Targeting viral dsRNA for antiviral prophylaxis

Zhou Fei,*†,1 Yang Liu,*† Zhen Yan,† Daming Fan,§ Alice Alexander,* and Jing-Hua Yang*‡

*Department of Surgery, Boston Veterans Affairs Healthcare System, Boston University School of Medicine, Boston, Massachusetts, USA; and †Department of Neurosurgery, ‡Department of Pharmacology, and §Department of Gastroenterology, Xijing Hospital, Xi’an, China

Key Words: apoptosis · double-stranded RNA · pathogen-associated molecular pattern

Double-stranded (ds)RNA in the infected cells is a trait shared by most if not all viruses. While humans have developed variable immune responses, viruses have also developed countermeasures to defeat dsRNA-induced antiviral strategies. Thus, we proposed a broad antiviral strategy to antagonize the countermeasures of viruses and bypass the dsRNA-induced signals that are readily defeated by viruses. By rewiring the dsRNA-binding proteins in the dsRNA complex and reconnecting them to apoptosis signaling, we created several dsRNA-dependent caspase recruiters, termed dsCAREs, to bypass dsRNA-induced antiviral signals that would otherwise be targeted by viruses. Adenovirus and vesicular stomatitis virus, representing viruses of the dsDNA and negative-stranded RNA viral groups, were used to infect HEK293 cells. The dsCARE chimera was added in medium to evaluate its antiviral activity. The truncated dsCAREs were used as controls. We demonstrate that dsCARE suppresses viral infection starting at 0.1 μg/ml and reaches the peak at 2 μg/ml. The EC50 was ~0.2 μg/ml. However, it had an undetectable effect on uninfected cells. Further data show that both dsRNA binding and apoptosis activation of dsCARE are essential for its antiviral activity. We conclude that dsRNA is a practical virus-associated molecular pattern that can be targeted for broad and rapid antiviral prophylaxis.—Zhou Fei, Z. Liu, Y. Yan, Z. Fan, D. Alexander, A., Yang, J.-H. Targeting viral dsRNA for antiviral prophylaxis. FASEB J. 25, 1767–1774 (2011). www.fasebj.org

ABSTRACT Double-stranded (ds)RNA in the infected cells is a trait shared by most if not all viruses. While humans have developed variable immune responses, viruses have also developed countermeasures to defeat dsRNA-induced antiviral strategies. Thus, we proposed a broad antiviral strategy to antagonize the countermeasures of viruses and bypass the dsRNA-induced signals that are readily defeated by viruses. By rewiring the dsRNA-binding proteins in the dsRNA complex and reconnecting them to apoptosis signaling, we created several dsRNA-dependent caspase recruiters, termed dsCAREs, to bypass dsRNA-induced antiviral signals that would otherwise be targeted by viruses. Adenovirus and vesicular stomatitis virus, representing viruses of the dsDNA and negative-stranded RNA viral groups, were used to infect HEK293 cells. The dsCARE chimera was added in medium to evaluate its antiviral activity. The truncated dsCAREs were used as controls. We demonstrate that dsCARE suppresses viral infection starting at 0.1 μg/ml and reaches the peak at 2 μg/ml. The EC50 was ~0.2 μg/ml. However, it had an undetectable effect on uninfected cells. Further data show that both dsRNA binding and apoptosis activation of dsCARE are essential for its antiviral activity. We conclude that dsRNA is a practical virus-associated molecular pattern that can be targeted for broad and rapid antiviral prophylaxis.—Fei, Z., Liu, Y., Yan, Z., Fan, D., Alexander, A., Yang, J.-H. Targeting viral dsRNA for antiviral prophylaxis. FASEB J. 25, 1767–1774 (2011). www.fasebj.org

