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Review

AP-1 transcription factors in cytotoxic lymphocyte development and antitumor immunity☆

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The proper functioning of cytotoxic lymphocytes, such as natural killer and CD8+ T cells, is essential for effective cancer-immunity and immunotherapy responses. The differentiation of these cells is controlled by several transcription factors (TFs), including members of the activator protein (AP)-1 family. The activity of AP-1 family members is regulated by various immune signaling pathways, which can be triggered by activating or inhibitory receptors as well as cytokines. The target genes controlled by AP-1 TFs are central to generate immunity to pathogens or malignancies. Here, we provide an overview of the current understanding of how AP-1 TFs regulate cytotoxic lymphocytes.

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Activator protein-1 transcription factors

The activator protein (AP)-1 family consists of Jun (c-Jun, JunB, and JunD), Fos (c-Fos, FosB, Fra-1, and Fra-2), Maf (c-Maf, MafB, MafA, Mafg/f/k, and Nrl), and ATF (activating transcription factor) (ATF2, LRF1/ATF3, BATF, BATF2, BATF3, JDP1, and JDP2) [1]. AP-1 transcription factors (TFs) are basic region leukine zippers that act as dimers: Jun proteins are able to form homo- and heterodimers, while Fos and ATF proteins cannot homodimerize and require dimerization with Jun proteins. AP-1 TFs are rapidly transcribed in response to extrinsic stimuli and regulate various cellular processes, including differentiation, proliferation, transformation, migration, inflammation, or apoptosis. The composition of AP-1 dimers determines their specificity and whether target gene expression is positively or negatively regulated [1]. While AP-1 TFs are commonly associated with tumor promotion, also tumor-suppressive functions have been reported [2]. Similarly, anti- and pro-inflammatory effects of AP-1 members are known, and they can act upstream and downstream of inflammatory mediators. Despite their known role in regulating inflammation and function of several immune cell types, the functions of AP-1 TFs in natural killer (NK) and (chimeric antigen receptor [CAR])-T cells are incompletely understood.

Activator protein-1 in natural killer cells

NK cells are innate lymphocytes, essential to kill infected or malignant cells. Their function is regulated by activating and inhibiting receptors and cytokines. Tight regulation of several TFs, including the AP-1 family, is essential for NK cell development and function. In unstimulated human NK cells, c-Jun and JunD are highly expressed, but AP-1 expression changes upon stimulation [3]. After NK cell activation by IL-2, c-Jun- and JunD expression remains unaltered, but c-Fos and JunB expression and DNA-binding activity increases [4]. Additionally, TNF-α (tumor necrosis factor alpha), an important cytokine for proliferation and function of NK cells [5], exerts its effects through AP-1 and MAPK (Mitogen-activated protein kinase) pathways [6]. NK cell activation via CD16 results in elevated levels of c-Fos [3], indicating an important role of AP-1 in regulating NK cell function. Current knowledge is summarized below and in Figure 1.

