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T-bet-expressing B cells during HIV and HCV infections

James J. Knox^a, David E. Kaplan^{b,c}, and Michael R. Betts^{a,*}

^aDepartment of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

^bMedicine and Research Services, Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA, USA

^cDivision of Gastroenterology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

T-bet-expressing B cells, first identified as perpetrators of autoimmunity, were recently shown to be critical for murine antiviral responses. While their role in human viral infections remains unclear, B cells expressing T-bet or demonstrating a related phenotype have been described in individuals chronically infected with HIV or HCV, suggesting these cells represent a component of human antiviral responses. In this review, we discuss the induction of T-bet in B cells following both HIV and HCV infections, the factors driving T-bet⁺ B cell expansions, T-bet's relationship to atypical memory B cells, and the consequences of T-bet induction. We propose potential antiviral roles for T-bet⁺ B cells and discuss whether this population poses any utility to the HIV and HCV immune responses.

Keywords

T-bet; B cells; HIV; HCV; Humoral immunology

1. B cells and the transcription factor T-bet

T-bet (T-box expressed in T cells) is a member of the T-box transcription factor family that promotes type 1 (Th1) immunity against intracellular pathogens and is expressed exclusively within the immune system [1]. While T-bet was first described as a critical regulator of naïve CD4 T cell differentiation into the T helper 1 (Th1) lineage, subsequent studies have identified the importance of this transcription factor in regulating development and effector functions of various cell types, including CD8 T cells, NK cells, B cells, dendritic cells, and monocytes (reviewed in detail in [1]). T-bet can drive similar gene expression programs between these different lineages by binding the same gene targets in each cell type (e.g. induction of interferon gamma (IFN γ) production; [2]). T-bet also drives separate lineage-specific Th1-associated programs in these cells (e.g. cytolytic potential in CD8 T cells but not B cells) and represses development of alternative lineages relevant for dissimilar immune

*Corresponding author. Betts@upenn.edu (M.R. Betts).

responses (e.g. Th2 response for extra-cellular pathogens; [3]). Thus, by performing its functions simultaneously across immune cell types, T-bet serves to broadly coordinate the promotion of a Th1-type response.

The earliest studies examining T-bet in B cells assessed both the stimulators and consequences of T-bet expression. T-bet expression was first identified in mouse B cells by Glimcher et al., who found that T-bet regulates IFN γ production by B cells following Th1 cytokine (IL-12, IL-18) and CD40 stimulation [4]. This B cell-produced IFN γ was suggested to play an important immunoregulatory role by promoting Th1 CD4 T cell development, and, in an analogous way, autocrine Th1-type polarization of B cells [5,6]. However, subsequent studies identified perhaps the most important B cell-specific function of T-bet: regulation of immunoglobulin (Ig) isotype switching to the IgG2a/c isotype [7,8], the signature antibody isotype of humoral Th1-type immune responses [9]. Following completion of isotype switching, IgG2a⁺ memory B cells and plasmablasts continue to depend on T-bet for their survival and functionality [10]. Altogether, T-bet appears to function as a Th1 master regulator within the B cell compartment of mice by controlling the early development of IgG2a⁺ B cells from naïve precursors and actively maintaining the integrity of mature IgG2a⁺ memory B cells.

To extend these observations to humans, our group and others have begun to characterize T-bet expression in peripheral B cells in various cohorts of human subjects [11–14]. T-bet⁺ B cells are now recognized as significant players in various immunological processes, including Th1-type immunity, autoimmune diseases, and immunosenescence. This review focuses on the role of T-bet⁺ B cells during chronic human viral infections, specifically HIV and HCV.

2. T-bet⁺ B cells as an antiviral population

The roles of T-bet in regulating IFN γ production and IgG2a class switching suggest that this transcription factor primes B cells for antiviral responses. Early *in vitro* studies of cytokines or other factors promoting B cell T-bet induction, such as IL-12, IL-18, and anti-CD40 stimulation, have further supported this hypothesis [4]. Subsequent studies have additionally identified IFN γ and IL-21 as potent inducers of T-bet expression and IgG2a isotype switching in B cells, particularly when paired with TLR7 or TLR9 stimulation [7,8,15–18]. As viral nucleic acids can stimulate TLR7 and/or TLR9, and IFN γ and IL-21 are produced by the immune system in response to viral infections, these experiments suggested that viral infection would be ideal for development of the T-bet⁺ B cell subset.

