B cell lineages: documented at last!

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Functional and phenotypic distinctions characterize B-1 cells versus B-2 cells. The identification of adult bone marrow cells that give rise exclusively to B-1 B cells support earlier ideas regarding the existence of dedicated B-1 progenitors.

In this issue of *Nature Immunology*, Montecino-Rodriguez, Leathers and Dorshkind put an end to a controversy that has simmered and bubbled for nearly 15 years. In what should become a landmark paper, these authors report the isolation of phenotypically distinct progenitors for mouse B-1a, B-1b and B-2 cells at an early developmental stage, well before rearrangement of the gene encoding the immunoglobulin heavy-chain complex (*Igh*) is complete[1]. Thus, despite heated arguments to the contrary, these functionally distinct B cells are now firmly located in separate developmental lineages.

The physical and ontological differences that distinguish B-1a, B-1b and B-2 cells are fully consistent with the assignment of these B cells to separate lineages. Similarly, the repertoire and functional differences that distinguish the lineages support the idea that they evolved sequentially to meet the increasingly complex immunological challenges encountered by more highly evolved organisms[2,3]. B-1a cells, the earliest B cells, develop almost exclusively in the neonate and persist thereafter as a self-replenishing population[3,4]. These cells produce most of the 'natural' IgM in serum and participate in innate immunity[3]. They typically do not participate in responses to immunization with 'laboratory' antigens. However, they have been shown to produce IgM responses to certain types of antigenic stimulation and to rapidly produce specific IgM antibodies that are essential for the initiation of delayed-type hypersensitivity responses and reactive airway disease[5].

B-1b B cells are distinguished phenotypically from B-1a cells (at present) only by the absence of surface CD5; that is, B-1a cells are $sIgM^{hi}CD5^{lo}$, whereas B-1b cells are $sIgM^{hi}CD5^{lo}$. However, functional and developmental differences distinguish these two types of B-1 cells. Notably, B-1b cells are required for protection against certain parasites and bacteria[6,7]. B-1a cells, in contrast, contribute minimally (if at all) to this protection but produce other antibodies, notably to phosphatidylcholine, that B-1b cells do not produce. The cytokine responses of B-1a and B-1b cells also differ: B-1b cells respond to interleukin 9 (IL-9), whereas B-1a cells respond to IL-4 and IL-5.

Finally, although both B-1a and B-1b cells develop initially as self-replenishing populations in the neonate, progenitor activity for B-1a wanes after neonatal life, whereas substantial progenitor activity for B-1b can be found in both neonatal and adult bone marrow. Montecino-Rodriguez *et al.* directly document this developmental shift by showing that B-1 progenitors ($Lin^{-}CD45R^{lo-neg}CD19^{+}$) isolated from adult bone marrow mainly give rise to B-1b cells, whereas the phenotypically similar B-1 progenitors isolated from fetal liver mainly give rise to B-1a B cells[1]. A similar age distinction has been reported for B-1 progenitor activity[8].

B-2 cells, apparently the last to appear on the evolutionary scale, begin to develop in bone marrow toward the end of the neonatal period and are by far the predominant population of B cells developing in adult bone marrow[3,4]. Montecino-Rodriguez *et al.* confirm this time scale by showing that progenitors for these cells ($Lin^{-}CD45R^{+}CD19^{-}$) are rare in fetal liver but can be readily isolated from adult bone marrow[1]. Unlike B-1a and B-1b B cells, B-2 cells are relatively short-lived (several months, at best) and are known to persist as self-replenishing cells only when triggered by antigen to differentiate into IgG-producing memory B cells[3,4]. They produce most of the commonly studied IgG antibody responses and the only antibody responses in which substantial affinity maturation occurs. Nevertheless, B-1 cells also produce IgG antibodies and in fact produce perhaps most of the IgG found in sera from unimmunized animals.

Those developmental and functional differences suggested a model in which at least three B cell lineages evolved sequentially, resulting in a 'layered' immune system in which successive layers are adapted to meet increasingly complex immunological challenges[9]. B-1a, B-1b and B-2 cells were proposed to belong to separate (layered) lineages because the progenitors for these B cells are differentially enriched in fetal-neonatal versus adult lymphoid progenitor sources and the transfer of fetal and adult progenitor sources together does not alter the developmental fate of the transferred progenitors[4]. However, this multilineage model remained hypothetical because there was no known way, at the time, to separate the progenitors of the proposed B cell lineages.

