[Frontiers in Bioscience 13, 4379-4392, May 1, 2008]

Does the understanding of immune activation by RNA predict the design of safe siRNAs?

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1. ABSTRACT

The innate immune system uses pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) to recognize conserved pathogen-associated molecular patterns (PAMPs) expressed by microbes and to activate the initial phase of immune response. Both bacterial and viral nucleic acids activate TLRs resulting in cytokines and interferon production. Recent studies indicated that unmodified small interfering RNAs (siRNAs) corresponding to either mammalian (self) or microbial (non-self) RNA sequences can activate immunity through TLR7/8 in a sequence-dependent manner. Hence, the use siRNAs in humans will require understanding the mechanisms involved in the discrimination between self and non-self RNAs. In the case where immunostimulation is not wanted, chemical modifications can prevent immune activation and reduce off-target effects, while preserving siRNA silencing potency. Interestingly, 2'-*O*-methyl modified RNAs not only evade immune sensing, but antagonize with immunostimulatory single- or double-stranded siRNAs to activate innate immunity. This review highlights the recent progress in understanding the siRNA sensing by innate immunity and presents a range of strategies allowing either the design of siRNAs with minimal or maximal immunostimulatory potency for therapeutic applications.

2. INTRODUCTION

Although RNA-based gene silencing was first described in plants, its importance come from the demonstration that RNA interference (RNAi) can be used in the nematode *Caenorhabdtis elegans* to specifically inhibit gene expression (1-3). In all of these organisms long dsRNAs are used as triggers of RNAi. Most mammalian cells, however, do react to long double-stranded (ds) RNA (>30 bases) by activating the interferon pathway leading to apoptosis (4). DsRNA is synthesized during the replication of many viruses, which includes RNA and DNA viruses, and is a potent activator of innate immune cells. Notably, fundamental insights into mammalian RNAi came from biochemical studies showing that the main components of the RNAi machinery were conserved across species and that short RNA duplexes of 21 nt in length, known as small interfering RNAs (siRNAs) are the effectors of RNAi (5,6). These observations led to the demonstration that siRNAs, mimicking the Dicer cleavage products, are able to mediate efficient and specific RNAi upon tansfection into mammalian cells without triggering the IFN response (7), Figure 1). Subsequent to this discovery, the use of siRNAs to study gene functions in mammalian systems has become a standard laboratory technique (8, 9).