Despite this suggestive body of work, T-bet⁺ B cell involvement in specific antiviral responses was not directly demonstrated until several years later. Using the gamma herpes virus 68 (ghv68) mouse model of viral infection, Rubtsova et al. showed that T-bet⁺ B cells acutely expand, produce anti-ghv68 antibodies, and are necessary to control viremia to low levels [19]. More recently, Barnett et al. (2016) used inducible B cell-specific T-bet knockout mice to show that T-bet⁺ B cells are critical for maintaining control of chronic lymphocytic choriomeningitis infection, through both virus-specific IgG2a production and Ig-independent functions [20]. By connecting viral loads to the presence of this population,

these studies together have established T-bet⁺ B cells as an antiviral subset, with a direct role in controlling multiple viral infections, and raised the possibility that this subset is also critical for human antiviral immunity.

To identify an analogous human population, we characterized human T-bet-expressing B cells from peripheral blood and identified two primary T-bet⁺ memory B cell populations: a T-bet^{low} subset of resting memory B cells (CD21⁺CD27⁺), and a T-bet^{high} subset expressing inhibitory receptors with reduced or negative CD21 expression (CD21⁻CD85j^{high}; Fig. 1 and [13]). This latter population was characterized as a transcriptionally distinct memory B cell subset with an activated phenotype and a specific homing receptor profile (CD11c⁺CXCR3⁺; [13]). Further, we observed enrichment of IgG1 and IgG3 isotypes (human homologues of mouse IgG2a/c) in total T-bet⁺ B cells, suggesting a similar regulation of antiviral isotype switching in human B cells by T-bet and parallel roles for T-bet⁺ B cells in humans and mice [13]. In order to investigate potential involvement of T-bet⁺ B cells during human antiviral responses, our group examined the acute induction of these cells after administration of the attenuated replicating yellow fever (YFV) and vaccinia (VV) virus vaccinations in experimentally vaccinated humans [13,21,22]. Individuals receiving each vaccine demonstrated an expansion of T-bet⁺ B cells peaking between weeks three and four post-vaccination, with a concomitant reduction in population size following pathogen clearance [13]. Notably, T-bet induction was not restricted to memory B cells, as plasmablasts also transiently expressed increased levels of T-bet during the early acute response [13]. These findings establish that, like in mice, human viral infections actively drive T-bet expression in the B cell compartment.

3. B cells and HIV infection

HIV remains a global health crisis over 30 years after its initial discovery as the causative agent of AIDS [23]. The high mutation/replication rate and ability to induce systemic immunopathology enable HIV to evade host immunity and establish a chronic infection [24]. HIV is characterized by its ability to directly infect and kill CD4 T cells, but the virus also induces additional developmental and functional perturbations in multiple immune cell types [25].

From the earliest descriptions of HIV infection, B cell hyperactivity was evidenced in viremic individuals by lymphadenopathy, hypergammaglobulinemia, and increased activation marker expression, cell turnover, and cell death [26,27]. The B cell compartment is significantly impacted by HIV infection, demonstrating drastic alterations in cell phenotype, functionality, and the representation of particular subsets [27]. Many of these changes are due to the effects of excessive infection-induced cytokines and viral replication products on B cells and other immune cells that regulate B cell development [28]. HIV-induced peripheral B cell subset alterations include numerical decreases in naïve and resting memory B cells, the major B cell subsets in human peripheral blood, and overrepresentation of normally rare CD21⁻ B cell subsets, including transitional B cells, plasmablasts, and atypical memory B cell subsets (activated memory and “tissue-like” memory B cells; reviewed in detail in [28]). Hyperactivation and many of the B cell subset imbalances can be normalized by lowering immune activation with antiretroviral therapy (ART); however,

ART-treated individuals exhibit instances of deficient humoral recall responses and an incomplete restoration of resting memory B cell numbers by ART, suggesting that chronic viremia irreversibly depletes a portion of humoral memory either directly or indirectly through the loss of CD4 T cell help [27,29]. Many of these HIV infection-induced perturbations may impede the development and maintenance of protective B cell and antibody responses, contributing to chronic persistence of the virus in untreated individuals. However, it is important to note that HIV infection does in fact elicit a particularly strong and evolving humoral response that can put significant immunological pressure on the virus [30,31].

