A Critical Role of IL-21-Induced BATF in Sustaining CD8-T-Cell-Mediated Chronic Viral Control

Graphical Abstract

Highlights
- CD4-derived IL-21 sustains CD8 T cell antiviral response in a temporal manner
- IL-21-induced BATF is required to sustain virus-specific CD8 T cell function
- BATF overexpression sufficiently rescues “unhelped” CD8 T cells
- BATF and IRF4 cooperatively regulate Blimp-1 expression and effector T cell function

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In Brief
Xin et al. identify a pathway that connects CD4-derived IL-21 to a BATF-mediated transcriptional program in CD8 T cells, which plays a key role in the sustained effector function of CD8 T cells during chronic viral infection.

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A Critical Role of IL-21-Induced BATF in Sustaining CD8-T-Cell-Mediated Chronic Viral Control

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SUMMARY

Control of chronic viral infections by CD8 T cells is critically dependent on CD4 help. In particular, helper-derived IL-21 plays a key role in sustaining the CD8 T cell response; however, the molecular pathways by which IL-21 sustains CD8 T cell immunity remain unclear. We demonstrate that IL-21 causes a phenotypic switch of transcription factor expression in CD8 T cells during chronic viral infection characterized by sustained BATF expression. Importantly, BATF expression during chronic infection is both required for optimal CD8 T cell persistence and anti-viral effector function and sufficient to rescue “unhelped” CD8 T cells. Mechanistically, BATF sustains the response by cooperating with IRF4, an antigen-induced transcription factor that is also critically required for CD8 T cell maintenance, to preserve Blimp-1 expression and thereby sustain CD8 T cell effector function. Collectively, these data suggest that CD4 T cells “help” the CD8 response during chronic infection via IL-21-induced BATF expression.

INTRODUCTION

Chronic viral infections, such as HIV-1 and hepatitis C virus (HCV), affect hundreds of millions of people worldwide (Virgin et al., 2009). Viral persistence is usually associated with T cell exhaustion, a progressive differentiation process that is characterized by diminished T cell survival and effector functions (Virgin et al., 2009; Wherry, 2011). Early during chronic infection with the persistent Clone 13 (Cl13) variant of lymphocytic choriomeningitis virus (LCMV) in mice, virus-specific effector CD8 T cells clonally expand normally and are phenotypically equivalent to their counterparts in an acute infection (Brooks et al., 2006; Doering et al., 2012; Fuller and Zajac, 2003). However, as chronic infection progresses, virus-specific CD8 T cells gradually lose the ability to produce important effector molecules, such as interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin-2 (IL-2), and concomitantly upregulate multiple inhibitory surface molecules, including programmed cell death protein 1 (PD-1), LAG-3, CD160, and 2B4 (Barber et al., 2006; Blackburn et al., 2009; Wherry, 2011). Nevertheless, “exhausted” CD8 T cells are indispensable, because they retain sufficient function to limit viral replication such that even transient CD8 T cell depletion causes a substantial rise in viral load (Jin et al., 1999; Letvin and Walker, 2003; Schmitz et al., 1999).

The outcome of chronic viral infection also greatly depends on the quantity and quality of CD4 T cells. A vigorous CD4 T cell response often correlates with better viral control in HIV patients and spontaneous recovery in HCV and hepatitis B virus-infected individuals, whereas decreased CD4 T cell number or function is tightly associated with failure of viral eradication and disease progression (Porichis and Kaufmann, 2011; Rehermann and Nascimbeni, 2005; Virgin and Walker, 2010). Besides direct killing of infected cells, CD4 T cells provide critical help to B cell- and CD8 T cell-mediated antiviral responses. In the absence of CD4 help (i.e., deletion or severe reduction of CD4 T cells), exhausted CD8 T cells develop greater dysfunction and lose the ability to contain virus (Battegay et al., 1994; Matloubian et al., 1994; Zajac et al., 1998). Recent studies have elegantly shown that IL-21, produced mainly by CD4 T cells, enhances CD8 T cell-mediated control of persistent viruses (Elsaesser et al., 2009; Fröhlich et al., 2009; Yi et al., 2009). Nevertheless, how IL-21 and its downstream signaling and genetic pathways regulate effector CD8 T cell differentiation and effector function remains poorly defined. Here, we report that IL-21-induced basic leucine zipper transcription factor,
ATF-like (BATF), expression is critically required to sustain antiviral function of CD8 T cells during chronic infection.

