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Bone Marrow Lymphocytes

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Summary

Some aspects of bone marrow lymphocyte production are reviewed. Attention is drawn in particular to fundamental histogenetic differences in the production of B and T lymphocytes. B lymphocyte precursors are transitional cells.

The great interest now being shown in bone marrow, as a major source of B lymphocytes, makes it all the more remarkable that for more than half a century, the generally accepted view was that lymphocytes were not an integral part of the marrow, and that in fact there were no lymphocytes in the bone marrow proper. It is true that there were a few who opposed this view, and who attached considerable importance to the 'lymphoid' cells of the marrow. But they were a very small minority. The majority view was advocated very forcibly for many years by *Naegeli* (1), who held that lymphocytes in the marrow were 'extraparenchymatous', and not really part of the marrow parenchyma. He maintained that if any lymphocytes were present, they must be embryonic or pathological forms. All this sounds very strange to us at the present time, yet such indeed was the case.

With such a conceptual background, the problem of lymphocyte production in the marrow could not be taken seriously, and in fact it rarely called for consideration. This attitude was reinforced by the existence of a striking morphological difference between the bone marrow and the remainder of the lymphomyeloid complex. In other parts of the complex – lymph nodes, thymus, spleen, and lympho-epithelial tissues – there are organized masses of lymphoid tissue, in which mitotic figures are often conspicuous. The germinal centres in lymph nodes, for example, were one of the first areas in which cell mitosis was studied in animal tissues (2). In contrast, normal bone marrow rarely contains any organized lymphoid tissue. At the most, there may be occasional aggregates of cells, as noted by *Sundberg* (1953) (3). The occurrence of lymphoid nodules under pathological conditions is of course a very different matter and has been noted repeatedly, from 1902 onwards (4, 5, 6, 7).

In discussing the problems of lymphocyte production in the marrow, it is helpful to see them in their wider perspective, against a quantitative background. The most extensive data on marrow lymphocyte production have been obtained in young adult guinea-pigs (8). These data indicate that for every lymphocyte present in the blood stream, three are daily entering the blood via the thoracic duct, while twenty are present in the marrow. Furthermore, thoracic duct lymphocytes are for the most part a recirculating population, while marrow lymphocytes in the main are newly formed, with a rapid turnover time of 2-3 days. Since the majority of marrow lymphocytes leave the marrow to enter the blood stream, they do so in numbers sufficient to replace those in the circulation about six times per day, quite apart from lymphocytes entering the blood form other sources. It should be emphasised at this point that these facts are much more obvious in the marrow of smaller laboratory animals than in normal human adult marrow. However, during foetal life, and the postnatal growth period, human marrow seems to be more in line with that of experimental animals.

The pioneer experiments of *Osmond and Everett* (1964) (9) demonstrated clearly that lymphocytes in the marrow are formed by the division of cells which had previously been designated 'transitional' (10). Transitional cells are a spectrum of cells, of varying sizes and degrees of basophilia, which are an essential constituent of bone marrow. They have been given a variety of names, among which the commonest are 'lymphoid', 'lymphocytoid', and 'lymphocyte-like', and they are important as a source both of lymphocytes and of other marrow cells.

From an analysis of labelling curves and grain counts, *Osmond and Everett* (loc. cit.) concluded that in the guinea-pig the population of marrow lymphocytes is maintained in a dynamic steady state, with an average turnover time of 3 days or less. This view was subsequently confirmed in the marrow of the rat (11, 12), and recently in the mouse (13), and it seems to be generally true, making the necessary allowances for age and species. It is clear that if the sole function of transitional cells is to divide and give rise to small lymphocytes, they would soon disappear, and lymphocyte production would be at an end. This does not happen, because the transitional cells are capable of intrinsic proliferation, by the simple process of smaller transitional cells enlarging, and larger transitional cells dividing to form the smaller cells.