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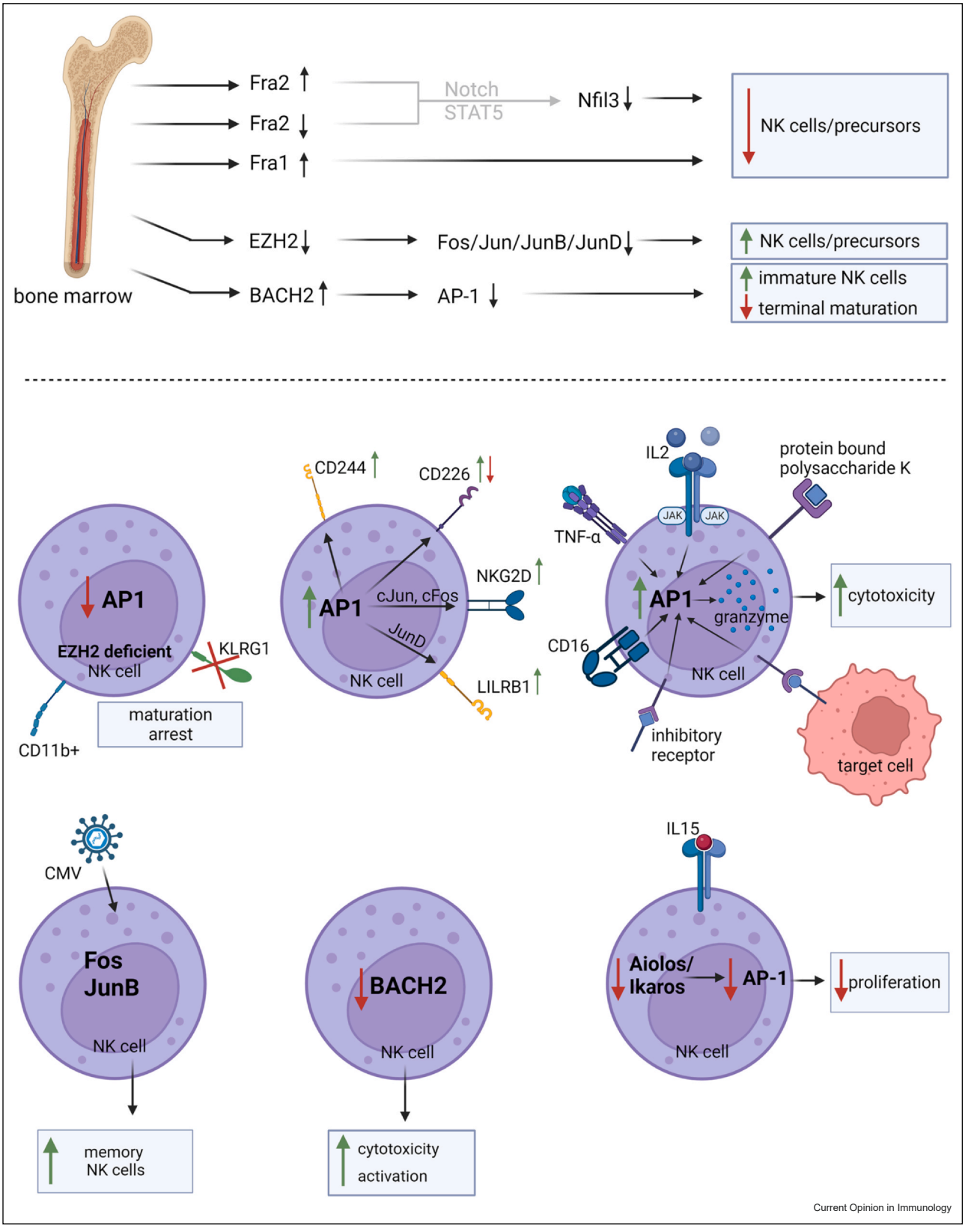
Figure 1


bone marrow
Fra2
Fra2
Fra1
Notch
STAT5
Nfil3
NK cells/precursors
EZH2
Fos/Jun/JunB/JunD
NK cells/precursors
BACH2
AP-1
immature NK cells
terminal maturation
AP1
EZH2 deficient
NK cell
KLRG1
CD11b+
maturation
arrest
CD244
CD226
AP1
cJun, cFos
JunD
NKG2D
LILRB1
NK cell
IL2
protein bound
polysaccharide K
JAK
JAK
TNF-α
AP1
granzyme
cytotoxicity
CD16
NK cell
inhibitory
receptor
target cell
CMV
Fos
JunB
NK cell
memory
NK cells
BACH2
NK cell
cytotoxicity
activation
IL15
Aiolos/
Ikaros
AP-1
proliferation
NK cell
Current Opinion in Immunology

AP-1 TFs affect NK cell development and function. Overexpression and silencing of Fra-2 lead to decreased NK cell numbers, potentially via STAT5 and/or Notch signaling, leading to downregulation of NFIL3. Loss of EZH2 leads to decreased levels of AP-1 family members, resulting in elevated numbers of NK cells and their precursors, while the AP-1 antagonist, BACH2, restrains terminal NK cell maturation and leads to a higher abundance of immature NK cells.

