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Regulation of the type I IFN induction: a current view

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Abstract

The type I IFN-α/β gene family was identified about a quarter of a century ago as a prototype of many cytokine gene families, which led to the subsequent burst of studies on molecular mechanisms underlying cytokine gene expression and signaling. Although originally discovered for their activity to confer an antiviral state on cells, more evidence has recently been emerging regarding IFN-α/β actions on cell growth, differentiation and many immunoregulatory activities, which are of even greater fundamental biological significance. Indeed, much attention has recently been focused on the induction and function of the IFN-α/β system regulated by Toll-like receptors (TLRs), which are critical for linking the innate and adaptive immunities. The understanding of the regulatory mechanisms of IFN-α/β gene induction by TLRs and viruses is an emerging theme, for which much new insight has been gained over the past few years.

Introduction

IFN-α and -β were originally identified as humoral factors that confer an antiviral state on cells, and these cytokines constitute a family, termed type I IFNs, which encompasses a group of structurally related genes (1–11). In humans and mice, multiple functional IFN-α gene subtypes exist, whereas a single gene exists for IFN-β (1–11). The pleiotropic roles of IFN-α/β in various biological systems have been uncovered further in recent studies.

Most notably, the IFN-α/β system gained much attention in the context of Toll-like receptor (TLR) signaling that modulates the development of innate and adaptive immune systems (12–16). In brief, the stimulation of antigen-presenting cells (APCs) by pathogen-associated molecules, such as LPS, unmethylated DNA (CpG DNA) and double-stranded RNA (dsRNA), via distinct TLR family members leads to the expression of various effector molecules, including IFN-α/β (15–20). IFN-α/β then contribute to the induction of the expression of co-stimulatory molecules, such as CD40 and CD86, and the functional maturation of APCs (12, 21, 22). Recombinant IFN-α/β potently enhance the antibody response (including the induction of isotype switching) through the stimulation of dendritic cells (DCs) (23). Furthermore, the cross-presentation of antigens on MHC class I molecules, the induction of CTL responses and the subsequent memory $CD8^+$ T cell survival are also dependent on IFN-α/β (24–28).

In addition to immune modulation, IFN-α/β affect cellular development and homeostasis. In the bone marrow, type I IFNs are weakly produced and regulate the homeostatic differentiation of hematopoietic cells, such as B cells, T cells, osteoclasts and myeloid DCs (29–33). Although the underlying mechanisms are still unknown, in most cases, IFN signaling negatively affects hematopoietic cell development. Evidence has also been provided that IFN-α/β contribute to anti-tumor activities and the control of cell growth (34, 35). These findings underscore the broad biological activities of type I IFNs in maintaining the normal immune homeostasis as well as in preserving the integrity of many cell types.

The induction of IFN-α/β is regulated primarily at the transcriptional level, wherein IFN regulatory factors (IRFs) play central roles (36–38). Our knowledge concerning the mechanism underlying the transcriptional regulation of IFN-α/β genes has rapidly expanded over the past few years, particularly in the context of TLR signaling. The mechanism by which pathogen-derived ligands and respective host receptors, including TLRs, trigger the IFN-α/β induction is becoming clearer. Furthermore, the availability of newly generated mice deficient in one or two transcription factors of the IRF family has allowed us to obtain clearer pictures of the contribution of each IRF member to the transcriptional regulation of these genes. In this review, we focus on recent studies on the transcriptional regulation of IFN-α/β genes and its immunological significance.

IFN-α/β signaling and induction of target genes; an overview

The IFN-α/β signaling pathway and the activation of the target genes have been reviewed in depth elsewhere (3–11, 29, 39,

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40), and only a brief description on the cardinal features will be made below. All IFN-α/β species interact with the same receptor complex, termed the IFN-α/β receptor (IFNAR), which consists of at least two subunits, IFNAR1 and IFNAR2. The intracellular domains of IFNAR1 and IFNAR2 are associated with the Janus family of protein tyrosine kinases (Jak kinases), Tyk2 and Jak1, respectively. The binding of IFN-α/β to IFNAR results in the cross-activation of these Jak kinases, which then phosphorylate IFNAR1, Stat1 and Stat2. These Stats recruited to the phosporylated IFNAR1 form two distinct transcriptional activator complexes, namely, IFN-α-activated factor (AAF) and IFN-stimulated gene factor 3 (ISGF3). AAF is a homodimer of Stat1, whereas ISGF3 is a heterotrimeric complex of Stat1, Stat2 and IRF-9 (also known as p48 or ISGF3γ). AAF and ISGF3 translocate into the nucleus, and bind to specific DNA sequences, named the IFN-γ-activated sequence (GAS) and the IFN-stimulated response element (ISRE), respectively. IFN-signaling results in the transcriptional induction of hundreds of target genes (IFN-stimulated genes), which include the genes for dsRNA-activated serine/threonine protein kinase, 2′,5′-oligoadenylate synthetase and the p53 tumor suppressor (5–11, 34). Other target genes critical for the induction of IFN-α/β genes include IRF-7, TLR3, TLR7 and the dsRNA-recognition molecule retinoic acid-inducible gene I (RIG-I) (41–44).

IFN-α/β gene enhancers and IRFs

The promoter region of the IFN-β gene contains at least four regulatory *cis*-elements: the positive regulatory domains (PRDs) I, II, III and IV (45–47) (Fig. 1). The PRD I and PRD III elements are activated by members of the IRF family (36–38, 46, 48–55). On the other hand, PRD II and PRD IV elements, as yet not identified in the IFN-α promoters, are activated by nuclear factor κB (NF-κB) and ATF-2/c-Jun, respectively (47, 48, 56–60). As for the promoter region of IFN-α genes, PRD I- and III-like elements (PRD-LEs) that bind IRFs have been identified (61–64), but it still remains elusive whether or not other transcription factors contribute to the induction of these genes.

The prototype IRF family molecule IRF-1 was first discovered as a transcriptional activator that binds to PRD I or III of the human *IFNB1* gene (50). Subsequently, other IRFs have been discovered and the mammalian IRF family now comprises nine members (36–38). It has been demonstrated that many of these IRF family members play a pivotal role in diverse biological processes, including immunity, inflammation and apoptosis (36–38, 65). All these members are characterized by the presence of a well-conserved N-terminal DNA-binding domain of about 120 amino acids, which recognizes similar DNA sequences (consensus; 5′-GAAANNGAAAG/CT/C-3′), termed ISRE, which is similar to the PRD and PRD-LEs found in the IFN-α/β promoters (39, 66).

Among IRFs, at least four members, namely, IRF-1, IRF-3, IRF-5 and IRF-7, have been implicated as the regulators of IFN-α/β gene transcription (36–38, 50, 67). Although IRF-1 is the first discovered IRF member in the context of IFN gene induction (50), the induction of both IFN-α and IFN-β mRNAs by Newcastle disease virus (NDV) remains essentially normal in *Irf1*-deficient embryonic fibroblasts (MEFs) (68). In addition, it has also been demonstrated that the dsRNA- or virus-mediated IFN induction in MEFs derived from *Irf5*-deficient mice is normal (69), indicating that neither IRF-1 nor IRF-5 is essential for gene induction. As will be described below, it appears that IRF-7 is the master regulator of IFN-α/β gene induction, whereas IRF-3 also critically participates depending on the nature of the stimuli (70, 71). It must be mentioned, however, that IRF-1 may be involved in the induction of IFN-α and -β genes by dsRNA in fibroblasts (68) and by CpG DNA in some DCs (K.H., unpublished results).

IFN induction pathway in virus-infected fibroblasts: the classical pathway

IRF-3-mediated IFN gene induction (early view)

In the 1990s, the studies of the transcriptional regulation of IFNs were mainly carried out using virus-infected fibroblasts. In this context, the role of IRF-3 has been extensively studied among IRF family members. IRF-3 is expressed constitutively in a variety of cells and localizes in the cytoplasm as an inactive monomer (54, 72–77). IRF-3 has potential virus-mediated phosphorylation sites in the C-terminal region (Ser385, 386, 396, 398, 402 and 405, and Thr404 of human IRF-3). Phosphorylation of Ser396 was first reported by using phospho-specific antibody (78). Another report demonstrated that phosphorylation of Ser386 is the critical determinant for the activation of IRF-3 (79). No direct evidence of phosphorylation for the remaining five serine/threonine sites has been reported. The phosphorylation event induces IRF-3 activation and homodimerization (54, 72–79). Based on the crystal structure of IRF-3, there are two models of IRF-3 activation and dimerization. One is 'the phosphorylation-induced dimerization model', in which phosphorylation at Ser385 or Ser386 of IRF-3 induces dimerization (80). The other model is 'the autoinhibitory model', in which two regions corresponding to residues 380–427 and 98–240 of IRF-3 mutually interact to form a closed structure in the inhibited state. This structure is opened by the introduction of massive negative charges following the multiple phosphorylation of C-terminal serine/threonine residues, resulting in IRF-3 activation and dimerization (81). Whatever the mechanism, the dimeric form of IRF-3 then translocates to the nucleus, forms a complex with co-activator p300/CBP and binds to the PRD I or PRD III element (54, 72–79). The active IRF-3 is also known to directly induce chemokine genes such as RANTES and IP10 during viral infection (82–84).

