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REVIEW

# Interferon regulatory factor 3 in adaptive immune responses

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**Abstract** Interferon regulatory factor (IRF) 3 plays a key role in innate responses against viruses. Indeed, activation of this transcription factor triggers the expression of type I interferons and downstream interferon-stimulated genes in infected cells. Recent evidences indicate that this pathway also modulates adaptive immune responses. This review focuses on the different mechanisms that are implicated in this process. We discuss the role of IRF3 within antigen-presenting cells and T lymphocytes in the polarization of the cellular immune response and its implication in the pathogenesis of immune disorders.

**Keywords** T cell differentiation · Type I IFNs · IL-12 family · CD8 T lymphocyte · Th17 · TLR

## Introduction

Interferon regulatory factor (IRF) 3 has been discovered nearly 20 years ago as a regulatory component of virus-infected cells [1]. This transcription factor is constitutively expressed in most tissues. Just like other members of the family, IRF3 possesses an amino (N)-terminal DNA-binding domain (DBD) that is characterized by a series of well-conserved tryptophan-rich repeats [2]. The DBD forms a helix-turn-helix domain and interacts with interferon-stimulated response element (ISRE) sites (GAAANNGAAANN). Together with the closely related IRF7, IRF3 plays an important role in type I IFNs production and the control of viral infections [3]. These soluble mediators display direct antiviral action but also regulate multiple aspects of both innate and adaptive immune responses.

With the discovery of Toll-like receptors (TLR), an unappreciated role for IRF3 in the context of Gram-negative bacterial recognition by antigen-presenting cells (APCs) was also demonstrated. Indeed, IRF3 was found to be a major component of the "MyD88-independent" pathway triggered downstream of TLR4 in response to lipopolysaccharide (LPS) [4, 5]. In LPS-stimulated macrophages or dendritic cells (DCs), several important polarizing cytokines were found to be direct or indirect targets of IRF3. Hence, through modulation of APCs function, activation of IRF3 influences T cell differentiation. Moreover, several IRFs, including IRF3, were shown to directly interact with key transcription factors responsible for T cell differentiation. In this article, we discuss the complex role of IRF3 in shaping cellular immune responses and its implication in immune-mediated disorders.

## Different signaling pathways lead to IRF3 activation

IRF3 is constitutively expressed in most cell types. It displays both nuclear export and nuclear localization sequences (NES and NLS, respectively). In steady state, IRF3 continuously shuttles between the cytoplasm and the nucleus. As the effect of the NES is dominant, it mainly resides in the cytoplasm in basal conditions [6]. It is activated upon recognition of specific pathogen-associated molecular patterns (PAMPs) such as nucleic acids or LPS [7]. These cytoplasmic or endosomal sensors recruit specific adaptor molecules and the downstream IKK-related kinases, TANK-binding kinase (TBK1) and IκB kinase ε (IKK ε) [8, 9]. Carboxy-terminal phosphorylation of serine

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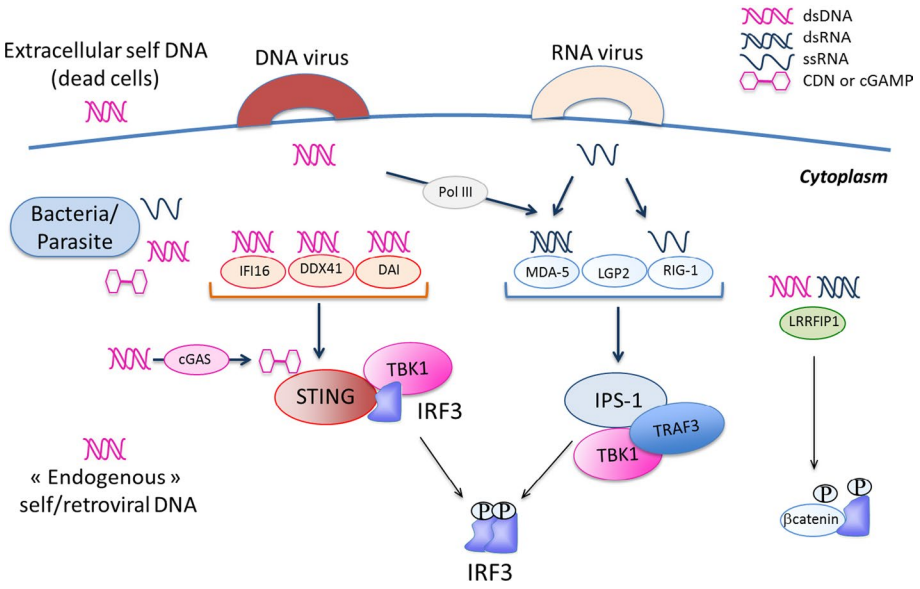


