progressed past the $G_{1a}$ stage, as Jaleco et al report in their letter. We are in the process of determining whether this small population reflects the infected fraction of the naive CD4+ T cells. Nonetheless, this 4% cycling naive subpopulation in response to IL-7 has not acquired CD45RO expression (unpublished data). Preliminary phenotypic analysis from our laboratory of IL-7–treated naive T cells also indicates that they do not up-regulate CD69 or HLA-DR expression.

Jaleco et al suggest that the infected population may be that which initially expressed HLA-DR, which is an attractive hypothesis. But, though in the report published we have not depleted for HLA-DR expression, phenotypic analysis of the purified naive T-cell population reflected less than 2% of CD45RO-depleted cells to express HLA-DR. Others have depleted for HLA-DR expression, to isolate resting cells, and have been able to detect intracellular p24 expression within resting/quiescent naive T cells.[3,4] Jaleco et al also suggested that maintaining CD8+ T cells in these experiments may impact the ability of IL-7 to modulate HIV infection of CD4+ naive T cells. But even in experiments where CD8+ T cells were depleted, we were still able to demonstrate IL-7 enhancement of HIV replication in the naive CD4+ T-cell compartment, as defined by CD4+CD45RA+CD45RO− expression (unpublished data). Additionally, we were able to also demonstrate that IL-7 can induce HIV replication in CD4+ memory (CD4+CD45RA−CD45RO+) and even total peripheral blood mononuclear cells (PBMCs).

IL-7 is an attractive immune-modulator. But prior to clinical studies utilizing this cytokine, a clear understanding of its role in modulating HIV replication in resting/naive T cells and even on other cellular fractions needs to be elucidated.

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# To the editor:

## Heme inhibits the mitochondrial import of coproporphyrinogen oxidase

Heme has a variety of catabolic and regulatory functions within cells. It serves as a prosthetic group in hemeproteins, such as cytochromes, globins and catalases. The need for heme is greatest during erythropoiesis, where large amounts of heme are required in the synthesis of hemoglobin. The induction of heme biosynthesis is an early event in erythroid differentiation, and the expression of each enzyme of the pathway increases sequentially. Among the 8 enzymes of the pathway, δ-aminolevulinate synthase (ALAS) is the rate-limiting enzyme. Therefore, the finely tuned regulation of the synthesis of ALAS, or an erythroid-specific form (ALSE), is necessary for erythroid differentiation. Several mechanisms involved in this regulation have been reported.[1,2] Among them, feedback inhibition of transcription of the gene and mitochondrial import of the protein by heme seem to be important.[2] Recently, coproporphyrinogen oxidase (CPO), the sixth enzyme of the pathway, has been reported to represent rate-limiting steps downstream from ALASE in the regulation of heme biosynthesis.[3,4] The regulation of the synthesis of CPO has been reported,[5] but the regulation by heme has not been reported. Here, we examined whether heme inhibits the mitochondrial import of CPO as in the case of ALASE.

Full-length human CPO cDNA was obtained by polymerase chain reaction (PCR) using the human liver cDNA library as template. The forward and reverse primers used were 5′-TGCGGGAACATGGCCT-TGCAGCTGGG-3′ and 5′-CTCCAAACCCCTGCACAGCCAT-TCTG-3′, respectively. PCR products were then cloned in vitro in the transcription vector pGEM-5Z under the control of the SP6 RNA polymerase. Using this cDNA, the precursor protein of CPO (preCPO) was synthesized in the transcription-translation–coupled system in the presence of ${}^{35}$S-methionine and then was imported into rat-liver mitochondria as previously reported.[6] We used Sub9-DHFR in which the presequence of subunit 9 (Sub9) of the F0-ATPase (residue 1-69) was fused at the C-terminus with dihydrofolate reductase (DHFR) and a precurser protein of AAC (ATP/ADP carrier; preAAC) as controls. The mitochondrial imports of these precursor proteins are known not to be influenced by heme.

Transport of preCPO into isolated rat mitochondria was 99% inhibited in the presence of 20 μM exogenous hemin (Figure 1). In contrast, the transport of Sub9-DHFR and preAAC, assayed under identical conditions, was not inhibited at low hemin concentrations and was below 20% inhibited by 20 μM hemin (Figure 1). These results clearly indicate that heme inhibits the mitochondrial import of preCPO.
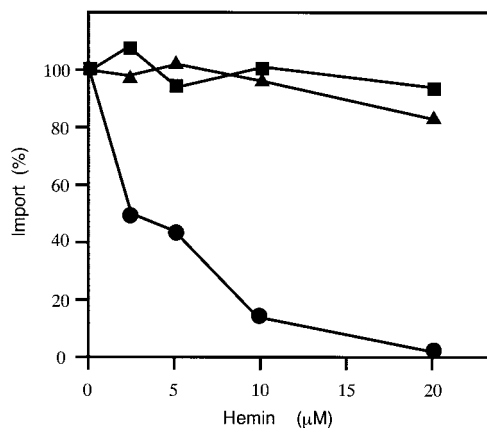


**Figure 1. Inhibition of the mitochondrial import of preCPO by high hemin concentration.** Import reaction was performed in the presence or absence of hemin at the indicated concentrations for 30 minutes at 30°C. Then mitochondria were treated with trypsin and CPO, and Sub9-DHFR and AAC were analyzed by fluorography. The bands of mature CPO, mature Sub9-DHFR, and AAC were quantified by densitometry of exposed film. ● represents CPO; ■, Sub9-DHFR; and ▲, AAC.

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.

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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]

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 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.

**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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