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Research paper

Signals that drive T-bet expression in B cells

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ABSTRACT

Transcription factors regulate various developmental and functional aspects of B cells. T-bet is a recently appreciated transcription factor associated with "Age-associated B cells" or ABCs, the development of autoimmunity, and viral infections. T-bet expression is favored by nucleic acid-containing antigens and immune complexes and is regulated by interplay between various cytokines, notably, the TFH cytokines IL-21, IL-4 and IFNγ. Adaptive signals by themselves cannot upregulate T-bet; however, they have a synergistic effect on induction of T-bet by innate receptors. The functional role of T-bet+ B cells is unclear, although it is known that T-bet promotes class switching to IgG2a/c. It is likely T-bet serves dichotomous roles in B cells, promoting pathogenic autoreactive antibodies on one hand but mediating microbial immunity on the other, making it a target of interest in both therapeutic and prophylactic settings.

Host-pathogen interactions and environmental cues collectively shape the quality of primary adaptive immune responses by initiating circuits that enable effector and memory lymphocytes to provide protective immunity and react effectively to subsequent challenges. Inappropriate differentiation can result in a failure to protect the host, and can engender immune pathologies associated with autoimmunity, allergy, and chronic inflammatory disorders. Accordingly, a complete understanding of the signaling networks underlying the establishment of discrete effector and memory cell pools is key to developing effective vaccines and therapeutic strategies.

Shifts in transcriptional programs are fundamental to the direction of cell fate, and these shifts are determined by the aggregate of extrinsic signals received during activation. Herein, we briefly summarize key aspects of transcriptional regulation within the B lineage, followed by a more detailed consideration of the signals that drive antigen-experienced B cells to adopt fates associated with T-bet expression.

1. Transcription factors guide B cell differentiation and function

As in all cell lineages, B cell genesis and differentiation require turning on appropriate developmental programs and silencing those that foster other fates. Detailed reviews about the nature and interactions of transcription factors that orchestrate late B cell development can be found elsewhere [1-4], and are thus treated briefly here. The Pax5, EBF1 and E2A proteins are some of the earliest controllers that establish the transcriptional network responsible for promoting B cell development and suppressing other lineages. For example, Pax5 expression is critical for commitment to B cell fate; Pax5-deficient pro-B cells retain the potential to develop into non-B cell lineages [5,6]. Pax5 regulates the expression of many B cell surface molecules and receptors, including CD19, CD21, CD79a, as well as other relevant transcription factors like IRF4/8 and BACH2. All mature B cells continue to express Pax5, and deletion even at these mature stages yields reversion to a multipotent progenitor-like state, highlighting the role of this transcription factor in maintaining B cell character [7]. Exogenous signals that activate key transcriptional regulatory pathways also govern triage into different pre-immune B cell pools; for example, Notch-2 transcriptional activities are required for marginal zone B cell differentiation. Once a B cell is within the quiescent mature follicular (FO) or marginal zone (MZ) pools, these transcriptional programs are maintained at steady state unless activating signals are received.

2. Activation initiates transcriptional program shifts

Analogous to the differentiation of pre-immune B cells, the fates of activated B cells are also guided by shifts in transcription factor representation. In accord with the tenets of clonal selection, B cells require engagement of their antigen receptor – the BCR – to initiate activation. The immediate consequences of BCR signaling involve modification or further activation of pre-existing transcriptional regulatory systems, such as NF-κB, NFAT, and AP-1. The strength and duration of the BCR signal per se can impact eventual cell fate. For example, strong BCR signaling is associated with a higher propensity to rapidly adopt a plasma cell fate [8,9]. Despite the influence of BCR

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ligation on these relatively short-term outcomes, the ultimate fate of BCR-activated cells is also strongly influenced by additional exogenous signals. These signals include co-stimulation received during cognate T helper interactions, cytokines within the activating milieu, and signals from Pathogen Associated and Danger Associated Molecular Patterns (PAMPs and DAMPs) via innate receptors such as toll-like receptors (TLRs). The permutations, kinetics, and downstream integration of these signaling cues prompt the establishment of distinct transcription factor landscapes, which in turn drive fate choice and effector function. Two archetypical examples of this in antigen-activated B cells are Bcl6 and BLIMP1, transcription factors required for germinal center (GC) formation and GC B cell proliferation versus plasma cell (PC) differentiation, respectively. Thus, BCL6 expression is upregulated in response to cognate helper T cell interactions, and represses the activity of cell cycle regulators and molecules involved in DNA damage response. As a result, GC B cells are able to proliferate rapidly and undergo somatic hypermutation. In contrast, BLIMP1 promotes the development of plasma cells. BCL6 and BLIMP1 are reciprocally antagonistic – so BCL6/Blimp1 mutual repression is essential for B cells to commit exclusively to either GC or PC fate. These functions are clearly evidenced by the phenotype of Bcl6-deficient mice; GC development is blocked but plasma cells secreting low-affinity antibodies still develop [10].

These examples illustrate how fundamental and master transcriptional regulators act to govern major fate and differentiation choices within the pre-immune and antigen-experienced B cell pools, based on the aggregate of initiating upstream signals. While the existence of broad categories – such as GC versus plasma cell fates – have been appreciated for some time, recent findings indicate that further functional subsets exist among antigen-experienced B cells – and this diversification is similarly established through engagement of key transcriptional regulators. One such example is T-bet, encoded by the *tbx21* gene. This transcription factor was first described in helper T cells – hence the moniker “T-Box Expressed in T cells” – in studies that showed T-bet promotes IFNγ production, but suppresses IL-4 and IL-5. Thus, T-bet skews the differentiation of naive CD4 cells to a Th1 profile while repressing the Th2 program [11,12]. Subsequent studies revealed that T-bet is required for differentiation and function of effector CD8+ T cells [13], and interactions between T-bet and other transcription factors play key roles in the development immune cell subsets. For example, the T-bet versus Eomes axis is critical to CD8 effector versus memory differentiation [14-16].

