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CELLULAR MIGRATION STREAMS. THE INTEGRATION OF THE LYMPHOMYELOID COMPLEX*

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Mr. President, may I start off by thanking you and your colleagues for the great honor you have done me in inviting me to be Honorary President of the Ninth International Congress of Lymphology? As an "old lymphatic war horse"—to use a description often employed by Cecil Drinker — it has given me the welcome opportunity of meeting many old friends, and also making a number of new ones.

CONSTITUENTS OF THE LYMPHOMYELOID COMPLEX

The term "Lymphomyeloid" has been in use since the early days of hematology. It was applied to tissues in which lymphoid and myeloid cells were intermingled. The term "Lymphomyeloid Complex", which we introduced in 1970 (1) is something very different. It comprises a number of discrete tissues, lymphoid, myeloid, or mixed, dispersed throughout the body, with no immediately obvious interrelationship. The Lymphomyeloid Complex (LMC) has six major constituents: bone marrow, thymus, spleen, lymph nodes, lymphoepithelial tissues and connective tissues. The term "connective tissue" we use in its widest sense, comprising both connective tissue as usually understood, and also the body fluids, which we regard as connective tissue with a fluid matrix.

CELLULAR MIGRATION STREAMS

The scattered parts of the LMC are connected to one another by what in 1959 we termed "CELLULAR MIGRATION STREAMS" (2), but in a brief lecture one has time to deal with only a few of these streams of migrating cells, and even then not in very great detail. Furthermore, in addition to the known migration streams, it seems very likely that there are a number of others which we have not as yet been able to identify. The blood is the most rapid channel for cell migrations between the different parts of the complex. In addition, there are slower migration streams via the lymph and the various connective tissues.

Fig. 1 is a diagram which I made many years ago, in an attempt to represent in a single figure the various components of the LMC and some of its migration streams. In order to get the whole complex into one figure, some misleading adjustments had to be made. For one thing, the diagram gives no indication of the primacy of the bone marrow in relation to the other constituents of the LMC. Another misleading adjustment is the way in which the connective tissues are depicted, as a very narrow and apparently insignificant strip-labelled

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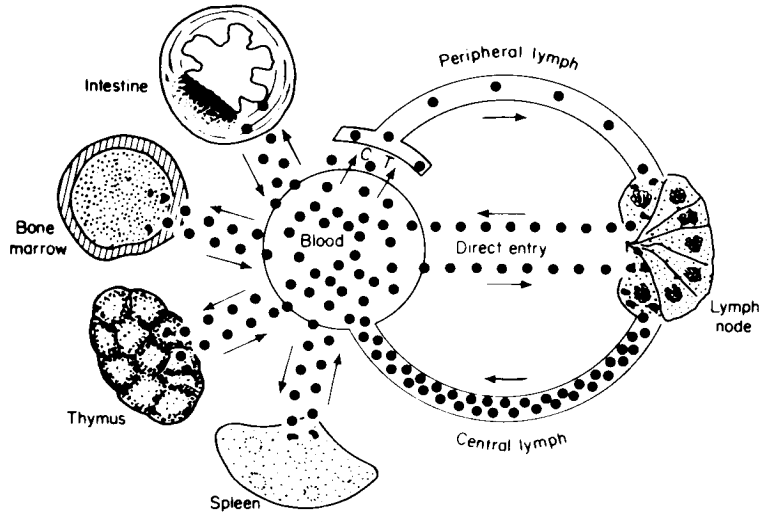

Fig. 1: Scheme to illustrate the main constituents of the lymphomyeloid complex and some of the main cellular migration streams. Most of the migrating cells are lymphocytes, but as emphasized in the text, many other cell types are involved, though in much smaller numbers. Cells can leave or enter the blood stream by directly traversing the walls of the blood vessels ("direct entry"). "Indirect entry" cells are those which first obtain access to the lymph stream, in which they then reach the blood. Connective tissue (CT) includes blood and serous cavities. From Yoffey and Courtice (1970).

CT in the diagram — between blood and peripheral lymph. This is misleading, because the extent of the connective tissues is very great, though we are unfortunately unable to quantitate either the size or the cell population of the less fluid part of these tissues, one of the major blind spots in our knowledge of the LMC. Methods of connective tissue study are difficult. One approach is via the study of lymph immediately after its formation, when it contains cells which have just passed through or been formed in the connective tissues. So the study of what we termed *peripheral lymph* can give us some information. Another dynamic approach is the skin window technique (4).

In making this diagram (Fig. 1) I had in mind primarily the migration of lymphocytes. But the lymphomyeloid complex contains a great variety of cells, and these too are involved to a widely varying extent in the numerous migration streams — macrophages and their precursors, granulocytes, plasma cells and their precursors, NK cells, Langerhans cells, dendritic cells, and as we have recently come to learn, a wide range of committed and uncommitted hemopoietic stem cells.