In the early view, IRF-3 was thought to be primarily responsible for the initiation of IFN-β induction: the IFN-β gene is first activated by signals that induce the cooperative binding of IRF-3 with other transcription factors, namely, NF-κB and c-Jun/ATF-2, to the IFN-β promoter (Fig. 2, upper panel). This (early) view was supported by several lines of evidence. Mice carrying a null mutation in *Irf3* alleles are vulnerable to encephalomyocarditis virus (EMCV) infection, and IFN-α/β mRNA expression induced by NDV is markedly impaired in *Irf3*$^{-/-}$ MEFs (71). It was also shown that IFN-α gene induction is affected in MEFs from mice deficient in *Ifnb1* (85). These results led to the notion that IFN-α/β gene induction occurs sequentially, wherein the initial IFN-β

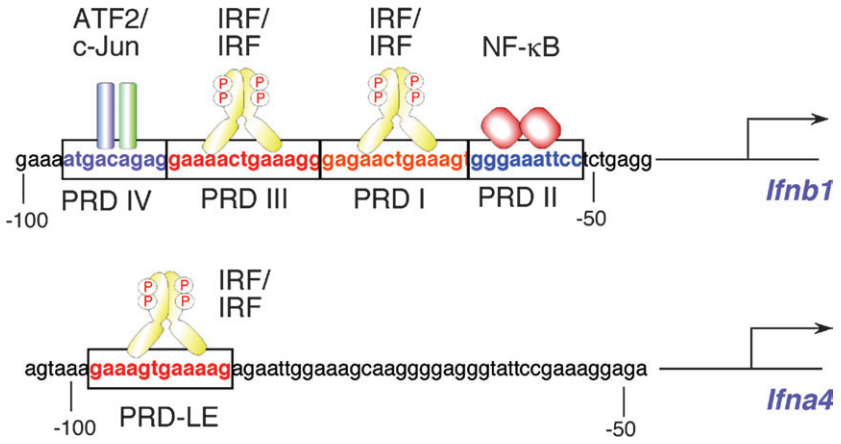

Fig. 1. Schematic representation of murine IFN-β and -α gene (*Ifnb1* and *Ifna4*, respectively) promoters. The IFN-β gene contains at least four positive regulatory *cis*-elements: PRD I, II, III and IV. NF-κB and ATF-2/c-Jun bind to the PRD II and PRD IV elements, respectively. The PRD I and PRD III elements are recognized by members of the IRF family. The promoter region of the IFN-α gene contains one PRD-LE, which can serve as a binding site for IRFs.

induction by IRF-3 (first phase) triggers the positive-feedback loop regulation of the gene induction mediated by IFN-inducible IRF-7 that can activate both IFN-α and -β genes (second phase) (41, 42) (Fig. 2, upper panel). Although still applicable to some cells, such as early-passage MEFs expressing IRF-7 at very low levels (86), this two-step induction model needs to be reconciled with recent findings on mice lacking *Irf7* (70) (see below).

IRF-7-mediated IFN gene induction in fibroblasts (current view)

IRF-7 was first described to bind and repress the Qp promoter region of the EBV-encoded gene EBNA-1, which contains an ISRE-like element (87). Similar to IRF-3, IRF-7 mainly resides in the cytoplasm and requires the phosphorylation of C-terminal serine residues for its activation and nuclear translocation (41, 42, 88, 89). The Ser437 and 438 residues of murine IRF-7 are the primary targets of phosphorylation, but an additional phosphorylation of the Ser425–426, Ser429–431 or Ser441 residue is required to fully activate IRF-7 in virus-infected cells (90). It has been shown that, similar to IRF-3, IRF-7 also undergoes dimerization to activate its target genes (41, 42, 88, 89). As described above, the expression of the IRF-7 gene is regulated by IFN-α/β-activated ISGF3 (41, 42). In addition, it has been shown that IRF-3 is potent in activating the IFN-β gene rather than most of the IFN-α genes (except for the IFN-α4 gene), whereas the ectopic expression of IRF-7 causes the activation of both IFN-α and IFN-β genes (41, 42, 71, 83). Therefore, it was considered that IRF-7 is involved in the late phase of IFN-α/β gene induction, contributing to the positive-feedback regulation for the robust IFN-α/β production in antiviral immunity.

Only very recently, *Irf7*-deficient mice ($Irf7^{-/-}$ mice) have been generated, allowing the rigorous assessment of the above view of the positive-feedback regulation (70). In MEFs from $Irf7^{-/-}$ mice, IFN-α/β gene induction by viruses [vesicular stomatitis virus (VSV), herpes simplex virus-1 (HSV-1) and EMCV] is more severely impaired than in $Irf3^{-/-}$ MEFs. Consistently, $Irf7^{-/-}$ mice are more vulnerable than $Irf3^{-/-}$ mice to viral infections, which correlates with a marked decrease in serum IFN level (70). These results, therefore, finally proved the critical role of the IRF-7-dependent pathway in IFN-α/β gene induction in MEFs; IRF-7 plays the major role, functioning even in the absence of IRF-3. Although IRF-3 also participates in IFN-β gene induction, it contributes little in the absence of IRF-7. Thus, we have to reconsider the positive-feedback model described above: IRF-7, expressed at low levels in unstimulated cells [for example by constitutive IFN signaling (29)], is critical for activating the initial phase of gene induction; this induction indeed occurs even in the absence of IRF-3 (Fig. 2, lower panel). Although IRF-3 also participates in this pathway, it perhaps needs to interact with IRF-7 for its full function. In other words, the homodimer of IRF-7 or the heterodimer of IRF-7 and IRF-3, rather than the IRF-3 homodimer, is perhaps very critical for inducing IFN-α/β in MEFs infected by viruses. Once the initial activation of IFN genes is achieved by IRF-7 (and IRF-3), the positive-feedback regulation becomes fully operational, wherein IFN-induced IRF-7 fully participates (Fig. 2, lower panel).

IRF kinases

Recently, TANK-binding kinase 1 (TBK1; also known as T2K and NAK) and inducible IκB kinase (IKK*i*; also known as IKKε) have been identified as virus-activated IRF-3 and IRF-7 kinases (91, 92). Indeed, in MEFs derived from *Tbk1*-deficient mice, IFN-α/β mRNA induction was shown to be diminished in response to VSV or Sendai virus infection (93–95). An *in vitro* study suggests that cyclophilin B (CypB), a member of the immunophilin family of *cis–trans* peptidyl-prolyl isomerases, is also involved in virus-mediated IRF-3 phosphorylation (96). Although the exact function of CypB has remained unclarified, considering the fact that CypB has an endoplasmic reticulum (ER)-directed signal sequence (97), ER might be involved in the IFN induction pathway.

As a key upstream regulator of the virus-mediated IRF-3 or IRF-7 activation, RIG-I was recently identified (44). RIG-I mediates the recognition of dsRNA, the main sign of replication for many viruses (98), and the subsequent activation of TBK1

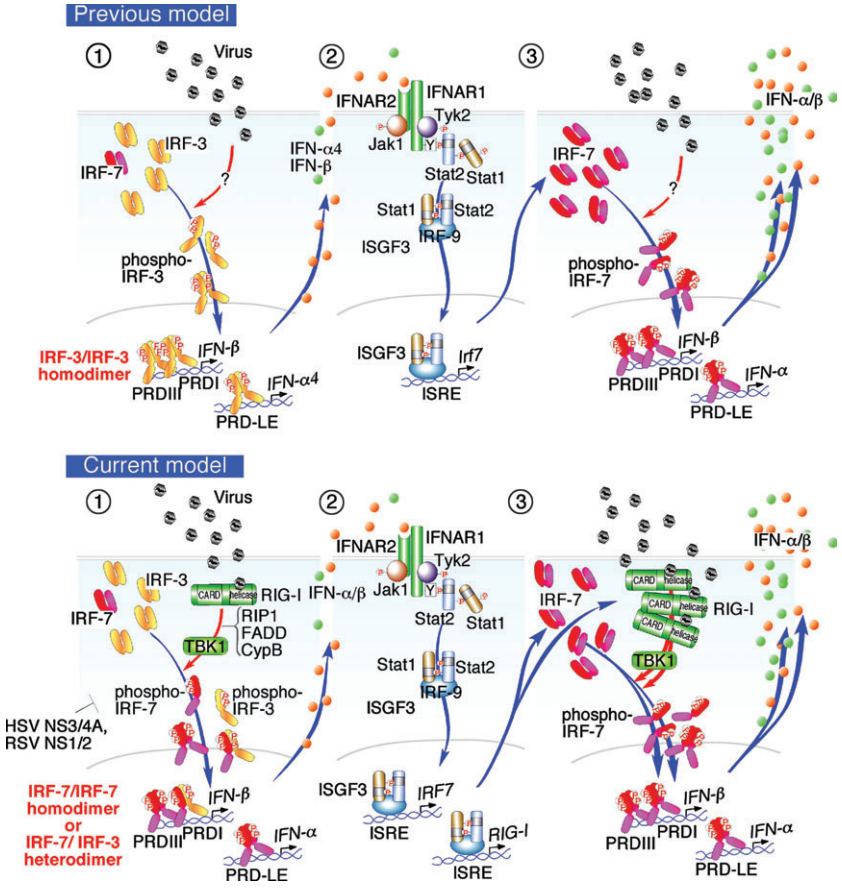

Fig. 2. Virus-mediated IFN-α/β gene induction in fibroblasts (early versus current view). In the previous model (upper panel), the initial induction of IFN-β is mediated mainly by IRF-3, and then a positive-feedback loop becomes operational following IRF-7 induction by the IFNAR-Tyk2/Jak1-ISGF3 pathway. In the current model (lower panel), IRF-7 plays a pivotal role in both the first and second phases of IFN induction. In addition, as the crucial components of the cytosolic virus detection system, RIG-I and TBK1 were recently identified. The interaction of the helicase domain of RIG-I with viral RNA or dsRNA may induce protein–protein interactions between the RIG-I CARD and other unknown CARD-containing adaptor proteins, resulting in the activation of TBK1. In addition, FADD and RIP1 have also been implicated in this activation pathway, but it remains to be clarified how these factors precisely contribute to the TBK1 activation. Activated TBK1 induces the phosphorylation of the specific serine residues of IRF-3 and IRF-7, resulting in the homodimerization of IRF-7 or the heterodimerization of IRF-7 and IRF-3. These dimmers then translocate to the nucleus and activate the IFN-α/β genes. IRF-7 and RIG-I are induced by IFN signaling, which is an essential aspect for the amplification of IFN-α/β signaling. Some viruses are known to block the activation of this pathway.

