ACTA STEREOL 1998; 17/1: 3-11
ORIGINAL SCIENTIFIC PAPER

LYMPHOID INFILTRATES IN THE BONE MARROW: 3D ASPECTS

Jonathan Salisbury

Department of Histopathology, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ, United Kingdom

ABSTRACT

The distinction between benign and malignant lymphocytic infiltrates present in bone marrow trephine biopsies is important for primary diagnosis of malignancy, lymphoma staging and prognosis. No single criterion exists to make this distinction and so discrimination is made on the basis of a number of morphological observations, often supplemented by immunohistochemical staining of tissue sections. In recent years, attention has been given to the pattern of infiltration shown by benign and malignant lymphoid infiltrates in the bone marrow. Three-dimensional reconstruction studies and, to a lesser extent, stereological investigations have shed new light on the microarchitecture of the marrow and provided three-dimensional models of pathological states. The three-dimensional reconstruction studies have shown that benign lymphoid aggregates occupy the central marrow spaces, away from the bone trabecular surfaces (non-paratrabecular). In contrast, bone marrow infiltrates of follicular lymphoma, a low-grade non-Hodgkin's malignant lymphoma, always occupy, at least in part, the zone next to the bone marrow trabeculae. This is described as a paratrabecular pattern of infiltration. Other types of low-grade non-Hodgkin's lymphoma may show either or both patterns i.e. non-paratrabecular and/or paratrabecular. That different types of lymphoid infiltrates show different patterns of marrow infiltration could be explained either by an intrinsic difference in the lymphoid cells or by the way in which they are responding to regulators of the haematopoietic microenvironment. Possible regulators of marrow microenvironments include the haematopoietic precursors themselves, the marrow stromal cells, the extracellular marrow matrix, haematopoietic cytokines and growth factors. Because the pattern of paratrabecular infiltration shown by follicular lymphomas is similar to the paratrabecular zoning demonstrated by normal granulopoiesis, similar regulator(s) of the haematopoietic microenvironment may be important in determining this spatial architecture.

Keywords: bone marrow pathology, lymphoma pathology.

INTRODUCTION

Human bone marrow normally contains a population of mature lymphoid cells. Many of these are aggregated together to form distinct structures within the bone marrow that are termed benign lymphoid aggregates (Hashimoto et al., 1957). In some non-neoplastic diseases, the marrow lymphoid population increases and these benign lymphoid aggregates become

more numerous and enlarged. This state has been described as a reactive nodular lymphoid hyperplasia of the bone marrow (Navone et al., 1985). Many of the connective tissue diseases, particularly rheumatoid arthritis, are examples of diseases where this occurs.

The bone marrow may also become a site of involvement by a variety of lymphoid malignancies including Hodgkin's disease and the non-Hodgkin's malignant lymphomas. Definitive histopathological criteria for the diagnosis for marrow involvement by Hodgkin's disease are (1) Reed-Sternberg cells in a background typical of Hodgkin's disease or (2) mononuclear Hodgkin's cells in a background typical of Hodgkin's disease if Reed-Sternberg cells have been identified in other specimens. The non-Hodgkin's lymphomas may be classified as either low-grade or high-grade malignancies using the REAL (Revised European-American Lymphoma) Classification (Harris et al., 1994). Involvement of the bone marrow by high-grade non-Hodgkin's lymphoma is morphologically very different from benign lymphoid infiltrates and confusion is unlikely. However, it is well recognised that there may be discordance between the appearance of non-Hodgkin's lymphoma in different sites in the same patient, typically with high-grade non-Hodgkin's lymphoma in a lymph node (arising by transformation from a low-grade non-Hodgkin's lymphoma) and low-grade non-Hodgkin's lymphoma in the bone marrow (representing the original tumour) (Conlan et al., 1990; Deverell et al., 1997).

