Histone acetyltransferase CBP is critical for conventional effector and memory T-cell differentiation in mice

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Compared with naïve T cells, memory CD8+ T cells have a transcriptional landscape and proteome that are optimized to generate a more rapid and robust response to secondary infection. Additionally, rewired kinase signal transduction pathways likely contribute to the superior recall response of memory CD8+ T cells, but this idea has not been experimentally confirmed. Herein, we utilized an MS approach to identify proteins that are phosphorylated on tyrosine residues in response to Listeria-induced T-cell receptor (TCR) stimulation in both naïve and memory CD8+ T cells from mice and separated by fluorescence- and flow cytometry–based cell sorting. This analysis identified substantial differences in tyrosine kinase signaling networks between naïve and memory CD8+ T cells. We also observed that an important axis in memory CD8+ T cells couples Janus kinase 2 (JAK2) hyperactivation to the phosphorylation of CREB-binding protein (CBP). Functionally, JAK2-catalyzed phosphorylation enabled CBP to bind with higher affinity to acetylated histone peptides, indicating a potential epigenetic mechanism that could contribute to rapid initiation of transcriptional programs in memory CD8+ T cells. Moreover, we found that CBP itself is essential for conventional effector and memory CD8+ T-cell formation. These results indicate how signaling pathways are altered to promote CD8+ memory cell formation and rapid responses to and protection from repeat infections.

During infection, memory CD8+ T cells are generated to provide protective immunity against subsequent reinfection. Memory CD8+ T cells respond more rapidly than naïve T cells and provide a more robust response upon repeat infection. Likely, this rapid response is in part driven by alterations in their signal transduction pathways. Because each adaptive T-cell immune response requires T-cell receptor signaling, it is likely that downstream kinase signaling networks are reprogrammed in memory cells to elicit more efficacious responses.

Previous studies demonstrated that there are indeed differences in the proximal TCR4 signaling proteins between naïve and memory T cells (1, 2): however, at the systems level it is completely unknown how signaling differs between naïve and memory CD8+ T cells.

There are multiple mechanisms that can enable memory cells to respond more efficaciously to secondary infection, including alteration of cytokine receptor expression level, localization, and phosphorylation of signaling proteins. Indeed, memory T cells have elevated expression of cell adhesion molecules, including CD44, that promote interactions with antigen-presenting cells (3). In addition to cell-surface proteins, there are notable differences in the levels of signaling proteins in memory cells that can contribute to a rewired signaling network, including higher levels of LCK and ZAP-70 (4). Increased localization of LCK to the CD8 coreceptor and elevated phosphorylation of LCK can also contribute to lowering TCR signaling thresholds in memory T cells (5). Memory CD8+ T cells have more extensive lipid rafts enriched with phosphoproteins that can more efficiently activate mitogen-activated protein kinase signaling pathways (1). However, the level of most TCR proximal signaling events are similar between naïve and memory T cells, including the extent of CD3 phosphorylation (1). This raises the possibility that other signaling pathways can be differentially activated by a TCR stimulus in memory T cells.

Previous work focused on tracking differences in canonical TCR signaling pathways, which revealed subtle differences between naïve and memory T cells (1, 6). A more global approach could help identify additional pathways that are rewired in response to a TCR stimulus in memory CD8+ T cells. Proteomic changes that occur in CD8+ T cells are hampered by a requirement for large cell numbers, particularly at memory time points where few cells remain. To enable proteomic-level profiling of phosphotyrosine signaling from low cell numbers, we developed an approach that uses antibodies to

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**CBP is essential for conventional memory T cells**

To determine whether our infection model recapitulated differences in phosphotyrosine signaling networks observed previously (1, 2), naïve and memory T cells obtained from OT-I mice were activated in vitro with plate-bound anti-CD3 and soluble anti-CD28 antibodies. Western blotting was performed with an antibody that binds phosphorylated tyrosine residues. In addition, we performed mass spectrometry to identify tyrosine-phosphorylated proteins. The results showed that TCR activation resulted in qualitative differences in the phosphotyrosine protein networks between activated naïve and memory T cells (Fig. 1).