In Fra-1-overexpressing mice, numbers of liver NK cells are significantly decreased [7]. Additionally, precise regulation of Fra-2 is particularly important as ectopic overexpression leads to systemic NK cell deficiency in mice. Conversely, Fra-2 silencing in human CD34+ cells results in decreased NK cell numbers following in vitro differentiation [8,9]. In IL-2-stimulated CD4+ T cells, Fra-2 is a STAT5 target [10], which is also essential for NK cell development, downstream of IL-7 and IL-15 [11]. This suggests a possible STAT5–Fra-2 axis regulating NK cell development and/or survival. Alternatively, Fra-2 can be activated by Notch signaling [12], which is essential for transition from common lymphoid progenitors to pre-NK cells. Global Fra-2 overexpression leads to a cell-intrinsic defect at this developmental stage due to a downregulation of E4bp4/Nfil3 (Nuclear Factor, Interleukin 3 Regulated) [8] — an essential TF for NK cell development [13]. The effects of Fra-2 in mature NK cells and their function have not yet been studied.

Enhancer of zeste homolog 2 (EZH2)-deficient mice, lacking a histone methyltransferase, associated with silenced chromatin [14], have increased numbers of NK cells and their precursors [15]. The maturation process of these cells is however impaired. These EZH2-deficient NK cells express lower levels of Fos, Jun, JunB and JunD, suggesting that EZH2 deletion affects NK cell maturation and function through AP-1 attenuation [16]. In murine NK cells, AP-1 expression is induced by the family of Ikaros TFs (Ikzf) and mice lacking Ikzf-1 display reduced levels of all AP-1 components, leading to defective IL-15 signaling in NK cells. Additionally, Ikzf-3 knockout further decreases AP-1 levels and leads to a complete absence of peripheral NK cells, highlighting the importance of AP-1 in IL-15-mediated regulation of NK cell survival and function [17]. In contrast, the AP-1 repressor BTB Domain And CNC Homolog 2 (BACH2) is highly expressed in immature NK cells in mouse bone marrow and spleen and decreases during final maturation [18]. Similar findings have been reported in human NK cells, where BACH2-expression levels are higher in immature $CD56^{bright}$ than in mature $CD56^{dim}$ cells [19]. In mice, both global BACH2 deletion and its loss in Ncr1-expressing cells, leads to development of more mature NK cells in the spleen and lung along with increased granzyme-B expression. BACH2-deficient NK cells also exhibit elevated levels of Inteferon (IFN)-γ and granzyme B following IL-15 stimulation, but this effect diminishes upon additional stimulation with IL-12 and IL-18 [20].

