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MDA5 Participates in the Detection of Paramyxovirus Infection and Is Essential for the Early Activation of Dendritic Cells in Response to Sendai Virus Defective Interfering Particles

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Defective interfering (DI) particles are byproducts of virus replication that potently enhance dendritic cell (DC) maturation by virus infection. DI particles have been reported for many different viruses and are strong inducers of type I IFNs. The cellular mechanisms involved in the response to DI particles are not known. In this study, we show that 1) DI particles are recognized by more than one viral sensor independently of TLRs and type I IFN signaling; 2) The helicase MDA5 participates in the detection of DI genomes as MDA5-deficient DCs respond inefficiently to Sendai virus stocks containing DI particles; 3) DI particles stimulate the expression of IRF3-responsive genes by a uniquely potent mechanism when compared with other prototypic viral stimulus; and 4) the efficient detection of DI particles overcomes virus immune antagonism. These data highlight the outstanding adjuvant capacity of DI particles in stimulating mouse and human DCs. They also offer biological relevance to the previously reported inhibition of MDA5 by different paramyxovirus V proteins. The unique mechanism by which DI particles trigger the maturation of DCs represents a novel strategy that could be further exploited for the development of potent adjuvant molecules. The Journal of Immunology, 2008, 180: 4910 – 4918.

D endritic cell (DC) maturation is a pivotal event in the generation of adaptive immunity. In response to virus infection, DCs up-regulate costimulatory and major histocompatibility molecules on their surface, allowing effective T cell stimulation; DCs also produce chemokines, proinflammatory cytokines, and type I IFNs that promote lymphocyte recruitment, polarization of the immune response, and the generation of an anti-viral state. DC maturation is initiated upon recognition of microbial signature molecules by specialized pattern recognition receptors. Two types of molecules have emerged as the primary sensors of virus infection: TLRs and cytosolic RNA helicases. TLRs 3, 7, 8, and 9 recognize viral nucleic acids in endosomai compartments and signal either through the Toll/IL-1 receptor domain-containing adapter inter-
(DI) particles, known to enhance the induction of type I IFN in infections with many different viruses (21–24). DI particles arise as mistakes of viral replication that occur when the virus polymerase releases from the genomic template and resumes synthesis after either skipping a segment of the genome (internal deletion type) or using the nascent strand as template (copy-back type) (25). Since DI particles contain incomplete viral genomes, they are incapable of replication, unless the full complement of viral proteins necessary for a functional replication machinery is provided by coinfection with standard virus (26). It has been demonstrated that DI particles specifically containing copy-back genomes provide the enhanced type I IFN induction ability to SeV stocks (27). The properties of their antigenomic promoter also give copy-back DI particles enhanced replicative ability as compared with standard viruses or internal deletion DI genomes (28).

Though a role for DI particles in promoting type I IFN production has been known for decades, the cellular components that participate in DI genome recognition and response are unknown. As DI particles are one of the most potent viral stimuli described to induce DC maturation, they are important candidates for consideration in the development of novel vaccines against a variety of pathogens. This study aims to investigate the cellular components involved in the detection of SeV DI particles by DCs.

Materials and Methods

Cell lines and mice

Cell lines were grown in tissue culture medium consisting of DMEM (Invitrogen Life Technologies), 10% FBS (heat inactivated, endotoxin level 0.25 EU/ml; HyClone), 1 mM sodium pyruvate, 2 mM l-glutamine (Invitrogen Life Technologies), and 50 μg/ml gentamicin (Boehringer Mannheim). DC2.4 cells were transduced using retroviruses produced from the murine Maloney Leukemia Virus-based LgPW vector kindly provided by Domenico Tortorella (Mount Sinai School of Medicine, New York, NY). TLR3 mice (Sv129 background) were obtained from B&K Universal. Mice DCs were prepared according to a previously published protocol and maintained in the same concentration of brefeldin A or DMSO-absence of serum. Human DCs were cultured according to a standard protocol (30). Briefly, CD14+ monocytes were positively selected using magnetic beads and cultured with GM-CSF and IL-4 (500 and 1000 U/ml, respectively; PeproTech). Nonadherent DCs were collected and infected after 6 days in culture. Mouse DCs were prepared according to a previously published protocol ensuring the production of immature DCs (20, 31). Bone marrow cells isolated from mouse tibias and femurs were depleted of CD4, CD8, B220, and MHC class II positive cells by magnetic bead separation. Remaining cells were cultured in RPMI 1640 supplemented with 1% normal mouse serum or 5% FBS and 25 μM GM-CSF (PeproTech). In some experiments, DCs were treated with 1 μg/ml brefeldin A (Golgi Plug; BD Pharmingen) or equal volumes of DMSO as a control for 1 h before infection and maintained in the same concentration of brefeldin A or DMSO for the 6-h course of infection.