The concept of cellular migration streams owes its inception to an early mistaken working hypothesis. When I first started to be interested in lymphocytes, in 1929, I did not know quite where to begin, but thought it would be a good starting point to obtain some idea of the magnitude of lymphocyte production. At the time I thought that for the most part newly-formed lymphocytes entered the blood through the thoracic duct, and that by counting thoracic duct lymphocytes I was measuring lymphocyte production. One then had to explain why the level of the blood lymphocytes remained fairly cons-

tant, despite the fact that large numbers of what were thought to be newly formed lymphocytes were constantly entering it. As there was no evidence of large-scale destruction of lymphocytes while in the blood stream, the maintenance of the blood lymphocyte level was mistakenly attributed to the constant migration of lymphocytes out of the blood stream into the bone marrow to act as stem cells. This of course was later shown to be wrong, partly because of the demonstration by Osmond and Everett (1964) (5) of the large-scale new formation of lymphocytes in the marrow. But my mistaken working hypothesis had at least one merit, for it led to formulating the concept of cellular migration which, though not valid in this particular instance, nevertheless subsequently underwent such considerable development.

One point needs clarification at the outset. The general concept that cells can migrate has of course been with us since the early days of pathology and hematology. In the classical studies on inflammation, for example, an enormous amount of work was done on the local migration of cells in inflammatory exudates. But when we introduced the term CELLULAR MIGRATION STREAMS (2) we were trying to pinpoint streams of a different kind, of cell migrations which were not an ad hoc response to obvious external stimuli, but were taking part under normal physiological, not pathological conditions. The main channel for most of the migration streams is the blood, in which the white cells seem to be predominantly in transit. Incidentally, despite the large numbers of cells constantly migrating through the blood, we still have no idea how the level of the blood lymphocytes is normally kept relatively constant.

THE DYNAMO OF THE LYMPHOMYELOID COMPLEX — BONE MARROW

When we look at all these migrating cells passing through the blood, some recirculating, some newly-formed, the question arises whether the various streams are completely independent, or whether there is somewhere a definite starting point from which all the streams diverge. We now know that there is such a starting point, namely the bone marrow.

This conclusion was reached largely after a series of quantitative studies of bone marrow and other parts of the LMC, extending over a number of years, and conducted in collaboration with numerous colleagues, to whom I am greatly indebted. We developed a technique which gave us absolute counts of the various cells in unit volume of marrow, as seen in Table 1.

Table 1
Absolute Counts (Thousands per mm^3) of Main Cell Groups in Guinea Pig Marrow

	Mean ± SD
Early neutrophils	49 ± 21
Late neutrophils	405 ± 120
Total eosinophils	73 ± 22
Total basophils	17 ± 9
Myeloblasts	25 ± 13
Proerythroblasts	18 ± 9
Basophilic erythroblasts	358 ± 128
Polychromatic erthroblasts	53 ± 31
Orthochromatic erythroblasts	53 ± 31
Reticulocytes	197 ± 58
Lymphocytes	502 ± 114
Transitional cells	48 ± 16

Early neutrophils — promyelocytes and myelocytes; late neutrophils — metamyelocytes, band, and segmented forms. From Hudson et al [1963].

These counts, together with measurements of marrow volume, made it possible to estimate the total populations of the different cell groups in the marrow as a whole, and the changes they underwent in various experimental situations. Though the most numerous observations were made on the bone marrow of the guinea pig, the same general pattern was found in rats and mice. The data thus obtained could be compared with quantitative and qualitative studies of cells in the blood and other parts of the

lymphomyeloid complex.

In the adult guinea pig the red marrow contains about 2,000,000 nucleated cells per cu.mm., and the majority of these fall into three main groups, erythroid, myeloid, and lymphoid. There is also a small group, which we originally termed "transitional" cells, for reasons which were partly wrong, though later work showed that in ways other than those originally intended the term was a very appropriate one.

THE TRANSITIONAL (STEM) CELL COMPARTMENT

Though transitional cells are only about 2% of the total nucleated cells in the adult guinea pig, they are in fact the stem cells from which most of the other cells of the marrow arise. Furthermore, as we now know, the integrity of the entire lymphomyeloid complex depends ultimately on the transitional cells or the cells derived from them. As far as lymphocytes are concerned, the bone marrow is not only the primary source of the B lymphocytes. It is also the source of the prothymocytes. upon which the thymus depends for its maintenance. In addition, it may well be that the transitional cells give rise to all the antigen-presenting cells formed in the marrow, namely macrophages, Langerhans cells, and dendritic cells. The "monoblast" of Van der Meer et al. (1982) (6) is a typical transitional cell.

A full account of the transitional cell compartment will be found elsewhere (7, 8). Very briefly, one may here note that it consists of a spectrum of cells of different sizes, and differing degrees of basophilia. The size range is from a little over 6 to 10μ+. The nucleus of transitional cells is predominantly leptochromatic, the leptochromasia being more marked in the larger than in the smaller transitionals. The larger transitionals, especially the basophilic ones, have a high labeling index with thymidine.

Transitional cells and colony-forming units

The various colony-forming units appear to be both morphologically and kinetically members of the transitional cell compartment. It took some time to establish this, since the early colony-forming studies were performed by investigators who had a strong antimorphology bias. Again and again one comes across the statement, which was almost an article of faith, a religious dogma, that stem cells could not be morphologically identified. For quite a while, therefore, no attempts at morphological identification were made. But when finally such attempts were made, it became clear that the dogma was untenable. Moore, Williams, and Metcalf (1972)(9) appear to have been the first to identify CFU-C as transitional cells. Since then a number of investigators have described similar stem cell morphology, though the terminology has been confused.

