Therefore, it is likely that heme regulates its own synthesis by inhibiting the import of not only ALASE but also CPO. The mechanisms of this feedback inhibition of mitochondrial import of CPO by heme are not clear. The heme,[2] regulatory motif, which was identified in the presequence of ALASE and was shown to be involved in the feedback inhibition by heme,[2] was not identified in preCPO. Therefore, heme seems to regulate the mitochondrial import of CPO by the mechanisms that differ from that involved in ALASE. These results shed light on the field of clarifying the importance of heme on the regulation of the synthesis of CPO.

**Shinji Susa, Makoto Daimon, Hideyu Ono, Song Li, Tadashi Yoshida, and Takeo Kato**

*Correspondence: Makoto Daimon, The Third Department of Internal Medicine, Yamagata University School of Medicine, 2-2-2 Iida-Nishi, Yamagata 990-9585, Japan; e-mail: mdaimon@med.id.yamagata-u.ac.jp*

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# To the editor:

## Of mice and men . . . and elephants

The debate about hematopoietic stem cell numbers and productive capacity is relevant to many areas of clinical and experimental hematology. Recently, Abkowitz et al[1] reported that the total number of stem cells in mammals is conserved so that mice, cats, humans, and possibly elephants have the same-sized stem cell pools. They emphasized that, since humans live longer than mice and need to produce more blood cells, human stem cells must be capable of producing many more blood cells than murine stem cells. A corollary of this is that the frequency of stem cells in human bone marrow will be lower than the frequency in murine bone marrow. Since we have made similar measurements in mice and calculations in humans,[2,3] we were interested in determining whether our data are consistent with those of Abkowitz et al[1] and whether the calculated numbers of stem cells in humans can account for the daily requirement to replace mature blood cells lost through senescence.

Table 1 compares earlier data, obtained using $^{59}$Fe ferrokinetics to estimate marrow cellularity and the spleen colony (CFU-S) assay[4] to measure hematopoietic stem cells in 3 strains of mice,[2] with the values reported by Abkowitz et al.[1] Our data indicate slightly greater values than Abkowitz et al,[1] but they are of the same order of magnitude. Table 1 also shows data for the derived frequency of human hematopoietic stem cells, assuming that the human hematopoietic stem cell population is the same size as that of the mouse, which again is consistent with that reported by Abkowitz et al.[1]

**Table 1. Numbers and frequencies of hematopoietic stem cells (HSCs)**

| | Number | Frequency of HSCs |
|---|---|---|
| Mice | | |
| Abkowitz et al[1] | 11 200-22 400 HSCs/mouse* | 4-8 HSCs/$10^5$ BM cells |
| Coggle et al[2] | 36 000-125 000 HSCs/mouse† | 7-14 HSCs/$10^5$ BM cells |
| Humans | | |
| Abkowitz et al[1] | 11 200-22 400 HSCs/person | 0.7-1.5 HSCs/$10^8$ BM cells‡ |
| (mean) | (16 800 HSCs/person) | |
| Gordon et al[3] | 36 000-125 000 HSCs/person | 2.4-8.3 HSCs/$10^8$ BM cells‡ |
| (mean) | (81 000 HSCs/person) | |
| Elephants[8] | | |
| Abkowitz et al[1] | 11 200-22 400 HSCs/elephant | 0.8-1.5 HSCs/$10^{10}$ BM cells‡ |
| (Mean) | (16 800 HSCs/elephant) | |
| Gordon et al[3] | 36 000-125 000 HSCs/elephant | 2.4-8.3 HSCs/$10^{10}$ BM cells§ |
| (Mean) | (81 000 HSCs/elephant) | |

*Calculated from frequency by $\times$ 2.8 $\times$ $10^8$ BM cells/mouse.
†Calculated from frequency by $\times$ 5-9 $\times$ $10^8$ BM cells/mouse.
‡Calculated from number by $\div$ 1.5 $\times$ $10^{12}$ BM cells/person.
§Calculated from number by $\div$ 1.5 $\times$ $10^{14}$ BM cells per elephant, under assumption that elephants have the same number of stem cells as mice and humans. See Table 2.

