Correspondence

To the editor:

In utero fetal liver hematopoietic stem cell transplantation: is there a role for alloreactive T lymphocytes?

The use of hematopoietic stem cells for in utero transplantation to create permanent hematochimerism represents a new concept in fetal therapy, although this approach has provided quite heterogeneous results. Flake and Zanjani have provided an excellent updated review of the current knowledge of in utero stem cell transplantation and have formulated diverse possible reasons for its poor clinical success.1 In fact, the only clear success, or claims of success, has been obtained in fetuses affected by immunodeficiency syndromes such as severe combined immunodeficiency (SCID) or bare lymphocyte syndrome. Flake and Zanjani strongly support the possibility that in such immunodeficiency disorders there is a selective advantage for donor cells, which then overcome a biological barrier to engraftment in fetuses. To date, the nature of this biological barrier remains unknown.

Alternatively, or perhaps additionally, we have raised the possibility that the fetus may develop an alloimmune response that ultimately accounts for graft failure. Accordingly, it has been reported that fetal liver stem cells are capable of engraftment in a syngeneic, nonallogeneic host,2 and that successful alloge neic in utero transplantation of fetal stem cells in sheeps and monkeys occurs if both the donor and the recipient fetuses are in a preimmune condition.3 Finally, we have reported a case in which in utero fetus-to-fetus transplantation performed at the 19th week of gestation resulted in neither engraftment nor tolerance induction; rather, 2 years after birth, the recipient had developed a highly increased cytotoxic T-lymphocyte precursor (CTLp) frequency against donor cells.4 Toward this possibility, we have recently undertaken molecular, phenotypic, and functional studies aimed at identifying the presence of fully competent T lymphocytes in samples of fetal livers and cord blood. We have found the presence of mature VDJ TCRβ chain transcripts in fetal liver and cord blood cells taken from 7 to 16 weeks of gestation. T-cell clones obtained from fetal liver stem cells showed a mature TCR αβ+, CD8+ phenotype and displayed strong alloreactivity against alloge neic HLA class I molecules.5 The very low yield of such clones from fetal liver-derived T lymphocytes strongly supports the view that their frequency is rather low and we have estimated it at around 0.2/10⁶ cells. Similar low, yet significant alloreactive response has been found within CD8+ T lymphocytes taken from cord blood.6

Based on our results, as well as experimental data from the literature, we favor the possibility that the presence of alloreactive T lymphocytes may explain the failure to engraft in fetuses older than 13 to 16 weeks. Overall, our results may provide useful informations on the stages of fetal T-cell development and can help in devising new strategies and planning further clinical trials in intrauterine transplantation.

References


Response:

Role of the alloimmune response after in utero hematopoietic stem cell transplantation

We appreciate Renda et al’s comments regarding their important investigations into the presence of alloreactivity in the early gestational human fetus. We agree that the immune response likely plays an important role in the barrier to engraftment following in utero transplantation, particularly after the appearance of phenotypically mature T lymphocytes in the peripheral circulation. The question is not whether the immune system presents a barrier to engraftment but, rather, when the human immune response
becomes an important consideration and what components of the immune response play an important role. It is clear from animal studies in sheep\(^1,2\) and mice\(^3\) that engraftment can occur across full allogeneic or xenogeneic barriers if transplantation is performed early enough in gestation. In studies that we have presented (manuscripts in preparation or submission),\(^5,6\) it is also clear that tolerance to the mouse model of in utero hematopoietic stem cell (HSC) transplantation can be achieved across full major histocompatibility complex (MHC) barriers and involves deletion of host-against-donor-reactive T cells, as well as donor-against-host-reactive T cells, and can be mediated by either direct or indirect antigen presentation, by donor- or host-derived antigen-presenting cells, respectively. Thus if the transplant is performed early enough in gestation and there is adequate antigen presentation in the thymus, it appears that T-cell mediated alloreactivity is not prohibitive to engraftment.

The natural immune system may also play a role in fetal immune response. NK cells are present early in gestation, but their function is poorly understood. We are in the process of investigating the effect of prenatal transplantation on the inhibitory receptor profile of host- and donor-derived NK populations.\(^7\) Until better studies are performed in defined animal models, the importance of the immune response and the parameters required for tolerance or immune sensitization will remain conjectural. There is a strong need for further direct studies, as Renda et al are pursuing, to define the immune response of the early gestational human fetus. These studies will allow informed extrapolation of findings in animal models to the human fetus for optimization of clinical in utero HSC transplantation.

