Self-renewal of thymocytes in the absence of competitive precursor replenishment

Thomas Boehm

Soon after transplantation of wild-type thymi into immunodeficient mice lacking functional T cell receptors, productive T cell development in the donor thymus ceases. This observation underlies one of the central dogmas of T cell biology: because thymocytes are seemingly short-lived, intrathymic T cell development depends on continuous import of lymphoid progenitors from the bone marrow. New work reinterprets the outcome of this classical experiment as being the result of competition for intrathymic niches specifically supporting the DN3 stage of early T cell development. Surprisingly, when this niche space is uncontested by immigrating host progenitors, development of T cells in the thymus grafts continues. These new findings suggest that early thymocytes do indeed have substantial self-renewing potential.

The thymus is an evolutionarily conserved primary lymphoid organ that is essential for T cell development. Its immunological function was discovered in mice ~50 yr ago (Miller, 1961). Thymopoietic tissue is present in all vertebrates (Boehm et al., 2012), including jawless fish (lamprey and hagfish; Bajoghli et al., 2011), the sister group of jawed vertebrates that encompasses species as diverse as sharks and humans. The thymic microenvironment consists mostly of epithelial cells that are capable of attracting lymphoid progenitor cells, specifying these cells to the T cell lineage, and orchestrating a complex series of selection events that culminate in the generation of a self-tolerant and diverse repertoire of T cell receptors (TCRs) that is clonally expressed on T cells.

A central dogma of thymus biology

Because the thymus is an anatomically well-defined structure, surgical manipulations have played a decisive role in elucidating key aspects of its biology. Surgical removal unequivocally demonstrated the requirement of the thymus for the generation of T cells in different species (Miller, 1961; Horton and Manning, 1972). Likewise, transplantations of thymus tissue from one animal to another proved to be an extremely informative means of examining more complex questions. After transplantation of wild-type thymi into hosts unable to complete the somatic assembly of TCR genes, donor T cells in the transplants were substituted within a few weeks by developmentally incapacitated host-derived lymphocyte progenitors (Frey et al., 1992; Takeda et al., 1996). These findings, verified repeatedly in many laboratories, were made into one of the central dogmas of thymus biology, namely that thymocytes are short-lived, and thus continuous T cell differentiation in the thymus depends on the constant supply of lymphocyte progenitors from extrathymic sources (e.g., the bone marrow). Indeed, this basic tenet has been stated thousands of times in the introductory paragraphs of papers dealing with various aspects of thymus function. But this ritual will have to change, as a result of two papers published in this issue of *The Journal of Experimental Medicine*.

Through the clever use of genetically modified mice, Peaudecerf et al. and Martins et al. have struck this decades old dogma off the list of unquestioned principles in thymus biology. In doing so, they introduce the concept of cellular competition to explain important aspects of the T cell differentiation process. What exactly have Peaudecerf et al. and Martins et al. done?

Transplantation of thymi into immunodeficient hosts

Using a recently established mouse model exhibiting a severe reduction of hematopoietic stem cells (HSCs) in the bone marrow, Martins et al. (2012) reexamined the issue of whether the thymus harbors and exports hematopoietic progenitor cells. The use of *Rag2*$^{-/-}\gamma c^{-/-}$ *Kit*$^{W/Wv}$ mice as recipients of wild-type donor thymi provides a particularly sensitive assay system for studying this question because the many empty HSC niches in the bone marrow of these mice are readily occupied by transplanted HSCs without the need for prior conditioning (Waskow et al., 2009); however, in the overwhelming majority of mice, the authors detected no bone marrow engraftment by donor-derived HSCs emanating from the thymus transplants, indicating that export of HSCs from the thymus is a rare event. Nevertheless, when Martins et al. (2012) examined T cell development in the grafted thymus under these conditions of failing progenitor supply, they unexpectedly observed sustained donor T cell development over much longer periods of time than previously observed in similar, but not identical, experimental paradigms (Frey et al., 1992; Takeda et al., 1996; Berzins et al., 1998). Of the three mutations co-introduced into their host animals, Martins et al. (2012) established that γ_c deficiency (Cao et al., 1995), rather than lack of c-kit (Rodewald et al., 1995) or Rag2 (Shinkai et al., 1992),

T. Boehm is at the Department of Developmental Immunology, Max Planck Institute of Immunobiology and Epigenetics, D-79108 Freiburg, Germany.