RESULTS

IL-21 Sustains Effector CD8 T Cell Function and BATF Expression

To understand the interplay between CD4 and CD8 T cells, we used IL-21 turbo red fluorescent protein (IL-21-tRFP) knockin reporter mice (Shulman et al., 2014) to examine the production of IL-21, a critical CD4-derived cytokine that sustains CD8 T cell response during chronic viral infection (Elsaesser et al., 2009; Frohlich et al., 2009; Yi et al., 2009). Concurrent with the rapid loss of IL-2-producing CD4 T cells, as reported previously (Brooks et al., 2005; Crawford et al., 2014; Fuller and Zajac, 2003; Oxenius et al., 1998), IL-21-expressing CD4 T cells began to emerge around day 5 and continuously increased at later phases of chronic infection with LCMV Cl13 (Figures 1A and S1A). In contrast, acute infection with the LCMV Armstrong (Arm) strain only led to a transient induction of IL-21 around day 5–8 post-infection (p.i.) (Figure 1A). To dissect the temporal effects of CD4-derived IL-21 on CD8 T cell differentiation, we infected Il21+/+ and Il21−/− mice with LCMV Cl13 and compared the virus-specific CD8 T cell responses at either the initial stages of infection (day 5) or later during the response when the exhausted CD8 T cell phenotype first appears (day 10).

IL-21 deficiency did not significantly alter IFN-γ production and CD107a expression in CD8 T cells during the initial phase of Cl13 infection (Figure 1B). Conversely, deletion of IL-21 led to a significant reduction of IFN-γ+CD107a+ CD8 T cells in the GP33- and NP396-specific effector cells at day 10 p.i. (Figure 1B). Likewise, granzyme B was rapidly lost by day 10 p.i. in the absence of IL-21 (Figure 1C). Intriguingly, exacerbated CD8 T cell exhaustion in Il21−/− mice correlated with a significant reduction of Blimp-1 and BATF expression in effector CD8 T cells at day 10 but not day 5 p.i., whereas other transcriptional regulators, such as Eomes, T-bet, and IRF4, remained largely unchanged (Figures 1D and S1B). Lastly, we found that IL-21-induced BATF expression primarily depended on the signal transducer and activator of transcription 3 (STAT3) signaling pathway (Figure 1E). Together, these data suggested that the IL-21-STAT3-BATF axis might play a critical role in the maintenance rather than the establishment of effector CD8 T cell function and survival.
BATF Is Intrinsically Required for Sustained Effector Function in CD8 T Cells