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Double-stranded RNA (dsRNA) may exist as a result of RNA replication in the “RNA world” and has apparently evolved differently in lower vs. more advanced species. While long stretches of dsRNA are widely found in plants and other lower species (1), they are not readily detected in mammals. Although bidirectional transcripts are widely encoded in human and mouse genomes (2) and complementary RNA transcripts are also expressed (3), attempts to clone their dsRNA forms in healthy mammalian cells have not been successful. However, most viruses produce dsRNA intermediates during infection in host cells. Thus, generation of dsRNA during infection is a trait shared by dsRNA viruses and also dsDNA viruses, positive-strand RNA viruses, and negative-strand RNA viruses (4–6). Thus, dsRNA may still be considered a pathogen-associated molecular pattern and danger signal for innate immune responses against viral infection in mammalian systems. Unlike in lower species, where they may be involved in gene regulation through RNA interference, long stretches of dsRNA provoke predominantly innate immune responses and ultimately lead to growth inhibition or apoptosis (7, 8). Typically, dsRNA of >30 bp tends to interact with intracellular dsRNA-binding proteins (DRBPs). For instance, dsRNA binds to intracellular Toll-like receptor 3 (TLR3) and activates IFN transcription via interferon regulatory factor 3 (IRF-3) and nuclear factor κB (NF-κB) (9). This activation is essential for IFN production in response to viruses. The dsRNA-activated protein kinase (PKR) and 2’5’-oligoadenylate synthetase are important DRBPs that cause immediate translation inhibition and ribosomal RNA degradation (10). Other DRBPs, including dsRNA-dependent PKR activator (PACT/RAX), TAR RNA-binding protein (TRBP), the dsRNA-specific adenosine deaminase (ADAR), and the nuclear factor NF90 (11–14), are also involved in mediation of dsRNA-induced PKR activation. In addition, the cytoplasmic RNA helicases melanoma differentiation-associated protein 5 (MDA-5) and retinoic acid inducible gene I (RIG-I) are 2 new pathogen receptors that may involve intracellular dsRNA (15).

While humans have developed variable immune responses against the universal dsRNA pattern, viruses have also developed countermeasures to defeat dsRNA-induced antiviral responses (16–19). It has become increasingly clear that viruses encode DRBPs to counteract dsRNA and dsRNA-induced signaling. For instance, many viruses are shown to encode proteins that interfere with IFN signaling by inhibiting IFN synthesis, inactivating secreted IFN molecules, and blocking the action of IFN-induced antiviral proteins (18, 19). Thus, both the host and virus apply a range of measures to deal with dsRNA, indicating that dsRNA is a vulnerable target for pro- and antiviral mechanisms. The question

1 These authors contributed equally to this work.
2 Correspondence: Department of Surgery, Boston University School of Medicine, 150 S. Huntington Ave., Boston, MA 02130, USA. E-mail: jyang@bu.edu
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is how to antagonize the countermeasures of viruses or simply bypass the dsRNA-induced signals that are readily defeated by viruses. Recently, we have shown that dsRNA is recruited by a few functionally important factors (13, 14), including the dsRNA editase ADAR1, the dsRNA effectors PKR and NF90 (20), and the dsRNA regulator PACT (11). Interactions in the complex are demonstrated to play an important role in regulation of host susceptibility to viral infection. Notably, the proteins in the complex are either dsRNA receptors or mediators, and their functions are centered at translation and transcriptional regulation of dsRNA-induced responses in viral infection, suggesting a typical “dsRNA signalosome” (13, 14) that regulates dsRNA-induced signaling. This dsRNA complex is reasoned to be the early cellular response to virus infection. To address the problem of how to antagonize viral countermeasures or bypass dsRNA-induced signals that are overcome by viruses, we rewired the dsRNA signalosome by creating several dsRNA-dependent caspase recruiters, termed dsCAREs. These chimeras are designed to prevent viral infection by providing a shortcut that connects the virus-induced dsRNA signaling directly to growth inhibition or cell death (21).

MATERIALS AND METHODS

Plasmids and constructs

The cDNAs of NF90 and PACT were amplified from the human cDNA library (Invitrogen, Carlsbad, CA, USA) by RT-PCR and confirmed by DNA sequencing. The cDNAs of human PKR (GeneBank BC093676), ADAR1 (GeneBank BC038227), and Apa-f (GeneBank DN998849) were directly purchased from Invitrogen. Two oligonucleotides, AGCTTG-GATGCTACGCCCCCTGGGGCGCCGTCAGGCCGGTCG-CACTCGGT and CCATCTCGAGACCATGGCACGGCCGTG, were annealed, filled by PCR, and cleaved with BamHI and XhoI to produce the cDNA for protein transduction domain (PTD). The DNA cassette coding for the dsRNA binding domain of NF90, PACT, or ADAR1 was generated by PCR, using primers with the XhoI and KpnI cleavage sites at their 5' and 3' ends, respectively. The DNA fragment coding for the caspase recruitment domain (CARD) signal was generated by PCR, using primers with the KpnI and HindIII cleavage sites at their 5' and 3' ends, respectively. Thus, the DNA of dsCARE was sequentially linked with these restriction cleavage sites in the order of BamHI-XhoI-KpnI-HindIII and cloned into the pcDNA3 vector (Invitrogen) for transient expression or pRSET B for recombinant protein production in bacteria. All DNAs were confirmed by restriction endonuclease digestion and sequencing.

