mRNA Is an Endogenous Ligand for Toll-like Receptor 3*  

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Toll-like receptors (TLRs) are the basic signaling receptors of the innate immune system. They are activated by molecules associated with pathogens or injured host cells and tissue. TLR3 has been shown to respond to double stranded (ds) RNA, a replication intermediary for many viruses. Here we present evidence that heterologous RNA released from or associated with necrotic cells or generated by in vitro transcription also stimulates TLR3 and induces immune activation. To assess RNA-mediated TLR3 activation, human embryonic kidney (HEK) 293 cells stably expressing TLR3 and containing a nuclear factor-κB-dependent luciferase reporter were generated. Exposing these cells to in vitro transcribed RNA resulted in a TLR3-dependent induction of luciferase activity and interleukin-8 secretion. Treatment with in vitro transcribed mRNA activated nuclear factor-κB via TLR3 through a process that was dose-dependent and involved tyrosine phosphorylation. Furthermore, in vitro transcribed natural or 2'-fluoro-substituted mRNA induced the expression of TLR3, interferon regulatory factor-1, tumor necrosis factor-α, and interleukin-1 receptor-associated kinase-M mRNA in human dendritic cells (DCs). DCs responded to mRNA treatment by expressing activation markers, and this maturation was inhibited by antagonistic TLR3-specific antibody. Endogenous RNA released from or associated with necrotic cells also stimulated DCs, leading to interferon-α secretion, which could be abolished by pretreatment of necrotic cells with RNase. These results demonstrate that RNA, likely through secondary structure, is a potential host-derived activator of TLR3. This finding has potential physiologic relevance because RNA escaping from damaged tissue or contained within endocytosed cells could serve as an endogenous ligand for TLR3 that induces or otherwise modulates immune responses.

Mammalian Toll-like receptors (TLRs)† play a key role in host defense during pathogen infection by regulating and linking innate and adaptive immune responses. TLRs belong to a family of receptors that recognize pathogen-associated molecular patterns (for review, see Refs. 1–4). DCs are the primary antigen-presenting cells and the only antigen-presenting cells capable of sensitizing naive T cells. TLRs expressed by immature DCs, upon binding their respective ligands, deliver activation and maturation signals that cause the DCs to migrate to lymphoid tissue and switch from antigen acquisition to antigen presentation. Ligands for most of the TLRs have been identified and consist of bacterial and viral constituents such as unmethylated CpG DNA, daRNA, lipopolysaccharide (LPS), and flagellin (2–5). Host-derived ligands for the TLRs have also been identified, and these include heat shock proteins, extracellular matrix breakdown products, chromatin-IgG complexes, pulmonary surfactant, and necrotic cells (for review, see Refs. 3, 6, and 7).

Upon binding of ligand, TLRs have been shown to activate a variety of signaling pathways, including phosphoinositide 3-kinase, an N-terminal kinase, p68, NF-κB, extracellular signal-related kinase, and interferon (IFN) regulatory factor-3 (IRF-3), each leading to the induction of numerous target genes involved in inflammation, cellular differentiation, and direct antimicrobial activity (8, 9). A TLR3/4-specific antiviral gene program mediated by the adaptor protein TRIF and involving the activation of IRF-3 has recently been identified (8), with TLR3 being the more potent mediator (10). IRF-3 transactivates a set of primary genes including IFN-β. Secreted IFN-β then participates in an autocrine/paracrine loop leading to the production of a set of secondary genes that are involved in antiviral and antimicrobial responses (10). Thus, TLRs are capable of recognizing both pathogen-associated molecular patterns, as well as certain endogenous stimuli, which may function as danger signals (11, 12). In this report, we identify free and cell-associated RNA as a new host-derived ligand of TLR3 and demonstrate its importance in modulating the phenotype of activated DCs.