**Fig. 1** Cytosolic recognition of nucleic acids leads to IRF3 activation. The presence of single/double-stranded RNA (ss/dsRNA) or double-stranded DNA (dsDNA) in the cytosol triggers TBK1 activation through specific cytosolic pattern-recognition systems involving IPS-1 or STING adaptor molecules, respectively. TBK1 induces the phosphorylation of IRF3 on specific serine residues, resulting in homo- (or hetero-) dimerization. Cytoplasmic dsDNA can be of microbial (intracellular bacteria or parasite, DNA virus such as HSV) or host (exogenous or endogenous DNA) origin. Several DNA sensors, such as IFI16, DDX41 or DAI have been reported. In addition, cytoplasmic DNA induces the production of cGAMP by the cyclase enzyme cGAS. cGAMP and the structurally related cyclic dinucleotides from intracellular bacteria directly activate the STING pathway. RNA will access the cytosol upon entry of RNA viruses in the cell or when genomic DNA from DNA viruses or intracellular bacteria is transcribed by an RNA polymerase such as RNA pol III. dsRNA is also a replication intermediate of both positive and negative RNA viruses. Cytoplasmic RNA is sensed by RLR helicases, such as RIG-I, MDA-5 or LGP2, that trigger IRF3 activation through the IPS-1 adaptor molecule (also known as MAVS). Several cofactors, such as β catenin, activated by LRRFIP1 upon recognition of cytoplasmic DNA or RNA, positively regulate IRF3 activity

residues located in the regulatory domain of IRF3 allows homo- and heterodimerization, nuclear translocation and association with chromatin modifiers such as CREB-binding protein (CBP) and p300 to promote the transcription of its target genes [10] (Fig. 1).

Several sensing pathways, including RIG-I- and Toll-like receptors (RLRs and TLRs), converge to IRF3 activation. In a broad range of cell types, intracellular viral RNA and synthetic dsRNA are recognized by retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5), two cytoplasmic DExD/H box helicases [11, 12]. RIG-I senses blunt-end base-paired RNA with a 5′-triphosphate and is involved in the recognition of antisense (−) ssRNA viruses or sense (+) ssRNA/dsRNA viruses. MDA5 recognizes higher-order structured RNA that contains long dsRNA but also ssRNA, produced during the replication cycle of some viruses such as picornaviruses [13, 14]. These two RNA-sensing molecules activate the adaptor molecule IFN β promoter stimulator 1 (IPS-1, also known as MAVS/Cardif/VISA) which is required for IRF3 activation and appropriate type I IFN production [15–18].

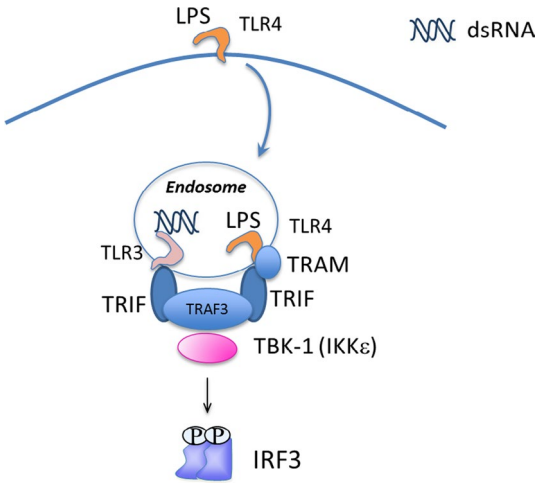


**Fig. 2** TLR3 and TLR4 activation leads to IRF3 activation. Activation of TLR3 (by dsRNA) in the endosomal compartment leads to TBK1 activation in a TRIF-dependent fashion. Upon stimulation by LPS, TLR4 coupling to TRIF requires internalization of the receptor in the endosomal compartment in a CD14- and PI(3)K-dependent fashion

dsRNA is also recognized by TLR3, expressed in the endoplasmic reticulum and endosomal compartment (Fig. 2). It is highly expressed by resting mouse conventional CD8α$^+$ and human BDCA3$^+$ DCs but also by other leukocytes such as CD8 T and NK cells, epithelial cells or central nervous system-resident cells [19]. TLR3 signals exclusively through TIR domain adaptor-inducing IFNβ (TRIF), which activates IRF3 pathway in a TNFR-associated factor (TRAF)3-dependent fashion [20]. Notably, in addition to dsRNA generated during viral replication, TLR3 also recognizes endogenous self mRNA that is released during necrosis [21].