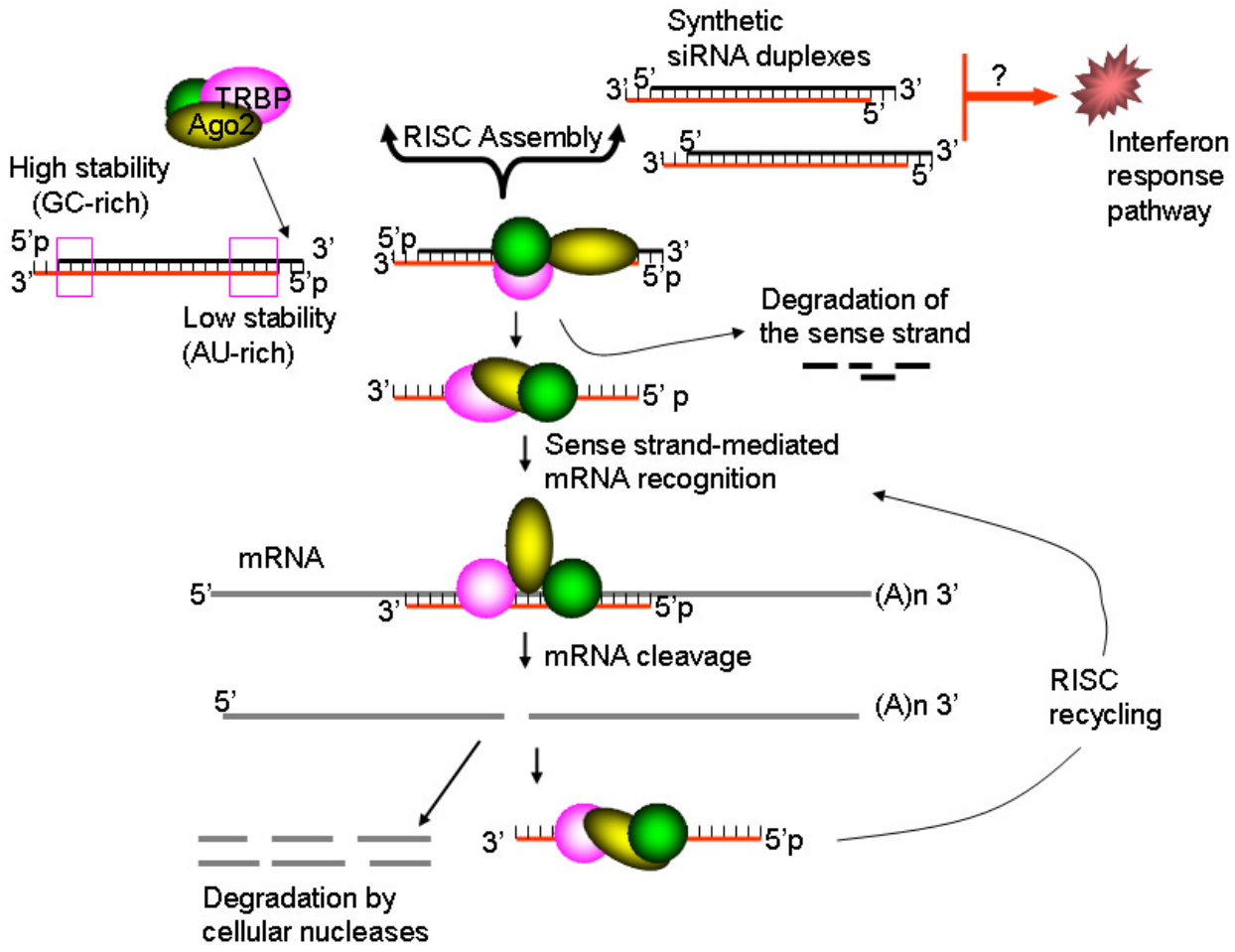

Figure 1. Schematic representation of gene silencing by siRNAs. In contrast to long double-stranded RNAs, siRNAs are directly loaded into a multi-protein complex termed RNA-induced silencing complex (RISC, where the sense strand with high 5'-stability) is cleaved by the nuclease AGO2. This will lead to strand separation. Subsequently, the RISC containing the antisense strand (guide strand) seeks out and binds to complementary mRNA sequences. Bound mRNA molecules are then cleaved by AGO2 and cleaved mRNA fragments are rapidly degraded by cellular nucleases. Following dissociation, the active RISC is able to recycle and cleave additional mRNA molecules.

Notably, one important feature of RNAi is the processing of long dsRNAs by an RNase III-like protein, known as Dicer, into 21-24 nucleotides (nt) ds siRNAs. Like all RNase III enzymes, Dicer leaves two nucleotide (nt) 3'overhangs and 5' phosphate groups. These siRNA duplexes are then incorporated into a multiprotein complex, the RNA-induced silencing complex (RISC). Subsequently, the antisense strand guides the RISC to recognize and cleave target mRNA sequences (9). The catalytic activity of RISC, which leads to the cleavage of target mRNAs at the site opposite to the 10^{th} and 11^{th} positions of the guide siRNA strand, is mediated by argonaute 2 (AGO2) protein, the only AGO family member that is cleavage competent (10, 11). Members of argonaute family protein are highly basic proteins containing two common domains, PAZ and PIWI domains. The PIWI domain is essential for interaction with Dicer and contains the nuclease activity that cleaves of target mRNAs. It should be noted that during RISC activation the passenger siRNA strand is also cleavage by AGO2, thus facilitating the formation of functional RISC complexes (12, 13). Interestingly, analysis of the crystal structure of a siRNA guide strand associated with PIWI domain showed that nucleotides 2–8 form a seed sequence that directs target mRNA recognition by RISC (14).

Although initial studies demonstrated that siRNAs are specific and small enough to evade the immune system, recent reports demonstrated that they can activate innate immunity by inducing cytokine and type I interferon expression (15-18). Additionally, some investigators showed that transcripts having some homology with a given siRNA can be downregulated by RNAi pathway, a phenomenon known as off-target effects (16). Although some of the off-target effects can be reduced using lower siRNA concentrations, the interferon response was observed even at low concentrations (8, 15). Therefore, there is a need to examine the immunostimulatory effects of any potential therapeutic siRNA in human immune cells prior to clinical applications. Additionally, it is important to

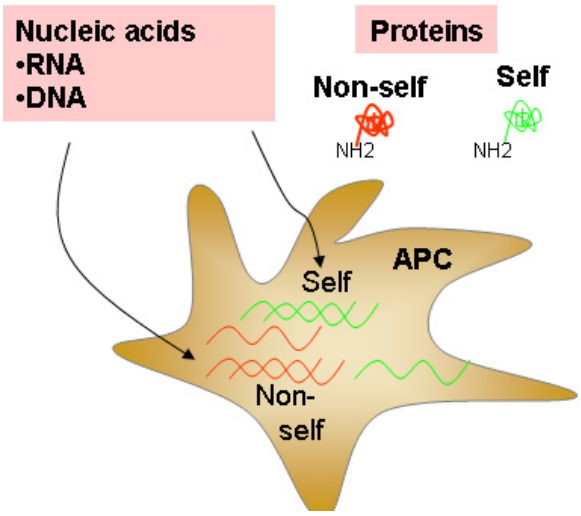

Figure 2. How antigen presenting cell (APC) discriminate between self and non-self nucleic acids or proteins? APC such as dendritic cells can capture antigens derived from self and non-self compounds with the same efficiency. Also, self and non-self nucleic acids can be sensed by the same receptors.