**Experimental Procedures**

Plasmids and Reagents—NF-κB-dependent ELAM-1-luciferase reporter plasmid (pELAM-luc) (Jesse Chow, Eisai Research Institute, Andover, MA) and pTEVluc (Daniel Gallie, University of California at Riverside) were used. Generation of pTEVgag (p55 core protein of HIV-1) was described previously (13). pUNO-TLR3 and pSFV1 were purchased from InvivoGen (San Diego, CA) and Invitrogen, respectively. Expression plasmid pEF-BOS-TRIFANAC for dominant negative TRIF was a gift of S. Akira (Osaka University, Osaka, Japan). Plasmid containing Renilla luciferase-encoding sequences (pSVRen) were generated from pGL2uc (John Atkins, University of Utah) (14) following the removal of the firefly luciferase coding sequence with BamHI and NotI digestions, end-filling, and religation. Cells were treated with the following reagents: 1 ng/ml TNF-α (R&D Systems, Minneapolis, MN); 0.1–1 μg/ml LPS (Escherichia coli 055:B5) (Sigma); CD40L trimer (a kind gift from Elaine Thomas, Immunex, Seattle, WA); poly(A), poly(C), poly(G), and poly(U) single strand (ss) RNA and poly(I)poly(C) dsRNA (Sigma); 2 μg/ml lipoteichoic acid, 5 μM CpG oligodeoxynucleotide, and 1 μg/ml R-848 (InvivoGen).
mRNA Is an Endogenous Ligand for Toll-like Receptor 3

Cell Culture—Human embryonic kidney (HEK) 293 cells (ATCC, Manassas, VA) were propagated in Dulbecco’s modified Eagle’s medium supplemented with glutamine (Invitrogen), and 10% fetal calf serum (Hyclone, Ogden, UT) (complete medium). A cell line, TLR3-293, derived from HEK293 cells, which was first stably transfected with pELAM-luc and then with pCMV-6-XL5 containing human TLR3 cDNA (Origene Technologies, Rockville, MD) was generated. TLR3-293 cells were grown in complete medium with 125 μg/ml Zeocin and 400 μg/ml G418 (Invitrogen). Leukophoresis samples were obtained from HIV-uninfected volunteers through an IRB-approved protocol. Peripheral blood mononuclear cells were purified by Ficol-Hypaque density gradient purification. DCs were produced as described previously (13). The resulting immature DCs were used between 6 and 9 days after the initial culture of monocytes.

Treatment of TLR3-293 Cells—TLR3-293 cells were seeded into 96-well plates (5 × 10^3 cells/well) 1 day prior to stimulation and cultured without antibiotics. The following day, the cells were exposed to RNA without or with prior complexing to Lipofectin (Invitrogen) as described previously (15). When the treatment was performed in the absence of Lipofectin, cells were exposed to 50 μg/ml mRNA (F-mRNA1 and F-mRNA2) containing 2’-deoxy-2’-fluoro-substituted nucleotides or mRNA1, or poly(I)/poly(C) that was added to complete medium and incubated for 8 or 16 h. When Lipofectin-complexed RNA was used, cells were exposed to 5 μg/ml poly(C), poly(A), poly(U), poly(G), poly(I)/poly(C), or in vitro transcribed mRNA with or without a cap structure (Roche Diagnostics), for 1 h. The RNA was then removed, and the cells were incubated further in complete medium for 7 or 15 h. Where noted, cells were pretreated with 3 μg staurosporine, 0.5 μg genistein, 5 μM 2-aminopurine (Sigma), or 40 μM (Origene Technologies, Rockville, MD) was generated. TLR3-293 cells (Hyclone, Ogden, UT) (complete medium). A cell line, TLR3-293, derived from the parental HEK293 cells, contains considerable secondary structure, including double stranded regions. This led us to test whether mRNA signaled through TLR3 and might be the TNF-α activating ligand for this receptor.

RESULTS

In Vitro Transcribed mRNA Activates the NF-κB Signaling Pathway through TLR3 in TLR3-293 Cells—We demonstrated previously that mRNA containing a poly(A) tail signaled and activated DCs. Two mechanisms were described. The first acted through a P2Y nucleotide receptor and was mediated by the poly(A) tail. The second induced TNF-α and was independent of the poly(A) tail (18). TLR3 has been demonstrated to signal in response to poly(I)/poly(C) dsRNA but not ssRNA (poly(C) homopolymer) (5). Although mRNA is a single strand, it differs from synthetic poly(C) homopolymer in that it contains considerable secondary structure, including double stranded regions. This led us to test whether mRNA signaled through TLR3 and might be the TNF-α-inducing activity observed in our earlier experiments (18). mRNA is intrinsically
unstable in tissue culture because of the presence of RNases, so different formulations of ssRNA and dsRNA were complexed to Lipofectin, which provides protection against RNase-mediated degradation. These Lipofectin-complexed RNAs were added to HEK293 cells stably transfected with human TLR3 and the ELAM-luc reporter plasmid, which responds to NF-κB activation by production of luciferase. Neither Lipofectin alone nor poly(C) nor poly(U) homopolymer complexed to Lipofectin acti-