(44). RIG-I contains a C-terminal RNA helicase domain as well as an N-terminal caspase recruitment domain (CARD) (44). The interaction of the helicase domain with viral RNA or dsRNA may induce a conformational change of RIG-I and promote protein–protein interactions between the RIG-I CARD and other downstream CARD-containing proteins (Fig. 2, lower panel). Definitive evidence for the essential role of RIG-I for the IFN gene induction by RNA viruses has recently been obtained by generating MEFs deficient in *Ddx58* (RIG-I gene) (99). It has also been reported that the loss of the Fas-associated protein with the death domain (FADD) or the receptor-interacting protein 1 (RIP1) leads to a defect in IFN-β production against VSV infection (100). These reports point to the importance of RIP1 and FADD, which may be recruited to viral dsRNA recognizing RIG-I, in the regulation of the TBK1-mediated activation of IRF-7 and IRF-3 [the RIG-I–RIP1–FADD–TBK1–IRF-7(-3) pathway], although this possibility is yet to be rigorously assessed.

The IFN induction pathway described above is operational in various cells, such as MEFs, and has been extensively studied in the context of innate antiviral immunity. Hence, it may be called 'the classical pathway' *vis-à-vis* the recently discovered TLR pathways of IFN induction (described below). Intriguingly, the RIP1–FADD–TBK1–IRF-7(3)-mediated IFN induction pathway is reminiscent of the Imd pathway in *Drosophila* (101, 102). In *Drosophila*, Imd (a homologue of the mammalian RIP1) and *Drosophila* (d)FADD are required for the stimulation of the induction of anti-microbial gene expression through the activation of the NF-κB homologue Relish via an IKK complex (101, 102). Therefore, this pathway may be highly conserved and may also be classical in the context of evolution. In turn, viruses have developed mechanisms for counteracting this classical pathway to evade from the host's immune responses (9). For example, hepatitis C virus non-structural proteins 3 and 4A (NS3/4A) interfere with the functions of RIG-I and TBK1, thereby inhibiting the

activation of IRF-3 during its infection (103–105). Human respiratory syncytial virus NS1 and NS2 were also shown to suppress the activation and nuclear translocation of IRF-3 (106). A better understanding of this pathway is therefore critical to establish an efficient therapeutic regulation of viral infections.

TLR signaling and induction of IFN genes

General overview

An exciting direction of IFN-α/β research was spawned by the discovery of TLRs. Indeed, the activation of many TLRs results in the induction of IFN-α and/or -β and this induction has received much attention in the context of linking innate and adaptive immunity (12–16, 20). The TLR family consists of as many as 13 germline-encoded receptors in mammals that recognize various pathogen-associated molecules derived from bacteria, viruses, fungi and protozoa (15, 20, 101, 107). All TLRs contain intracellular Toll/IL-1 receptor (TIR) domains, which transmit downstream signals via the recruitment of adaptor proteins such as myeloid differentiation primary response gene 88 (MyD88) (20, 101, 108–110). MyD88 is linked to several effector molecules, such as IL-1R-associated kinases 1/4 (IRAK1/4), tumor necrosis factor receptor-associated factor 6 (TRAF6) and transforming growth factor-β-activated kinase 1, and these molecules are linked to the activation of NF-κB, mitogen-activated protein kinases, extracellular signal-related kinases, p38 and c-Jun N-terminal kinase (JNK) (20, 101, 109–111). Recent studies revealed that two IRF members, IRF-5 and IRF-7, are also activated by some of the TLRs via the MyD88 pathway (69, 112, 113).

Although less studied than the above-mentioned MyD88 pathway, some TLRs also utilize additional adaptors, such as TIR-associated protein (TIRAP; also called MAL), TIR-domain-containing adaptor-inducing IFN (TRIF; also called TICAM-1) and TRIF-related adaptor molecule (TRAM; also called TIRP or TICAM-2) (20, 108, 114–119). Thus, the versatility of the response may be mediated at least in part by the differential utilization of those adaptor proteins that activate overlapping but distinct downstream signaling pathways. TLR4 signaling is one of the best studied in this context, and it utilizes two signaling pathways, namely, the MyD88–TIRAP and TRAM–TRIF pathways (20, 108, 114–119). On the other hand, TLR9 subfamily members, TLR7, TLR8 and TLR9, transmit signals by solely utilizing MyD88 (20, 120–122). Notwithstanding the utilization of distinct signaling pathways, the activation of TLR3, TLR4 and the TLR9 subfamily commonly triggers the IFN response (15–20, 108, 121, 122).

TLR4-mediated IFN induction

TLR4 is activated by LPS or the lipid A component of Gram-negative bacteria, as well as by some viral components such as the fusion protein of respiratory syncytial virus or the envelope proteins of mouse mammary tumor virus and Moloney murine leukemia virus (123–125). Although it is not clear whether TLR4 is involved in the IFN response during viral infections, the TLR4 signal-mediated IFN-β gene induction (IFN-α is not induced *in vitro*) is best studied by LPS stimulation (17, 19, 20, 126–129).

It has been shown by gene-targeting studies that the LPS-stimulated IFN-β induction via TLR4 is mostly if not entirely MyD88 independent (17), but TRAM–TRIF dependent (127–129), whereas the induction of pro-inflammatory cytokine genes, such as tumor necrosis factor-α and IL-6, is dependent on both MyD88 and TRAM–TRIF (127–129). The gene-targeting studies have revealed that the TBK1 but not IKK*i* is critical in this pathway (93, 94). In TLR4 signaling, NAK-associated protein 1 (NAP1), which is recruited to TRIF, may be required to induce the oligomerization and activation of TBK1 (130). It was shown that IRF-3, rather than IRF-7, is essential for this pathway (70, 84). *Irf3*-deficient mice exhibited resistance to LPS-induced endotoxin shock (84), and a central role of IFN-β in this shock was previously reported (131). These reports indicate that IFN-β induction by TLR4 is mediated by the homodimer of IRF-3, which is activated by the TRAM–TRIF–NAP1–TBK1 pathway (Fig. 3). Interestingly, however, if IRF-7 is up-regulated by the pre-treatment with recombinant IFN-β, IFN-β mRNA induction by LPS can be observed even in *Irf3*$^{-/-}$ DCs (84). Therefore, IRF-7 can be activated by TLR4 signaling, if expressed prior to the stimulation, and the LPS-activated IRF-7 has a potential to activate IFN-β gene induction.

TLR3-mediated IFN induction

TLR3 recognizes dsRNA, which is commonly produced during viral replication, and it is indeed required for the full induction of IFN-α/β and pro-inflammatory cytokines in response to exogenous stimulation with synthetic dsRNA or reovirus-derived dsRNA (18). Similar to the case of TLR4, TLR3 activation can induce IFN-α/β expression via a MyD88-independent, TRIF-, NAP1- and TBK1-dependent signaling pathway (93, 94, 127, 128, 130). Indeed, evidence has been provided that the activation of IRF-3 and the subsequent IFN-α/β induction are completely abolished in *Trif*- or *Tbk1*-deficient cells in response to stimulation by dsRNA (93, 94, 127, 128), and *Trif*-deficient mice are highly susceptible to mouse cytomegalovirus infection (128). However, unlike the TLR4-mediated IFN induction, the dsRNA-mediated induction of IFN-α/β mRNAs is still observed in *Irf3*-deficient DCs (84); this residual induction is completely abolished in DCs from *Irf3* and *Irf7* doubly deficient mice (K.H., unpublished result). Therefore, IRF-7 is also required for TLR3 signaling to fully induce the genes, although it is currently unknown which signaling pathway is linked to IRF-7 activation.

Difference between TLR3- and TLR4-mediated IFN inductions

From the above-mentioned observations, it can be interpreted that the mechanisms of TRIF-mediated IFN gene induction via TLR4 and via TLR3 are not the same. Indeed, TLR3 activation results in the induction of IFN-α as well as IFN-β, whereas TLR4 induces only IFN-β (17, 18, 132). Although the precise mechanism underlying this interesting difference is currently unknown, it is possible that an additional signaling event might occur in the TLR3–TRIF pathway. In this context, it is noteworthy that TLR3 signaling up-regulates TLR3 expression via IFN signaling; type I IFNs induced by TLR3 signaling transcriptionally induces *Tlr3* gene via ISGF3 activation, so as