Scientific dogma

The question of dogma is an interesting one in the history of science in general. I happen to be especially familiar with it in the field of immuno-hematology, where there are many striking examples. One of the most outstanding examples is the view, held by the vast majority of observers for well over half a century, that the small lymphocyte was a "mature" cell, incapable of growth and with no known future. I can still recall being widely critised for stating, in "Lymphatics, Lymph, and Lymphoid Tissue" (Drinker and Yoffey, 1940)(10) that all the evidence pointed to the small lymphocyte being capable of growth. This view was for many years rank heresy, accepted by only a very small minority, until Hungerford et al. (1959)(11) began their classical studies on the action of PHA on small lymphocytes in vitro. This established beyond any doubt that small lymphocytes were capable of growth, and as I have previously written: "When this fact finally came to be accepted, an army of investigators moved in and suddenly became interested in lymphocytes and everything appertaining to them. The first trickle of papers swelled into an ever-widening stream

which soon became a raging torrent, sweeping away many of the old boundaries and opening up many exciting new fields of research. It was not only the lymphocyte which had become transformed, but to a very remarkable extent the climate of opinion surrounding it."

Another example is the identity of the stem cell, which again for more than half a century was maintained by most observers to be the singularly ill-defined reticulum cell. This was repeated in paper after paper, year in year out, and copied from one text book to another, so everybody thought it must be true — until it was shown to be otherwise.

An even more striking example is the occurrence of lymphocytes in the bone marrow. From the start of the century Naegeli, one of Europe's foremost hematologists, maintained for many years (12) that lymphocytes in the bone marrow were not really part of the true marrow parenchyma, but were what was termed "extraparenchymatous." This sounds very odd to us now, but it was the dogma accepted by the great majority of haematologists for over half a century until, like other immutable truths based on unsatisfactory evidence, it was shown to be a fallacy. When one contemplates all these dogmas, based on very flimsy evidence but proclaimed with great certainty, one cannot help but be reminded of the famous lines of Hilaire Belloc: "Oh never, never let us doubt, what nobody is sure about."

After this digression let it be noted that the transitional cell compartment, as usually identified with the light microscope, comprises both the committed and the uncommitted stem cells. In response to appropriate stimuli the uncommitted cells are capable of differentiating in various directions. By the use of more sophisticated techniques than simple light microscopy, some of the committed cells can be clearly identified. In virtue of its transitional cell content, the bone marrow may be regarded as the dynamo, the cellular power house, not only of the bone marrow, but also of the entire lymphomyeloid complex.

The proliferation gradient

At first sight it seems strange that this small group of transitional cells in the bone marrow should be responsible for maintaining the integrity not only of the marrow, but also of the entire lymphomyeloid complex. But in this connection three important points must be noted. First, even in the normal steady state the cells of the compartment are proliferating actively.

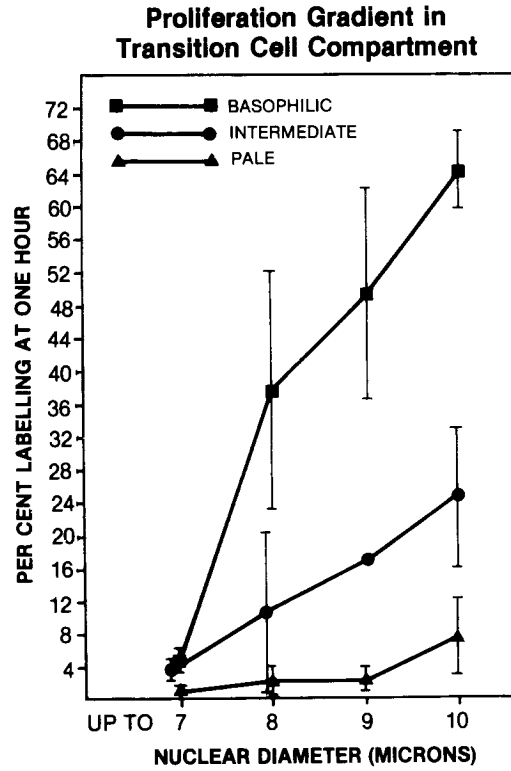

Fig. 2: The cells of the transitional cell compartment exhibit a characteristic proliferation gradient, 1 hour after in vivo labeling of bone marrow cells with tritiated thymidine. The labeling percentage varies with the degree of basophilia and cell size. The highest labeling index is to be found in the basophilic transitionals, the lowest in the pale cells. Between these two groups are the cells with intermediate degrees of basophilia. In all three groups, the labeling index is lowest in the small cells, and highest in the large. From Tavassoli and Yoffey (1983).

Taking the compartment as a whole, in the young adult guinea pig the labeling index is

35%, while in the rat it is even higher, around 50%. Second, the compartment has a marked, and variable, proliferation gradient. If we measure the labeling index in relation to cell size, we find that in the normal steady state very few cells are proliferating at the small end, while at the large end, especially among the basophilic cells, there is a labeling index of around 70% (Fig. 2). When there is increased demand for stem cells, a greater number of the small cells enlarge and enter the more rapidly growing large end of the compartment. In some recent rat experiments, for example, when we stimulated erythropoiesis, which requires additional stem cells to differentiate, the labeling index of the TC compartment as a whole rose to over 70% (13). Thirdly, once the transitional cells begin to differentiate, an amplifying mechanism is introduced, whether the differentiation is intra- or extra-myeloid. Thus, when thymocyte precursors migrate to the thymus, they have been thought to undergo as many as 8 mitoses before the final T lymphocyte stage is reached. So on this basis, one prothymocyte could give rise to over 200 T cells. In the case of the B lymphocytes, the amplification is also quite marked, possibly even more so than in the case of the T cells. But it is difficult to measure accurately, partly because the differentiation can occur in two stages, in the primary and secondary responses. Within the marrow amplification also occurs during the differentiation of stem cells into erythrocytes, granulocytes or monocytes.