Sensing of Gram-negative bacteria LPS by TLR4, expressed mostly by monocytes, myeloid DCs and macrophages also triggers TRIF-dependent signaling (Fig. 2). This requires internalization of the receptor in the endosomal compartment in a CD14- and phosphatidylinositol-3-OH kinase (PI(3)K)-dependent fashion [22, 23].

Accumulation of cytosolic dsDNA activates IRF3-dependent type I IFN response (Fig. 1) [24, 25]. This happens in the context of infections with intracellular pathogens but also upon impaired ability to clear exogenous DNA normally metabolized in endo-lysosomes or imbalanced control of endogenous DNA products [26]. DAI, the first DNA sensor identified, recognizes nucleic acids from various pathogens such as Listeria *monocytogenes*, cytomegalovirus or HSV1. However, cells from DAI-deficient mice are still able to produce type I IFN in response to foreign DNA [27]. These data suggest the existence of alternative pathways for DNA sensing. Indeed, RNA polymerase III uses AT-rich dsDNA from various pathogens to produce RNA containing a 5′triphosphate group that activates the RIG-I/IPS-1-dependent pathway. Several groups have reported the existence of other cytosolic proteins, such as DDX41 or IFI16, that are responsible for type I IFN production in response to dsDNA in different cell types [28–30]. In contrast to the redundancy of DNA sensors, the adaptor protein stimulator of interferon genes (STING) appears to be central for the production of type I IFN in response to cytosolic dsDNA [31]. In response to cytosolic dsDNA, formation of an active STING dimer provides a scaffold to assemble IRF3 in close proximity to TBK1, through its C-terminal domain [32]. Thus, STING directs TBK1-dependent phosphorylation of IRF3 for DNA-sensing pathways. In addition, STING functions as a direct sensor of cyclic dinucleotides (CDN), a second messenger produced by bacteria and archaea [33]. Stimulation of cells with cytosolic DNA induces the synthesis of cyclic di-GMP-AMP (cGAMP) from ATP and GTP by a cyclase enzyme called cGAMP synthetase (cGAS). cGAMP is structurally related to CDN and also leads to STING-dependent induction of type I IFN [34, 35]. Furthermore, cGAMP can be transferred from producing to neighboring cells through gap junctions, thereby spreading the antiviral state [36]. STING could function both as an adapter for DNA sensing and a receptor for cyclic dinucleotides [26, 37].

In macrophages, leucine-rich repeat flightless-interacting protein 1 (LRRFIP1) promotes IFNβ production in response to vesicular stomatitis virus and Listeria *monocytogenes*. This dsDNA and dsRNA sensor activates β catenin, which acts as a cofactor of IRF3 [38].

## Direct and indirect involvement of IRF3 on APCs function

Once activated, IRF3 is recruited to promoter regions of multiple target genes. In particular, IRF3 is central for the induction of IFNβ in response to viral infections or LPS. It is part of the enhanceosome that binds to the proximal promoter region and initiates local histone acetylation and nucleosomal repositioning [39]. Importantly, other IRFs such as IRF1, IRF5 or IRF7 contribute to this process in a cell type- and stimulus-specific fashion. For example, in plasmacytoid DCs, IRF7 rather than IRF3 is implicated in type I IFN production in response to TLR7 or TLR9 stimulation [40]. MyD88 directly interacts with IRF7 through its death domain [41]. These observations indicate that IRF3 may be dispensable for systemic type I IFN induction in the course of infections.

Intriguingly, TLR9 activation in B lymphocytes also triggers production of type I IFNs in a MyD88-dependent fashion [42]. It does not involve TBK1, but in sharp contrast with TLR9 signaling in pDCs, this effect was found to be IRF3-dependent. This could be due to the fact that IRF7 is preferentially expressed in pDCs.