Quantitative RT-PCR (qRT-PCR), microarrays, and cytokine ELISAs

RNA was extracted using the High Pure RNA isolation kit (Roche) according to the manufacturer’s protocol for cultured cells. RNA was concentrated using ammonium acetate precipitation and washed with 80% ethanol. Equivalent masses of RNA from each sample (1–2.5 μg) were reverse transcribed using Affinity Script RT enzyme (Stratagene) and oligo dT (Roche). cDNA was diluted 50-fold with water, and PCR reactions were performed in triplicate with specific primers using SYBR green (Roche) and Platinum polymerase (Roche). Reactions were run on a 384-well plate using the Roche Lightcycler. Normalization was performed using β-actin, α-tubulin, and rps11 levels. Sequences for mouse and human primers used in this study are shown in Table I. Primers used for detection of SeV NP mRNA were previously published (20). For analysis, fold inductions were calculated over mock-infected values and representations of copy number are based on an empirical estimate of 2500 for the number of β-actin transcripts per cell (32). For specific detection of positive sense DI RNA genomes, reverse transcription was performed using the primer 5'-CATCATCAATACGGCTTCG TG-3' and PCR was done with the following primers: 5'-TCATATGG ATAAGTCCAAGA-3' and 5'-TCTACGGGATGTAATGAA-3'. For microarray analysis, type I IFN receptor knockout mice were mock-infected or infected with viruses for 2 or 6 h. Cells were pelleted, frozen using a dry ice/ethanol bath, and submitted to Miltenyi Biotec for Agilent microarray analysis. ELISA kits were used for measurement of
Results

SeV DI particles can efficiently enhance the maturation of human DCs

Our studies of mouse DCs have shown that the ability of these cells to optimally mature in response to SeV is dependent upon the presence of DI particles in the virus stocks (20). In this study, we extended this observation to human DCs. DCs prepared from human blood precursors are unable to up-regulate cytokine and costimulatory molecule transcripts after infection with a SeV stock low in its content of DI particles, herein referred to as SeV low DI (LD) (Fig. 1). Conversely, SeV LD gains the ability to up-regulate the expression of cytokine and chemokine transcripts, the amount of protein secreted to the culture supernatants, and the expression of costimulatory molecules on the cell surface when purified (p) DI particles are added back to the infection or when infected with SeV high in DI particle content (HD). This effect of DI particles cannot be mimicked by increasing the amount of SeV LD (Fig. 1).

DC activation by SeV containing DI particles does not require TLR signaling

SeV HD potently induces maturation of DCs lacking either TLR3 or the TLR adaptor molecule MyD88 (7). Nevertheless, compensation between these two pathways has not been ruled out, nor have we tested the ability of DI particles purified from SeV HD stocks to trigger TLR signaling. Thus, we examined the induction of cytokines and chemokines by DI particles in DCs deficient in both MyD88 and TLR3. Similarly to wild-type cells, DCs prepared from MyD88/TLR3 double knockout mice responded weakly to SeV LD but robustly up-regulated TNF-α, IP10, IFN-β, and the IFN-inducible viral sensors RIG-I and MDA5 when pDI particles were added to this virus (Fig. 2). The gene up-regulation in response to SeV with DI particles was quantitatively comparable or higher than that of the TLR ligands CpG DNA (TLR9), polyinosinic-polycytidylic acid (TLR3), LPS (TLR4), and respiratory syncytial virus (TLR4), yet qualitatively different in that TLR-independent activation of DCs did not stimulate IL-10 production while TLR4 and 9 ligands induced IL-10 gene transcription at 6
hours post infection (hpi) (Fig. 2). These results demonstrate that SeV containing DI particles triggers DC activation, including induction of chemokines, proinflammatory cytokines, type I IFNs and type I IFN-responsive genes independently of the known viral TLRs.

