Chapter 1: Understanding blood and its components

A Guide for Nurses

Leukaemia Care
Nurse Academy

Introduction

Haematopoiesis is the process by which blood cells develop. It begins with the emergence of haematopoietic stem cells (HSCs) from the major arteries of a developing embryo, which eventually seed the bone marrow. After birth, a steady 'pool' of HSCs from which all blood cells arise is maintained in the bone marrow by the carefully orchestrated regulation of HSC self-renewal and differentiation.

List of abbreviations

Abbreviations that appear more than once in the chapter:

- **AML:** acute myeloid leukaemia
- **GM-CSF:** granulocyte–macrophage colony stimulating factor
- **HSC:** haematopoietic stem cell
- **M-CSF:** macrophage colony-stimulating factor

If you would like any information on the sources used for this booklet, please email **communications@leukaemiacare.org.uk** for a list of references.

In this booklet

About Leukaemia Care

Leukaemia Care is a national charity dedicated to ensuring that people affected by blood cancer have access to the right information, advice and support.

Our services

Helpline

Our helpline is available 9.00am - 10.00pm on weekdays and 9.30am - 12.30pm on Saturdays. If you need someone to talk to, call **08088 010 444**

Nurse service

We have two trained nurses on hand to answer your questions and offer advice and support, whether it be through emailing **nurse@leukaemiacare.org.uk**, over the phone on **08088 010 444** or via LiveChat.

Patient Information Booklets

We have a number of patient information booklets like this available to anyone who has been affected by a blood cancer. A full list of titles – both disease specific and general information titles – can be found on our website at **www.leukaemiacare.org.uk/support-and-information/help-and-resources/information-booklets/**

Support Groups

Our nationwide support groups are a chance to meet and talk to other people who are going through a similar experience. For more information about a support group local to your area, go to **www.leukaemiacare.org.uk/support-and-information/support-for-you/find-a-support-group/**

Buddy Support

We offer one-to-one phone support with volunteers who have had blood cancer themselves or been affected by it in some way. You can speak to someone who knows what you are going through. For more information on how to get a buddy call

08088 010 444 or email **support@leukaemiacare.org.uk**

Online Forum

Our online forum, **www.healthunlocked.com/leukaemia-care**, is a place for people to ask questions anonymously or to join in the discussion with other people in a similar situation.

Patient and carer conferences

Our nationwide conferences provide an opportunity to ask questions and listen to patient speakers and medical professionals who can provide valuable information and support.

Website

You can access up-to-date information on our website, **www.leukaemiacare.org.uk**, as well as speak to one of our care advisers on our online support service, LiveChat (9am-5pm weekdays).

Campaigning and Advocacy

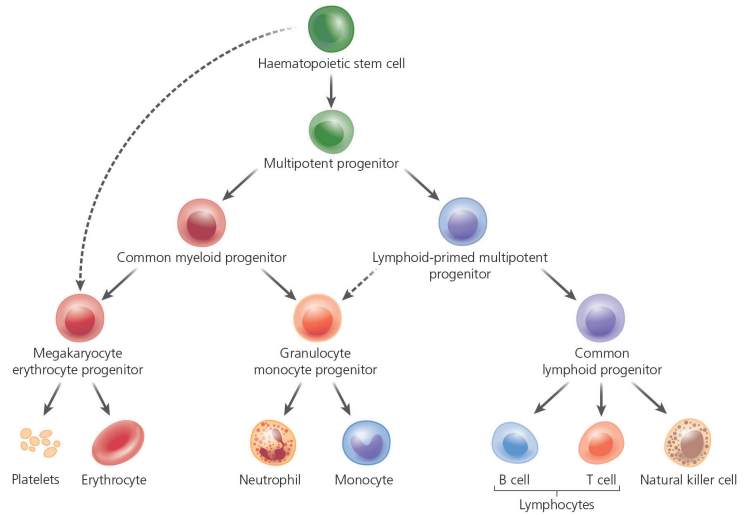
Leukaemia Care is involved in campaigning for patient well-being, NHS funding and drug and treatment availability. If you would like an update on any of the work we are currently doing or want to know how to get involved, email **advocacy@leukaemiacare.org.uk**

Patient magazine

Our quarterly magazine includes inspirational patient and carer stories as well as informative articles by medical professionals. To subscribe go to **www.leukaemiacare.org.uk/communication-preferences/**