INDIVIDUAL MIGRATION STREAMS

Having touched briefly on what I have termed the dynamo, the cellular power house, of the lymphomyeloid complex, let me discuss some individual migration streams — to the extent that our time will allow — namely: (1) Marrow to thymus (2) Connective tissue and lymph node streams (3) Plasma cell precursors and plasma cells (4) Stem cell migration.

1. Migration from marrow to thymus

The migration of cells from marrow to thymus was suggested by the early marrow shielding experiments (14). Evidence that the thymus received stem cells from the circulation emerged from a study of thymic grafts, in which a graft of a chromosomally distinct thymus made it possible to identify the immigrant cells (15, 16). In experiments of this nature the central part of the graft undergoes necrosis, but the peripheral rim, in contact with blood vessels, survives, and for a time is the site of active proliferation of the precursor cells present in the graft. However, as shown by chromosomal analysis, from around the 10th day onwards the donor cells fall sharply and become replaced by host cells. Schlesinger and Hurwitz (1968) (17) came to the same conclusion on the basis of immunological differences in the grafted thymuses between host and donor cells. In themselves, of course, these experiments show that the thymus is repopulated by cells from an extrathymic source, but give no information about the actual source itself. However, taken in conjunction with marrow-shielding experiments (14) and the results of marrow transfusion (18, 19) and marrow-labeling (20) studies, it is difficult to envisage any other source for the prothymocytes than the bone marrow.

There is now a good deal of evidence to indicate the presence of T cell precursors in the marrow by experiments restricted to the marrow itself. Komuro and Boyse (1973) (21) noted that cells from the bone marrow — as also from the spleen or fetal liver — of nu/nu mice could be induced, by a product of mouse thymus, to express TL and Thy-1 antigens in vitro. Scheid et al (1973) (22) showed that non-thymic products could also induce T cell differentiation in the marrow of both normal and nude mice, and they concluded (cf. 23) that the T cell precursors in the marrow were already specifically committed. Working with rat bone marrow, Goldschneider (1976) (24) came to the conclusion that T cells could be derived from what he regarded as null lymphocytes. Morgan et al (1976) (25) and

Gillis et al (1978) (26) observed the formation of T cells in long term cultures of human bone marrow. Press et al (1977) (27) reported that null lymphocytes in murine bone marrow acquired T cell surface antigens during the course of the blastogenic response to PHA, while Press and Rosse (1977) (29), in continuation of these studies, concluded that both B cells and T cell precursors develop from uncommitted transitional cells.

Later work seems to fit in with the view that there is a very close relationship, if not actual identity, between thymocyte precursors and transitional cells. Thus, Rusthoven and Phillips (1980)(30) noted that hydroxyurea destroyed 70% of the CFU-T in murine bone marrow. From the proliferation gradient (Fig. 2) it is clear that this result would also hold good for the basophilic large transitional cells. There are in fact no other cells in the bone marrow for which this would hold good. Boersma et al (1981) (31) studied the postirradiation regeneration of thymocytes after bone marrow transplantation, and observed that the CFU-T ("thymocyte precursors") had the same velocity-sedimentation rate, buoyant density, and cell surface charge as CFU-S. Greiner et al (1982) (32) performed studies of rat bone marrow with the fluorescence-activated cell sorter. Forty percent of the marrow prothymocytes were dexamethasone sensitive, and 60% resistant. The dexamethasone-resistant prothymocytes displayed large amounts of Thy-1 antigen, like the CFU-S. Furthermore, the dexamethasone-resistant prothymocyte coisolates with the CFU-S in the FACS. Though they were not prepared to equate prothymocytes with CFU-S, the possibility seems to remain open that the CFU-S give rise to the prothymocytes.

Another line of approach has been the long-term culture of marrow stem cells, which have typical transitional cell morphology (33). Though these cells possess neither B nor T cell characteristics, it has been shown that irradiated hosts develop full functional B and T lymphocytes when transfused with these marrow stem cells (34, 35).

Finally, one may note that the thymus affords a particularly good example of amplification during the process of hemopoietic stem cell differentiation. In work with guinea pigs (36) the lymphocyte production pathway was followed over a period of 6 to 8 days in radioautographs of thymic smears after a single in vivo pulse of tritiated thymidine. The sequential labeling pattern of: Lymphoblasts →large →medium →small lymphocytes was associated with progressive, and finally very marked label dilution (see also 37). In the mouse, Metcalf (1967) (38) reported almost 100% labeling of thymocytes in the course of three days, and calculated cell cycle times of 6.8 hours for the large and 8.2 hours for the small lymphocytes (cf. Claesson and Hartmann (1976) (39). Over a period of 3 days this would mean at least 8 mitoses in the course of the lymphocyte production pathway, so that there would be need for only a small number of precursor cells, which could be difficult to identify among the large number of lymphocytes produced. Kadish and Basch (1976) (40), in a study of the repopulation of the thymus of irradiated mice, have also emphasized the small number of precursors required to effect this repopulation.