In LPS-stimulated macrophages or myeloid DCs, direct IRF3 binding is required for the recruitment of SWI/SNF complexes to distinct sets of remodeling-dependent genes, such as CXCL10, RANTES, IL-27a, IL-12a or IL-15 allowing their selective activation [43]. Furthermore, IFNβ will then signal in an autocrine/paracrine fashion and lead to the induction of a specific module of genes that depends on other transcriptional regulators such as ISGF3 (STAT1/STAT2/IRF9), STAT1/STAT1 and downstream IRF1, IRF7 or IRF8. It provides an important feedback loop that conditions APC state at the single-cell level [44]. Thus, IRF3 activation in APCs will elicit both direct and secondary, type I IFN-dependent transcriptional programs [5].

The involvement of these different waves has been well studied for the IL-12 family. These heterodimeric cytokines are critical regulators of both innate and adaptive immune responses (see Box 1). They are mostly produced by APCs in response to pathogens. The balance between IL-12,

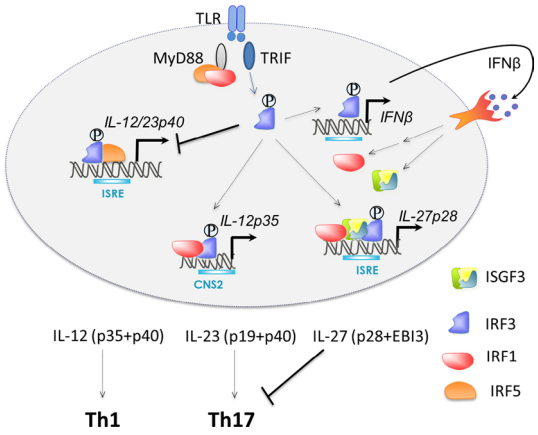


**Fig. 3** IRF3 contributes to the balance between IL-12 family members in antigen-presenting cells. IL-12 and IL-23 share a common chain (p40). IL-12 induces secretion of IFNγ by CD4 T (Th1), CD8 T and NK cells. IL-23 promotes IL-17 production by several T cell types including the Th17 subset. IL-27 shares homology with IL-12p70 and IL-23 and limits Th17 differentiation. Upon activation downstream of TLRs, IRF3 activates the transcription of *IL-12p35* and *IL-27p28* through direct recruitment to ISRE-binding sites within the promoter regions. IRF3 is also recruited to the IL-12/23p40 promoter and enhancer regions. For this gene, it leads to repression through competition with IRF5, a related transcription factor that is activated downstream of the MyD88-dependent pathway. Autocrine/paracrine IFNβ signaling further supports *IL-12p35* and *IL-27p28* gene expression through the upregulation of IRF1 (for both genes) and activation of ISGF3 (for *IL-27p28* gene only)

IL-23 and IL-27 production is strongly dependent on the set of IRF family members that are activated upon contact with specific pathogen-associated molecular patterns (Fig. 3).

Direct recruitment of IRF3 to both *IL-12p35* and *IL-27p28* promoter regions was demonstrated in LPS-stimulated DCs [45, 46]. Autocrine type I IFNs also contribute to the activation of both genes through the upregulation of IRF1 and sustained recruitment of this transcription factor [47, 48]. However, formation of the ISGF3 complex is critical for amplification of *IL-27p28* gene expression but is dispensable for the *IL-12p35* gene activation. ISGF3 activation also leads to expression of IRF7, which in some settings could participate in late activation of the *IL-27p28* gene either directly or through amplification of type I IFN synthesis. Recent evidences indicate that IRF3 is directly recruited to the *IL12/23p40* promoter and enhancer regions in the context of RLR signaling [49]. This leads to repression of this gene through competition with IRF5, thereby limiting the production of both IL-12 and IL-23 in these specific settings. Taken together, these data indicate that the balance between IL-12, IL-23 and IL-27 production is strongly dependent on IRF3, thereby affecting ensuing adaptive immune responses (see Fig. 3).

## The role of IRF3-dependent signaling on CD4 T cell polarization

Polarization of CD4 T helper into distinct effector lineages is determined by the expression of master regulators, such as T-bet, Foxp3, GATA-3 or the orphan nuclear receptor RORγt, acting in close interaction with transcription factors from the STAT family. For example, STAT4 (activated by IL-12), STAT6 (activated by IL-4), STAT3 (activated by IL-6, IL-21 or IL-23) and STAT5 (activated by IL-2) are directly implicated in Th1, Th2, Th17 or Treg development, respectively [50]. Several bodies of evidences indicate that through its function in APCs, IRF3-dependent pathway influences the polarization of CD4 T cells.