Replication of DI particles is required for potent induction of DC maturation by SeV

Although pDI particles can enhance DC maturation triggered by other strains of SeV (20), they do not affect DC maturation induced by other negative-strand RNA viruses, such as NDV or influenza virus (data not shown). These data suggest that the replication of DI particles by cognate SeV polymerase machinery is required for enhancement of DC maturation. Supporting this contention, the ability of pDI particles to enhance cytokine secretion in response to SeV LD is lost if the virus is UV-inactivated (Fig. 3B). This presumably occurs because UV-inactivated virus is unable to generate the viral replication machinery needed for its own replication and for the replication of DI particles, as suggested by the loss of viral NP mRNA production (Fig. 3A). To better evaluate whether the replication of DI genomes is needed for the enhancement of DC maturation by these particles, we treated DCs with replication-competent SeV LD together with UV-inactivated pDI particles. When pDI particles were UV-inactivated DCs did not secrete cytokines nor did they highly up-regulate the costimulatory molecule CD80 in response to the infection (Fig. 3, B and C; SeV LD plus pDI UV), indicating that the simple presence of DI particles is not enough to elicit robust DC maturation. Rather, replication of the DI genomes is essential for their stimulatory capacity. To confirm the replication of DI particles by the standard virus machinery, we developed a qRT-PCR assay specific for the positive sense RNA of a copy-back DI species cloned and sequenced from DCs infected with SeV HD stocks. This DI genome of 546 nucleotides likely corresponds to a previously described predominant DI genome involved in type I IFN induction by SeV-Cantell (27). RNA from cells infected with SeV LD did not contain this copy-back DI species, whereas addition of pDI particles to SeV LD increases the amount of the copy-back RNAs (Fig. 3D). Confirming the specificity of the PCR, DI species were only detected when reverse transcription was performed using a primer specific for positive SeV strands and positive DI genomes as opposed to a primer specific for mRNA poly(A) tails (oligo dT), which does not prime reverse transcription of DI genomes (Fig. 3D).

SeV containing DI particles triggers DC maturation independently of type I IFN signaling

Type I IFN signaling has been shown to be essential for the maturation of DCs induced by some viruses, such as NDV (33, 34). Moreover, the elimination of the type I IFN antagonist proteins from SeV results in enhanced production of antiviral cytokines from infected cells (15). However, we have reported type I IFN-independent maturation of DCs by SeV-C (35) known to produce high levels of DI particles (20, 27). Thus, we performed a side by side comparison of the type I IFN requirement for the induction of DC maturation by SeV HD, SeV LD, NDV (detected by RIG-I (15)), and EMCV (detected by MDA5 (14, 15)). In agreement with previous reports, DCs produced a suboptimal cytokine response to NDV in the absence of type I IFN signaling (Fig. 4A), whereas SeV LD does not induce detectable DC maturation. In contrast, EMCV induction infected a normal production of antiviral cytokines (TNF-α and IL-6) in IFN receptor knockout DCs, similar to the response to SeV HD. MDA5, IFNβ, TNF-α, IL-12p40, and IP10 mRNAs were also efficiently up-regulated by SeV HD when the secretion of cytokines from infected DCs was inhibited by Brefeldin A ((16) and data not shown). To rule out the direct activation of the type I IFN signaling mediator STAT1 by DI particles (36), we infected STAT1 knockout DCs with SeV LD, SeV HD, NDV, and EMCV. The results show that DCs are able to produce proinflammatory cytokines, such as TNF-α and IL-6 as well as IFNβ, independently of STAT1 in response to SeV HD (Fig. 4A). These results demonstrate that SeV DI particles can be efficiently detected and can potently induce DC maturation in the absence of signaling by type I IFN or other cytokines.

SeV DI particles uniquely activate IRF3-dependent gene transcription independently of type I IFN signaling

As shown above, DI particles provide SeV with the ability to induce the production of TNF, IL-6, and the IRF3-dependent genes IFNβ, IP10, and MDA5, independently of type I IFN. In contrast, EMCV induces the expression of IL-6 and TNF but is unable to trigger the IRF3-dependent genes in the absence of type I IFN.
The expression of IFN-γ/H9252, IP10, and MDA5 induced by SeV HD at 2 hpi is largely lost in IRF3 knockout DCs, whereas TNF-α/H9251 up-regulation, an IRF3-independent gene, remains normal (Fig. 4C). This result suggests that DI genome detection leads to a uniquely potent and early activation of IRF3-dependent genes in the absence of type I IFN.

