**Transcriptional regulation and T cell exhaustion**

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**Purpose of review**
This review highlights the control of transcriptional networks, including induction of inhibitory receptors, by T cell-specific transcription factors in exhausted T cells that accumulate in chronic viral infections including HIV.

**Recent findings**
Transcriptional profiling has established distinct molecular phenotypes for exhausted CD4+ and CD8+ T cells in chronic viral infection models. There exists a subset of transcription factors associated with exhaustion, notably Blimp-1, basic leucine zipper transcription factor, ATF-like and Helios. Epigenetic phenomena are likely important in regulating gene expression networks during exhaustion as illustrated by programmed death 1 promoter methylation patterns.

**Summary**
Following chronic viral infections, CD4+ and CD8+ T cells defined functionally and phenotypically as exhausted have distinct transcriptional profiles. These studies have identified a core set of transcription factors that have been implicated in promoting exhaustion. However, no single factor appears to be an exhaustion determining factor, suggesting that T cell exhaustion reflects a combinatorial mechanism with multiple transcription factors interacting to influence the development of functionally exhausted T cells as well as different T effector populations.

**Keywords**
exhaustion, gene regulation, HIV, transcription factors, transcriptional profiling

**INTRODUCTION**
The adaptive immune response is usually insufficient to control HIV infection, replication, dissemination and host progression to AIDS. As with other chronic viral infections, T cell exhaustion is implicated in the persistence of HIV. Exhaustion is a hierarchical process that results in diminished T cell effector functions, fewer polyfunctional T cells and eventual deletion of antigen-specific T cells [1,2]. Exhaustion is also characterized by the expression of inhibitory receptors, including programmed death 1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), Tim-3 and lymphocyte activation gene-3 (LAG-3) [3]. The induction of inhibitory receptors as well as the repression of effector molecules during chronic infection suggests that exhaustion involves complex regulation of gene networks coordinated by key signalling events and transcription factors that influence cell cycle, maturation and T cell function. This review will focus on recent work examining the factors responsible for the coordinated control of gene expression during the establishment of T cell exhaustion with an emphasis on T cell dysfunction in HIV infection.

**Transcriptional regulation of exhaustion markers**
Coordinated gene expression is a combinatorial mechanism that includes the recruitment of transcription factors to cis-acting elements, such as promoters and enhancers, RNA polymerase II processivity and efficient transcription elongation, as well as epigenetic modifications to the DNA, nucleosomes and other regulators of chromatin organization. The factors and events regulating inhibitory receptor expression have not been well defined. For example, the promoters and other cis-elements for these genes as well as the transcription factors that target these elements have not been carefully characterized. There are several reports, too many...
to highlight in detail for this review, describing promoter polymorphisms for PD-1, Tim-3 and CTLA-4 that correlate with increased susceptibilities to autoimmune diseases and cancers [4–6]. However, the mechanisms by which these polymorphisms contribute directly to disease remain unclear. These reports in sum do suggest the possibility that even modest changes in the expression of these inhibitory receptors can lead to T cell dysfunction and disease.

DNase 1 hypersensitivity mapping identified putative transcriptional elements upstream of PD-1. The most critical cis-element for PD1 expression binds the inducible transcription factor NFATc1 [7]. Furthermore, histone marks associated with transcriptional activity such as H3 histone acetylation and H3K4 trimethylation were increased in the promoter region in cells expressing PD-1 [7], indicating that chromatin organization limits PD-1 expression. The importance of epigenetic regulation of the PD1 gene was also suggested by mapping the methylation pattern of guanine and cytosine rich (GC-rich) regions in the PD-1 promoter. HIV-specific PD1⁺ T cells had unmethylated DNA within the upstream cis-elements compared with naive T cells that did not express PD-1 [8**]. Interestingly, this methylation pattern was independent of HIV viral load, with the PD-1 promoter remaining hypomethylated despite several years of successful antiretroviral treatment. This suggests that long-term epigenetic reprogramming of the PD-1 gene during chronic HIV infection is maintained and contributes to persistent T cell exhaustion. As for other markers of exhaustion, even less is known about the elements that regulate these genes. NFAT has been shown to positively regulate CTLA-4 transcription [9] and TIM-3 is induced by the Th1 differentiation factor T-bet, the latter raising the possibility of a negative feedback loop that balances T cell activation with inhibitory pathways associated with tolerance and exhaustion [10].