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**Results**

**Tyrosine kinase signaling networks are rewired in memory CD8⁺ T cells**

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ting was performed with antibodies specific for the phosphorylated T-cell signaling proteins. The level of phospho-JAK2, phospho-STAT3, and phospho-Tyr-396 LCK were increased in stimulated memory CD8^+ T cells compared with naïve cells (Fig. 1B).

Because TCR stimulation resulted in qualitative differences in tyrosine phosphorylation, we utilized an antibody enrichment proteomic screen to identify proteins that were tyrosine-phosphorylated in memory and naïve CD8^+ T cells at the global level (Fig. 1C). In this protocol, proteins containing a phosphorylated tyrosine residue were enriched by immunoprecipitation with antibodies that bind to phosphorylated tyrosine. Following immunoprecipitation, tyrosine-phosphorylated proteins were digested with trypsin, and MS was utilized to identify proteins in the IP. From this analysis, there were 323 proteins identified in activated naïve T cells, 370 proteins identified in the activated memory cell IP, and 119 proteins that were shared between naïve and memory CD8^+ T cells (Fig. 1D). These data identified specific protein targets that may be differentially regulated by tyrosine phosphorylation between naïve and memory CD8^+ T cells.

JAK2 and STAT3 were specifically identified in the memory phosphotyrosine IP by mass spectrometric screen (supporting File S1). To confirm these data, Western blotting was performed with antibodies that recognize JAK2 phosphorylated on Tyr-1007 and STAT3 phosphorylated on Tyr-705 (Fig. 1E). Tyr-1007 is autophosphorylation by JAK2 and thus serves as a marker of JAK2 activation (10), which was elevated in memory CD8^+ T cells. Tyr-705 on STAT3 is phosphorylated by JAK2 and is also hyperphosphorylated in memory CD8^+ T cells, suggesting that JAK2 is more active in memory CD8^+ T cells.

A statistical overrepresentation analysis was performed on proteins identified in the phosphotyrosine IPs to identify pathways that are differentially regulated by tyrosine phosphorylation in response to TCR signaling in naïve and memory CD8^+ T cells (Fig. 1F). Multiple signaling pathways associated with T-cell responses, including granyme A, JAK/STAT, OX40, AMPK, and iNOS, were more enriched in the phosphotyrosine IPs from memory CD8^+ T cells. These pathways have established roles in the context of memory cell function where JAK/STAT signaling maintains homeostasis (11), OX40 signaling enhances both memory T-cell survival and recall responses (12), AMPK signaling is required for memory formation (13), and iNOS signaling enhances the persistence of memory CD8^+ T cells postvaccination (14). Taken together, this analysis demonstrates that our proteomic screen was able to identify specific proteins that were differentially tyrosine-phosphorylated and pathways that were differentially regulated in the reactivation of memory CD8^+ T cells by TCR stimulation.

**CBP is phosphorylated on Tyr-1126 by JAK2 only in memory CD8^+ T cells**

One protein that was identified only in the memory CD8^+ phosphotyrosine IP was CBP, which functions as a transcription factor coactivator. CBP is a central hub in the iNOS (15), granzyme A, and AMPK pathways, which were all differentially targeted by tyrosine phosphorylation in memory T cells (Fig. 1F). These data illustrate that phosphorylated CBP is important for CD8^+ memory T-cell recall responses. The ScanSite program was then utilized to identify potential kinases that could phosphorylate CBP. This approach identified putative JAK2 phosphorylation sites in CBP, which is interesting given that JAK2 is hyperactivated in memory T cells (Fig. 1E). To determine whether JAK2 directly phosphorylated CBP, naïve and memory CD8^+ T cells were activated in the presence or absence of a JAK2-specific inhibitor (Fig. 2, A–C). Proteins that contained phosphotyrosine residues were immunoprecipitated using an anti-phosphotyrosine antibody and Western blotted with antibodies specific for CBP, phosphorylated JAK2 (p-JAK2), and p-STAT3. In naïve T cells, phosphorylated CBP was not detected; however, both p-JAK2 and p-STAT3 were observed (Fig. 2, A and C). As expected, inhibition of JAK2 diminished JAK2 autophosphorylation and STAT3 phosphorylation. In memory T cells, phosphorylated CBP was observed, and JAK2 inhibition diminished phosphorylated CBP (Fig. 2, B and C). As anticipated, JAK2 inhibition resulted in reduced p-JAK2 and p-STAT3. These results demonstrated that JAK2 phosphorylated CBP in stimulated CD8^+ memory T cells.