define the molecular basis for self and non-self discrimination of nucleic acids in order to develop strategies that facilitate the design of safe therapeutic siRNAs.

3. IMMUNE RECGNITION OF PATHOGENS

Immunity refers to the global ability of the host to resist the predation. The innate immune system provides the first line of protection against infection. Through a limited number of germline encoded PPRs, innate cells sense the presence of pathogens and respond specifically through the identification of conserved structures expressed by a large group of microorganisms, known as PAMPs (19, 20). PAMPs are unique to microorganisms such as lipopolysaccharide, peptidoglycan, capsular structures, bacterial flagellin, bacterial DNA, bacterial lipids, viral RNAs, and viral glycoproteins. Among PRRs, Toll-like receptors (TLRs) are crucial for pathogen-derived products and activation of innate and adaptive immunity (21, 22). They owe their name to the *Drosophila melanogaster* protein Toll, which controls the dorsal-ventral patterning of the fly embryo (23). In flies such as *Drosophila melanogaster,* the innate immune response to fungal and gram-positive bacterial infections is mostly under the control of the Toll signaling pathways (24). Their discovery in humans as sensors of microbial compounds has transformed the views of discrimination between self and non-self components, a key requirement of any immune system. TLRs expressed by vertebrates are type 1 transmembrane proteins bearing an intracellular Toll/IL-1R homology domain (25).

The conservation of Toll receptors in diverse organisms as humans and nematodes along with similarities in their signaling pathways shows that TLRs represent an early and successful response to detect microbes and initiated immune responses, leading to the specific activation of antigen presenting cells. To date, 13 members of the TLRs family have been identified and are expressed predominantly on cells of the immune system (25). TLR1-9 are common to humans and mice, while TLR10 appears to be functional only in humans and TLR11-13 are found only in the mouse. All TLRs contain extracellular leucine-rich repeat domains, which recognize pathogens and a cytoplasmic signaling domain known as Toll-interleukin receptor (TIR) domain, which links the recognition signal with intracellular signaling pathways. Upon ligation of TLRs with their cognate ligand, signaling cascades are activated resulting in the production of innate effector response that is responsible for the activation of adaptive immune response. With exception of TLR3, all TLRs recruit the adaptor protein MyD88, a cytoplasmic protein containing a TIR domain and a death domain. Upon activation, the TIR domain on the intracellular region of the TLR binds to MyD88 TIR domain, leading to the recruitment and activation of IRAK1 and IRAK4, and subsequently TRAF6 (25). This signaling pathway eventually leads to the activation of signal transduction cascades, including recruitment of adaptor molecules, tyrosine phosphorylation and activation of transcription factors that result in the expression of the host defense immune genes. Notably, the mitogen-activated protein kinases are activated downstream of TRAF leading to the production of proinflammatory cytokines such as TNF-α, IL6, IL-1β, and IL12.

Although normally present at the plasma membrane to detect extracellular pathogens, particularly bacterial products, TLR7, TLR8 and TLR9 are not expressed at the plasma membrane but, instead, sequestered in the intracellular compartments such as endosomes to sense viral components. A small fraction of TLR3 can be found in the endosomes. The intracellular localization of these receptors may prevent them from recognizing potentially dangerous self-ligands, which would trigger activation of the innate immunity in the absence of infection (see below). Despite our understanding of immune responses against foreign antigens, it is still unclear how an antigen presenting cell (APC) can differentiate between self and non-self antigens (Figure 2).