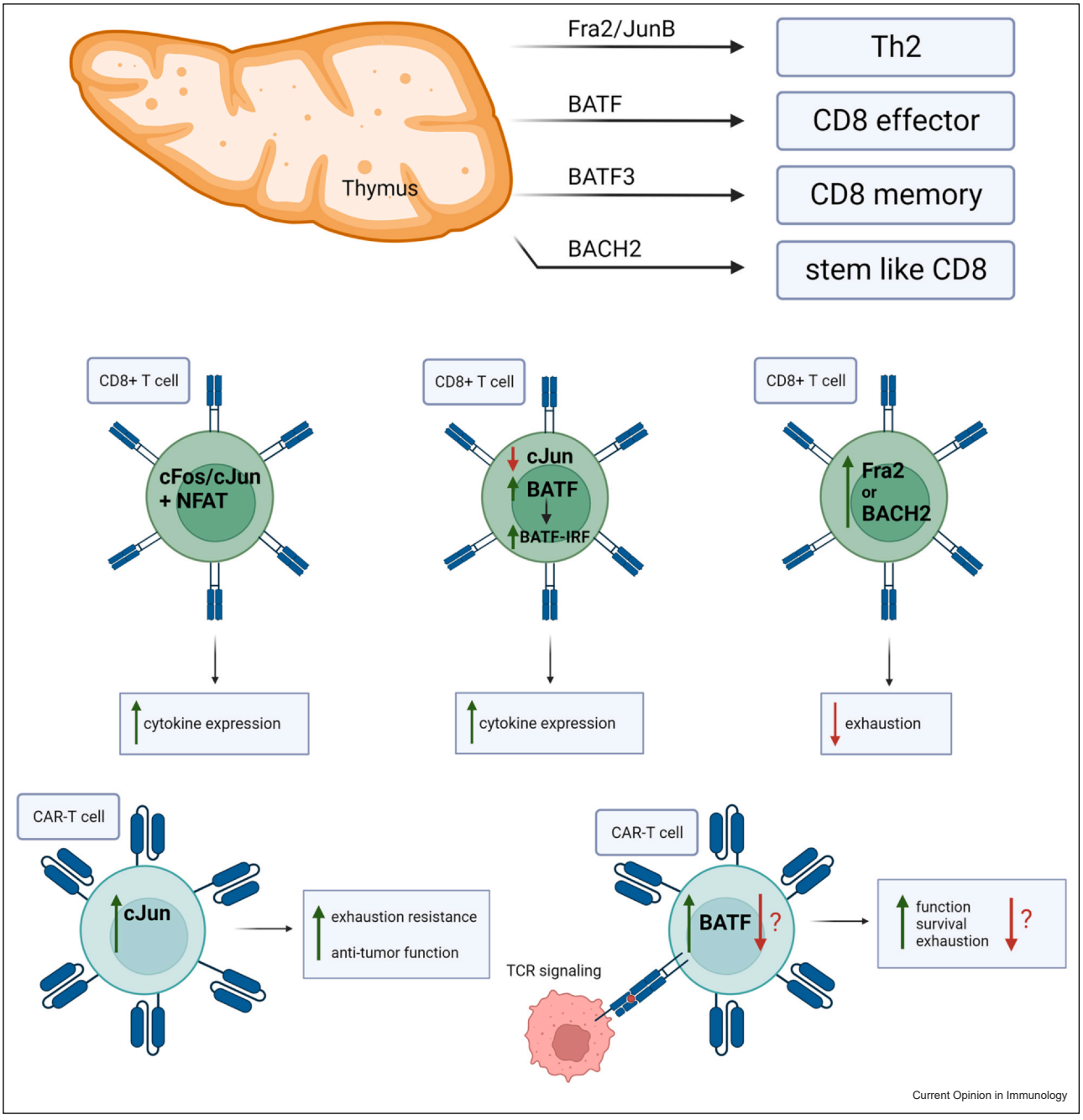
AP-1 factors play a critical role in the regulation of NK cell surface receptor expression. C-Jun or c-Fos overexpression enhances the levels of the activating receptor NKG2D in NK and T cells [24], while the inhibitory receptor LILRB1 is induced by JunD [25]. The receptor 2B4/CD244 has dual roles: in human NK cells, it is activating, leading to increased cytotoxicity and IFN-γ production, but in mice, it triggers an inhibitory effect [21,22]. In a human NK cell line, its expression is induced through Ets-1/AP-1 interactions [22]. Likewise, in human hepatocarcinoma cells, two different Ets-1/AP-1-binding sites have been identified on the promoter of the human-activating receptor DNAM-1/CD226. These binding sites provide separate regulatory mechanisms: at one site, transcriptional activity of a c-Jun/c-Fos dimer is enhanced by its interaction with Ets-1, while at the other site, Ets-1 inhibits AP-1- dependent transcription by competing for the binding site [23]. This indicates a very fine- tuned and tight regulation of AP-1-induced target gene transcription.