4. B cells and HCV infection

Similar to HIV, chronic hepatitis C infection is characterized by high level antigen exposure in humans, with analogous exhaustion-related changes like increased inhibitory receptor expression on T cells [32]. The influence of chronic antigen exposure on the B cell compartment is less clear, but hepatitis C-infected individuals demonstrate humoral aberrations that are similar to HIV⁺ individuals: profound hypergammaglobulinemia consisting of non-virus-specific antibodies [33,34] produced by oligoclonally-activated B-cells [35,36] and relatively late emergence of HCV humoral immunity, with antibodies to hepatitis C envelope and core proteins appearing by 6–8 weeks after infection [37–40]. A portion of these antibodies exhibit neutralizing capacity through interference with viral binding to cellular targets such as SRBI, CD81 and claudin-1 [41,42], but the presence of neutralizing antibodies is not clearly associated with early resolution of infection in chimpanzee experiments and human series [39,40,43–55]. In the absence of early resolution, evolving viral mutations drive the development of novel virus-specific B cell clones and somatic hypermutation [44,56–59]. Some of these clones include rheumatoid factor-producing V_H1-69 and V_K3-20-rearranged IgM-secreting B-cells [35,36] that clinically may present with type II cryoglobulinemia. Traditional memory B cell expansion does not occur in HCV-infected individuals [60–62], and typically the CD27⁺ memory B cell pool has been found to be similar [60] or slightly reduced in frequency [61] due to defects in proliferation [61,62], enhanced differentiation into IgG-secreting plasmablasts [61,62], compartmentalization to the liver [63], and/or enhanced memory B cell apoptosis [61,64], the latter of which is more likely related to advanced liver disease than HCV infection itself [64–66]. Instead, as in HIV, tissue-like memory B cells are expanded in HCV-infected individuals; this is discussed further below [67–69].

5. HIV and HCV promote T-bet expression in B cells

The acute HIV-specific humoral response develops in a sequential manner, with early emergence of binding antibodies and somewhat delayed development of Env-specific neutralizing antibodies [70]. Acute antibody induction suggests early involvement of B cells in the response, and given that routine vaccination with live virus vectors could induce the expansion of T-bet⁺ B cell populations in humans, it was perhaps unsurprising that we similarly found that HIV stimulates T-bet expression in both B cells and plasmablasts during the early acute phase of infection (estimated 3–4 weeks post infection; [13]). However, unlike these live virus vaccine responses, during which the pathogen is cleared and T-bet⁺ B

cell numbers decline, an abnormally large T-bet⁺ B cell population is retained into chronic HIV infection that eventually dominates the peripheral memory B cell compartment [13]. Phenotypic analyses suggest this T-bet induction accounts for the previously described activated memory and tissue-like memory B cell expansions in viremic individuals (further described below; [71–73]); however, T-bet⁺ B cell expansion involves additional subsets as well. Treatment of chronically infected viremic subjects with ART, which greatly reduces viremia and the resulting immune activation, substantially decreases the T-bet⁺ B cell population size [13]. These findings highlight that continuous viral replication and chronic immune activation may be primary factors responsible for establishing and maintaining expanded T-bet⁺ B cells in viremic individuals, particularly the T-bet^{high} subset. In addition to peripheral blood, B cell T-bet expression has also been observed in lymphoid tissue of HIV infected individuals with lymphadenopathy: Johrens et al., (2006) observed low levels of T-bet in germinal center and interfollicular B cells and high expression in a curious parafollicular population termed monocytoid B cells [74]. While the relationship between these subsets and peripheral T-bet⁺ memory B cells has not been defined, T-bet induction and maintenance in B cells occurs systemically during the course of HIV infection.

