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Seminars in Immunology 17 (2005) 347–355

seminars in IMMUNOLOGY

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Bone marrow microenvironmental changes in aged mice compromise V(D)J recombinase activity and B cell generation

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Abstract

B cell generation and immunoglobulin (Ig) diversity in mice is compromised with aging. Our recent work sought to understand mechanism(s) that contribute to reduced B cell production in aged mice. Using in vivo labeling, we found that reduction in marrow pre-B cells reflects increased attrition during passage from the pro-B to pre-B cell pool. Analyses of reciprocal bone marrow (BM) chimeras reveal that the production rates of pre-B cells are controlled primarily by microenvironmental factors, rather than intrinsic events. To understand changes in pro-B cells that could diminish production of pre-B cells, we evaluated *rag2* expression and V(D)J recombinase activity in pro-B cells at the single cell level. The percentage of pro-B cells that express *rag2* is reduced in aged mice and is correlated with both a loss of V(D)J recombinase activity in pro-B cells and reduced numbers of pre-B cells. Reciprocal BM chimeras revealed that the aged microenvironment also determines *rag2* expression and recombinase activity in pro-B cells. These observations suggest that extrinsic factors in the BM that decline with age are largely responsible for less efficient V(D)J recombination in pro-B cells and diminished progression to the pre-B cell stage. These extrinsic factors may include cytokines and chemokines derived from BM stromal cells that are essential to the development of B cell precursors. The changes during aging within the BM hematopoietic microenvironment most likely are linked to the physiology of aging bone. Bone degrades with age (osteoporosis) due to decreased formation of new bone by osteoblasts. Marrow stem cells (MSC) are considered the progenitor of both adipocytes, osteoblasts and hematopoietic stromal cells and a controlled reciprocal regulation exists of osteoblast versus adipocyte differentiation; with age adipocytes increase, and osteoblast decrease. It is possible that stromal cell generation from MSC is compromised during aging. Currently, understanding of BM microenvironmental factors that regulate *rag* gene expression is very limited. However, as early progenitors differentiate, it is increasing clear that a limited set of transcription factors (e.g. *ikaros*, PU.1, E2A, EBF, *pax5*) regulate B-lineage specific genes, and that expression and stability of these factors is responsive to the microenvironment. Current and future work by several groups will strive to understand mechanisms that regulate these factors and how aging impacts these regulatory circuits.

Keywords: Aging; B cell; Immunoglobulin; V(D)J recombination; Bone marrow stromal cells; Osteoporosis

1. Humoral immunity, aging and B cell development

The renewal of the immune system in mice declines during aging: B and T cell generation are reduced [1,2] and as a result, old mice are more susceptible to pathogens [3]. The human immune system is also compromised during aging, resulting in increased susceptibility to pathogens and reduced vaccine efficacy [2,4]. Given the critical role of humoral immunity for both vaccines and responses to infection, we have used murine models to ask whether the generation of the primary B cell repertoire is compromised during aging.

New B cells with a diverse immunoglobulin (Ig) repertoire must be generated throughout life to seed the peripheral B cell pool. In aged mice, the number of mature peripheral B cells is similar to that of young mice, however, the rate of turnover is lower as fewer mature B cells are generated [1]. Increasingly, evidence indicates that the age-associated decline in humoral immunity may be due in part to reduced generation of B cells and reduced Ig diversity. Previous work has in fact shown that Ig heavy chain gene V_H to DJ_H joining and Ig diversity is reduced in aged mice [1–11].

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doi:10.1016/j.smim.2005.05.012

B cell development occurs continuously during life. In adult mice, this process initiates in the bone marrow as multipotent progenitors (MPPs) derived from hematopoietic stem cells (HSC) lose myeloid potential and become common lymphoid progenitors (CLPs) (reviewed in [12–14]). CLPs are particularly efficient B cell progenitors [15–17] that progress as pre-pro-B, pro-B, pre-B and immature B cells in the bone marrow, and are then exported primarily to the spleen where they progress through stages of immature transitional B cells and develop into mature naïve B cells.

2. V(D)J recombination in B cell development

The generation of new B cells is completely dependent on the assembly of Ig genes by V(D)J recombination. In each B cell progenitor, a unique combination of V_H, D_H and J_H gene segments (one of each segment) are joined together to encode the IgH variable region, and either V_κ and J_κ or V_λ and J_λ gene segment are joined to encode the IgL variable region. Each V, D and J segment is flanked by a conserved recombination signal sequence (RSS) that are the targets of the V(D)J recombinase (reviewed in [18,19]).