To further confirm that JAK2 phosphorylates CBP, in vitro kinase reactions were performed (Fig. 2D). Recombinant JAK2 was incubated with recombinant CBP and ATP. The reaction was resolved by SDS-PAGE, and Western blotting was performed using an antibody that specifically binds to phosphotyrosine residues. The intensity of the band corresponding to the phosphorylated CBP increased as a function of time and plateaued by the 5-min time point. The in vitro kinase reaction was also analyzed by MS (supporting File S2), which mapped the specific JAK2 phosphorylation site to Tyr-1126, which is located in the bromodomain of CBP (Fig. 2E). Taken together, these data demonstrated that signaling through the TCR results in hyperactivation of JAK2, which can phosphorylate CBP in memory CD8^+ T cells.

**Phosphorylation increases the affinity of CBP for multiple acetyl-lysine marks on histones**

To predict how phosphorylation affects CBP function, phosphorylated Tyr-1126 was modeled using the crystal structure of the CBP bromodomain bound to an acetylated peptide (Fig. 3A) in which Tyr-1126 is in a solvent-exposed loop. In this model, the phospho group on Tyr-1126 would contact the acetyl group of the acetylated ligand, suggesting that phosphorylation of the CBP bromodomain could influence the interaction between CBP and acetylated ligands.

The CBP bromodomain is known to bind acetylated histones, including H3 acetylated on Lys-36 (H3K36Ac) (16). One possibility is that JAK2 phosphorylation of CBP alters its specificity for acetylated ligands. To test this hypothesis, recombinant CBP was phosphorylated by JAK2. Either p-CBP or CBP alone was then incubated with histone arrays that contained multiple acetylated histone peptides (Fig. 3B). This assay reproduced known CBP interactions, including H2aK9Ac (17) and H3K36Ac (16), and the profile between p-CBP and CBP was similar for most of the tested acetylated peptides. However, p-CBP was able to bind to additional acetylated histone peptides on the array, including H3K14Ac, H3K9Ac, and H2K4Ac. This screen suggested that JAK2 phosphorylation of the CBP
bromodomain enhanced the ability of CBP to bind to a greater diversity of acetylated histone peptides.

To confirm the results of the histone array, we performed binding measurements with p-CBP and CBP using H3K4Ac, H3K9Ac, H3K27Ac, and H3K36Ac peptides (Fig. 3, C–F).Phosphorylated CBP bound to all of the acetylated histone peptides tested. However, dephosphorylated CBP was only able to bind H3K36Ac. This suggested that JAK2 phosphorylation functions to promote CBP binding to acetylated targets and increases the number of acetylated histone marks that are recognized by CBP.

CBP-deficient CD8⁺ T cells are similar to innate CD8⁺ T cells in mixed bone marrow chimeras

In vivo, CBP deficiency has previously been shown to result in an abundance of innate CD8⁺ memory T cells that are derived in the thymus, express the transcription factor eomesodermin (EOMES), and can produce IFNγ upon stimulation and in response to their cognate antigen (7, 8, 18). To determine the function of CBP in conventional CD8⁺ T cells during a response to infection, we first generated conditionally deficient CBP mice (CBP CKO mice) by crossing CBP floxed mice to the CD4Cre recombinase transgenic line. We next generated mixed bone marrow (BM) chimeras in which we reconstituted sublethally irradiated host animals with a 1:1 ratio of wildtype (WT) BM and CBP CKO BM that were congenically distinct. At 8 weeks after reconstitution, we determined the frequency of total CD8⁺ T cells in the peripheral blood and demonstrated that the frequency of CBP CKO CD8⁺ T cells was reduced by >50% relative to the WT cells (Fig. 4A). CBP CKO CD8⁺ T cells in peripheral tissue expressed higher CD122 and EOMES relative to their WT counterparts, indicating that these cells were phenotypically like innate CD8⁺ T cells, as described previously (Fig. 4B). We examined CD44, CD62L, KLRG1, and CD127 expression on the naïve cell populations and determined that WT and CBP CKO CD8⁺ T cells from the mixed BM chimeras had approximately equal expression of these markers (Fig. 4C). We activated WT and CBP CKO CD8⁺ T cells in vitro with anti-CD3 and anti-CD28 for 48 h. We determined that CD44 expression was unaffected by loss of CBP, whereas CD25 and CD69 expression was significantly down-regulated with loss of CBP (Fig. 4D). Interestingly, expression of CBP has previously been implicated in positive regulation of IL-2 (7), and this may account for the reduction we observed in the high-affinity IL-2 receptor CD25. We concluded that CBP
CKO CD8⁺ T cells were phenotypically more like innate CD8⁺ T cells with regard to CD122 and EOMES expression and that the CBP CKO CD8⁺ T cells were activated in vitro with anti-CD3 and anti-CD28.