Hierarchical relationship of blood cell development

Figure 1.1



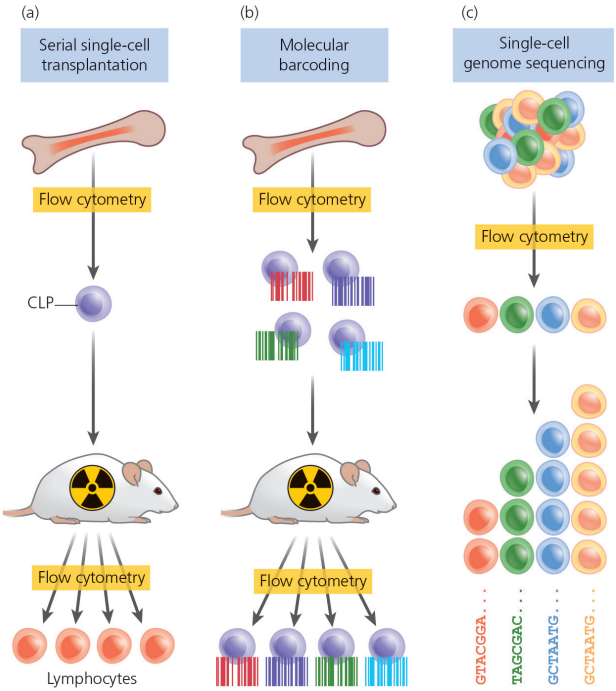
Differentiation

HSCs in the bone marrow subsequently develop into other terminally differentiated cells such as erythrocytes, granulocytes and monocytes (Figure 1.1). HSCs give rise to both myeloid and lymphoid lineages of blood cells. The commitment of differentiated cells is irreversible: for example, monocytes are unable to form erythrocytes.

Self-renewal

The second fundamental attribute of HSCs is the ability to self-renew to provide a continuous source of blood cells throughout the human lifespan. The ability to self-renew is maintained through a number of tightly regulated mechanisms that are gradually being elucidated. The incidence of uncontrolled proliferation (as occurs in cancer) is rare compared with the number of times the haematopoietic system has to respond by controlled proliferation to injury or infection. One way in which this is regulated is through the loss of self-renewal properties in differentiated cells such as neutrophils and monocytes. For example, vast numbers of neutrophils are drawn to sites of infection, but they have a limited lifespan and have to be replaced by upstream progenitors. This requires the

Figure 1.2



HSCs to exit dormancy and generate intermediate progenitors (see Figure 1.1), which are able to divide rapidly and replenish these peripheral cells.

Identification of upstream progenitor cells

The precursors of fully differentiated neutrophils and erythrocytes bear intermediate properties between them and the HSCs. They have an increasingly restricted developmental potential as they complete their development. Traditionally, these precursor cells have been identified by labelling cell surface markers with antibodies conjugated to fluorescent proteins, which can then be identified by flow cytometry. Cells sorted on the basis of these cell surface markers have been transplanted into irradiated mice, and only specific populations of cells have been found to develop from them. For example, when common lymphoid progenitors are transplanted into irradiated mice they give rise only to lymphocytes (Figure 1.2a). Similarly, upstream intermediate progenitors of myeloid and erythroid cells have been identified. However, the exact

Hierarchical relationship of blood cell development (cont.)

lineages and potential of different intermediaries have been revised over the years.

More recent work based on single-cell analyses has revealed novel insights into the process of haematopoiesis (Figures 1.2b,c). Normal blood cells can be sorted using flow cytometry into individual cells, and RNA can be extracted from them. From this, the expression levels of different genes can be identified using next-generation sequencing and, in combination with traditional transplantation studies, the ultimate fate of these cells can be determined. These studies have further revised the models of haematopoiesis, with some suggesting that haematopoietic development is a continuous process rather than one of sequential subpopulations that become increasingly restricted in terms of lineage potential.

Regulation of normal blood cell development

Above, we have described the differentiation of HSCs through various oligopotent, and eventually unipotent, terminal effector cells. A considerable body of work has emerged to explain how this is controlled, so that sufficient but not excessive numbers of fully differentiated cells are generated in response to infection and inflammation.