The degree to which expanded T-bet⁺ B cells are HIV-specific remains unclear. The sheer size of the T-bet⁺ population (comprising up to 80% of the memory B cell compartment in some viremic individuals) suggests bystander activation accounts for a portion of this population, and polyclonal activation of non-specific memory B cells is known to be characteristic of HIV infection [13]. However, we also found that the principal B cell response to HIV Env, the virus' surface glycoprotein and antibody target of interest, is regulated by T-bet, as nearly all memory B cells recognizing the HIV Env protein highly express the transcription factor [13]. Whether the infected individual is acutely or chronically viremic, displays natural control of infection (viremic and elite controllers), or controls viremia using ART, the HIV Env-specific B cell response is consistently dominated by T-bet-expressing cells [13]. These findings highlight T-bet as a critical mechanism regulating the HIV humoral response and identify the T-bet⁺ B cell population as an important consideration during the development of vaccines and therapeutic strategies to influence HIV humoral immunity.

Viremic HCV-infected individuals also demonstrate expansion of virus-specific atypical B cells during chronic infection, as confirmed by several groups [67–69]. Recent work confirms both CD27⁺CD21⁺ and CD27⁺CD21⁺ B cells highly express T-bet in chronic HCV-infected individuals independent of liver disease stage and generally co-express CD11c, CD95, CD267, and CXCR3, with a minority expressing the inhibitory receptor FcRL5 [20]. A unique feature of the T-bet⁺ B cells in chronic HCV infection relative to age-matched healthy donors was expression of a predominantly class-switched IgG⁺ memory phenotype. Therapeutic resolution of chronic hepatitis C infection reduced the overall frequency of T-bet⁺ B cells, particularly the IgG⁺ T-bet⁺ B cell subset. Notably, re-exposure of convalescent B cells to autologous or similar subtype virus promptly re-induced T-bet expression, further supporting a relationship between the presence of virus and T-bet induction.

6. Mechanisms underlying T-bet induction by B cells in HIV infection

The protective nature of a Th1 response to HIV was proposed over two decades ago [75], and multiple lines of evidence demonstrate significant production of HIV-induced Th1-associated cytokines that drive various aspects of Th1-type immunity (Table 1). Several of these cytokines, including IL-12, IL-15, IL-18, TNF, and IFN γ , are detectable in plasma shortly after the rise in peak viremia begins [76]. While levels of some acutely expressed cytokines wane as infection progresses, viremic individuals notably maintain elevated IFN γ levels during chronic infection [77,78]. IFN γ is a potent inducer of T-bet expression in B cells [7,18], and the early appearance and continued presence of this cytokine could promote T-bet⁺ B cell development during HIV infection. Interferon alpha (IFN α), which is produced at high levels by plasmacytoid dendritic cells (pDCs) during HIV infection [79,80], could also support T-bet⁺ B cell differentiation: IFN α supports antiviral B cell responses in mouse models [81], and is known to induce T-bet expression in human B cells and prime them for IFN γ production [82]. The HIV virus itself provides sufficient quantities of TLR7 and TLR9 ligands during infection that could indirectly support B cell T-bet induction by stimulating pDCs to produce IFN α [79,83–86]. These TLR ligands can also directly promote T-bet expression when recognized by B cells [15–18]. It remains an open question regarding the relative contribution of these and other T-bet-promoting signals to T-bet⁺ B cell development during HIV infection, and the degree to which each of these signals contributes may differ during acute versus chronic phases of infection.

The anatomical location where B cells receive these signals is also unclear. B cell responses are generally initiated in lymphoid tissue, where maturation can occur via T cell-dependent (germinal center) or –independent (extrafollicular) mechanisms [87]. T follicular helper (Tfh) cells, which provide B cell help in germinal centers, produce the cytokine IL-21 to support antibody class switching and differentiation [88]. Tfh cells in HIV- and SIV-infected lymph nodes produce IL-21 [89,90], and as IL-21 strongly induces T-bet, particularly in combination with TLR7 or TLR9 ligands [18], this cytokine may selectively promote development of T-bet⁺ B cells at the expense of other lineages. Additionally, several studies have demonstrated the capacity of Tfh cells to produce IFN γ during SIV and other viral infections [90,91]. These Tfh-derived cytokines could support T-bet⁺ B cell development locally within germinal centers; however, extrafollicular development of T-bet⁺ B cells is also possible, as a recent murine study assessing influenza humoral immunity showed the development of a protective IgG2-based antibody response arising in the absence of germinal centers. Indeed, T-bet-dependent class switching to the IgG2a isotype in mice can occur in the absence of T cell help [8]. Additionally, peripheral development of T-bet⁺ B cells from naïve or other memory precursors in non-lymphoid tissues is also possible, as HIV-induced cytokines such as IFN α and IFN γ are detectable systemically [76]. Regardless of the location, the milieu of cytokines and viral-derived products during HIV infection clearly provide multiple signals that could drive T-bet⁺ B cell differentiation, and similar mechanisms may also promote T-bet induction during HCV infection.

