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T-bet+ B cells: A common denominator in protective and autoreactive antibody responses?

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T-bet+ B cells have emerged as a key component of the humoral immune response in both infections and autoimmune disorders, with many of their phenotypic and functional attributes conserved between mice and humans. They are protective (infections) and pathogenic (autoimmunity), although the associated commonalities and differences remain unclear. Heterogeneity within this pool, in terms of origin, fate and function may underlie these divergent roles. Their significance is context-dependent- they may constitute a persistent effector memory cell pool, or products of recent primary responses. In both cases however, T-bet+ cells likely represent antigen-experienced progenitors of antibody-secreting cells with multipotent properties. Given their key contributions to both immunity and disease, T-bet+ B cells are an attractive target for vaccination and therapeutic strategies.

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Introduction

While known for its role in antibody isotype switching since 2002 [1], an appreciation for T-bet as a marker and driver of B cells with key roles in both protective immunity and autoimmunity has emerged within the last several years. Early studies identified a population of B cells, lacking CD21 and CD23 [2] and expressing CD11c [3], which accumulated in aging mice and humans and was also detected in young female mice predisposed to autoimmunity. These age-associated B cells or ABCs were subsequently found to be associated with T-bet expression [4], and revealed as a component of both pathogen-specific and autoreactive immune responses. These dichotomous properties reflect an escalating convergence of observations in both mice and humans that span seemingly unrelated areas: microbial infection and immunity [5••,6,7,8•,9,10], aging [2,3,5••,11], and autoimmunity [3,12••,13–16]. Together, these findings have established T-bet+ B cells as important players in all of these settings, and have sparked growing interest in the origin and nature of these cells. This is evidenced by several recent reviews [17,18] and collections [19], as well as a steadily increasing frequency of primary manuscripts focused on this B cell subset. Herein, we consider the aggregate of recent findings, with emphasis on features that are either common or disparate to T-bet+ B cells in both pathogen-specific and self-reactive antibody generation.

T-bet+ B cells are antigen-experienced cells in mice and humans

A growing body of literature indicates that most, if not all, T-bet+ B cells are antigen-experienced cells and in at least some settings constitute a persistent antigen-specific effector memory B cell subset. Foremost, in the mouse, they are generated during the early expansion phase of pathogen-driven immune responses and persist indefinitely, thus fulfilling the fundamental criteria for a memory B cell population. Studies of *Ehrlichia muris* infection were among the first to identify T-bet+ B cells as a feature of pathogen-specific responses. These studies showed that CD11c+ B cells arise at the peak of infection and CD11c+ T-bet+ B cells could be detected in the spleen as late as 30 days post infection [20]. Subsequently, T-bet+ B cells have been reported in a variety of mouse and human microbial infections. In mouse models, LCMV and MHV infection lead to the appearance of persistent T-bet+ B cell populations. Moreover, a key role in viral control or clearance was shown for LCMV and murine gamma herpes virus respectively [8•,21]. Analogous populations of T-bet+ B cells have now been documented in several chronic and acute human viral infections [6,7,22,23,24•]. Although T-bet per se was not directly interrogated, the earliest studies to detect an 'atypical' memory B cell (Bmem) that in retrospect corresponds phenotypically to T-bet+ Bmem were from HIV infected individuals [10], and a similar if not identical population of Bmem are also observed in malaria infection [24•]. More recent findings show T-bet+ B cells as a consistent feature of HCV infection [7].

Current research has focused on understanding what roles T-bet+ B cells play in sustaining protective immunity. In LCMV infection these cells are implicated in control of

