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Determination of the Probability of Self-Renewal in Haemopoietic Stem Cells: A Puzzle

R. SCHOFIELD and L. LAJTHA

Probability of Self-Renewal: Assumptions and Limitations

A Commentary

H.E. WICHMANN and M. LOEFFLER

ABSTRACT. **The probability of self-renewal of stem cells cannot be measured directly. However, for the growth of haemopoietic stem cells in spleen colonies of mice it can be estimated in two different ways. The assumptions and limitations of these two methods have been investigated for the following conditions: a) heterogeneous populations, b) settling of additional stem cells in existing colonies, c) removal of stem cells from spleen colonies. This analysis has been stimulated by a paper by Schofield and Lajtha (1983) in which puzzling discrepancies between the estimated self-renewal probabilities have been presented. Possible solutions to their 'puzzle' are discussed.**

KEY WORDS: **Haemopoiesis – Stem cells – Self-renewal – Mathematical analysis**

INTRODUCTION

The probability of self-renewal is a very fundamental property of stem cells. It corresponds to the fraction of daughters of stem cells which remain stem cells for the next cell cycle. Unfortunately, it cannot be measured directly, and only in few situations can it be estimated.

For exponentially growing colonies of haemopoietic stem cells in the spleen of mice (Colony forming Units of the spleen, CFU-S) two estimates of the 'true' self-renewal probability are available. One uses the

Medizinische Universitätsklinik Köln, Jos. Stelzmann Str. 9, D-5000 Köln 41, FRG

slope of the growth curve, the second considers the mean and variance of the number of CFU-S/colony.

Schofield and Lajtha (1983) have collected several examples in which both estimates are different although they should be equal. This puzzle has stimulated our investigation.

THE PROBABILITY OF SELF-RENEWAL AND ITS ESTIMATES

In the following, p denotes the 'true' self-renewal probability of stem cells in spleen colonies, p_S denotes its estimate from the slope of exponential growth curves and p_V denotes its estimate according to the approach of Vogel et al. (1968). The mathematical relations between these values are investigated in the first three sections. The reader who is mainly interested in the biological consequences might omit this part and continue with section IV where the applications are discussed.

I. The 'True' p

If asymmetric divisions are excluded, one finds after mitosis of a stem cell with the 'true' self-renewal probability p either no stem cell (with the probability $1-p$) or 2 stem cells (with the probability p). In the next generation these 2 stem cells result in either no stem cells (with $(1-p)^2$) or 2 stem cells (with $2\,p(1-p)$) or 4 stem cells (with p^2). If this procedure is continued one ends up with the recursion formula

$$P_n(2k) = \sum_{\substack{m \geq k \\ m \text{ even}}}^{2^{n-1}} P_{n-1}(m) \binom{m}{k} (1-p)^{m-k} p^k$$
$$P_1(0) = 1-p, \qquad P_1(2) = p. \tag{1}$$

where $P_n(2k)$ is the probability of finding $2k$ stem cells in the n-th generation. This formula has been given by Vogel et al. (1969, page 252) for colonies which have developed from single stem cells with constant p. However, it can easily be generalized for p varying with time, for heterogeneous subpopulations with respect to p or for migration of stem cells to or from colonies. For all these cases it is possible to calculate the mean number M_n of stem cells per colony in the n-th generation according to

$$M_n = \sum_{\substack{m=0 \\ \text{even}}}^{2^n} m \times P_n(m). \tag{2}$$

If p is greater than 0.5, M_n increases from one generation to the next. The growth curve is exponential for homogeneous stem cells with a constant p but otherwise might have a different shape.

Furthermore, from formulae (1) and (2) one finds the coefficient of variation V_n by

$$V_n^2 = \sum_{\substack{m=0 \\ \text{even}}}^{2^n} (m - M_n)^2 \times P_n(m)/M_n. \tag{3}$$

If one is used to thinking in units of time rather than in generations, one might substitute n by t/T_C where t is the time and T_C is the cell cycle time. Schofield and Lajtha (1983) assume that T_C is approximately 6 h.

II. Slope of Growth Curves (p_S)

If the 'true' p cannot be measured, indirect information must be used to estimate the probability of self-renewal. This information is available in exponentially growing colonies. Here one finds for the mean number of stem cells in the n-th generation (Lajtha et al., 1971)

$$M_n = M_0(2p_S)^n. \tag{4}$$

M_0 is the mean number in the 0-th generation and p_S represents the estimate of p from the slope which equals $\ln(2p_S)$. For a homogeneous population with constant p, $p_S = p$. However, in situations where p is variable it is not clear what p_S really measures.

