nature medicine

Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF

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CD8+ T cells in chronic viral infections such as HIV develop functional defects including loss of interleukin-2 (IL-2) secretion and decreased proliferative potential that are collectively termed 'exhaustion'[1]. Exhausted T cells express increased amounts of multiple inhibitory receptors, such as programmed death-1 (PD-1)[2,3], that contribute to impaired virus-specific T cell function. Although reversing PD-1 inhibition is therefore an attractive therapeutic strategy, the cellular mechanisms by which PD-1 ligation results in T cell inhibition are not fully understood. PD-1 is thought to limit T cell activation by attenuating T cell receptor (TCR) signaling[4,5]. It is not known whether PD-1 also acts by upregulating genes in exhausted T cells that impair their function. Here we analyzed gene expression profiles from HIV-specific CD8+ T cells in individuals with HIV and show that PD-1 coordinately upregulates a program of genes in exhausted CD8+ T cells from humans and mice. This program includes upregulation of basic leucine transcription factor, ATF-like (BATF), a transcription factor in the AP-1 family. Enforced expression of BATF was sufficient to impair T cell proliferation and cytokine secretion, whereas BATF knockdown reduced PD-1 inhibition. Silencing BATF in T cells from individuals with chronic viremia rescued HIV-specific T cell function. Thus, inhibitory receptors can cause T cell exhaustion by upregulating genes—such as *BATF*—that inhibit T cell function. Such genes may provide new therapeutic opportunities to improve T cell immunity to HIV.

We hypothesized that inhibitory receptors such as PD-1 function to inhibit T cells not only by reducing TCR signaling but also by inducing the expression of genes that impair T cell function. To test this hypothesis, we analyzed gene expression profiles from HIV-specific CD8+ T cells for upregulation of PD-1–induced genes.

The majority of individuals infected with HIV show chronic elevation of viral load in the absence of antiretroviral therapy (progressors), which is associated with defects in HIV-specific T cell cytokine secretion, proliferation and survival[6,7]. In contrast, spontaneous control of viral replication has been documented for a small minority of individuals (controllers)[8]. Analysis of CD8+ T cell responses to HIV in progressors and controllers therefore allows a comparison of populations of human antigen-specific T cells at the extremes of functional competence.

We sorted CD8+ T cells specific for epitopes from the Gag protein (hereafter termed HIV-specific CD8+ T cells) from 18 progressors and 24 controllers (**Fig. 1a**, **Supplementary Fig. 1** and **Supplementary Table 1**). The gene expression profiles of HIV-specific CD8+ T cells from progressors showed marked differences compared to those from controllers ($n = 518$ genes, moderated t statistic <-2.0, **Fig. 1b** and **Supplementary Table 2**). Genes upregulated in HIV-specific CD8+ T cells from progressors were enriched for those involved with the interferon response and major histocompatibility complex expression (**Supplementary Table 3**), consistent with a higher viral load in progressors. HIV-specific CD8+ T cells from controllers were enriched for genes involved in mRNA transcription and protein translation, consistent with previous observations of defects seen in the mouse model of chronic lymphocytic choriomeningitis virus (LCMV) infection[9] (**Supplementary Table 4**). We therefore compared the expression profiles of HIV-specific CD8+ T cells to exhausted LCMV-specific CD8+ T cells from the mouse model[9]. Using an analytic technique called gene set enrichment analysis[10–12] (**Supplementary Methods**), we analyzed the expression profiles of mouse virus-specific CD8+ T cells during infection with each of

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Received 13 July; accepted 7 September; published online 3 October 2010; doi:10.1038/nm.2232