Transfection

Cell lines used in this study include human HEK 293 and 293T (ATCC SD-3515; American Type Culture Collection, Manassas, VA, USA). Cells were maintained in DMEM with 10% (v/v) FBS and penicillin-streptomycin-anphoterin B (Gibco-BRL, Gaithersburg, MD, USA) at 37°C with 5% CO₂. For transient expression, ~6 × 10⁴ cells were seeded (~70% confluence) and transfected with 4 µg of either the pcDNA3 vector or testing plasmids using 10 µl Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. The cells were allowed to recover for 6 h at 37°C in a CO₂ incubator before viruses were applied to the cells.

Adenovirus infection

HEK293 or 293T cells were maintained in 6-well plates for 8 h. Recombinant Adeno X AcGFP1 virus (632504; Clontech, Palo Alto, CA, USA) was added to the medium at a multiplicity of infection (MOI) of 10⁻⁴ or 1. The low MOI was used to mimic the condition of early infection under which the dCARE’s prophylactic effect could be evaluated. The high MOI, however, represented the late stage of infection that might be used to elucidate dsCARE-induced growth inhibition of the infected cells. The viruses were allowed to absorb for 30–60 min. The cells were washed with warm PBS twice to remove excess viruses and continuously cultured for 12 h in DMEM with 10% FBS. Cells infected with adenovirus were analyzed under the fluorescent microscope (BX60; Olympus, Tokyo, Japan). The virus titer was determined by infection of semiconfluent 293T cells with a series of virus dilutions; GFP-positive cells were counted under the fluorescent microscope at 24 h postinfection by 3 independent people.

Vesicular stomatitis virus (VSV) infection

HEK293 cells were seeded in 6-well plates for 8 h at 1.5 × 10⁵ cells/well. VSV-EGFP1 viruses (22) were added to the medium at a MOI of 10⁻⁴. Viruses were allowed to absorb for 30–60 min. Cells were washed with warm PBS twice to remove excess viruses and continuously cultured for 6 d in DMEM with 5% FBS. Cells infected with VSV-EGFP1 were visualized under fluorescent microscopy. To quantitate the titer, culture medium was collected and passed through a 0.2-µm filter. Tenfold dilution series were made with PBS to infect semiconfluent NIH3T3 cells. GFP-positive plaques were counted under fluorescent microscope 12 h postinfection, and the viral titer was calculated accordingly.

Cell viability

Cell viability was determined in the absence of viral infection or in the presence of high titer of viruses (MOI>1) by hemocytometer counting. Typically, cells were seeded in 6-well plates for 8 h at 1.5 × 10⁵ cells/well. To determine the toxicity of dsCARE, 0, 0.2, 0.4, 1.0, 2.0, and 4.0 µg/ml of dsCARE was added to the medium and incubated for 48 h. To determine the toxicity of dsCARE specific to infected cells, a high titer of viruses (MOI>1) was added to the medium 24 h before addition of dsCARE. Cells were collected and mixed with an equal volume of 0.4% trypan blue and added to the hemocytometer chamber. Stained and unstained cells were counted under microscope to calculate the percentage of viable cells.

Protein expression and purification

The cDNA coding for the chimera proteins was cleaved with BamHI and HindIII and subcloned into the pRSET expression vector (Invitrogen). The plasmid was confirmed by restriction enzyme digestion and retransformed into bacterial strain BL21 (Invitrogen). A single clone was selected to grow in 2 ml of Luria-Bertani medium with 100 µg/ml of ampicillin at 37°C overnight. It was then amplified in 200 ml of Luria-Bertani medium and shaken at 37°C overnight in the same medium without induction. Bacteria were collected by centrifugation (5000 rpm, 4°C, 10 min) and resuspended in 100
mL of lysis buffer (PBS containing 0.1% Triton X-100 and 8 M urea pH 7.2). After sonication, the supernatant was collected and applied to nickel resin (Qiagen, Valencia, CA, USA) 2 h at 4°C. The bound proteins were washed with the lysis buffer and eluted with lysis buffer containing 300 mM imidazole. The protein was renatured by dialysis against gradually decreasing concentrations of urea until it reached zero. Its concentration was determined by Nanodrop, and quality was confirmed on 12% SDS-PAGE.