By contrast with high-grade lymphoma, involvement of the bone marrow by low-grade non-Hodgkin's lymphoma may show close morphological resemblance to benign lymphoid aggregates (Mennemeyer and Kjeldsberg, 1976). Making this distinction between benign lymphoid aggregates and low-grade non-Hodgkin's lymphoma involving the bone marrow has therefore become a major topic of interest in diagnostic haematopathology. The distinction is important for staging and prognosis as well as for primary diagnosis. This article reviews how the three-dimensional features of the lymphoid infiltrate may contribute to this distinction and discusses mechanisms which may contribute to the formation of the observed three-dimensional architecture.

THE NORMAL LYMPHOID POPULATION OF HUMAN BONE MARROW

The morphological criteria for recognising lymphoid aggregates as benign are that they should be well-defined collections of lymphoid cells (Figure 1) (Bartl et al., 1982; McKenna and Hernandez, 1988); the aggregate should be less than 600 μm in diameter (Rwylin et al., 1974); and there should be a mixed population of lymphoid cells with a delicate reticulin framework (Schmid and Isaacson, 1992). Whilst it is true that there is an increase in the numbers and coarseness of reticulin fibres in marrow deposits of follicular and lymphoplasmacytoid lymphoma, no morphometric differences have been found between the reticulin content of benign lymphoid aggregates and chronic lymphocytic leukaemia/lymphocytic lymphoma showing a nodular pattern of marrow infiltration (Thiele et al., 1990). Germinal centres may occur in the marrow and seem to be particularly common in anaemia or eosinophilia (Fahri, 1989).

Immunohistochemical studies using B- and T-cell markers permit an easy recognition and exact evaluation of the size of benign, reactive lymphoid and malignant infiltrates but do not allow distinction between benign lymphoid aggregates and low-grade non-Hodgkin's lymphoma (Horny et al., 1989; Pich et al., 1991). The majority of cells in benign and reactive lymphoid aggregates are B-cells but T-cells are always admixed.

A diffuse, loosely scattered infiltrate of lymphocytes is also present in normal bone marrow and constitutes 1-5% of all nucleated cells. Immunohistochemical staining of bone marrow trephine sections shows that the majority of these cells are T lymphocytes as opposed to B lymphocytes (Horny et al., 1989). An increase in these small mature lymphoid cells may occur diffusely in the bone marrow as a reactive phenomenon in many non-neoplastic diseases, especially in viral infections.

The bone marrow mast cell content shows a direct relationship with the percentage of marrow lymphoid cells but is not related to any specific lymphoproliferative processes (Yoo et al., 1978).

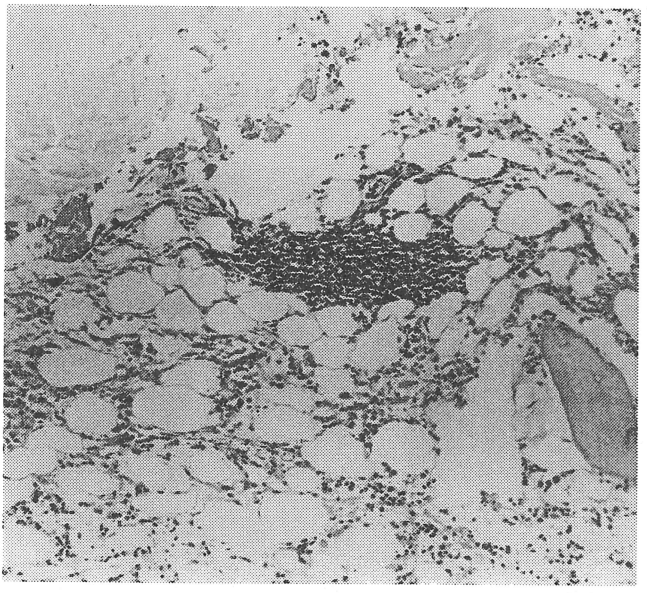

Fig. 1. Human bone marrow showing a benign lymphoid aggregate in the central marrow space.