DI particles provide SeV stocks with a distinctive ability to induce gene transcription in infected DCs

To more broadly study the transcriptional profiles induced directly by infection with SeV LD and HD in the absence of type I IFN feedback, we performed genome-wide microarray analysis on RNA obtained from type I IFN receptor-deficient DCs after 2 and 6 h of infection. NDV infection was used as a control (Fig. 5A).

SeV LD induced the transcription of only three genes after 2 h of infection, while 54 genes were up-regulated in DCs infected with SeV LD in the presence of DI particles. The strength of the DI particle stimuli was confirmed at 6 hpi by their type I IFN-independent induction of 697 genes, whereas only 16 genes were induced by SeV LD. NDV infection up-regulated 11 and 141 genes at 2 and 6 hpi, respectively, in the type I IFN receptor knockout DCs. Looking more closely at the genes induced in these conditions, it was evident that a transcriptional program involving type I IFN related genes was the first and most potently triggered program after 2 h of infection with SeV containing DI particles. This program was very weakly induced by SeV LD or NDV at this time point (Fig. 5B). Additional transcription programs, including a DC maturation program that involves chemokines, proinflammatory cytokines, and costimulatory molecule genes, were robustly operating by 6 hpi in DCs coinfected with SeV LD and DI particles (Fig. 5C). The activation of these additional programs was virtually absent in DCs infected with SeV LD or NDV (Fig. 5C). Confirming our previous data (16), the viral sensor MDA5 is highly up-regulated after 2 h of infection with SeV in the presence of DI particles independently of type I IFN, whereas RIG-I expression was barely induced in the absence of type I IFN signaling. Thus, DI particles provide SeV stocks with the ability to potently trigger the type I IFN-independent induction of IRF3-dependent genes, including the viral sensor MDA5, followed by genes associated with DC maturation.

Impairment of MDA5 function reduces DC activation in response to infection with SeV HD

To evaluate the role of MDA5 in the response to SeV HD infection, we established a stable DC2.4 cell line highly expressing the SeV V protein, a known inhibitor of MDA5 but not RIG-I (11, 37). DC2.4 cells expressing the V protein were impaired in their response to SeV LD plus pDI particles, particularly early in infection (Fig. 6A and B). DC2.4 cells expressing the SeV V protein were infected at the same level as wild-type cells, as they express similar levels of the viral NP protein (Fig. 6C). The decrease in type I IFN production in DC2.4 cells expressing the V protein was ~50% at 6 hpi in multiple experiments. This effect was specific for the V protein since the SeV P protein that contains an identical N-terminus of 317 amino acids with the V protein is incapable of this inhibition (Fig. 6D).

We next infected wild-type and MDA5 knockout DCs with SeV HD, NDV, or EMCV. At 2 hpi, up-regulation of IFNβ mRNA was impaired in MDA5 knockout DCs infected with SeV HD (Fig. 7A). This decrease in IFNβ induction by SeV HD in MDA5 knockout DCs was less pronounced at 6 hpi and was also seen for NDV at this time point (Fig. 7B). The induction of IFNβ by EMCV at 6 hpi
was entirely dependent on MDA5 as previously reported. IL-6 transcriptional induction was also decreased for all viruses tested (Fig. 7C), whereas TNF-α induction was independent of MDA5 (Fig. 7D), suggesting that these cytokines are regulated through different pathways. Viral replication as measured by SeV and NDV NP mRNA production was consistent in wild-type and knockout cells confirming that the infections were equivalent (Fig. 7E). Overall, these data demonstrate that MDA5 participates in the detection of paramyxovirus infection and is essential for the early activation of DCs in response to SeV DI particles.

Efficient detection of DI particles overwhelms the virus immune antagonism

As DI particle replication interferes with the synthesis of standard virus proteins, potent induction of type I IFN by SeV DI particles has been proposed to result from interference with production of the SeV type I IFN induction antagonistic V protein (27). As expected, interference with the expression of SeV NP, P, and V proteins (denoted P/V since the primers used amplify mRNA for both proteins) by DI particles at 10 hpi was manifested by a marked decrease in NP and P/V mRNAs compared with cells infected with virus lacking DI particles (Fig. 7F). This interference was not due to increased levels of type I IFN, as it was still observed in DCs derived from type I IFN receptor knockout mice. Interestingly, the expression of viral NP and P/V mRNAs was not inhibited by DI particles at 2 hpi (Fig. 7F) while robust induction of chemokine and cytokine genes was already present (Figs. 5 and 7A). Though overexpression of V can inhibit early activation of DCs by DI genomes (Fig. 6), infection of wild-type cells leads to low levels of V at 2 hpi that do not block DC activation effectively. Consistent