Mature NK cells have two important effector functions: cytotoxicity and the production of cytokines and chemokines such as IFN-γ, TNF-α, or CXCL8 [26]. The roles of AP-1 factors in regulating NK cell cytokine production are not yet understood, but AP-1 factors regulate IFN-γ secretion in T cells [27]. It is of interest to study if there is a potential similar regulation in NK cells.

TGF-β, an immunosuppressive cytokine, impairs NK cell cytotoxicity by suppressing activity of several TFs, including AP-1 activity [28]. As it has been suggested that TGF-β acts in feedback loops with AP-1 factors in epithelial cells and myofibroblasts, it would be of relevance to study a potential similar regulation in cytotoxic immune cells.

The cytotoxic potential of NK cells is currently widely exploited in immunotherapy [29]. Clinical trials involving an IL-15-receptor agonist increased expansion and activation of NK cells and showed improvement of patients suffering from non-Hodgkin lymphoma. Notably, after the first dose, these NK cells exhibited already-elevated expression of Jun and Fos [30]. In a human NK cell line, cytotoxicity correlated with accumulation of JunB, FosB, and c-Fos and inhibitory receptor signaling impaired the activation of the AP-1 factors [31]. Additionally, stimulation of NK cells with IL-2, protein-bound polysaccharide K, or tumor cells increased AP-1 DNA-binding activity [32]. Interestingly, the granzyme-B promoter displays an AP-1- binding site [33].

