Hematopoiesis: **Wandering progenitor cells**

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The generation of mice that are chimeric for expression of α_4 integrin has revealed a critical role for this adhesion molecule, specifically in postnatal lymphopoiesis.

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Like the Jews of old wandering in the desert, longing to find a permanent homeland, hematopoietic stem cells spend much of their time roaming throughout the embryo during vertebrate development [1]. Embryonic hematopoiesis in the mouse begins at about day 7.5 of gestation in both an extraembryonic site, the yolk sac blood islands, and an intraembryonic site, the para-aortic splanchnopleur or aortic–gonad–mesonephros region. Stem cells, potentially from both of these disparate sites, then migrate to the fetal liver, where they take up temporary residence from approximately day 11 through day 15 of fetal development. Ultimately, the cells emigrate to the bone marrow, where they finally arrive at their permanent site of residence for the rest of the lifetime of the organism. These migratory waves during embryonic development probably serve to move the hematopoietic stem and progenitor cells to different stromal microenvironments, where diverse growth and differentiation factor milieus can exert their appropriately timed effects.

As these diasporas require that the hematopoietic stem and progenitor cells lodge in highly specific anatomic sites, it is likely that they involve one or more adhesion systems. Because of the difficulty of studying hematopoiesis *in vivo*, the adhesion systems that might potentially be involved in either embryonic or adult hematopoiesis have been studied mostly in *in vitro* stromal systems. The approach has been to analyze the effects of blocking antibodies on the adhesion and expansion of progenitor cell types *in vitro*. The results of such experiments have suggested that the $\alpha_4\beta_1$ integrin may be involved in several aspects of hematopoiesis. For example, this integrin has been found to play a role in the attachment of hematopoietic progenitor cells to bone marrow stromal cells, and it has also been implicated in B-cell development [2]. Both the immunoglobulin-G-like vascular cell adhesion molecule-1 (VCAM-1) and fibronectin ligands for the $\alpha_4\beta_1$ integrin have been associated with these effects [3–5].

Interestingly, *in vivo* studies have revealed that administration of blocking antibodies directed against the $\alpha_4\beta_1$ integrin result in a profound release of progenitor cells from bone marrow, and this antibody also inhibits the engraftment of progenitor cells in this hematopoietic compartment [6]. Lastly, the *in vivo* engraftment of fetal liver by hematopoietic progenitors has been found to be inhibited by lack of the β_1 integrin subunit [7]. Together, these results argue strongly that the $\alpha_4\beta_1$ adhesion molecule, and possibly other β_1 integrins, play a role in adult, and conceivably also fetal, hematopoiesis.

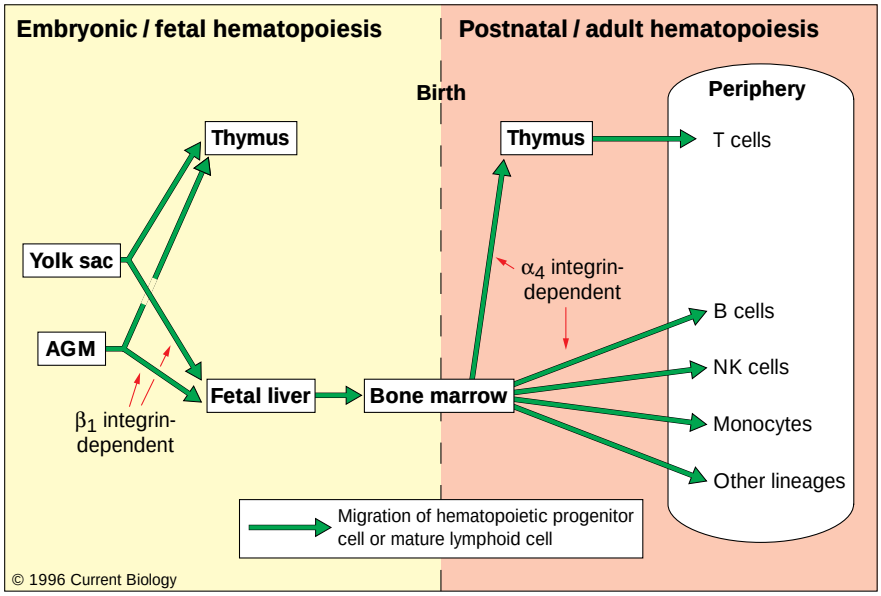
The role of mammalian gene products *in vivo* is currently best analyzed using what is now a standard procedure: the generation of 'knockout' mice by targeted gene inactivation in totipotent embryonic stem (ES) cells. Although this technique is extraordinarily powerful, the analysis of such mutant animals is hindered if they die during embryogenesis. In the case of the α_4 integrin knockout mice, the homozygous animals die early during embryogenesis, and the lethality is manifested by a failure of the chorion to fuse with the allantois, as well as cardiac defects [8]. This lethality thus precludes the analysis of the role of this integrin in embryonic and adult hematopoiesis.

