Differentiation antigens in acute leukaemia

DENIS J. REEN
Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland

The heterogeneity of the haemopoietic cell system has been recognized for many years because of the diversity of functional and morphological differences between various cell types. This is particularly well exemplified in the clinical expression of various leukaemias where the morphological appearance, clinical presentation and response to therapy are a reflection of the leukaemic cell type. Cells of the haemopoietic system and their malignant offspring may be classified into different types and subtypes based on their expression of specific or selective cell markers. These cell types. This is particularly well exemplified in the Children, Crumlin, Dublin 12, Ireland of maturation and differentiation-associated cell antigens of the haemopoietic system and their malignant offspring may be classified into different types and subtypes based on their expression of specific or selective cell markers. These markers are various structural antigens such as the sheep erythrocytes, complement receptors or insulin. A wide range of enzyme analyses describing a variety of enzyme abnormalities or alternations has also been used to distinguish subsets of leukaemia (Drexler et al., 1981). The most extensive use of cell-specific markers for the study of leukaemia, however, has been the application of monoclonal antibodies for the identification and characterization of differentiation and maturation-associated cell antigens of the haemopoietic system. The advent of monoclonal antibody technology has had a major impact on our understanding of the immunobiology of human leukaemia since it is now recognized that leukaemias, especially those of the lymphoid system, can be subdivided into clinically relevant subgroups based on the expression of normal cell differentiation antigens (Thierfelder et al., 1976). This immunological approach to the classification of normal and leukaemia cell differentiation and maturation antigens is based on the concept that as a cell differentiates it must change its molecular content so as to acquire new functional capabilities and discard old ones. Thus, each cell type has a fixed differentiation pathway plotted out along which it loses or acquires various structural antigens as it accurately mirror the genetically determined commitment of the cell. Marker studies in normal haemopoiesis and in leukaemia are therefore used to gain some insight into the development biology and cell lineage relationship of normal haemopoietic tissue and by using this knowledge to gain an understanding of the biology, evolution and clinical heterogeneity of haemopoietic tissue.

The phenotypic profile and cellular characteristics of 'pluripotent stem cells', which are capable of differentiating to all blood cell lineages, are not known. The first recognizable stem cell which is already lineage committed expresses Ia-like (HLA-DR) antigens. These HLA-DR antigens are lost on all cell lineages of the haemopoietic system upon maturation, with the exception of B-lymphocytes and a subset of the monocytes/macrophage series. The appearance of myeloid-specific cell-surface antigens corresponds to the loss of HLA-DR antigens on maturing myeloblast cells. A number of monoclonal antibodies recognizing a strongly immunogenic myeloid-specific cell-surface antigen expressed on all cells of the myeloid lineage have been characterized (Majdic et al., 1981). This antigen which is recognized by the monoclonal antibody VIM-D5 consists of two polypeptide chains of molecular mass 150 and 105 kDa respectively (Knapp, 1982).

Much more detailed knowledge has been acquired on the expression of differentiation- and maturation-phase-specific antigens of lymphoid cells than for cells of other lineages through the use of monoclonal antibodies. The most immature thymocyte precursor cells which originate in the bone marrow express the sheep erythrocyte receptor (E) and its analogous T11 differentiation antigen. Reinherz has described four stages of lymphocyte maturation, each stage being identified by a restricted phenotype expression (Reinherz et al., 1980). The most immature thymocytes express the OKT9 and OKT10 antigens while more mature lymphocytes are present as two separate populations expressing either the T4 or T8 mutually exclusive antigens of the functionally distinct helper and suppressor lymphocyte subsets. Cells of the B-lymphocyte lineage have been identified by using a combination of heterologous and monoclonal antibodies as well as by DNA probes for the analysis of immunoglobulin gene re-arrangement as a marker of lineage commitment to the B-cell line. A number of monoclonal antibodies, e.g. BA-1, BA-2, VIB-C5 and B1, are available which recognize early or pre-B cells in advance of their expression of surface immunoglobulin (Stashenko et al., 1980; Abramson et al., 1981; Knapp, 1982). The use of monoclonal antibodies for the identification of differentiation antigens is most valuable when the functional role of the identified antigen is known and when it is related to the function of the cell type. OKT9 which reacts with immature thymocytes and with transformed cells recognizes the transferin receptor on the cell surface (Sutherland et al., 1981). The OKT11 monoclonal antibody which reacts with a 40 kDa glycosylated polypeptide recently identified as the sheep erythrocyte receptor on T-lymphocytes (Verbi et al., 1982). However, monoclonal antibodies are not yet known. The majority of differentiation antigens presently recognized by monoclonal antibodies on leukaemic cells is not known.