In the early 1990s, alternative views of B cell origins began to appear[10,11]. Single-lineage models were proposed in which the functional, phenotypic and developmental differences of the three types of B cells were ascribed to the effects of antigen-driven differentiation and selection. Focusing on evidence indicating that B-1 cells tend to produce antibodies to self and repetitive antigens, whereas B-2 cells tend to produce antibodies to exogenous antigens, these authors[10,11] argued that the 'decision' to differentiate to the B-1 rather than the B-2 phenotype reflects different responses of mature B cells to stimulation with the different types of antigens: repetitive self antigens encountered during neonatal life drive B cells with IgM receptors that recognize these antigens to differentiate to the B-1 phenotype; exogenous antigens encountered in adult life drive B cells whose receptors recognize these antigens to differentiate to the B-2 phenotype. The simplicity of this 'function-begets-phenotype' hypothesis had much to recommend it, particularly in an era in which the focus had shifted to molecular studies and younger investigators had little patience for interpreting results from what tended to be considered complex 'old-style' reconstitution studies. Thus, over time (and despite substantial evidence to the contrary[2,3,9]) the single-lineage view became the favored context for the consideration of B cell origins.

Based on that paradigm, studies moved toward determining how or when B-2 cells 'turn into' B-1 cells or vice versa. B-2–to–B-1 phenotypic conversions were reported for B-2 cells stimulated via immunoglobulin receptors in certain conditions or forced by molecular means to shift to the production of antibodies with typical B-1 specificities[9]. Those findings, which generated valuable insights concerning the plasticity of mature B-1 and B-2 cells, were interpreted as confirming the single-lineage hypothesis and, by implication, negating the multilineage view. However, as Dorshkind and colleagues now teach, data from those conversion studies were perhaps interpreted more zealously than wisely.

Dorshkind and colleagues present evidence that satisfies crucial criteria for distinguishing

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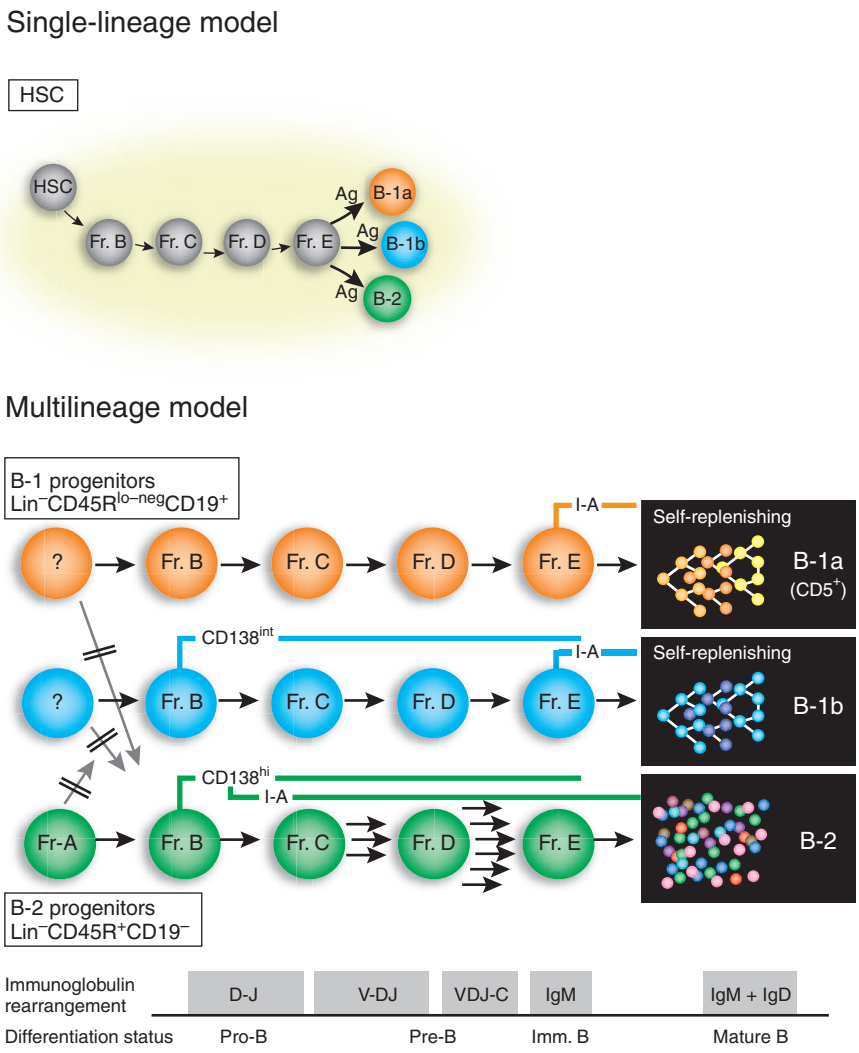

