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Studies of Myelopoiesis Using Monoclonal Antibodies and Variant Lines from the Promyeloid Cell Line HL60*

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Surface marker studies of human leukaemia cells argue in favour of a normal cell surface phenotype and that unique surface changes are not essential to malignancy. This has led to speculation that leukaemia may result "from a subtle uncoupling of the controls which integrate proliferation with maturation" [1]. Studies using in vitro assays for haemopoietic progenitor cells (for example, GM-CFUc assay) have shown that specific humoral factors regulate normal haemopoiesis (GM-CSFs). Thus, the process of differentiation can in part be explained in terms of the expression of discriminatory surface receptors which are essential to alter (or maintain) the maturation status of cells. However, in order to investigate the underlying set of intracellular regulatory mechanisms which integrate proliferation with maturation it will be necessary to (a) work with cloned populations of cells and (b) have convenient markers for structures which are essential to cell proliferation and maturation within a particular lineage.

Various haemopoietic cell lines, which can be induced to differentiate, are available and can be used to study the process of differentiation. The continuous human promyeloid cell line HL60 can be induced to differentiate into neutrophils by 1.25% dimethylsulphoxide (DMSO) and related compounds [2]. When treated with 12-0-tetradecanoylphorbol-13-acetate (TPA) [3] or T-lymphocyte conditioned medium [4] the HL60 cells mature into monocytes/macrophages. The bipotential nature of this cell line may also provide insight to the processes which ultimately restrict the differentiation capacities of cells to a particular lineage.

Monoclonal antibodies to myeloid-associated surface antigens are convenient markers for the differentiated properties and the "maturation programme" of myeloid cells. The availability of the HL60 line provides the means of readily obtaining large numbers of early myeloid cells necessary for raising and characterising monoclonal antibodies. Hybridomas were produced using mice immunised with HL60 cells coated with antibody raised in mice against normal peripheral blood leucocytes [5]. After immunising with coated cells, 7 out of 22 (32%) hybridomas produced antibodies which identify antigens selectively expressed by myeloid cells. Six of these hybridomas have been cloned and the specificities of the antibodies AGF4.36 and 4.48 have been described in detail previously [5].

The distribution of myeloid antigens at different stages of differentiation was investigated using autoradiographs on bone marrow and peripheral blood cells. The results are shown in Fig. 1. Five of the antibodies (AGF4.48, 8.19, 8.29, 8.41 and 9.47) identify an antigen(s) expressed at the promyeloid to blood neutrophil stages of myeloid maturation. In contrast, one of the antibodies (AGF4.36) defines an antigen which is transiently expressed on cells at the promyeloid to metamyeloid stages and is absent from most bone marrow and blood neutrophils. Loss of the AGF4.36 antigen during differentiation was also observed when HL60 cells were induced to

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mature using 1.25% DMSO. The disappearance of the AGF4.36 antigen was compared with the loss of the BK19.9 antigen, which is a marker for proliferating cells and identifies the transferrin receptor [6]. As shown in Fig. 1, the decrease in the number of AGF4.36 antigen-positive cells (from 87% to 37%) correlates with the maturation, as opposed to proliferation of the HL60 cells. The above panel of antibodies identify differentiated properties of neutrophils. In particular, the transient expression of the AGF4.36 antigen suggests that this antibody may identify a membrane change pertinent to differentiation.

The regulation of the expression of the aforementioned surface properties of HL60 cells can be revealed through its alteration and the use of cloned variant lines from HL60. Seven variants of the HL60 line have been isolated which do not mature in response to 1.25% DMSO. These lines were isolated, in medium containing 1.25% DMSO, after culturing the HL60 cells in the presence of SV40 virus (HL60m2 and m4) [7], treating HL60 cells with alpha (α) particle radiation (HL60 Ast1, 3, 4 and 25) or from untreated HL60 cultures (HL60Sp1). The variant cells are morphologically and cytochemically similar to HL60 and are able to differentiate along the monocyte series when treated with TPA [7]. The expression of myeloid antigens by the variant lines is shown in Fig. 2. In contrast to HL60, four of these lines fail to express the 'transient' myeloid antigen (AGF4.36) and show a reduced expression of myeloid antigens. HLA class 1 (2A1), a leucocyte-associated antigen (BK19.45) and the transferrin receptor (BK19.9) are expressed in similar amounts on the HL60 and variant cell lines. The lines Ast3, 4 and Sp1, which express myeloid antigens to be same extent as HL60, indicate that the absence or reduced expression of myeloid antigens is not an artefact of prolonged growth in DMSO. These lines are currently being karyotyped with a view to identifying chromosomal aberrations relating to the above changes.