Fig. 1. TLR3-mediated activation of NF-κB and IL-8 secretion by mRNA in TLR3-293 cells. HEK293 cells stably expressing TLR3 and the reporter plasmid ELAM-luc were exposed to different RNAs complexed with Lipofectin (A, C, and D) or without (B). A, luciferase activity in RNA-stimulated cells. Cells were incubated with Lipofectin complexed to poly(C), poly(U), poly(I)-poly(C), and to in vitro transcribed gag-encoding mRNA with or without a cap structure or poly(A) tail (5 μg/ml), or Lipofectin alone (control) for 1 h. Lipofectin-complexed RNA was removed, the medium was replaced, and luciferase activity was assayed 7 h later. B, luciferase activity in cells treated with mRNA containing 2'-deoxy-2'-fluoro-substituted nucleotides. F-mRNA1, F-mRNA2, or poly(I)-poly(C) (50 μg/ml) was added to medium containing 10% serum without Lipofectin complexing for 8 h. C, IL-8 secretion by cells treated with Lipofectin-complexed RNA homopolymers, poly(I)-poly(C), or in vitro transcribed mRNA2 and mRNA3 as described for A. IL-8 was measured in supernatants collected 8 h later. D, dose-dependent IL-8 secretion in cells exposed to different concentrations of mRNA2 or poly(I)-poly(C). Cell treatment with the Lipofectin-complexed RNA was performed as described for A. IL-8 was measured in supernatants collected 8 h after RNA stimulation. Values for luciferase and IL-8 in quadruplicate were averaged and S.E. calculated. Experiments were performed at least three times for each data point.

The instability of mRNA in tissue culture systems required the use of Lipofectin as a stabilization reagent to be able to measure mRNA signaling. Lipofectin did not seem to affect TLR3 signaling because similar induction was seen with both Lipofectin-complexed and free poly(I)-poly(C) dsRNA (Fig. 1, A and B). However, it is conceivable that Lipofectin complexing might lead to structural alteration of mRNA, causing it to signal through TLR3. To exclude this possibility, we generated RNase-resistant mRNA containing 2'-deoxy-2'-fluoro-substituted nucleotides (2'-F-deoxycytidine and 2'-F-deoxyuridine). Two mRNAs, encoding luciferase and the Semliki Forest virus nonstructural genes, were made with fluoro-substituted nucleosides. We found that neither of these mRNAs was trans-

"K. Kariko, unpublished observation."
We were also concerned about the use of a synthetic promoter reporter system. Transcriptional regulation, even of the same promoter, can differ considerably between a stably integrated promoter versus the endogenous locus because of differences in chromatin structure and other variables in the promoter environment (19, 20). Additionally, the luciferase transcription unit has been reported to confer ambiguous promoter-independent responsiveness in certain cell lines (21).

Therefore, it was important to demonstrate that stimulation of TLR3 by mRNA could induce transcription of an endogenous locus. It was reported previously that HEK293 cells transfected with TLR4 produced IL-8 in response to specific ligand (22). Therefore, we analyzed supernatants from TLR3-expressing HEK293 cells for IL-8 production after stimulation with RNA. Interestingly, in the absence of Lipofectin, these cells exhibited a high basal secretion of IL-8 (typically 100–400 pg/ml) with a significant increase in IL-8 after poly(I)−poly(C) or mRNA stimulation. In the presence of Lipofectin, endogenous IL-8 secretion was greatly reduced (less than 10 pg/ml), although ligand-induced secretion remained intact. We found that RNAs encoding HIV gag or Renilla luciferase induced the secretion of IL-8 at levels comparable with that observed for poly(I)−poly(C) (Fig. 1C). Using IL-8 as a readout of TLR3 signaling allowed us to observe more easily concentration-dependent induction by mRNA (Fig. 1D). These data demonstrate that mRNAs can efficiently signal through TLR3 and are functionally different from synthetic ssRNA homopolymers, which cannot signal through TLR3.