MARROW INVOLVEMENT BY LOW-GRADE NON-HODGKIN'S LYMPHOMA

Morphological criteria for recognising marrow involvement by low-grade non-Hodgkin's lymphoma (Bartl et al., 1984) are that they should be large collections of lymphoid cells (greater than 600 μm in diameter)(Rwylin et al., 1974) with poorly defined margins. Lymphoid cells may show interstitial infiltration at the periphery of the deposits. Inclusion of fat cells within the deposits is very suggestive of lymphoma. An incomplete rim of eosinophils at the periphery of the deposits is a moderately frequent finding, particularly in trephine biopsies taken after chemotherapy. The location of the deposits is important because both paratrabecular and parasinusoidal deposits are more likely to be lymphoma. Paratrabecular deposits are seen particularly with follicular lymphoma (Figure 2) (Schmid and Isaacson, 1992; Bishop, 1993). Simple morphometric measurements such as nuclear morphology,

nuclear area and nuclear contour index are insufficient, in most cases, to distinguish reactive from neoplastic lymphoid infiltrates (Deverell et al., 1997).

Demonstration of light chain restriction by immunostaining for kappa and lambda immunoglobulin light chains will secure a diagnosis of low-grade B-cell non-Hodgkin's lymphoma. In practice, however, this is often not achievable because of the technical difficulty of light chain immunostaining on decalcified, paraffin-embedded sections. The demonstration that immunostaining for the proto-oncogene protein bcl-2 could be useful in distinguishing between reactive follicular hyperplasia and follicular lymphoma in lymph nodes led to suggestions for its use in distinguishing between benign lymphoid aggregates and deposits of follicular lymphoma in bone marrow trephines (Neilson et al., 1995). However, not all authors have confirmed this is a useful immunostain to distinguish between benign and malignant lymphoid nodules in bone marrow biopsies because bcl-2 can be demonstrated in 60% of reactive nodules (Skalova and Fakan, 1997).

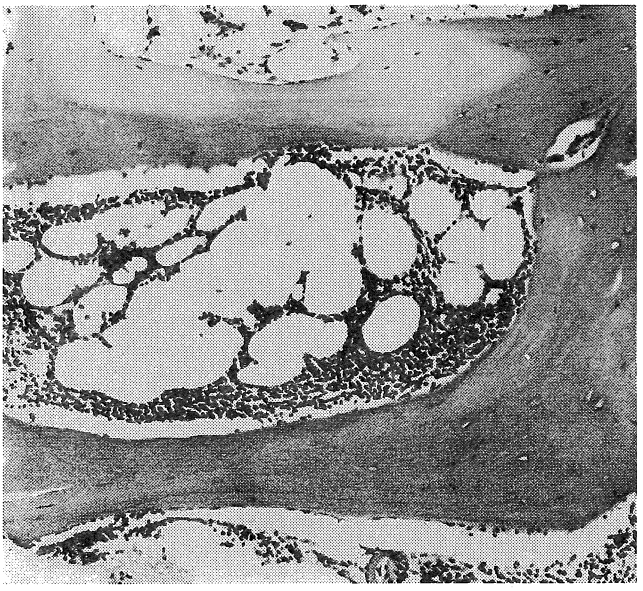

Fig. 2. Human bone marrow showing infiltration by non-Hodgkin's lymphoma (follicular lymphoma) in the paratrabecular space.

The demonstration of aberrant T-cell immunophenotypes can be useful in distinguishing bone marrow involvement by T-cell lymphomas, especially if these aberrant immunophenotypes are identical to those observed in other sites of involvement (Gaulard et al., 1991).

THE THREE-DIMENSIONAL ORGANISATION OF THE BONE MARROW

That there is a spatial arrangement to the organisation of the bone marrow has been long understood (Bartl et al., 1985; Frisch et al., 1985) and models of the arrangement were being made twenty years ago (Mohandas and Prenant, 1978). Understandably, these initially concentrated on the 3D organisation of the major haematopoietic cell lines. The granulocytic precursors were shown to concentrate near the surfaces of the cancellous bone trabeculae (the paratrabecular location), the erythroid precursors to be most concentrated in the central marrow space away from the bone surfaces, and the megakaryocytes to be found exclusively in the immediate vicinity of the bone marrow sinusoids. Because of this zoning phenomenon, use of the appropriate sampling techniques becomes important when performing differential counts of haematopoietic cells in bone marrow trephines (Wilkins and O'Brien, 1988).