Experimental autoimmune encephalomyelitis (EAE) is a classical Th17-dependent model. IRF3$^{-/-}$, IFNβ$^{-/-}$, TRIF$^{-/-}$ and IFNAR$^{-/-}$ mice display more severe disease with exacerbated Th17-type response, while administration of polyI:C was found to be beneficial [51–53]. Several cellular/molecular mechanisms have been incriminated for the anti-inflammatory action of type I IFNs. Their receptor is broadly expressed at the surface of immune and non-immune cells. However, in the EAE model, Prinz and coworkers showed that the beneficial role of endogenous and locally produced type I IFN requires signaling in myeloid cells rather than in B, T or neuroectodermal CNS cells [54]. Type I IFN signaling in macrophages and microglial cells led to a reduction of chemokine production, phagocytosis, and MHC II expression and reduced NLRP3-dependent inflammasome activity through the induction of suppressor of cytokine signaling (SOCS)1 [55] Furthermore, Guo and coworkers showed that the beneficial role of the TRIF/IRF3/IFN pathway in the course of EAE was mediated by IL-27 [52]. In these experiments, endogenous type I IFN-dependent induction of IL-27 negatively regulated Th17 development and inflammation. Along this line, type I IFN signaling also regulates the expression of intracellular osteopontin, which was shown to influence production of IL-27 by DCs [56]. In contrast, the therapeutic effect of IFNβ in EAE appears to be independent of IL-27 signaling [57].

In the context of viral infections, triggering of RLR-dependent IRF3 activation attenuates IL-12/23p40 expression. Hence, Th1 (driven by IL-12) and Th17 (driven by IL-23) responses that develop upon Listeria *monocytogenes* infection were strongly decreased by coinfection with vesicular stomatitis virus [58]. This inhibition was found to be IRF3-dependent and was responsible for the increased susceptibility of virus-infected mice to sublethal doses of bacteria.

High-affinity antibody responses are critically dependent on the help provided by a specialized subset of follicular helper CD4 T ($T_{FH}$) lymphocytes. Type I IFNs enhance T

cell-dependent antibody responses in vivo [59]. Both LPS and polyI:C strongly support the development of antigen-specific $T_{FH}$ cells and their homing to B cell follicles. This effect was shown to be mediated by type I IFN signaling in DCs, presumably through enhanced IL-6 production [60]. In vitro, TLR9 signaling in B lymphocytes was found to promote IgG2a production in an IRF3-dependent manner. This observation indicates that IRF3 and type I IFN could also be implicated in the induction of T cell-independent antibody secretion in the context of infection or autoimmune disease [42].

Recent data indicate that in the context of house dust mite challenge or immunization in the presence of alum, IRF3-dependent signaling is required for the initiation of Th2 response and airway hyperresponsiveness [61]. This effect is independent of type I IFNs. Upon injection of alum, DNA release from dying cells triggers TBK1–IRF3 activation, probably in a STING-dependent fashion. In this context of sterile inflammation, IRF3 was required for the local production of IL-12p40 dimers which induce the migration of inflammatory monocytes/DCs to the draining lymph node [62]. Interestingly, papain-induced Th2 responses were also shown to be dependent on TLR4–TRIF signaling [63].

Taken together, these observations indicate that activation of IRF3 within APCs and subsequent autocrine type I IFNs signaling limit the induction of Th17 (and Th1) responses. This can be favorable (in the context of autoimmune inflammation) or deleterious for the host (in the context of bacterial infection). Furthermore, triggering this pathway might also support $T_{FH}$ and Th2 differentiation, a finding with potential implication for the design of vaccine adjuvants and in the context of allergy.