CBP-deficient CD8⁺ T cells cannot form conventional effector or memory T cells in response to Listeria infection

Our MS screen revealed that phosphorylated CBP was up-regulated in stimulated memory CD8⁺ T cells. To further explore the function of CBP in effector and memory CD8⁺ T cells, we performed Western blotting on OT-I cells isolated from uninfected hosts (naïve) or from OT-I cells isolated at day 7 (effector) or day 25 (memory) after infection (Fig. 5). We noted that both effector and memory CD8⁺ T cells showed increased phosphorylated CBP expression relative to naïve T cells (Fig. 5A). We next infected the mixed WT and CBP CKO BM chimeras with 5 × 10⁵ cfu of L. monocytogenes OVA (Lm-OVA). Analysis of OVA-tetramer⁺ CD8⁺ T cells in the peripheral blood of infected mice revealed that although WT cells mounted a robust effector T-cell response, CBP-deficient cells did not (Fig. 5B). Additionally, when we gated on total WT or CBP CKO CD8⁺ cells and determined CD127 and KLRG1 expression, we noted that the phenotype of WT cells skewed more toward an effector KLRG1hi CD127lo population than did the CBP CKO cells at the peak and throughout the course of the infection (Fig. 5, C and D). We stimulated total CD8⁺ T cells on day 7 after infection with OVAp in vitro to determine inflammatory cytokine production. We determined that WT cells were adept at producing IFNγ and TNFα, whereas the CBP-deficient cells were not, presumably due to a paucity of OVA-tetramer⁺ CD8⁺ T cells in the absence of CBP (Fig. 5E). As expected, CBP CKO cells expressed more EOMES, CD122, and EGR2 (Fig. 5F) than WT cells at day 8 after infection, strongly indicating that these cells had adopted an innate memory T-cell phenotype as reported previously. However, stimulation of WT or CBP CKO cells with PMA and ionomycin at day 40 after infection revealed that total CBP CKO CD8⁺ T cells could produce IFNγ and IL-2 at levels equal to those in WT cells, whereas loss of CBP expression resulted in impaired ability of these memory cells to produce TNFα after stimulation (Fig. 5G). Our data show that CBP expression is critically required by conventional CD8⁺ T cells to mount an antigen-specific response to bacterial infection and as such is required for conventional effector and memory T-cell formation.

Discussion

Herein, we characterized how phosphotyrosine signaling networks differ between naïve and memory CD8⁺ T cells in response to TCR activation. This analysis revealed specific proteins that have differential tyrosine phosphorylation between
naïve and memory T cells. The presumption is that memory T cells are optimized to generate more efficacious immune responses, and our results suggest that the observed changes in tyrosine-phosphorylated proteins contribute to a more expeditious immune response. One signaling network that may facilitate rapid immune responses by memory T cells consists of JAK2 and CBP (Fig. 6). Following activation through the TCR, JAK2 is hyperactivated and phosphorylates CBP in memory T cells. Phosphorylation enables CBP to bind to more acetylated histone marks, which we predict would more efficiently activate transcriptional programs necessary for cytotoxic T-cell function. With loss of CBP, both the effector and memory T-cell responses to infection are perturbed, indicating a requirement for CBP throughout infection and for robust memory T-cell formation.

