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The Dual Functions of IL-1 Receptor-Associated Kinase 2 in TLR9-Mediated IFN and Proinflammatory Cytokine Production

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Bone marrow-derived plasmacytoid dendritic cells (pDCs) from IL-1R–associated kinase (IRAK)2-deficient mice produced more IFNs than did wild-type pDCs upon stimulation with the TLR9 ligand CpG. Furthermore, in CpG-stimulated IRAK2-deficient pDCs there was increased nuclear translocation of IFN regulatory factor 7, the key transcription factor for IFN gene transcription in these cells. In IRAK2-deficient macrophages, enhanced NF-κB activation and increased expression of CpG-induced genes were detected within 2 h after treatment. However, at later times, NF-κB activation was decreased and, in contrast to the results with IFN, there was less secretion of other proinflammatory cytokines (such as TNF-α and chemokines in CpG-stimulated IRAK2-deficient pDCs and macrophages). Therefore, although IRAK2 is a negative regulator of TLR9-mediated IFN production through its modulation of the transcriptional activity of IFN regulatory factor 7, it is also a positive regulator of TLR9-mediated proinflammatory cytokine and chemokine production at some level subsequent to transcription. The Journal of Immunology, 2011, 186: 000–000.

The IRAK1–TRAF6 complex then dissociates from the receptor to interact with and activate the downstream kinases transforming growth factor β-activated kinase 1 (TAK1) and MEK kinase 3 (17), which then activate NF-κB and MAPK, resulting in the production of proinflammatory cytokines and chemokines (18). Interestingly, some TLR-induced proinflammatory cytokines, such as TNF-α, in turn activate NF-κB through autocrine or paracrine pathways, which further promote the additional production of proinflammatory cytokines and chemokines at late times after TLR ligand stimulation (19, 20).

We and others recently reported that IRAK2 is essential for TLR-mediated proinflammatory cytokine and chemokine production (21, 22). IRAK2-deficient macrophages showed normal early, but reduced late activation of NF-κB and late activation of NF-κB in response to the TLR2 ligand Malp-2 and the TLR7 ligand R848, suggesting that IRAK2 is critical for the late transcriptional response to the activation of TLR2 and TLR7 (21, 22). Importantly, a reduction of cytokine and chemokine mRNA stability and translation was observed in IRAK2-deficient bone marrow (BM)-derived macrophages in response to LPS, demonstrating that IRAK2 is also involved in the posttranscriptional control of TLR-mediated cytokine and chemokine production (22).

It is important to note that the activation of TLR-mediated signaling can also lead to the production of type I IFN, including IFN-α and IFN-β (23). Plasmacytoid dendritic cells (pDCs) are the major cell type that produces type I IFN and regulates antiviral immunity in vivo (24). pDCs express high levels of TLR7 and TLR9, which detect microbial-derived ssRNA and unmethylated CpG dinucleotides, respectively. Previous studies showed that TLR9 activation in pDCs leads to high levels of type I IFN through the formation of a MyD88–IRAK1–TRAF6 complex (25, 26) and the subsequent activation of IFN regulatory factor 7 (IRF7), a master transcription factor for type I IFN expression (27).

We now unexpectedly find that IRAK2 is actually a negative regulator of CpG-induced IFN production at the transcriptional level.
level. IRAK2-deficient pDCs displayed increased nuclear translocation of IRF7 and IFN production in response to stimulation with TLR9 ligands. In contrast, IRAK2 is still required for TLR9-mediated posttranscriptional control of proinflammatory cytokine and chemokine production. CpG-stimulated IRAK2-deficient pDCs and macrophages both produced smaller amounts of proinflammatory cytokines and chemokines than did wild-type cells, which coincided with reduced NF-κB secretion and decreased late NF-κB activation in IRAK2-deficient macrophages. Taken together, the results demonstrate the dual functions of IRAK2 in TLR9-mediated IFN and proinflammatory cytokine production.