The role of cytokine signalling

Cytokines provide a signal to cells to proliferate and differentiate. For example, dormant HSCs can be stimulated by the cytokine interferon-alpha (IFN-alpha) to produce more proliferative oligopotent stem cells that can then differentiate into other cells such as neutrophils. Other cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) drive differentiation of progenitor cells into neutrophils and monocytes.

Permissive versus instructive signalling

An ongoing debate on the role of cytokine signalling is whether cytokines merely provide a permissive environment for HSCs to differentiate into a specific role (permissive model) or whether they have a more direct role, driving the HSCs down a specific differentiation lineage (instructive model).

Permissive signalling

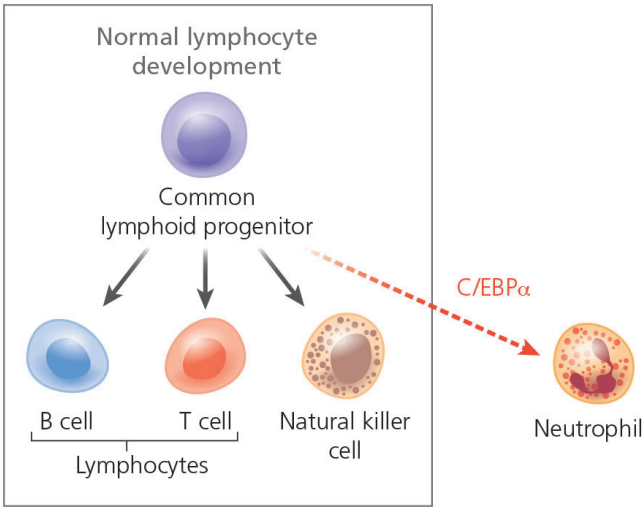
Mice in which the receptor for M-CSF had been removed produced only low numbers of monocytes. However, when the myeloid cells were rescued by the expression of the anti-apoptotic gene BCL2, the monocyte numbers increased. This study suggests that the role of cytokines is to allow the survival of HSCs, which enables them to fully differentiate.

Instructive signalling

In one study, the exogenous expression of specific cytokine receptors (interleukin-2 and

Regulation of normal blood cell development (cont.)

Figure 1.3



GM-CSF) in progenitor cells that had already commenced lymphoid development enabled transdifferentation of the cells into myeloid development. This study suggests that cytokine signalling can regulate cell-fate decisions.

Overlapping role of cytokines

Different cytokines can activate the same receptors, and different receptors can have overlapping downstream effects. For example, although knockout of the erythropoietin receptor results in the absence of mature erythrocytes, early erythroid progenitors can still persist, in part because of the likely compensatory effect of thrombopoietin signalling, which normally regulates platelet production.

The role of specific transcription factors

Transcription factors are vital in the regulation of haematopoiesis. Evidence for this is seen through the disruption of haematopoiesis in both mouse models and in familial patterns of disease. Haematopoietic cells are exquisitely sensitive to subtle variations in expression levels of transcription factors. For example, a simple twofold increase in the levels of the

transcription factor GATA binding protein 2 blocks differentiation of haematopoietic cells in mice. Powerful experimental data also show the ability of ectopically expressed transcription factors to transdifferentiate committed haematopoietic cells into different lineages (Figure 1.3).

One important master regulator of haematopoiesis is the gene RUNX1 (AML1). This gene is essential for the emergence of HSCs in the developing embryo. A complete absence of RUNX1 results in the death of the developing embryo. A dysfunctional copy of RUNX1 is inherited in familial platelet disease. Affected family members, who inherit this condition in an autosomal dominant manner, are thrombocytopenic with a predisposition to the development of acute myeloid leukaemia (AML).

C/EBP-alpha is another important transcription factor in haematopoiesis Mice with a knockout of the CEBPA gene lack mature neutrophils, suggesting that this gene is vital for their development. Recent studies have shown that germline mutations in CEBPA are associated with an increased risk of developing AML with a documented penetrance rate of 100%. The importance of transcription factors in haematopoiesis is underlined by the ability of C/EBP-alpha to transdifferentiate cells into neutrophils (see Figure 1.3). Using retroviral vectors, overexpression of CEBPA can rapidly force lymphoid progenitors, lymphocytes and even AML cells into mature myeloid cells such as neutrophils.