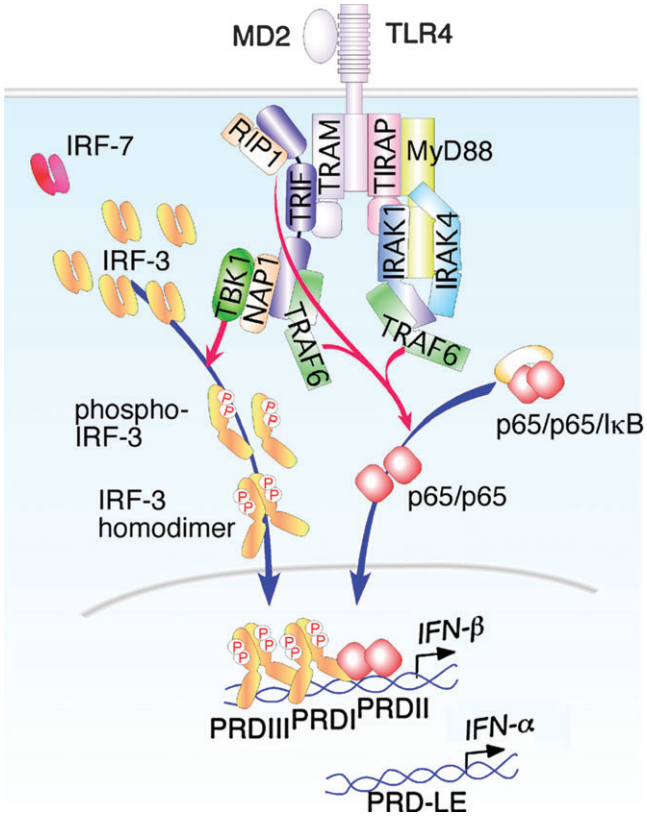

Fig. 3. TLR4-mediated IFN induction pathway. TLR4 signals through at least four adaptors: TIRAP, MyD88, TRAM and TRIF. These adaptors associate with many different signaling components and serve as platforms for the initiation of divergent signaling pathways. The oligomerization of MyD88 induces the recruitment and autophosphorylation of IRAK1 and IRAK4, which then associate with TRAF6, leading to the activation of NF-κB. On the other hand, TRAM and TRIF mediate the activation of IRF-3 as well as NF-κB. The C-terminal portion of TRIF associates with RIP1, which is responsible for TRIF-mediated NF-κB activation. The N-terminal portion of TRIF associates with TRAF6, which is also involved in NF-κB activation. In addition, the N-terminal portion of TRIF also associates with TBK1 through NAP1, which mediates the phosphorylation of IRF-3 (and IRF-7, if present). Phosphorylated IRF-3 forms homodimers, which associate with CBP/p300 in the nucleus (data not shown) and bind to the IFN-β promoter.

to amplify and maintain TLR3 signaling (21, 43, 132). Thus, this positive-feedback mechanism may account, at least in part, for IRF-7-dependent IFN-α induction during TLR3 signaling.

Unlike TLR4 that is expressed on the cell membrane, TLR3 is compartmentalized in intracellular organelles, such as endosomes (133). It has been reported that endosomal acidification is necessary for TLR3 signaling (133). Therefore, an additional molecule present in the endosomal compartment may also participate in the response in collaboration or in parallel with TLRs to induce IFN-α production. In this context, it is worth noting that tyrosine residues in the cytoplasmic domain of TLR3 need to be phosphorylated for the signaling (134). The phosphorylated tyrosine residues then recruit phosphatidylinositol-3 kinase (PI3K), and PI3K activity might be necessary for the endosomal trafficking of TLR3 as well as the activation of the Akt pathway to fully activate IRF-7 (135).

Additional evidence for the difference between TLR3 and TLR4 signalings was provided by the observation that the IRF-3-mediated activation of ISRE by TLR4, but not TLR3, requires the p65 subunit of NF-κB (RelA) (136). TLR4 stimulation fails to activate ISRE in *Rela*-deficient MEFs, whereas the response to TLR3 in these cells is normal (136). More recently, it has been shown that LPS-activated IRF-3 is recruited to the NF-κB site of IP10 (*Cxcl10*) and is necessary for the transcriptional activity of RelA (137). These reports indicate that NF-κB and IRF-3 must cooperate in TLR4 signaling, but not TLR3 signaling, to a gene whose promoter contains an ISRE.

New IFN induction pathway activated by TLR9 subfamily

Recently, much attention has been focused on the high-level induction of IFN-α/β upon the activation of TLR9 subfamily members in plasmacytoid dendritic cells (pDCs), a small subset of DCs (122, 138–143). Accumulating evidence indicates that certain viruses induce the IFN-α/β gene via the activation of the TLR9 subfamily members TLR7, TLR8 and TLR9 (122, 144–149). For example, DNA viruses such as HSV contain a high number of unmethylated CpG motifs in their genomes, which are recognized by TLR9 and induce robust IFN production in pDCs (144, 145). Likewise, TLR7/8 signaling is essential for IFN induction against influenza virus or VSV infection by recognizing viral genomic single-stranded RNA (ssRNA) (146–148).

In contrast to TLR3- or TLR4-mediated TRIF-dependent IFN induction, the TLR9 subfamily members exclusively use MyD88 as their signaling adaptor for IFN induction (122, 144–149). Recently, evidence has been provided that MyD88 interacts with IRF-7 but not with IRF-3 in the cytoplasm (112, 113). Fluorescence microscopy studies showed that a significant fraction of IRF-7 co-localizes with MyD88 in endosomal vesicles, whereas diffusely expressed IRF-3 does not co-localize with MyD88 (112, 113, 150). Furthermore, fluorescence resonance energy transfer analysis revealed a direct interaction between IRF-7 and MyD88 (112, 113). IRF-7 was also found to interact with TRAF6, another adaptor molecule functioning downstream of MyD88 (112, 113). When cells expressing fluorescently tagged IRF-7 were stimulated with an IFN-inducing TLR9 ligand, A- or D-type unmethylated CpG DNA (CpG-A), the nuclear translocation of IRF-7 was observed (112). Furthermore, upon co-transfection of expression plasmids for MyD88 and IRF-7 together with the IFN-β promoter-driven reporter gene, the reporter gene was strongly induced (112, 113). Similar observations were made by co-expressing TRAF6 and IRF-7 (112, 113). These *in vitro* studies suggest that IRF-7, but not IRF-3, interacts with and is activated by MyD88 and TRAF6 upon TLR9 stimulation to induce IFN gene induction (Fig. 4).

Definitive evidence has been provided for the selective requirement of IRF-7 in IFN-α/β gene induction in pDCs via TLR9 subfamily activation *in vivo* (70). Splenic pDCs derived from *Irf7*$^{-/-}$ mice exhibit a profound defect in the induction of IFN-α/β stimulated either by viral infections (HSV and VSV) or by synthetic TLR ligands (CpG-A and ssRNA) (70), whereas the induction is normal in pDCs from the mice deficient for previously implicated transcription factors, such as IRF-1, IRF-3, IRF-5 or Smad3 (36, 67, 151). Therefore, a robust IFN

gene induction in pDCs is totally dependent on the activation of IRF-7. As mentioned above, IRF-7 is also essential for IFN induction mediated by TLR–MyD88-independent cytosolic detection of viruses in fibroblasts (classical IFN induction pathway) and appears to be the master regulator of the entire IFN-dependent defense mechanism against viral infection (70).

Activation of IRF-7 by TLR9 signaling

The mutation studies of MyD88 revealed that the death domain of MyD88 is responsible for MyD88 interaction with IRF-7 (112). The death domain also interacts with the IRAK family of serine/threonine kinases, the signal transducer between MyD88 and TRAF6, suggesting the potential involvement of IRAKs in the IRF-7 pathway (20, 101, 109, 110, 152). Indeed, pDCs derived from *Irak4*-deficient mice have a defect in IFN-α production, indicating the involvement of IRAK4 in IRF-7 phosphorylation (112). More recently, it has been shown that IRAK1 directly phosphorylates IRF-7 (153). *In vitro* kinase assay revealed that IRAK1, but not IRAK4, phosphorylates recombinant IRF-7. Furthermore, *Irak1*$^{-/-}$ pDCs exhibit a severe impairment of IFN-α induction but a normal induction of pro-inflammatory cytokines, when stimulated by ligands for TLR7 or TLR9 (153). Considering the fact that IRAK4 is essential for both IFN-α and pro-inflammatory cytokine induction (153), IRAK4 may act upstream of IRAK1 in the signaling and participate in the IRF-7 pathway via the phosphorylation of IRAK1. This IRF-7 activation pathway mediated by MyD88–IRAK4–IRAK1–TRAF6 appears to be powerful for IFN induction (Fig. 4).

MyD88 signaling complex: the cytoplasmic transductional–transcriptional processor

In contrast to IFN-α/β gene induction, the activation of NF-κB, JNK and p38 occurs normally in *Irf7*-deficient DCs (70). Furthermore, the induction of pro-inflammatory cytokines, such as IL-12 and IL-6, is not inhibited in *Irf7*-deficient DCs (70). Therefore, the function of a cytoplasmic molecular complex anchored by MyD88 in the activation of NF-κB/MAP kinases is controlled independently of IRF-7, which selectively regulates the IFN limb of the MyD88-dependent cytokine gene induction program in TLR signaling. That is, the MyD88-dependent signaling may be modified by MyD88-interacting molecules that direct activation of their target genes in response to a given pathogen.

In addition to IRAKs and IRF-7, IRF-5 was also shown to interact with MyD88 to regulate the induction of pro-inflammatory cytokines (69) (Fig. 4). Furthermore, the Toll-interacting protein (Tollip), Pellino1, Pellino2 and Pellino3 interact with IRAKs (154–157). Therefore, it is plausible that a highly ordered multimolecular complex organized around MyD88 exists in the cytoplasm and regulates the gene induction program. In analogy to the computing terminology, we named this multimolecular complex the cytoplasmic transductional–transcriptional processor (CTTP) (112). Depending on the nature of the input signal, CTTP may dynamically change its composition and determine the specificity, strength and longevity of the output, that is, transcriptional events. Further investigations are clearly required for determining the regulation of the proposed CTTP complex.

