURPHY, D.B., MCDEVITT, H.O. & HOOD, L. (1981) Structure of murine Ia antigens. Two dimensional electrophoretic analyses and high pressure liquid chromatography tryptic peptide maps of products of the I-A and I-E subregions and of an associated invariant polypeptide. *J. exp. Med.* **153,** 936.

MINOWADA, J., JANOSSY, G., GREAVES, M.F., TSUBOTA, T., SRIVASTAVA, B.I.S., MORIKAWA, S. & TATSUMI, E. (1978) Expression of an antigen associated with acute lymphoblastic leukaemia in human leukemia-lymphoma cell lines. *J. Natl. Cancer Inst.* **60,** 1269.

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

O'FARRELL, P.Z., GOODMAN, H.M. & O'FARRELL, P.H. (1977) High resolution two-dimensional electrophoresis of basic as well as acid proteins. *Cell*, **12,** 1133.

OWEN, M.J., KISSONERGHIS, A.M. & LODISH, H.F. (1980) Biosynthesis of HLA-A and HLA-B antigens *in vivo*. *J. biol. Chem.* **255,** 9678.

SCHLOSSMAN, S.F., CHESS, L., HUMPHREYS, R.E. & STROMINGER, J.L. (1976) Distribution of Ia-like molecules on surface of normal and leukaemic human cells. *Proc. Natl. Acad. Sci. USA.* **73,** 1288.

SNARY, D., BARNSTABLE, C.J., BODMER, W.F., GOODFELLOW, P. & CRUMPTON, M.J. (1977) Cellular distribution, purification and molecular nature of human Ia antigens. *Scand. J. Immunol.* **6,** 439.

SPRINGER, T.A., KAUFMAN, J.F., TERHORST, C. & STROMINGER, J.L. (1977) Purification and structural characterisation of human HLA-linked B cell antigens. *Nature*, **268,** 213.

WINCHESTER, R.J. & KUNKEL, H.G. (1979) The human Ia system. *Advances in Immunology*, Vol. 28, p. 221. Academic Press, New York.

WINCHESTER, R.J., ROSS, G.D., JAROWSKI, C.I., WANG, C.Y., HALPER, J. & BROXMEYER, H.E. (1977) Expression of Ia-like antigen molecules on human granulocytes during early phases of differentiation. *Proc. Natl. Acad. Sci. USA.* **74,** 4012.

VERBI, W., GREAVES, M.F., KOUBEK, K., JANOSSY, G., STEIN, H., KUNGE, P. & GOLDSTEIN, G. (1982) OKT11 and OKT11A: monoclonal antibodies with pan T reactivity which block sheep erythrocyte 'recptors' on T cells. *Eur. J. Immunol.* **12,** 81.