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Dynamics of haemopoiesis across mammals

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Haemopoiesis is a fundamental physiologic process found in many animals. Among mammals, the diversity in size and function required suitable adaptations of this process. In this work, we use allometric principles to determine whether this required a change in the basic architecture of haemopoiesis. We show that it is possible to express both the number and rate with which haemopoietic stem cells replicate as well as total marrow output across all mammals as a function of adult mass. This unified view, which is compatible with the existing data, suggests that there was no need for major adaptations in the architecture of haemopoiesis across mammals.

Keywords: haemopoiesis; allometry; scaling; stem cells

1. INTRODUCTION

The emergence of large multicellular organisms required the development of systems for mass transport of oxygen and nutrients to cells far removed from exchange surfaces. The problem was solved by the evolution of the circulatory system and haemopoiesis. At the root of haemopoiesis, one finds haemopoietic stem cells (HSC), from which a hierarchy of cell types unfolds through several branches leading to progressively more committed cell lineages (Weissman *et al.* 2001; McCulloch & Till 2005; Dingli *et al.* 2007). Many biological observables related to the circulation (generally designated by Y) scale with the mass M of the organism (perhaps the simplest surrogate of an organism's complexity) as $Y = Y_0 M^a$, where Y_0 is a constant and the exponent a consistently being a multiple of 1/4 (Banavar *et al.* 1999). Recently, it has been found (Dingli & Pacheco 2006) that at least in mammals, the number of HSC (N_{SC}) that actively contribute to haemopoiesis scales allometrically with mass (M) with the same universal exponent (3/4) as the basal metabolic rate (BMR) of the organism (West *et al.* 1997; Banavar *et al.* 1999), i.e. $N_{SC} = N_0 M^{3/4}$. The 3/4 exponent has been interpreted as resulting from either hierarchical networks organized in a fractal-like manner so as to minimize energy loss (West *et al.* 1997) or directed networks organized to minimize flow (Banavar *et al.* 1999). More recently, it has been proposed that there may be an evolutionary drive towards the emergence of self-similar, fractal-like complex networks (Song *et al.* 2006).

The validity of the assumptions underlying the allometric scaling relationship obtained ultimately relies on the availability of experimental data. In this context, the recent data on baboons (*Papio* sp.) and macaques (*Macaca mulatta*), published by Shepherd *et al.* (2007), provide important additional information regarding the principles that regulate haemopoiesis across mammals. In the following, we provide a unifying framework that captures the essential features of haemopoiesis from HSC to circulating blood across all mammals.

2. MATERIAL AND METHODS

For the purpose of this analysis, mammalian species are characterized by their average adult mass M. The size of their active stem cell pool, N_{SC} is assumed constant in time and given by $N_{SC}(M) = N_0 M^{3/4}$ ($N_0 \approx 15.9\ \mathrm{kg}^{-3/4}$; Dingli & Pacheco 2006). We note that during ontogenic growth, $N_{SC}(M)$ also scales allometrically (Dingli & Pacheco 2007). In each species, HSC replicate at a rate $R(M)$ given by $R(M) = R_0 M^{-1/4}$ ($R_0 \approx 2.9\ \mathrm{kg}^{1/4}\ \mathrm{yr}^{-1}$; Rufer *et al.* 1999; Dingli & Pacheco 2006).

Data for mammalian lifespan for a large number of species (http://www.centralpets.com/pages/mammals/other_exotics.html) were fitted to the empirical function $L(M) = L_0 M^{1/4}$ (figure 1*b*) to obtain $L_0 \approx 8.6\ \mathrm{kg}^{-(1/4)}\ \mathrm{yr}$ (Lopes *et al.* 2007). We assume that the *fundamental* architecture of haemopoiesis remains unchanged across mammalian species and consider that haemopoiesis is composed of a total of $K = 32$ different stages of cell replication/differentiation in mammals. These stages or 'compartments' should not be considered as discrete in space but more as a convenient way to account for the number of cell divisions that link HSC with the circulating blood. Thus, a cell may divide and move from compartment k to $k+1$ and functionally still be the same (e.g. myeloblast). The size of each compartment k ($k = 1, \ldots, K = 32$) grows as $N(k) = N_{SC}(M)\gamma^k$ ($\gamma = 1.93$; Dingli *et al.* 2007) while the rate of replication in each compartment scales as $r(k) = R(M) r^k$ ($r = 1.26$; Dingli *et al.* 2007), such that the average time between cell divisions in each compartment is $\tau(k) = r(k)^{-1}$. In our model, short-term repopulating cells (STRC) are represented by cells in compartments $k = 1$–5 ($N_{STRC} \approx 1.2 \times 10^4$ cells for humans). With probability $\varepsilon \approx 0.85$, cell division leads to differentiation into the next compartment. In each of the k compartments, the average number of cell divisions is then given by $\sum_{j=0}^{\infty} \varepsilon \cdot (1-\varepsilon)^j \cdot (j+1) = 1/\varepsilon$. Hence, the average time a cell remains in compartment k is $\tau(k)/\varepsilon$ and the average time ($\tau_{av}(M)$) that an STRC contributes