2. Connective tissue and lymph node streams

(a) Channels of migration

In lymph nodes there are a number of interesting and varied migration streams. Their analysis is complicated by the fact that there are two channels for the entry of cells into the node, and three for exit. In a node such as the popliteal, one of the most extensively studied, cells reach the node either through the afferent peripheral lymph or from the blood stream through the post-capillary (high endothelium) veins. Cells leave the node (1) through the efferent lymph (2) through the post-capillary veins, and (3) via the thin-walled veins into which the post-capillary veins drain. In the case of the post-capillary and thin-walled veins, the cells in transit usually appear to be small lymphocytes. The larger cells entering and leaving the node are to be

found predominantly in the afferent and efferent lymph.

Substantial numbers of small lymphocytes appear to be capable of leaving the node via the thin-walled veins, which are often very full of these cells — a phenomenon described as "lymphocyte loading" (l). As far back as 1929, Ehrich (41) illustrated thin-walled veins full of lymphocytes, and commented on a similar observation by previous investigators. In freshly fixed material, one can frequently observe what appear to be small lymphocytes in transit through the vein wall.

In the case of the thin-walled veins, the movement of lymphocytes seems to be in one direction only, namely out of the lymph node into the blood stream, but the position with regard to the post-capillary veins has been open to dispute. Gowans and Knight (1964) (42) were of the opinion that the lymphocytes in the walls of the post-capillary veins were migrating from the blood into the lymphoid tissue. Sainte-Marie et al (1967) (43) thought that the lymphocytes in the walls of the post-capillary veins were moving in the opposite direction, from the lymphoid tissue into the blood stream. Rydgren et al (1976) (44), in careful studies of the locomotory pattern of lymphocytes in the wall of the post-capillary vein, concluded not only that there was movement of lymphocytes in both directions, but that more lymphocytes were actually leaving the node in this situation than were entering it. Some aspects of the problem have recently been reviewed by Ford and Smith (1982) (45).

The exit of lymphocytes from the node via the post-capillary and thin-walled veins makes it difficult to assess the cell output of lymph nodes on the sole basis of the cell content of efferent lymph.

(b) The cells in peripheral lymph

The first differential counts of the cells in peripheral lymph were made by Yoffey and Drinker (1939) (3), mainly in leg lymph of cats and dogs, and to a lesser extent in intestinal lymph. They found that peripheral lymph contained about 50% of lymphocytes, 20% of monocytes and macrophages, and 20% of neutrophils. Small numbers of basophils and eosinophils were occasionally present. Since then a number of investigators have studied the cells of peripheral lymph from a variety of tissues (46-49). For the most part the relatively high content of monocytes and macrophages (the 'mononuclear phagocytes' of later authors) has been amply confirmed.

There appears to be a constant passage of monocytes and macrophages to the node via the afferent peripheral lymph, yet in the lymph draining the node they are rarely to be found, nor are they present in thoracic duct lymph under normal conditions (48, 50), though they may be found there in appreciable numbers when the reticulo-endothelial system is stimulated (51). It is still not clear what happens to the macrophages when they reach the node.

A notable feature of the macrophages in peripheral lymph is that they are often closely associated with lymphocytes, suggesting the possibility of an immune reaction. The occasional presence in peripheral lymph of large basophilic lymphoid cells, resembling lymphoblasts, seems to support this view (46,48).

(c) Passage of lymphocytes from blood into lymph

The passage of lymphocytes from blood into the connective tissues and peripheral lymph does not appear to be a random process, for if it were one would expect the lymphocyte content of peripheral lymph to be more or less proportional to the concentration of lymphocytes in the blood. This is not the case. In our own experiments (3) the lowest lymphocyte count in peripheral lymph was found in an animal which had the highest level of lymphocytes in the blood. A more recent study (48) also reports a similar lack of correlation between lymphocytes in blood and peripheral lymph.

(d) Distribution of B and T cells

The position with regard to the lymph node migration streams is further com-

plicated by the relative distribution of B and T cells. Normal human blood has a preponderance of T lymphocytes. Brown and Greaves (1974) (52) estimated that 81.2% of the blood lymphocytes were T cells, 15% B cells. Eremin et al (1976) (53) gave a figure of 69% T and 29% B cells. Lukomska et al (1980) (54) found that human peripheral lymph has relatively few B cells, most of its lymphocytes being T cells. The excess of T cells in blood and lymph is reflected in the normal node, though it may change somewhat in nodes which are reacting to a variety of stimuli. Thus, according to Black et al (1980) (55) normal human lymph nodes contained 68% T and 18% B cells. However, reactive nodes averaged 44% T and 32% B cells. Gery et al (1977) (56) found a marked accumulation of B cells in nodes stimulated with different immunogens, including T-specific stimulants.

The use of monoclonal antibodies (57, 58) has made it possible to identify in lymph nodes not only the location of B and T cells, but also of T cell subsets. These newer observations confirm, inter alia, the preponderance of B cells in the follicles and of T cells in the paracortical areas.

According to Antonelli et al (1981) (59) there are no NK cells in lymph nodes or thymus.