7. T-bet and tissue-like memory B cells

The striking induction of T-bet in B cells by HIV and HCV serves to explain several well-described anomalies in the B cell compartment in individuals infected with these pathogens. Much of the peripheral expanded T-bet⁺ B cell population during HIV infection demonstrates a distinct CD21⁺T-bet^{high} phenotype with concurrent expression of several activation markers and inhibitory receptors [13]. These cells phenotypically resemble activated memory (AM) B cells and tissue-like memory (TLM) B cells, two atypical memory populations poorly represented in healthy individuals but greatly expanded during viremic HIV infection [71–73]. As described above, AM and TLM B cells also highly express T-bet during chronic HCV infection [20]. TLM B cells have specifically been described as anergic and are hypothesized to represent an exhausted population that either formed or lost functionality due to chronic antigen stimulation, analogous to the phenomenon of T cell exhaustion [73]. The development of this potentially hypo-responsive population, whose antibodies have also been shown to bear decreased rates of antibody somatic hypermutation and *in vitro* HIV neutralization capacity compared to those derived from resting memory B cells, is considered to be a potential factor preventing humoral immunity from clearing HIV in infected individuals [73,92].

When considering the role of this population during HIV infection, a contradiction arises: TLM B cells comprise a significant fraction of the T-bet⁺ B cell population, but murine studies clearly identify T-bet⁺ B cells as critical components of a successful immune response to various viruses. There are several explanations that could potentially resolve these seemingly contradictory findings: first, it is possible that the TLM B cell subset (CD21⁺CD27⁺ phenotype) represents an aberrant developmental stage that exists at low levels even in non-inflamed individuals and is relatively dysfunctional regardless of infection status; in this scenario, different T-bet⁺ B cell subsets would mediate antiviral immunity. However, our findings from acute yellow fever and vaccinia vaccination show infection-induced activation of the T-bet⁺ population's TLM subset, suggesting TLM B cells actively participate in the acute antiviral response [13]. Conversely, T-bet⁺ B cells with a TLM phenotype may effectively participate in acute viral infections, but specifically undergo exhaustion and become dysfunctional if they are abnormally maintained for an extended period of time, such as in the context of chronic HIV and HCV infections.

However, a third possibility also exists: T-bet-expressing TLM B cells and other T-bet^{high} subsets may not be dysfunctional per se and instead represent a distinct type of antigen-experienced B cell that is weakly responsive to traditional *in vitro* stimuli (i.e. BCR and CD40 stimulation) and occupies a different biological niche compared to classical resting memory B cells. In support of this possibility, murine studies show that, unlike classical memory B cells, T-bet-expressing B cells respond poorly to B cell receptor (BCR) stimulation and instead are particularly responsive to TLR7 and TLR9 stimulation, particularly when paired with cytokines such as IFN γ or IL-21 [18]. Analyses of TLM B cells have largely failed to assess the TLR ligand/cytokine stimulation combinations known to efficiently activate T-bet⁺ B cells and may therefore misrepresent the cells' responsiveness. Comparative analyses further support this hypothesis, as the phenotype of human T-bet^{high} B cells (including both TLM and activated memory subsets) during chronic

HIV infection is nearly identical to those during yellow fever and vaccinia responses and the small populations of T-bet⁺ B cells in healthy individuals at rest, and this similarity extends to transcriptional analyses as well [13]. The presence of similar T-bet⁺ B cells (including TLM phenotype cells) in individuals with vastly different immunological environments and outcomes suggests that T-bet⁺ B cells are a normal component of the immune compartment that becomes activated and expands in response to viral infection.