Materials and Methods

Reagents

LPS (Escherichia coli 055:B5) was purchased from Sigma-Aldrich, R848 from GLSynthesis, and CpG A (ODN 1585) and CpG B (ODN1826) from InvivoGen. Abs against phospho-ERK, phospho-p38, and phospho-IκBα were purchased from Cell Signaling Technology. Abs against IRAK1, IRAK2, and β-actin were from Santa Cruz Biotechnology. Abs against IRAK2 were from Abcam. Allophycocyanin-conjugated TNF-α Ab, brezelfin D, and permeabilization buffer were from ebioscience. IRAK2 knockout and control mice were generated, as previously described (22). TNF-α knockout and control mice were purchased from Taconic Farms. IL-1R, IL-18R, and IL-33R knockout and control mice were generated, as previously described (22).

Western analysis

Cells were harvested, washed once with PBS, and lysed for 30 min at 4°C in 1.0% Nonidet P-40, 100 mM Tris hydrochloride (pH 8.0), 20% glycerol, and 0.2 mM EDTA. Cellular debris was removed by centrifugation at 10,000 × g for 5 min. For immunoblotting, cell extracts were fractionated by NaDodSO4-PAGE and transferred to Immobilon-P transfer membranes (Millipore), using a wet transfer apparatus (Bio-Rad Laboratories). Immunoblot analysis was performed, and the bands were visualized with HRP-coupled goat anti-rabbit, goat anti-mouse, or donkey anti-goat Ig as appropriate (Rockland), using the ECL chemiluminescence Western blotting detection system (GE Healthcare).

Murine herpesvirus 68 infection

BM-derived pDCs were plated in 12-well plates at 5 × 10^5 cells/ml and infected with murine herpesvirus 68 (MHV68) (multiplicity of infection [MOI] = 5) for 24 h. IFN, proinflammatory cytokine, and chemokine concentrations in the supernatant media were measured by ELISA. To analyze the proliferation of virus in pDCs, GFP protein was measured by the Western method.

ELISA

BM-derived pDCs were stimulated with MHV68 (MOI = 5) or CpG A (1 μg/ml), and supernatant media were collected 24 h after stimulation. BM-derived macrophages were stimulated with LPS (1 μg/ml), R848 (1 μg/ml), or CpG B (1 μg/ml) for 24 h, and supernatant media were collected 24 h after stimulation. IL-6, KC, and TNF-α concentrations were measured by ELISA kits from R&D Systems. IFN-α and IFN-β concentrations in pDC culture media were measured by using ELISA kits from PBL Biomedical Laboratories. One million cells were used for ELISA.

Preparation and fractionation of nuclear extracts

BM-derived pDCs were stimulated with CpG A (1 μg/ml) for the indicated times. Cell pellets were collected and washed with ice-cold PBS twice, resuspended in 6 vol buffer A (10 mM HEPES [pH 7.9], 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, 1 mM PMSF, 0.3 mM Na3VO4, and 5 mM NaF), and incubated on ice for 10 min. The cell suspension was transferred to a Dounce homogenizer, and the cells were disrupted with 45 strokes. After centrifugation at 500 × g for 1 min at 4°C, the supernatants were collected and measured as cytoplasmic fractions. The nuclear pellets were washed with 1 ml Nuclei EZ Lysis buffer (Sigma-Aldrich Nuclei EZ Prep kit), followed by washing with 1 ml buffer A. Nuclear pellets were collected by centrifugation at 500 × g for 1 min at 4°C, resuspended in 50 μl buffer B (20 mM HEPES [pH 7.9]), 500 mM NaCl, 1.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM DTT, 1 mM PMSF, 0.3 mM Na3VO4, and 5 mM NaF), and incubated

**FIGURE 1.** IRAK2-deficient pDCs are more resistant to the infection by MHV68. Wild-type and IRAK2-deficient BM-derived pDCs were infected with MHV68 (MOI = 5) for 24 h. IFN-α and IFN-β (A) TNF-α and KC (B) concentrations in culture media were measured by ELISA. Results shown are the means and SD of triplicate determinations. *p < 0.05. C. Cell lysates were analyzed by the Western method with Abs against GFP, IRAK2, and actin.
on ice for 30 min. After centrifugation at 14,000 × g for 10 min at 4°C, the supernatant nuclear fractions were collected.