The transitional cell compartment gives rise not only to lymphocytes, but also to all other blood cells, mainly erythrocytes and granulocytes. The smaller transitional cells divide to form small lymphocytes, while the larger ones develop an increasing amount of basophilic cytoplasm and give rise to blast cells. The size of the transitional cell compartment at any given time is thus the expression of a very dynamic equilibrium. On the one hand, the intrinsic proliferation of the transitional cells would result in their progressive increase, while on the other, their division to form small lymphocytes, or their differentiation into other blood cells, would have the reverse effect and reduce the size of the compartment. In quantitative terms, there are three major cell groups which can make heavy demands on the transitional cell compartment, namely lymphocytes, granulocytes, and erythrocytes. A call for greatly increased production of one of these three main groups has repercussions both on the number of transitional cells produced, and on the production of the other two groups. This is shown very clearly in the response of the marrow to a strong hypoxic stimulus, when increased red cell production is associated with a marked fall in marrow lymphocytes and granulocytes (14).

During the period of post-hypoxic polycythaemia, marrow granulocytes rebound to normal or slightly above-normal levels, while lymphocytes rebound to markedly above normal levels. During this rebound period, the transitional cells greatly increase in number, and rebound marrow is therefore particularly suitable for the study of transitional cells and their properties. *Rosse* (1971) (15) and *Rosse and Trotter* (1974) (16) have used rebound marrow to demonstrate that the smaller transitional cells are capable of switching into either lymphocyte formation by dividing, or into erythropoiesis by enlarging and developing haemoglobin.

The formation of small lymphocytes in the marrow does not in itself constitute evidence of B cell formation. For this one needs to demonstrate the presence of surface immunoglobulin, shown by the uptake of labelled antiglobulin on the lymphocyte surface (17, 18). This surface immunoglobulin is possibly being continuously secreted in small amounts by B lymphocytes (19), which thus have features in common with plasma cells (20).

Osmond and Nossal (1974a, 1974b) (21, 22) combined labelling studies of surface immunoglobulins with kinetic studies of cell proliferation by means of tritiated thymidine, and their initial immunoglobulin studies showed that, in adult mice, approximately half the marrow lymphocytes did not have surface immunoglobulin, though from the observations of *Rosse* (1975) (23) in the guinea-pig, a number of transitional cells had already begun to acquire immunoglobulin receptors. The development of immunoglobulin-bearing small lymphocytes from transitional cell precursors would appear to be the last stage in the maturation of a type of cell radically different from the other major cell groups in the marrow, since the latter are

post-mitotic, without further growth potential, whereas the B lymphocyte is capable of further growth and development, though probably of a limited nature.

The bone marrow is a major if not the exclusive source of B lymphocytes. This has been shown in experiments such as those of *Unanue* et al. (1971) (24), who reported that when adult mice were thymectomised, lethally irradiated, and then transfused with bone marrow cells, they were devoid of Ig negative lymphocytes, but showed a normal number of lymphocytes with surface Ig.

Finally, it must be noted that the production of B lymphocytes in the marrow differs strikingly in its histogenesis from the formation of T lymphocytes in the thymus. B cells in the marrow are formed in the course of what has been termed the Short Production Pathway (7, 9), consisting of 2-3 mitoses, whereas T cells are produced in the thymus via the Long Production Pathway, consisting of about 8 mitoses (25). Furthermore, the starting point for the short production pathway is a large transitional cell (8, 9), whereas the starting point for the long production pathway has been thought to be a reticulum cell (25), or a 'monocytoid' cell (26). An additional difference depends upon the access of foreign proteins to the developing lymphocytes. Proteins in the circulation have free access to the cells of the short production pathway in the bone marrow, since the endothelium of the marrow is freely permeable to large molecules, and permits the rapid passage of proteins and even particulate matter into the marrow parenchyma (7, 27). In the case of the thymus, on the other hand, there seems to be a barrier to the free entry of proteins into the thymic parenchyma (28). It is conceivable that the differential access of proteins to the developing B and T cells is a significant factor in deciding how they develop.

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