4. CYTOPLASMIC AND ENDOSOMAL SENSING OF VIRAL NUCLEIC ACIDS

The immune system has evolved cellular and molecular strategies to discriminate between foreign and self nucleic acids. Among the cytoplasmic sensors of long ds RNA, is the dsRNA-dependent protein kinase (PKR) that phosphorylates serine and threonine residues of target proteins (25). Most human cells constitutively express a low level of PKR that remain inactive. Upon binding to dsRNA, PKR forms a homodimer leading to its autophosphorylation and activation. Activated PKR phosphorylates the translation initiation factor elF-2α causing the inhibition of protein synthesis, an essential step in antiviral resistance (25). Also, PKR can phosphorylate

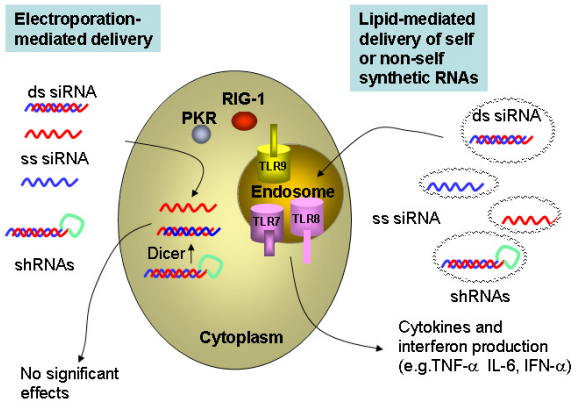

Figure 3. Immune response to self and non-self chemically made RNAs. Lipid delivery of immunostimulatory siRNAs (self or non-self sequences) activates innate immunity through endosomal TLR7/8, leading to cytokines and type I interferon production. The delivery of the same siRNA sequences into the cytoplasm through electroporation did not induce cytokine production (35, 38).

IKK-β, leading to the activation of NF-κB signaling pathway. It should be noted that PKR's binding to ds RNA is sequence-independent and the presence of interferon upregulates PKR expression. Although as little as one helical turn of dsRNA (about 11 bp) has been shown to interact and activate PKR, optimal activation requires at least 30-80 bp dsRNA (26).

A second protein that is stimulated by dsRNA is 2'-5' oligoadenylate synthetase (OAS), which is expressed constitutively and upregulated through IFN-α and -β signaling during antiviral responses (27). OAS activates the endoribonuclease RNAase L through the synthesis of short oligoadenylates leading to the cleavage of both cellular and viral RNAs. Although both OAS and PKR are implicated in antiviral immunity, PKR and RNAse L are mainly IFN effectors and not absolutely required for IFN production. Therefore, other kinases may be involved. More recently two additional intracellular helicases, retinoid-acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5), that sense viral dsRNA were identified (28). These helicases are widely expressed in inactive form and like other antiviral protein; they are upregulated by IFN □/□. RIG-I encodes a caspase recruitment domain (CARD) at the N terminus, in addition to an RNA helicase domain. The RNA helicase domain requires ATPase activity and is responsible for viral dsRNA recognition and binding, resulting in a conformational change that exposes the CARD domain that interacts with another CARD-containing adaptor protein, known as IPS-1, MAVS, Cardif or VISA. (28). IPS-1 is an outer mitochondrial membrane binding protein. IPS-1 activates IRF3 and IFR-7 through TBK1/IKKi. This signaling pathway leads to the activation of NF-kB and interferon regulatory factor 3 that trigger IFN-β production (29). Mitochondrial retention of IPS-1 is essential for IRF-3, IRF-7 and NF-kB activation by RIG-1 (29).

In addition to cytoplasmic sensors, recent studies have also shown that TLRs are crucial in sensing viral and bacterial nucleic acids (22). It should be noted that the subcellular localization of TLRs correlates with the nature of their ligands, rather than their sequence similarity. Whereas most TLRs are expressed in the plasma membrane for detecting bacterial components, TLR3, TLR7, TLR8 and TLR9, are expressed in intracellular compartments (endosomes, lysosomes) (21). The immune function of this cellular localization is to sense viral RNAs. TLR3 is also expressed on the cell surface and it is believed to recognize viral double-stranded RNA released during cell lysis (30). TLR7 and TLR8 recognize viral single-stranded RNA (ssRNA) and small synthetic antiviral compounds referred to as imidazoquinolines (31). TLR9 recognizes unmethylated CpG-DNA motifs, which are frequently found in bacterial DNA (32). The presence of CpG dinucleotides is suppressed in the vertebrate genomes and, when present, the cytosine is usually methylated. However, the structural differences of eukaryotic versus prokaryotic DNA are presumably not the only mechanism for distinguishing self from non-self nucleic acids. Indeed, the sequestration of self-DNA in the nucleus and TLR9 in the intracellular compartments (e.g. endosomes) should provide an additional safety mechanism for avoiding contact with each others. The sequestration of TLR7 and TLR8 in the endosomes, most likely limit their reactivity to self nucleic acids (22). However, despite these tolerance mechanisms, endosomal TLRs seem to play an important role in the induction and/or perpetuation of autoimmune diseases (33). It should be noted that intracellular NOD-like receptors detect bacteria, whereas viruses are detected by Toll-like receptors (which also recognize bacteria, fungi, and protozoa) and RIG-like receptors. The virus-detecting Toll-like receptors operate mainly in plasmacytoid dendritic cells by responding to viral nucleic acids that have been ingested by the cell through phagocytosis and incorporated into endosomal compartments. In these cells, the major immune response is production of type 1 interferon (34).