To investigate the cell autonomous role of BATF in effector T cell differentiation, we first generated bone marrow (BM) chimeric mice with CD8 T cell-specific Batf deletion. In this model, CD8 T cells were reconstituted solely from either wild-type (WT) or Batf<sup>−/−</sup> BM cells, whereas most other immune cells were WT (Figure S2). Next, we infected these chimeric mice with LCMV Cl13 and examined the virus-specific CD8 T cell responses. Early during infection (day 5), the expansion, effector function (production of IFN-γ and granzyme B), inhibitory molecule, and transcription factor expression of virus-specific CD8 T cells in Batf<sup>−/−</sup> BM chimeric mice were largely indistinguishable from those in Batf<sup>+/+</sup> control cells (Figures S3A–S3F). This led to the similar viral loads in these two groups of mice (Figure S3G). In contrast, the frequency and number of GP33, NP396, and GP276 tetramer<sup>+</sup> CD8 T cells were drastically reduced in Batf<sup>−/−</sup> BM chimeric mice by day 8 p.i. and through the later phases of infection in comparison with their WT counterparts (Figures 2A and 2B). Likewise, IFN-γ-producing virus-specific CD8 T cells were significantly reduced in the absence of BATF (Figure 2C). Batf<sup>−/−</sup> virus-specific CD8 T cells also expressed significantly lower amounts of granzyme B and the inhibitory molecules PD-1 and 2B4, as well as the transcription factor Blimp-1 (Figures 2D–2F). Lastly, these impaired effector T cell responses were accompanied by poor viral containment in Batf<sup>−/−</sup> BM chimeric mice (Figure 2G). Overall, these data demonstrated that cell-intrinsic BATF expression was critically required for the maintenance of virus-specific CD8 T cell effector function and survival at late stages of chronic viral infection.

BATF Overexpression Enhances CD8 T Cell Response

To explore whether BATF was sufficient to augment effector function, the MSCV-IRES-Thy1.1 (MIT) retroviral vector (RV) was used to overexpress BATF in CD8 T cells, with transduced cells identified by Thy1.1 expression. Most P14 cells transduced with empty MIT RV underwent contraction from day 8–21 p.i., whereas P14 cells transduced with BATF/MIT RV significantly increased in frequency over time (Figure 3A). To...
dissect whether BATF-mediated enrichment of P14 cells at the later stages of infection resulted from increased expansion or survival, we assessed the proliferation and apoptosis of Thy1.1+ RV-transduced cells. BATF-overexpressing cells (BATF-Thy1.1+) contained a significantly higher proportion of proliferating cells (Ki67+) and had increased survival (Annexin-V/7-AAD) compared with empty MIT-transduced cells (control-Thy1.1+) (Figures 3B and 3C). In addition, BATF overexpression clearly increased the frequency of CD107a+ and IFN-γ+ cells in total transferred P14 cells compared to empty MIT at day 21 p.i. (Figure 3D). Furthermore, BATF overexpression led to higher expression of granzyme B and Blimp-1 (Figures 3E and 3F) and better viral control (Figure 3G). Collectively, these data suggest that BATF enhanced both the quantity and the quality of virus-specific CD8 T cells.

**BATF Overexpression Rescues “Unhelped” CD8 T Cells**

Given that CD4 T cells are the main producers of IL-21, BATF expression in GP33-specific effector CD8 T cells was significantly reduced in the absence of CD4 cells (Figure 3H). Next, we examined whether BATF overexpression could restore the effector function of virus-specific CD8 T cells in the absence of CD4 help. Consistent with the preceding observations, BATF overexpression significantly enhanced the expansion, survival, and effector function of P14 cells in comparison with control MIT RV-transduced cells (Figure 3I and data not shown). These heightened CD8 T cell responses mediated by BATF overexpression led to significantly improved viral control (Figure 3J). Similar observations were obtained in IL-21 receptor (IL-21R)-deficient CD8 T cells when BATF was overexpressed in Il21r−/− P14 cells (Figures 3K and 3L). Collectively, these results suggested that BATF overexpression could bypass the requirement...
for CD4 help and IL-21 signaling in the maintenance of effector CD8 T cell responses during chronic viral infection.