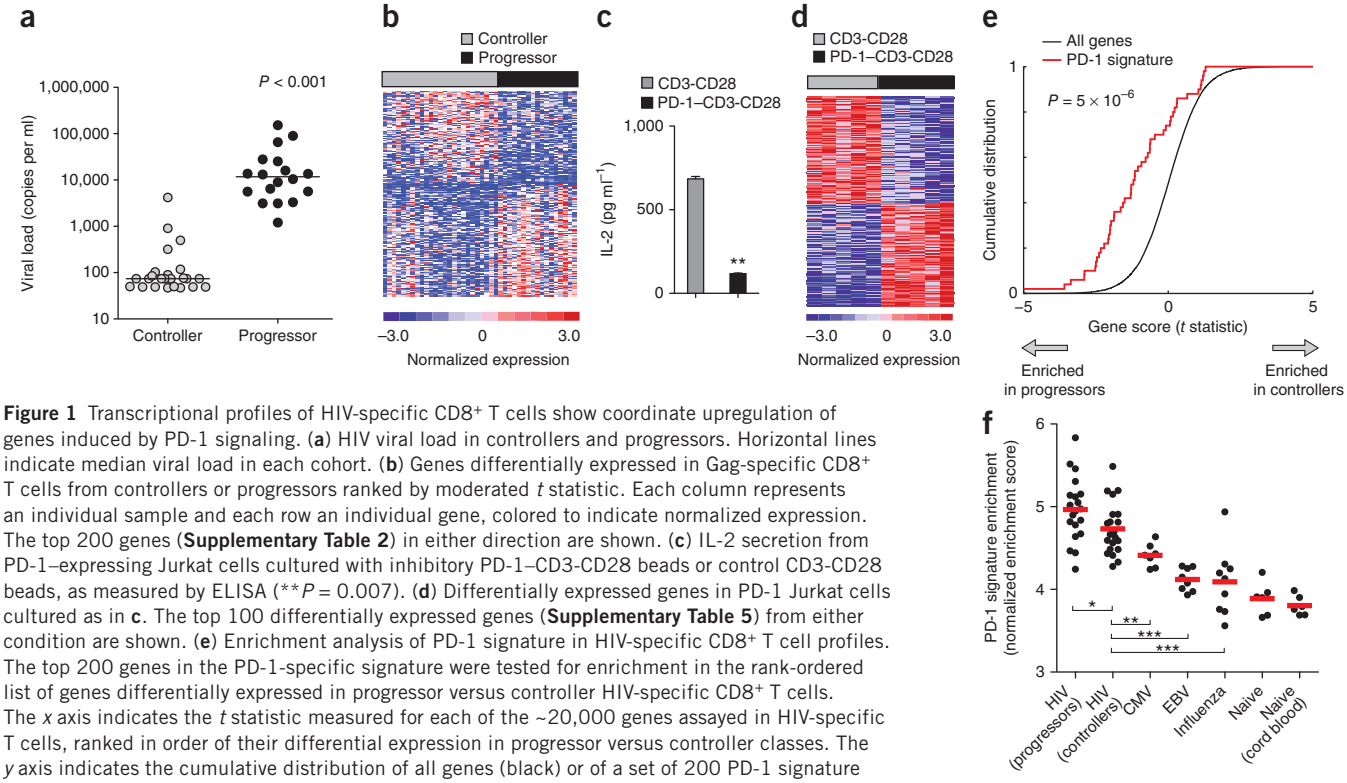

Figure 1 Transcriptional profiles of HIV-specific CD8+ T cells show coordinate upregulation of genes induced by PD-1 signaling. (**a**) HIV viral load in controllers and progressors. Horizontal lines indicate median viral load in each cohort. (**b**) Genes differentially expressed in Gag-specific CD8+ T cells from controllers or progressors ranked by moderated *t* statistic. Each column represents an individual sample and each row an individual gene, colored to indicate normalized expression. The top 200 genes (**Supplementary Table 2**) in either direction are shown. (**c**) IL-2 secretion from PD-1–expressing Jurkat cells cultured with inhibitory PD-1–CD3-CD28 beads or control CD3-CD28 beads, as measured by ELISA (**$P = 0.007$). (**d**) Differentially expressed genes in PD-1 Jurkat cells cultured as in **c**. The top 100 differentially expressed genes (**Supplementary Table 5**) from either condition are shown. (**e**) Enrichment analysis of PD-1 signature in HIV-specific CD8+ T cell profiles. The top 200 genes in the PD-1-specific signature were tested for enrichment in the rank-ordered list of genes differentially expressed in progressor versus controller HIV-specific CD8+ T cells. The *x* axis indicates the *t* statistic measured for each of the ~20,000 genes assayed in HIV-specific T cells, ranked in order of their differential expression in progressor versus controller classes. The *y* axis indicates the cumulative distribution of all genes (black) or of a set of 200 PD-1 signature genes (red). Gene sets that are related to the class distinction on the *x* axis would be expected to deviate from the dotted line (that is, shifted toward the left if enriched in profiles of CD8+ T cells from progressors). (**f**) Enrichment of PD-1 signature in tetramer-sorted CD8+ T cells specific for various human viral pathogens. PD-1 signature genes were tested for enrichment by single-sample enrichment analysis in gene expression profiles from sorted tetramer+CD8+ T cells specific for the indicated pathogens or in naive CD8+ T cells. Each point represents the relative enrichment of PD-1 signature genes in an individual sample. The *y* axis indicates normalized enrichment score (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ by Wilcoxon ranked-sum test).

two strains of LCMV: clone 13 (Cl13), which gives rise to chronic infection with T cell exhaustion, and Armstrong (Arm), an acute infection that does not cause T cell exhaustion[9,13]. We found that the HIV progressor signature was significantly enriched in the profiles of exhausted LCMV-specific CD8+ T cells from Cl13 infection ($P = 4.8 \times 10^{-5}$, **Supplementary Fig. 2**), suggesting a global similarity between the transcriptional profiles of exhausted CD8+ T cells in humans and in the mouse model.