Transcriptional profiling of exhausted CD8⁺ cells and key transcription factors

What signals and transcriptional events control the development of T cell exhaustion downstream of inhibitory receptor expression during chronic infection remains an outstanding question. Although specific transcription factors such as FoxP3, T-bet, GATA-3 and RORγT have been associated with the differentiation of Treg, Th1, Th2 and Th17 lineages, respectively [11], no ‘master regulator’ of T cell exhaustion has been described. Recent efforts have employed transcriptional profiling to characterize gene networks and critical regulators of CD8⁺ T cell exhaustion. Studies in mouse models of chronic infection have shown that exhausted CD8⁺ T cells display characteristic gene expression patterns in broad domains of cellular function that are clearly distinct from that of other populations such as memory T cells and anergic T cells [3,12**]. On the basis of the relative abundance of transcripts, several candidate transcription factors have been identified, including Pbx3, Eomes, Prdm1, NFATc1 [12**] and basic leucine zipper transcription factor, ATF-like (BATF) [13], which may be centrally important in coordinating gene expression networks in exhausted CD8⁺ T cells (Table 1) [14–17, 18**,19,20**,21,22*,23–25]. Furthermore, FOXO3a, through its ability to regulate apoptosis, negatively regulates CD8 T cell activation, expansion and survival following lymphocytic choriomeningitis virus (LCMV) infection in mice. Knocking out FOXO3a improves viral control by decreasing apoptosis and increasing the number of polyfunctional antigen-specific CD8⁺ T cells [21,12**–25]. Experiments that directly establish FOXO3a as part of a regulatory network controlling T cell exhaustion have not been performed. Although confirmation of these ‘exhaustion-promoting factors’ is still required, some candidates have emerged as critical regulators of exhaustion.

Blimp-1 (Prdm1) was initially recognized for its role in regulating terminal differentiation of B cells into antibody-secreting plasma cells [14] but more recently has been appreciated as a regulator of T cell maturation [15]. It is critical for the generation of CD8⁺ effector memory T cells [16]. For example, CD8⁺ T cells from Blimp-1 deficient mice secrete reduced amounts of effector cytokines and granzyme, exhibit compromised cytotoxic activity and traffic poorly to peripheral tissues when responding to secondary challenges [26]. Blimp-1 also coordinates the expression of other T-lineage transcription factors such as T-bet, Eomesodermin and Bcl-6 [17]. The role of Blimp-1 in exhaustion is supported by lower expression of several inhibitory receptors in Blimp-1 deficient T cells and the rescue of effector
functions in exhausted CD8⁺ T cells from mice with a conditional deletion of Blimp-1 [27]. Blimp-1 may be part of a negative feedback loop in which its expression is induced by T cell receptor signalling and interleukin (IL)-2 stimulation [16] and its actions limit IL-2 secretion and other effector functions during persistent antigen exposure. Importantly, Blimp-1 is elevated in T cells from patients with chronic HIV infection compared with long-term nonprogressors [28*] and is induced in T cells primed by HIV-pulsed dendritic cells in association with other markers of exhaustion, including PD-1, Tim-3, LAG-3 and CTLA-4 [29]. Additional studies are required to identify the genes that are targeted by Blimp-1 and its impact on CD8⁺ T cell function in HIV-infected patients.

Experiments with HIV-positive individuals have implicated the transcription factor BATF in CD8⁺ T cell exhaustion during chronic HIV infection [13]. BATF belongs to a family of basic leucine zipper transcription factors that are part of the larger AP-1 family factors. BATF can dimerize with AP-1 subunit Jun to inhibit AP-1 target gene activation [19]. The suspected role of BATF in exhaustion is based on the observation that it is upregulated in response to PD-1 signalling. Interestingly, individuals with progressive HIV infection (progressors) have elevated levels of BATF compared with individuals who are able to control their infection. Similar to observations in HIV-positive patients, BATF expression correlates with PD-1 expression in murine models of chronic viral infection. Overexpression of BATF inhibits T cell proliferation and effector cytokine secretion, whereas knocking down BATF in CD8⁺ T cells from HIV progressors restores these functions [13]. However, BATF impacts the maturation of multiple T cell lineages. It is necessary for Th17 generation [30] and plays an important role in follicular helper T cell and Th2 development [31]. BATF also promotes CD8⁺ T effector cell differentiation via Sirt1-mediated histone acetylation to upregulate T-bet in response to IL-12 stimulation [32]. In the LCMV chronic infection model, BATF induces transcription factors that drive an effector phenotype such as T-bet and Blimp-1 while inhibiting interferon-gamma (IFN-γ) and granzyme B expression. BATF-deficiency leads to smaller effector T cell populations in viral infection resulting in higher viral loads, which can be reversed by BATF complementation [20**]. Thus, BATF may provide a checkpoint during T cell maturation that ensures sufficient stimulatory signals are present to merit a full-fledged effector T cell response; but when antigen exposure is prolonged, BATF may belong to a conglomeration of factors that favours T cell exhaustion.