III. The Approach of Vogel et al. (p_V)

Vogel et al. (1968, 1969) and Matioli et al. (1968) have proposed a second estimate of the self-renewal probability, which in the following shall be denoted by p_V. The calculation of p_V is based on the mean M_n and the coefficient of variation V_n of the number of CFU-S, determined after n generations. The method can be applied either in spleens or in isolated spleen colonies but not in the bone marrow.

For sufficiently large spleen colonies (more than 15 generations, p greater than or equal to 0.55) they find

$$V_n^2 = \frac{2 - 2p_V}{2p_V - 1} + \frac{1}{M_n}, \tag{5}$$

and for suspensions of total spleens

$$V_n^2 = \frac{1}{C(2p_V - 1)} + \frac{1}{M_n}. \tag{6}$$

Here C is the average number of colonies per spleen. If a homogeneous stem cell population with a constant self-renewal probability is given,

p_V also represents the 'true' p: $p_V = p$. However, if not, it is again unclear what p_V measures.

IV. Simulation of Colony Growth if the 'True' p is Known

The estimates p_S and p_V have been developed because p is not measurable. The values of p_S and p_V are equal to p only in homogeneous stem cell populations with a constant self-renewal probability. However, it is not clear what p_S and p_V measure if this condition does not hold. In this situation the mathematician is in a better position than the experimentalist. He can pretend to know the 'true' p and use it for his calculations. Thus it is possible to simulate different hypotheses about stem cell properties in the early phase after settling in the spleen and to determine the consequences of these hypotheses on p_S and p_V. This has been done for some of the most frequent speculations on stem cell behaviour at the beginning of colony growth (see below).

For these simulations, specific assumptions about the 'true' p are made and the mean number M_n of stem cells in the n-th generation and the coefficient of variation V_n in this generation are calculated. From these values p_S and p_V can be derived using formulae (4) and (5).

In the following, p_V is evaluated in the 20th generation and p_S is calculated from the slope between the 19th and 20th generation. The choice of 20 generations is arbitrary and has no influence on the results which are presented in Figure 1 and Table 1. These results are stable after more than 15 generations. The following conclusion can be drawn:

1) For a homogeneous stem cell population, both p_V and p_S are equal to the 'true' p.

2) For a heterogeneous population, we find that p_S represents the maximum p. This follows because after 20 generations the most rapidly growing subpopulation contributes the majority of stem cells. On the other hand, the calculations show that p_V represents the minimum p in the population. This can be understood if one realizes that in a mixed population the total coefficient of variation is mainly determined by the subpopulation with the maximum variability, and that is the subpopulation with the minimum p.

In total, p_V represents the subpopulation with the minimum p, and p_S represents the subpopulation with the maximum p. Both cover the range of the 'true' self-renewal probabilities in a heterogeneous population.

3) If there is an initial removal of stem cells from the spleen colony, this can be represented mathematically by a lower p for the first generations. The initially lower p leads to a delay in the growth curve but has no influence on p_S since the slope after 20 generations is determined by the p value which is effective at that time. However, p_V is significantly reduced by the initial cell removal. As shown in Table 1, a

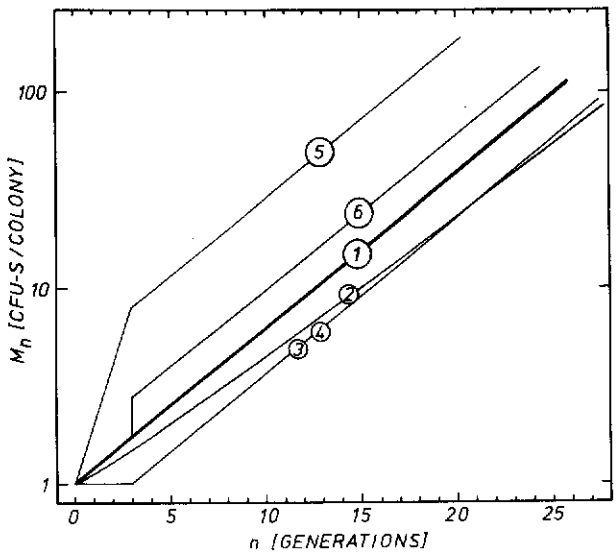

Fig. 1. Theoretical growth curves of CFU-S in spleen colonies for different self-renewal probabilities. Explanation of the symbols in Table 1