We next asked whether this exhausted CD8+ T cell signature was influenced by PD-1 signaling. To determine this, we first identified the genes upregulated after PD-1 ligation. We incubated PD-1–expressing Jurkat cells with beads coated with a cross-linking antibody to PD-1 together with antibodies to CD3 and CD28 (PD-1–CD3-CD28 beads); or with beads coated with equivalent amounts of control antibody together with CD3 and CD28 (CD3-CD28 beads). Incubation with PD-1–CD3-CD28 beads resulted in significantly decreased production of IL-2 compared to cells incubated with CD3-CD28 beads ($P = 0.007$, **Fig. 1c**), as previously observed[14,15]. Microarray analysis identified over 1,000 genes that were significantly upregulated in cells functionally inhibited by PD-1 ($n = 1179$, $t > 2.0$, **Fig. 1d** and **Supplementary Table 5**). A similar number of genes had reduced expression after PD-1 ligation ($n = 1{,}361$, $t < -2.0$, **Fig. 1d** and **Supplementary Table 5**). We validated 13 representative genes that were upregulated in PD-1–ligated Jurkat cells. Incubation of human CD4+ T cells with PD-1 ligand-immunoglobulin fusion protein (PD-L1–Ig)–CD3-CD28 beads led to the coordinate upregulation of these representative PD-1 signature genes in a PDL1-Ig dose-dependent manner (**Supplementary Fig. 3**). Thus, ligation of PD-1 in CD3- and CD28-stimulated cells induces a specific transcriptional program in both Jurkat cells and primary human T cells.

We sought to determine whether the transcriptional program induced by PD-1 signaling defined *in vitro* could be detected in gene expression profiles from exhausted CD8+ T cells *ex vivo*. We therefore tested whether PD-1–induced genes were coordinately upregulated in HIV-specific CD8+ T cells from HIV progressors. By enrichment analysis, we found that a set of PD-1 signature genes was significantly upregulated in the HIV progressors compared with controllers ($P = 5 \times 10^{-6}$, **Fig. 1e**). Similarly, we found that PD-1 signature genes were significantly upregulated in exhausted LCMV-specific CD8+ T cells from Cl13 infection compared with Arm infection ($P = 2 \times 10^{-4}$, **Supplementary Fig. 4**). Thus PD-1 ligation results in upregulation of a consistent pattern of genes in exhausted CD8+ T cells in humans and mice.

The upregulation of PD-1 signature genes in exhausted CD8+ T cells contrasted with that seen in profiles of human virus-specific CD8+ T cells associated with functional T cell responses. Using single-sample gene set enrichment analysis (**Supplementary Methods**), we found that the PD-1 signature was significantly more enriched in HIV-specific CD8+ T cells than in antigen-specific CD8+ T cells specific for cytomegalovirus (CMV) ($P < 0.01$), Epstein-Barr virus (EBV) ($P < 0.001$) or influenza virus ($P < 0.001$) from healthy HIV-uninfected donors (**Fig. 1f**). Notably, the PD-1 signature was significantly more enriched in HIV-specific T cells than in EBV-specific T cells, despite the fact EBV-specific T cells express PD-1 (refs. 16,17). This suggests that the upregulation of PD-1 signature genes may not occur in all cells that express PD-1 but may reflect