**mRNA Treatment Induces IRF-1, IRAK-M, TNF-α, and TLR3 mRNAs in Human DCs**—To confirm the role of extracellular mRNA as a TLR3 ligand in primary cells, we treated DCs with various RNA constructs and used Northern blot analysis to monitor changes in the expression of genes known to be involved in dsRNA-mediated signaling. First, we examined IRF-1, a transcription factor whose mRNA accumulates in response to stimulation by various cytokines, including type I IFN, after poly(I)−poly(C) treatment, suggesting the involvement of IRF-1 in type I IFN induction by dsRNA (26). To test whether TLR3 stimulation with different RNA constructs would lead to the transcriptional up-regulation of IRF-1, Northern blot analysis was performed on DCs that were treated for 8 h with RNA. These RNAs included in vitro transcribed conventional and 2′-deoxy-2′-fluoro-substituted mRNA, poly(C) homopolymer, and poly(I)−poly(C). We observed that IRF-1 mRNA was induced in response to both mRNA and poly(I)−poly(C), but not to RNA homopolymer (Fig. 2A). IRF-1 mRNA was known to induce IRF-1, but we found that addition of cycloheximide did not block IRF-1 induction (Fig. 2A), suggesting that IRF-1 induction by mRNA does not require new protein synthesis and is therefore a direct result of primary signal transduction.

Although the above results suggest that the exogenous mRNAs may utilize TLR3 to signal DCs, we cannot exclude the possibility that other mediators such as PKR (27, 28) or nucleotide receptors may be involved in mRNA-induced signaling (18). The IRAK-M kinase was originally identified in cells of monomyeloid origin and shown to be induced upon TLR stimulation with LPS and negatively regulate TLR signaling to subsequent LPS challenge (29). We analyzed monocyte-derived DCs and found that they also produced and up-regulated IRAK-M mRNA subsequent to treatment with various TLR ligands, including lipopolysaccharide (TLR2), poly(I)−poly(C) (TLR3), LPS (TLR4), and R-848 (TLR7) (Fig. 2B). However, IRAK-M was not up-regulated by other non-TLR stimulators of DCs including CD40L and ATP (data not shown). Interestingly,
monocyte-derived DCs did not respond to nonmethylated CpG DNA (TLR9). Both human type A and B CpG oligonucleotides and supercoiled and linear plasmids were added to DCs, but no induction of IRAK-M mRNA or increase in DC activation markers was detected (data not shown), in agreement with previous findings (30). Next, we tested whether in vitro transcribed RNA could induce IRAK-M. We used mRNA containing 2′-deoxy-2′-fluoro-substituted nucleotides because conventional mRNA is unstable in cell culture conditions in the absence of the protective Lipofectin carrier, but, as we showed in earlier experiments, mRNA stabilized with fluoro-derivatized C and U nucleotides was able to induce luciferase (Fig. 1B) and IL-8 (data not shown) in TLR3-293 cells. Fig. 2C shows that mRNA containing 2′-deoxy-2′-fluoro-substituted nucleotides (F-mRNA) induced IRAK-M and TNF-α mRNA to levels similar to poly(I)poly(C) induction. This induction did not require new protein synthesis because cycloheximide was not inhibitory (Fig. 2C). Conventional mRNA complexed with Lipofectin delivered to DCs also induced IRAK-M mRNA accumulation (data not shown).

Human TLR3 mRNA has been demonstrated to be induced by dsRNA and IFN-α (10, 31). DCs stimulated with poly(I)poly(C) or Lipofectin-complexed mRNA induced TLR3 mRNA, whereas homopolymer RNA and other DC-activating agents, including TNF-α and CD40L, did not (Fig. 2D). These data demonstrate that mRNA activates DCs similar to the TLR3 ligand poly(I)poly(C) (5), and induces IRF-1, IRAK-M, TNF-α, and TLR3 mRNA accumulation.

mRNA-triggered IL-8 Secretion Is Tyrosine Phosphorylation-dependent and PKR-independent—Signaling from the TLR receptor is modulated by the differential use of adaptor proteins and may be MyD88-dependent and/or -independent (8, 32, 33). TLR3 requires tyrosines in its cytoplasmic tail for signaling (34). TLR3 can also signal in an IRAK-independent manner through PKR (33). The use of these various signaling pathways by TLR3 has not been fully elucidated. In the simple NF-κB readout system, all of these activities, TRIF (8, 32), IRAK, and PKR, can lead to reporter activation. To discern better the signaling pathways used by mRNA, several inhibitors of TLR signaling were employed. The tyrosine phosphorylation inhibitors, genistein and staurosporine, completely blocked NF-κB-driven luciferase and IL-8 production by poly(I)poly(C) and mRNA (Fig. 3, A and B, respectively). The inhibitory TIRAP peptide (35, 36), which potently blocks TLR4 but not TLR2 or TLR9 signaling (36), did not block TLR3 signaling by poly(I)poly(C) or mRNA (Fig. 3, A and B, respectively). Signaling by dsRNA, as well as by TLR3, has been demonstrated to activate PKR which leads to NF-κB induction (33). To rule out the possibility that mRNA caused increased reporter and IL-8 production by acting directly on PKR instead of signaling through TLR3, the PKR inhibitor 2-aminoarabinoside was employed. Both poly(I)poly(C) and mRNA-mediated induction of ELAM-luc activity and IL-8 production was unaffected by 2-aminoarabinoside treatment (Fig. 3, A and B). Similar data were obtained whether mRNA with Lipofectin or F-mRNA without Lipofectin was used. These data support the idea that mRNA signals through TLR3 in a manner similar to dsRNA and is dependent on tyrosine phosphorylation and independent of PKR.

