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CONCEPT OF HUMAN STEM CELL KINETICS*

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Running Head: Concept of Human Stem Cell Kinetics

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Introduction:

The concept of a stem cell for hemopoiesis goes back to at least the turn of the century. Maximow and Bloom⁽¹⁾ considered hemopoiesis to be divided into homoplastic hemopoiesis--the production of mature cells by young elements of the same type. Under physiologic conditions, the needs of the adult organism are supplied, usually by homoplastic hemopoiesis. When the requirements for hemopoiesis are increased after hemorrhage, during infection and regeneration from injury, homoplastic hemopoiesis does not suffice and new erythroblasts and myelocytes develop from a common stem cell and this is called heteroplastic hemopoiesis. To modern techniques for study of the stem cell and its properties, add new parlance and the stem cells are now divided into the pluripotent and committed stem cells⁽²⁾. Almost all of the information on the stem cells derives from studies on mice.

The pluripotent hemopoietic stem cell (HSC) is the reserve precursor of at least the erythrocytic, granulocytic, and megakaryocytic committed stem cells. In the mouse the HSC is characterized by studying the colony formation in the spleen of mouse. From the elegant studies initiated by the Canadian group of Siminovitch, Till, and McCulloch⁽³⁻⁵⁾ confirmed by numerous investigators, the colony forming cell spleen (CFC_s) in the bone marrow has the following characteristics:

1) About 10-15 colonies are formed in the spleen per 10^5 bone marrow cells injected. With a seeding factor of about 0.17⁽⁶⁾ and rounding off, the abundance of CFC_s in murine bone marrow is circa 1 per 1000.

2) "Thymidine suicide" studies show that the fraction of CFC_s varies among strains of mice from nearly 0 to 20%. Therefore nearly 0-20% are

in DNA synthesis and 80-100% are in G_0 ^(6a).

3) From early studies of Bond et al. ⁽⁷⁾ and Brecher and Cronkite ⁽⁸⁾ the pluripotent stem cell migrates through the blood and in fact has been isolated from the blood.

4) The morphologic identity is unclear. Its morphology may depend on its functional state (G_0 or in cycle). It has been described as a "small lymphocyte" ⁽⁹⁾, a monocytoid ⁽¹⁰⁾ transitional cell ^(10a).

It is generally considered that the erythroid committed stem cell is the erythropoietin responsive cell. Furthermore the in vitro blood and bone marrow culture systems are believed to measure the abundance of the erythroid and granuloid committed stem cells (CSC) since both erythrocytic and granulocytic colonies are grown and enumerated in culture. The cell producing colonies in culture is called the colony forming cell (CFC_c).

The CFC_c has the following characteristics:

a) In the mouse--

- 1) Abundance in blood of about 1 per 10^4 , and in bone marrow of 2 per 1000 bone marrow cells ⁽¹¹⁾
- 2) Thymidine suicide of about 0.35 in normal mice and 0.80 in mice with regenerating marrow ⁽¹¹⁻¹³⁾

b) In man--

- 1) Abundance of about 0.1 to 1×10^4 blood cells and 0.1 to 1×10^3 bone marrow cells ⁽¹⁴⁾
- 2) Thymidine suicide of 0.35 ⁽¹⁵⁾

The preceding numbers are rounded off from published papers.

Study of the human stem cell is very difficult. Human cells do not produce spleen colonies. It has been suggested by several investigators

that the colonies formed in culture of human peripheral blood or bone marrow by CFC₀ are CSC since they produce differentiated cells (erythrocytic, granulocytic, and macrophage colonies).

The organizing chairman of this symposium specifically asked for some information on the radiosensitivity of the stem cell and whether there are any new techniques for its study. My colleagues have produced data that bear on these subjects.

D₀ of Human Stem Cells:

The D₀ of murine CFU₀ is circa 95 rads⁽¹⁴⁾. However, the D₀ varies from 100 rad for bone marrow and 140 rad for blood cells^(14a). The D₀ of human CFC's in culture is circa 137 rads and mouse CFC's circa 160 rads⁽¹⁴⁾. Another approach to the measurement of human stem cell D₀ is the irradiation of human bone marrow and its growth in diffusion chambers implanted in the murine peritoneal cavity⁽¹⁶⁾.