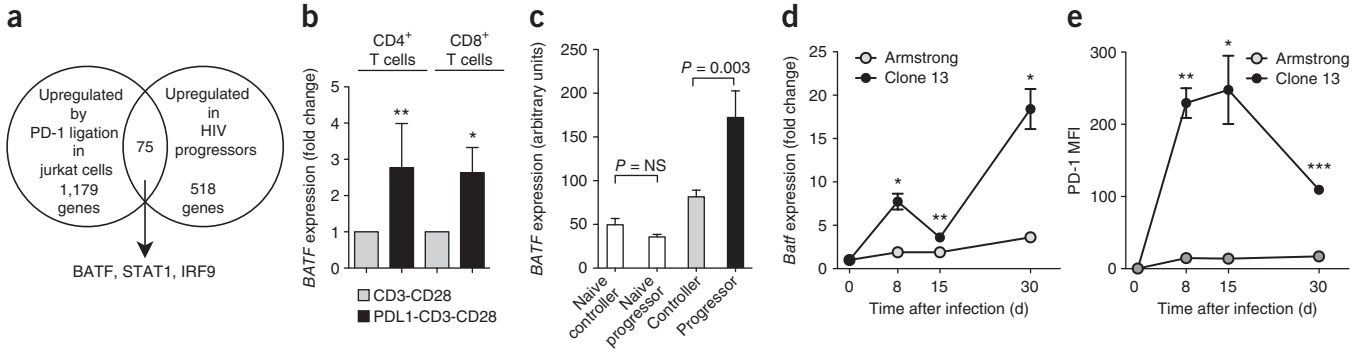

Figure 2 Expression of BATF is upregulated by PD-1 and increased in exhausted T cells. (**a**) A Venn diagram representation of three transcription factors upregulated in Gag-specific T cells from HIV progressors and Jurkat cells after PD-1 ligation ($t > 2.0$). (**b**) *BATF* expression measured by real-time quantitative PCR in primary human CD4+ and CD8+ T cells cultured with CD3-CD28 beads or PD-L1–Ig–CD3-CD28 beads for 4 d. Data represent independent experiments with four to ten normal donors and are shown as expression relative to the CD3-CD28 bead condition (**$P = 0.001$; *$P = 0.02$; paired t test). (**c**) Relative *BATF* expression in arbitrary expression units from Affymetrix analysis of sorted naive ($CD62L^+CD45RA^+$) or HIV Gag-specific CD8+ T cell populations from controllers and progressors. (**d**) *Batf* expression measured by real-time quantitative PCR in LCMV-specific CD8+ T cells from mice infected with LCMV Armstrong or LCMV clone 13 relative to naïve mice (*$P < 0.05$; **$P < 0.01$). (**e**) PD-1 expression on LCMV-specific CD8+ T cells measured by flow cytometry after infection with the indicated viruses (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Error bars represent means ± s.e.m.

increased strength and duration of PD-1 signaling experienced by CD8+ T cells in the setting of chronic infection.

We reasoned that genes upregulated by PD-1 in exhausted CD8+ T cells might include those involved in the inhibition of T cell function. To refine the list of candidate genes, we identified genes that were both upregulated by PD-1 ligation in Jurkat cells and upregulated in HIV progressors (**Fig. 2a** and **Supplementary Table 6**). We focused on transcription factors because of their broad effect on the cell state. Of the 75 genes common to both gene sets, only three encoded transcription factors: *BATF*, signal transducer and activator of transcription-1 (*STAT1*) and interferon regulatory factor-9 (*IRF9*) (**Fig. 2a** and **Supplementary Table 6**). We selected BATF for further analysis because it has been shown to function as a negative regulator of AP-1 activity[18,19]. Moreover, we have previously observed it to be upregulated during CD8+ memory differentiation in humans and mice[20], suggesting that it may have a conserved role in regulating T cell function. *BATF* expression showed a two- to threefold increase in both primary human CD4+ and CD8+ T cells after incubation with PD-L1–Ig–CD3-CD28 beads compared with CD3-CD28 beads, indicating that *BATF* expression is increased by PD-1 ligation *in vitro* ($P = 0.001$ and $P = 0.02$, respectively, **Fig. 2b**).

We observed high *BATF* levels in antigen-specific T cells with the greatest degree of dysfunction (**Fig. 2c**,**d**). *BATF* expression measured by microarray was significantly higher in exhausted HIV-specific CD8+ T cells from progressors than in HIV-specific T cells from controllers ($P = 0.003$, **Fig. 2c**). However, *BATF* expression was not significantly different in naive CD8+ T cells from controllers or progressors (**Fig. 2c**). A significant correlation existed between microarray and RT-PCR measurements of *BATF* expression ($r = 0.53$, $P = 0.02$, Spearman's rank test, **Supplementary Fig. 5**).

To define the kinetics of *Batf* expression after infection with a persistent virus, we compared *Batf* expression in mouse virus-specific CD8+ T cells during acute and chronic LCMV infection (**Fig. 2d**). As early as day 8 after infection, epitope GP33 (DbGP33)-specific CD8+ T cells in LCMV Cl13 infection expressed significantly higher amounts of *Batf* than in Arm infection ($P = 0.02$). *Batf* expression was maintained at higher levels in virus-specific cells in Cl13 infection at day 15 and by day 30 was approximately sevenfold higher than in DbGP33-specific CD8+ T cells generated during Arm infection ($P = 0.02$). The higher expression of *Batf* during chronic versus acute infection was coincident with the upregulation of PD-1, as GP33-specific T cells in Cl13 infection showed increased levels of both PD-1 and *Batf* by day 8 (**Fig. 2d**,**e**). Increased *Batf* expression is therefore an early and persistent feature of exhausted CD8+ T cells in the setting of chronic viral infection *in vivo* and temporally correlates with upregulation of PD-1.

We next tested whether BATF could inhibit T cell function. Overexpression of BATF in primary human T cells (**Supplementary Fig. 6a**) markedly reduced proliferation in response to CD3-CD28 beads compared to vector-transduced cells ($P = 0.002$, **Fig. 3a**,**b**). Apoptosis was also slightly increased in BATF-overexpressing cells after stimulation (29% versus 20%, $P = 0.013$, **Fig. 3a**,**b**), consistent with the previously defined role of PD-1 signaling in reducing cell survival[21]. However, the majority of BATF-overexpressing cells were viable, suggesting that reduced proliferation was not simply from cell death. Overexpression of BATF also significantly reduced IL-2 secretion after CD3 and CD28 stimulation, ($P = 4.5 \times 10^{-5}$) but was not overtly toxic, because interferon-γ (IFN-γ) secretion was not significantly reduced compared with vector controls (**Fig. 3c**). Thus, increased expression of BATF reduces proliferation and IL-2 secretion in primary human T cells.