CORRESPONDENCE
T.B.: boehm@immunbio.mpg.de

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was the critical factor facilitating sustained intrathymic donor T cell development.

In a parallel study, Peaudecerf et al. (2012) observed sustained donor T cell development originating from transplanted wild-type thymi in host *Rag2*$^{-/-}$ $\gamma c^{-/-}$ or *Rag2*$^{-/-}$*Il7r*$^{-/-}$ recipient mice; persistent donor T cell development was not, however, observed in *Rag2*$^{-/-}$ recipients. These findings directly implicate impaired IL-7 signaling as a mechanism facilitating autonomous T cell development in the thymus.

Why does failure of IL-7 signaling cause such a different outcome to failure of TCR signaling? The answer lies in the sequence of events underlying intrathymic T cell development (Fig. 1 A). Under the influence of various chemokines emanating from the thymic microenvironment, early thymic progenitors enter the thymic rudiment (Calderón and Boehm, 2011) and proliferate upon exposure to IL-7 and SCF, the ligand of the c-kit receptor (Rodewald et al., 1997). During these early stages of T cell development, thymocytes are positive for CD44 and transit from a CD25-negative (DN1) stage to a CD25-positive stage (DN2). At the subsequent DN3 stage, T cells rearrange their *Tcrb* gene segments, and generation of a functional TCRβ chain enables expression of the so-called pre–TCR complex on the cell surface. When V(D)J recombination fails (as in mice lacking *Rag2* [Shinkai et al., 1992], *Prkdc* [Blunt et al., 1995], or *Cd3ε* [DeJarnette et al., 1998]), thymocytes do not develop beyond the DN3 stage. In contrast, mutations impairing IL-7 signaling (caused by loss of either α [Peschon et al., 1994] or γ_c [Cao et al., 1995] chains of the IL-7 receptor) arrest development at the earlier DN2 stage.

Competition for niche occupancy

Martins et al. (2012) and Peaudecerf et al. (2012) show that only when host-derived progenitors are capable of differentiating further than the DN2 stage, do resident donor thymocytes eventually disappear from the thymus graft (Fig. 1 B). In contrast, when there is no continuous progenitor supply (as is the case in recipients deficient for both SCF and IL-7 signaling), or when progenitors arrest before