Details of this technique will not be discussed since all are published. Briefly, one makes dilutions of bone marrow containing fewer and fewer cells. Graded number of cells are placed in diffusion chambers and implanted into the peritoneal cavity of the mouse. After a stated number of days the diffusion chambers are removed, the cells harvested from the chamber, counted, smeared on slides and characterized morphologically. By irradiating the host mice containing human bone marrow in diffusion chambers and measuring cell recovery, one can establish a radiation dose survival curve for human granulocytic stem cells. Carsten et al.⁽¹⁶⁾ have investigated the D₀ of human stem cells by this technique. Figure 1 shows three studies of radiation of human granulocytic stem cells. The D₀ is 72.6 rads.

A New Approach to Study of Stem Cells:

The existing techniques for study of stem cells have yielded a large mass of data and considerable insight into the PHSC and CSC in the mouse. An innovative approach for stem cell study has been introduced by Reincke et al. (17). This approach avails itself of the low energy and short range of Auger electrons from ^{55}Fe . By administering enough ^{55}Fe one can deliver a lethal intracellular dose of radiation to iron incorporating erythropoietic precursors. The selective intramedullary death is demonstrated by absolute dose dependent diminution of erythropoietic cells in the bone marrow and dose dependent decrease in the subsequent incorporation of ^{59}Fe into circulating red blood cells. This dose dependent hypoplasia of differentiated erythropoiesis makes possible the study of potential feedback loops from the differentiated marrow compartment to the erythroid CSC and to the PHSC. The ^{55}Fe erythropoietic cytocide continues because of reutilization of ^{55}Fe . This cytocide is followed by a prompt decrease in the number of CFU_E in the bone marrow of the ^{55}Fe treated mice and a significant decrease in the capability of this marrow to protect against lethal irradiation injury of other mice, a measure of the number of pluripotent stem cells in the bone marrow.

It can be shown that the dose to other cells from ^{55}Fe in erythropoietic precursors is miniscule compared to the dose to the differentiated red cell precursors. At this moment one cannot eliminate the possibility that sufficient ^{55}Fe is incorporated into the PHSC to cause the CFU_E reduction. It is unlikely. It is tentatively concluded that the pluripotent stem cell pool was depleted because of an increased demand for differentiation along the erythropoietic pathway. This approach has

considerable promise in testing the capability of the PHSC to undergo sustained mitotic activity by continual drainage down the erythropoietic pathway and to search for intramedullary feedback loops regulating erythropoiesis and the relationship of erythropoiesis to granulopoiesis. One will also in principle be able to test whether Hayflick's hypothesis applies to PHSC since the latter will be forced to divide at a maximum rate for prolonged periods. This is a clinically important subject since repetitive courses of chemotherapy for leukemia destroys PHSC and leukemic cells in parallel and hemopoietic regeneration requires extensive proliferation from a few surviving PHSC. If Hayflick's hypothesis applies to the PHSC one might expect marrow hypoplasia to take place at a later date after cessation of chemotherapy.

Characterization of Human Stem Cell Pools from Known Kinetics of Erythro- and Granulopoiesis:

Another approach to study the human stem cell pool is to start from the peripheral blood where the turnover rates of red cells and granulocytes are well known. From the sum of these turnover rates, the structure of human bone marrow (amplification from stem cell to non-dividing cell), absolute cellularity, DNA synthesis time and fraction of cells in DNA synthesis, one can estimate the minimum flux of CSC into the erythrocytic and granulocytic differentiated pools in the marrow. The data from which the calculations will be made are reviewed⁽¹³⁾ and summarized here:

- 1) Red cell average life span 120 days
- 2) Red cell turnover rate (RTR) $12 \times 10^7/\text{kg/hr}$
 $(288 \times 10^7/\text{day})^{(18)}$
- 3) Granulocyte life span (random loss) half time of 6.8 hrs⁽¹⁹⁾

- 4) Granulocyte turnover rate (GTR) $6.8 \times 10^7/\text{kg/hr}$
($163.2 \times 10^7/\text{day}$)⁽¹⁹⁾
- 5) RTR + GTR equals $18.8 \times 10^7/\text{kg/hr}$
- 6) Erythroid marrow cellularity (N^E) equals $536 \times 10^7/\text{kg}$ ⁽²⁰⁾
- 7) Granulocytic marrow (N^G) $1140 \times 10^7/\text{kg}$ ⁽²⁰⁾
- 8) $N^E + N^G = 1676 \times 10^7/\text{kg}$ ⁽²⁰⁾
- 9) DNA synthesis time in human bone marrow is about 12 hrs for erythrocytic and granulocytic proliferating pool⁽²¹⁾
- 10) Amplification from the committed stem cell to the non-dividing erythrocytic and granulocytic cell averages 16 ⁽¹⁸⁾

The application of the above data to estimate the number of CSC in DNA synthesis, the CSC pool size and its potential relationship to a PHSC pool is shown in Figure 2.