RESULTS AND DISCUSSION

We first rewired dsRNA signaling directly to apoptosis by modifying the protein components in the dsRNA complex (Fig. 1A). Because ADAR1 in the dsRNA complex is known to antagonize the antiviral response (13, 14), its deaminase domain was removed and the dsRNA-binding domain reconnected to the CARD of Apaf-1 (23). This modification would transform ADAR1 into dsCARE, which should be activated by long stretches of dsRNA during infection. Adenovirus, a typical dsDNA virus, was first used to test the antiviral effect of the chimera because adenovirus is known to generate large amounts of dsRNA during replication (4). The construct (AD123, Fig. 1A) and a control vector were transiently expressed in 293T cells. After transfection, cells were infected with adenovirus carrying the GFP marker at a low virus titer (MOI = 10^{-4}) to mimic a prophylactic condition at the early stages of viral infection. Green cells were monitored under fluorescent microscopy 3–7 d after infection to determine virus multiplication. Typically, the virus replicated exponentially and became widespread in 1 wk, when cells were transfected with the control vector (Fig. 1B) or not transfected (data not shown). However, significantly fewer viruses were detected starting from 2 d after transfection of the dsCARE construct (AD123), suggesting that virus infection was suppressed by dsCARE in the early stage of infection. Please note that because very low MOI was used in these experiments, cytopathic effects were not seen until 7 d postinfection when viruses were spread, indicating low toxicity of dsCARE in uninfected cells.

To avoid the low transfection efficiency (typically <50%) of the dsCARE plasmid, recombinant dsCARE protein was produced in bacteria. To make the recombinant dsCARE protein permeable through the cell membrane, a PTD (24) was added at the N termini of dsCARE. The PTD was originally modified from HIV Tat peptide and further optimized for cell uptake of large exogenous proteins (25). Recombinant dsCARE was directly added in the culture medium of 293T cells before adenovirus infection. Consistently, we found that adenovirus infection was efficiently suppressed at ~1 µg/ml of recombinant dsCARE (AD123, Fig. 1C). These data indicate that the dsCARE chimera suppresses viral infection but has little effect on total cell population.

To confirm that the antiviral effect requires dsRNA binding, dsCARE (AD123) was modified by substituting the dsRNA-binding repeats 1, 2, and 3 with the repeats 1 and 2 (AD12), the repeats 2 and 3 (AD23), or the repeat 2 (AD2) (Fig. 2A). A deaminase region of indicating low toxicity of dsCARE on uninfected cells. Green indicates positive cells infected with adenovirus. C) Antiviral activity of recombinant dsCARE (AD123). dsCARE (AD123) was subcloned into pRSET vector, and a fragment of synthetic DNA coding for the protein transduction domain was added as indicated. Chimera was produced in bacteria and added to the culture medium at a final concentration of ~1 µg/mL. Cells were then infected with GFP-positive adenovirus. Again, no cytopathic effects were seen under the experimental conditions because of the low MOI. Photographs were taken under light and fluorescent microscope (FL) microscope (left panel), and GFP-positive cells were counted (right panel) 7 d after infection (n = 3). No cytopathic effects were seen at the low MOI used in this experiment,
ADAR1 containing no dsRNA-binding repeats was used to construct a negative control (ADcat). In addition, a control containing only a dsRNA-binding domain without the CARD sequence (dsRBD) was also included to confirm the requirement of the caspase recruiter. All chimeras had the PTD fused at their N termini for cell delivery and were produced in bacteria for direct use in cell culture (Fig. 2). Typically, 1 μg/ml of each chimera was added directly to culture medium following adenovirus infection (MOI = 10^4). Our data showed that the chimeras with dsRNA-binding repeats 1 and 2 (AD12) and repeats 2 and 3 (AD23) also suppressed adenovirus infection (Fig. 2C). In contrast, significantly less activity was observed when only repeat 2 (AD2) was used, and no significant antiviral activity was detected for the chimera without dsRNA-binding domain (ADcat).