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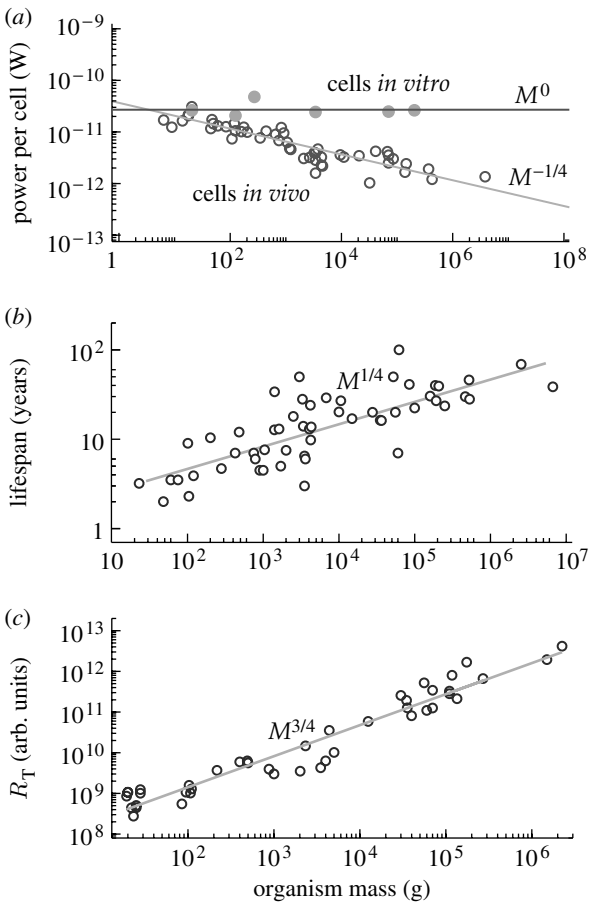

Figure 1. Allometric relationships between mammalian mass and several observables. (*a*) *In vitro*, cells isolated from different mammals have similar metabolic rates, but this is altered when studied *in vivo* (data digitized from West *et al.* 2002) where the BMR decreases with mass as $M^{-(1/4)}$. (*b*) $M^{1/4}$ scaling fit of data for the lifespan of mammals adapted from Lopes *et al.* (2007). (*c*) The total reticulocyte count (R_T) also scales with mass as $M^{3/4}$. As shown in Dingli & Pacheco (2006), the active stem cell pool scales in the same way with mammalian mass.

to haemopoiesis is given by Dingli *et al.* (2007)

$$\tau_{av}(M) = \frac{\sum_{k=1}^{5} N(k)\frac{\tau(k)}{\varepsilon}}{\sum_{k=1}^{5} N(k)} = \underbrace{\frac{1}{R_0 \cdot \varepsilon}\frac{\sum_{k=1}^{5}\left(\frac{\gamma}{r}\right)^k}{\sum_{k=1}^{5}\gamma^k}}_{A_{STRC}} \cdot M^{1/4} \qquad (A_{STRC} \approx 59 \text{ d kg}^{-1/4}).$$

In other words, the average time a cell remains in a given compartment, k, is obtained computationally by counting the total number of such cell divisions for the cell populations occupying the relevant compartments. Since the focus of this work is mainly in the STRC, we restrict the analysis to compartments 1–5, which represents the cell population in our model.

3. RESULTS AND DISCUSSION

A paradigmatic example of allometric scaling is the mass-specific BMR, which scales with mass as $B(M) = B_0 M^{-1/4}$ across 27 orders of magnitude (West *et al.* 2002; figure 1*a*). It appears that the BMR dictates the rate of replication of cells *in vivo* since the rate of HSC replication across various mammals scales in the same way with their adult mass (Dingli & Pacheco 2006). *In vitro*, the cells isolated from different mammalian species replicate at an approximately constant rate (figure 1*a*), providing compelling evidence that it is the organism that regulates the cell's mitotic clock (West *et al.* 2002). The inverse relationship between rate and time suggests that animal lifespan $L(M)$ also follows qualitatively a 1/4 scaling relationship (Lopes *et al.* 2007), as shown in figure 1*b*. On the other hand, the 3/4 scaling of the size of the active HSC pool follows from the assumptions that (i) each HSC is equally represented in the blood such that the scaling exponent of the active HSC pool should be identical to that of the reticulocyte count across adult mammalian species and (ii) the haemopoietic tree (Dingli *et al.* 2007) remains invariant across mammals.

Taken together, these scaling relationships suggest that HSC replicate faster in a mouse than in a cat or a human. Given the mass of non-human primates such as baboons ($m \approx 20$ kg) and macaques ($m \approx 6.5$ kg), allometric scaling predicts that their HSC replicate at rates intermediate between that of humans and smaller animals such as mice ($m \approx 25$ g) and cats ($m \approx 4$ kg): we obtain that HSC replicate, on average, once in every 29 weeks in macaques and once in every 36 weeks in baboons, in excellent agreement with the data reported by Shepherd *et al.* (2007; see also table 1).