5. TLR7/8 ARE THE MAIN SENSOR OF SIRNAS

As mentioned above, siRNAs have emerged as a powerful experimental tool to study gene functions. Although siRNAs were initially thought to be small enough to avoid the activation of the IFN pathway (7), we and others have shown that they could activate innate innate immunity in mammalian cells (35-38). Initial studies indicated sequence-independent activation of PKR and TLR3 signaling pathways by siRNAs (18, 39). However, recent studies demonstrated that PKR and TLR3 do not represent the major pathways by which chemically synthesized siRNAs activate immunity (35-38). Indeed, certain siRNA sequences stimulated monocytes via TLR8 or dendritic cells via TLR7 to produce proinflammatory cytokines and large amounts of interferon α, respectively (Figure 3). Also, the data indicate that a high concentration of self-RNA (sense strands) or non-self RNA (antisense strands) within the endosomes does not necessarily activate TLR signaling because the effects were found to be sequence-dependent (35). This response is mainly mediated

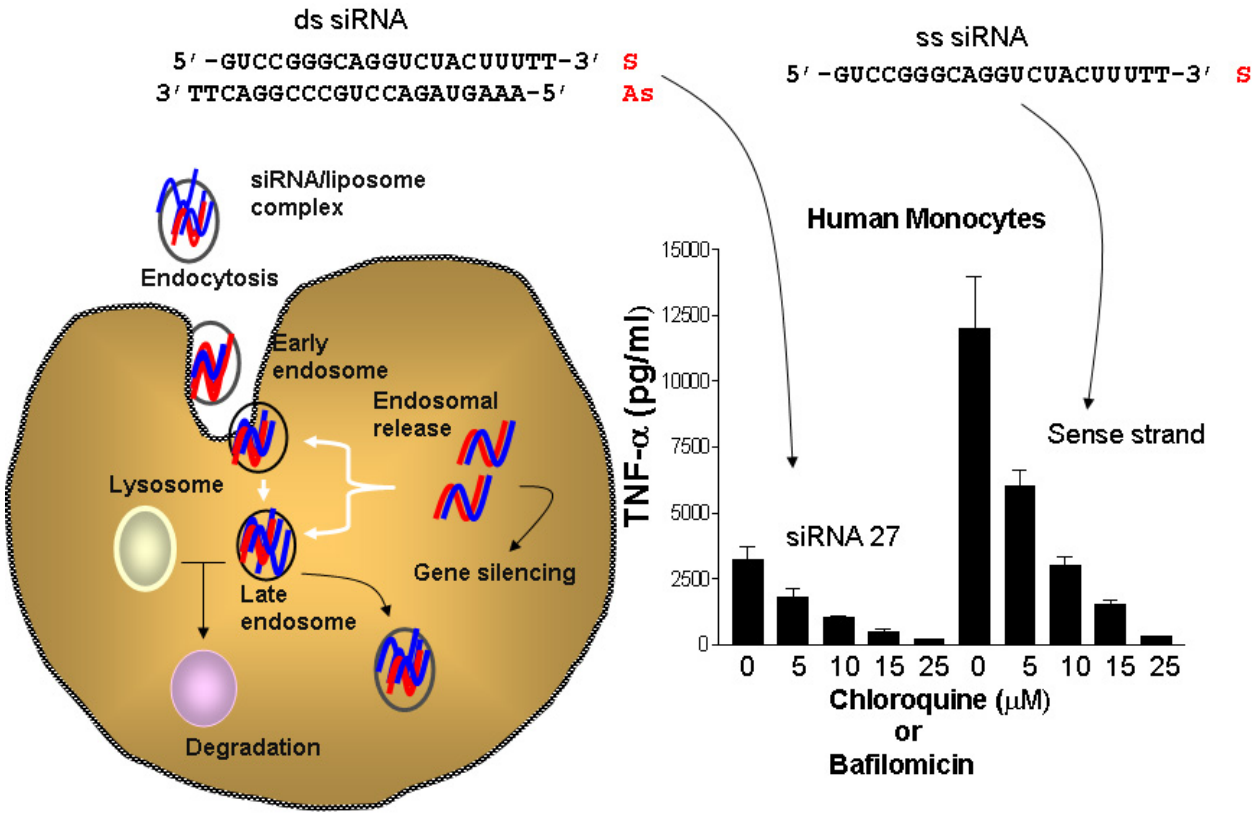

Figure 4. Inhibition of endosome maturation using chloroquine or bafilomycin blocks immune activation by immunostimulatory ss siRNAs and dsRNAs.

through TLR7 in mice and TLR7/8 in humans. Indeed, TLR7 knockout mice did not mount immune activation in response to siRNAs (36). We also found that ss siRNAs are more effective than ds siRNA in triggering TLR7 and TLR8 responses (35, 38). Altogether, these findings highlight the importance of analyzing the immunostimulatory potential of siRNAs prior to clinical applications. For each target gene, it is therefore wise to screen for several siRNA sequences.