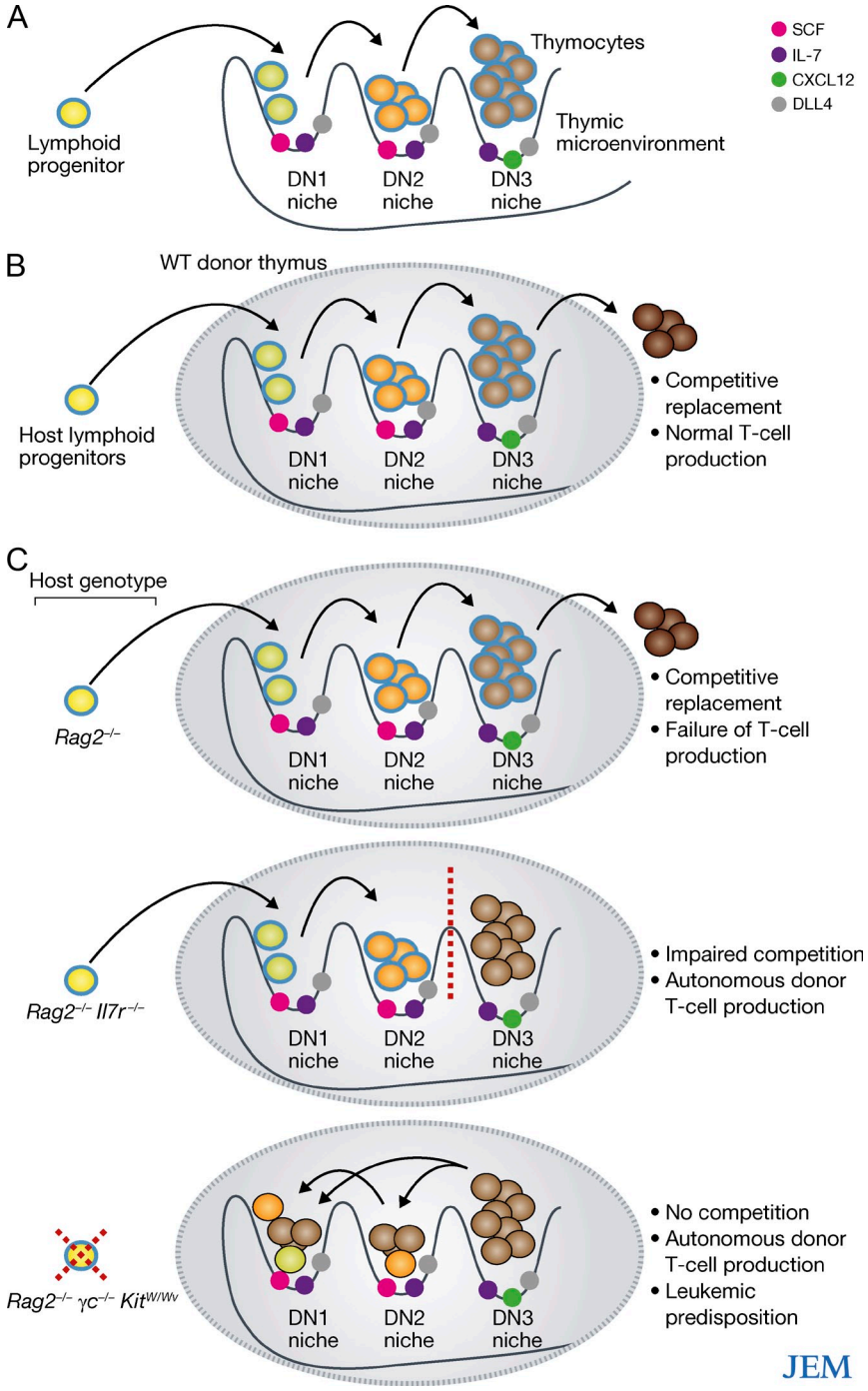

Figure 1. Competition for T cell progenitor niches in the thymus. (A) A schematic depiction of early thymocyte development. Incoming lymphoid progenitors home to the thymic rudiment, where they are sequentially exposed to stromal factors (e.g., SCF, IL-7, CXCL12, and DLL4) in different types of niches, here designated by the stage of thymocytes occupying them. (B) After transplantation into a wild-type (WT) host, thymocytes in the donor thymus are competitively replaced by a continuous stream of host-derived progenitor cells. Host-derived progenitor cells are outlined in blue. (C) Transplantation of WT thymi into hosts bearing different mutations alters the outcome of intrathymic T cell development. In *Rag2*$^{-/-}$ hosts, host progenitor cells competitively replace donor thymocytes in DN1, DN2, and DN3 niches even though host thymocytes fail to generate a functional pre–TCR or TCR. In *Rag2*$^{-/-}$*Il7r*$^{-/-}$ hosts, competitive replacement is restricted to DN1 and DN2 niches. Additional abrogation of SCF signaling eliminates competition in all niches, perhaps facilitating donor thymocyte occupancy of inappropriate niches. In the latter two situations, sustained generation of donor T cells occurs in the WT thymus graft.

the DN3 stage of development (as in the case of impaired IL-7 signaling), donor thymocytes switch into a mode in which they continue to cell autonomously generate T cells in the thymus graft (Fig. 1 C). Hence, rather than explaining the disappearance of donor thymocytes observed in previous experiments (Frey et al., 1992; Takeda et al., 1996) as a sign of their short life-span, Martins et al. (2012) and Peaudecerf et al. (2012) implicate competition between newly emerging/imported host-derived DN3 thymocytes and resident donor-derived thymocytes as the force driving donor thymocyte disappearance. Therefore, lack of competition for space in the "DN3 niche" appears to be critical to enable autonomous T cell production.