(e) Non-lymphoid cells

Finally, in this brief account of lymph node migration streams, one may note the increased interest in previously unrecognized non-lymphoid cells. Pugh et al (1983) (49), in a study of the non-lymphoid cells in rat peripheral (intestinal) lymph, thought that they resembled the dendritic cells which Steinman and Cohn (1973) (60) had described in mice. The presence of dendritic cells in peripheral lymph, which they presumably enter from the blood stream (61), would explain their presence in lymph nodes. Langerhans cells also may be found in peripheral lymph (62-64). Silberberg-Sinakin et al (1976) (62) gave intradermal injections of ferritin, and within a few hours identified antigen-bearing Langerhans cells in the dermal lymphatics and regional nodes. It is noteworthy that both Langerhans cells and dendritic cells are believed to be of bone marrow origin, in which case the bone marrow, which also gives rise to macrophages, is a source both of antigen-presenting and antibody-forming cells.

3. Plasma cell migration

Without going at length into all aspects of plasma cell production, I would like to devote a little time to the formation of plasma cell precursors and plasma cells in lymph nodes, and their migration via the lymph stream into the blood. It must be emphasized in this connection that some measure of plasma cell production occurs even in the normal animal. This is part of the normal process of "subinfection," and some of the literature has been reviewed elsewhere (1). Surprisingly, a certain amount of plasma cell formation occurs even in germ-free animals (66, 67). In the case of the mouse, this may result from the difficulty in excluding viruses.

Most information about plasma cell production and migration has been derived from experiments in which plasmacytopoiesis has been stimulated by a variety of antigens, usually in the popliteal node. Many antigens on reaching lymph nodes evoke a characteristic immune response, with the formation both of plasma cells, and of even larger numbers of plasma cell precursors, the plasmablasts. These precursors are the large basophilic cells which one finds leaving the node in the efferent lymph and later appearing in the thoracic duct. The mature plasma cells have already passed the peak of antibody production, and appear to have only a short life span in the lymph nodes (68). But only a small number of plasmablasts develop into mature plasma cells in the reacting node. Several studies (69, 70) have emphasized the massive discharge of plasmablasts from the lymph node into its efferent lymph. It appears that the many plasmablasts which migrate from the antigenically stimulated

node are the major factor in the development of general immunity, much more so than the plasma cells which develop in the lymph node itself.

Nossal and Makela (1961) (72) made a detailed study of the kinetics of plasma cell proliferation. Cunningham et al (1964) (70) noted that at the peak of the antibody response far more antibody-forming cells leave the node than are present in it. In their experiments on the popliteal node, Hall et al (1967) (71) showed that, if the efferent popliteal lymph was collected, and its plasmablasts thereby prevented from entering the blood stream, no general immunity developed even though the popliteal node contained large numbers of plasma cells.

The large basophilic plasmablasts ('lymphoblasts') leaving a node may pass through one or more intermediate nodes to reach the blood stream via the thoracic and right lymph ducts. But some may be held up in intermediate nodes and there undergo maturation into fully formed plasma cells. Thus, Turk and Heather (1965) (73) injected antigen into the paws of the foot, and 4-6 days later found appreciable numbers of plasma cells in inguinal, femoral and abdominal nodes. However, when there is massive production of plasmablasts, large numbers can traverse even a chain of nodes without being held up, and can be identified in the thoracic duct immediately before entering the blood stream. As the large basophilic cells in thoracic duct lymph (50) the plasmablasts can readily be recognized with the light microscope.

Wesslen (1952) (74) obtained thoracic duct lymph from rabbits three days after the subcutaneous injection of living typhoid bacilli, and found that 5-6% of its cells were of the large basophilic variety. He was then able to show that while these lymphoblasts did not contain specific agglutinin, it was present after 48 hours in culture. Wesslen therefore concluded that he was dealing with potential antibody-forming cells in thoracic duct lymph.

A large number of the plasmablasts which enter the blood stream are filtered out in the first capillaries which they reach, namely the lung, where they undergo their final maturation.

The changes involved in plasma cell formation and the migration of plasma cell precursors became more readily recognizable following the electron microscope studies of Braunsteiner et al in 1953 (75). They discovered the characteristic arrangement of the endoplasmic reticulum in the mature plasma cell, and also noted that this arrangement was beginning to be evident in the earlier stages of its development, i.e. in the immature plasma cell, which could now also be unmistakably identified on ultramicroscopic examination. Braunsteiner and Pakesch (76) were able to identify these immature plasma cells in thoracic duct lymph, while in the same year Schooley and Berman (77) cultured thoracic duct lymphocytes in diffusion chambers and observed the formation of mature plasma cells from the immature precursors. It is pertinent to note that small numbers of plasmablasts are to be found even in normal thoracic duct lymph (78, 79). These are possibly derived from the intestinal and mesenteric lymphoid tissue, constantly bombarded by immunological stimuli.

Until relatively recently, immune responses in lymph nodes were envisaged in terms primarily of changes in cells which were thought to be present in the node from the outset of antigenic stimulation. Kinetic studies however have brought out the fact that some B lymphocytes are constantly migrating from marrow to lymph nodes, and that this migration is especially evident in the primary immune responses occurring in the nodes (80). It should be noted that at the same time as the B cell population of the node is increasing (53, 55, 56), the T cells in the nodes still have an essential part to play, even though their numbers may have diminished relative to the increase in B cells.

Since it is now generally accepted that plasma cells are derived from activated B lymphocytes, it would appear that the bone marrow is the ultimate source of the migrating plasma cell population. Plasmacytopoiesis in the lymph node

represents one of the stages in the life history of the plasma cell which starts life in the bone marrow.