FIGURE 5. DI particles provide SeV with the ability to potently activate multiple transcriptional programs independently of type I IFN signaling. A–C Type I IFN receptor knockout DCs were infected with SeV LD, SeV HD, or NDV (MOI 1.5) or mock-infected for 2 or 6 h. Cells were then snap-frozen and delivered to Miltenyi Biotech for Agilent microarray analysis of extracted mRNAs. A, Individual genes being up-regulated or down-regulated as compared with mock-infected cells are shown as lying above (red) or below (green) the diagonal blue line representing unchanged genes. The number of up-regulated genes for each condition is shown in the upper left corner. The fold change in expression of selected genes at (B) 2 or (C) 6 hpi are color coded, and genes are functionally divided into classes including those associated with type I IFN, DC maturation, viral sensing, or TNF signaling. The scale for fold change in B was calculated relative to mock-infected cells and also applies to C.
with the necessity of viral proteins for DI genome replication, these results indicate that interference with viral gene expression early in infection is not a requirement for potent induction of DC maturation by DI genome replication, but rather DI particles provide a uniquely potent stimulus for the triggering of a transcriptional program in DCs.

**Discussion**

In this work, we describe a highly efficient mechanism for the triggering of DC maturation in response to virus infection: the detection of byproducts of viral replication, the DI particles. Although the in vivo significance of DI particles has not yet been established, their potency in the triggering of DC maturation makes them excellent candidates for novel adjuvants. Triggering of DC maturation in response to DI particle genomes is initiated by non-TLR virus sensors, including MDA5, and does not rely on cytokine signaling. Additionally, DI genome replication is essential for their stimulatory capacity. This is supported by our published observations that even high doses of DI particles alone cannot supply the stimulatory capacity seen when coinfected with a live virus (20). The mechanism of DC activation by DI particles is different from the mechanism used by SeV LD, NDV, and EMCV in that the up-regulation of the viral sensors for these viruses depends on type I IFN signaling, whereas infection with SeV HD increases MDA5 transcription even in type I IFN receptor knockout DCs. MDA5 transcription in response to SeV HD is dependent upon the transcription factor IRF3 (16).

It was recently reported that DI genomes of the copy-back type and not internal deletion DI genomes are responsible for enhancing IFNβ induction by SeVs (27). We were able to clone and sequence

**FIGURE 6.** The SeV V protein inhibits early DC activation by DI particles. Wild-type DC2.4 cells or cells stably transduced using retroviruses derived from the LgPW vector and expressing the proteins of interest were infected with SeV LD or SeV LD plus different doses of pDIs (100, 50, and 20 hemagglutinating unit) at an MOI of 1.5 or mock-infected. A–C, qRT-PCR on RNA from infected cells (D) IFNβ secretion at 6 hpi was measured by ELISA. Error bars indicate the SD of triplicate measurements in a representative experiment.

**FIGURE 7.** MDA5 is involved in the detection of SeV DI genomes. Wild-type and MDA5 knockout DCs were mock-infected or infected with SeV HD, NDV or EMCV for 2 (A) or 6 h (B–E). RNA was extracted from cells, and (A and B) IFNβ, (C) IL-6, (D) TNF-α, and SeV NP and (E) NDV NP mRNA up-regulation was determined by qRT-PCR. Fold induction was calculated relative to mock-infected cells. Relative expression levels for viral genes were calculated as fold induction over background levels of the PCR in noninfected cells. Error bars indicate the SD of triplicate measurements in a representative experiment. F, Wild-type or type I IFN receptor knockout BM-DCs were infected with SeV LD or HD at an MOI of 1.5. RNA was extracted at 2 or 10 hpi as indicated, and viral NP and P/V mRNAs were measured by qRT-PCR.
FIGURE 8. Representation of DC activation by SeV LD and HD. A. SeV LD encodes for immune antagonists that inhibit the activation of infected DCs. The V protein interferes with MDA5 signaling inhibiting the transcription of type I IFN and other genes. The C protein inhibits the response to type I IFNs in the infected cell. As a result of infection with SeV LD, DCs produce minimal levels of type I IFNs and a very limited number of related proteins. B. SeV HD provides a strong stimulus for DC activation that is efficiently sensed by intracellular proteins including RIG-I and MDA5. The inhibitory effect of the V protein is overwhelmed by the stimulus provided by DI genomes resulting in strong activation of transcription factors and the production of high levels of type I IFN, the viral sensor MDA5, and other DC maturation-related genes, independently of feedback cytokine signaling.