**Illumina BeadChip microarray analysis**

A total of 250 ng RNA was reverse transcribed into cRNA and labeled with biotin-UTP, using the TotalPrep RNA amplification kit (Ambion). cRNA was quantified using a nanodrop spectrophotometer, and the cRNA quality (size distribution) was further analyzed in a 1% agarose gel. cRNA was hybridized to the Illumina MouseRef8 v1.1 Expression BeadChip using standard protocols (provided by Illumina). The microarray data were submitted to the Gene Expression Omnibus at National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo/, accession number GSE24264).

**Intracellular staining**

BM-derived macrophages were stimulated with CpG B (1 μg/ml) for indicated times and fixed with formaldehyde (2% in PBS) on ice for 20 min. Two hours before fixing, brefeldin A was added to block protein secretion and enrich intracellular TNF-α. The fixed cells were collected by centrifuge at 1000 × g for 5 min at 4°C, washed with PBS twice, and blocked with PBS containing 2% serum on ice for 30 min. The cell pellets were

![Image](https://example.com/image.png)

**FIGURE 2.** Increased responses to CpG A stimulation in IRAK2-deficient pDCs. Wild-type and IRAK2-deficient BM-derived pDCs were stimulated with CpG A (1 μg/ml) for 24 h. IFN-α and IFN-β (A) TNF-α and KC (B) concentrations in culture media were measured by ELISA. Results shown are the means and SD of triplicate determinations. *p < 0.05. C, Wild-type and IRAK2-deficient BM-derived pDCs were treated with CpG A (1 μg/ml) for the indicated times, and total RNAs (2 μg) were analyzed by real-time PCR. *p < 0.05. D, Nuclear and cytoplasmic fractions prepared from wild-type or IRAK2-deficient pDCs, either untreated or treated with CpG A (1 μg/ml), were analyzed by the Western method with Abs against IRF7, histone H3, and GAPDH. E, Wild-type or IRAK2-deficient pDCs were treated with CpG A (1 μg/ml) for the indicated times, and cell lysates were analyzed by the Western method with Abs against IRAK2, phospho-IκBα, IκBα, phospho-ERK, phospho-JNK, phospho-p38, and actin. The protein levels were analyzed by ImageJ 1.43u and normalized to actin.
collected by centrifuge at 1000 × g for 5 min, resuspended in permeabilization buffer containing 1% serum, and incubated for 30 min at room temperature. Allophycocyanin-conjugated TNF-α Ab (1 μg/ml) was added to the cell suspension, followed by incubation for 45 min. The cell pellets were collected by centrifuge at 1000 × g for 5 min, washed with 1× permeabilization buffer containing 1% serum twice, and resuspended in PBS. The TNF-α–stained cells were analyzed by flow cytometry by using a BD FACSCalibur instrument.

Results

IRAK2 is a negative regulator of TLR9-induced IFN production in pDCs

To examine the role of IRAK2 in IFN production, BM-derived wild-type and IRAK2-deficient pDCs were infected with MHV68 and examined for virus-induced IFN production by

FIGURE 3. Increased responses to CpG B stimulation in IRAK2-deficient macrophages. A, Wild-type and IRAK2-deficient BM-derived macrophages were treated with LPS (1 μg/ml), R848 (1 μg/ml), or CpG B (1 μg/ml) for the indicated times. Cell lysates were analyzed by the Western method with Abs against IRAK2, phospho-IKBα, IKBα, phospho-ERK, phospho-p38, and actin. B, Wild-type or IRAK2-deficient BM-derived macrophages were treated with R848 (1 μg/ml) or CpG B (1 μg/ml) for 2 h. A heat map is shown for some genes from Illumina microarray analyses that were induced less by R848, but more by CpG in IRAK2-deficient macrophages. C, Real-time PCR analysis of selected genes from microarray analysis. Wild-type and IRAK2-deficient BM-derived macrophages were treated with R848 or CpG B for the indicated times or were untreated. *p < 0.05.
ELISA. Interestingly, IRAK2-deficient pDCs produced more IFN (IFN-α4 and IFN-β; Fig. 1A), but less proinflammatory cytokines (TNF-α and KC; Fig. 1B), compared with wild-type pDCs in response to infection. Consistent with the increased amounts of IFN, virus-mediated GFP expression (an indication of MHV68 proliferation) was decreased in IRAK2-deficient pDCs compared with wild-type cells (Fig. 1B). Because MHV68 activates IFN production through TLR9, we examined the role of IRAK2 in TLR9-mediated IFN production by treating the cells with TLR9 ligands. A group of synthetic TLR9 ligands, termed CpG A, are potent IFN inducers in pDCs (29). CpG A is characterized by phosphodiester backbone CpG motifs and a phosphorothioate-modified poly G stretch at the 5' and 3' ends, and is capable of forming stable higher-order structures. In contrast, conventional phosphorothioate-modified CpG (CpG B) generally does not form higher-order structures (30, 31). Compared to CpG A, CpG B is