Table 1. Influence of different hypotheses on the 'true' self-renewal probability p on the estimates p_V and p_S

Stem cells	p	p_V[a]	p_S[a]
1.[b] Homogeneous population	0.6	0.602	0.600
	0.7	0.700	0.700
2. Heterogeneous subpopulations	0.55/0.6	0.560	0.592
	0.6/0.7	0.612	0.693
3. Initial cell removal from the colony	0.6 thereafter	0.564	0.600
($p=0.5$ for 3 generations)	0.7 thereafter	0.591	0.700
4. Initial delay in commencement of growth (3 generations)	0.6	0.602	0.600
	0.7	0.700	0.700
5. Initially higher self-renewal	0.6 thereafter	0.838	0.600
($p=1$ for 3 generations)	0.7 thereafter	0.922	0.700
6. Multiple settling (a second stem cell with the same p settles after 3 generations in an already existing colony)	0.6	0.599	0.600
	0.7	0.681	0.700

[a] M_n and V_n are calculated from formulae (1)–(3), p_V from Vogel's formula (5) for $n=20$ generations and p_S from the slope between generations 19 and 20 according to formula (4)

[b] The numbers refer to the curves in Figure 1

removal of 20% of the stem cells from the colony for the first 3 generations ($p=0.5$ initially and $p=0.7$ thereafter) leads to $p_V=0.59$ although p has been 0.7 nearly all the time. Thus, p_V represents not the actual p at the time of measurement but includes the history of p during colony growth.

4) If there is an initial delay before the stem cells start to divide, this leads to a shift of the growth curves. However, neither p_S or p_V are affected since this delay reduces only the number of generations.

5) If the self-renewal probability p is initially enlarged, this influences p_V and the growth curve both of which are enlarged. p_S is not affected by the initial behaviour since the slope reflects the later value of p. This situation is the reverse of 3.

6) If a colony is not derived from a single stem cell but additional circulating stem cells settle in existing colonies, this also influences the growth curve. As shown in Figure 1, a settling of a new cell leads to a higher growth curve but the slope and thus p_S is unchanged. Interestingly p_V is also unchanged as long as the new settled stem cell has the same 'true' p as the other stem cells in the colony.

In summary, the early behaviour of the self-renewal probability p has no influence on p_S. p_S represents the value of p at the time of measurement and that is after about 20 generations. In all cases discussed above, the growth curves are parallel after 15 generations and thus p_S is equal.

On the other hand, p_V does not only represent the actual value but also the history of p. The initial behaviour during the first few generations has a particularly severe impact on p_V. Two types of influences are found: Either p_V represents the p for late times (and thus p_S, situations 4 and 6) or secondly p_V reflects the shift of the growth curves (situations 2, 3, 5). In the latter cases, p_V is smaller than p_S if the growth curves are below the standard curve 1 and p_V is enlarged if the growth curves are above curve 1.

DISCUSSION

The essential point in the paper by Schofield and Lajtha (1983) is that for parallel growth curves of stem cells (which correspond to equal p_S) different values for p_V have been found. This 'puzzle' cannot be understood if the stem cells under consideration have the same 'true' p throughout colony growth and if each colony is derived from a single CFU-S. If that were the case, one would expect p_S and p_V to be equal (and equal to p), which is not so.

The authors present four examples. In their first example they consider bone marrow recovery. Since Vogel's formulae are only applicable for spleens or spleen colonies this example shall be neglected here.

In their second example, Schofield and Lajtha show two parallel growth curves of CFU-S in spleens of serivally transplanted CFU-S.

The curves are parallel and the curve after four transfers is below that after the first transfer. In addition, after four transfers p_V is below 0.62, while $p_V = 0.66$ after one transfer.

