Antiviral defense: interferons and beyond

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Mice lacking the adaptor protein that initiates an antiviral response downstream of the RNA helicases retinoic acid–inducible gene 1 (RIG-I) and melanoma differentiation–associated gene 5 (MDA5) have recently been described. These studies highlight the essential and nonredundant role of nucleic acid recognition in the induction of type I interferon production and raise important questions regarding the nature of cell-autonomous virus detection in coordinating the antiviral response.

Viruses are the most abundant pathogens on earth, and the survival of many organisms depends on their ability to sense and restrict viral infection. Detection of nucleic acids is a fundamental means of viral recognition in both prokaryotes and eukaryotes, although the types of receptors that recognize nucleic acids and the responses induced by these receptors vary greatly across phyla. In jawed vertebrates, nucleic acid–sensing receptors induce the production of type I interferons (IFNs). These cytokines, originally identified as a soluble activity that blocked virus spread among cultured cells (1, 2), have several essential functions in the antiviral response. Type I IFNs act in both an autocrine and paracrine fashion by inducing the expression of hundreds of genes that together establish an “antiviral state,” which restricts the spread of virus among neighboring cells. In addition, type I IFNs enhance the function of natural killer (NK) cells and the differentiation of virus–specific cytotoxic T lymphocytes (CTLs), both of which recognize and eliminate virus-infected cells.

Despite the attention that type I IFNs have received since their identification, the receptors that trigger their production remained mysterious until quite recently. Two types of receptors are now known to link pathogen detection to the type I IFN response. The first type is Toll-like receptors (TLRs), which are expressed primarily by macrophages and dendritic cells (DCs) (for review see reference 3). Several features of TLR-mediated nucleic acid recognition indicate that these receptors are only part of the overall antiviral response. First, TLRs specific for viral nucleic acids are expressed by only a small fraction of specialized cells, whereas almost all cells produce type I IFNs when infected with virus or transfected with nucleic acids. Second, the TLRs that sense nucleic acids are localized in the endosomes of sentinel cells that are generally not infected with virus. These cells sample endocytosed material but do not detect the presence of intracellular infection. Third, mice lacking the adaptor protein MyD88, which are unable to activate most TLR signaling pathways, are remarkably resistant to infection with several viruses (4). Thus, although TLRs can detect viral infection, a second, more widely expressed receptor system must signal the presence of virus from within all cells.

The missing link

Two years ago, Fujita and colleagues published a landmark study that sparked the current advances in our understanding of antiviral immune responses (5). The group screened for cDNAs that could enhance the activation of an IFN regulatory factor 3 (IRF3) reporter after transfection with polyinosinic–polycytidylic acid (poly I:C)—a synthetic double-stranded (ds)RNA polymer that potently induces the production of type I IFNs. Their screen turned up the caspase-recruitment domain (CARD)–containing cytoplasmic RNA helicase RIG-I and the closely related MDA5 (5). Shortly afterward, Balachandran et al. demonstrated that the signaling proteins Fas–associated via death domain (FADD) and receptor-interacting protein 1 (RIPI) were essential for the production of type I IFNs in response to infection with vesicular stomatitis virus (VSV) and transfection with poly I:C (6). Another signaling protein, TNF receptor–associated factor 3 (TRAF3), was also shown to be involved (7). But the link between the RNA helicases and these signaling molecules remained undefined.

Less than a year ago, four groups independently identified this missing link as a CARD–containing adaptor protein, naming it IFN-β promoter stimulator-1 (IPS-1) (8), mitochondrial antiviral signaling protein (MAVS) (9), virus-induced signaling adaptor (VISA) (10), and CARD adaptor–inducing IFN-β (CARDIF) (11). Here we refer to this protein as IPS-1. Overexpression of IPS-1 activated IRF3 and NF-kB, and knockdown of IPS-1 expression blocked signaling in response to both RNA viruses and poly I:C. Interestingly, one group found that IPS-1 is localized to the outer membrane of mitochondria (9), and another group showed that it is cleaved and inactivated by the hepatitis C virus (HCV) protease NS3/4A (11), which had previously been shown to prevent dsRNA–activated signaling to IRF3 and IFN-β (12, 13).