To the editor:

**Proliferative history and hematopoietic function of ex vivo expanded human CD34\(^+\) cells**

We read with interest the paper of Glimm and Eaves,\(^1\) in which the effect of in vitro division on the function of primitive human hematopoietic progenitor cells (HPC) was examined. The interest stemmed from the fact that this paper investigated an issue that we previously examined in a series of 5 papers\(^2,4,5\) and that was also addressed by other laboratories in a similar fashion.\(^6,7\) Data from Glimm and Eaves\(^1\) confirmed results we first described in 1995,\(^2\) which were similar in scope to previous\(^8\) and subsequent findings\(^9,10\) illustrating that a small fraction of cultured human CD34\(^+\) cells remains quiescent in culture with associated cytokine nonresponsive (CNR) characteristics while maintaining primitive hematopoietic potential. Although the paper by Glimm and Eaves reiterates and corroborates previously published results from many laboratories, the authors construed their results to support specific conclusions that may not be directly borne by the experimental evidence presented in their report.\(^1\) This letter is intended to demonstrate that, viewed differently, data presented by Glimm and Eaves can be interpreted to be in full support of the previous work rather than in disagreement with earlier findings that were directly and strongly criticized by the authors.

At the center of this controversy is the hematopoietic potential of groups of cells of different proliferative history following their maintenance in ex vivo expansion cultures. Specifically, these are cells that we previously defined as cytokine nonresponsive (CNR) cells,\(^7\) which remain undivided for several days in expansion cultures, as compared with those that undergo 1, 2, 3, or more cell divisions, which Glimm and Eaves referred to as postmitotic.\(^1\) We described in several publications\(^2,4,5\) that the frequency of long-term hematopoietic culture-initiating cells (LTHC-ICs)\(^2\) was higher among CNR cells than among those proliferating extensively in expansion cultures. Similarly, Glimm and Eaves demonstrated that, in at least 3 out of 6 samples examined, the frequency of long-term culture-initiating cells (LTC-ICs) was several-fold higher in the undivided fraction than in the postmitotic fraction.\(^10_{26}\) When cells that had undergone no more than 2 divisions were tested against those dividing at least 3 times, the frequency of LTC-ICs among the former group was in excess of 80-fold higher in 4 of 5 samples tested.\(^10_{26}\) In their discussion, Glimm and Eaves argue that in our studies,\(^2\) in which a similar approach was taken to isolate cells of different proliferative history, we may have isolated, in the same group, cells undergoing up to 4 divisions (rather than CNR cells only) due to the “lower resolution of PKH2 staining.”\(^1(p2165)\) According to Glimm and Eaves, the insensitivity of our protocol may explain why an increased frequency of LTHC-ICs was detected among cells we “thought to have remained quiescent”\(^10_{26}(p2165)\) in culture.

It is unfortunate that Glimm and Eaves did not examine all of the published literature on this same issue. In a paper published in 1998,\(^8\) we clearly demonstrated that the frequency of LTHC-ICs, although relatively high among CNR cells (0 divisions), was highest among cells undergoing 1 division in vitro. LTHC-IC frequency among cells dividing twice was as high as that observed for nondividing cells (CNR cells). Only when cells divided more than 3 times did the frequency of LTHC-ICs decline considerably. That we were able to dissect the proliferative history of cultured cells into single cell division cycles and to examine the frequency of LTHC-ICs among each fraction demonstrating the persistence of LTHC-IC frequency through 2 divisions confirms important points: first, that our methodology was sensitive enough to examine single divisions rather than groups of cells undergoing no more than 2 or at least 3 divisions as achieved by Glimm and Eaves\(^1\); second,

### References

In utero fetal liver hematopoietic stem cell transplantation: is there a role for alloreactive T lymphocytes?

Maria Concetta Renda, Emanuela Fecarotta, Aurelio Maggio, Francesco Dieli, Guido Sireci, Alfredo Salerno, Lola Markling, Magnus Westgren, Gianfranca Damiani, Cristina Jakil and Francesco Picciotto