Systemic versus local IFN induction and action

Systemic IFN induction for innate antiviral immunity

A series of gene disruption studies revealed the contribution of the classical and TLR9 subfamily–MyD88-dependent IFN induction pathways, both mediated by IRF-7, to innate antiviral immunity. *Irf7*$^{-/-}$ mice are highly vulnerable to infection by HSV or EMCV, and IFN-α induction is markedly inhibited in the sera of *Irf7*$^{-/-}$ mice infected with either of these viruses (70). On the other hand, *Irf3*$^{-/-}$ mice and *Myd88*$^{-/-}$ mice are more resistant to these viral infections, and the IFN-α induction level is almost the same as that of the wild-type mice (70). In view of the fact that IFN induction is entirely dependent on the TLR9–MyD88 pathway in HSV-infected splenic pDCs (144, 145), these results suggest that the classical IFN-α/β induction pathway, which is less effective than the MyD88–IRF-7-dependent induction pathway in pDCs but operational in many cell types, constitutes a critical part of the innate antiviral defense, wherein IRF-7 also plays an essential role. On the other hand, it is possible that, depending on the virus type or viral load, the TLR–MyD88-dependent pathways may also participate in the antiviral response.

Local IFN induction for antigen-specific T cell response

The importance of the MyD88-dependent IFN induction pathway was underscored by the finding of the *in vivo* induction of antigen-specific CD8$^+$ T cell response. When *Myd88*$^{-/-}$ or *Irf7*$^{-/-}$ mice were immunized with soluble ovalbumin (OVA) and CpG-A, the induction of antigen-specific CD8$^+$ T cells was severely impaired (70). This CpG-A-dependent, OVA-specific CD8$^+$ T cell response is dependent on pDCs, because the pre-treatment of wild-type mice with 120G8, the antibody that allows a selective depletion of pDCs (158), inhibits the response (70). These results collectively demonstrate the selective and essential role of the MyD88–IRF-7 pathway in pDCs in the TLR9-mediated triggering of the CD8$^+$ T cell response, which is mediated by IFN production. In this context, it is interesting to note that pDCs are found predominantly in the T cell areas of secondary lymphoid organs (139, 143), implying that IFN-α/β produced by pDCs may act 'locally' to induce DC maturation, which effectively couples with the induction of CD8$^+$ T cell-mediated adaptive immunity. It is worth noting that the requirement of the MyD88–IRF-7–IFN pathway is not applicable for other TLRs, which are also involved in the induction of T cell response via the MyD88-dependent signaling pathway: When the adjuvant used was a mycoplasmal lipopeptide, which activates TLR2 (and TLR6) (159), the deficiency in T cell response was observed in *Myd88*$^{-/-}$ mice but not in *Irf7*$^{-/-}$ mice (70), indicating the operation of the MyD88-dependent, IRF-7-independent gene activation program for this TLR-induced T cell response.

Future prospects

There is accumulating evidence for multiple signaling pathways for IFN induction. The immune system makes proper use of the IRF–IFN system to induce diverse responses. In addition to the classically known signaling pathways, the list of newly identified IFN induction signaling pathways is rapidly growing

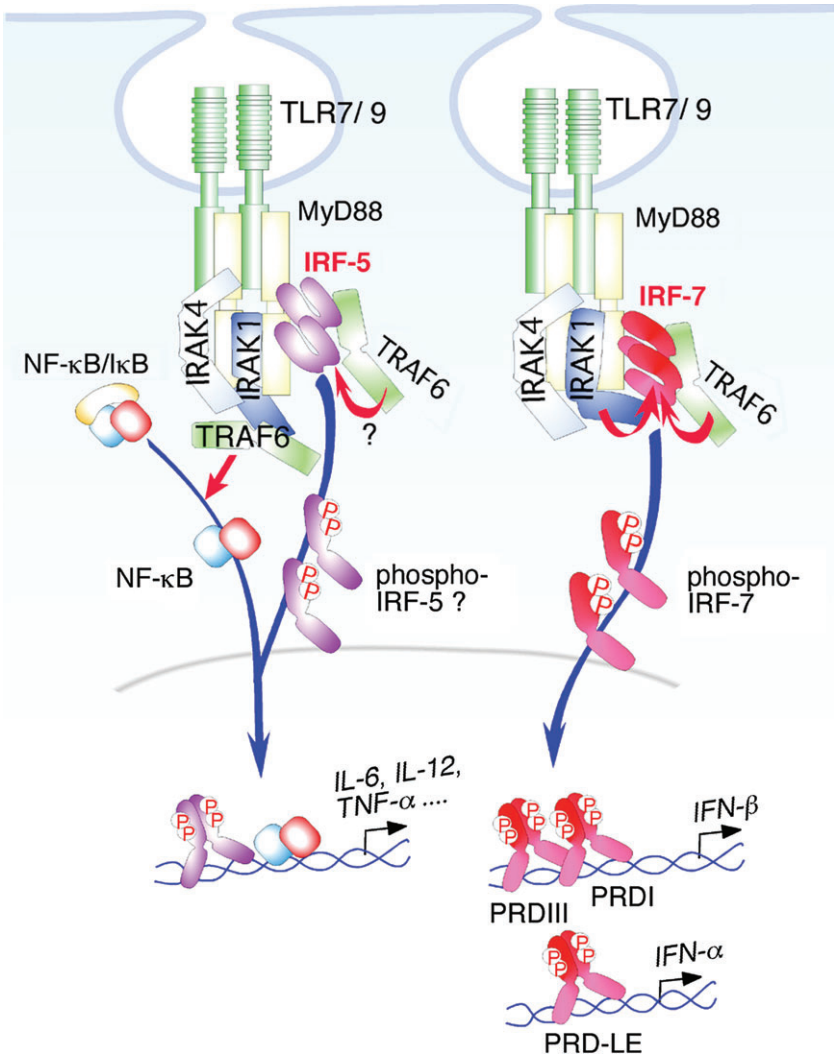

Fig. 4. TLR7/9–MyD88-dependent signaling pathways. The transcription factors IRF-5 (left) and IRF-7 (right) directly bind to MyD88 and regulate the gene induction program for pro-inflammatory cytokines and IFN-α/β, respectively. IRF-5 interacts with and is activated by MyD88 and TRAF6 by an as yet unknown mechanism. Activated IRF-5 translocates to the nucleus to activate pro-inflammatory cytokine gene transcription, presumably in cooperation with NF-κB. IRF-7 also interacts with MyD88 and is activated by IRAK4, IRAK1 and TRAF6 for a robust IFN-α/β induction. Depending on the cell type or the nature of ligands, the composition of complexes forming around MyD88 dynamically changes to properly evoke downstream transcriptional events. In pDCs, the signaling complex consisting of IRF-7, as shown in the right, is readily formed and efficiently induces IFN-α/β production (the 'new' IFN induction pathway).

and will continue to grow. The classical pathways will also continuously need to be re-evaluated.

In contrast to the beneficial aspects of the IFN system on the host defense against viral infection or oncogenesis, accumulating evidence also suggests that an aberrant activation of immune systems by high levels of IFN-α/β contributes to the development of autoimmune diseases, such as systemic lupus erythematosus (SLE) (160–162). An aberrant activation of the MyD88–IRF-7 pathway might be responsible for pathogenesis of this disease. In this context, it is interesting that the TLR9–MyD88–IRF-7 signaling pathway is under spatiotemporal regulation, wherein a prolonged signaling in endosomes is critical for a robust IFN induction (150), and that in patients with SLE, a complex of anti-DNA antibodies and DNA is present and it binds to and enters into the endosomes of pDCs via FcR-mediated endocytosis (163). Further understanding of the IRF–IFN system should provide important insights into improvements in therapeutic interventions for numerous diseases related to infection and immunity.

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Abbreviations

AAF	IFN-α-activated factor
APC	antigen-presenting cells

CARD	caspase recruitment domain
CTTP	cytoplasmic transductional–transcriptional processor
CypB	cyclophilin B
DC	dendritic cells
dsRNA	double-stranded RNA
EMCV	encephalomyocarditis virus
ER	endoplasmic reticulum
FADD	Fas-associated protein with the death domain
HSV-1	herpes simplex virus-1
IFNAR	IFN-α/β receptor
IKK*i*	inducible IκB kinase
IRAK1/4	IL-1R-associated kinases 1/4
IRF	IFN regulatory factor
ISGF3	IFN-stimulated gene factor 3
ISRE	IFN-stimulated response element
JNK	c-Jun N-terminal kinase
MEF	*Irf1*-deficient embryonic fibroblast
MyD88	myeloid differentiation primary response gene 88
NAP1	NAK-associated protein 1
NDV	Newcastle disease virus
OAS	oligoadenylate synthetase
NF-κB	nuclear factor κB
NS	non-structural protein
OVA	ovalbumin
pDC	plasmacytoid dendritic cell
PI3K	phosphatidylinositol-3 kinase
PRD	positive regulatory domains
PRD-LE	PRD I- and III-like elements
RIG-I	retinoic acid-inducible gene I
RIP1	receptor-interacting protein 1
SLE	systemic lupus erythematosus
ssRNA	single-stranded RNA
TBK1	TANK-binding kinase 1
TIR	Toll/IL-1 receptor
TIRAP	TIR-associated protein
TLR	Toll-like receptor
TRAF6	tumor necrosis factor receptor-associated factor 6
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain-containing adaptor-inducing IFN
VSV	vesicular stomatitis virus

