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Lineage Determination During Haemopoiesis

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"How do cells differentiate into one type or another? is one of the most fundamental questions in cell biology and is also pertinent to the problem of cell transformation, which may often involve progenitor cells [1] and derangement of developmental processes. Commitment of haemopoietic progenitor cells to differentiation along at least five distinct pathways during haemopoiesis continues throughout life, and the haemopoietic system provides an ideal model for studying the above problem. At present, it is not clear whether the haemopoietic stem cell can transform directly into a cell committed to differentiation along any one of five succeeding lines or whether this cell undergoes a series of binary decision-making steps throughout various cell cycles.

In a new model for the development of haemopoietic progenitor cells we have suggested that potentials for development along a pathway of differentiation are expressed individually, consecutively and in a particular order determined within the genome [2]. The model considers the fate of a haemopoietic stem cell which is induced to differentiate and gives rise to a cell(s) committed to decision-making that has lost its self-maintaining capacity. In the model, the committed progenitor cell first acquires a capacity for, and is then restricted to, megakaryocyte differentiation. As this cell divides it gives rise to cells able to develop towards megakaryocytes and to progress to the next stage of commitment to erythroid differentiation. This division process(es) may generate a cell(s) which is channelled towards megakaryopoiesis and a cell(s) which progresses to the next stage of commitment. Alternatively, progeny able to respond to inducers of megakaryopoiesis which fail to receive a signal for differentiation towards megakaryocytes progress to the next stage in the sequence of commitment during progenitor cell development. In this case, as the progenitor cell ages it loses the ability to respond to inducers of megakaryopoiesis as the potential for erythropoiesis is expressed. Subsequently and as above, in a genetically predetermined order, the potentials to respond to inducers of neutrophil, monocyte, B-cell and T-cell differentiation are expressed.

The notion that lineage potentials may be sequentially determined during haemopoiesis arises from studies of variant lines derived from the promyeloid cell line HL60 [2]. The variant cell lines were selected in medium containing 1.25% DMSO, which gives optimal induction of neutrophil differentiation within HL60 cultures. When cultured in 1.5%–2.0% DMSO, the lines show a variable capacity for differentiation induction into neutrophils. In contrast, when treated with inducers of monocyte differentiation, such as 12-0-tetradecanoylphorbol-13-acetate (TPA), the lines either differentiate like HL60 cells or fail to respond to a wide range of concentrations (4–50 n*M* TPA) of inducers of monocyte differentiation [2]. The variant lines are assumed to reflect the inher-

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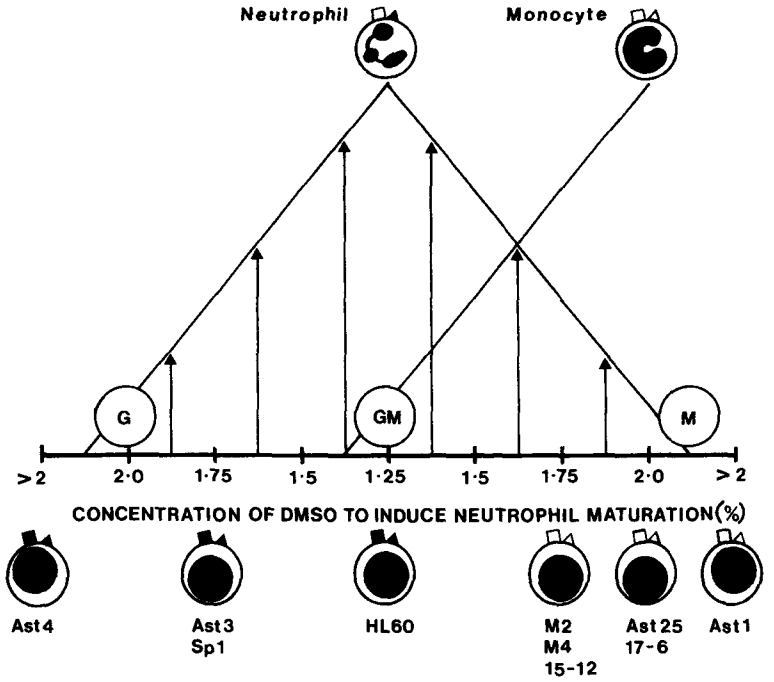

Fig. 1. Positions of variant cell lines derived from HL60 within a proposed developmental sequence. They are arranged in sequence as regards their responsiveness to inducer of neutrophil and monocyte differentiation and as to whether they express the AGF4.48 (▲) and AGF4.36 (■) myeloid antigens or fail to express these antigens (△, □)

ent heterogeneous responsiveness of HL60 cells to inducers of neutrophil and monocyte differentiation. As shown in Fig. 1, these data, together with analyses of surface antigen expression [2], suggest that the variant lines typify a developmental progression within HL60 cultures. During this process cells first acquire a capacity to be induced to differentiate terminally to neutrophils, as reflected by the concentrations of DMSO and other inducers required to induce differentiation. Subsequently, as this capacity for neutrophil differentiation is lost, cells acquire responsiveness to inducers of monocyte differentiation. Thus, variant lines unable to differentiate towards monocytes typify cells which have not yet undergone the differentiation step necessary to acquire the ability to respond to inducers of monocyte differentiation.