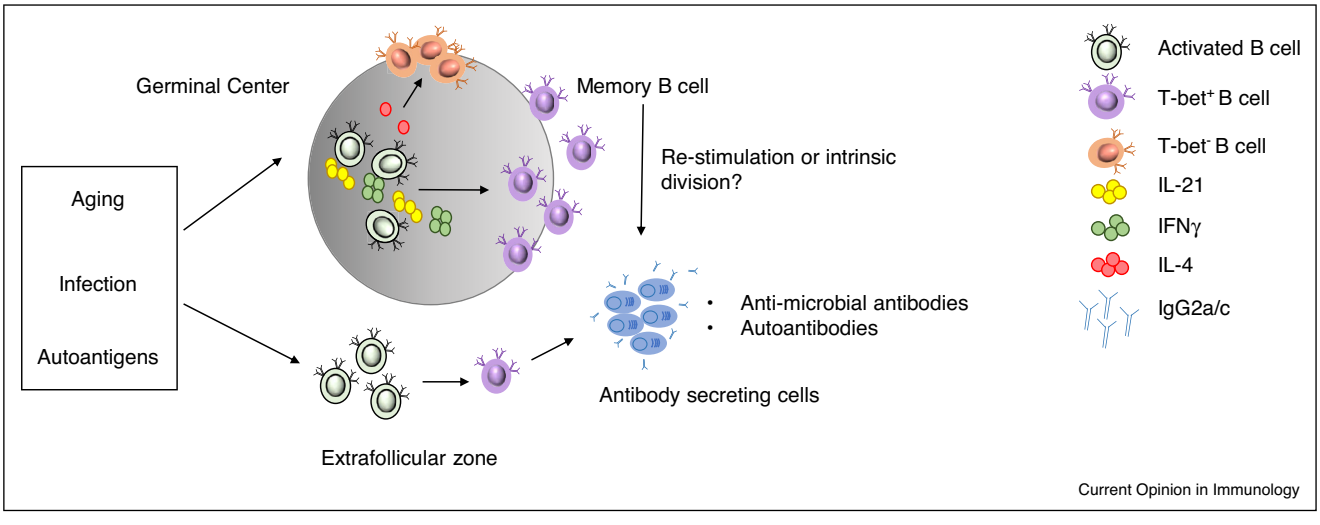
chronic infection through mechanisms that are both-dependent on and independent of viral-specific IgG2a production [8•]. T-bet$^+$ B cells that develop early on in response to *E. muris* infection give rise to IgM$^+$ memory cells that self-renew and possess stem cell-like attributes, presumably maintaining long-term immunity and responding to re-challenge [25••]. The persistent T-bet$^+$ B cells also expressed canonical memory markers CD73, PD-L2, and CD80 [26] and had little or no expression of the GC marker GL-7, suggesting that they are a subset of memory B cells and not a long-lived GC-derived population [20]. These studies provide basis for a role of T-bet$^+$ B cells by maintaining protective antibodies in response to *E. muris* infection.

Studies to date have focused on T-bet$^+$ B cells in the spleen (mice) or peripheral blood (humans). Hao *et al.* also detected ABCs in bone marrow, but not the lymphatics or peritoneum, suggesting a restricted tissue distribution [2]. Work from the Blomberg laboratory identified ABCs in murine visceral adipose tissue [27], and B cells with T-bet$^+$CD11c$^+$ transcriptional signature in human subcutaneous adipose tissue [28], reporting an association with IgG autoantibodies in both cases. These reports collectively suggest that T-bet$^+$ B cells might be anatomically restricted, similar to resident memory T cells. The implications of such sequestration are not clear. Since T-bet expression in B cells is antigen-driven, anatomical restriction could be representative of nature of antigen/signals received. Alternatively, this could be due to location-specific progenitors, such as, marginal zone B cells of the spleen or peritoneal B1 B cells. Finally, T-bet could regulate expression of integrins causing retention of B cells in a particular location and preventing/limiting their circulation.

T-bet$^+$ cells in primary effector B cell responses

In addition to their contribution to memory responses, T-bet$^+$ cells constitute the dominant component of B cell responses generated by the activation of naïve B cells and their differentiation into plasmablasts through an extrafollicular pathway [29]. This pathway has been documented in Systemic Lupus Erythematosus (SLE), a chronic relapsing autoimmune condition with prominent activation of naïve B cells during acute disease flares [30] despite the presence of strong memory responses. This informative context indicates that T-bet$^+$ cells, also bearing additional ABC markers such as upregulation of CD11c and downregulation of CD21, are particularly prominent among activated cells within the IgD$^+$CD27$^-$ naïve compartment as well as in the IgD$^-$CD27$^-$ cells (double negative; DN) frequently included within the atypical tissue-based memory compartment. A close developmental relationship between these compartments is documented by both extensive sharing of phenotypic markers; a very closely related transcriptome; and extensive BCR repertoire sharing. These studies also demonstrate that ABC-like T-bet$^+$ cells are readily generated from human naïve cells upon TLR7 and IL-21 stimulation and document that activated naïve and DN T-bet$^+$ cells are poised for plasmablast differentiation (Figure 1).

Figure 1



T-bet$^+$ B cells promote protective or pathogenic humoral immune responses: aging-related inflammation, microbial infection, or autoantigens can trigger signaling downstream of nucleic acid-sensing receptors in B cells, either in the germinal center (GC) or extrafollicularly. Together with a specific cytokine milieu favored by IL-21 and IFNγ but suppressed by IL-4, this gives rise to T-bet$^+$ B cells, which may differentiate into antibody-secreting cells or form a persistent memory subset. The latter, either via self-renewal, or subsequent re-stimulation, differentiate into antibody secreting cells to maintain protective or autoreactive antibody.

