fold axis probably runs perpendicular to the stripes[4], is markedly perturbed by the binding of groES. One of the previously flat surfaces is bowed out, forming a dome in the side view. This clearly shows the asymmetry of groES binding, and suggests a structural change in one disk of the groEL oligomer to produce an increase in volume greater than would be caused by the simple addition of the M_r 70,000 groES 7-mer to the M_r 840,000 groEL. This may be an indication of conformational changes in groEL suggested to occur as part of ATP-driven protein folding[3].

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Stem cells

SIR — Jones *et al.* have demonstrated[1] that cells can be separated from mouse bone marrow which contribute 50–80% of the DNA in myeloerythroid, B and T cells 60 days after irradiation, but which possess no radioprotective and little CFU-S activity. They conclude "that there are two vital classes of engrafting cells: committed progenitors, which provide initial, unsustained engraftment, and pluripotent haematopoietic stem cells, which produce delayed, but durable, engraftment." Iscove in his News and Views article[2] contrasted these results with our report[3] that Thy-1^{lo} $Lin^-Sca\text{-}1^+(Th^{lo}L^-S^+)$ cells representing only about 0.05% of all bone marrow cells, are 1–2,000-fold enriched in radioprotection, CFU-S (day 12), and long-term multilineage reconstituting (LTMR) activity. Iscove concluded "the extent of possible overlap of day-12 CFU-S and long-term reconstituting units appears to be very low indeed and seems incompatible with conclusions drawn by Spangrude *et al.*".

Iscove's comments could be taken to mean that the $Th^{lo}L^-S^+$ cell type we described[3] is not the most highly enriched fraction of haematopoietic stem cell yet obtained, or that the fraction does not even contain haematopoietic stem cells by the definition of LTMR. That is not so. We have shown that 1–20 Ly–5 marked $Th^{lo}L^-S^+$ cells mixed with 100 unmarked $Th^{lo}L^-S^+$ cells will allow the assay of a single clonogenic event *in vivo*[4]. In most cases, marked clones were multi-lineage (T, B, myeloid). About 30% of the clones gave sustained engraftment for more than 9 weeks. The rest stopped producing new progeny before the 9-week time point. These two outcomes could result from a primary heterogeneity in the stem cells, or a chance event such as location in a self-renewing microenvironment. We tested[5] whether the subset could be divided into subsets using the dye Rh123. $Rh123^{lo}$ cells contain both measurable day-12 CFU-S and were highly enriched for LTMR, whereas $Rh123^{hi}$ cells contained fourfold greater CFU-S day-12 activity than the $RH123^{lo}$ cells, but were significantly less effective at giving rise to pre CFU-S and LTMR.

We believe that any differences between the results of Jones *et al.* and ours are likely to be obscured by the problems of studying a highly enriched, extremely rare population compared with studying populations in which the separation technology is not designed to isolate rare subsets, and which may either alter cellular potentials or contain regulatory cells.

Iscove concluded "for the cells of greatest practical interest — those with long-term reconstituting ability — there still seems to be no satisfactory substitute for long-term assays *in vivo*." We agree and have done such assays[3–6]. If we assume that Iscove means by the term "practical" that one principal objective of the isolation of haematopoietic stem cells is to restore lethally irradiated or myelo-ablated humans, we would say that long-term reconstitution alone is not sufficient: Jones *et al.* reported that none of 16 mice survived with 1×10^5 25 ml per minute cells (the fraction capable of long-term reconstitution), certainly an unacceptable outcome for bone marrow transplantation in man.

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ISCOVE REPLIES — Weissman and colleagues could mean that they consider the Thy-$1^{lo}Lin^-Sca\text{-}1^+$ fraction of mouse bone marrow to be purified to a similar degree in both long-term multilineage reconstituting stem cell (LTMRSC) activity as well as CFU-S. The issue is important: since their fraction is nearly pure in CFU-S, near purity of LTMRSC would also be implied — the end of the road, as it were. In the News and Views piece in question[2], I pointed out the apparent discrepancy between that interpretation and the findings of Jones *et al.*[1], showing virtually complete physical separation of CFU-S and radioprotective activity away from the bulk of the LTMRSC in marrow. If correct, the numbers were strong enough to imply that the majority cell type in the $Th^{lo}L^-S^+$ fraction (CFU-S) had to be something *other* than LTMRSC. The discrepancy was more than trivial, and to explain it away as "likely to be hidden in the quantitation of studying a highly enriched, extremely rare population. . ." seems less than sufficient.

In support of the claim for extensive co-purification of LTMRSC In $Th^{lo}L^-S^+$ fraction, Weissman *et al.* cite their more recent study[4]. In every case of successful clonal reconstitution achieved with their fraction, they write, the response was multilineage, "in 20–35% of the cases the clone gave sustained. . . engraftment" and, in the latter case, "retransplantation. . . was the rule". However, the statements lose in impact when the actual numbers are examined. Only 1/40–1/13 $Th^{lo}L^-S^+$ cells yielded detectable blood cell production up to 3–7 weeks, fewer continued much longer, and in only a solitary instance (mouse C7) of 280 mice transplanted with purified cells was reconstitution durable enough to survive transfer into secondary hosts.

If nothing else, Smith *et al.*[4] and Spangrude and Johnson[5] confirm that the $Th^{lo}L^-S^+$ fraction is considerably more heterogeneous than was originally appreciated. LTMRSC may be present, but the experiments to date establish their presence only as a very small minority. The presence of a few is not terribly informative. What does matter is how they *distribute* in fractionation experiments, and whether the presence of a few in the $Th^{lo}L^-S^+$ fraction could represent an experimental contaminant whose true phenotype could differ significantly from that of the bulk of the fraction. Reconstitution assays have been available for some time for measurment of LTMRSC specifically, quantitatively and independently of short-term radioprotection (see, for example, ref. 7). Appropriate application of such assays will clarify the current uncertainties and one can only look forward to publications in the refereed literature which do so.

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