Figure 1 The development of three distinct B cell lineages in the layered immune system in the mouse. The lineage commitment of B cell progenitors for each of the lineages is defined early in B cell development, before the initiation of immunoglobulin heavy-chain rearrangement. Top, previous single-lineage model, now negated by new findings presented in this issue[1]. Bottom, multilineage model for B cell development, which best fits the present data, with the surface phenotypes now known to distinguish the progenitors for three B cell lineages. Fr. A–Fr. E represent the Hardy scheme for B cell development; gray boxes (bottom) indicate the sequential immunoglobulin heavy-chain rearrangements that occur as B cells mature through the Hardy sequence (D, diversity; J, joining; V, variable; C, constant). B-1 progenitors ($Lin^{-}CD45R^{lo-neg}CD19^{+}$) are located in a 'new' early progenitor fraction distinguished from Fr. A or Fr. B by the absence of the B220-6B2 CD45R determinant (other CD45R determinants are expressed)[1]. B-2 progenitors ($Lin^{-}CD45R^{+}CD19^{-}$) are located in the 'classical' Hardy Fr. A. The developmental pathways for each of the lineages is further distinguished by the expression of CD138 and the stage at which I-A (major histocompatibility complex class II) is initiated[12]. The progenitors for B-1a, B-1b and B-2 are also distinguished by the time at which they appear in bone marrow[1]. Ag, antigen; B, B cell; Imm., immature.

cell lineages[1]. They show that B-1 and B-2 progenitors can be physically separated from each other and that the two progenitors differ in their requirements for cytokine support; that is, from thymic stromal lymphopoietin and IL-7. The studies presented also show that although B-1a and B-1b progenitors are phenotypically similar, the times at which they appear during ontogeny separate them from one another. Those lineage distinctions have been confirmed and extended by experiments showing that CD138 and major histocompatibility complex class II molecule (I-A) expression distinguish the developmental pathways for B-1a, B-1b and B-2 cells from the beginning of immunoglobulin rearrangement onward[12] (**Fig. 1**). These findings collectively provide the initial characterization of B lineage developmental differences in neonates and adults. Thus, they open the way for exploration of the evolutionary and functional mechanisms that have established and maintained this multilineage architecture in the mouse immune system.

There is still some question as to whether evolutionary homologs of the mouse B cell lineages are present in the human immune system. Comparison of B cell development and function in other species indicates that a variety of solutions have been found for generating antibody diversity and for populating the immune system with antibody-producing cells. Most (perhaps all) B cells in the chicken and the rabbit seem to be similar to B-1 cells, including the expression of surface molecules similar to CD5. The rat, in contrast, may not have a B cell population homologous to B-1 cells, whereas humans do have a $CD5^{+}$ B cell population in peripheral blood and other organs.

In mouse and man, the B cells that express CD5 share many similarities. They develop early and are eventually overshadowed as a population by B cells that do not express this surface marker. They express a restricted antibody repertoire that is enriched for antibodies that may be useful for innate defense against invading pathogens. In both species, this repertoire seems to be positively selected during prenatal and early neonatal life, perhaps by exposure to internal (self) antigens. However, whereas mouse B-1 cells persist as a self-replenishing B cell population, the origins and self-replenishing capabilities of human $CD5^{+}$ B cells are unclear. Thus, the crucial criteria for determining whether human B cells subsets are homologous to the mouse B-1a, B-1b and B-2 lineages have yet to be met. In any event, the demonstration by Dorshkind and colleagues that distinct progenitors define three B cell lineages in the mouse[1] brings order to what has been a chaotic discussion and establishes a new paradigm for B cell development in the mouse. In addition, these seminal findings provide a new impetus for understanding how or whether the human immune system is similarly constructed and, on a more global level, how B cell development mechanisms came to vary so broadly during evolution.

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