T-bet$^+$ B cells in humoral autoimmunity

Both direct and indirect evidence links T-bet$^+$ B cells with autoimmunity. Rubtsov *et al.* showed that they appear earlier in autoimmune prone mouse strains and that such cells were also in the peripheral blood within small cohorts of scleroderma and RA patients [3]. This study showed that ABCs were involved with autoantibody production both *in vitro* and *in vivo*, suggesting a pathogenic role. Interestingly, a likely analogous pool of cells was also described by Adlowitz *et al.* [16], although not using T-bet per se as the identifying marker. These cells lacked the canonical CD27 memory marker, but nonetheless had features of memory B cells and lacked CD21 expression – similar to the T-bet$^+$ ABCs in mice. More recent studies have confirmed and extended the notion that T-bet$^+$ B cells are a consistent feature of a growing list of autoimmune diseases. Wang *et al.*, in a cohort of over 200 SLE patients, showed that T-bet$^+$CD11c$^+$ B cells were elevated in SLE and the degree of expansion correlated with disease activity [12••]. Moreover, *ex vivo* stimulation of this subset revealed an enrichment for SLE-associated autoreactive antibody specificities. Thus, T-bet$^+$ B cells or their equivalents clearly play a pathogenic role in autoimmunity, in direct opposition to the protective attributes they display in infections.

Why are T-bet$^+$ B cells a feature of both protective and self-reactive immunity?

A fundamental question revolves around why this B cell subset is a feature shared by protective humoral immune memory as well as autoreactive and autoinflammatory disorders. A point of convergence is the constellation of signals that drive the T-bet$^+$ B cell fate. In general, T-bet fate is regulated by a combination of innate, adaptive and cytokine signals received during initial activation. Thus, neither BCR nor CD40 costimulatory signals — either alone or in conjunction with IL-21 and IFNγ — result in T-bet$^+$ B cells. Thus, while endogenous nucleic acid sensing TLRs, are necessary to poise naïve B cells for the T-bet$^+$ fate [4,31••,32], but alone are insufficient and must be accompanied by a promoting cytokine milieu. Among these, IL-21 and IFNγ promote T-bet, whereas IL-4 blocks its expression. These relationships have been established through cytokine receptor knockout studies both *in vitro* and *in vivo* [31••], as well as in strong associations in murine autoimmune models and human autoimmune disease [12••,13,15].

There are also areas of divergence, which are still under investigation, but may shed some light on why T-bet$^+$ B cells can mediate both protective as well as pathogenic outcomes. Importantly, T-bet$^+$ B cells may not constitute a monolithic population. For instance, CD11c expression is often associated with these cells, however, not all T-bet$^+$ B cells express CD11c. It is likely that other molecules involved in signaling, trafficking, and metabolism further subdivide the T-bet$^+$ B cell subset into multiple functionally and anatomically distinct pools. This points to heterogeneity within the T-bet$^+$ B cell pool itself, and raises two important questions: do progenitor-successor relationships exist within these pools, or do they have distinct origins, for example, extrafollicular and GC-derived? Secondly, are the various T-bet$^+$ subsets contributing to different aspects of immunological memory? It is conceivable that one subset differentiates into plasma cells while another is a persistent, self-renewing memory population that responds upon re-challenge. While T-bet expression is predominantly associated with the isotypes IgG2c in mice and IgG1 in humans, a significant proportion of these cells express IgM. This could represent another level of heterogeneity in the pool, or the IgM$^+$ T-bet$^+$ B cells could be a transient stage that is yet to switch. A recent study by Kenderes *et al.* suggests that the IgM$^+$ T-bet$^+$ B cells are a stem-cell-like memory pool, with multipotent differentiation abilities; however, this study does not address whether isotype switched T-bet$^+$ B cells behave similarly.