It is important to point out that studies have failed to describe a unique transcription factor or set of factors that drive CD8⁺ T cells to exhaustion. As illustrated by Blimp-1 and BATF, these factors have overlapping expression patterns and influence multiple T cell lineages. Blimp-1 and BATF expression correlates with expression of inhibitory receptors but also are required for memory and T effector activities. Even T-bet, the ‘master regulator’ of Th1 cells, is required for T cell exhaustion. These apparently contradictory findings indicate that transcription factors are often both positive and negative regulators and their activity is dependent on the cell type, levels of expression, intracellular localization and posttranslational modifications. Moreover, they may function at target sites in a combinatorial fashion. For example, BATF binds widely in CD8⁺ effector cells, often in combination with interferon regulatory factor-4 (IRF4) [20**,33]. Accordingly, BATF DNA binding is decreased in IRF4⁻/⁻ T cell systems, indicating that these two transcription factors often work in coordination to mediate transcription in T cells [33]. Taken together, these transcription factors would be well

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**Table 1.** Transcription factors implicated in T cell exhaustion

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>Expression in exhausted T cell</th>
<th>Target genes in exhaustion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blimp-1 (Prdm1)</td>
<td>Upregulated</td>
<td>T-bet, Bcl-6, Eomes</td>
<td>[12*,14–17]</td>
</tr>
<tr>
<td>T-bet</td>
<td>Downregulated low expression</td>
<td>Tim-3</td>
<td>[10,18**]</td>
</tr>
<tr>
<td>Pbx3</td>
<td>Upregulated</td>
<td>N.D.</td>
<td>[12**]</td>
</tr>
<tr>
<td>Eomes</td>
<td>Upregulated</td>
<td>N.D.</td>
<td>[12**,18**]</td>
</tr>
<tr>
<td>BATF</td>
<td>Upregulated</td>
<td>T-bet, Blimp-1, Id-2, Eomes, cytokine receptors</td>
<td>[13,18**,19,20**]</td>
</tr>
<tr>
<td>Klf4</td>
<td>Upregulated</td>
<td>N.D.</td>
<td>[18**]</td>
</tr>
<tr>
<td>Foxo3a</td>
<td>N.D.</td>
<td>Proapoptotic genes</td>
<td>[21,22*,23–25]</td>
</tr>
<tr>
<td>NFATc1</td>
<td>Upregulated</td>
<td>PD-1, CTLA-4</td>
<td>[7,9,12**]</td>
</tr>
<tr>
<td>Helios</td>
<td>Upregulated [exhausted CD4⁺ cells]</td>
<td>N.D.</td>
<td>[18**]</td>
</tr>
</tbody>
</table>

N.D., not determined.
positioned to balance the generation of effector populations with the necessary negative signals associated with exhaustion to properly initiate and resolve immune activation.

**CD4+ T cell exhaustion**

Functional CD4+ T cells are critical for CD8+ antiviral responses and have been demonstrated to be protective against CD8 exhaustion [3,34,35]. Through direct infection and abortive infection-mediated pyroptosis [36], HIV decreases the number of CD4+ cells [3], and CD4+ T cell populations also show signs of exhaustion during chronic infections including diminished production of IFNγ, tumour necrosis factor-alpha (TNFα) and IL-2 [3] and elevated expression of PD-1, LAG-3 and CTLA-4 [37–41]. In order to identify transcriptional networks and pathways associated with CD4+ T cell exhaustion, the Wherry group did genome-wide transcriptional profiling of antigen-specific CD4+ T cells in the LCMV model of chronic infection. In general, these studies described a heterogeneous population of CD4+ cells that share many common features with exhausted CD8+ cells, but are clearly distinct in their expression of gene categories, phenotypic markers of exhaustion and transcription factors [18]. Several factors were expressed in both exhausted CD4+ and CD8+ T cells, including Eomes, BATF, Klf4 and Blimp-1 (Table 1). T-bet expression was diminished but was necessary to retain the CD4+ exhaustion phenotype. The most highly regulated factor was the Ikaros family member Helios. Although the mechanism by which Helios influences CD4+ T cell exhaustion is unclear, it may be inhibiting IL-2 or IL-12 receptor expression by sequestering Ikaros or inducing epigenetic changes associated with transcriptional repression [42].