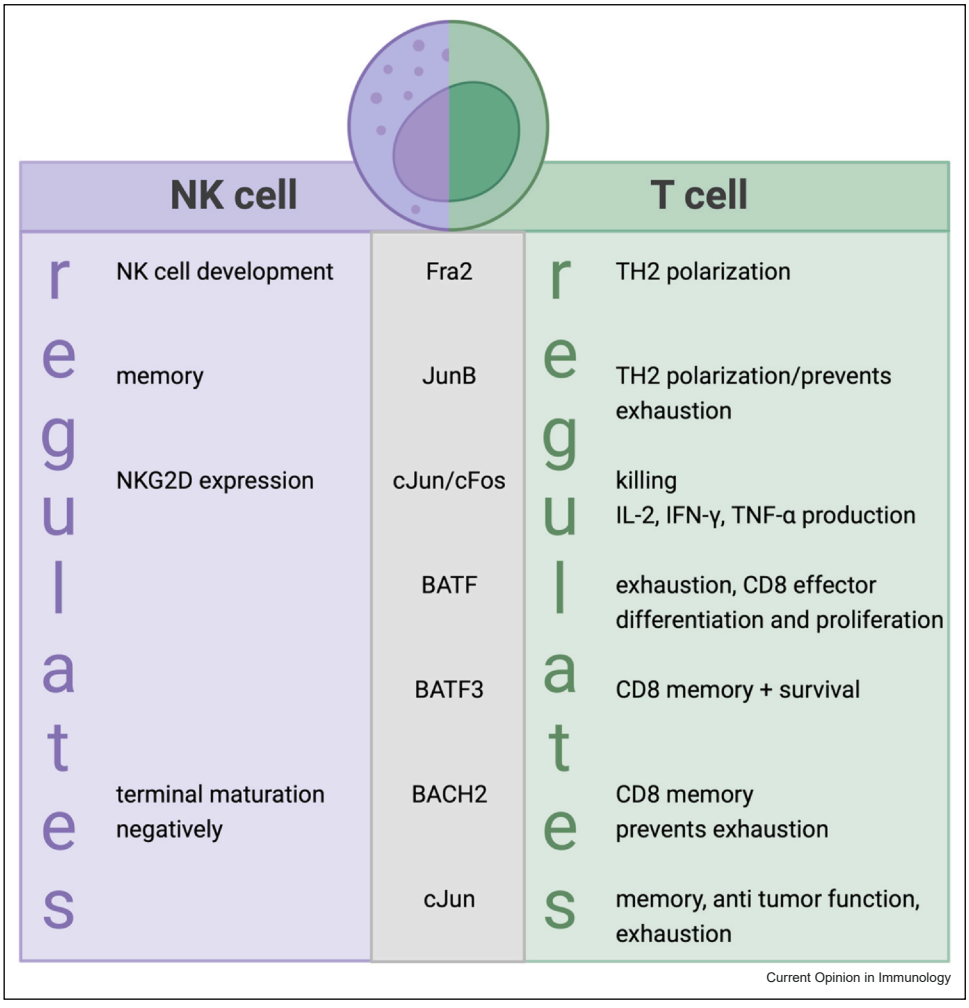
Figure 2



AP-1 regulating T- and CAR-T-cell development, function, and exhaustion. Development and polarization are driven by different AP-1 TFs: Fra-2 and JunB drive Th2 polarization, while BATF, BATF3, and BACH2 regulate the development of different CD8+ T-cell subsets. C-Fos and c-Jun can cooperate with NFAT TFs to induce cytokine production in activated T cells. C-Jun, Fra-2, and BACH2 play a protective role against exhaustion, whereas the role of BATF in exhaustion regulation remains uncertain due to conflicting findings.

NK cells are also the first defense in viral infections [34] and human cytomegalovirus (CMV) infection can trigger the generation of memory NK cells. A recent study revealed the enriched presence of AP-1-binding motifs — particularly for Fos–JunB in memory NK cells [35]. Given that mice also acquire NK cell memory following CMV infection [36], and enrichment of Jun- binding sites in chromatin regions that are highly accessible in memory

Figure 3



Shared and distinct effects of AP-1 in NK and (CAR)-T cells.

CD8+ and NK cells [37], it is of interest to explore the role of AP-1 TFs in the establishment, maintenance, and functionality of memory NK cells.

Activator protein-1 in T cells

T cells develop in the thymus into regulatory, helper (CD4+), or cytotoxic (CD8+) T cells. The interactions between AP-1 and NFAT TFs play a significant role in the regulation of lineage-specific genes and T-cell differentiation [38]. C-Jun and c-Fos cooperate with NFAT factors to induce cytokine expression in activated T cells. It has been hypothesized that NFAT TFs have different effects and target genes depending on the availability of AP-1 factors, thereby controlling T-cell development, function, and anergy [39].

AP-1 components, in particular JunB, accumulate in all subsets, but high transcriptional activity was only shown in Th2 cells [40]. Several studies have demonstrated that AP-1 factors function downstream of T cell receptor (TCR) signaling and drive CD8+ effector differentiation [41]. BATF (Basic leucine zipper transcription factor, ATF-like) is essential for the differentiation of CD8+ effector T cells, by promoting lineage- determining TFs, but represses effector molecules and BATF-deficient CD8+ T cells display defective proliferation and metabolism [42]. In contrast, BATF3 promotes CD8+ T- cell survival and memory [43]. CD8+ T-cell memory can also be promoted by BACH2, acting via AP-1 attenuation, thereby preventing terminal effector differentiation [44].