From these allometric relationships, the total number of divisions (T) a typical HSC undergoes during the lifetime of the mammal in which it resides scales as $T \sim M^{-1/4} \cdot M^{1/4} \sim M^0$. Hence T becomes independent of mass, and the average number of replications of each HSC should remain approximately constant for all mammals and compatible with the Hayflick hypothesis of a limited number of divisions for a given cell (Hayflick & Moorhead 1961). This result has been recently proposed by Shepherd *et al.* (2007) based on their experimental data. Our scaling analysis provides a natural explanation for this finding. More generally, this behaviour derives from the principle that it is the organism (and its self-regulatory complexity) that regulates the rate of cell replication and not vice versa; otherwise it would be impossible to rescue a lethally irradiated mouse with human HSC: the *intrinsic* rate of replication of the human HSC would be too slow to allow haemopoietic reconstitution in the time frame necessary for the recovery of the mouse. Rather, the human HSC transplanted in the mouse will replicate at a rate dictated by the murine BMR. Allometric scaling also allows us to predict that the length of time that HSC contribute to haemopoiesis varies across species, following a 1/4 scaling relationship with mammalian mass. Indeed, since each cell roughly replicates the same number of times during the lifetime of the organism, the length of time will scale with the same power as the average lifespan, e.g. $M^{1/4}$.

We further investigated the robustness of the allometric predictions by combining them with our recently developed multi-compartment model of haemopoiesis in humans. In this model, cell division is associated with either differentiation or self-renewal (Dingli *et al.* 2007), along a cascade of progressive stages of cell commitment. Using this model in combination with the allometric scaling of N_{SC} (see §2), we determined the average daily

Table 1. Some haemopoiesis-related properties of mammalian species derived from the allometric scaling relationships studied in this work. (M, average mass of mammalian species; N_{SC}, size of the active stem cell pool; N_{SC}^{T}, size of the active stem cell pool contributing to haemopoiesis after bone marrow transplantation; $R(M)$, rate of replication of HSC; $\tau_{av}(M)$, average time STRC contribute to haemopoiesis; Ω, daily bone marrow output.)

property	*Mus musculus*	*Rattus norvegicus*	*Felis catus*	*Macaca mulatta*	*Canis familiaris*	*Papio* sp.	*Homo sapiens*	*Gorilla gorilla*	*Elephas maximus*
M (kg)	0.025	0.250	4.0	6.5	*12.5*	18.0	70.0	135.0	4500.0
N_{SC}	1	6	45	65	105	139	385	630	8736
N_{SC}^{T}	1	2	13	20	32	42	116	190	2634
$R(M)^{-1}$ (in weeks)	7[a]	13	25	29	34	37	52	61	147
$\tau_{av}(M)$ (in weeks)	3.3	5.9	11.9	13.4	15.8	17.3	24.3	28.7	68.8
Ω (cells)	$10^{8.91}$	$10^{9.66}$	$10^{10.6}$	$10^{10.72}$	$10^{10.93}$	$10^{11.05}$	$10^{11.49}$	$10^{11.71}$	$10^{12.85}$

[a]Data for mice, for which the active stem cell pool made up of a *single* HSC is the one that deviates most from available estimates (Spangrude *et al.* 1991). This is not surprising, given the average nature of the allometric scaling relationship, although it conforms with the notion that haemopoiesis in the mouse may not reflect that characteristic of larger mammals (Abkowitz *et al.* 1995).

marrow output for various species, together with the average time that STRC contribute to haemopoiesis. The results are presented in table 1, where a synopsis of HSC properties across mammals is provided. Our estimates show that the total marrow output produced by a mouse during its lifetime is similar to what a human produces in a day, or a cat in a week, in agreement with prior evidence (Abkowitz *et al.* 1995).

HSC are usually considered to be divided into two broad compartments: an active pool of cells that are contributing to haemopoiesis and a quiescent reserve (Phillips 1991). There is evidence that once an HSC is selected to the active pool, it may remain contributing to haemopoiesis for a long time (McKenzie *et al.* 2006). Our allometric scaling predicts the number of active HSC as a function of mammalian mass. As expected, the number of active HSC increases with mass, yet even for the largest land mammals (the Asian elephant), the number of active HSC is still below 10 000 (Dingli & Pacheco 2006) and in keeping with a recent proposal that the total number of HSC is conserved across mammals and may be as low as 10 000 cells (Abkowitz *et al.* 2002). Therefore, it appears that smaller mammals have a larger pool of reserve HSC. This is perhaps one reason why HSC from mice can be transplanted serially so many times without loss of self-renewal capability.

These results and the overall agreement with the data for HSC replication in non-human primates published by Shepherd *et al.* (2007) suggest that haemopoiesis is not *qualitatively* different across mammals. The number of active HSC and their rate of replication scale in relation to the mass of the host mammal to match the demands of the organism in which they reside. In this context, it is not necessary to propose differences in the number of stages of differentiation for different mammalian species. These observations on haemopoiesis are compatible with a central tenet in evolutionary biology that nature retains what is effective and adapts it to situations of higher complexity.

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