6. TLR7/ TLR8 RECOGNITION RNA MOTIFS

TLR7 and TLR8 recognize certain siRNA sequences, provided they are delivered to the endosomes. Thus, what is the nature of IFN-inducing motif present in one sequence but absent in another? Initial experiments indicate that some types of secondary structures and/or specific nucleotides are responsible for the activation of NF-κB signaling pathway by siRNAs in human monocytes (15). Monocytes are circulating peripheral blood cells that can be differentiated by cytokines into macrophages of different phenotypes as well as into dendritic cells. As mentioned above, siRNA effects are sequence-dependent and can occur with ds siRNAs and ss siRNAs (35). Judge *et al.* found that the 5'-UGUGU-3' motif was indispensable for the immune activation by a siRNA in human blood cells (37). However, Hornung *et al.* identified a 9 nt motif RNA motif (5'-GUCCUUCAA-3') that is recognized by TLR7 in the context of siRNA duplexes and the activity does not depend on GU content (36). However, our studies indicated that interferon induction by siRNAs cannot be easily suppressed by selecting siRNA sequences without the GU dinucleotides (35). Indeed, several siRNA sequences without GU induced TNF-α production in human PBMC and monocytes. Although the precise nature of the RNA motifs responsible of innate immune activation is not known, we found that the ability of siRNAs to activate TNF-α production is largely dependent on the uridine content because the replacement of uridines with adenosines abrogated immune activation (38).

7. OVERCOMING SiRNA IMMUNE ACTIVATION

Considering the high frequency of uridines in messenger RNAs it is more likely that a high proportion of siRNAs will activate innate immunity. Therefore, it would be desirable to develop strategies that evade immune activation. At least 3 distinct ways to avoid immune activation by siRNAs. The first would be to use delivery agents that avoid the delivery and/or retention of siRNA within the endosomes. Indeed, inhibition of endosomal maturation and acidification blocked both single-and double-stranded siRNA-induced TNF-α production (Figure 4).

Song and colleagues showed that an antibody-based delivery strategy can result in gene silencing without