a copy-back DI genome from our SeV HD stock similar to that described by others to be the primary DI genome produced by SeV strain Cantell. Furthermore, we designed a specific qRT-PCR reaction to confirm the presence and replication of this copy-back DI species in infected DCs. Analysis of the primary sequence of this DI genome reveals complimentary end regions spanning 93 nucleotides. This DI genome is theoretically capable of providing dsRNA, the reported ligand for MDA5, as well as the 5’ phosphates that activate RIG-I (12–15). In addition, the efficient replication of copy-back genomes, the generation of free trailer RNA, or their effect on the replication of standard virus may lead to the generation of viral stimulus for DC activation. It is tempting to speculate that the potency of DC activation by SeV DI particles is due to initial triggering of both RIG-I and MDA5. In fact, the type I IFN response to SeV HD can be enhanced by overexpression of RIG-I (20, 38), suggesting that RIG-I is a second sensor for DI genomes.

Recent work demonstrated that RIG-I, but not MDA5, plays a significant role in the triggering of type I IFN expression in response to SeV lacking its antagonistic proteins (15). Notably, the wild-type SeV used in this study did not stimulate cells, indicating that it did not contain DI particles. Curiously, although MDA5 was found to be superfluous for the detection of paramyxoviruses, it is well established that a highly conserved interaction of paramyxovirus V proteins with MDA5 limits the activity of this helicase (11, 37). In this study, we demonstrate that V protein overexpression inhibits the induction of type I IFN by SeV HD and that knockout of MDA5 results in a similar decrease in type I IFN production after infection with SeV containing DI particles. Interestingly, the efficiency of DI genome detection overrides the virus antagonistic abilities in wild-type cells.

In addition to the V protein, SeV also encodes four C proteins that inhibit STAT1 phosphorylation needed for type I IFN signaling (39, 40). We can speculate that the C proteins impair the IFN-stimulated expression of RIG-I (10, 16, 41), and that the indirect inhibition of RIG-I up-regulation is an additional strategy used by SeV to escape immune recognition (Fig. 8). This strategy for inhibiting RIG-I up-regulation, coupled with the inhibition of MDA5 by the V protein, likely confers SeV LD with the immune evasion capacity that we observed in our microarray experiments in which only three genes were induced by this virus early in infection (Fig. 5A). The requirement for type I IFN signaling in the cytokine induction by NDV may also be explained by the observation that low constitutive RIG-I expression is highly increased by type I IFN signaling (10, 41). NDV, an avian virus not adapted to mouse cells, cannot efficiently block type I IFN synthesis or signaling (42), and this cytokine is then used by the host to amplify factors required for the induction of DC maturation. In fact, pretreatment of type I IFN receptor knockout DCs with retinoic acid increases RIG-I transcription and also increases the type I IFN response of these cells to NDV infection (unpublished observations). Cells infected with SeV HD, in contrast, do not depend on type I IFN to up-regulate the viral sensor MDA5 since DCs are extremely sensitive to the presence of DI particles. This also implies that virus containing DI particles cannot escape immune recognition.

In summary, the recognition of DI genomes by the host cell represents an additional mechanism for the triggering of DC maturation in response to virus infection. DI genomes are detected by TLR-independent sensors, including MDA5, that sequentially and efficiently trigger the transcription of a variety of genes involved in the anti-viral immune response. DI particles represent a novel tool for the study of the mechanisms that govern this response and can be envisioned as a potent natural adjuvant.

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Disclosures
C. B. Lopez, T. M. Moran, and J. S. Yount along with Mount Sinai School of Medicine, have a pending grant that relates to the use of methods for enhancing immune responses in vertebrate animals whereby DI animals are utilized to promote DC maturation/activation.

References
DI PARTICLES POTENTLY TRIGGER IRF3-DEPENDENT GENE EXPRESSION