Enforced expression of BATF *in vitro* did not increase the expression of PD-1 or of two other inhibitory receptors (CD244 or CD160) in CD8+ or CD4+ T cells (**Supplementary Fig. 6b–e**). This suggests that BATF does not mediate inhibition by modulating the expression of these inhibitory receptors. Future studies will be required to determine whether BATF regulates other components of the PD-1–induced expression signature.

We next asked whether depletion of BATF enhances T cell function by using shRNA-mediated gene silencing (**Fig. 3d**,**e**). Compared with control hairpins, depletion of BATF in Jurkat cells with two different shRNA sequences (**Fig. 3d**) significantly increased *IL2* expression in cells cultured with PD-1–CD3-CD28 beads ($P < 0.01$, **Fig. 3e**), reversing the IL-2 inhibition to levels seen in cells stimulated with CD3-CD28 beads. Testing of additional hairpin sequences showed that there was a strong correlation between the extent of knockdown and the degree of increase in IL-2 secretion, confirming the on-target specificity of BATF silencing ($r = -0.82$, $P = 0.056$, Spearman's rank test; **Fig. 3f**).

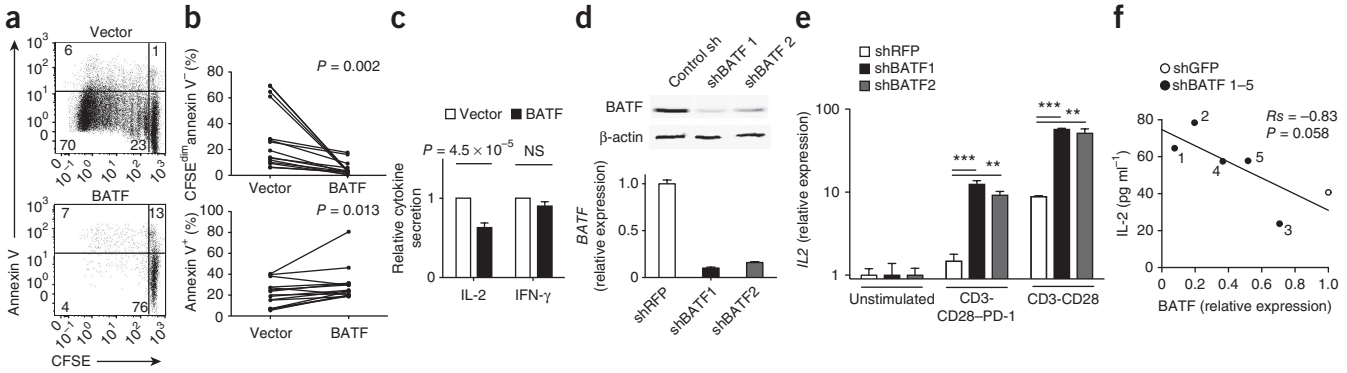

Figure 3 BATF inhibits T cell function. (**a**) FACS analysis of carboxyfluorescein succinimidyl ester (CFSE)-labeled primary human CD4$^+$ or CD8$^+$ T cells from healthy volunteers transduced with a lentivirus expressing BATF (bottom) or with control vector (top) and cultured for 4 d with CD3-CD28 beads. (**b**) Summary data of proliferation (percentage CFSE^{dim}annexin V$^-$, top) and cell death (percentage annexin V$^+$, bottom) in primary human CD4$^+$ or CD8$^+$ T cells (n = 14) transduced as in **a** and cultured for 4 d with CD3-CD28 beads. (**c**) IL-2 and IFN-γ secretion by primary human CD4$^+$ T cells (n = 10) transduced as in **a** and cultured with CD3-CD28 beads. Data are shown normalized to the empty vector condition. NS, not significant. (**d**) *BATF* expression in PD-1–expressing Jurkat cells lentivirally transduced with shRFP (control) or two separate shBATF sequences measured by western blotting (top) or quantitative PCR (bottom). (**e**) *IL2* expression by PD-1 Jurkat cells transduced with shRFP or shBATF cultured with no beads or either PD-1–CD3-CD28 or CD3-CD28 beads for 18 h. Data show *IL2* expression (± s.e.m.) measured by quantitative PCR (***$P < 0.001$; **$P = 0.01$). Data are normalized to the gene encoding β-actin and are presented as fold change with respect to unstimulated conditions. (**f**) Correlation between BATF silencing and IL-2 secretion in PD-1–expressing Jurkat cells transfected with five sequence-independent shBATF constructs or a control hairpin and cultured with PD-1–CD3-CD28 beads. BATF expression was measured by quantitative PCR and presented as fold change relative to control hairpin. Error bars represent means ± s.e.m.

BATF silencing also increased IL-2 expression in cells stimulated with CD3-CD28 beads without exogenous PD-1 cross-linking ($P < 0.01$, **Fig. 3e**), suggesting that pathways in addition to the PD-1 pathway can inhibit cell activation via BATF. Silencing BATF may therefore have the effect of increasing IL-2 expression not only by relieving PD-1–mediated inhibition but also by impairing other negative feedback pathways. Consistent with this, the expression of BATF across 42 samples of HIV-specific CD8$^+$ T cells significantly correlated with expression of several receptors with known or putative inhibitory function (**Supplementary Fig. 7**)[22,23].