We concluded that the dsRNA-binding feature was essential for the antiviral infection activity, although each unit of the dsRNA-binding repeats did not contribute equally (Fig. 2D). As expected, no antiviral activity was observed when CARD was removed from dsCARE (dsRBD), confirming that the caspase recruitment feature was also essential. Therefore, both dsRNA binding and apoptosis activation are required for the antiviral activity of dsCARE. These results support our hypothetical mechanism that virus-induced dsRNA activates dsCARE to initiate apoptosis of the infected cells and consequently prevent virus multiplication.

Next, we compared the antiviral activity of dsCAREs that were made using other components of the dsRNA complex, including PKR, NF90, and PACT (Fig. 3A). As
these proteins are also DRBPs, connecting them directly to apoptosis would create a shortcut to bypass the signal cascades that would otherwise be targeted by viruses. To test their antiviral activity, the chimeric dsCARE (PKR), dsCARE (NF90), and dsCARE (PACT) were produced in bacteria and purified to homogeneity (Fig. 3B). We found that all of the chimeras suppressed virus multiplication in cell culture (Fig. 3C). Notably, ADAR1 and PKR in the dsRNA complex have been shown to play opposite roles in regulating host susceptibility (14). Removal of their enzymatic activity and reconnection of their dsRNA-binding domain with the caspase recruitment domain synchronized their signals to the antiviral effect. The role of NF90 and PACT in regulation of host susceptibility is not known; however, they were transformed into dsCARE with similar efficiency. In agreement with previous results, these data indicate that the dsRNA-binding activity is essential for dsCARE to bypass the signal cascades that would otherwise be targeted by viruses.

Furthermore, dose-dependent experiments demonstrated that the antiviral activity was detected at \( \sim 0.1 \) \( \mu \)g/ml and reached the peak at \( \sim 2 \) \( \mu \)g/ml (Fig. 4A, B). To estimate the toxicity, increasing amounts of dsCARE were applied on uninfected 293T cells. The cell viability was determined by trypan blue exclusion test and counted with a hemacytometer under the microscope. No significant toxicity was observed in the testing dose range of dsCARE when cells were not infected with virus (Fig. 4C). In the presence of high titers of virus (MOI>1), however, the number of viable cells quickly decreased when the amounts of dsCARE increased. These results indicated that dsCARE specifically discriminated between the infected and uninfected cells. It efficiently destroyed virus-infected cells but had no significant toxicity on uninfected cells. Based on this finding, we also investigated the potential use of dsCARE as an antiviral prophylactic agent. To this end, 293 cells were cultured in the medium containing 2 \( \mu \)g/ml of dsCARE or 100 \( \mu \)g/ml of BSA for 1 wk and then infected with low titters of adenovirus (Fig. 4D, E). At 1 wk postinfection, the virus reached nearly 100% of infection in the control group but was not detectable in the testing group. At 10 d postinfection, cells in the control group were completely killed by viruses, whereas cells in the test group were still viable.

Because dsRNA is a universal molecular pattern for virus infections, we reasoned that dsCARE could be used as a broad antiviral prophylactic against medically important human pathogens. VSV was then tested as a typical negative-sense RNA virus in the Rhabdoviridae family. This was because VSV represents a group of many lethal viruses, such as Ebola virus (filoviruses), Nipah virus (paramyxoviruses), Lassa virus (arenaviruses), and rabies virus, as well as influenza virus (orthomyxoviruses). Although dsRNA was not demonstrated in La Crosse virus-infected cells (26), other studies (18, 19) have provided evidence that small amounts of dsRNA are, indeed, produced by negative-strand RNA viruses, leading to activation of cellular antiviral countermeasures. In addition, some negative-strand RNA viruses, such as Ebola virus, Nipah virus, and influenza A virus, have evolved mechanisms to inhibit cellular defense mechanisms that are activated by dsRNA, indicating that dsRNA is present in infected cells (27–30). Thus, negative-strand RNA viruses are likely the targets of dsCARE, and the result will be particularly important because of the potential public threats of these viruses in pandemic situations. In the experiment, NIH293 cells were infected with a laboratory-adapted strain of VSV carrying the GFP marker (22) (a gift from Dr. Rose, Department of Pathology, Yale School of Medicine, New Haven, CT, USA) at \( \sim 10^{-4} \) MOI. Typically, widespread infection (GFP-positive cell) was observed at 3 d postinfection; however, no infection was seen when \( >3.6 \) \( \mu \)g/ml of dsCARE was present in the culture medium, and a clear dose dependence was demonstrated at 3 and 5 d postinfection (Fig. 5). After 6 d of infection, cells without dsCARE were lysed by the virus, whereas no sign of cytolysis or viral infection was detected when dsCARE was \( >3.6 \) \( \mu \)g/ml. Consistently, cell viability was proportional to dsCARE. The effective concentration of dsCARE that caused 50% virus reduction was \( \sim 1.5 \) \( \mu \)g/ml. Notably, \( 3.6 \) \( \mu \)g/ml is a realistic concentration for prophylactic applications. For in-
stance, because VSV is an airborne virus that can infect both insects and mammals via aerosols of respiratory droplets. dsCARE may be developed as a rapid antiviral spray and used to prevent infections from the viruses in this family.