Adult humans need to produce $1 \times 10^{11}$ neutrophils and $2 \times 10^{11}$ red blood cells per day throughout life. Assuming a lifespan of 80 years, this amounts to a total of $80 \times 365 \times 3 \times 10^{11}$ ($8.8 \times 10^{15}$) for these 2 lineages alone, which must be generated by 16 800 (Abkowitz et al[1]) or 81 000 (our data) stem cells. Assuming steady-state stem cell kinetics[5] and 100 stem cell replications per lifetime, stem cell division and self-renewal with a probability of 0.5 will result in $1.68 \times 10^6$ to $8.1 \times 10^6$ new stem cells available for differentiation and, ultimately, mature blood cell formation. Consequently, each of these stem cells must be responsible for producing $1.1 \times 10^9$ to $5.2 \times 10^9$ mature cells. It is noteworthy therefore that Brummendorf et al[6] reported that single stem cells generate, on average, nearly $10^9$ $CD34^+CD38^-$ cells in vitro and the greatest number observed was more than $10^{12}$. The corresponding stem cell frequency would be 1 in $5 \times 10^8$ to $10 \times 10^8$. As noted by Abkowitz et al,[1] these frequencies are much lower than the frequencies of candidate human stem cells measured in the NOD/SCID transplantation model.[7] Clearly, if stem cells did not self-renew, they would need to be capable of producing many more cells to sustain hematopoietic cell production throughout life. If this were the case, each of the 16 800 to 81 000 stem cells would be required to produce on average $1.1 \times 10^{11}$ to $5.2 \times 10^{11}$ mature cells and their frequencies would be commensurately 2 orders

**Table 2. Asian and African elephant characteristics**

| | Asian | African |
|---|---|---|
| Body weight, kg | 3000-4000 | 6000-7500 |
| Blood volume, L | 300-400 | 600-750 |
| White cell count, $\times$ $10^9$/L | 9.8-25.3 | 4.4-12.8 |
| Total white cells per elephant, $\times$ $10^{12}$ | 2.9-10.1 | 2.6-9.6 |

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of magnitude lower. Moreover, at such low frequencies a total marrow cellularity of $1.5 \times 10^{12}$ could accommodate only a very few stem cells.

Insufficient data are available to estimate the size of the stem cell pool and kinetic parameters in elephants accurately (Table 2). But simple calculations of the possible frequency and productive capacity of elephantine stem cells can be made. The data in Table 1 and Abkowitz et al[1] indicate that mice, cats, and humans have on the order of $1 \times 10^{10}$ to $2 \times 10^{10}$ bone marrow cells per kilogram of body weight. If the same is true of elephants, a large African elephant of 7 500 kg (Table 2) could have as many as $1.5 \times 10^{14}$ bone marrow cells. The corresponding frequencies of stem cells in such animals would be 1 to 8 per $10^{10}$ if all species have the same number.[1] Furthermore, elephants have many more blood cells than mice or humans (Table 1), up to $10 \times 10^{12}$. If red and white cell kinetics are similar in humans and elephants, who live about the same length of time, a large African elephant will need to produce $17.5 \times 10^{20}$ cells in a lifetime. This number corresponds to $2 \times 10^{14}$ to $10 \times 10^{14}$ cells per stem cell.