Studies of mice lacking either RIG-I or MDA5 showed that the two helicases are essential for type I IFN production in response to distinct classes of RNA virus. RIG-I responds to in vitro–transcribed dsRNA and is required for the in vivo response to VSV, Newcastle disease virus (NDV), Sendai
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MDA5 deficiency (15, 16),

with VSV (18). Furthermore, as with

knockout mice (17, 18). In cells from

IPS–1–deficient mice, RNA virus– and
dsRNA-induced signaling and type I

IFN production were profoundly im-
paired. The only exception to this rule

was in plasmacytoid (p)DCs, which ex-
cursively use TLRs to link RNA recog-
nition to type I IFN production (14,

18). IPS–1–deficient cells were unable
to activate IRF3 and NF-kB, failed to
produce type I IFNs and did not acti-
ivate IFN-inducible genes downstream
of RIG-I and MDA5 (17, 18). In vivo,

Ips–1– mice were highly susceptible
to infection with VSV (17, 18), despite
normal serum levels of type I IFNs
(probably induced by pDCs through
TLR–dependent mechanisms) (18).
Interestingly, Ips–1 was highly haplo-
sufficient in vivo: heterozygous
(Ips–1+/−)

and homozygous (Ips–1−/−)

mice were equally susceptible to infection
with VSV (18). Furthermore, as with

MDA5 deficiency (15, 16), Ips–1−/−
mice were more susceptible to lethal
infection with EMCV (17). Unlike VSV
infection, however, EMCV infection
failed to induce the early production
of type I IFNs in the serum of IPS–1–
deficient mice, suggesting that this non-

encapsidated picornavirus might evade
TLR–dependent detection by pDCs.

These studies show that IPS–1 is es-

sential for antiviral responses to RNA
viruses and synthetic dsRNA. In con-
trast, IPS–1 was not required for type I

IFN production in response to trans-
fected DNA (17, 18), or to infection

with the intracellular bacterium

Listeria monocytogenes (18) or the Vaccinia

poxvirus (17). These data, together with

recent reports demonstrating a TLR–
independent response to intracellular

DNA (19–21), suggest that cytosolic

RNA and DNA recognition activate

the production of type I IFNs via dis-


tinct signaling pathways that converge

on IRF3.

The characterization of IPS–1–
deficient cells and mice is an important
advance in our understanding of how
cells respond to infection with RNA
viruses. But these studies also raise in-

teresting questions regarding the nature
of nucleic acid recognition and the dif-

ferent ways in which infected cells co-

ordinate antiviral immunity.

Are nucleic acids the sole initiators

of antiviral type I IFN production?

The phenotype of IPS–1–deficient cells
formally demonstrates that RIG-I and

MDA5 are the primary, nonredundant

sensors that link cytosolic dsRNA
detection to the type I IFN response.

Before the characterization of RIG-I

and MDA5, the IFN-inducible protein

kinase R (PKR) had been thought to be
important for the type I IFN response
to dsRNA, because PKR–deficient
cells and animals show reduced type I

IFN production and are susceptible to
infection with VSV (22). However, in

light of the identification of the RNA

helicase pathway of viral recognition, it

now appears that PKR does not induce

the type I IFN response to cytosolic
dsRNA. Instead, PKR appears to have

a separate, but still important, role in

cell–autonomous control of viral infec-

tion. The function of PKR most likely

involves its ability to inhibit translation
of host cell mRNA by phosphorylating
the translation initiation factor eIF2α—

the only known substrate of PKR (22).

Perhaps the reduced type I IFN pro-
duction by PKR–deficient cells reflects
the possibility that type I IFNs are more
efficiently translated under conditions
where eIF2α is limiting, as would oc-
dur during viral infection.