It is now clear that T-bet expression defines a unique, antigen-experienced B cell subset. Early studies suggested that T-bet played a role in inflammatory cytokine production and immunoglobulin isotype switching [17,18], and more recent observations have expanded these findings to show that T-bet is a key player in determining the nature and quality of effector and memory B cell subsets. Studies from Szabo et al. established a link between T-bet expression and IFNγ production in B cells. While these authors did not examine the exact signals driving T-bet expression, they laid the groundwork for other studies that went on to identify activation requirements and cytokine circuits that are instrumental in inducing T-bet expression in B cells. Subsequent work revealed that immunoglobulin isotype switching to $IgG_{2\,a/c}$ is facilitated by T-bet [18–21], as are some instances of anti-viral and anti-bacterial immunity which, incidentally, rely on $IgG_{2a/c}$-mediated protection [22–24]. More recently, T-bet was found to be important for the emergence of age-associated B cells (ABCs), and T-bet expressing B cells have been described in a variety of infections and autoimmune scenarios. While these at first glance these may seem disparate and poorly connected phenomena, they likely provide clues to common signals that initiate the T-bet transcriptional program in activated B cells.

3. Age-associated B cells, a T-bet driven subset

The discovery of a B cell subset that accumulates with age, and also arises in infection and autoimmunity, led to questions about what transcriptional programs direct its differentiation. Phenotypically, these naturally occurring ABCs express B220, CD19, and are negative for CD43 and CD93, indicating that they are mature B2 cells. However, they lack CD23 and CD21/35, canonical pre-immune B cell markers that discriminate between FO and MZ B cell subsets. Also distinct from FO and MZ subsets, roughly half of all ABCs defined by these criteria express T-bet, and among these, about one-third also express CD11c. ABCs do not proliferate (but still survive) in response to BCR ligation in vitro. Instead, ABCs proliferate in response to endosomal TLR signals, particularly from TLR7 and TLR9. In accord with increased T-bet expression, they tend to secrete antibodies of the $IgG_{2a/c}$ isotype when activated [25,26]. Since T-bet positive B cells are a subset of ABCs, it is pertinent to summarize what is known about development of ABCs prior to addressing factors inducing T-bet expression

4. The genesis of ABCs

The origin of naturally arising ABCs remains incompletely understood, although increasing evidence suggests that most, if not all, are the result of antigen-driven activation. It remains possible that age-related alterations in B cell lymphopoiesis foster the generation of a pre-immune B cell subset with these characteristics. However, sublethal irradiation and autoreconstitution of aged mice resulted in a splenic B cell profile similar to young mice, with a marked absence of ABCs [25]. Thus, the aged bone marrow microenvironment is not fundamentally predisposed to generating ABC-like cells. Nonetheless, increasing evidence suggests that ABCs themselves may dampen overall B lymphopoiesis ([27], Riley et al. this volume). Cell cycle analysis revealed that ABCs themselves are quiescent, leading to the conclusion that they accumulate with age, rather than self-renew [25]. To explore whether ABCs can be derived from existing mature B cell subsets, FO B cells were CFSE-labelled and adoptively transferred into young congenic hosts. A month later, some of the transferred cells had divided, and those that had undergone the most exhaustive division had also acquired an ABC phenotype. Thus, ABCs can arise from pre-immune pools such as FO B cells, consistent with the notion that they reflect antigen-driven differentiation, and accumulate over time. The observation that FO B cells underwent several divisions before giving rise to ABCs led to the question of what cell intrinsic and microenvironmental requisites were necessary for this process. To address this, Russell Knode et al. modified the adoptive transfer system described above, and used donor CD23+ B cells from either MHC $II^{-/-}$ or $CD154^{-/-}$ mice. While WT donor cells proliferated, and adopted ABC characteristics ($CD23^{-}$ and $T\text{-}bet^{+}$), the knockout cells failed to do either. Additionally, aging $CD154^{-/-}$ mice did not develop ABCs [28]. Together, these observations indicate that the development of T-bet expressing ABCs from pre-immune B cells requires antigen presentation and cognate help.

These observations make it tempting to speculate that most ABCs are derived from antigen-driven events, and several further observations favor this possibility. First, our recent analyses of the Ig heavy and light chains from sorted, naturally occurring ABCs revealed a largely stochastic representation of VL and VH gene segment usage, suggesting that these ABCs reflect an aggregate of immune experiences over the life of the individual, thus drawing from the full repertoire of BCRs. Second, these analyses revealed clear evidence of somatic hypermutation among ABCs, strengthening the case for a germinal center origin [29]. Nonetheless, it is worth remembering that T-bet expression is a characteristic of only about half of $CD23^{-}CD21^{-}$ B cells. It is as yet unknown what prompts the dichotomy of T-bet expression in the mature $CD23^{-}CD21^{-}$ pool. Perhaps the overall ‘natural’ $CD23^{-}CD21^{-}$ ABC population includes both naïve and antigen experienced cells, the latter being characterized by T-bet expression (see Swain et al., this volume, for a discussion of this idea). Little is known about whether and how the T-bet positive and negative fractions differ functionally, or if T-bet expression is more easily induced in the T-bet negative fraction of