Finally, we tested whether silencing BATF would improve the function of HIV-specific T cells. We assessed HIV-specific T cell function after BATF knockdown (**Fig. 4a**,**b**) by measuring cytokine secretion or proliferation in response to Gag peptides. BATF knockdown caused a significant increase in CD8$^+$ Gag-specific IFN-γ secretion (**Fig. 4c**) compared to a control siRNA pool, increasing IFN-γ secretion by an

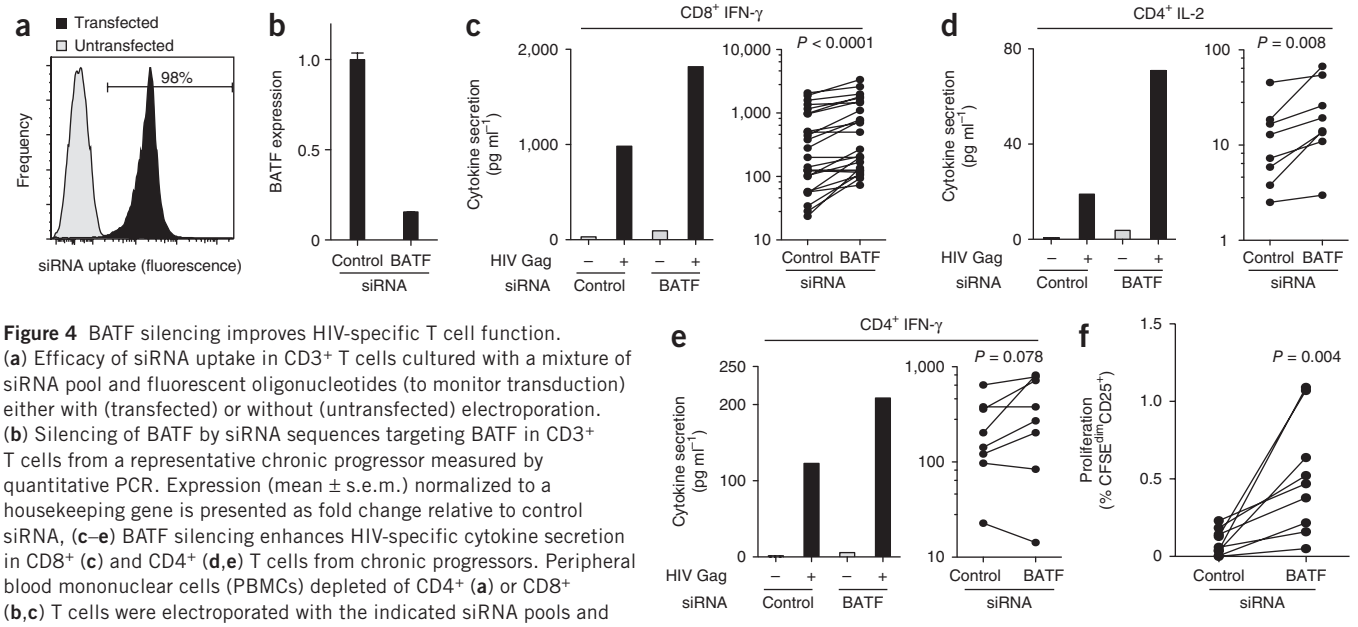

Figure 4 BATF silencing improves HIV-specific T cell function. (**a**) Efficacy of siRNA uptake in CD3$^+$ T cells cultured with a mixture of siRNA pool and fluorescent oligonucleotides (to monitor transduction) either with (transfected) or without (untransfected) electroporation. (**b**) Silencing of BATF by siRNA sequences targeting BATF in CD3$^+$ T cells from a representative chronic progressor measured by quantitative PCR. Expression (mean ± s.e.m.) normalized to a housekeeping gene is presented as fold change relative to control siRNA, (**c**–**e**) BATF silencing enhances HIV-specific cytokine secretion in CD8$^+$ (**c**) and CD4$^+$ (**d**,**e**) T cells from chronic progressors. Peripheral blood mononuclear cells (PBMCs) depleted of CD4$^+$ (**a**) or CD8$^+$ (**b**,**c**) T cells were electroporated with the indicated siRNA pools and cultured with or without HIV Gag peptides for 4 d, and IFN-γ (**c**,**e**) or IL-2 (**d**) amounts were measured by a highly sensitive cytokine bead assay. In each panel, the left graph shows results from a representative subject, and the right graph shows summary data (CD8$^+$ responses, 26 HIV epitope responses in four subjects; CD4$^+$ responses, HIV Gag peptide pool in seven subjects). Cytokine amounts shown are adjusted for background secretion, and statistical significance was evaluated with the paired t test. (**f**) Proliferation of CFSE-labeled CD8$^+$ T cells, as measured by the fraction of CFSE^{dim}CD25$^+$ cells 6 d after transfection and peptide stimulation of PBMCs. Data represent nine HIV epitope-specific responses in four subjects.