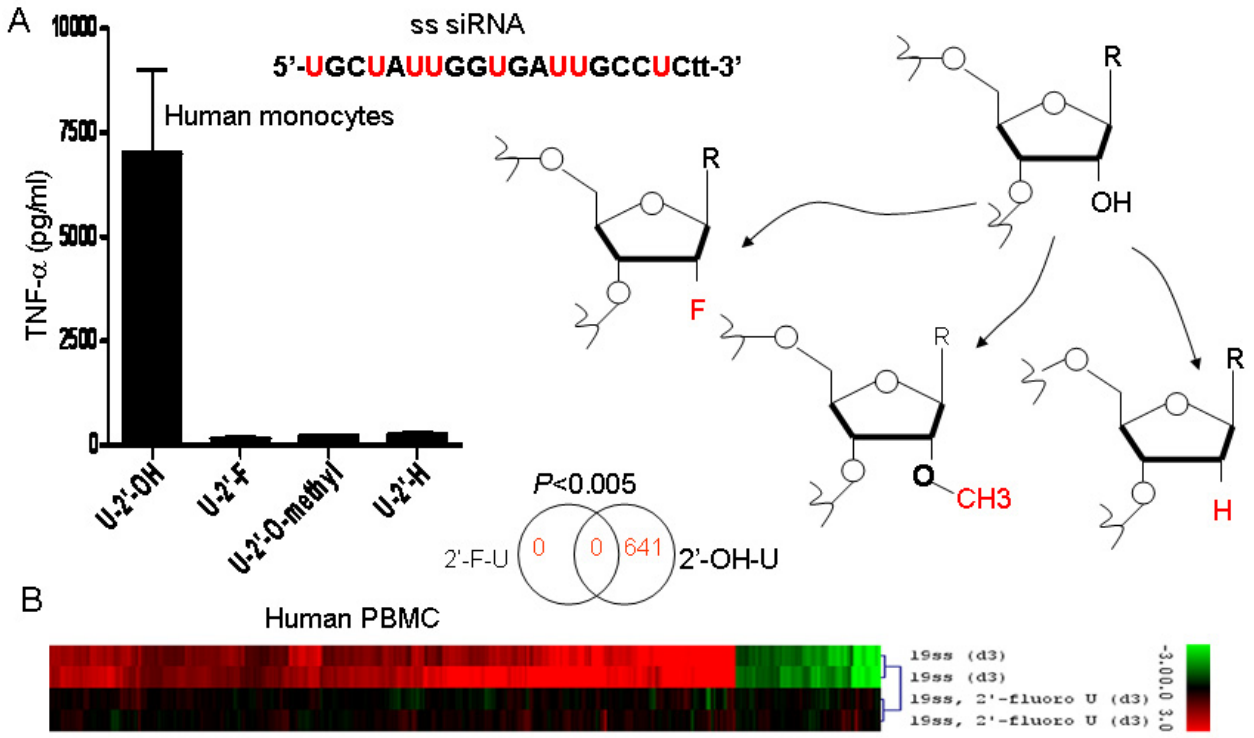

Figure 5. Modified RNAs evade TLR recognition. A) Unmodified single- or double-stranded siRNAs activate innate immunity, whereas their 2'-uridine modified counterparts did not. B) Analysis of global gene expression in response to unmodified and 2'-fluoro uridine modified ss siRNA (38, 43).

immune activation (40). The second way relies on the use of modified nucleotides that are essential to protect RNA from degradation by nucleases *in vivo* (41). However, the chemical modifications that block immune activation must be chosen carefully so as not to inhibit siRNA silencing potency. Thus, finding the appropriate chemical modifications for blocking siRNA immune activation will be important for exploring their therapeutic applications. In this respect, the incorporation of various 2'-modified nucleotides in siRNA sequences abrogated their immunostimulatory potency (42). However, the chemical modifications that block immune activation must be chosen carefully so as not to inhibit siRNA silencing activity. Thus, finding the appropriate chemical modifications for inhibiting siRNA immune activation will be important for exploring their therapeutic applications. Fortunately, we have shown that replacement of only uridines with their 2'-fluoro, 2'-deoxy, or 2'-*O*-methyl modified counterparts can abrogate immune recognition of siRNAs by TLRs without reducing their silencing potency (38, Figure 5). These findings have subsequently confirmed by gene expression analysis using microarray technology (43). Interestingly, TLR 7/8 ligands such as immunostimulatory siRNAs induced the expression of a large number of genes that are involved in both innate and adaptive immunity. Furthermore, several set of genes involved in different pathways such as antigen presentation, cell motility, and endosome maturation are induced (Figure 6). For example, the genes encoding for V1 ATPase H subunit and p38 mitogen-activated protein kinase are upregulated. This would indicate that endosomal maturation can be controlled by RNA-induced TLR7/8 signaling. In the presence of exogenous antigens, endosome acidification and maturation are expected to enhance the presentation of antigens in the context of MHC class II that result in the activation of CD4+ T cells. A fraction of endocytosed exogenous antigens escape from early endosomes and enter the MHC class I presentation pathway, a process known as cross-presentation (Figure 7).

Regarding chemical modifications, Judge and colleagues also demonstrated that the incorporation of 2'-*O*-methyl-uridine or 2'-*O*-mehtyl guanosine residues into siRNAs can abrogate their immunostimulatory potency (44). Collectively, the available data offer the possibility of choosing the appropriate chemical modifications that evade immune activation without reducing siRNA-silencing activity. When designing chemically modified siRNAs, it is also important to consider some rules in order to avoid the inhibition of siRNA cleavage activity. First, the 5' end of the sense strand must have a free hydroxyl or phosphate group. Second, the 5' end of the sense strand can be modified in order to block its incorporation into the RISC. Third, the 3' ends of the sense and antisense strands can be modified with any fluorochrome. These 3'- end modifications would facilitate the examination of siRNA uptake and imaging.

The finding that 2'-modified RNAs can evade immune activation suggest that naturally modified RNAs