If one assumes that detection of the most immature erythrocytic and granulocytic precursor establishes a cytologic boundary between the CSC pool, one can then estimate the flux of CSC into the differentiated cell pool (red and white) by dividing the RTR + GTR (K_{out} of marrow) by the average amplification of 16.

$$K_{\text{out}} = 18.8 \times 10^7/\text{kg/hr}$$

hence K_{in} from CSC pool = $1.17 \times 10^7/\text{kg/hr}$.

In a stem cell pool the birth rate (K_B) is twice the K_{out} ($1.17 \times 10^7/\text{kg/hr}$ or $2.35 \times 10^7/\text{kg/hr}$). From K_B and DNA synthesis time (t_g) one can calculate the number of cells in DNA synthesis (N_g^{CSC}). Since most human cells have a DNA synthesis time close to 12 hours, we assume this applies to CSC.

$$\begin{aligned}
 N_s^{CSC} &= t_s \times K_B \\
 &= 12 \times 2.34 \times 10^7 = 28 \times 10^7/\text{kg}
 \end{aligned}$$

The total population of CSC (N^{CSC}) is obviously larger than the N_s^{CSC} . Assume that thymidine suicide of 0.35 for colony forming cells pertains to the total CSC pool. Then

$$N^{CSC} = \frac{28 \times 10^7}{0.35} = 80 \times 10^7/\text{kg}$$

Accepting this as the absolute value for CSC the ratio of

$$N^{CSC} + N^E + N^G = 80 + 1676 = \frac{1}{21} \quad \text{or there is}$$

1 CSC to every 21 erythroid-granuloid cells.

Total bone marrow cellularity in man has been experimentally determined to be $1800 \times 10^7/\text{kg}$ (22). Another approach to estimating the total bone marrow cellularity is from the mass of the red bone marrow of 1.3 kg in standard man and assuming an average volume for bone marrow cells of 1000×10^{-12} ml giving $2000 \times 10^7/\text{kg}$. However, for this calculation Donohue's determined number is used. $N^E + N^G$ is 1676×10^7 , hence other cells constitute $124 \times 10^7/\text{kg}$ or 7% of the marrow. If $80 \times 10^7/\text{kg}$ is the true value for CSC then 4.4% of total marrow is CSC and over half of the non-erythroid non-granuloid cells are CSC. If true, we have all seen but didn't recognize what we saw.

Are these estimates valid? Does a thymidine suicide of 0.35 or 0.35 of the CSC in DNA synthesis apply? If the fraction in DNA synthesis is smaller, then the fraction of the total marrow occupied by CSC becomes larger. If the time for DNA synthesis is overestimated the N_s^{CSC} is overestimated. If the DNA synthesis time is really less than the measured

time for differentiated marrow cells the estimate of N_s^{CSC} is too high. Even reducing t_s to a probably unrealistically low value of 1 hour for man reduces N_s^{CSC} to $6.6 \times 10^7/\text{kg}$ or 1 in 300 marrow cells are CSC. The above line of logic and assumption leads to a ratio of CSC to total marrow cells totally incompatible with the in vitro bone marrow culture of 5 per 10^4 colony forming cells in human bone marrow.