What could be the mechanism by which resident cells become less competitive? One possibility is that exposure to external signals such as cytokines and chemokines changes their adhesive properties, reducing their competitive fitness for one niche and increasing it for another; a similar mechanism operates in the testis stem cell niche of *Drosophila* (Issigonis et al., 2009). In the context of different precursor niches in the thymus, successive changes in adhesive properties would result in competitive replacement of resident cells from one niche to the next as a result of continuous import of lymphoid progenitors. In this view, competitive niche occupancy not only fosters stage-specific differentiation but also provides the basis for directed progression along the known developmental trajectory of thymocytes.

The observations of Martins et al. (2012) and Peaudecerf et al. (2012) ascribe special importance to a niche specifically supporting DN3 thymocytes. This particular thymic microenvironment furnishes additional signals, such as those exemplified by the co-stimulatory activity of CXCL12 (Janas et al., 2010; Trampont et al., 2010). In hindsight, the important role of the DN3 niche is perhaps not surprising, given that the DN3 stage, during which the pre–TCR is formed and expressed on the cell surface, is a critical checkpoint in thymocyte development. Thymocytes failing this so-called β selection abort further development and succumb to apoptosis (von Boehmer et al., 1998). With respect to niches for earlier stages of thymocyte development, potentially important differences might exist between recipients entirely lacking endogenous lymphoid progenitors (owing to the combined effects of impaired SCF and IL-7 signaling, for example) and recipients supplying DN2-arrested progenitors to the donor thymus (as a result of impaired IL-7 signaling but normal SCF signaling). When host lymphoid progenitors unable to receive IL-7 signals colonize the DN1 and DN2 niches of the thymus graft, they compete with resident donor cells; however, such competition does not occur in the DN3 niche (Fig. 1 C). In contrast, when the host does not supply any lymphoid progenitors, donor cells are uncontested even in the DN1 and DN2 niches (Fig. 1 C). Abnormally long residence time might have unpredictable effects on the phenotype and genomic stability of such early T cell precursors that are endowed with considerable proliferative potential, perhaps underlying the changes in the TCR repertoire observed by Martins et al. (2012). This underscores the importance of precisely delivered environmental signals from the thymic microenvironment that support distinct trajectories of hematopoietic differentiation (Calderón and Boehm, 2012).

Predisposition to leukemia?

Activation of the *LMO2* oncogene (Boehm et al., 1991) is an initiating event for the development of human T cell leukemia (Rabbitts, 1994). Interestingly, when expressed in mouse hematopoietic cells, *Lmo2* confers stem cell–like properties on developing thymocytes; serial transfer experiments revealed that this self-renewal potential is associated with cells of the DN3 phenotype (McCormack et al., 2010). Therefore, it is conceivable that *Lmo2*-expressing cells are particularly effective in competing for DN3 niche space and might spend an extended residence period in this niche, possibly constituting a predisposing factor for the development of leukemia. Considering these observations and the results of Martins et al. (2012) and Peaudecerf et al. (2012), any situation leading to unphysiologically long DN3 niche residence times might be leukemogenic.

In this view, the gated nature of thymus colonization (Foss et al., 2001) represents an endogenous risk factor predisposing to leukemic development. For example, after retrovirus-mediated restoration of the expression of missing or faulty IL-7 receptor signaling components in patients undergoing gene therapy for genetic defects in this pathway (Fischer et al., 2011), the inevitable somatic mosaicism of mutant and corrected HSCs might lead to only intermittent importation of differentiation-competent corrected progenitor cells into the thymus. This situation would essentially phenocopy the situation in the mice studied by Martins et al. (2012).

If combined, the leukemia-predisposing effect of temporary failure of niche competition may synergize with the proleukemogenic effects of retrovirally activated *LMO2*; this might lead to the rapid development of leukemia that was observed in patients (Hacein-Bey-Abina et al., 2003).

In conclusion, the iconoclastic results reported by Peaudecerf et al. (2012) and Martins et al. (2012) clarify an important aspect of thymus biology and T cell differentiation, and moreover reaffirm the importance of cellular competition for precious niche space in these processes. In addition, their system might provide a unique opportunity to examine the role of cell competition and niche occupancy in the genesis of T cell leukemia.

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