References

1 Taniguchi, T., Mantei, N., Schwarzstein, M., Nagata, S., Muramatsu, M. and Weissmann, C. 1980. Human leukocyte and fibroblast interferons are structurally related. *Nature* 285:547.
2 Weissmann, C. and Weber, H. 1986. The interferon genes. *Prog. Nucleic Acid Res. Mol. Biol.* 33:251.
3 Pestka, S., Langer, J. A., Zoon, K. C. and Samuel, C. E. 1987. Interferons and their actions. *Annu. Rev. Biochem.* 56:727.
4 Taniguchi, T. 1988. Regulation of cytokine gene expression. *Annu. Rev. Immunol.* 6:439.
5 Vilcek, J. and Sen, G. S. 1996. Interferons and other cytokines. In Fields, D. M., Knipe, P. M. and Howley, P. M., eds, *Fields Virology*, 3rd edn, p. 375. Lippincott-Raven, Philadelphia, PA.
6 Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H. and Schreiber, R. D. 1998. How cells respond to interferons. *Annu. Rev. Biochem.* 67:227.
7 Samuel, C. E. 2001. Antiviral actions of interferons. *Clin. Microbiol. Rev.* 14:778.
8 Levy, D. E. and Garcia-Sastre, A. 2001. The virus battles: IFN induction of the antiviral state and mechanisms of viral evasion. *Cytokine Growth Factor Rev.* 12:143.
9 Katze, M. G., He, Y. and Gale, M., Jr. 2002. Viruses and interferon: a fight for supremacy. *Nat. Rev. Immunol.* 2:675.
10 Pestka, S., Krause, C. D. and Walter, M. R. 2004. Interferons, interferon-like cytokines, and their receptors. *Immunol. Rev.* 202:8.
11 Platanias, L. C. 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat. Rev. Immunol.* 5:375.
12 Gallucci, S., Lolkema, M. and Matzinger, P. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med.* 5:1249.
13 Biron, C. A. 2001. Interferons α and β as immune regulators—a new look. *Immunity* 14:661.
14 Le Bon, A. and Tough, D. F. 2002. Links between innate and adaptive immunity via type I interferon. *Curr. Opin. Immunol.* 14:432.
15 Iwasaki, A. and Medzhitov, R. 2004. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* 5:987.
16 Theofilopoulos, A. N., Baccala, R., Beutler, B. and Kono, D. H. 2005. Type I interferons (α/β) in immunity and autoimmunity. *Annu. Rev. Immunol.* 23:307.
17 Hoshino, K., Kaisho, T., Iwabe, T., Takeuchi, O. and Akira, S. 2002. Differential involvement of IFN-β in Toll-like receptor-stimulated dendritic cell activation. *Int. Immunol.* 14:1225.
18 Alexopoulou, L., Holt, A. C., Medzhitov, R. and Flavell, R. A. 2001. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 413:732.
19 Hertzog, P. J., O'Neill, L. A. and Hamilton, J. A. 2003. The interferon in TLR signaling: more than just antiviral. *Trends Immunol.* 24:534.
20 Takeda, K. and Akira, S. 2005. Toll-like receptors in innate immunity. *Int. Immunol.* 17:1.
21 Honda, K., Sakaguchi, S., Nakajima, C. *et al.* 2003. Selective contribution of IFN-α/β signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. *Proc. Natl Acad. Sci. USA* 100:10872.
22 Hoebe, K., Janssen, E. M., Kim, S. O. *et al.* 2003. Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-independent pathways. *Nat. Immunol.* 4:1223.
23 Le Bon, A., Schiavoni, G., D'Agostino, G., Gresser, I., Belardelli, F. and Tough, D. F. 2001. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells *in vivo*. *Immunity* 14:461.
24 van den Broek, M. F., Muller, U., Huang, S., Zinkernagel, R. M. and Aguet, M. 1995. Immune defence in mice lacking type I and/or type II interferon receptors. *Immunol. Rev.* 148:5.
25 Tough, D. F., Borrow, P. and Sprent, J. 1996. Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* 272:1947.
26 Zhang, X., Sun, S., Hwang, I., Tough, D. F. and Sprent, J. 1998. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells *in vivo* by IL-15. *Immunity* 8:591.
27 Le Bon, A., Etchart, N., Rossmann, C. *et al.* 2003. Cross-priming of $CD8^+$ T cells stimulated by virus-induced type I interferon. *Nat. Immunol.* 4:1009.
28 Ahonen, C. L., Doxsee, C. L., McGurran, S. M. *et al.* 2004. Combined TLR and CD40 triggering induces potent CD8+ T cell expansion with variable dependence on type I IFN. *J. Exp. Med.* 199:775.
29 Taniguchi, T. and Takaoka, A. 2001. A weak signal for strong responses: interferon-α/β revisited. *Nat. Rev. Mol. Cell. Biol.* 2:378.
30 Lin, Q., Dong, C. and Cooper, M. D. 1998. Impairment of T and B cell development by treatment with a type I interferon. *J. Exp. Med.* 187:79.
31 Takayanagi, H., Kim, S., Matsuo, K. *et al.* 2002. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-β. *Nature* 416:744.
32 Honda, K., Mizutani, T. and Taniguchi, T. 2004. Negative regulation of IFN-α/β signaling by IFN regulatory factor 2 for homeostatic development of dendritic cells. *Proc. Natl Acad. Sci. USA* 101:2416.
33 Ichikawa, E., Hida, S., Omatsu, Y. *et al.* 2004. Defective development of splenic and epidermal $CD4^+$ dendritic cells in mice deficient for IFN regulatory factor-2. *Proc. Natl Acad. Sci. USA* 101:3909.
34 Takaoka, A., Hayakawa, S., Yanai, H. *et al.* 2003. Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 424:516.
35 Takaoka, A. and Taniguchi, T. 2003. New aspects of IFN-α/β signalling in immunity, oncogenesis and bone metabolism. *Cancer Sci.* 94:405.

36 Taniguchi, T., Ogasawara, K., Takaoka, A. and Tanaka, N. 2001. IRF family of transcription factors as regulators of host defense. *Annu. Rev. Immunol.* 19:623.
37 Nguyen, H., Hiscott, J. and Pitha, P. M. 1997. The growing family of interferon regulatory factors. *Cytokine Growth Factor Rev.* 8:293.
38 Mamane, Y., Heylbroeck, C., Genin, P. *et al.* 1999. Interferon regulatory factors: the next generation. *Gene* 237:1.
39 Darnell, J. E., Jr., Kerr, I. M. and Stark, G. R. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264:1415.
40 Taniguchi, T. and Takaoka, A. 2002. The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr. Opin. Immunol.* 14:111.
41 Marie, I., Durbin, J. E. and Levy, D. E. 1998. Differential viral induction of distinct interferon-α genes by positive feedback through interferon regulatory factor-7. *EMBO J.* 17:6660.
42 Sato, M., Hata, N., Asagiri, M., Nakaya, T., Taniguchi, T. and Tanaka, N. 1998. Positive feedback regulation of type I IFN genes by the IFN-inducible transcription factor IRF-7. *FEBS Lett.* 441:106.
43 Miettinen, M., Sareneva, T., Julkunen, I. and Matikainen, S. 2001. IFNs activate toll-like receptor gene expression in viral infections. *Genes Immun.* 2:349.
44 Yoneyama, M., Kikuchi, M., Natsukawa, T. *et al.* 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol.* 5:730.
45 Goodbourn, S. and Maniatis, T. 1988. Overlapping positive and negative regulatory domains of the human β-interferon gene. *Proc. Natl Acad. Sci. USA* 85:1447.
46 Leblanc, J. F., Cohen, L., Rodrigues, M. and Hiscott, J. 1990. Synergism between distinct enhanson domains in viral induction of the human β interferon gene. *Mol. Cell. Biol.* 10:3987.
47 Du, W. and Maniatis, T. 1992. An ATF/CREB binding site is required for virus induction of the human interferon β gene. *Proc. Natl Acad. Sci. USA* 89:2150.
48 Panne, D., Maniatis, T. and Harrison, S. C. 2004. Crystal structure of ATF-2/c-Jun and IRF-3 bound to the interferon-β enhancer. *EMBO J.* 23:4384.
49 Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K. and Taniguchi, T. 1987. Interferon-β gene regulation: tandemly repeated sequences of a synthetic 6 bp oligomer function as a virus-inducible enhancer. *Cell* 49:357.
50 Miyamoto, M., Fujita, T., Kimura, Y. *et al.* 1988. Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-β gene regulatory elements. *Cell* 54:903.
51 Fujita, T., Sakakibara, J., Sudo, Y., Miyamoto, M., Kimura, Y. and Taniguchi, T. 1988. Evidence for a nuclear factor(s), IRF-1, mediating induction and silencing properties to human IFN-β gene regulatory elements. *EMBO J.* 7:3397.
52 Fan, C. M. and Maniatis, T. 1989. Two different virus-inducible elements are required for human β-interferon gene regulation. *EMBO J.* 8:101.
53 Escalante, C. R., Yie, J., Thanos, D. and Aggarwal, A. K. 1998. Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. *Nature* 391:103.
54 Wathelet, M. G., Lin, C. H., Parekh, B. S., Ronco, L. V., Howley, P. M. and Maniatis, T. 1998. Virus infection induces the assembly of coordinately activated transcription factors on the IFN-β enhancer *in vivo*. *Mol. Cell* 1:507.
55 Fujii, Y., Shimizu, T., Kusumoto, M., Kyogoku, Y., Taniguchi, T. and Hakoshima, T. 1999. Crystal structure of an IRF-DNA complex reveals novel DNA recognition and cooperative binding to a tandem repeat of core sequences. *EMBO J.* 18:5028.
56 Fujita, T., Miyamoto, M., Kimura, Y., Hammer, J. and Taniguchi, T. 1989. Involvement of a *cis*-element that binds an H2TF-1/NF κB like factor(s) in the virus-induced interferon-β gene expression. *Nucleic Acids Res.* 17:3335.
57 Lenardo, M. J., Fan, C. M., Maniatis, T. and Baltimore, D. 1989. The involvement of NF-κB in β-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. *Cell* 57:287.
58 Visvanathan, K. V. and Goodbourn, S. 1989. Double-stranded RNA activates binding of NF-κB to an inducible element in the human β-interferon promoter. *EMBO J.* 8:1129.
59 Du, W., Thanos, D. and Maniatis, T. 1993. Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. *Cell* 74:887.
60 Chu, W. M., Ostertag, D., Li, Z. W. *et al.* 1999. JNK2 and IKKβ are required for activating the innate response to viral infection. *Immunity* 11:721.
61 Ryals, J., Dierks, P., Ragg, H. and Weissmann, C. 1985. A 46-nucleotide promoter segment from an IFN-α gene renders an unrelated promoter inducible by virus. *Cell* 41:497.
62 Raj, N. B., Israeli, R., Kellum, M. and Pitha, P. M. 1989. Upstream regulatory elements of murine α4-interferon gene confer inducibility and cell type-restricted expression. *J. Biol. Chem.* 264:11149.
63 Genin, P., Braganca, J., Darracq, N., Doly, J. and Civas, A. 1995. A novel PRD I and TG binding activity involved in virus-induced transcription of IFN-A genes. *Nucleic Acids Res.* 23:5055.
64 Braganca, J., Genin, P., Bandu, M. T., Darracq, N., Vignal, M., Casse, C. *et al.* 1997. Synergism between multiple virus-induced factor-binding elements involved in the differential expression of interferon A genes. *J. Biol. Chem.* 272:22154.
65 Lohoff, M. and Mak, T. W. 2005. Roles of interferon-regulatory factors in T-helper-cell differentiation. *Nat. Rev. Immunol.* 5:125.
66 Tanaka, N., Kawakami, T. and Taniguchi, T. 1993. Recognition DNA sequences of interferon regulatory factor 1 (IRF-1) and IRF-2, regulators of cell growth and the interferon system. *Mol. Cell. Biol.* 13:4531.
67 Barnes, B. J., Moore, P. A. and Pitha, P. M. 2001. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon α genes. *J. Biol. Chem.* 276:23382.
68 Matsuyama, T., Kimura, T., Kitagawa, M. *et al.* 1993. Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. *Cell* 75:83.
69 Takaoka, A., Yanai, H., Kondo, S. *et al.* 2005. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 434:243.
70 Honda, K., Yanai, H., Negishi, H. *et al.* 2005. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 434:772.
71 Sato, M., Suemori, H., Hata, N. *et al.* 2000. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-α/β gene induction. *Immunity* 13:539.
72 Yoneyama, M., Suhara, W., Fukuhara, Y., Fukuda, M., Nishida, E. and Fujita, T. 1998. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J.* 17:1087.
73 Lin, R., Heylbroeck, C., Pitha, P. M. and Hiscott, J. 1998. Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *Mol. Cell. Biol.* 18:2986.
74 Servant, M. J., Grandvaux, N. and Hiscott, J. 2002. Multiple signaling pathways leading to the activation of interferon regulatory factor 3. *Biochem. Pharmacol.* 64:985.
75 Sato, M., Tanaka, N., Hata, N., Oda, E. and Taniguchi, T. 1998. Involvement of the IRF family transcription factor IRF-3 in virus-induced activation of the IFN-β gene. *FEBS Lett.* 425:112.
76 Lin, R., Mamane, Y. and Hiscott, J. 1999. Structural and functional analysis of interferon regulatory factor 3: localization of the transactivation and autoinhibitory domains. *Mol. Cell. Biol.* 19:2465.
77 Suhara, W., Yoneyama, M., Iwamura, T. *et al.* 2000. Analyses of virus-induced homomeric and heteromeric protein associations between IRF-3 and coactivator CBP/p300. *J. Biochem. (Tokyo)* 128:301.
78 Servant, M. J., Grandvaux, N., tenOever, B. R., Duguay, D., Lin, R. and Hiscott, J. 2003. Identification of the minimal phosphoacceptor site required for *in vivo* activation of interferon regulatory factor 3 in response to virus and double-stranded RNA. *J. Biol. Chem.* 278:9441.