average of 60% ($P < 0.0001$). We observed similar results in HIV-specific CD4$^+$ T cells, where silencing BATF caused a twofold increase in Gag-specific IL-2 secretion ($P = 0.008$, **Fig. 4d**) and a trend toward an increase in IFN-γ secretion ($P = 0.078$, **Fig. 4e**). HIV-specific CD8$^+$ T cell proliferation was also increased by BATF knockdown, with a fivefold increase in the fraction of proliferating cells incubated with Gag peptides ($P = 0.004$, **Fig. 4f**). Reducing BATF expression therefore increases the function of exhausted HIV-specific T cells.

We show that exhausted T cells specific for HIV in humans and for LCMV in mice share a common expression signature that reflects the transcriptional consequences of PD-1 receptor ligation. Our data therefore suggest a model in which inhibitory receptors such as PD-1 may mediate T cell exhaustion not only by limiting TCR signaling but also by inducing the expression of proteins such as BATF that inhibit T cell function.

BATF is a highly conserved member of the AP-1 family, a group of transcription factors that regulate many aspects of cellular function in the immune system[24]. Recent studies show that BATF is required for T helper type 17 and follicular T helper cell differentiation[25,26]. BATF may therefore be one of a number of transcription factors, such as B lymphocyte–induced maturation protein-1 (refs. 27,28), that have distinct, context-dependent roles both in regulating the function of T cells responding to chronic viral infection and in CD4$^+$ lineage decisions. Our studies do not identify the mechanism by which PD-1 ligation induces BATF upregulation, or whether this happens by direct or indirect pathways. However, our findings give impetus to further studies of how BATF regulates T cell state.

Blockade of PD-1–PDL-1 interactions partially reverses T cell dysfunction[16,17] and improves control of viral replication[2,29], indicating that the function of exhausted T cells can be rescued even in settings of viral persistence. BATF and the pathways that control its activity may provide new opportunities to reverse CD8$^+$ T cell exhaustion. These findings suggest that integrated genomic analysis of T cell responses in humans can be used to identify new regulators of T cell function that are potential therapeutic targets for improving T cell immunity in chronic infection.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Accession codes. Data have been deposited in Gene Expression Omnibus with accession code GSE24082.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We would like to thank the subjects for taking part in the study; E. Cutrell, B. Baker, K. Moss, A. Rathod and C. Brume for coordinating sample management; and A. Sharpe, T. Golub, G. Lauer, M. Altfeld and H. Joffe for valuable discussions. This work was supported by US National Institutes of Health grants AI082630, AI56299, HHSN26620050030C and HL092565, the International HIV Controllers Study and the Foundation for the National Institutes of Health through the Grand Challenges in Global Health Initiative.

AUTHOR CONTRIBUTIONS

M.Q. designed and performed experiments, analyzed data and helped write the paper. F. Pereyra designed the clinical components of the study. B.N. and J.L.J. designed and performed computational experiments. F. Porichis, D.S.K., J.Z. and D.E.K. designed and performed siRNA experiments in samples from subjects with HIV. C.F., Q.E., B.J., K.B., S.I., K.R., I.T., A.P.-T., D.D. and L.F. all performed experiments. G.J.F. designed experiments and developed PD-L1–Ig. J.A., A.C., H.S. and E.J.W. designed and performed mouse experiments and analyzed data. W.N.H., B.E. and B.D.W. conceived of the study and designed the experiments. W.N.H. analyzed data and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. Subjects were recruited from outpatient clinics at local Boston hospitals, or referred from providers throughout the US, following institutional review board approval (Partners Institutional Review Board) and with written informed consent. HIV controllers included elite controllers (n = 20) with HIV RNA below the level of detection for the respective available ultrasensitive assay (<75 copies per ml by DNA or <50 copies per ml by ultrasensitive PCR); and viremic controllers (n = 4) with HIV RNA levels <2,000 copies per ml. Chronic progressors (n = 18) were defined as having HIV RNA levels above 2,000 copies (**Supplementary Table 1**). All subjects were off therapy and had detectable HIV-specific $CD8^+$ T cells in the peripheral blood, allowing a median of 21,500 HIV Gag $tetramer^+$ T cells (range 3,00 –85,000 cells) to be isolated for microarray analysis from each subject.