Not only do these findings clarify

the relative contribution of PKR to

the antiviral response, they also imply a

more general principle with fundamen-
tal implications for antiviral responses

and autoimmunity: nucleic acid recog-
nition might be the only way for most
cells to induce type I IFN production.

In models of infection with various
classes of RNA virus, IPS–1–deficient

cells are completely unable to mount a
type I IFN response. This suggests that

no component of these viruses other

than the RNA, including envelope
glycoproteins and capsid proteins, can
induce the production of type I IFNs.

Moreover, it indicates that stress re-
sponses, which are activated in cells sup-
porting rapid viral replication, are also

insufficient to induce the production
of type I IFNs. In this regard, it would
be interesting to examine mitogen-

activated protein kinase activity during

viral infection in IPS–1–deficient cells,
as it is likely that intact stress response

pathways are activating the expression
of genes other than type I IFNs.

If nucleic acid recognition is the

only way for most cells to induce the

production of type I IFNs in response
to infection with RNA viruses, does a

similar principle apply to recognition of

DNA viruses? Until recently, dsRNA

was thought to be the main signature of

viral replication inside cells, produced
by replicating RNA viruses and bidi-

rectional transcription of DNA viruses.

However, the finding that IPS–1–
deficient cells mount a normal antiviral
response to transfection with DNA, and
to infection with L. monocytogenes or a

poxvirus (17, 18), indicates that cyto-
solic dsRNA is not the only trigger of

the type I IFN response. This raises the

question of why two signaling pathways
are needed to link nucleic acid detec-
tion to the antiviral response. The sim-

plest explanation is that RNA and DNA

viruses activate different cell–intrinsic

effector responses that are tailored to

combating the particular type of virus.

Importantly, neither type I IFNs nor ge-

neric IFN-inducible genes account for

the specificity of this response, as trans-
fection with DNA and poly I:C acti-
vates a similar profile of IFN-inducible

genes (21). Instead, the two signaling

pathways might drive the transcription

of distinct IFN-independent genes and
might also activate different transcrip-
tion-independent antiviral mechanisms.

If so, this would have important impli-
cations for the design of antiviral thera-
pentics, which would need to target the

IFN-independent response appropriate

for the type of virus infection.
Another implication of these studies relates to autoimmune disorders, which are often associated with the production of type I IFNs (23). Most cells tested respond to RNA viruses and dsRNA in an IPS-1–dependent manner, and a similarly ubiquitous response exists to cytosolic DNA (20, 21). Thus, an inappropriate response of these sensor proteins to self-derived, intracellular nucleic acids might be a critical contributor to autoimmunity. This scenario implies a cell-autonomous initiation of autoimmunity, which is distinct from several current models in which defective clearance of apoptotic cells results in the accumulation of extracellular nucleic acids (24). TLRs might have a key role in the detection of these extracellular self-nucleic acids (25), particularly chromatin and RNA–protein complexes (26–30), whereas the cytosolic RNA and DNA sensors might be responsible for a distinct set of autoimmune disorders that may only sometimes be associated with the failed clearance of apoptotic cells (19). Breeding IPS-1–deficient mice with autoimmune-prone mouse strains would be a simple way to begin to address this hypothesis.

Cell-autonomous defense: beyond type I IFNs?

The observation that IPS-1–deficient mice are highly susceptible to infection with VSV despite generating a normal systemic type I IFN response (18) is reminiscent of the phenotype of FADD-deficient fibroblasts, which are unable to control VSV replication even after pretreatment with type I IFNs (6). It is possible that local concentrations of type I IFNs are too low to control viral replication in peripheral tissues in the absence of IPS-1. But there is another possible explanation that would suggest a role for IPS-1 signaling beyond the induction of type I IFNs: detection of cytosolic nucleic acids might trigger cell-intrinsic apoptosis and/or flag the infected cells—but not their uninfected neighbors—for elimination by NK cells and CTLs.