This example might correspond to the situations 1 and 2 in Table 1 and Figure 1. If the CFU-S population is homogeneous after one transfer (situation 1) but becomes heterogeneous due to damage by serial transplantation (situation 2) the theoretical results are quite similar to the experimental findings: (a) The growth curves are parallel, (b) the curve of the heterogeneous population is below that of the homogeneous one and (c) the value of p_V in the heterogeneous population is significantly smaller than in the homogeneous population (0.61 compared to 0.7 or 0.56 compared to 0.6, Table 1). In total, if one speculates that serial transplantation produces subpopulations with decreased self-renewal capacity, p_S measures the higher p of the undamaged population while p_V represents the p of the subpopulation with the lowest self-renewal probability.

As a third example Schofield and Lajtha consider drug-induced stem cell damage. They show that a longer exposure of stem cells to isopropylmethane sulphonate (IMS) before grafting leads to parallel but delayed growth curves. The corresponding values of p_V are 0.56 after 24 h and 0.59 after 2 h exposure.

Here the same hypothesis as before might be discussed. If the drug damages not all but only some stem cells such that their self-renewal properties are reduced, one would find subpopulations with normal p values and others with smaller p values. Again, p_S would measure the normal p and p_V would measure the self-renewal probability of the most severely damaged subpopulation. One would find theoretically that (a) the growth curves are parallel, (b) the curve after a long exposure to the drug is below that after a short exposure and (c) the value of p_V after a long exposure is smaller than after a short exposure. This again corresponds to the experimental results.

In their final example, Schofield and Lajtha compare the effect of different inoculum sizes of normal bone marrow cells on spleen colony growth. They find parallel growth curves. The smaller inoculum leads to a shift of the curve, and a smaller p_V (0.59 compared with 0.69) is measured in this case.

Before considering this puzzling example, we would like to discuss a different problem which in part can be answered from Table 1 and Figure 1: What happens to spleen colony growth in lethally irradiated recipients if the CFU-S in bone marrow and spleen are being constantly exchanged via the circulation? The removal of stem cells from the spleen corresponds to situation 3 and leads to a delay in the growth curve and a reduction in p_V. The settling of CFU-S in the spleen may occur in two ways. If the new cells settle in already existing colonies, situation 6 is found, if they settle separately we find situation 4 because this corresponds to a delayed growth. In both situations the growth curves are shifted but p_V is not affected.

In summary, the exchange of CFU-S between bone marrow and spleen leads to a parallel shift in the growth curve for CFU-S per colony. It remains unclear whether the shift will be to the left or to the right, since the relative amount of migration and settling is unknown. As a second consequence, p_V becomes smaller. This reduction is due to migration, since settling in the spleen has no influence on p_V.

Now let us consider two cases, one in which settling and migration is at a low rate and a second in which settling is at a low rate but migration is at a high rate. If settling and migration are low, colony growth will not be influenced very much. Therefore, we might relate this to situation 1 in Table 1. If settling is low and migration is high one might neglect settling and consider only migration. This would correspond to situation 3 in Table 1. Comparing situations 1 and 3 one finds (a) parallel curves, (b) a lower growth curve for the high migration rate and (c) a small value of p_V for high migration (0.59 compared with 0.7 or 0.56 compared with 0.6, Table 1).

This result is quite similar to the findings of Schofield and Lajtha (1983) in their final example and suggests the following hypothesis: For a large inoculum the exchange of CFU-S between bone marrow and spleen is low. For a small inoculum where the number of CFU-S transferred is small the repopulation of the bone marrow is delayed. Therefore, a significant removal of CFU-S from the spleen to the bone marrow might occur until bone marrow has sufficiently recovered. This initial removal of CFU-S from the colony would be responsible for a shift of the growth curve (Fig. 1) and a reduced p_V (Table 1).

Although these comments on the 'puzzle' of Schofield and Lajtha are self-consistent they obviously are speculative. Therefore, they cannot be interpreted as answers, but only as stimulation for further discussion and specific experimental investigations. However, the conjecture of Schofield and Lajtha (1983) that p_S and p_V may measure different stem cell properties, is strongly supported by our calculations. Their measurements and our calculations suggest that there might be a heterogeneity of stem cells and complex interactions during the early phase of colony growth, depending on the experiments performed. Similar results have been found by David and MacWilliams (1978) for hydra stem cell colonies.

If this is true, p_S and p_V in fact measure different aspects of self-renewal and therefore are complementary. p_S would represent the 'true' p which is effective at the time of measurement while p_V would be an integrative measure of the total history of p. Only in the idealized case of a homogeneous stem cell population with constant p, would p_S and p_V be identical. In many realistic situations, this does not seem not to be the case.

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