Finally, there is an appreciation that transcription factors regulate each other's activities and form a network that defines the identity and function of each cell. Elucidating these networks will allow the de novo generation of different components of the haematopoietic system (e.g. artificial blood) and help us to understand how they might be subverted in leukaemia.

Key points

Understanding blood and its components

- Haematopoietic stem cells (HSCs) develop into terminally differentiated myeloid and lymphoid cells through intermediate progenitor cells.
- The lineage of haematopoietic cells has been extensively studied using flow cytometry and traditional transplantation techniques.
- The precursors of fully differentiated blood cells have intermediate properties between them and the HSCs. Developmental potential becomes increasingly restricted as blood cells complete their development.
- The self-renewal of HSCs is a highly regulated process that provides a continuous source of blood cells in adult humans.
- Cytokines such as interferon-alpha and granulocyte–macrophage colony-stimulating factor provide signals to HSCs to proliferate and differentiate.
- Transcription factors are also vital in the regulation of haematopoiesis.

Glossary

Cytokine

A small protein (e.g. interferon, interleukin) secreted by cells in the immune system, which act as a signal to the cells around them to behave differently.

Differentiation

The process by which cells change to acquire specialized features that serve a specific function.

Flow cytometry

An analytical cell-biology technique that utilises light to identify, separate and characterise cells in a heterogeneous fluid mixture containing live cells. In addition, antibodies tagged with fluorescent dyes, and raised against highly specific cell surface antigens (e.g. clusters of differentiation markers), can be used to better identify and separate subpopulations of cells within a larger group.

Granulocyte

A type of white blood cell that has granules in its cytoplasm (e.g. basophil, eosinophil, neutrophil).

Haematopoiesis

Blood cell development.

Next-generation sequencing

Also known as high-throughput sequencing, is the term used to describe a number of different gene sequencing technologies.

Oligopotency

The ability of a cell to differentiate into several other cell types.

Progenitor cell

A cell that can differentiate into several types of cell and is pushed to differentiated into its target cell. (It is usually more limited than a stem cell in the kinds of cells it can become).

Thrombocytopenia

Low platelet count.

Transcription factor

A protein that controls the rate of conversion of genetic information in DNA into messenger RN.

Transdifferentiation

The conversion of one cell type into another without going through an oligopotent cell state.

Unipotency

Capable of giving rise to only one cell type.

Figures

Figure 1.1 Hierarchy of haematopoiesis – the multiple stages of blood cell development from haematopoietic stem cells to terminally differentiated cells through intermediate progenitors. The dashed lines represent an alternative differentiation pathway proposed by Adolfsson et al. 2005, based on the presence of lymphoid-primed multipotent progenitors.

Figure 1.2 Experimental haematopoietic models used to query the fate of different cell populations. Flow cytometry is the most widely applied method for characterising and, in combination with cell sorting, isolating stem cells. (a) Flow cytometry can isolate populations of lymphoid or myeloid stem cells in vivo. First, a population of cells with the same surface cell markers (for example, common lymphoid progenitors [CLP]) is isolated from a bone marrow sample by flow cytometry. This is transplanted into an irradiated mouse. The resulting cell line is then analysed by flow cytometry, in this case showing differentiation into lymphocytes only. (b) The in vivo differentiation of individual haematopoietic progenitor cells can be tracked by labelling each cell with a unique genetic barcode. Its progeny can then be tracked by high-throughput sequencing, permitting the contribution of clonal populations to the overall haematopoietic system to be identified. (c) Single-cell genome sequencing has helped to refine traditional views of cell differentiation. Single cells isolated from blood or bone marrow samples by flow cytometry can then be grouped according to their gene expression to establish the clonal relationship between individual cells.

Figure 1.3 Transcription factors can transdifferentiate cells committed to other lineages. For example, the overexpression of the CEBPA gene in common lymphoid progenitor (CLP) cells makes the transcription factor C/EBP-alpha, which at high enough levels can reprogram CLPs into mature myeloid cells such as neutrophils, rather than normal lymphocytes.

Leukaemia Care is a national charity dedicated to providing information, advice and support to anyone affected by a blood cancer.

Around 34,000 new cases of blood cancer are diagnosed in the UK each year. We are here to support you, whether you're a patient, carer or family member.

Want to talk?

Helpline: **08088 010 444**
(free from landlines and all major mobile networks)

Office Line: **01905 755977**

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YOUR Blood Cancer Charity