Previous work identified signaling proteins in TCR proximal signaling, costimulatory receptor signaling, and downstream signaling cascades that contribute to memory CD8+ T-cell recall responses (1, 4). At the level of TCR proximal signaling, early events, including phosphorylation of CD3 components, are similar between naïve and memory CD8+ cells. However, memory T cells have more lipid rafts containing phosphorylated proteins that could better signal through linker for T-cell activation (LAT) and other downstream pathways (1). Additionally, PDK1 was hyperphosphorylated in memory cells, consistent with its role in memory recall responses. Our data also support a model where coreceptor and TNF signaling pathways are poised to be more sensitive to stimulation as we observed hyperphosphorylation of TRAF5 in memory T cells. Indeed, deletion of OX40, which signals through TRAF5, impairs CD8 memory recall responses.

In addition to differential TCR signaling cascades in naïve, effector, and memory T cells, cytokine signaling also plays a critical role in primary versus secondary CD8+ T-cell immune response to infection (19). CD127 (IL-7Rα) is expressed on naïve T cells, and IL-7 signaling is critical for naïve T-cell survival (20, 21). In contrast, conventional memory T cells in lymphoid organs rely on IL-7 and predominantly IL-15 signaling for their survival (20, 21). Thus, it is certain that cytokine signaling, resulting in downstream STAT1, -3, and -5 transcriptional activation, also profoundly influences the expediency with which memory T cells respond to secondary challenge. Future challenges will be in understanding how differential signals from the TCR and cytokine receptors together rewire memory T cells for protection from repeat infection.

It has been proposed that memory T cells are poised to respond to stimulation and generate cytokines with faster kinetics, similar to innate-like cells. However, the biochemical basis for how memory CD8+ T cells transduce signaling inputs for faster cytokine production relative to naïve cells is not well established. We found that components of cytokine signaling pathways were hyperphosphorylated in memory T cells, which we propose could contribute to more expeditious engagement of
CBP is essential for conventional memory T cells

transcription. For example, signaling through the IL-10 receptor is critical for memory CD8 formation (25–27). We found that the IL-10 receptor is hyperphosphorylated in memory T cells, which would be predicted to promote STAT dimerization and activation of downstream transcriptional activation. Additionally, signaling through the IL-6 receptor is also critical for CD8 memory T cells (28, 29). We found that GP130 was expressed increased EOMES and CD122 levels and enhanced cytokine production in memory T cells versus their naïve counterparts. Activation of JAK2 could drive multiple downstream signaling events, leading to more efficient cytokine production. Indeed, we identified that JAK2 hyperactivation in memory T cells regulated the CBP transcriptional activator. JAK2 phosphorylation enabled CBP to bind to a greater variety of acetylated histone marks. We posit that JAK2-catalyzed phosphorylation of CBP is part of a signaling circuit that enables memory T cells to more efficiently activate transcriptional programs and immune responses.

stream of the IL-6 receptor. We also found that JAK2 was a critical kinase that was more avidly activated in memory CD8+ T cells versus their naïve counterparts. Activation of JAK2 could drive multiple downstream signaling events, leading to more efficient cytokine production. Indeed, we identified that JAK2 hyperactivation in memory T cells regulated the CBP transcriptional activator. JAK2 phosphorylation enabled CBP to bind to more types of acetylated histone modifications, which could lead to expedited transcriptional activation. We therefore posit that JAK2 phosphorylation of CBP is a critical switch that enables CD8+ memory T cells to more expediently produce cytokines. Taken together, our data help to support a model that kinase signaling networks are better poised to transduce signals to rapidly generate cytokine production in memory T cells responding to secondary challenge.

The function of CBP in the thymic development of T cells has been described previously (7, 8). Using conditionally deficient CBP mice, it was shown that loss of CBP expression resulted in innate-like CD8+ T cells that indeed produced increased EOMES and CD122 levels and enhanced IFNγ production (7–9). Here, we discovered that phosphorylated CBP was expressed in conventional effector and memory T cells but not naïve cells. Using mixed bone marrow chimeras, we confirmed previous findings and showed that loss of CBP in T cells resulted in innate-like CD8+ T cells that indeed expressed higher levels of EOMES, CD122, and IFNγ. However, during infection, we noted that CBP-deficient CD8+ T cells could not mount an antigen-specific immune response to bacterial infection and were deficient in IFNγ and TNFα production, although CBP deficiency resulting in decreased TNFα production was previously described (7, 8).
CBP is essential for conventional memory T cells

