Occasional articles

Histology of normal haemopoiesis: Bone marrow histology I

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Introduction

The bone marrow trephine biopsy specimen holds an unusual position among pathological specimens. Having been obtained in most cases by a haematologist, the biopsy specimen is processed in a histopathology laboratory and is then reported either by a haematologist or a histopathologist, depending on local custom. Individual practitioners may acquire great skill in the interpretation of trephine biopsy specimens, but others in both branches of pathology are sometimes perplexed when faced with tissue appearances for which other experience within their individual disciplines may not have prepared them fully. Ideally, interpretation should be a collaborative procedure, combining the clinical and cytological knowledge of the haematologist with the histopathologist's skills in analysis of normal and abnormal tissue structure.

An important starting point for all concerned is to have a good appreciation of the normal appearances of haemopoiesis as represented in a trephine biopsy specimen, against which abnormal changes can then be assessed. The purpose of this article is to describe these normal appearances and to indicate briefly some uses and limitations of various processing and staining techniques in bone marrow biopsy specimen interpretation.

General structure of bone marrow

It is impossible to describe the haemopoietic tissue of bone marrow without at least a brief reference to the underlying bony structure. A trephine biopsy specimen may have a quantity of dense, cortical bone (and even extraneous elements such as cartilage or tendon) at its outer end, but for the most part consists of trabecular bone—a meshwork of delicate bony plates and strands within which the haemopoietic tissue and its stroma are suspended.¹ The precise distribution of bony trabeculae, in the iliac crest as at other sites, is determined primarily by mechanical aspects of bone function, but the spaces of varying size and shape which lie between adjacent trabeculae comprise the basic structural units of haemopoiesis.

The trabecular surfaces are covered by a layer of endosteal cells, usually inconspicuous but sometimes recognisable as osteoblasts. Occasional multinucleate osteoclasts are also a normal finding at trabecular margins. A sinusoidal network arborises throughout the inter-

trabecular spaces,² but little is known about the dynamics of blood flow through human bone marrow. Arterioles and venules tend to lie towards the centres of intertrabecular spaces. They are usually seen in only a small proportion of intertrabecular spaces in any one biopsy specimen, suggesting that each may supply or drain a number of such spaces.

The trabeculae, arterioles, and venules form the structural framework around which granulopoiesis develops. Erythropoiesis and megakaryopoiesis occur in apposition to the fine, branching sinusoids.

MARROW STROMA

The trabecular and vascular architecture of medullary bone provides the basic framework, nutritional supply, and waste removal system for haemopoiesis, but specialised support of the self-renewal and differentiation of haemopoietic precursors is provided by the bone marrow stroma. This stroma consists of a heterogeneous mixture of adipocytes, fibroblasts, and macrophage-like cells, together with a complex extracellular matrix of reticulin, binding proteins, including fibronectin and haemonection, and proteoglycans.³ In culture systems specific haemopoietic microenvironments—for example, for erythropoiesis or granulopoiesis—can be shown to be determined by the nature of the stromal cells and the binding of growth factors to restricted matrix sites.⁴ How this correlates with the spatial distribution of haemopoiesis within intact marrow *in vivo* is not yet understood.

Despite this evidence of its important contribution to normal haemopoiesis, bone marrow stroma appears disappointingly bland on conventional histological examination. Adipocytes are readily apparent and small numbers of scattered fibroblasts can usually be discerned. Reconisable macrophages are remarkably few: those which contain storage iron can be demonstrated by Perl's stain, but they are not numerous in normal marrow and only occasionally can they be seen to lie in close proximity to developing erythrocytes. Immunostaining of paraffin wax embedded trephine biopsy sections using macrophage reactive monoclonal antibodies such as Mac387 (anti-calgranulin; Dako UK Ltd) and KP1 (CD68; Dako UK Ltd) also shows only small numbers of macrophages in normal marrow (unpublished observation).

Spindle cells expressing alkaline phosphatase, butyrate esterase, or fibroblast associated

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Figure 1 Normal paratrabecular zone of promyelocytes and myelocytes. Streptavidin-biotin complex immunoperoxidase technique using the monoclonal antibody NP57, reactive with neutrophil elastase.

Figure 2 Normal clusters of proerythroblasts and normoblasts. Streptavidin-biotin complex immunoperoxidase technique using the monoclonal antibody Ret40f, reactive with β -sialoglycoprotein (glycophorin C).

antigens have been shown in frozen or resin embedded sections⁵⁻⁷ and in cultured marrow stroma, but the precise functions of these cells remain uncertain.

BONE MARROW CELLULARITY

This term is usually used with reference to the haemopoietic cell content of a bone marrow biopsy specimen relative to its adipocyte content. Biopsy specimens are generally considered to be of normal cellularity if 40–60% of the non-bony tissue is composed of haemopoietic cells. Cellularity varies widely with age and site, however, so that in a neonate normal cellularity may be 100% and in an elderly patient it may be 20%.⁸ Haematologists and pathologists who regularly assess trephine biopsy specimens will also be well aware that intertrabecular spaces immediately beneath the bony cortex are frequently hypocellular, especially in the elderly, and may be unrepresentative of the cellularity deeper in the biopsy specimen.