## The role of IRF3-dependent signaling on CD8 T cell differentiation

Defense against intracellular pathogens requires efficient cytotoxic responses. CD8 cytotoxic T lymphocytes (CTLs) recognize antigenic peptides derived from proteins synthetized within the cells and presented by a MHC class I expressed in most cell types. Thus, CTLs recognize antigens from intracellular pathogens or tumors and kill them to limit the spread of the infection. Initiation of cytotoxic responses requires education of naïve CTLs by APCs. In the case of antigens that are not expressed by APCs, CTL activation requires delivery of engulfed exogenous antigens to a distinct endosomal/lysosomal pathway that allows the processed peptides to be presented on MHC-I molecules (cross-presentation). Type I IFNs have been shown to play a major role in promoting cross-priming against both soluble proteins and cell-associated antigens, derived for example from apoptotic tumor cells. Lebon and colleagues have shown that efficient cross-priming of viral or soluble antigens in vivo is dependent on Type I IFN signaling in DCs [64]. Whether type I IFNs affect the cross-presentation process or the capacity of DCs to efficiently cross-prime CD8 T cells, which also relies on their maturation status and the expression of costimulatory molecules, remains, however, to be firmly established. Lorenzi et al. [65] observed that type I IFNs promote intracellular Ag persistence in CD8α + DC that have engulfed apoptotic tumor cells, regulating intracellular pH and MHC-I–peptide surface expression. These results suggest a direct role of type I IFNs on cross-presentation.

Although this has not been thoroughly studied, the specific role of IRF3 in this process is likely to be context-dependent. For example, mouse $CD8\alpha^+$ DCs display a unique capacity for cross-priming and express high levels of TLR3. Efficient immunization with cells infected with Semliki Forest virus (dsRNA virus) requires TLR3 signaling in vivo. Absence of TLR3 in host cells results in weak upregulation of costimulatory molecules by DCs and generation of few Ag-specific CD8 T cells with low cytotoxic capacities [66]. Both TLR3 and MDA5 appear to participate in polyI:C-dependent CTL induction against soluble antigens [67]. However, the antitumor activity of polyI:C seems to be restricted to the TRIF pathway. Indeed, CTL responses elicited by polyI:C are mostly abrogated in $TRIF^{-/-}$ and $IRF3/7^{-/-}$ animals but not in $IPS\text{-}1^{-/-}$ or $IFNAR^{-/-}$ mice [68]. Hence, primary sensing of dsRNA and activation of IRF3/7 in CD8α + DCs is sufficient to induce cross-presentation, which minimally involves the IPS-1 or IFNAR amplification pathway. In sharp contrast, upon injection of CpG oligonucleotides, pDCs are the main source of type I IFNs. The antitumor effect mediated by CTL cross-priming will be mediated by TLR9, which signals in an IRF3-independent fashion [69].

In addition to their effect on APCs, type I IFNs also directly act on CD8 T cells. This was initially shown in the context of infection with acute lymphocytic choriomeningitis virus (LCMV), a virus that induces high levels of type I IFNs. In this model, type I IFN signaling in T cells was mandatory for the expansion and survival of antigen-specific CD8 T cells [70]. Thus, along with antigen and costimulation (signals 1 and 2), type I IFNs (and other innate cytokines) act as the so-called third signal, required for productive response and to avoid death and/or tolerance induction [71]. Transcriptomic analysis of in vitro-activated CD8 T cells demonstrates that, similar to IL-12, type I IFNs strongly influence their differentiation program [72]. Type I IFNs and IL-12 act directly on CD8 T cells to induce the expression of IL-2Rα (CD25), IL-2/15Rβ (CD122), IL-15Rα or IL-18R providing a proliferative advantage and

enhanced CD8 T cell response [73–75]. In vivo, sustained CD25 expression and IL-2 signaling in the late expansion phase favor the division of CD8 T cells through activation of the PI3K pathway and expression of FoxM1, a positive regulator of cell cycle progression genes [76].

Type I IFNs may also affect long-term differentiation into memory cells. In the LCMV model, despite decreased initial expansion, functional memory cells can still be generated from IFNAR$^{-/-}$ CD8 T cells [77]. By contrast, in the course of Listeria *monocytogenes* (LM) infection, impaired type I IFN signaling in CD8 T cells leads to a markedly reduced generation of memory cells despite strong primary expansion [78]. However, the role of type I IFNs is complex in this context as they globally play a deleterious role for the resistance to the infection, partly by sensitizing CD8 T cells to apoptosis [79]. Indeed, Archer et al. [80] recently showed that secretion of cyclic diadenosine monophosphate (c-di-AMP) constrains long-term CD8 T cell response by triggering the STING/IRF3 pathway. This suppression was partially mediated by the action of type I IFNs, but CD8 T cells were not found to be direct targets.