79 Mori, M., Yoneyama, M., Ito, T., Takahashi, K., Inagaki, F. and Fujita, T. 2004. Identification of Ser-386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. *J. Biol. Chem.* 279:9698.
80 Takahasi, K., Suzuki, N. N., Horiuchi, M. *et al.* 2003. X-ray crystal structure of IRF-3 and its functional implications. *Nat. Struct. Biol.* 10:922.
81 Qin, B. Y., Liu, C., Lam, S. S. *et al.* 2003. Crystal structure of IRF-3 reveals mechanism of autoinhibition and virus-induced phosphoactivation. *Nat. Struct. Biol.* 10:913.
82 Lin, R., Heylbroeck, C., Genin, P., Pitha, P. M. and Hiscott, J. 1999. Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription. *Mol. Cell. Biol.* 19:959.
83 Nakaya, T., Sato, M., Hata, N. *et al.* 2001. Gene induction pathways mediated by distinct IRFs during viral infection. *Biochem. Biophys. Res. Commun.* 283:1150.
84 Sakaguchi, S., Negishi, H., Asagiri, M. *et al.* 2003. Essential role of IRF-3 in lipopolysaccharide-induced interferon-β gene expression and endotoxin shock. *Biochem. Biophys. Res. Commun.* 306:860.
85 Erlandsson, L., Blumenthal, R., Eloranta, M. L. *et al.* 1998. Interferon-β is required for interferon-α production in mouse fibroblasts. *Curr. Biol.* 8:223.
86 Hata, N., Sato, M., Takaoka, A., Asagiri, M., Tanaka, N. and Taniguchi, T. 2001. Constitutive IFN-α/β signal for efficient IFN-α/β gene induction by virus. *Biochem. Biophys. Res. Commun.* 285:518.
87 Zhang, L. and Pagano, J. S. 1997. IRF-7, a new interferon regulatory factor associated with Epstein-Barr virus latency. *Mol. Cell. Biol.* 17:5748.
88 Marie, I., Smith, E., Prakash, A. and Levy, D. E. 2000. Phosphorylation-induced dimerization of interferon regulatory factor 7 unmasks DNA binding and a bipartite transactivation domain. *Mol. Cell. Biol.* 20:8803.
89 Lin, R., Mamane, Y. and Hiscott, J. 2000. Multiple regulatory domains control IRF-7 activity in response to virus infection. *J. Biol. Chem.* 275:34320.
90 Caillaud, A., Hovanessian, A. G., Levy, D. E. and Marie, I. J. 2005. Regulatory serine residues mediate phosphorylation-dependent and phosphorylation-independent activation of interferon regulatory factor 7. *J. Biol. Chem.* 280:17671.
91 Sharma, S., tenOever, B. R., Grandvaux, N., Zhou, G. P., Lin, R. and Hiscott, J. 2003. Triggering the interferon antiviral response through an IKK-related pathway. *Science* 300:1148.
92 Fitzgerald, K. A., McWhirter, S. M., Faia, K. L. *et al.* 2003. IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nat. Immunol.* 4:491.
93 Hemmi, H., Takeuchi, O., Sato, S. *et al.* 2004. The roles of two IκB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J. Exp. Med.* 199: 1641.
94 Perry, A. K., Chow, E. K., Goodnough, J. B., Yeh, W. C. and Cheng, G. 2004. Differential requirement for TANK-binding kinase-1 in type I interferon responses to toll-like receptor activation and viral infection. *J. Exp. Med.* 199:1651.
95 McWhirter, S. M., Fitzgerald, K. A., Rosains, J., Rowe, D. C., Golenbock, D. T. and Maniatis, T. 2004. IFN-regulatory factor 3-dependent gene expression is defective in *Tbk1*-deficient mouse embryonic fibroblasts. *Proc. Natl Acad. Sci. USA* 101:233.
96 Obata, Y., Yamamoto, K., Miyazaki, M., Shimotohno, K., Kohno, S. and Matsuyama, T. 2005. Role of cyclophilin B in activation of interferon regulatory factor-3. *J. Biol. Chem.* 280:18355.
97 Price, E. R., Zydowsky, L. D., Jin, M. J., Baker, C. H., McKeon, F. D. and Walsh, C. T. 1991. Human cyclophilin B: a second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence. *Proc. Natl Acad. Sci. USA* 88:1903.
98 Jacobs, B. L. and Langland, J. O. 1996. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. *Virology* 219:339.
99 Kato, H., Sato, S., Yoneyama, M. *et al.* 2005. Cell type-specific involvement of RIG-I in antiviral response. *Immunity* 23:19.
100 Balachandran, S., Thomas, E. and Barber, G. N. 2004. A FADD-dependent innate immune mechanism in mammalian cells. *Nature* 432:401.
101 Medzhitov, R. 2001. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1:135.
102 Hoffmann, J. A. 2003. The immune response of Drosophila. *Nature* 426:33.
103 Foy, E., Li, K., Sumpter, R., Jr *et al.* 2005. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. *Proc. Natl Acad. Sci. USA* 102:2986.
104 Otsuka, M., Kato, N., Moriyama, M. *et al.* 2005. Interaction between the HCV NS3 protein and the host TBK1 protein leads to inhibition of cellular antiviral responses. *Hepatology* 41:1004.
105 Breiman, A., Grandvaux, N., Lin, R. 2005. Inhibition of RIG-I-dependent signaling to the interferon pathway during hepatitis C virus expression and restoration of signaling by IKKε. *J. Virol.* 79:3969.
106 Spann, K. M., Tran, K. C. and Collins, P. L. 2005. Effects of nonstructural proteins NS1 and NS2 of human respiratory syncytial virus on interferon regulatory factor 3, NF-κB, and proinflammatory cytokines. *J. Virol.* 79:5353.
107 Beutler, B. 2004. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 430:257.
108 Barton, G. M. and Medzhitov, R. 2003. Toll-like receptor signaling pathways. *Science* 300:1524.
109 Medzhitov, R., Preston-Hurlburt, P., Kopp, E. *et al.* 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell* 2:253.
110 Wesche, H., Henzel, W. J., Shillinglaw, W., Li, S. and Cao, Z. 1997. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7:837.
111 Hacker, H., Vabulas, R. M., Takeuchi, O., Hoshino, K., Akira, S. and Wagner, H. 2000. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. *J. Exp. Med.* 192:595.
112 Honda, K., Yanai, H., Mizutani, T. *et al.* 2004. Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. *Proc. Natl Acad. Sci. USA* 101: 15416.
113 Kawai, T., Sato, S., Ishii, K. J. *et al.* 2004. Interferon-α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat. Immunol.* 5:1061.
114 Horng, T., Barton, G. M. and Medzhitov, R. 2001. TIRAP: an adapter molecule in the Toll signaling pathway. *Nat. Immunol.* 2:835.
115 Fitzgerald, K. A., Palsson-McDermott, E. M., Bowie, A. G. *et al.* 2001. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. *Nature* 413:78.
116 Oshiumi, H., Matsumoto, M., Funami, K., Akazawa, T. and Seya, T. 2003. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-β induction. *Nat. Immunol.* 4:161.
117 Yamamoto, M., Sato, S., Mori, K. *et al.* 2002. Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the Toll-like receptor signaling. *J. Immunol.* 169:6668.
118 Oshiumi, H., Sasai, M., Shida, K., Fujita, T., Matsumoto, M. and Seya, T. 2003. TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-β. *J. Biol. Chem.* 278:49751.
119 Fitzgerald, K. A., Rowe, D. C., Barnes, B. J. *et al.* 2003. LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. *J. Exp. Med.* 198:1043.
120 Hemmi, H., Takeuchi, O., Kawai, T. *et al.* 2000. A Toll-like receptor recognizes bacterial DNA. *Nature* 408:740.
121 Hemmi, H., Kaisho, T., Takeuchi, O. *et al.* 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol.* 3:196.
122 Wagner, H. 2004. The immunobiology of the TLR9 subfamily. *Trends Immunol.* 25:381.
123 Haynes, L. M., Moore, D. D., Kurt-Jones, E. A., Finberg, R. W., Anderson, L. J. and Tripp, R. A. 2001. Involvement of toll-like