GRANULOPOIESIS

The earliest recognisable granulopoietic precursors, the promyelocytes and myelocytes, lie adjacent to the surfaces of bony trabeculae (fig 1) and the adventitial aspect of small blood vessels.⁸⁻⁹ With increasing distance from the trabecula or vessel, increasing numbers of metamyelocytes and mature polymorphs are

seen. This radial orientation of granulopoiesis can be appreciated easily in reactive granulocytic hyperplasia or chronic granulocytic leukaemia but is not always obvious in normal marrow, in which the zone of promyelocytes and myelocytes may only be one or two cells thick. In undecalcified resin-embedded biopsy specimens the immature granulopoietic zone can be highlighted by Leder's stain for chloroacetate esterase,¹⁰ which tends to react more strongly with promyelocytes and myelocytes than with later granulocytes. Unfortunately, this visually impressive staining technique cannot be performed successfully with biopsy specimens decalcified by formic or acetic acids, as the enzyme activity is lost. However, a very similar demonstration of the distribution of early granulocytes can be achieved in decalcified, wax-embedded biopsy specimens by immunohistochemical staining for neutrophil elastase¹¹ (fig 1).

In general, using routine stains, little granularity can be appreciated in granulocytes of trephine biopsy specimens which have undergone acid decalcification, with the exception of eosinophils, whose granules are well preserved.

Occasional promyelocytes and myelocytes, scattered singly or in small clusters, occur at sites distant from trabeculae and vessels, and it is therefore not necessarily abnormal to see immature granulocytes in the centres of intertrabecular spaces.

MONOPOIESIS

The close association of granulopoiesis and monopoiesis in bone marrow culture systems *in vitro*¹² suggests that within intact marrow monocyte precursors should be found in a similar distribution to that of granulocytes. Monocytes, however, are difficult to recognise in trephine biopsy sections and little is known about the spatial organisation of their developing precursors. No staining technique is currently widely available for reliable and specific demonstration of monocytes in fixed trephine biopsy specimens. Butyrate esterase enzyme activity does not survive routine processing, although it may be preserved by some resin-embedding protocols.⁶ Most immunohistochemical agents which will react in decalcified, wax embedded trephine biopsy specimens for demonstration of monocytes (Mac387, CD15, C68) also react strongly with granulocytes,¹³⁻¹⁵ which predominate in normal marrow and tend to obscure any monocytes present.

ERYTHROPOIESIS

Erythrocytes develop in small clusters which are dispersed throughout the intertrabecular spaces⁹ but which tend not to encroach on the paratrabecular and perivascular zones of early granulocytes. In a trephine biopsy section these clusters measure about 5–10 cells in diameter. Erythroid clusters have a radial organisation, with proerythroblasts in the centre and progressively more mature forms towards the periphery (fig 2). Convincing evidence of a close spatial association between

(A)

(B)

Figure 3 (A) Appearance of normal erythroid cells following decalcification and wax-embedding: shrinkage artefact causes perinuclear halo formation. (B) Normal erythroid cells in an undecalcified, resin-embedded trephine biopsy specimen. Their cytoplasmic margins are indistinct, giving the clusters a syncytia-like appearance.

Figure 4 Normal megakaryocyte in close association with a marrow sinusoid (the retraction is artefactual). Inset A: small, mononuclear megakaryocyte, presumably immature. Inset B: irregular bare nucleus, presumed to represent a senescent megakaryocyte.

such clusters and marrow sinusoids can rarely be seen in routine histological sections.

Erythroid clusters are usually readily apparent in normal marrow trephine biopsy specimens by virtue of the dense blue-black colour of the compact, round nuclei of mid- and late normoblasts in sections stained with haematoxylin and eosin. Giemsa staining shows intense cytoplasmic basophilia in proerythroblasts and early normoblasts. Immunohistochemical staining of α and β sialoglycoproteins (glycophorins A and C)^{16 17} can also be used to demonstrate erythropoiesis (fig 2).

A helpful artefact occurs in decalcified, wax embedded trephine biopsy specimens which contributes to the ease of recognition of erythroid cells. Shrinkage of the nucleus leaves a clear perinuclear halo in each cell (fig 3A). This can assist in distinguishing erythroid cells from lymphocytes, which have darkly staining nuclei of a similar size to those of late normoblasts. Fortunately, although perinuclear haloes are absent from undecalcified, resin-embedded biopsy specimens, the morphology of red cell precursors is usually distinctive in haematoxylin and eosin or Giemsa stained sections from bone marrow biopsy specimens processed in this way (fig 3B).