Aside from TLM B cells themselves, antibodies derived from HIV-infected individuals' TLM cells have been described as inferior, demonstrating decreased somatic hypermutation and HIV neutralization capacity compared to resting memory cells; however, further studies are necessary to determine whether this trend is consistent across infections or specifically related to altered B cell help during HIV infection. For example, antibodies derived from *Plasmodium falciparum*-specific TLM B cells (including the T-bet⁺ populations) are more highly mutated and show comparable neutralization capacity in relation to antigen-specific resting memory B cell counterparts [93], and T-bet⁺ B cells in mice demonstrate significant levels of somatic hypermutation [94]. TLM B cells tend to be enriched for polyreactivity and are expanded in several autoimmune diseases, suggesting this population may contribute to pathology [14,92,95]; however, polyreactivity has also been hypothesized to enhance antiviral humoral immunity and may be a beneficial property during antiviral responses [96]. Lastly, with regard to the aforementioned points, it becomes difficult to extrapolate from TLM B cell findings as most TLM B cell studies are complicated by CD21⁺CD27⁺ population heterogeneity: we and others identified significant differences between the T-bet⁺ and T-bet[−] subsets that together comprise the TLM B cell population [13,97], but studies have generally assessed the TLM population as a whole. Accordingly, additional studies are necessary to further define the stimuli that best activate T-bet⁺ TLM B cells, the relationship of this population during HIV infection to its counterpart in non-inflamed individuals, and any potential role for T-bet⁺ TLM B cells during HIV.

8. Ramifications of viral-induced T-bet expression for humoral immunity

T-bet regulates hundreds of gene targets in T cells, altering characteristics such as cytolytic capacity, cytokine production, and trafficking potential [1], and T-bet may function to a similar extent in B cells. Therefore, HIV and HCV's induction and maintenance of T-bet in B cells likely reshapes the character of the B cell compartment, directly impacting B cell functionality and the quality of humoral immune responses as a whole. Antibody isotype modification represents perhaps one of the primary consequences of T-bet induction, as T-bet plays a critical role regulating isotype switching and in human B cells is associated with IgG1 and IgG3 antibody isotypes [13]. During chronic viremic HIV infection, the continuous stimulation of a T-bet response results in isotype changes to the antibody repertoire, potentially affecting both HIV-specific and non-specific clones: the frequencies of IgG1⁺ and IgG3⁺ memory B cells are increased in viremic individuals compared to HIV-negative donors, and this biasing extends to soluble antibody as plasma IgG1 and IgG3 titers are also significantly higher [13]. The cells from which this plasma IgG1 and IgG3 derives is unclear, but plasmablasts, the antibody-secreting cells present in peripheral blood, also express T-bet at elevated levels in viremic individuals and may contribute to increased production of these serum antibodies [13]. These observations suggest viremic HIV-infected

individuals harbor a Th1-biased antibody isotype repertoire, and this biasing also extends to virus-specific antibodies as numerous studies have demonstrated that the vast majority of HIV Env-specific serum antibodies express the IgG1 isotype, with IgG3 being the second most common [13,98–103]. Our study used a soluble form of the HIV Env protein, gp140, to identify Env-specific B cells and simultaneously examine isotypes of soluble Env-specific antibodies in chronically infected individuals. We connected the T-bet-driven Env-specific B cell response with a gp140 serum antibody response dominated by the IgG1 isotype, suggesting that T-bet serves to coordinate a Th1-focused Env-specific antibody response during HIV infection [13].

In addition to isotype switching, a murine study and ours suggest, via RNA expression analyses, that T-bet may regulate additional factors influencing antibody properties [13,20]. Transcript of activation-induced cytidine deaminase (AID, or *AICDA* as transcript), a B cell-specific enzyme critical for both antibody isotype switching and somatic hypermutation, was highly expressed in both human and mouse T-bet⁺ B cells [13,20], and we demonstrated, using siRNA knockdown of T-bet in primary human peripheral B cells, that *AICDA* transcript levels are diminished in T-bet's absence [13]. *AICDA* expression is tightly regulated by a number of factors [104], but these findings suggest T-bet promotes expression of *AICDA* and, in doing so, could indirectly support further antibody mutation to potentially improve affinity. Indeed, while mutation rates of human T-bet⁺ B cells have not been thoroughly assessed, surface-expressed antibodies on murine T-bet⁺ B cells display evidence of hypermutation [94]. A role for T-bet promoting antibody maturation would be of interest to HIV vaccine efforts, as broadly neutralizing antibodies to HIV, which develop after years of infection in only a portion of individuals and can neutralize diverse viruses, often display abnormally high mutation rates [105]. Increased or chronic AID expression is likely required to produce the extensive mutation levels described, so maintenance of T-bet expression may facilitate development of broad neutralization in these individuals. RNA analyses in mice and humans also identified differential expression of several enzymes regulating antibody glycosylation in T-bet⁺ B cells [13,20]. Antibody glycosylation patterns have not been directly interrogated in T-bet⁺ B cells, but transcriptional analyses suggest T-bet could potentially alter Fc region glycosylation, which strongly influences antibody functionality by impacting interactions with immune effector cells and even antibody half-life [106]. Additional studies are necessary to understand the degree to which T-bet influences antibody development and maturation within the human immune system.