FIGURE 4. Impaired activation of NF-κB at late times after CpG B stimulation in IRAK2-deficient macrophages. A, Wild-type and IRAK2-deficient BM-derived macrophages were treated with LPS (1 μg/ml), R848 (1 μg/ml), or CpG B (1 μg/ml) for 24 h. TNF-α, KC, and IL-6 concentrations in culture media were measured by ELISA. Results shown are the means and SD of triplicate determinations. *p < 0.05. B, Wild-type and IRAK2-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) or LPS (1 μg/ml) for the indicated times, and total RNAs (2 μg) were analyzed by real-time PCR to determine TNF-α, KC, and IL-6 expression levels. *p < 0.05. C, Wild-type and IRAK2-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) for the indicated times. Cell lysates were analyzed by the Western method with Abs against IRAK2, IRAK1, phospho-IκBα, IκBα, and actin.
a much weaker inducer of IFNs in pDCs, but a much stronger inducer of proinflammatory cytokines in macrophages and conventional DCs (32). Multimeric CpG A tends to be retained in early endosomes, whereas monomeric CpG B typically resides in late endosomes. Although it has been suggested that the endosomal localization of CpGs is critical for their functions, the mechanisms for the differential properties of CpG A and CpG B are still not clear (33). Consistent with the results of virus infection, more IFN was detected in IRAK2-deficient pDCs than that in wild-type pDCs upon CpG A stimulation (Fig. 2A). Furthermore, IRAK2-deficient pDCs showed markedly increased IFN mRNA and IRF7 nuclear translocation in response to CpG A stimulation (Fig. 2C, 2D), suggesting that IRAK2 probably inhibits the TLR9-induced transcription of IFN genes. Moreover, TLR9-induced phosphorylation of IκBα, p38, ERK, and JNK was also increased in IRAK2-deficient pDCs compared with wild-type cells, demonstrating increased NF-κB and MAPK signaling (Fig. 2E). Taken together, these results indicate that IRAK2 is a negative regulator of TLR9-mediated signaling in pDCs, functioning at a receptor-proximal level to inhibit the activation of all branches of downstream signaling.

**FIGURE 5.** Impaired activation of NF-κB at late times after CpG B stimulation in TNF-α-deficient macrophages. A, Wild-type and TNF-α-deficient BM-derived macrophages were treated with LPS (1 μg/ml), R848 (1 μg/ml), or CpG B (1 μg/ml) for 24 h. TNF-α, KC, and IL-6 concentrations were measured by ELISA. Results shown are the means and SD of triplicate determinations. *p < 0.05. B, Wild-type or TNF-α-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) for the indicated times, and total RNAs (2 μg) were analyzed by real-time PCR. *p < 0.05. C, Wild-type or TNF-α-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) for the indicated times. Cell lysates were analyzed by the Western method with Abs against IRAK2, IRAK1, phospho-IκBα, IκBα, and actin.
R848 and CpG B. A total of 334 genes was induced 2-fold in wild-type macrophages after 2 h of stimulation with CpG B. The majority (213, 63%) of the genes induced by CpG B were expressed at higher levels in IRAK2-deficient macrophages than in wild-type cells. In contrast, the majority of the R848-induced genes (594 of 716; 83%) were expressed at lower levels in IRAK2-deficient macrophages. A large group of genes was induced by both R848 and CpG B, most of which showed increased expression in response to CpG B, but decreased expression in response to R848 in IRAK2-deficient macrophages (Fig. 3B). The expression patterns of several genes shown in Fig. 3B were confirmed by real-time PCR (Fig. 3C).