receptor 4 in innate immunity to respiratory syncytial virus. *J. Virol.* 75:10730.
124 Kurt-Jones, E. A., Popova, L., Kwinn, L. *et al.* 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 1:398.
125 Rassa, J. C., Meyers, J. L., Zhang, Y., Kudaravalli, R. and Ross, S. R. 2002. Murine retroviruses activate B cells via interaction with toll-like receptor 4. *Proc. Natl Acad. Sci. USA* 99:2281.
126 Kawai, T., Takeuchi, O., Fujita, T. *et al.* 2001. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. *J. Immunol.* 167:5887.
127 Yamamoto, M., Sato, S., Hemmi, H. *et al.* 2003. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 301:640.
128 Hoebe, K., Du, X., Georgel, P. *et al.* 2003. Identification of *Lps2* as a key transducer of MyD88-independent TIR signalling. *Nature* 424:743.
129 Yamamoto, M., Sato, S., Hemmi, H. *et al.* 2003. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat. Immunol.* 4:1144.
130 Sasai, M., Oshiumi, H., Matsumoto, M. *et al.* 2005. Cutting edge: NF-κB-activating kinase-associated protein 1 participates in TLR3/Toll-IL-1 homology domain-containing adapter molecule-1-mediated IFN regulatory factor 3 activation. *J. Immunol.* 174:27.
131 Karaghiosoff, M., Steinborn, R., Kovarik, P. *et al.* 2003. Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. *Nat. Immunol.* 4:471.
132 Doyle, S., Vaidya, S., O'Connell, R. *et al.* 2002. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. *Immunity* 17:251.
133 Funami, K., Matsumoto, M., Oshiumi, H., Akazawa, T., Yamamoto, A. and Seya, T. 2004. The cytoplasmic 'linker region' in Toll-like receptor 3 controls receptor localization and signaling. *Int. Immunol.* 16:1143.
134 Sarkar, S. N., Smith, H. L., Rowe, T. M. and Sen, G. C. 2003. Double-stranded RNA signaling by Toll-like receptor 3 requires specific tyrosine residues in its cytoplasmic domain. *J. Biol. Chem.* 278:4393.
135 Sarkar, S. N., Peters, K. L., Elco, C. P., Sakamoto, S., Pal, S. and Sen, G. C. 2004. Novel roles of TLR3 tyrosine phosphorylation and PI3 kinase in double-stranded RNA signaling. *Nat. Struct. Mol. Biol.* 11:1060.
136 Wietek, C., Miggin, S. M., Jefferies, C. A. and O'Neill, L. A. 2003. Interferon regulatory factor-3-mediated activation of the interferon-sensitive response element by Toll-like receptor (TLR) 4 but not TLR3 requires the p65 subunit of NF-κB. *J. Biol. Chem.* 278:50923.
137 Leung, T. H., Hoffmann, A. and Baltimore, D. 2004. One nucleotide in a κB site can determine cofactor specificity for NF-κB dimers. *Cell* 118:453.
138 Kadowaki, N., Ho, S., Antonenko, S. *et al.* 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* 194:863.
139 Nakano, H., Yanagita, M. and Gunn, M. D. 2001. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J. Exp. Med.* 194:1171.
140 Asselin-Paturel, C., Boonstra, A., Dalod, M. *et al.* 2001. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat. Immunol.* 2:1144.
141 Krug, A., Rothenfusser, S., Hornung, V. *et al.* 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-α/β in plasmacytoid dendritic cells. *Eur. J. Immunol.* 31:2154.
142 Krieg, A. M. 2002. CpG motifs in bacterial DNA and their immune effects. *Annu. Rev. Immunol.* 20:709.
143 Colonna, M., Trinchieri, G. and Liu, Y. J. 2004. Plasmacytoid dendritic cells in immunity. *Nat. Immunol.* 5:1219.
144 Lund, J., Sato, A., Akira, S., Medzhitov, R. and Iwasaki, A. 2003. Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. *J. Exp. Med.* 198:513.
145 Krug, A., Luker, G. D., Barchet, W., Leib, D. A., Akira, S. and Colonna, M. 2004. Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood* 103:1433.
146 Diebold, S. S., Kaisho, T., Hemmi, H., Akira, S. and Reis e Sousa, C. 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303:1529.
147 Heil, F., Hemmi, H., Hochrein, H. *et al.* 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303:1526.
148 Lund, J. M., Alexopoulou, L., Sato, A. *et al.* 2004. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc. Natl Acad. Sci. USA* 101:5598.
149 Krug, A., French, A. R., Barchet, W. *et al.* 2004. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 21:107.
150 Honda, K., Ohba, Y., Yanai, H. *et al.* 2005. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature* 434:1035.
151 Qing, J., Liu, C., Choy, L., Wu, R. Y., Pagano, J. S. and Derynck, R. 2004. Transforming growth factor β/Smad3 signaling regulates IRF-7 function and transcriptional activation of the β interferon promoter. *Mol. Cell. Biol.* 24:1411.
152 Suzuki, N., Suzuki, S., Duncan, G. S. *et al.* 2002. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature* 416:750.
153 Uematsu, S., Sato, S., Yamamoto, M. *et al.* 2005. Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-α induction. *J. Exp. Med.* 201:915.
154 Zhang, G. and Ghosh, S. 2002. Negative regulation of toll-like receptor-mediated signaling by Tollip. *J. Biol. Chem.* 277:7059.
155 Yu, K. Y., Kwon, H. J., Norman, D. A., Vig, E., Goebl, M. G. and Harrington, M. A. 2002. Cutting edge: mouse pellino-2 modulates IL-1 and lipopolysaccharide signaling. *J. Immunol.* 169:4075.
156 Jiang, Z., Johnson, H. J., Nie, H., Qin, J., Bird, T. A. and Li, X. 2003. Pellino 1 is required for interleukin-1 (IL-1)-mediated signaling through its interaction with the IL-1 receptor-associated kinase 4 (IRAK4)-IRAK-tumor necrosis factor receptor-associated factor 6 (TRAF6) complex. *J. Biol. Chem.* 278:10952.
157 Jensen, L. E. and Whitehead, A. S. 2003. Pellino3, a novel member of the Pellino protein family, promotes activation of c-Jun and Elk-1 and may act as a scaffolding protein. *J. Immunol.* 171:1500.
158 Asselin-Paturel, C., Brizard, G., Pin, J. J., Briere, F. and Trinchieri, G. 2003. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. *J. Immunol.* 171:6466.
159 Takeuchi, O., Kawai, T., Muhlradt, P. F. *et al.* 2001. Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int. Immunol.* 13:933.
160 Blanco, P., Palucka, A. K., Gill, M., Pascual, V. and Banchereau, J. 2001. Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus. *Science* 294:1540.
161 Ronnblom, L. and Alm, G. V. 2001. A pivotal role for the natural interferon α-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. *J. Exp. Med.* 194:F59.
162 Ronnblom, L. and Alm, G. V. 2003. Systemic lupus erythematosus and the type I interferon system. *Arthritis Res. Ther.* 5:68.
163 Bave, U., Magnusson, M., Eloranta, M. L., Perers, A., Alm, G. V. and Ronnblom, L. 2003. Fc gamma RIIa is expressed on natural IFN-α-producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG. *J. Immunol.* 171:3296.