There is, however, another way of investigating at least the effects on hematopoiesis of a mutation that is lethal to the whole animal. This is to produce chimeric mice whose blood system is derived wholly or in part from ES cells in which both alleles of the gene of interest have been knocked out [9]. Using surface markers specific for the ES-derived hematopoietic cells, the contribution of these cells to various mature hematopoietic lineages can be assessed. An additional twist is to use mice deficient in RAG1 and RAG2, enzymes involved in the recombination events that construct functional immunoglobulin and T-cell receptor genes from their germline parts in B and T cells, respectively. Such mice cannot make their own B and T cells, so when they are used as ES-cell recipients, all of the B and T cells of the resulting chimeric animals are derived from the ES cells. It is this system that Arroyo *et al.* [10] have recently used to analyze the *in vivo* functions of the α_4 integrin in fetal and adult hematopoiesis.

Using this chimeric mouse system, Arroyo *et al.* [10] have made a number of interesting observations. Perhaps unexpectedly, given previous *in vitro* data, they found that T cells lacking the α_4 integrin developed normally during embryogenesis. However, these cells disappeared with time after birth, so that by one month they were absent. At this age the thymus was also affected and became atrophic,

Figure 1

Integrin involvement in embryonic and adult hematopoiesis. The diagram shows the migratory pathways taken by hematopoietic progenitor cells before and after birth, highlighting the steps that have recently been shown to require integrins. Hematopoiesis begins during embryogenesis in the yolk sac and aorta–gonad–mesonephros (AGM) region; hematopoietic progenitor cells then migrate to the fetal liver, where a β_1 integrin appears to be required for engraftment [7]. Migration to the bone marrow occurs late in fetal development, and this is a site of hematopoiesis postnatally and in adult life. The α_4 integrin appears to be required for the development of lymphoid lineages postnatally, but not prenatally [7].



again suggesting a deficiency in T-cell production. These observations indicate that the embryonic development of these hematopoietic cells was normal, but that their postnatal maintenance was deficient. The development of B cells derived from the mutant ES cells was found to be severely compromised in the chimeric mice, and this deficiency in peripheral B cells was mirrored by a deficiency of bone marrow B-cell progenitors.

Interestingly, the reconstitution of sub-lethally irradiated recipient animals with bone marrow derived from the chimeric animals revealed that T cells can develop without this integrin, although B-cell development was again severely compromised. This latter result suggests that thymic homing and differentiation of T cells does not require the α_4 integrin, although this molecule does appear to have a critical role in B-cell development. Importantly, this effect was not found in all hematopoietic lineages. Thus, monocytes and natural killer cells underwent apparently normal development in the absence of this adhesion system, suggesting that the defect occurs at a stage after the development of the partially committed hematopoietic stem cell.

What are we to make of these interesting observations? First, they strongly suggest that there is a switch in the requirement for the α_4 integrin in lymphopoiesis when the animal is born (Fig. 1). Preliminary *in vitro* data suggested that interactions between hematopoietic progenitor cells and the surrounding stroma are critical for lymphoid development and that these interactions apparently involve the α_4 integrin, and the new *in vivo* experiments provide further compelling evidence for an important role for this adhesion system in adult lymphopoiesis. As Arroyo *et al.* [10] point out, this requirement could reflect an adhesive mechanism, a signalling mechanism, or both. The interesting observation that α_4 integrin is not required during embryonic lymphoid development suggests that the bone marrow compartment changes during the first postnatal month. Alternatively, another adhesion molecule may compensate for the loss of the α_4 integrin during embryonic lymphoid development, and this compensatory state is lost during postnatal growth.

The earlier *in vitro* studies suggested a role for the α_4 integrin in the development of early hematopoietic progenitor cells [11] as well as of myeloid progenitor cells [2]. As Arroyo *et al.* [10] found that only the lymphoid compartment is affected by the absence of α_4 integrin, these earlier observations might have reflected a purely *in vitro* phenomenon, but it is also possible that other adhesion systems come into play during embryogenesis to compensate for the loss of the α_4 integrin. And, as mentioned above, embryonic hematopoietic progenitor cells lacking the β_1 integrin were recently shown [7] to be unable to contribute to the hematopoietic system of chimeric mice and to colonize the fetal liver, although they were capable of differentiating *in vitro* into all three hematopoietic lineages (Fig. 1). Again, Arroyo *et al.* [10] found no such defect in their chimeric mice, suggesting that another integrin α chain must be involved with fetal liver engraftment by hematopoietic stem cells during embryogenesis.

In summary, the elegant work of Arroyo *et al.* [10] has revealed a crucial role for one of the integrins in postnatal lymphoid development. Together with the work of Hirsch

et al. [7], the results suggest that integrins may play critical roles in hematopoiesis, both during embryogenesis and postnatally. The use of such chimeric systems to examine otherwise lethal knockouts should be manna for the legions of cell biologists interested in hematopoiesis.

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