**BATF and IRF4 Cooperatively Regulate Blimp-1 Expression**

Recent studies have elegantly demonstrated that BATF and IRF4 cooperatively bind to AP1-IRF4 composite elements (AICEs), and contribute to the chromatin accessibility of a set of lineage-specific genes that regulate Th17 and CD8 T cell development (Ciofani et al., 2012; Kurachi et al., 2014; Li et al., 2012; Murphy et al., 2013; Schraml et al., 2009). In agreement with these observations, we found that IRF4 was also intrinsically required for CD8 T cell differentiation and antiviral function during chronic LCMV infection (Figure S4). Analyzing the chromatin immunoprecipitation sequencing datasets obtained from both CD4 and CD8 T cells (Kurachi et al., 2014; Li et al., 2012), we identified AICEs in multiple genes associated with late effector CD8 T cell differentiation, metabolism, and function, such as Prdm1, Tbx21, Hif1a, and Gzmb (data not shown). Given the crucial role of Blimp-1 (encoded by Prdm1) in maintaining effector function in virus-specific CD8 T cells, we tested how Blimp-1 expression was regulated by the cooperative activity of BATF and IRF4. First, we established an in vitro effector T cell differentiation model to mimic the cytokine environment at early (high IL-2 [IL-2hi], low IL-21) and later (low IL-2 [IL-2lo], high IL-21) phases of effector T cell differentiation during chronic infection in vivo. Under IL-2hi culture conditions, CD8 T cells maintained Blimp-1 expression as reported previously (Figure 4A) (Pipkin et al., 2010) and IL-21 was able to further augment Blimp-1 (Figure 4A). Conversely, in IL-2lo culture conditions, Blimp-1 expression was largely diminished, although BATF and IRF4 were still modestly expressed (Figure 4B). IL-21 induced Blimp-1 expression in IL-2lo-skewed CD8 T cells, which was accompanied by strong BATF induction (Figure 4B). However, this IL-21-induced Blimp-1 expression was diminished in Batf−/− CD8 T cells (Figure 4B). We further observed that IL-21 supplementation in IL-2lo-cultured CD8 T cells sustained IFN-γ production, degranulation (i.e., CD107a expression), and granzyme B expression (Figures 4C and 4D), which was accompanied by elevated BATF and Blimp-1 expression (Figure 4E). Thus, BATF-dependent induction of Blimp-1 by IL-21 that occurs in IL-2lo environments is crucial for sustained effector function in CD8 T cells.

Given that decreased Blimp-1 expression was also found in IRF4-deficient CD8 T cells (Figure S4F), we next directly examined whether BATF might need to cooperate with IRF4 to...
regulate Blimp-1 expression. To test this idea, we skewed acti-
vated CD8 T cells in IL-2lo culture for a longer time (3 days) until
IRF4 expression was mostly diminished (Figure 4F). Even though
IL-21 promoted BATF expression (Figure 4F) and antigen stimu-
lation (anti-CD3 antibody) induced IRF4 expression as reported
previously (Man et al., 2013; Yao et al., 2013), either stimulus
alone failed to potently induce Blimp-1 expression (Figure 4F).
Only when BATF and IRF4 were simultaneously induced by
combined IL-21 and antigen treatment was Blimp-1 expression
strongly upregulated (Figure 4F). Collectively, these results sug-
gest that IL-21 and antigen transcriptionally synergize, by the
respective induction of BATF and IRF4, to maintain Blimp-1
expression and CD8 T cell effector function.

**DISCUSSION**

In this study, we identified a pathway that connects CD4-derived
IL-21 to a BATF-mediated transcriptional program in CD8 T cells,
which plays a key role in the maintenance of the effector CD8
T cell response. Moreover, the IL-21-STAT3-BATF pathway is
likely the molecular mechanism of CD4 help during chronic viral
infection, because BATF overexpression could rescue unhelped
CD8 T cell function during chronic viral infection. Finally, we
found that the transcriptional partner of BATF, IRF4, was criti-
cally required for the sustained expression of Blimp-1, as well
as the maintenance and function of effector T cells. These results
unexpectedly reveal that CD4 help (via IL-21-BATF) may syner-
gize with antigen engagement (via TCR-IRF4) to maintain the
CD8 T cell response during chronic infection.