The main observation arising from the investigation of antigen expression on leukaemic cells is the absolute reflection of normal differentiation cell antigens on leukaemia-transformed cells. This arises because of the restricted or dual origin of these cells, the imposition of maturation arrest and the fairly broad conversion of a qualitatively normal phenotype on leukaemic cells. It is now accepted that ALL consists of two broad cell subtypes along the lines of normal lymphocyte differentiation, both of which originate in lymphocyte progenators of the B-cell and T-cell lines. Within these two categories, subtypes can be defined which reliably reflect sequential stages of maturation by early or pre-B cells and the early compartments of these two distinct cell lineages. The subtypes of ALL may be divided into four clinically relevant groups based on the expression of a restricted or dominant phenotype. These are: 'common'-ALL (cALL), null-cell ALL, T-cell ALL and B-cell ALL, which have an increasing favourable clinical prognosis in the order B-cell ALL < T-cell ALL < null-cell ALL < cALL. In a study of 39 children with ALL we found that 73% of cases express the dominant cALL phenotype, cALL+* Ia+, TdT+, E-, SmI-*. This 'common' subtype is defined by at least two monoclonal antibodies, J5 and V1L-A1, which recognize a 98 kDa polypeptide (cALLA) on the cell surface (Ritz et al., 1980; Knapp et al., 1982). This antigen, though not present on mature blood lymphocytes as it is expressed on a minority of bone marrow precursor cells which are also TdT+ and express the Ia antigen. This antigen has also been demonstrated on some clusters of subcapsular thymocytes but the simultaneous expression of T-cell differentiation antigens on these cells has not yet been determined (Hoffmann-Fezer et al., 1983). The presence of elevated levels of TdT measured by DNA probes for the analysis of immunoglobulin gene re-arrangement is generally accepted as confirmation of malignant transformation of lymphoid cells even though some myeloid leukaemias are also TdT-positive (Srivastara et al., 1978).
More recently a single case of a non-haemopoietic malignancy demonstrating TdT-positive blast cells involving neuroblastoma has been observed (O’Meara et al., 1985). Our results using a panel of monoclonal antibodies, including the B-cell-associated antibodies BAI and VIB-C5, suggest that ALL leukaemias are more correctly classified as pre-B-cell leukaemias (Table 1). All cALLA⁺ individuals reacted with one or both of these antibodies but failed to express cytoplasmic immunoglobulin. This categorization is already supported by Korsmeyer et al. (1981) who has shown that ‘common’ ALL blast cells undergo immunoglobulin gene re-arrangements, suggesting a commitment to B-cell lineage differentiation. There is, however, considerable heterogeneity of expression of individual antigens within this cALLA subgroup, as is evident from our results (Table 1).

Another major clinical subclass of ALL expressing the dominant phenotype Ia⁺, TdT⁺, cALLA⁺, SmIg⁻, E⁻, is classified as null-cell leukaemia. This subclass cannot at present be allocated with certainty to any particular cell line based on its reactivity with monoclonal antibodies. However, using DNA probes heavy chain gene re-arrangement has been observed but in the presence of germ-line light chains in blast cells with the Null-cell phenotype. These cells may represent B-cells ‘trapped’ within the B-cell precursor stage, possessing ineffectively re-arranged immunoglobulin genes. Whether differentiation antigens exist which reflect this aberrant cell phase remains to be established.

Table 1. Classification of ‘common’ ALL and null-cell ALL subtypes in childhood ALL (n = 29)

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Tdt</th>
<th>Ia</th>
<th>cALLA*</th>
<th>BA-1</th>
<th>VIB-C5</th>
<th>Cyt μ</th>
<th>SmIg</th>
<th>T-cell*</th>
<th>OKT9</th>
<th>OKT10</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Common’</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Null</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

More recently a single case of a non-haemopoietic malignancy demonstrating TdT-positive blast cells involving neuroblastoma has been observed (O’Meara et al., 1985). Our results using a panel of monoclonal antibodies, including the B-cell-associated antibodies BAI and VIB-C5, suggest that ALL leukaemias are more correctly classified as pre-B-cell leukaemias (Table 1). All cALLA⁺ individuals reacted with one or both of these antibodies but failed to express cytoplasmic immunoglobulin. This categorization is already supported by Korsmeyer et al. (1981) who has shown that ‘common’ ALL blast cells undergo immunoglobulin gene re-arrangements, suggesting a commitment to B-cell lineage differentiation. There is, however, considerable heterogeneity of expression of individual antigens within this cALLA subgroup, as is evident from our results (Table 1).