When produced systemically, type I IFNs also influence the function and homeostasis of bystander CD8 T cells. They upregulate the effector functions of memory CD8 T cells in the absence of antigenic stimulation [81]. Furthermore, type I IFNs are able to "prime" naïve CD8 T cells for enhanced effector functions upon later contact with the antigen [82]. In contrast, several reports indicate that type I IFNs also induce the apoptosis of bystander memory CD8 T cells [83, 84]. This memory attrition that occurs in the first moments of an infection is likely to favor the expansion of the new Ag-responding CD8 T cells. In the context of chronic LCMV infection, these effects are detrimental to the host and participate in virus persistence by generating "hyper-immune" activation [85, 86].

In conclusion, activation of an IRF3-dependent program in APCs in the course of infection or immunization strongly affects the quality and the magnitude of CD8 T cell responses at initial and late phases, primarily through the induction of type I IFNs. These effects are very complex and context-dependent but have major implications in terms of vaccination and immunotherapeutic strategies against intracellular pathogens or tumor cells.

## IRFs within CD8 T cells influences their function and their polarization

Several IRFs, including IRF1, IRF4 and IRF8, are expressed in the lymphoid lineage. They cooperate with master transcription factors to regulate the expression of T cell effector genes. For example, IRF1 within CD4 T cells contributes to the stabilization of Th1 phenotype through the direct induction of IL-12Rβ1 required for their responsiveness to IL-12 [87].

IRF4 is central to the biology of T lymphocytes as its expression is regulated downstream of the T cell receptor (TCR) upon activation. It is required for the acquisition of effector functions of most CD4 subsets, except Th1, through its capacity to form heterodimers with lineage-specific partners.

In Th2 cells, IRF4 promotes expression of GATA-3 and acts synergically with NFATc2 and c-maf to promote IL-4 expression [88, 89]. In Th17 cells, IRF4 participates in the upregulation of RORγt expression [90]. Furthermore, IRF4-binding protein (IBP), a cytoplasmic protein rapidly phosphorylated upon TCR engagement, sequesters IRF4 in the cytoplasm and limits its recruitment to the promoters of IL-17 and IL-21 [91]. IRF4 is highly expressed in Th9 cells and directly promotes IL-9 expression [92]. Loss of IRF4 also impairs the ability of CD4 T cells to display follicular helper (Tfh) profile (ICOS, IL-21, BCL-6) [93] and the ability of Tregs to suppress Th2 differentiation through direct interaction with Foxp3 [94].

Within CD8 T cells, IRF4 is required for the expression of effector molecules, their expansion and survival after the initial phases of infection through the upregulation of Blimp-1 and T-bet and the repression of multiple inhibitors of cyclin-dependent kinases (Cdkn2a, Cdkn1a and Cdkn1c) [95].

IRF8 is a major regulator of IL-12 in APCs and is thereby critical for the induction of Th1 responses. Within

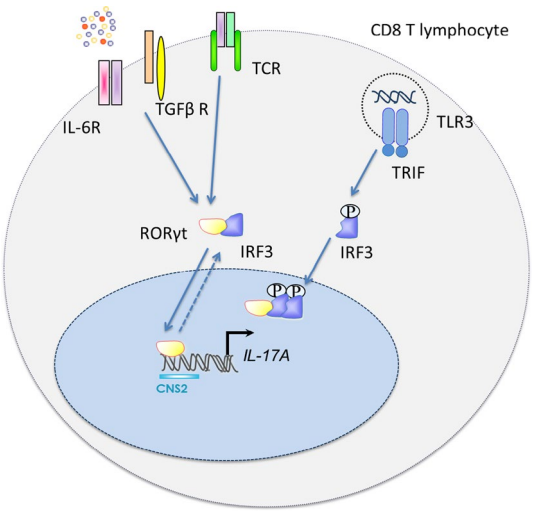


**Fig. 4** IRF3 regulates the differentiation of CD8 T cells into IL-17-producing cells. Activation of CD8 T cells in the presence of IL-6 and TGFβ leads to upregulation of RORγt and differentiation into IL-17-producing cells (Tc17). IRF3 limits IL-17 production through direct interaction with RORγt in the cytoplasm. We suggest that IRF3 interferes with the shuttling of RORγt from the cytoplasm to the nucleus and hence its recruitment to DNA-binding sites. Classical IRF3 activation by poly(I:C) potentiates this repressive effect on RORγt, presumably through increased interaction in the nucleus

T cells, IRF8 limits Th17 differentiation in vitro and in the course of T cell-mediated colitis [96]. Mechanistically, IRF8 physically interacts with RORγt and represses the transcription of IL-17 [96].