In response to persistent viral infection, CD4 T cells become
dysfunctional as measured by a diminished ability to co-produce
cytokines such as IFN-γ, TNF-α, and IL-2 (Brooks et al., 2005;
Crawford et al., 2014; Fuller and Zajac, 2003; Oxenius et al.,
1998). Interestingly, these exhausted CD4 T cells gradually
gain the ability to produce other cytokines, such as IL-21 (Fahey
et al., 2011; Harker et al., 2011, 2013). This developmental tran-
sition may alter the helper functions of CD4 T cells to enable in-
fected hosts to better adapt to chronic viruses. The immune
response to a chronic infection must balance anti-viral function
such that the infection is contained without causing fatal immu-
nopathology. Changes in the cytokine environment, including
changes in the cytokine profile of CD4 T cells, likely play an
important role in keeping this balance. Furthermore, a switch
from IL-2 to IL-21 production during chronic infection appears
to sustain sufficiently low levels of effector function for viral con-
rol without immunopathology. This notion has been supported
by recent studies in which IL-21 as a pleiotropic cytokine pro-
motes follicular helper T and B cell differentiation and anti-
body-mediated viral containment in the late phase of chronic
viral infection (Fahey et al., 2011; Harker et al., 2011; Petrovas
et al., 2012).

BATF, lacking a transcription activation domain, was initially
considered as a dominant-negative regulator of activator protein
1 activity (Echlin et al., 2000; Murphy et al., 2013). However,
recent discoveries unveiled that BATF-JUN heterodimers inter-
act with IRF4 to cooperatively bind to compound-binding
cis-regulatory elements of a distinct subset of target genes,
which activate transcriptional networks that govern Th17 cell dif-
ferentiation (Ciofani et al., 2012; Li et al., 2012; Murphy et al.,
2013; Schraml et al., 2009). BATF was also recently shown to
regulate effector CD8 T cell metabolism and survival (Kurachi
et al., 2014), which may explain the survival deficiencies we
see in Batf-deficient CD8 T cells during chronic infection. Despite
the essential function of BATF in the development of a range of
immune cells, little is known about how BATF expression is regu-
lated. Here, we identified IL-21 as a positive signal that regulates
BATF expression in CD8 T cells through a STAT3-dependent
pathway. This raises the question of whether other STAT3 cyto-
kines elevated during the later phases of LCMV Cl13 infection,
such as IL-10, IL-27, and IL-6, can also induce BATF expression
in CD8 T cells. In addition, other signals, such as IL-12 and PD-1
ligation, can induce BATF expression in CD8 T cells (Kuroda
et al., 2011; Quigley et al., 2010). The relative contribution of
these various pathways to the regulation of BATF expression re-
quires further study.

**EXPERIMENTAL PROCEDURES**

**Mice**

C57BL/6, Cd8a−/−, and Batf−/− mice were purchased from Jackson Labora-
tory. Il21−/− mice were purchased from Taconic. Mouse handling conformed
to the requirements of the Institutional Animal Care and Use Guidelines of
Medical College of Wisconsin.

**Flow Cytometry**

Lymphocytes harvested from spleen and other organs were stained and
analyzed by flow cytometry. All antibodies were purchased from eBioscience
and BD Biosciences except anti-granzyme B phycoerythrin (Invitrogen), BATF
(Cell Signaling Technology), and mouse IL-21 receptor Fc (R&D Systems). All
flow cytometry data were acquired on an LSR II (BD Biosciences) and analyzed
by FlowJo (Tree Star).

**Retrovirus-Mediated BATF Overexpression**

MIT RV was obtained from Dr. Susan Kaech. BATF was subcloned into MIT to
generate the BATF-MIT vector. To overexpress BATF in P14 cells, the spin
transfection method was used as described previously (Kao et al., 2011).

**Statistical Analysis**

Student’s t tests were performed by using the GraphPad Prism 6.0 software.
*p < 0.05, **p < 0.01 in all data shown.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures
and four figures and can be found with this article online at http://dx.doi.org/
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