Infection and autoimmunity themselves differ in the kinds of signals they could deliver to progenitors of T-bet$^+$ B cells, contributing to differential origins, which may in turn influence fate and function of the T-bet$^+$ B cells thus formed. Studies by Russel Knode *et al.* support GC origins for naturally occurring and influenza-specific T-bet$^+$ B cells, however in *Ehrlichia* infection these cells could also be extrafollicular. The abundance of evidence suggests that most murine T-bet$^+$ B cells arise from germinal centers, since – with the possible exception of *E. muris* infection, all of these pathogens engender T-dependent immune responses, generally skewed towards TH1. Consistent with this view, the T-bet$^+$ B cells that accumulate with age fail to appear in CD154 knockout mice and display somatic hypermutation [5••]. In addition, T-bet$^+$ B cells that emerge from adoptively transferred naïve B cells require cell-intrinsic MHC class II and CD40 expression [5••]. Together, these features implicate cognate T cell help, AID expression and participation in a germinal center reaction – all hallmarks of antigen-experienced cells. Contrary to the above, Racine *et al.* provided evidence for extrafollicular T cell-independent CD11c$^+$ B cells representing plasmablast precursors in *E. muris* infection [9] and it is well documented that AID expression, somatic hypermutation, and at least in some cases affinity maturation, also occurs in extrafollicular B cells [33–35]. Accordingly, it is possible that this reflects subthreshold self-reactivity within the pre-immune repertoire that is established through normal B cell positive selection [36–38], but that fail to lose self reactivity as somatically mutated variants, within either GC or extrafollicular contexts [39]. Alternatively, AID has also been implicated in the negative selection of developing B cells through mechanisms involving BCR and TLR signals [40–42], so these features might reflect rescue of newly formed B cells at the immature or

transitional stages that would otherwise face such AID-mediated deletion. These are not mutually exclusive possibilities, and future studies will likley assess their merit.

Whether alternative routes of generation give rise to functionally distinct populations is unknown, but a very likely possibility. Based on the nuances of origination, a population of T-bet$^+$ B cells could be programmed to form long-lived memory B cells, while another could be a short-lived pool that transitions into plasma cells. The association between origin, fate and function is unclear, but it could point to why T-bet$^+$ B cells have opposing outcomes in infection versus autoimmunity.

Implications for translational efforts

Insights into functional attributes and divergent fates of various T-bet$^+$ B cell populations raise questions about potential clinical aspects of these cells. Memory cells are critical for protection in infectious diseases, both as a result of prior exposure as well as in response to vaccination regimens. They also mediate pathology in an autoimmune setting. Thus, understanding the cellular sources, molecular programs and antigenic triggers responsible for the generation and survival of these pathogenic cells should lead to better strategies to promote the development of protective cells and regulate pathogenic populations of T-bet$^+$ B cells is of extreme translational importance.

The triad of cytokine milieu, co-stimulation and nucleic-acid-bearing antigens is a prerequisite for inducing T-bet$^+$ expression. This, in theory, provides a way to induce antigen-specific T-bet$^+$ B cells via vaccination. It would be interesting to compare the formation of T-bet$^+$ B cells in different vaccination regimens that are known to have different protection capabilities. If vaccination efficacy strongly correlates with presence of T-bet$^+$ B cells, this would be a strong argument in favor of promoting the formation of these cells to confer protection against infections. A foreseeable challenge would be to determine vaccine formulations and adjuvants that would provide the necessary signals, particularly for different pathogens.

Another consideration for vaccine development is the timing of booster doses. Data from the Schlomchik laboratory suggests that Bmems expressing different combination of markers respond differently to re-challenge. The expression of these markers on T-bet$^+$ B cells seems more heterogenous. This would imply that booster doses might favor one functional outcome over another, based on which T-bet$^+$ B cell population is predominant at the time of administration. This idea merits further investigation as it may help to hone vaccination regimens for more targeted outcomes.

Signals regulating development of T-bet$^+$ B cells are better characterized than ways to inhibit their production and/or function. This makes it challenging to not only interrogate the role of this population in autoimmunity but also to test if disease amelioration can be achieved by its regulation. Rituximab treatment, which leads to clinical improvement in SLE patients, is accompanied by reduction of ABC-like DN B cells now known to contain a large fraction of T-bet$^+$ cells [43]. In light of current knowledge, it is likely that the elimination of these PC precursors may have contributed to eliminate the expansion of plasma cells which are not a direct target of rituximab. However, whether the reduction of the CD27$^-$IgD$^-$ population and plasma cell expansion was a consequence of factors that caused clinical improvements in patients post-rituximab treatment, or a cause of said improvement has still not been formally determined, although the association of T-bet$^+$ B cells with antibody production would argue in favor of a causal relationship. Secondly, more research is needed to identify approaches to specifically target T-bet$^+$ B cells (or block their differentiation/function) to help regulate autoimmunity.

Taken together, T-bet$^+$ B cells have emerged as a unique population that plays both protective and pathogenic roles, thereby making it an attractive tool to manipulate immune responses in the desired direction. Future studies focused on understanding the origin and functions of different subsets within this population will further help to establish how best to exploit these cells in the clinic.

Conflict of interest statement

Nothing declared.

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