The hypothesis that sequential determination is not restricted only to granulocyte/macrophage commitment, but also applies to commitment to megakaryocyte, erythrocyte and neutrophil differentiation arises from consideration of the mature cell types produced in soft agar cultures by progenitor cells restricted to two differentiation pathways. Megakaryocyte/erythroid and erythroid/neutrophil progenitor cells, described in cultures of human and murine bone marrow cells [3], suggest close relationships between these lineages. Similarly, in the myelodysplastic and myeloproliferative disorders, the combinations of megakaryocyte and erythroid, erythroid and granulocytic, megakarycytic, erythroid and granulocytic dysplastic or proliferating cells argue in favour of an ordered, close relationship between progenitor cells during their development [4]. A close relationship between the potentials for macrophage and B-cell differentiation is revealed by experiments in which macrophage-like sublines were isolated from cultures of the murine pre-B lymphoma ABLS8.1 after treatment with 5-azacytidine [5]. These experiments can be interpreted in a manner similar to the HL60 studies [2]. The close relationship between B and T progenitor cells is inferred from the findings that immunoglobulin heavy chain and T-cell receptor genes are partially rearranged in T- and pre-B-cell leukaemias respectively [6]. Commitment to T-cell differentiation is placed last in the developmental sequence. Interestingly, chronic T-cell malignancies such as mycosis fungoides and Sezary's syndrome never terminate in anything other than a proliferative T-cell malignancy. Furthermore, pre-B blast crises in patients with chronic granulocytic leukaemia can be readily explained by the proposed maturation sequence of progenitor cells.

The model, if valid, has important genetic implications. Since DNA is duplicated semiconservatively, sequential determination of

lineage potentials during haemopoiesis would allow the older of the two DNA strands to be retained by cells which progress through the various stages of lineage commitment [7]. The newly synthesised strand, together with any errors occurring during DNA replication, is collected by the daughter progenitor cells, which differentiates along a pre-determined pathway. This process lowers the risk of spontaneous mutation occurring in the progenitor cell population [7]. Most stem cells reside in an "out-of-cycle" Go state [1], and Lajtha has proposed that this is a period when cells undertake "slow DNA repair" processes so as to correct errors in the genome [1]. The previous considerations reduce the requirement for DNA screening in order to maintain the integrity of the genome. Thus, if Go is not essential for "genetic housekeeping" [1] this poses the problem of what happens during Go? In describing the model for progenitor cell development we proposed that during sequential determination cells rearrange genes pre-requisite for differentiation towards a particular cell type [2]. This process of DNA rearrangement may be pertinent to the Go state, and inappropriate gene recombination events provide an explanation for malignancy. The majority of stem cells and lymphocytes which rearrange genes with respect to receptor diversity reside in Go, and they represent "target" cells for most if not all haemopoietic malignancies [1].

Of particular interest is whether the proposed sequence of lineage commitment parallels the pattern of recovery of bone marrow cells following damage. Early in vivo studies of bone marrow recovery in irradiated guinea-pigs showed that the marrow first contained megakaryocytes; this was followed by a wave of erythropoiesis and subsequent recovery of neutrophils [8]. These data support the model proposed. However, the circumstances in which marrow damage is induced, to what extent various progenitor cell populations are affected, and species differences may considerably affect the pattern of recovery seen. In studies of marrow recovery in rats following X-irradiation and sublethal doses of alkylating agents there was no suggestion of sequential recovery of lineages [9]. The sequential determination model does not necessarily predict a parallel sequential recovery or ontogeny of bone marrow cells. The minimal number of six cell cycles could generate cells with each potential for differentiation, and the relative growth rate kinetics of the various cell lineages have to be taken into account. Nevertheless, the work of Frindel and Vendrely [10] supports the sequential expression of potentials for erythropoiesis and granulopoiesis. Bone marrow from *Ara*-C treated mice was used to reconstitute irradiated recipients, and Frindel and Vendrely observed an increased number of erythroid colonies in the spleen, and found that erythroid colonies contained an increased number of granulocyte/macrophage colony forming cells (GM-CFC) when assayed in vitro. They suggested that "the CFU-S is channelled towards erythropoiesis for several generations. After a certain number of cell divisions, the instruction' to differentiate towards erythropoiesis is lost and CFU-S differentiate in a normal stochastic manner. When the recipient mice were killed 9 or 10 days after marrow injection GM-CFC had not yet matured and were capable of giving rise to in vitro colonies."

If a sequential process of stem cell commitment is conservatively followed, the steps in the sequence would have been grafted on in the order in which the cells appear in phylogeny. Macrophage function (phagocytosis) is a primordial defense mechanism – lymphocytes evolved later, and the coagulation function of platelets may have originated prior to phagocytosis as a mechanism of defense. This pattern of evolution of immune functions can be equated with the proposed sequence. Similarly, it is conceivable that T-lymphocytes evolved later to assist B cells in their function and as a feedback control mechanism for driving and limiting the process of progenitor cell maturation [2].

Resolution of the problem of whether the haemopoietic stem cell is pluripotent in a strict sense is essential to our understanding of regulation of haemopoiesis and the origin and progression of malignant disease. Studies of the potentiality of progenitor cells in vivo or grown in soft agar investigate the outcome after several cell divisions, and therefore do not reveal the mechanism of differentiation processes. An approach to this problem is suggested by studies of vari-

ant HL60 cell lines. The lines, arranged in a sequence of development, suggest that potentials for granulocyte and monocyte differentiation are sequentially expressed. If this notion and the order of the variant cell lines are correct, then near-neighbour comparison of proteins of each of the lines by 2-D gel electrophoresis should verify the hypothesis. The model predicts that HL60 Ast 4 is similar to HL60 Ast 3 and less related to HL60 Ast 1 (see Fig. 1). This analysis should also identify proteins which are developmentally regulated in relation to cell commitment.

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