In summary, the experimental and theoretical estimates of stem cell pool size in humans are not inconsistent with lifelong requirements for blood cell production sustained by a self-renewing stem cell population. Nor do the values we have derived appear to be inconsistent with the idea that mice, cats, humans, and elephants could have similar numbers of stem cells.[1]

**Myrtle Y. Gordon, John L. Lewis, and Stephen B. Marley**

*Correspondence: Myrtle Y. Gordon, LRF Centre for Adult Leukaemia, Department of Haematology, Imperial College Faculty of Medicine, DuCane Road London W12 ONN, United Kingdom; e-mail: myrtle.gordon@ic.ak.uk*

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# To the editor:

## Are PU.1 mutations frequent genetic events in acute myeloid leukemia (AML)?

Recently, Mueller et al[1] found PU.1 mutations in 7% of the 126 cases of acute myeloid leukemia (AML) they analyzed. DNA binding and transactivation of the M-CSF receptor promoter, a direct PU.1 target gene, were deficient in the 7 mutants that affected the DNA-binding domain of the PU.1. Those mutations also decreased the ability of PU.1 to synergize with proteins such as AML1 or c-Jun, possibly contributing to the differentiation block found in AML. We looked for study analysis of PU.1 gene mutations in 77 cases of primary AML (excluding therapy-related AML and AML following MDS): 30 M0, 10 M1, 11 M2, 11 M4, 13 M5, 1 M6 AML, and 1 M7 AML, according to FAB classification.[2] In all blood and marrow samples analyzed, more than 80% of blasts were present. Detection of PU.1 gene mutations was made on DNA by single-strand conformation polymorphism (SSCP) analysis[3] and direct sequencing of the 5 exons corresponding to the entire coding region of the PU.1 gene. The size of the polymerase chain reaction (PCR) products was 190 bp to 440 bp (Table 1). Except for a polymorphism in intron 3 (G>A transversion), no abnormal SSCP profile and no variation of sequence of the PU.1 gene was observed in the 77 de novo AML cases studied.

Our results are in contrast to those of Mueller et al[1] but in agreement with results of Vegesna et al,[4] who found no mutation in 60 AML patients tested. There are no clear explanations for these discrepancies. Vegesna et al[4] only used SSCP analysis for screening of mutations, but we also used, like Mueller et al, direct sequencing. We and Vegesna et al[4] used intronic primers and performed analyses on DNA, whereas Mueller et al[1] performed direct sequencing on cDNA of the PU.1 gene. As 2 of the 9 mutations reported by Mueller et al[1] corresponded to large deletions, probably not detectable by techniques using DNA, such mutations could have been overlooked in our study.

Mutations reported by Mueller et al[1] predominantly occurred in undifferentiated AML (M0 AML) or in AML of the monocytic lineage (M4 and M5 AML), but we included a large proportion of those cases in our study and none of them were mutated.

Other hypotheses for those discrepancies include ethnic differences, as patients studied by Mueller et al[1] were, to a large extent, part of the Japanese cohort studied by Osato et al[5] for AML1 mutations. Finally, we and Vegesna et al[4] included only cases of

**Table 1. Primers used for SSCP and direct sequencing analysis**

| Forward primers* | Reverse primers* | Hybridization temperature, °C | Size of PCR product, bp | Restriction enzyme used (sizes of products after digestion, bp) |
|---|---|---|---|---|
| (EX1S) 5′-GTTGGGCTGGTGGAGGAGT-3′ | (EX1AS) 5′-GACACCCCAAGGGGACTATC-3′ | 64 | 310 | N/A |
| (EX2S) 5′-CTCTCTCCAGACCCCAGGA-3′ | (EX2AS) 5′-CCTGACACCTCCTCTCTCA-3′ | 64 | 190 | N/A |
| (EX3S) 5′-ACAGGCCTGGCAGTCTC-3′ | (EX3AS) 5′-CTGAGCTCACCCCATACTG-3′ | 64 | 270 | N/A |
| (EX4S) 5′-TGGCTGCTGGGTCAGTT-3′ | (EX4AS) 5′-CTTCCTCCAGTCTCCTGTGC-3′ | 60 | 400 | *Rsa*I (180 and 220) |
| (EX5S) 5′-GGCGAGGGCTTAATGCTAT-3′ | (EX5AS) 5′-GGGGCGGCTCCCATGTGG-3′ | 60 | 440 | *Alu*I (140 and 300) |

*EX indicates exon, followed by exon number; S, sense; and AS, antisense.