production has been reported previously (30). The resulting loss of antigen-specific immune response of CD8$^+$ T cells in the absence of CBP was surprising as it has been previously shown that these innate CD8$^+$ memory T cells can respond to and protect from infection better than their naïve counterparts (31). A number of differences, including background strain (we used C57Bl/6 as the background strain; others have used BALB/c), use of 1:1 mixed bone marrow chimeras, and different infectious agent could help account for these differences in phenotype. Nevertheless, these innate memory CD8$^+$ T cells most likely play roles early during infection, prior to initiation of the adaptive immune response (18). In lieu of an ideal model system, such as a mouse line in which the phosphorylated bro-modomains of CBP are mutated, our results showed the surprising finding that loss of CBP in CD8$^+$ T cells results in innate memory T cells that cannot elicit an antigen-specific effector response to bacterial infection.

Although many challenges remain, using an MS approach, we have shown here that TCR signaling in naïve versus memory T cells is fundamentally different. Moreover, we have uncovered a number of previously unknown signaling pathways that are rewired for a more efficacious immune response to secondary infection. We have discovered that hyperactivated JAK2 is critical for CBP phosphorylation and that CBP itself is essential for an effective CD8$^+$ T-cell effector response and for memory CD8$^+$ T-cell differentiation.

Experimental procedures

Mice

Mice were bred and housed in specific pathogen-free conditions in accordance with and approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. CBP$^{f/f}$ and CD4Cre$^+$ mice on a C57Bl/6 background were originally purchased from The Jackson Laboratory. Mixed bone marrow chimeras were generated by transferring a mixture of 2.5 $\times$ 10$^6$ B220$^-$CD3$^-$NK1.1$^-$ bone marrow cells from a CD45.1.2 WT donor and 2.5 $\times$ 10$^6$ B220$^+$CD3$^+$NK1.1$^-$ bone marrow cells from a CD45.2 CBP$^{f/f}$CD4Cre$^+$ donor into sublethally irradiated (1000 rad) CD45.1 recipient mice. All chimeras were rested at least 8 weeks to allow reconstitution of the host. For infectious studies, mice were infected i.v. the following day with 5000 cfu Listeria-OVA. Listeria-OVA was grown to late-log phase.

Flow cytometry and cell sorting

Single-cell suspensions were prepared from specified tissues. Cells were counted prior to staining. The following antibodies were used: Viability (Biolegend and Thermo Fisher), ViabilityCD45.1-eFluor 450 (A20), CD45.1-BUV395 (A20, BD Biosciences), CD51-BV605 (A20, Biolegend) CD52-APC (104), CD4-eFluor 506 (53-6.7), CD8-APC-R700 (53-6.7, BD Biosciences), Vα2-PE (B20.1), CD44-PerCP-Cy5.5 (IM7), CD62L-APC-eFluor 780 (MEL-14), KLRG1-PE-eFluor 610 (2F1), CD127-FITC (A7R34), H-2 k B OVA-tetramer PE (MBL International), IFNγ-PE-eFluor 610 (XMG1.2), TNFα-eFluor 450 (MP6-XT22), IL-2-PE-Cy7 (JES5-5H4), CD4-PE (GK1.5), CD122-PE-Cy7 and -PE-Cy5 (TM-β1, Biolegend), CD69-PE (H1 2F3), CD25-eFluor 450 (PC61.5), EOMES-FITC (Dan11mag), and Ki-67-eFluor 506 (SolA15). All antibodies were purchased from Thermo Fisher (eBioscience) unless stated otherwise. Samples were fixed and permeabilized using a CytoFix/CytoPerm kit (BD Biosciences) according to the manufacturer’s instructions. Samples were filtered and then collected on an LSRII, LSRII Fortessa, or FACSaria (BD Biosciences) or Aurora instrument (Cytek) and analyzed using FlowJo software (Tree Star).

In vitro assays

CD8 T-cell stimulation assays were conducted using 10 μg/ml plate-bound anti-CD3 and 2 μg/ml soluble anti-CD28 (eBioscience) for the indicated time point. PMA and ionomycin stimulation assays were conducted using 1× Cell Stimulation Mixture for 6 h with brefeldin A and monensin added the final 3 h of stimulation using 1× Protein Transport Inhibitor Mixture (eBioscience). For restimulation assays using OVA, OVA p was added to the culture for the indicated time point.