IRAK2 is required for sustained CpG B-initiated NF-κB activation at late times

We next examined the overall effect of IRAK2 on TLR9-mediated cytokine and chemokine production. Despite the suppressive effect of IRAK2 in TLR9-mediated gene transcription at early times of CpG B stimulation (Fig. 3B), IRAK2-deficient macrophages still produced much less TNF-α, KC, and IL-6 than the wild-type macrophages (Fig. 4A). IRAK2-deficient macrophages showed decreased KC and IL-6 mRNA expression at late times of LPS stimulation (Fig. 4B), which is consistent with our previous report (22). Interestingly, although IRAK2-deficient macrophages showed increased TNF-α and KC mRNA expression at early times after CpG stimulation (0.5, 1, or 2 h), TNF-α and KC mRNA expression were dramatically decreased at late times (4 or 8 h; Fig. 4B). Moreover, IRAK2-deficient macrophages also showed decreased phosphorylation of IkBα at late times (4, 8, or 16 h; Fig. 4C), indicating that the activation of NF-κB is greatly decreased. Taken together, these results demonstrate that IRAK2 is essential for sustaining CpG B-initiated NF-κB activation at late times.

FIGURE 6. Impaired CpG B-mediated TNF-α secretion in IRAK2-deficient macrophages. A, Wild-type or IRAK2-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) for indicated times. TNF-α was assayed by intracellular staining, followed by flow cytometry analysis. The mean fluorescence intensities of TNF-α are shown, which is a representation of three independent experiments. B, The percentages of TNF-α-positive cells are shown. Results are the means and SD of triplicate determinations. *p < 0.05. C, Wild-type and IRAK2-deficient BM-derived macrophages were treated with CpG B (1 μg/ml) or were untreated. TNF-α concentrations in the supernatant media were measured by ELISA. Results shown are the means and SD of triplicate determinations. *p < 0.05.

IRAK2 deficiency reduces the efficiency of TNF-α secretion

Although previous studies have shown an important role of IRAK2 at late times of NF-κB activation in TLR2- and TLR7-dependent signaling, it was unclear how IRAK2 is involved in sustaining NF-κB activation for up to 16 h. Many TLR ligand-induced cytokines, such as IL-1 and TNF-α, can potentially activate NF-κB through...
autocrine or paracrine pathways, thereby promoting proinflammatory cytokine and chemokine production through positive feedback loops. Especially cytokines of the IL-1 family, including IL-1, IL-18, and IL-33, activate NF-κB through MyD88-dependent pathways that use IRAKs. We thus investigated the possibility that the reduced cytokine and chemokine production in IRAK2-deficient macrophages is due to decreased responses to the IL-1 family members. However, deficiencies in the IL-1R, IL-18R, or IL-33R had little impact on cytokine and chemokene production in response to LPS, R848, or CpG B, suggesting that these cytokines are not essential for regulating TLR ligand-induced cytokine and chemokene production (Supplemental Fig. 1).

In contrast, previous studies demonstrated that TNF-α is involved in TLR-mediated NF-κB activation at late times through a positive feedback mechanism (19, 20). To investigate the involvement of TNF-α in CpG B-mediated responses, we made BM-derived macrophages from TNF-α-deficient and wild-type mice. Compared with wild-type macrophages, TNF-α-deficient macrophages indeed produced less IL-6 and KC upon CpG B stimulation (Fig. 5A), indicating that TNF-α is involved in CpG B-induced cytokine and chemokene production. Specifically, TNF-α deficiency resulted in decreased KC and IL-6 mRNA expression and IkBα phosphorylation at late times (Fig. 5B, 5C), indicating that TNF-α participates in a second wave of CpG-induced proinflammatory cytokine and chemokene production. We then carefully examined TNF-α expression and production in wild-type and IRAK2-deficient macrophages. Importantly, consistent with the negative regulatory role of IRAK2 in TLR9 signaling, IRAK2-deficient macrophages actually showed a higher percentage of TNF-α-positive cells than did wild-type macrophages in response to CpG B (Fig. 6B). However, the amounts of secreted TNF-α were lower in the supernatant media from IRAK2-deficient macrophages than in medium from wild-type macrophages despite the fact that wild-type and IRAK2-deficient TNF-α-positive cells showed similar fluorescence intensities for TNF-α staining (Fig. 6A, 6C). These results suggest that IRAK2 deficiency probably has an impact on the efficiency of TNF-α secretion.