Necrotic Cell-associated RNA Induces IFN-α Secretion by DCs—We reported earlier (13) that DCs treated with in vitro transcribed mRNA up-regulate major histocompatibility complex and costimulatory molecules, secrete IL-12, IL-8, and IFN-α, and become potent T cell stimulators. These data were obtained using in vitro transcribed mRNAs produced from linearized plasmids by RNA polymerase. In the next series of studies, we tested whether endogenous RNA, either released from or associated with necrotic cells, can signal and activate DCs under physiologic conditions. It is well established that extracts of necrotic cells can activate DCs (for review, see Ref. 7). This activation is mediated at least in part by TLR2 and TLR4 and may involve heat shock proteins (6). DCs can mature into several types of cell with different effects on T cell development. The phenotype of activated DCs, broadly defined as Th0-, Th1-, Th2-, or Treg-inducing, are determined, in part, by the cytokines secreted in response to TLR stimulation (3, 4, 13, 37, 38). For example poly(I)poly(C) induces high levels of IL-12 and IFN-α, whereas LPS induces little IFN-α (13). We utilized HEK293 cells freeze-thawed four times as a model for necrotic cells and tested whether RNA from these cells stimulates TLR3 and modulates the DC phenotype. To confirm that the effector molecule in these experiments was RNA, necrotic cell lysates were also pretreated with Benzonase, a potent and nonspecific nuclease that degrades all RNA into oligomers of 2–5 nucleotides in length. Analysis of RNA isolated from necrotic cells that were incubated with or without Benzonase verified the effectiveness of nuclease digestion. Using ethidium bromide staining, intact ribosomal RNAs were still detectable in samples isolated from necrotic cells incubated without Benzonase, but not in those incubated with the nuclease (data not shown). When necrotic cells, with or without prior Benzonase treatment, were added to immature DCs, both resulted in

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activation of DCs as measured by an increase in CD83, CD80, CD86, and HLA-DR expression, suggesting that the removal of RNA did not alter the ability of necrotic cells to activate DCs (Fig. 4A and data not shown). However, measurement of IL-12 (p70), IL-8, and IFN-α from necrotic cell activated-DCs revealed that the presence or absence of RNA affected the pattern of cytokine production. Necrotic cells induced high levels of IL-12 and IFN-α secretion by DCs. The removal of RNA from the necrotic cells resulted in a decrease in IL-12 and a return to unstimulated levels of IFN-α (Fig. 4B). The level of secreted IL-8, which is induced by most activated DCs, was similar regardless of Benzonase treatment (Fig. 4B), suggesting that the decrease in IL-12 and IFN-α secretion by DC was selective and not a nonspecific cytotoxic effect. As we stated, we selected Benzonase because it can degrade all forms of RNA (ssRNA, dsRNA, and RNA bound to DNA) to small oligomers, but it also degrades all forms of DNA as well. Although mammalian DNA has been implicated in TLR9 activation (39), such activation is unlikely to occur because no form of DNA (CpG containing oligonucleotides or plasmid) was found to activate the DCs used in these studies, and this type of DC has been demonstrated not to express TLR9 (30). Therefore, we conclude that the loss of RNA and not the loss of DNA was responsible for the changes in DC activation. As an additional test, necrotic cells were treated with DNase, and no loss of IL-12 or IFN-α secretion was observed (data not shown). Thus, necrotic cells have been shown to activate DCs through TLR2 by some undefined ligand, to release heat shock proteins that signal through TLR2 and 4, and now have been shown also to signal through TLR3 by RNA. Although RNA does not increase the overall levels of DC activation in our in vitro system, it was observed to alter the phenotype of the activated DCs.