4. Stem cell migration

(a) Introduction

The variety of problems associated with the migration of stem cells has been studied extensively in recent years, more especially since the introduction of in vitro culture techniques. The rapidly expanding field has been reviewed from many aspects in the classical studies of Metcalf and Moore (1971), Metcalf (1977) (82) and others. The idea that stem cells can migrate to or from the hemopoietic tissues goes back to the early days of hematology. As long ago as 1890 Neumann (83), observing the development of marrow in ossified thyroid cartilage, raised the possibility that this marrow might be developing from stem cells present in the blood stream. In this concluding portion of my address — as indeed in all sections of my talk — it is not possible in the time at my disposal to touch on more than a small part of a vast field of research.

The modern period of the experimental study of migrating stem cells may be said to have begun with the work of Jacobson et al (1949) (84), who found in mice that death following irradiation could be prevented by shielding the spleen. Though the bone marrow was destroyed by the irradiation, it subsequently underwent complete regeneration, which was shown to be due to its recolonisation by stem cells migrating from the spleen through the blood stream. The 1960's saw the first demonstration of the presence of pluripotential stem cells — CFU-S — in the blood (85, 86), and the first demonstration that stem cells could be grown in vitro (87, 88). It has since been shown that the blood contains virtually all the different varieties of stem cell, committed and uncommitted.

In post-natal life the primary source of stem cells is the bone marrow, except for the small laboratory animals, where the spleen may also provide some stem cells. In the fetus, before the bone marrow assumes its definitive role, first the yolk sac, and then the liver, function as a source of stem cells.

(b) The role of the migrating stem cells

Some of the stem cells migrating through the blood stream serve an obvious purpose, as for example the CFU-T — otherwise known as T cell precursors or prothymocytes — which migrate from bone marrow in thymus, where they develop into thymocytes. On the other hand, the fate of many of the stem cells is far from clear. There is no obvious role for either the pluripotential or the unipotential stem cells which leave the blood stream for sites where they are not known to undergo further development.

Stem cells, committed or uncommitted, can conceivably migrate through the blood from the marrow of one bone to that of another, and to a small extent this does occur. But for the most part the stem cells remain in the marrow in which they first developed (20). From these latter — and other — experiments it would appear that the majority of stem cells in the normal animal are a relatively static population and tend to remain confined largely to their own marrow, despite the undoubted mobility of some of them when hemopoietic needs appear to warrant it (89).

Micklem et al (1975) (90) suggested as another possibility that many of the stem cells in the blood stream could be rejects from the bone marrow of cells which were not quite up to standard, perhaps suffering from clonal senescence. However, while it is quite conceivable that some of the circulating stem cells may be defective, there are always a number which are not (91). Furthermore, while it is true that stem cells may be found in some situations where they do not undergo any development, they may nevertheless be quite capable of doing so if transferred to a suitable environment. The connective tissues afford a good illustration of this, as was first shown in the peritoneal cavity, and later in the subcutaneous connective tissues.

Goodman (1963) (92) and Cole (1963) (93) showed that there were present in the murine peritoneal cavity cells which were

capable of conferring protection if transfused into lethally irradiated animals. However, in the actual peritoneal cavity from which the cells were obtained one can see no obvious signs of differentiation, and neither erythropoiesis nor granulopoiesis are to be found. Presumably an appropriate stromal background is required for differentiation to occur. We do not know how long the stem cells remain in the peritoneal cavity or what normally happens to them. It is pertinent here to note that there are present in the peritoneal cavity a number of cells which resemble typical transitional cells (94).

Hemopoietic stem cells may also be found in the subcutaneous connective tissues. Tyler et al (1972) (95) examined the subcutaneous inflammatory exudates formed in mice 18 hours after the implantation of coverslips. The cells of these exudates were found to be capable of promoting erythropoietic recovery when transfused into lethally irradiated recipients, as shown by ^{59}Fe incorporation in the spleen. They (95) were impressed by the presence in the exudate of cells which they termed 'monocytoid'. At a later date Scuderi et al (1977) (96) noted that the exudates also contained cells with transitional cell morphology. Cells with transitional cell morphology have also been illustrated recently in human skin window preparations from normal subjects (97). The presence of stem cells in connective tissue raises the possibility that, like some of the other mobile cells in connective tissue, they may pass via peripheral lymph into the regional nodes in which, if conditions were favorable, they could undergo differentiation. This could be one explanation of the phenomenon of myeloid metaplasia.

Normal adult lymph nodes are said to contain very small numbers of CFU-S, and suspensions of lymph node cells have in general not been found to confer protection (98). However, in the early transfusion literature Salvideo et al (1958) (99), who succeeded in conferring protection with lymph node suspensions, attributed the failure of other workers to the small number of cells transfused. If their findings can be confirmed, then presumably the more lymph node cells transfused, the greater the likelihood that sufficient migrating CFU-S would be present to confer protection.

The early work on stem cells was performed mainly in mice, and was concerned largely with migration between marrow and spleen, as measured by the spleen colony technique (100). But the development of the in vitro technique made possible the identification of a variety of stem cells in the marrow and blood stream not only of animals, but also of man, beginning with the early studies of Robinson and Pike (1970) (101), Chervenick and Boggs (1971) (102), Kurnick and Robinson (1971) (103), and Barr et al (1975) (104). Since then a voluminous literature has developed, and is continuing to grow with great rapidity.