Western blotting

PAGE was performed using Bio-Rad precast Protein TGX gels. Proteins were transferred to polyvinylidene difluoride membranes with a Bio-Rad Trans-Blot Turbo transfer system. Antibodies were purchased from Cell Signaling Technology: pTyr (P-Tyr-1000), CBP (D6C5), p-JAK2 (3771), JAK2 (D2E12), p-STAT3 (D3A7), STAT3 (124H6), and anti-FLAG tag (14793).

Mass spectrometry

One million naïve or memory CD8$^+$ T cells were activated using plate-bound anti-CD3 antibody and soluble anti-CD28 antibody for 10 min. Cells were lysed in 500 μl of ice-cold buffer containing 0.5% (v/v) Nonidet P-40, 1% (v/v) Triton X-100, 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, and Complete protease inhibitor and PhosSTOP (Roche Applied Science). The lysate was incubated at 4 °C overnight with magnetic beads coated with anti-phosphotyrosine antibodies (P-Tyr-1000, Cell Signaling Technology), and proteins containing phosphotyrosine residues were immunoprecipitated. The filter-aided sample preparation method was used to generate tryptic fragments (32). Samples were analyzed by reverse-phase LC in tandem with MS using a Dionex LC system with a New Objective PicoChip nanospray column in line with a Thermo Fisher LTQ XL mass spectrometer. Raw spectra were processed using the PEAKS 8 software package. The PEAKS 8 software package was used to identify proteins in the IPs using the UniProt mouse protein database and a 0.1% false discovery rate.

JAK2-CBP in vitro kinase assay

Recombinant JAK2 enzyme (0.1 μg; Active Motif) was incubated with 1 μg of recombinant CBP containing residues 518–1207 (Abcam, ab135297) in a buffer containing 60 mM HEPES (pH 7.5), 3 mM MgCl$_2$, 3 mM MnCl$_2$, 3 mM sodium orthovanadate, 1.2 mM DTT, and 1 mM ATP. The reaction was resolved by SDS-PAGE, and Western blotting using an antibody that binds to phosphotyrosine residues was utilized to monitor CBP phosphorylation (P-Tyr-1000, Cell Signaling Technology).
CBP is essential for conventional memory T cells

Equilibrium chemical assays to monitor CBP binding to acetylated histone peptides

Recombinant CBP bromodomain containing a FLAG tag (Active Motif) that was either phosphorylated or unphosphorylated was incubated with varying amounts of biotinylated histone peptides (Active Motif), which included H3K4Ac (ART-Kac-QTARK-STGGKAPRKQLA-GGYK(biotin)-NH2), H3K9Ac (ARTQTA-RKSTGGKAPRKQLA-GGYK(biotin)-NH2), H3K27Ac (ATK-AAR-Kac-SAPSTGGVKKPHRYPG-GGK(biotin)-NH2), and H3K36Ac (ATKAARKSAPATGGVKKPHRYPG-GGK(biotin)-NH2). The binding reactions were incubated at 37 °C for 1 h in a buffer comprising 50 mM HEPES-NaOH (pH 7.5) and 0.1% BSA. Magnetic beads coated with streptavidin were incubated with 5 μg of recombinant CBP containing residues 518–1207 (ab135297, Abcam) in a buffer containing 60 mM HEPES (pH 7.5), 3 mM MgCl₂, 3 mM MnCl₂, 3 μM sodium orthovanadate, 1.2 mM DTT, and 1 mM ATP. Reactions were quenched by adding a buffer containing 8 mM urea in 100 mM Tris-HCl (pH 8). Filter-aided sample preparation was utilized to prepare tryptic peptide fragments (32). Peptides were desalted using C₁₈ spin columns (Pierce). Samples were analyzed by reverse-phase LC in tandem with MS using a Dionex LC system with a New Objective PicoChip nanospray column in line with a Thermo Fisher LTQ XL mass spectrometer. Raw spectra were processed using the PEAKS 8 software package where phosphorylation was set as a variable modification with a 1% false discovery rate.

Statistical analysis

All graphs were created using GraphPad Prism 7, and statistical significance was determined with the two-tailed unpaired Student’s t test or using one-way analysis of variance adjusted for multiple comparisons where appropriate.

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