The V(D)J recombinase includes the products of the recombinase activating genes, *rag1* and *rag2*. During V(D)J recombination, RAG1 and RAG2 bind to RSSs and assemble a synaptic cleavage complex (SCC) that includes two Ig gene segments (e.g. a D_H and a J_H). Following formation of the SCC, RAG1 and RAG2 introduce DNA nicks and double-strand breaks between the RSS and the flanking coding end of the Ig gene segment. After coding end processing, non-homologous end joining (NHEJ) repairs the DNA breaks. This repair requires several ubiquitously expressed NHEJ proteins: Ku70, Ku80, DNA-PK, XRCC4, and DNA Ligase IV. The two coding ends are ligated to form a coding joint, and the two signal ends are ligated to form a signal joint. In most Ig recombination events, the signal joint forms a circular piece of DNA containing the region that was originally between the two recombining gene segments and the coding joint now connects two gene segments that encode part of a new Ig variable region.

IgH and IgL genes are assembled sequentially during B cell development. During the pro-B cell stage of development, two recombination events in the IgH locus produce a rearranged, intact IgH variable region gene. First D_H to J_H recombination occurs, followed by V_H to D_HJ_H recombination. During the pre-B cell stage, a light chain gene is formed by V to J recombination of either V_κ to J_κ or V_λ to J_λ. Expression of *rag1* and *rag2* are up-regulated both prior to and during pro-B cell stage, when the IgH gene is assembled, and are again up-regulated in the pre-B cell stage when Ig light (IgL) chain gene is rearranged [20–23].

rag1 and *rag2* are the only lymphoid-specific components of the V(D)J recombinase and their coordinate expression during B and T cell development is tissue- and developmental stage-specific [19–23]. *rag1* and *rag2* are located in one locus in the genome of all animals studied and are convergently transcribed [19–21,24]. Highly conserved core promoters and *cis*-elements control transcriptional regulation of *rag1* and *rag2* [25–36]. In addition to core promoters, *cis*-acting elements 5′ of *rag2* are essential to expression of both *rag1* and *rag2* and contribute to differential regulation in B and T cells [23,37,38]. For example, the *Erag* element located 22 kb 5′ of *rag2* is essential for *rag1* and *rag2* expression in B but not T lineage cells [39].

Transit from the pro-B to the pre-B cell stage is regulated by signals emanating from the pre-B cell receptor (pre-BCR). The pre-BCR is composed of Igα and Igβ, the IgH chain, and the surrogate light chain (SLC) that itself is composed of λ5 and Vpre-B. Signaling through the pre-BCR is dependent upon the presence of a rearranged functional IgH gene, and therefore productive V(D)J recombination is a limiting step for B cell development. Expression of both *rag1* and *rag2* are essential to V(D)J recombination; therefore, in their absence an intact IgH chain is not produced and B cell development is blocked at the pro-B cell stage [40,41]. If a functional heavy chain is made, signals through the pre-BCR promote developmental changes in the B cell precursor. Expression of *rag1* and *rag2* is turned off and the cell undergoes several rounds of proliferation. This accelerates the degradation of RAG1 and RAG2, as RAG2 is phosphorylated during the cell cycle resulting in ubiquitin-dependent degradation [42,43] and RAG1 half-life is shortened when associated with RAG2 [44]. These regulatory mechanisms stop further recombination of IgH and thus contribute to allelic exclusion. In addition, proliferation mediated by the pre-BCR expands the number of pre-B cells, resulting in greater numbers of pre-B cells than pro-B cells (3:1 ratio) in young mice.

3. B cell development is attenuated in aged mice due to decreased pre-B cell generation

B cell generation and frequencies of B cell subsets undergo age-associated attenuation in mice: B cell generation is markedly reduced [1,45], and fewer mature B cells are produced. However, the number of mature B cells is similar to that of young mice due to a significant increase in the half-life of mature cells [1,4]. Frequencies and numbers of B cell progenitor subsets in the bone marrow also change with age. The most noted change is the reduced frequency and number of pre-B cells, although the extent of reduction, and age at which this occurs varies [45–54]. Initial reports indicated that numbers of pro-B are not reduced in aged mice. However, we [55] and others find that numbers of pro-B cells are significantly reduced in aged mice [16,47,51]. The onset and severity of reduced pre-B cell numbers can vary substantially between mice, and interestingly, loss of pre-B cells within individuals is well correlated with reductions in pro-B cells [51,55].

Our recent work sought to understand mechanism(s) that contribute to the reduction in numbers of pre-B cells in aged