Transcriptional profiling also identified that Blimp-1 is highly upregulated in exhausted CD4+ T cells. Recently, Blimp-1 expression has been suggested to inversely correlate with HIV expression. CD4+ T cells from individuals with chronic HIV infection have significantly higher levels of Blimp-1, PD-1 and reduced IL-2 expression, a target of Blimp-1 transcriptional repression [28]. Blimp-1 has also been described to be associated with lower levels of HIV expression in different CD4+ memory T cell populations from nonprogressors [43]. Expression of Blimp-1 transcripts is targeted and limited by microRNA miR9. These findings, in addition to what is known of Blimp-1 function in exhausted CD8+ T cells, support a prominent role for Blimp-1 in T cell exhaustion, although exactly how it influences the course of HIV infection has not been resolved [16]. Furthermore, in addition to regulating specific cytokine networks and the expression of inhibitory receptors in CD4+ T cells, it is tempting to speculate that Blimp-1 is directly influencing HIV transcription and replication. We have observed that Blimp-1 binds within the HIV genome and represses HIV transcription [44]. It is unclear whether other factors associated with T cell exhaustion impact HIV transcription.

**CONCLUSION**

The lack of specific exhaustion T cell factors and the overlapping expression pattern of transcriptional regulators in multiple T cell subsets suggest that the determination of the exhaustion phenotype is a combinatorial process dependent on intracellular location, protein–protein interactions, posttranslational modifications, factor stoichiometry and epigenetic phenomena. Having an array of transcriptional regulators that participate in determining cell fate permits multiple pathways and checkpoints to integrate the range of signals coming from TCR signalling, costimulatory and inhibitory receptors and cytokines to assure a balanced and controlled immune response. It is interesting that many of the factors implicated in exhaustion, such as Blimp-1, T-bet and BATF, function as activators and repressors and determine the fate of multiple T lineages. Although this appears contradictory, especially upon reviewing the literature, it would be consistent if these transcription factors function as gatekeepers of T cell activation versus T cell suppression. Chronic infections, such as HIV, can alter the function of these transcriptional regulators, enhance the expression of inhibitory receptors and polarize cells towards exhaustion. Validating the role of specific transcription factors in exhaustion and determining their targets and mechanisms of action will provide general insights into the establishment of exhaustion as well as how viral infections mediate immune failure and viral persistence. Furthermore, understanding the biochemical processes that coordinate the gene networks activated and repressed during exhaustion may allow for better vaccine design and identify novel drug targets to eradicate persistent HIV infection.

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**Conflicts of interest**

*There are no conflicts of interest.*
REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

9. This study describes DNA methylation patterns at the PD-1 promoter in CD8+ T cells from infected patients.
14. This study demonstrates functional CD4+ T cell exhaustion.
22. This study demonstrates how FOXO3 can influence the size of T cell populations in the context of a chronic infection.
27. This study describes DNA methylation patterns at the PD-1 promoter in CD8+ T cells from infected patients.
30. This work provided evidence using cells from HIV-infected individuals that PD-1 expression correlates with Blimp-1 and that in long-term nonprogressors, a microRNA acts as a negative regulator of Blimp-1.
38. This study provides insights as to how aborted HIV infection eliminates CD4+ T cells.
41. This work described DNA methylation patterns at the PD-1 promoter in CD8+ T cells from infected patients.
43. A study using transcriptional profiling describes in detail the molecular characterization of exhausted CD4+ T cells compared with CD4+ memory cells and exhausted CD8+ T cells.
46. This study demonstrates that BATF has a dual role in promoting T cell receptor dependent induction of transcription factors but limiting the expression of effector molecules interferon-gamma (IFN-gamma) and granzyme B.
48. This study demonstrates how FOXO3 can influence the size of T cell populations in the context of a chronic infection.