The observation that the zoning phenomenon could be related the vascular network led to studies of the bone marrow vasculature. The vascular system of the bone marrow was studied in three-dimensions using scanning electron microscopy to examine micro-corrosion casts made with low viscosity resins (Draenert and Draenert, 1980). This showed that the nutrient arteries entering the medullary cavity gave rise to progressively smaller arteries, arterioles and arterial capillaries which then drained into the marrow sinusoids. Increasing in width, the sinusoids then drained into wider veins. Using three-dimensional reconstructions of serial resin sections of human bone marrow, Naito et al (1992) described intertwining sinuses and haematopoietic cords (or compartments). Their study allowed the definition of a unitary structure of the marrow - the haematopoietic cord - formed by a central arteriole surrounded by sinuses. Granulocytic precursors appeared distributed preferentially around the central arteriole whilst erythroid cells and megakaryocytes appeared close to the sinus walls. Interestingly, the erythroid "islands" that are clearly seen in bone marrow trephine sections were shown to be continuous cords of erythroid precursors when examined in three dimensions. Using a stereological approach, Lambertsen and Weiss (1986) studied haematopoietic colonies in the marrow of irradiated mice to investigate the microenvironmental organisation of marrow haematopoiesis. They showed that undifferentiated colony cells showed a constant predilection for endosteal and periarterial regions, with the majority of colony cells occurring along bone. Erythroid colony cells proliferated in the intermediate and central marrow zone sand along arteries. Granulocytic colony cells occurred in all areas at 3 days post-irradiation, but increased in density along bone surfaces thereafter.

THREE-DIMENSIONAL STUDIES OF LYMPHOID INFILTRATES IN THE BONE MARROW

Because paratrabecular deposits are seen in trephine biopsy specimens with low-grade non-Hodgkin's lymphoma, particularly follicular lymphoma (Schmid and Isaacson, 1992; Bishop, 1993), and the benign lymphoid aggregates appear in the central marrow space (non-paratrabecular location), we wished to test the hypothesis that benign lymphoid aggregates did not make contact with a bone trabecular surface. To investigate this hypothesis, we constructed three-dimensional models of five serially sectioned human bone marrow trephines, containing a total of 19 benign lymphoid aggregates (Salisbury et al., 1996). One hundred and forty-eight serial sections, each 10 μm thick, were cut from the decalcified, paraffin-embedded bone marrow trephine biopsies, numbered sequentially, and stained with haematoxylin and eosin. Drawings were made of the bone trabeculae and all recognisable lymphoid aggregates and

three-dimensional models were then reconstructed from the serial stack of drawings using recently developed computer-generated three-dimensional reconstruction techniques (Salisbury and Whimster, 1993; Salisbury 1994). The computer-generated images showed that benign lymphoid aggregates were located in the central marrow space and did not become localised, even in part, in the paratrabecular regions.

We then went on to analyse the three-dimensional location of deposits of low-grade non-Hodgkin's lymphoma in human bone marrow. This was achieved by making three-dimensional reconstruction models from serial tissue sections of five bone marrow trephines involved by lymphoma. The images of the computer-generated models showed that these deposits of low-grade non-Hodgkin's lymphoma involving the bone marrow always assumed a paratrabecular pattern of infiltration at some point (Salisbury et al., 1997). This was in direct contrast to the pattern of bone marrow infiltration shown by benign lymphoid aggregates.

REGULATORY FACTORS OF HAEMATOPOIETIC MICROENVIRONMENTS IN THE BONE MARROW

The observations that different haematopoietic cell lineages mature in different areas of the bone marrow led to the hypothesis that different haematopoietic microenvironments existed in these different locations i.e. paratrabecular, central marrow space, parasinusoidal. Possible regulators of haematopoietic microenvironments would include the three-dimensional structure of the marrow itself with its many cell types (particularly the marrow stromal cells), the abundant extracellular matrix, haematopoietic cytokines and growth factors.