IRF3 is also expressed in CD4 and CD8 T cells. Our group recently explored the specific role of this transcription factor in T cell polarization [97]. Since T lymphocytes also express innate receptors such as TLR3 and MDA-5, IRF3 can be classically activated by polyI:C. Addition of polyI:C on purified CD8 T cells limits their capacity to produce IL-17 in an IRF3-, TRIF- and IPS1-dependent fashion. Notably, even in absence of classical signs of IRF3 activation or exogenous PAMPs, the absence of IRF3 in CD8 T cells increased their capacity to produce IL-17 expression both in vitro and in vivo. As observed for IRF8, IRF3 directly interacts with RORγt through its IRF association domain, but this occurs in the cytoplasm rather than the nucleus (Fig. 4). It is therefore possible that IRF3 interferes with the shuttling of RORγt from the cytoplasm to the nucleus and hence its recruitment to DNA-binding sites. The role of IRF3 in Th17 differentiation is less marked than in Tc17, suggesting qualitative or quantitative differences in the transcriptional network involved in the differentiation of both cell types.

## Conclusions

It is now clear that the function of IRF3 extends beyond its role in the induction of an antiviral state. It is a critical node in TLR signaling pathways, which is central for the induction of important polarizing cytokines. IRF3 interacts with other transcription factors, coactivators and repressors, including other IRFs, NF-κB p65, Maf-B, β-catenin, RORγt or the proapoptotic molecule Bax [B-cell lymphoma 2 (Bcl2)-associated X protein] [2, 38, 98]. This broadens its capacity to influence different cellular processes such as cell death or metabolism. IRF3 may also act as a signaling platform, independent of its transcriptional activity [97–99]. It is therefore not surprising that this molecule participates in the regulation of adaptive immune response at different levels.

Several direct activators of the TBK1/IRF3 pathway display adjuvant properties with proven efficacy in preclinical models or clinical studies. For example, polyI:C is now evaluated in the context of cancer immunotherapy [100]. Along this line, cyclic di-AMP is a candidate mucosal adjuvant that triggers mixed Th1/Th2/Th17 response in mouse studies [101]. Monophosphoryl lipid A was suggested to be a TRIF-biased TLR4 agonist [102]. It is now used in conjunction with other adjuvants in order to increase cellular responses in the context of HPV or malaria vaccination [103]. The direct and indirect (type I IFN-mediated) roles of IRF3 in shaping the adaptive immune response in the context of vaccination or cancer immunotherapy still needs to be clearly defined.

Further work is also needed to understand the possible implications of these findings in human physiopathology. Indeed, *IRF3* gene polymorphisms have been linked to the severity of systemic lupus erythematosus or the clinical outcome of acute myeloid leukemia patients submitted to allogeneic stem cell transplantation [104, 105]. It will therefore be important to investigate how IRF3, upstream and downstream pathways can be manipulated to treat immune-related diseases.

### Box 1: the IL-12 family

*Interleukin (IL)-12*, composed of p35 and p40 subunits, directly activates NK cells and stimulates their IFNγ production. Furthermore, IL-12 plays a central role in the polarization of naive helper T lymphocytes into effector Th1 cells. The IL-12/Th1 axis is required for the development of protective responses against numerous intracellular pathogens and is involved in tumor immune surveillance. Until the discovery of IL-23, the IL-12/IFNγ axis was thought to be responsible for autoimmune inflammation in various experimental models. *IL-23* is another heterodimeric cytokine which shares its p40 subunit with IL-12 but favors the expansion and function of Th17 or Th22 inflammatory effector cells. The IL-23/IL-17 axis contributes to autoimmune inflammation and to the development of protective responses to extracellular pathogens such as Klebsiella *pneumoniae* or Citrobacter *rodentium*. It preferentially leads to the recruitment of neutrophils.

*IL-27* is composed of EBI3 and p28 subunits, which are homologous to p40 and p35 chains, respectively. In various infectious or autoimmune models, IL-27 limits Th1-, Th2- and Th17-type responses and favors the production of IL-10 by effector cells.

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