Mammals have evolved the immunity to eliminate virus-infected cells by suicide after sensing the viral dsRNA pattern. In some instances, the cell eliminates the virus through less drastic pathways that permit survival of the infected cell. Viruses, however, often encode dsRNA-binding proteins to counteract dsRNA-induced antiviral responses in order to replicate. This strategy is aimed at promoting apoptosis in response to virus toward the goal of eliminating virus-infected cells and thus also eliminating the virus. Thus, dsCARE may be developed into a rapid antiviral prophylactic and probably also an antiviral therapeutic that can be applied at the early stage of infection. As a limitation of this approach, it may not be applicable to infections that have been widely spread in organs. For instance, a potential intranasal dsCARE may be used to prevent upper respiratory tract viral infections, such as a preventive measurement for medical providers who need to contact the infected objects. Applying it to widely infected patients may cause erosion of the nasal mucosa. Animal studies are needed to determine its preventive efficacy and the proper period of time for therapeutic use.

Figure 4. Antiviral properties of the dsCARE in vitro. A, B) Dose-dependent antiviral prophylactic activity. Cells were infected with adenovirus at MOI = 10^{-4} to mimic the early stages of viral infection. Then, 0, 0.2, 0.4, 1.0, 2.0, 4.0, or 8 μg/ml of the dsCARE (PKR) protein was added. Typical photographs were taken 7 d after infection before the significant cytopathic effects were seen (A). Virus multiplication was estimated by GFP-positive cells and plotted against the dosage of dsCARE (PKR) (B). C) Toxicity and selectivity. 293T cells were cultured in the presence of 0, 0.2, 0.4, 1.0, 2.0, and 4.0 μg/ml of dsCARE (PKR) with no virus infection (circle) or high titer (MOI=1) of adenovirus (square). Total viable cells were counted 3 d postinfection using trypan blue exclusion test and plotted against the dose of dsCARE. Data are means ± sd; n = 3. D, E) Protective activity of dsCARE (PKR) against virus-induced cytopathic effects. Cells were infected with low dose of adenovirus (MOI=10^{-4}) and treated with 2 μg/ml of dsCARE or 100 μg/ml of BSA for 1 wk and then infected with adenovirus. A mock control without virus and dsCARE was also enclosed. Typical photographs were taken 7 d (D) and 10 d (E) after infection.
Nevertheless, virus-induced apoptosis of the infected cells is a natural mechanism of central importance for mammals to control viral infections. For instance, MDA5 and RIG-I are two recent examples that are structurally similar to dsCARE (15, 31). The difference is that MDA5/RIG-I is activated with a specific type of virus, whereas dsCARE is activated by long stretches of RNAs in general. Notably, although the host cells are known to encode a large amount of bidirectional transcripts, little cytotoxicity was observed in uninfected cells, suggesting that long stretches of endogenous dsRNA, if any, are below the level of detection by dsCARE and may be safe for targeting as a unique pathogen-associated molecular pattern against virus infections. In summary, dsCARE is shown to prevent infections from dsDNA and positive-sense RNA viruses and may be developed into a rapid antiviral prophylactic against different groups of virus as long as they produce dsRNA in the host.

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REFERENCES

28. Li, S., Min, J. Y., Krug, R. M., and Sen, G. C. (2006) Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA. Virology 349, 13–21

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