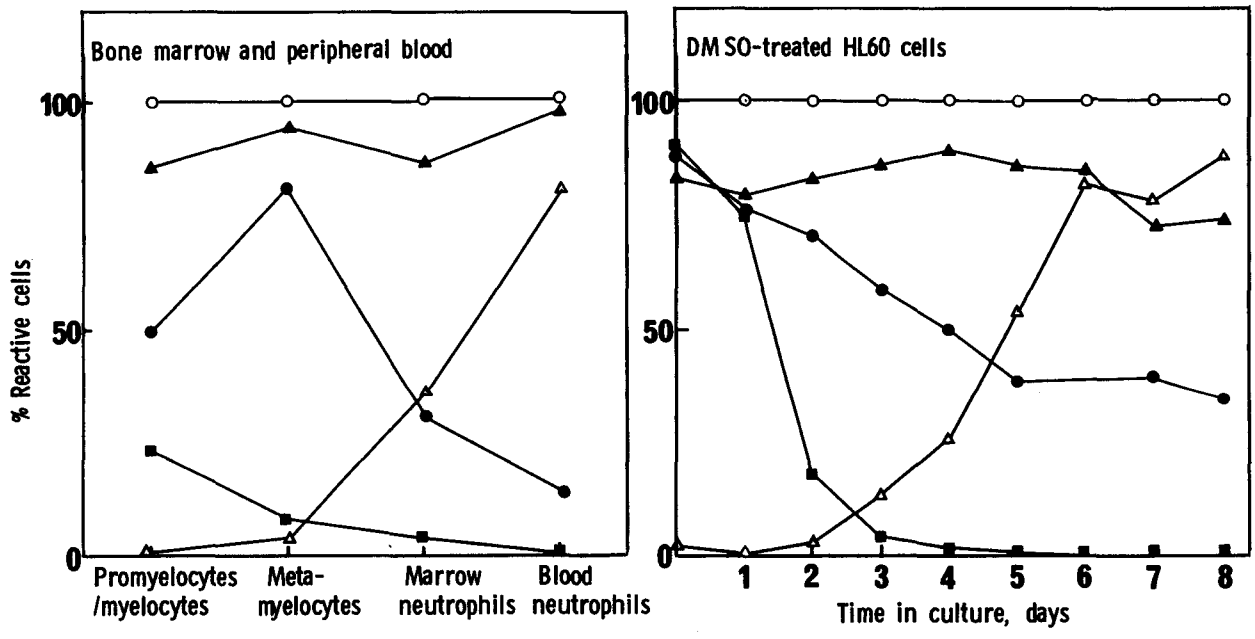

Fig. 1. The distribution of various antigens at different stages of myeloid maturation. The labelling by antibodies of marrow and peripheral blood cells was determined by autoradiography. Results are expressed as a percentage of morphological cell type. Reactivity of the antibodies with DMSO-treated HL60 cells was determined by indirect immunofluorescence. ●—●, AGF4.36 (anti-myeloid); ▲—▲, AGF4.48, 8.19, 8.29, 8.41 (anti-myeloid); ■—■, BK19.9 (anti-transferrin receptor); ○—○, BK19.45 (reacts with leucocytes only). The reactivity of antibody AGF9.47 against marrow and blood cells is similar to ▲—▲ except only 62% of blood neutrophils were reactive. Erythroid precursors were unreactive with the AGF series and monocytes (<1%–46%) were weakly stained. The percentage of antigen-positive cells is compared with the Fc-mediated phagocytosis of marrow and blood cells (△—△) (8) and the ability of the HL60 cells to phagocytose complement-coated yeast cells (△—△).