**Antagonistic TLR3-specific Antibody Inhibits mRNA-induced DC Activation**—To confirm that mRNA acts as a TLR3
activator of DCs, an antagonistic antibody that has been demonstrated to block poly(I)-poly(C)-induced IFN-β secretion in human fibroblasts (40) was used. Pretreatment of DCs with the anti-TLR3 mAb 3.7 inhibited up-regulation of CD83, CD80, and CD86 by flow cytometry. B, HEK293 cells were transfected with TLR3 and either a TRIF dominant negative (gray bars) expression vector or a control vector (■). The following day, cells were stimulated with Lipofectin alone (control) or poly(I)-poly(C) or mRNA1 complexed with Lipofectin. 24 h later supernatants were analyzed for IFN-β. Values obtained in triplicate were averaged and S.E. calculated. Experiments were performed three times.

**DISCUSSION**

TLRs play an important role in innate immunity by recognizing microbial infection and orchestrating an appropriate immune response. Mammalian TLRs respond not only to pathogen-associated structures but also to host-derived molecules that are released from injured tissues and cells. The growing list of endogenous ligands that activate TLR include heat shock proteins (6, 7, 41, 42), surfactant protein A (43), fibrinogen (44), hyaluronan (45), heparan sulfate (46), defensin (47), and chromatin-IgG complexes (39). In this report, we identify mRNA as a new endogenous ligand by demonstrating that RNA, associated or released from necrotic cells, can modulate DC activation and that RNA activation of DCs requires TLR3 signaling. We provide supporting evidence for this finding by demonstrating in a model system that in vitro transcribed mRNA can specifically activate TLR3 on DCs and on HEK293 cells overexpressing TLR3.

Alexopoulou et al. (5) were the first to report that dsRNA is the specific ligand for TLR3 by demonstrating that TLR3 recognizes and responds to viral or synthetic dsRNA but not to homopolymer ssRNA (5). Here, we present evidence that TLR3 also responds to stimulation by cellular or in vitro transcribed RNAs. Although these are typically regarded as ssRNA, cellular and in vitro transcribed RNAs do have dynamic secondary structures (e.g. hairpins) that contain double stranded sequences (27, 28). It is conceivable that the first component of the TLR3 signaling cascade, a putative dsRNA-binding protein, might interact efficiently with one helical turn of dsRNA (~11 bp) (27, 28), as has been suggested for other dsRNA-binding proteins (28), thus allowing short segments of dsRNA in mRNA to signal. This possibility is supported by secondary structure analysis, which predicted formation of multiple double stranded regions that were up to 14 bp long in each of the mRNA used in the present study (data not shown). Additionally, when mRNA1 was digested with S1 nuclease, which cleaves ssRNA but not dsRNA, the S1-resistant RNA products (most likely short dsRNA) signaled DCs with efficiency similar to that of the intact, undigested mRNA (data not shown).

**Induction of IRAK-M mRNA by TLR ligand** and mRNA have only been demonstrated for LPS in mouse macrophages (29). Here we demonstrate in human DCs and monocyte-derived macrophages (data not shown) that the expression of IRAK-M mRNA was upregulated by TLR ligands. Our results provide support for the model that in vitro transcribed mRNA specifically activates TLR3 on DCs and on HEK293 cells overexpressing TLR3.
mRNA Is an Endogenous Ligand for Toll-like Receptor 3

Was increased in response to other TLR ligands, including R-848, lipoteichoic acid, mRNA, fibrinogen, and poly(I):poly(C) as well as LPS (Fig. 2B and data not shown). Induction of IRAK-M mRNA by these ligands did not require new protein synthesis (Fig. 2C and data not shown). This demonstrates that IRAK-M is directly induced by multiple TLR ligands in human DCs and macrophages.

What could be the physiological relevance for TLR3 activation by cellular RNA? Examples certainly exist for nucleic acids being involved in induction of immune responses. For instance, it has been demonstrated recently that mammalian chromatin can induce the secretion of anti-DNA immunoglobulin by B cells through dual interaction with TLR9 and surface IgM (39). The autoimmune disease systemic lupus erythematosus is marked by autoantibodies against DNA and RNA (48). It therefore comes as no surprise that deficiencies in the removal of these host ligands during inflammation activates the immune system. Pathogens continuously mutate and evolve to escape immune surveillance, including detection by TLRs. Therefore, activation of TLRs by host-derived ligands released during injury and infection is very important to alleviate the sole need of pathogen for activation of the immune system. Whether the same cellular mRNA that activates DCs through TLR3 can also serve as a source of encoded antigen is currently under study.

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mRNA Is an Endogenous Ligand for Toll-like Receptor 3

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