MEGAKARYOPOIESIS

Mature megakaryocytes can easily be identified in tissue sections because of their large size, voluminous cytoplasm and nuclear multilobulation. They are found scattered singly throughout intertrabecular spaces,⁹ evenly distributed, except that they are usually absent from the immediate paratrabecular and perivascular zones occupied by early granulocytes. As with erythroid clusters, it is often difficult to see sites of intimate association between megakaryocytes and sinusoids (fig 4). However, electron microscopic studies have shown that peripheral cytoplasm of megakaryocytes protrudes into sinusoidal lumina and is shed into the intravascular compartment in the form of pro-platelets which subsequently fragment to give rise to platelets.^{9 18}

The precursors of megakaryocytes cannot always be readily identified within normal bone marrow trephine sections. Occasional, small, mononuclear forms can be found (fig 4; inset A), but their ontogenic relation to mature megakaryocytes is not known. The difficulty of recognising early megakaryocyte precursors exists because such cells are smaller than their progeny and may resemble promyelocytes morphologically. Giemsa staining can show cells in trephine biopsy sections which are probably megakaryocyte precursors: they have grainy lilac-pink cytoplasm resembling that of their mature counterparts. Some of the subtle variation in cytoplasmic coloration between haemopoietic cell lineages afforded by Giemsa staining is absent from decalcified biopsy specimens, and an alternative approach is to perform immunohistochemical staining for factor 8 related antigen or CD61 (platelet glycoprotein 3a).¹⁷ Low numbers of small, mononuclear cells, presumed to be megakaryocyte precursors, are present which

express these antigens. Such cells are apparently randomly distributed throughout intertrabecular spaces.

Also normally present in small numbers are scattered megakaryocyte nuclei with a convoluted outline and condensed chromatin pattern, apparently bare of cytoplasm (fig 4: inset B). These are considered to represent senescent megakaryocytes.

OTHER CELLS REGULARLY PRESENT IN NORMAL BONE MARROW

Lymphocytes

The distribution and morphology of lymphocytes within bone marrow biopsy specimens offer no clues as to whether these cells are undergoing some part of their development there or are simply in transit through the tissue.

Small lymphocytes of B and T phenotypes are present in low to moderate numbers scattered throughout the intertrabecular spaces. Clusters of a few lymphocytes, normally termed lymphoid aggregates, and larger collections termed lymphoid nodules are also frequently seen. The larger lymphoid nodules may incorporate a few macrophages and small vascular channels and they occasionally form true follicles with germinal centres. Lymphoid aggregates and nodules only rarely lie immediately adjacent to trabeculae; collections of lymphoid cells at such sites should raise a strong suspicion of lymphomatous infiltration. Criteria for distinguishing benign from malignant lymphoid infiltrates within bone marrow biopsy specimens have been described in detail by Rywlin and colleagues.¹⁹ However, even morphologically benign aggregates may be associated with subsequent lymphoid malignancy,²⁰ and they should always be assessed cautiously.

Plasma cells

These cells are usually present in small numbers, clustered at the adventitial margins of small blood vessels. They are also found scattered singly elsewhere in the intertrabecular spaces.

Mast cells

The relation between mast cells and basophil granulocytes remains uncertain. It is believed currently that both cell types arise by independent pathways from a common partially committed basophil stem cell.⁹ Metachromatic and immunohistochemical staining of mast cells in trephine biopsy specimens shows no spatial relation between developing basophils and mast cells, nor are cells found which exhibit transitional morphological, tinctorial, or immunohistochemical appearances.²¹

Mast cells can be detected in about 50% of trephine biopsy specimens by metachromatic staining. Their number is generally small in normal marrow and they tend to lie immediately adjacent to the endosteal surfaces and at the adventitial borders of blood vessels. They are also frequently found at the periphery of lymphoid nodules.^{8, 21} Morphologically, they are usually either spindle-shaped or oval with

homogeneous pink cytoplasm on haematoxy and eosin staining and a small, dense round nucleus. They stain metachromatically with Giemsa, but are difficult to discern among large numbers of developing granulocytes. Other metachromatic stains, such as toluidine blue or buffered thionine, are more useful for their demonstration. Immunostaining can be performed using the antibody AA1, which reacts with mast cell tryptase,²² which does not cross-react with basophils.

Conclusion

A great variety of cells, haemopoietic and non-haemopoietic, are present within normal bone marrow. In keeping with the highly specialised functions of the tissue, there is a complex but orderly spatial organisation of cells within it. Although we do not yet fully understand the relations between bone marrow structure and function, knowledge in this sphere is advancing as *in vitro* culture systems provide insights into the roles of cell-cell and cell-stroma interactions in haemopoiesis. Careful histological analysis of intact bone marrow biopsy specimens will also contribute to this increasing knowledge of normal haemopoiesis.

For haematologists, histopathologists and any other practitioners engaged in the diagnosis of bone marrow disorders, I hope that the foregoing account will be of practical use in clarifying current concepts of normal haemopoietic cell organisation and to set a threshold against which abnormal haemopoiesis can be assessed.

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