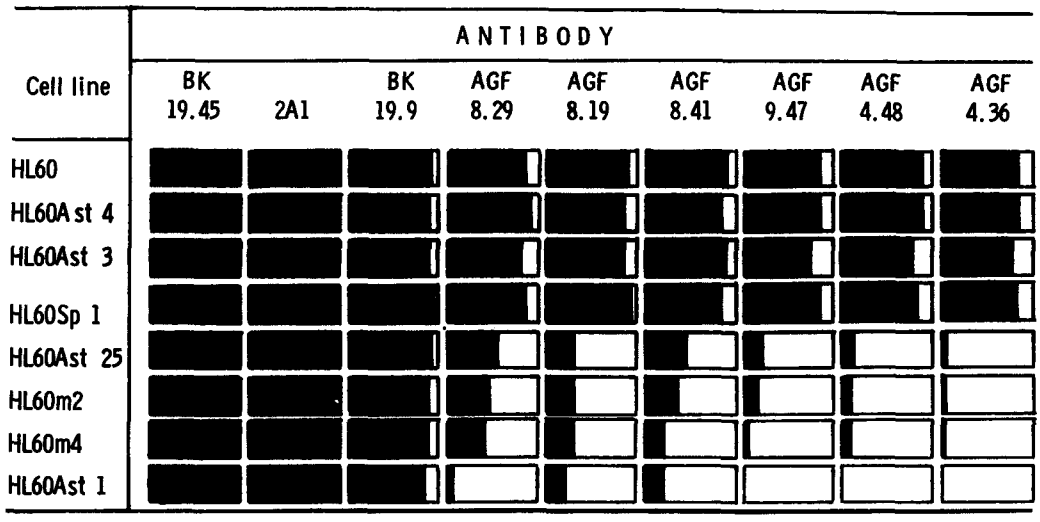

Fig. 2. Expression of surface antigens by variant cell lines from the human promyelocyte line HL60. The *shaded areas* indicate the proportion of reactive cells as determined by indirect immunofluorescence. The antibodies are described in the legend to Fig. 1, with the exception of 2A1 which reacts with class 1 HLA antigens.

The variant lines HL60m2, m4 and Ast25 can be induced to differentiate into neutrophils using higher DMSO concentrations (1.5%–1.75%). In the case of HL60m2 and m4, up to 70% of the cells matured and during this process failed to express the transient myeloid antigen (AGF4.36) and retained reduced amounts of the myeloid antigen (AGF4.48) [5]. The Ast 1 line, which is most affected in terms of expression of myeloid antigens, was insensitive to 2.0% DMSO, in which growth was also limited.

The use of the variant lines in studies of myelopoiesis is as follows. The HL60m2 and m4 appear to be intrinsically restricted in their ability to express the AGF4.36 and 4.48 antigens and can be used to investigate the regulation of expression per se of these antigens at the cell surface. Furthermore, these lines concomitantly show a reduced ability to mature. The line may reflect the interdependence of the controls which regulate the process of neutrophil maturation and the expression of myeloid antigens. Hence the expression of surface antigens can be studied in relation to the regulation of cell maturation. The Ast 1 line appears to be unable to differentiate into neutrophils and may provide insight into whether the presence of a certain surface component is essential for the development of a particular differentiated line. Finally, if physiological inducers of neutrophil maturation were available the lines can be used to investigate the role of the AGF4.36 antigen.

In conclusion, the combined use of cloned populations of cells and markers for the proliferative ability and maturation of cells may be used to investigate the regulation of myelopoiesis. A criticism is that in working with permanent cell lines the events may not reflect normal differentiation. However, the loss of the transferrin receptor as the HL60 cells stop proliferating and the AGF4.36 antigen as the cells mature parallels normal myeloid maturation. Insight, from cell line studies, to the intracellular regulation of these changes will be pertinent to normal myelopoiesis.

References

1. Greaves MF (1981) Biology of acute lymphoblastic leukaemia. Leukaemia Research Fund Annual Guest Lecture. Publ. Leukaemia Research Fund
2. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC (1978) Terminal differentiation of human promyelocytic leukaemia cells induced by dimethylsulphoxide and other polar compounds. Proc Natl Acad Sci USA 75:2458–2462

3. Rovera G, O'Brien TG, Diamond L (1979) Induction of differentiation in human promyelocytic leukaemia cells by tumor promoters. Science 204:868–870
4. Chiao JW, Freitag WF, Steinmetz JC, Andreeff M (1981) Changes of cellular markers during differentiation of HL60 promyelocytes to macrophages as induced by T-lymphocyte conditioned medium. Leuk Res 5:477–489
5. Fisher AG, Bunce CM, Toksoz D, Stone PCW, Brown G (to be published) Studies of human myeloid antigens using monoclonal antibodies and variant lines from the promyeloid cell line HL60. Clin Exp Immunol
6. Brown G, Kourilsky FM, Fisher AG, Bastin J, MacLennan ICM (1981) Strategy for screening for monoclonal antibodies against cellular antigens expressed on minor cell populations or in low amounts. Human Lymphocyte Differentiation 1:167–182
7. Toksoz D, Bunce CM, Stone PCW, Michell RH, Brown G (to be published) Variant cell lines from the human promyelocyte line HL60. Leuk Res
8. Barrett SG, Hansen KS, Bainton DF (1981) Differentiation of cell surface receptors on normal human bone marrow myeloid precursors. Br J Haematol 48:491–500