Another major clinical subclass of ALL expressing the dominant phenotype Ia⁺, TdT⁺, cALLA⁺, SmIg⁻, E⁻, is classified as null-cell leukaemia. This subclass cannot at present be allocated with certainty to any particular cell line based on its reactivity with monoclonal antibodies. However, using DNA probes heavy chain gene re-arrangement has been observed but in the presence of germ-line light chains in blast cells with the Null-cell phenotype. These cells may represent B-cells ‘trapped’ within the B-cell precursor stage, possessing ineffectively re-arranged immunoglobulin genes. Whether differentiation antigens exist which reflect this aberrant cell phase remains to be established.

Classification of the remaining ALL cell types into either T-cell or B-cell lineages is well established. The relatively few cases of B-cell ALL reported express a very homogeneous phenotype, SmIg⁺, Ia⁺, TdT⁺, E⁻. IgM is normally expressed on the surface of B-cell lymphoblasts with a distribution of κ/λ light chains of 2:1. IgD immunoglobulin, which is present on normal B-cells, is rarely expressed on B-cell lymphoblasts. However, this may represent an activated state of B-cells, which are known to lose their surface IgD during terminal maturation. A wide range of B-cell-associated monoclonal antibodies are available which react with B-cell leukaemic blasts (Headon & Reen, 1983). The T-cell group of malignancies identified by the dominant phenotype E⁺, Leu-1⁺, Ia⁺, cALLA⁺, SmIg⁺, are probably the most heterogenous of all leukaemic cells with respect to antigen expression. The heterogeneity of this group expressing both qualitative and quantitative phenotypic differences simply reflects the extent to which T-lineage differentiation antigens and maturation stage specific markers have been identified. The majority of T-ALL leukaemias correspond to an early common thymocyte type corresponding to Stage II normal thymocytes (Thiel, 1983).

The many detailed studies carried out on the characteri-
The study of the mammalian CNS has been particularly cultured nervous cells difficult due to three main features: (a) the complexity of its function, (b) the diversity of its cellular composition and (c) the relatively inaccessible location. These characteristics prevent, or at least gravely hamper, systematic manipulation of the CNS.

To avoid these problems we have, as one approach, cultured neuronal cells in vitro. Under defined culture conditions, a variety of development parameters have been observed, e.g. cell division, synapse formation and myelination. A major advantage of tissue-culture systems is their simplicity and easy manipulation. An essential adjunct to their unequivocal identification.

Methods

Cell suspensions and cultures. The cell suspensions and cultures were prepared as previously described (Abney et al., 1981, 1983; Raff et al., 1984).

Antibodies. All of the toxins, antisera and monoclonal antibodies used in this study have been previously described (Eisenbarth et al., 1979; Raff et al., 1979; Abney et al., 1981; Cohen & Selvendran, 1981; Ranscht et al., 1982).

Immunofluorescence. Cultured cells, unfractionated or fresh cell suspensions were labelled in indirect immunofluorescence assays as previously described (Raff et al., 1979; Abney et al., 1981).

Antibody- and complement-mediated cell killing. Cells from 10- or 13-day-old embryonic rat brain, growing on polylysine-coated coverslips in Linbro wells, were treated three consecutive times with antibody and complement by the method developed by Raff & Wortis (1969), as previously described (Abney et al., 1983).

The cultures were then allowed to develop for 10–15 days and strained with fluorescent antibodies.

Antibody-mediated rosette formation. Sterile suspensions were prepared from 13-day-old embryonic rat brains, treated with sterile monoclonal A4 antibody (Cohen & Selvendran, 1981) for 30 min on ice. Antibody-coated cells were then separated by adding 100 μl of 5% (v/v) Ox Red Blood Cells coated with affinity-purified goat anti-mouse immunoglobulin antibody.

The suspension was incubated for 30 min on ice, and rosettes were further stabilized by adding 100 μl of 5% (v/v) Ox Red Blood Cells coated with protein-A purified mouse immunoglobulin. After 30 min on ice, free and rosetted cells were separated according to the method developed by Mason (1981). The red cells were lysed in both fractions (positive and negative) by resuspending the centrifuged cells in 0.83% (w/v) ammonium chloride. After 3–5 min on ice, the cells were washed twice and plated on polylysine coverslips. When control cell suspensions were rosetted as described above but without addition of the mouse monoclonal antibody A4, no brain cells were isolated in the ‘positive fraction’.

All three types of culture (A4+, A4- and unfractionated) were allowed to grow and develop for 10 days in culture. Antibody-mediated cell separation by panning. Sterile cell suspensions were prepared from 13-day-old embryonic rat brains, treated with sterile monoclonal antibody A4 for 30 min on ice. Antibody-coated cells were then separated by placing the above suspension on a Falcon 1001 petri dish coated with affinity-purified goat anti-mouse immunoglobulin antibody, as described by Image et al. (1977). Both positive and negative fractions were cultured and allowed to develop for a further 10 days.

Results and discussion

Development in vitro and in vivo. Embryonic rat brain cell suspensions were prepared at day 10 and day 13, and