**Discussion**

We report two novel functions of IRAK2 in TLR9-mediated signaling. Although IRAK2 is a negative regulator of TLR9-mediated transcription, it is also involved in the regulation of cytokine production at the posttranscriptional level (Fig. 7). IFN production seems mainly to be affected by the negative effect of IRAK2 at the transcriptional level, and therefore IRAK2-deficient pDCs produce more IFN upon viral infection or CpG A stimulation (Figs. 1A, 2A). For proinflammatory cytokine and chemokene production in macrophages, we observed both increased CpG B-induced transcription (Fig. 3B) and reduced TNF-α secretion in IRAK2-deficient macrophages at early times (Fig. 6). Decreased proinflammatory cytokine and chemokene production in IRAK2-deficient macrophages 24 h after CpG B stimulation (Fig. 4A) demonstrates that the effect of reduced TNF-α secretion is dominant at late times. Interestingly, IRAK2-deficient pDCs also produced less proinflammatory cytokines and chemokines than did wild-type pDCs after virus infection or CpG A stimulation (Figs. 1B, 2B), suggesting that IRAK2 might play a similar role in pDCs in regulating cytokine and chemokene production.

IRAK2-deficient pDCs and macrophages show increased activation of both NF-κB and MAPK signaling cascades at early times after activation of TLR9-mediated signaling (Figs. 2B, 3A), demonstrating that the inhibitory effect of IRAK2 is at the receptor-proximal level. It is intriguing that IRAK1 is degraded faster and more completely in IRAK2-deficient macrophages than in wild-type cells (Fig. 4C). Because the modification and degradation of IRAK1 are essential for activation of TLR9-mediated signaling (34), it is conceivable that IRAK2 might compete with IRAK1 for an upstream kinase, such as IRAK4, and inhibit the activation of IRAK1. It is important to point out that the inhibitory effect of IRAK2 is specific for TLR9-mediated signaling. Interestingly, whereas IRAK2 was modified in response to several TLR ligands, including LPS and R848, IRAK2 modification was not detected at early times after stimulation with TLR9 ligands (Figs. 2D, 3A). In addition, we only observed ligand-induced interaction of IRAK2 with MyD88 and TRAF6 in response to R848 stimulation, but not in response to CpG B stimulation (Supplemental Fig. 2). Further study is required to map the modification sites of IRAK2 and to show how such modification affects its functions.

Many TLR-induced proinflammatory cytokines function not only as effectors in immune responses, but also as amplifiers of NF-κB signaling, inducing proinflammatory cytokine and chemokene production through positive feedback mechanisms (19, 20). Our study demonstrates the importance of the production of a second wave of the cytokine TNF-α for sustaining CpG-initiated NF-κB activation. Consistent with the critical role of IRAK2 in TLR4-induced cytokine production at posttranscriptional levels (22), our studies show that IRAK2 deficiency affected TLR9-induced TNF-α production through reduced TNF-α secretion. TNF-α is produced as a membrane-bound form, and its secretion requires the activation of TNF-α-converting enzyme, which cleaves TNF-α to release it (35). The activation of this enzyme is suggested to be mediated by activation of the MAPKs ERK and p38 (36). However, the activation of ERK and p38 is not impaired in CpG-stimulated IRAK2-deficient pDCs or macrophages (Figs. 2D, 3A), suggesting that IRAK2 may mediate TNF-α secretion through a different mechanism.

Although the innate immune response is crucial to suppress infection, aberrant immune responses to host Ags are frequently observed in many autoimmune diseases, such as systemic lupus erythematosus. Therefore, it is vital to keep immune responses at appropriate levels. Our study has revealed a new mechanism that regulates TLR9-mediated immune responses, in which IRAK2 plays multiple roles. IRAK2 suppresses TLR9-mediated signaling at early times after stimulation, thus increasing the threshold of immune responses induced by TLR9 ligands, and therefore potentially preventing overreaction to low-level interactions between TLR9 and host DNA. However, when TLR9-mediated proinflammatory cytokines are induced, IRAK2 promotes their secretion and increases cytokine production through positive feedback mechanisms, leading to an efficient immune response.

**Disclosures**

The authors have no financial conflicts of interest.

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