If the abundance of CFC_c in bone marrow represents the fraction of granulocytic CSC in bone marrow one can then calculate the influx of CSC into the granulopoietic pool from the following data:

- 1) Abundance of CFC_c 5 per 10^4 bone marrow cells (A)
- 2) Thymidine suicide for CFC_c 0.35 (I_L)
- 3) DNA synthesis time of 12 hours (t_s)
- 4) GTR $6.8 \times 10^7/\text{kg/hr}$
- 5) Absolute marrow cellularity of $1800 \times 10^7/\text{kg}$

The number of granulocytic CSC (N^{CSC}) is

$$\begin{aligned} N^{CSC} &= N \times A = 1800 \times 10^7 \times 0.0005 \\ &= 0.9 \times 10^7/\text{kg}. \end{aligned}$$

The number of granulocytic CSC in DNA synthesis (N_s^{CSC}) is

$$N_s^{CSC} = N^{CSC} \times I_L = 0.9 \times 10^7 \times 0.35 = 0.315 \times 10^7/\text{kg}.$$

The birth rate (K_B) of granulocytic CSC is

$$K_B = \frac{N_s^{CSC}}{t_s} = \frac{0.315 \times 10^7}{12} = 0.026 \times 10^7/\text{kg/hr}.$$

To maintain steady state conditions the flux out of the CSC pool is the sum of influx and half of the K_B or a minimum of $0.013 \times 10^7/\text{kg/hr}$

assuming influx is zero.

The amplification is the ratio of the efflux from the granulocytic CSC pool to the GTR or

$$6.8 \times 10^7 \div 0.013 \times 10^7 = 520.$$

Such an amplification would require about nine serial mitoses in the proliferating granulocytic pool. It is reasonably well established that the time from the myeloblast to the metamyelocyte is 130 hours⁽¹⁸⁾. If there is equal time for each successive multiplicative cell cycle there would be a generation time of 14 hours. If mitotic time is 0.75 hours the mitotic index would be about 5% or 5 times that observed by Killman et al.⁽²³⁾. With a DNA synthesis time of 12 hours⁽²¹⁾ the fraction granulocytic proliferating cells in DNA synthesis would be 12/14 or 0.85 compared to the observations in man of 0.15-0.30⁽¹⁸⁾. The latter fraction is compatible with the number of myelocytes having a diploid DNA content. From these lines of reasoning and calculations based on experimentally determined data, the notion of nine serial mitoses in the proliferating granulocytic pool must be rejected along with the notion that the in vitro culture of bone marrow determines the fraction of CSC in human bone marrow. Although murine data for RTR and GTR are not as good as human data, similar calculations lead to similar conclusions on the mouse.

For the sake of argument, let's assume that human marrow contains one pluripotent stem cell (PHSC) per 1000 bone marrow cells. Then there are 2×10^7 /kg. Feinendegen⁽¹⁸⁾ has shown that there is a slowly turning over compartment of mouse bone marrow, presumably PHSC with turnover time of about 33 days. Cell cycle time in man is 2-3 times longer than in the

mouse so an upper limit of PHSC turnover time in man may be 100 days and 1% per day go through cycle. DNA synthesis time is assumed to be 0.5 day (12 hrs).

$$\text{hence } N_s = \frac{N^{\text{PHSC}} \times t_s}{100} = 10^5/\text{kg}$$

$$K_B = \frac{N_s}{t_s} = \frac{10^5}{12} = 0.08 \times 10^5/\text{kg/hr}$$

and the flux out is half the K_B to maintain steady state size of PHSC pool. The steady state flux from PHSC to CSC is $0.04 \times 10^5/\text{kg/hr}$ or about 2900 cells leave the CSC pool for every cell that enters it from PHSC pool under steady state conditions.

Conclusion:

Until one can refute the logic of this approach or find a method of directly assaying the PHSC and CSC in man, one must doubt the in vitro bone marrow culture as an estimate of the abundance of CSC in human marrow.

Figure 1. The survival curve of human "stem cells" in irradiated bone marrow measured by growth of granulocytic cells in diffusion chamber. The growth probably represents proliferation of pluripotent hemopoietic, committed stem cells and their amplification into recognizable granulocytic series.

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FIGURE 1

Figure 2 A concept of human bone marrow cell proliferation constructed from the known erythrocytic and granulocytic turnover rate in human blood, absolute marrow cellularity, proportional cell distribution, and flow rates through the differentiated erythrocytic and granulocytic compartments.

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STRUCTURE OF HUMAN BONE MARROW HEMOPOIETIC CELL PROLIFERATION

$$(N^{PHSC} + N^{CSC} + N^E + N^G + \text{ALL OTHER CELLS} = 2000 \times 10^7/\text{kg})$$

FIGURE 2

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