Stem cell migration occurs throughout life, but appears to be taking place on a particularly massive scale in the fetus, in and between the rapidly growing constituents of the lymphomyeloid complex. The stem cell requirements of the fetal lymphomyeloid complex are enormous. During a period of several months, the marrow contains a higher concentration of transitional cells than at any other time of life (8). This must be attributed partly to the very great requirements of the bone marrow itself for cell production, and partly to the rapid growth of the other components of the LMC.

As far as erythropoiesis is concerned, one can calculate from the available data that between the twelfth and twenty-fifth week of gestation there is a massive increase in the circulating erythroid population (105). The erythrocyte content per unit volume of blood is more than doubled, while the body weight increases 17-fold. If the increase in blood volume during this period of development is of the same order as the increase in body weight, then the circulating erythrocyte population will increase more than 30-fold. This must be a conservative estimate, since it does not take into account the replacement of effete red cells, while in addition there is suggestive evidence that the mean red cell life is

shorter in the fetus than in the adult (106).

In the third trimester there is a further rapid increase in the number of erythrocytes per cu. mm. of blood (105). The relatively low volume of bone marrow in the fetus (107) makes the marrow inadequate to meet the great demand for red cells, so the contribution of the liver becomes essential. In fact, though the liver is known to possess pluripotential stem cells, its hemopoietic activity in the fetus seems to be directed almost entirely to erythropoiesis.

One sees no sign of lymphocytopoiesis, and only a very occasional transitional cell in the fetal liver, though both transitional cells and lymphocytes are very much in evidence in the bone marrow. Both lymphocytes and transitional cells are present in the bone marrow almost from the outset, and have been illustrated in photomicrographs (108), from which it is clear that the characteristic size spectrum of the TC compartment is already present. Lymphocytes and transitional cells average around 25% of the cells in fetal bone marrow (range 10-45%) and of these the transitional cells constitute about 15%. Thus there can be up to 7% of transitional cells in fetal marrow, a higher concentration than present at any other time.

The unusually larger number of stem cells in fetal marrow is associated with a much higher concentraton of stem cells — first estimated as CFU-S — in fetal than in adult blood. Barnes and Loutit (1964) (109) noted that when lethally irradiated mice were transfused with blood leukocytes to confer protection, 10^7 cells were required if the leukocytes were from the blood of adult mice, whereas only 10^4 or 10^5 were needed if the blood was taken from fetal mice. In other words, fetal blood contained more than 100 times as many stem cells as adult blood. It was subsequently noted, in man, that in midfetal life an appreciable number of blood 'lymphocytes' were in fact transitional cells (110), a representative selection of which were later illustrated in color (111). Although the percentage of transitional cells in fetal blood diminishes during the later stages of pregnancy, they are still present in the blood of the fetus at full term, when about 2% of these cells are in spontaneous DNA synthesis, as opposed to 0.2% in the adult (110-113). Prindull et al (1976) (114) also noted that cytoplasmic DNA synthesis, in all probability mitochondrial, occurs more frequently in cells of cord than of adult blood.

Fetal blood contains both committed unipotential and uncommitted pluripotential stem cells (115). From the recent in vitro experiments of Nakahata and Ogawa (116) it would appear that this is the case even in neonatal cord blood, and that it contains a number of the very primitive stem cells which gave rise to what were termed 'blast cell' colonies.

One possible destination for some of the many CFU-S in fetal blood may well be the thymus. The phenomenally rapid growth of the thymus in the fetus seems to be associated with the presence of considerable numbers of CFU-S (117), though hardly any are present in the neonate (90, 98). For reasons already noted (29-31) it would appear that, at the time when there are a number of CFU-S in the thymus, they could well function as thymocyte precursors. Galili et al (1980) (118) found a high proportion of prothymocytes in the human fetal thymus, but less than 1% in the postnatal thymus.

CONCLUDING REMARKS

In this address I have been able to touch on only a few of the cellular migration streams in the lymphomyeloid complex. Because of limitations of time, no mention has been made of the afferent and efferent migration streams connected with the spleen, or of the various lymphoepithelial tissues. Furthermore, in addition to those migration streams which we already recognize, there may well be many others of which we are as yet unaware.

In making these comments, I feel very much like the great German pathologist, Felix Marchand. At a meeting of the German Pathological Association in 1898, Marchand demonstrated some preparations which were seen by Virchow, who said: "I

realize now I shall not live to see the leucocyte problem solved". This seems to have spurred Marchand to a period of intensive research, and 15 years later he gave a lengthy communication (119) to the German Pathological Association on: "The origin and role of lymphocytes in inflammation". Marchand fully realized, even on the basis of what little was then known about lymphocytes, that there were many gaps in our knowledge, and he reacted in the same way as Virchow. "We too shall not live to see the solution of this problem, though we can affirm that the investigations of the last two decades have greatly clarified our knowledge of these matters. But as new results are obtained, new questions arise. I have hardly been able to do more than formulate some of these questions."

Thus Marchand in 1913. We, more than 70 years later, can but re-echo his sentiments. For although we now know a good deal more than Marchand did about lymphocytes — and related cells — we cannot but agree wholeheartedly that "as new results are obtained, new questions arise." Among these new questions are the identity and extent of the cellular migration streams, as well as the factors which control them.

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