T-bet additionally regulates the phenotype and function of B cells themselves. Expression of the homing receptors CD11c and CXCR3 is regulated by T-bet [13,19,107], conferring T-bet⁺ cells the ability to traffic into tissues and sites of inflammation where viral replication occurs. While our study did not assess cytokine production, mouse studies suggest T-bet⁺ B cells may be capable of producing Th1-associated cytokines during HIV such as IFN γ [6]. In doing so, T-bet⁺ B cells could help support a Th1-skewing of the immune response and directly promote antiviral gene expression in infected cells. T-bet⁺ B cells may also support the immune response via antigen presentation, as murine T-bet-expressing B cells demonstrate the ability to efficiently present antigen to T cells in the spleen [108]. Finally, evidence from the murine T-bet⁺ B cell response to ghv68, combined with intermediate RNA expression levels of several plasmablast- and plasma cell-associated transcription

factors (*PRDM1*, *IRF4*, and *XBPI*) in human T-bet⁺ B cells, suggests T-bet⁺ B cells may be primed to quickly differentiate into antibody-secreting cells and directly contribute to the antibody response [13,19]. T-bet⁺ B cells may be functionally heterogeneous depending upon their diverse tissue locations and immunological contexts, and future studies should address whether human T-bet⁺ B cells can execute the spectrum of functions described in mice.

9. T-bet⁺ B cells: contributing members of the antiviral response?

The excess numbers of T-bet⁺ B cells during HIV and HCV infections, their dominance of the HIV Env-specific response, and T-bet's potentially extensive impact on the character of humoral immunity raise an important question: do T-bet⁺ B cells meaningfully participate in HIV and HCV immune responses? Currently, there is no direct evidence for or against a protective role for T-bet⁺ B cells during HIV (or HCV) infection, which would be best assessed by specifically depleting T-bet-expressing B cells in a macaque model of SIV infection. However, as any humoral immunity-mediated control of HIV would likely derive from T-bet-expressing B cells and plasmablasts [13], it is informative to consider the more general role of B cells and antibodies in HIV control.

The degree to which humoral immunity impacts HIV viremia is unclear, but several lines of evidence support a role for B cells and antibodies in restricting HIV. Early studies assessing viral mutation and antibody diversity demonstrated neutralizing antibodies drive viral escape mutations [30,31], highlighting the extensive pressure placed on the virus by antibodies. The virus' ability to ultimately outpace the antibody response with these mutations and prevent clearance is often interpreted as proof of a dysfunctional humoral response; however, several studies suggest the virus is unable to completely evade this antibody response [109,110]. As such, instead of providing sterilizing immunity to HIV, humoral immunity may help to maintain low viremia: macaque studies assessing the effects of B cell depletion on SIV viral load demonstrated loss of viral control following depletion [111,112], although another produced conflicting results [113]. While B cell depletion in humans is generally not feasible, an interesting case study of a chronically infected HIV⁺ individual requiring rituximab (CD20⁺ cell depletion) for lymphoma treatment demonstrated a rise in plasma viremia following B cell depletion and concurrent decline of neutralizing antibody titers [114]. By directly tying the presence of B cells and neutralizing antibodies to maintenance of steady-state plasma viremia, these studies suggest that the humoral immune response helps to establish and preserve a balance between host and HIV/SIV. Lastly, while natural control of viremia is considered to be mediated mostly by CD8 T cells, humoral immunity may contribute in certain cases [115,116]. In summary, antibodies put significant pressure on the virus and appear to play a role in maintaining at least partial control of HIV viremia.