One of the key functions of type I IFNs is to provide a paracrine alarm signal and induce an antiviral state in uninfected neighboring cells. For example, RIG-I and MDA5 are induced by type I IFNs, which then increase the sensitivity of nucleic acid detection in neighboring cells. Another key role of type I IFNs is to enhance the cytosolic functions of NK cells and CD8+ T cells, which are important for inducing non–cell-autonomous apoptosis in infected cells. The recognition and elimination of infected cells must be carefully regulated such that only the infected cells—and not their immediate neighbors—are targeted. The IPS-1–dependent production of type I IFNs cannot supply the dominant “kill signal” for cytotoxic cells; this signal must be cell bound and it must be unique to infected cells (Fig. 1). Because RIG-I and MDA5 (and the currently unidentified DNA sensor(s)) detect viral nucleic acids within infected cells, they are perfectly suited to induce surface expression of the ligands that uniquely flag infected cells for elimination. This induction must be IFN independent and could even be IRF3 independent (Fig. 1).

What might these ligands be? Promising candidates are ligands for activating and inhibitory receptors that are expressed by NK cells and cytotoxic CD8+ T cells (31). Coordinated down-regulation of inhibitory ligands and up-regulation of activating ligands can determine the susceptibility of target cells to NK cell–mediated killing (31), but the mechanisms and signaling pathways responsible for altering the expression of these ligands remain largely unknown. These mechanisms must be cell autonomous, however, and could conceivably be controlled by IPS-1–dependent signals in infected cells. One activating receptor expressed by NK cells and some effector CD8+ T cells is NKG2D, which recognizes cell surface ligands induced by various stresses, including viral infection (32). One prediction...
of this model is that IPS-1–deficient mice would initiate adaptive immune responses normally after infection with RNA viruses but that effector CD8+ T cells and NK cells would be unable to kill infected cells. Another clinically relevant prediction is that treatment of virus-infected individuals with recombinant type I IFNs would only restore paracrine IPS-1–dependent immune responses and not the cell-autonomous effector functions downstream of IPS-1 (Fig. 1). For viruses such as HCV, which encodes the IPS-1–cleaving protease NS3/4A, pharmacological inhibitors that block NS3/4A would restore all functions of IPS-1 and would thus be far more effective than treatment with type I IFNs alone (33).

IPS-1 might also control induction of cell-intrinsic apoptosis in an IFN-independent manner (Fig. 1). Because IPS-1 is a transmembrane protein localized to mitochondria, it would be interesting to see if it interacts with or alters the function of the BCL2 family of proteins, which control mitochondrial–dependent apoptosis. However, infection with viruses other than VSV would be required to firmly establish this, as VSV-infected cells can be killed by the strong cytopathic effect of this virus, especially in the absence of type I IFN-induced signaling. Importantly, anchoring IPS-1 to other membranes in the cell impairs signaling, suggesting that mitochondrial localization, and not membrane attachment itself, is essential for its function (9). This observation, coupled with the fact that IPS-1 is not IFN inducible and is strongly haploinsufficient in vivo, implies that IPS-1 is coordinately regulated with the numbers of mitochondria and might be most abundant (and most sensitive to nucleic acid detection) in cells with the highest metabolic activity.

Conclusions and future directions

The description of IPS-1–deficient mice marks an important milestone in the rapidly advancing field of nucleic acid recognition. In just two years, the nonredundant cytosolic RNA sensors and several of the key signaling molecules that link RNA recognition to the antiviral response have been identified and characterized in vitro and in knock-out mice. Along the way, we have realized that an entirely separate system exists for the detection of intracellular DNA that is likely to be important for host responses to DNA viruses and intracellular bacteria. Together with the current knowledge of TLR–mediated nucleic acid recognition, we are now in a position to dissect the relative contribution of all of these systems to immunity and immunopathology.

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