In the context of chronic infections or cancer, c-Jun is downregulated in effector T cells. This is compensated by BATF upregulation, which is required for differentiation and function of effector T cells [45]. However, BATF–IRF4 (Interferon Regulatory Factor 4)

interactions result in expression of exhaustion-associated genes [41,46]. Exhaustion as a consequence of prolonged antigen stimulation leads to transcriptional and epigenetic alterations and reduced killing. Even at the initial stages of T-cell exhaustion, there is a decrease in chromatin accessibility in regions enriched with AP-1/bZIP motifs, causing an imbalance of AP-1 and IRF factors and decreased gene expression [47]. In tumor-infiltrating exhausted T cells, these AP-1 motifs are not transcriptionally active, but activity can be restored by immunotherapeutic co-stimulation [48]. In contrast to BATF, Fra-2 overexpression prevents CD8+ T-cell exhaustion [49]. Similarly, AP-1 antagonism by BACH2 overexpression enhanced the development of stem-like CD8+ T cells preventing their exhaustion [50]. It therefore seems conflicting that the TF EGR2 (Early Growth Response Protein) was reported to stabilize the phenotype of exhausted cells by repressing AP-1 [51]. This suggests different functions for different AP-1 members and highlighting the complexity and importance of AP-1 in regulation of T-cell development, survival, and function.

CAR-T cells are engineered with synthetic receptors that empower them to eliminate cells expressing specific antigens. One of their major limitations in immunotherapy is exhaustion [52]. C-Jun-overexpressing CAR-T cells lead to exhaustion resistance and increased antitumor functions in multiple in vivo models [53]. Similarly, BATF overexpression in CAR-T cells results in increased CAR-T function and survival and reduced exhaustion [47]. However, other studies reported a BATF-dependent upregulation of exhaustion genes and that BATF deletion improves tumor control [54]. These contradictory results underscore the significance of proper AP-1 regulation in tumor-controlling T cells. The effects of AP-1 TFs in T and CAR-T cells are depicted in Figure 2.

Activator protein-1–STAT interactions

The JAK-STAT (Januskinase-Signal Transducers and Activators of Transcription) pathway plays a central role in NK and T-cell development. AP-1 TFs can influence this signaling pathway on multiple levels. In cooperation with NFAT (nuclear factor of activated T-cells) TFs, AP-1 can trigger the production of STAT-activating cytokines such as IL-2, IL-4, or IL-12 [55], which play an important role for NK or T-cell functions. Additionally, AP-1 transcription or DNA- binding activity can be induced by STATs or their target genes. For instance, Fra-2 is a direct downstream target of STAT5 in IL-2-stimulated T cells [10] and AP-1 TFs may potentially mediate different STAT5-dependent functions in NK cells. Furthermore, AP-1 activation in response to IFN-γ signaling is strictly dependent on STAT1 [56].

In activated NK cells, parallel enrichment of STAT and AP-1 motifs has been shown, suggesting direct interactions of these TFs [57]. Similar findings have been reported in colorectal carcinoma and hematopoietic malignancies, where STAT3 and c-Jun form a complex and jointly regulate the expression of their target genes [58]. As STAT3 can also regulate effector molecule expression in NK and T cells [59,60], it would be interesting to evaluate if STAT3 and AP-1 might act together in the regulation of their responses. Understanding these complex interactions is important for modulating and fine-tuning NK and T cells in therapeutic settings.

Conclusions and future directions

Despite the similarities in their function and regulation, the effects of AP-1 TFs in NK and CD8+ cells are partially contrasting (Figure 3). The summarized literature also clearly indicates that our current knowledge in this area is strongly limited. Many conclusions have to be drawn from studies conducted in immortalized cell lines or other cell types, and genetically modified mice with global modifications. Additionally, numerous findings are based on indirect observations. As a result, the precise role of AP-1 in regulating these immune cells remains incompletely understood. This underscores the need for further research to comprehensively grasp their involvement. Gaining a deeper comprehension of how the different AP-1 factors affect cytotoxic immune cells is essential for harnessing them as potential targets for therapeutic interventions.

CRediT authorship contribution statement

D.S., A.H., N.H., and D.G. conceptualized the study and wrote the paper; D.S. and A.H. generated figures and tables.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

We confirm that the work is original research, has not been previously published, and has not been submitted for publication elsewhere. The authors declare no competing financial interests.

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- •• of outstanding interest

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