Flow cytometry and sorting. PBMCs were isolated by density centrifugation and were stained with a cocktail of antibodies to exclude irrelevant lineages (CD4, clone 13B8.2, Beckman Coulter; CD14, RMO52, Beckman Coulter; CD19, J3-119, Beckman Coulter; CD56, NCAM16.2, BD Biosciences) and dead cells (annexin V, BD Biosciences), antibody to CD8 (clone SFCI21Thy2D3, Beckman Coulter) and major histocompatibility complex class I HIV Gag-specific tetramers (generated in the Walker laboratory) to identify the antigen-specific populations, and antibodies against CD62L (clone DREG56, Beckman Coulter) and CD45RA (clone 2H4LDH11LDB9, Beckman Coulter) for memory phenotype characterization of the tetramer-positive fraction. $CD8^+tetramer^+$ cells were sorted with a FACSAria Cell Sorter (BD Biosciences). All experiments examining proliferation via CFSE dilution and survival via annexin V (BD Biosciences) staining were collected on a FC500 flow cytometer (Beckman Coulter). Analysis of flow cytometry data was carried out with FlowJo software (version 8.8.6, Tree Star).

Microarray data acquisition and analysis. Tetramer-sorted human $CD8^+$ T cells or Jurkat cells after 18 h of bead stimulation were pelleted and resuspended in TRIzol (Invitrogen). RNA was extracted with the RNAdvance Tissue Isolation kit (Agencourt). Concentrations of total RNA were determined with a Nanodrop spectrophotometer or Ribogreen RNA quantification kit (Molecular Probes/Invitrogen). RNA purity was determined by Bioanalyzer 2100 traces (Agilent Technologies). Total RNA was amplified with the WT-Ovation Pico RNA Amplification system (NuGEN) according to the manufacturer's instructions. After fragmentation and biotinylation, cDNA was hybridized to Affymetrix HT HG-U133A or HG-U133A2.0 microarrays. Microarray data for CMV-, EBV- and influenza-specific $CD8^+$ T cells and for LCMV-specific T cells were obtained from previous studies[9,20]. A detailed description of the microarray data analysis can be found in the **Supplementary Methods**.

Quantitative PCR. Expression of BATF after *in vitro* stimulation of primary human T cells, shRNA and overexpression experiments was determined by real-time quantitative PCR with Taqman gene expression assays for BATF (assay Hs00232390_m1) and β-actin (Hs00357333_g1), which served as a loading and normalization control. For LCMV mouse experiments, RNA was converted to cDNA with a high-capacity cDNA kit, and quantitative RT-PCR was performed with Taqman assay kits (*Batf*, *Gapdh*, *Hprt1*; Applied Biosystems). Expression levels were compared with the relative quantification method, comparing Batf expression to either Gapdh or Hprt1 housekeeping genes. Quantitative multiplex RT-PCR via ligation-mediated amplification was carried out as previously described[30]. PD-1 signature genes were selected for the multiplex validation panel based on previously established criteria[30], and sequences for primer sets are available upon request.

Mouse model of LCMV infection. Four-week-old C57BL/6 mice were purchased from Jackson Laboratory and were used in accordance with the Wistar Institute Institutional Animal Care and Use Committee guidelines. Mice were infected with 2×10^5 plaque-forming units of LCMV Arm intraperitoneally or 2×10^6 plaque-forming units LCMV Cl13 intravenously as previously described[13]. Viruses were grown and titered as previously described[13]. Groups of mice were infected with either LCMV Arm or LCMV cl13. At 8, 15 or 30 d after infection, spleens were collected and splenocytes pooled before CD19 depletion and sorting. LCMV tetramer–specific (DbGP33) $CD8^+$ T cells were sorted by a BD FACSAria directly into siliconized 1.5-ml tubes containing Trizol LS, followed by RNA extraction. Separate aliquots of cells were stained for PD-1 expression as described previously[9].

BATF small interfering RNA knockdown in HIV samples. PBMCs from untreated, chronically HIV-infected individuals were isolated by density-gradient centrifugation. Inhibition of BATF expression was achieved through siRNA transfection by electroporation on a Gene Pulser XCell (BioRad). Fifteen million cells were resuspended in 300 µl of Opti-MEM in a 2-mm cuvette and pulsed with 1 nmol of siRNA (ON-TARGET Non-Targeting pool or BATF ON-TARGETplus SMARTpool, Dharmacon). Pulse conditions were designed to maximize electroporation efficiency in T cells (a unique square wave with a pulse of 360 V and a duration of 5 ms), and transfection efficiency was assayed with siGlo fluorescent oligonucleotides (Dharmacon). For assessment of $CD4^+$ T cell cytokine responses, $CD8^+$ T cell–depleted PBMCs (RosetteSep $CD8^+$ depletion reagents; StemCell) were stimulated with an HIV Gag peptide pool (1 µg ml^{-1} per peptide) or left unstimulated. For HIV-specific $CD8^+$ T cell cytokine responses, nondepleted PBMCs were stimulated with 0.2 µg ml^{-1} of HIV optimal epitopes (Peptide Core Facility, Massachusetts General Hospital). After a 96-h incubation, IFN-γ and IL-2 levels were measured. Proliferation of $CD8^+$ T cells was measured 6 d after transfection and stimulation by a CFSE assay as published before[18].

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

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doi:10.1038/nm.2232