T-bet-regulated B cell and antibody responses could mediate control via a number of mechanisms: biased development of IgG1 and IgG3 isotype antibodies that efficiently engage immune effector cells, potential enhancement of antibody somatic hypermutation to enhance neutralization breadth, antibody-independent mechanisms such as cytokine production and antigen presentation that could promote T cell immunity to the virus, and acquisition of specific cell trafficking properties to position T-bet⁺ cells at peripheral sites of

inflammation. Future studies should delineate protective functions mediated by T-bet⁺ B cells during HIV and HCV infections and potential failings of this subset that might hinder chronic viral immunity.

10. Conclusion

Together, both murine and human studies identify T-bet⁺ B cells as contributors to viral immunity. T-bet⁺ B cells appear to be the primary humoral immune component activated by acute viral pathogens, and their activation is likely mediated by TLR ligands in combination with cytokines such as IFN γ and IL-21 and concurrent BCR stimulation *in vivo* (Fig. 2). Based on their temporal dynamics during acute infection and highly activated phenotype, we propose that human T-bet^{high} B cells represent the B cell compartment's effector subset, analogous to effector T cells that quickly respond to infection and efficiently mediate effector functions. The effector functions of T-bet^{high} B cells could include the switching from an early IgM-focused response to IgG1 and IgG3 antibody isotypes, efficient differentiation into antibody-producing cells, support of T cell immunity via cytokine production and antigen presentation, and potentially additional functions that have not yet been identified. Upon antigen clearance, this antigen- and/or inflammation-dependent population diminishes in size and may seed long-lived, resting or tissue-resident clones in preparation for antigen re-encounter. However, during chronic infection, T-bet⁺ B cells are instead maintained and begin to dominate the memory compartment as B cell differentiation is consistently shifted into this developmental stage by chronic antigen stimulation and inflammation. Still, this abnormally expanded population maintains much of the virus-specific memory B cell response and is the likely contributor to any beneficial humoral immunity acquired during HIV and HCV infections. Future efforts to understand mechanisms regulating humoral immunity to HIV and HCV should focus on elucidating the basic biology of this under-appreciated subset, as harnessing and manipulating T-bet⁺ B cells may hold promise for interventions to improve immunity to HIV, HCV, and other difficult viral infections.

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Fig. 1.

T-bet expression by B cell subsets of a healthy human donor. (A) Total B cells, defined by CD19 expression, from the peripheral blood mononuclear cells of an HIV-negative, HCV-negative human donor are depicted. Gates display the identification of naïve (black), resting memory (blue), and CD21-negative (purple) B cell subsets. (B) CD21-negative B cells are separated into CD85j high (red) and CD85j low (grey) subsets. (C) Histogram depicting T-bet expression of the aforementioned B cell subsets.

Fig. 2.

Model proposing the induction and differentiation of T-bet-expressing B cells by viral infections. T-bet is induced in B cells following TLR/cytokine/BCR stimulation during acute viral infection, leading to T-bet^{high}CD21⁻CD85 β ^{high} subset development. Upon viral clearance (yellow fever virus (YFV) and vaccinia virus (VV) vaccinations), the blood T-bet^{high} subset contracts in size, potentially due to trafficking of cells into tissues, differentiation into a resting phenotype (T-bet^{low}), or cell death. However, during chronic HIV and HCV infections, persistent viral replication and immune activation induces expansion and maintenance of a large T-bet^{high} B cell pool. Individuals cured of HCV offer a unique opportunity to assess the fate of HCV-induced T-bet⁺ B cells following antigen clearance.

Table 1

HIV infection broadly promotes a Th1 immune response. Various lines of evidence supporting a skewed Th1 immune response to HIV are listed.

Phenomenon	References
Th1 → Th2 switch hypothesis, proposing Th1 response as protective	[75]
Acute production of Th1-associated cytokines: IFN γ , TNF, IL-12, IL-15, IL-18	[76,117–119]
Chronic production of Th1-associated cytokines: IFN γ , TNF	[76,120]
Production of Th1 cytokines by Tfh cells during SIV	[90]
Biasing of humoral response to Th1-associated antibodies (IgG1 and IgG3)	[13,98–103]
Th1-type CD4 T cell differentiation	[121–123]
Induction of cytotoxic CD8 T cells	[124–127]