Haematopoietic precursors in the marrow have the potential for considerable cell-cell contact via adhesion molecules such as $\alpha_4\beta_1$ integrin (Clark and Keating, 1995). Experimental evidence for the importance of marrow stromal cells has been provided by an number of workers. There are morphological observations indicating that close functional co-operation exists between blast cells and marrow stromal cells in long-term mouse bone marrow culture (Harrison et el., 1984) and in mouse exogenous erythroid spleen colonies (Hofer et al., 1989). The immunophenotype of the marrow stromal cells is: CD10 +ve, CD45 -ve, collagen type IV +ve, HLA-DR -ve, laminin +ve, muscle actin +ve, Stro-1 +ve and vimentin +ve (Clark and Keating, 1995). Confocal images of the subset of marrow stromal cells defined by membrane-associated alkaline phosphatase (Westen-Bainton cells) have been produced showing their actual shape and organisation within the bone marrow architecture (Bianco and Boyle, 1993). Follicular dendritic cells, demonstrated by immunostaining for CD21, CD35 and DR53, are found rarely in benign lymphoid aggregates (2 out of 38) but frequently in low-grade B-cell lymphomas (92 out of 134), particularly in follicular lymphoma (Meuge-Moraw et al., 1996). The follicular dendritic cells form follicle-like networks with their number and size correlating directly to the tumour mass.

Interactions with components of the extracellular matrix, e.g. the glycosaminoglycan hyaluronic acid with cell-associated CD44, are also believed to be important in regulating the haematopoietic microenvironment (Clark and Keating, 1995). Interactions between B-cells and marrow stromal cells and between B-cells and marrow stromal cell-secreted extracellular matrix proteins are reviewed by Patrick et al. (1996). Both cell-cell and cell-matrix interactions can be studied by long-term bone marrow culture (Wang et al., 1995).

That the paratrabecular distribution of follicular lymphoma corresponds with the paratrabecular distribution of normal granulocytic precursors suggests that they may respond to a common haematopoietic microenvironment regulator. Whatever that regulator is, it may

not be the marrow stromal cells alone because their numbers are reduced in lymphoid malignancies compared with normal marrow. In contrast, granulocytic malignancies (acute myeloblastic leukaemia, chronic myeloid leukaemia) are associated with normal or increased numbers of marrow stromal cells (Dilly and Jagger, 1990).

PRACTICAL APPLICATIONS OF 3D ASPECTS OF MARROW LYMPHOID INFILTRATES

The location within the marrow space is an important factor that may help in distinguishing between benign and low-grade malignant infiltrates in the bone marrow. Both benign and malignant lymphoid aggregates may appear non-paratrabecular in a single section from a bone marrow trephine biopsy. Measurement of the distance that a malignant infiltrate may remain paratrabecular, made on the computer-generated three-dimensional models, showed that this distance was never more than 320 μm (in the trephines examined) (Salisbury et al., 1997). This means that by cutting and staining three levels from each trephine biopsy (with a distance greater than 320 μm between the first and third level) any malignant deposit that appears to be non-paratrabecular in the first level should be shown to be paratrabecular by the third level. This observation could then aid diagnostic discrimination. That three-dimensional reconstructions of human bone marrow have shown that benign lymphoid aggregates always remain non-paratrabecular is an important observation. However, it does not allow diagnostic discrimination between benign lymphoid aggregates and some lymphoid malignancies that may show identical patterns of marrow infiltration, such as chronic lymphocytic leukaemia (nodular pattern of infiltration) or lymphocytic lymphoma.

Stereological techniques are ideal for the exploration of three-dimensional space and it is surprising how few studies have used stereology to examine aspects of bone marrow physiology and pathology (Lambertson and Weiss, 1984, Heynen et al., 1985, Cullen and McDonald 1986).

In conclusion, the best discrimination between benign and malignant lymphoid aggregates in the bone marrow is achieved by a combination of the traditional morphological criteria, observations about aggregate location, and appropriate immunohistochemical staining. Like many other haematopathological entities, a full clinical history with peripheral blood and bone marrow aspirate findings, including the results of immunophenotyping and cytogenetics, may also prove crucial in reaching the correct diagnosis.

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