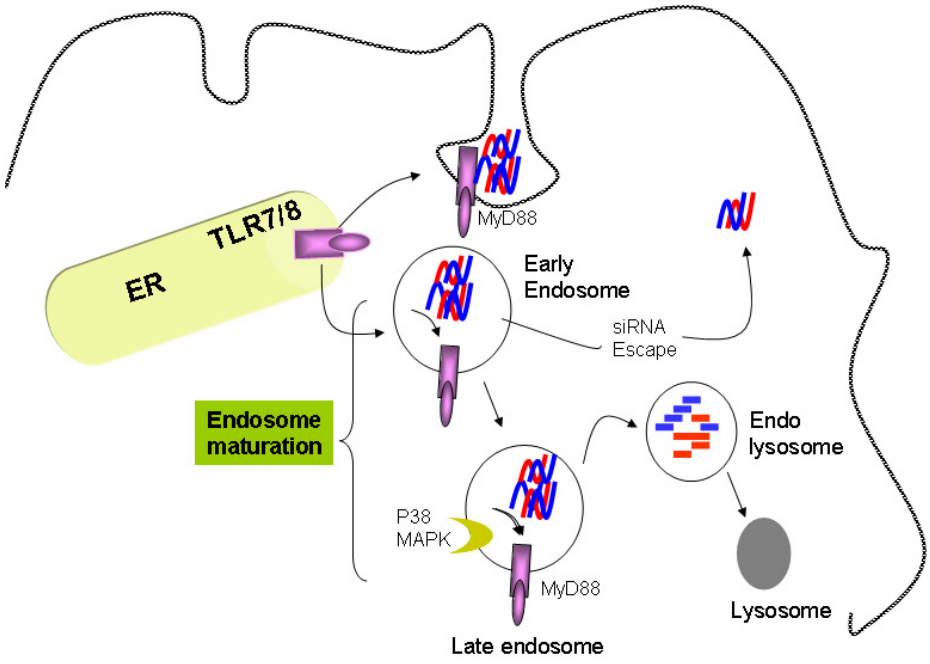

Figure 6. Liposome-formulated immunostimulatory siRNAs induce TLR7/8 signaling and endosomal maturation. Both processes are important for DC maturation, antigen processing, and presentation (35, 38).

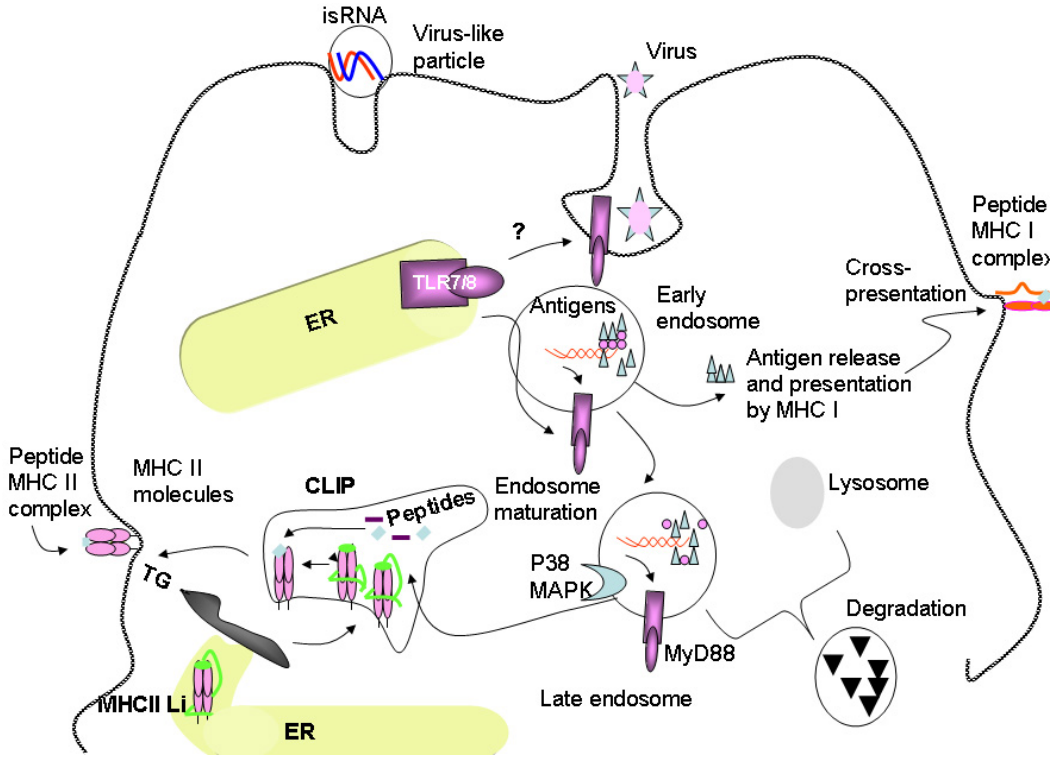

Figure 7. Subsequent to cell entry by receptor-mediated endocytosis, viral envelope proteins are suppose to be degraded by endosomal proteases leading to the release of viral nucleic acids that activate endosomal TLRs. Signaling via TLR7/8 or TLR9 is expected to induce endosome maturation and partial proteolytic degradation of the viral proteins to generate peptides that bind to nascent MHC molecules that are transported to the cell surface. A fraction of the captured antigens might enter the cytoplasm and join the class I antigen presentation pathway.

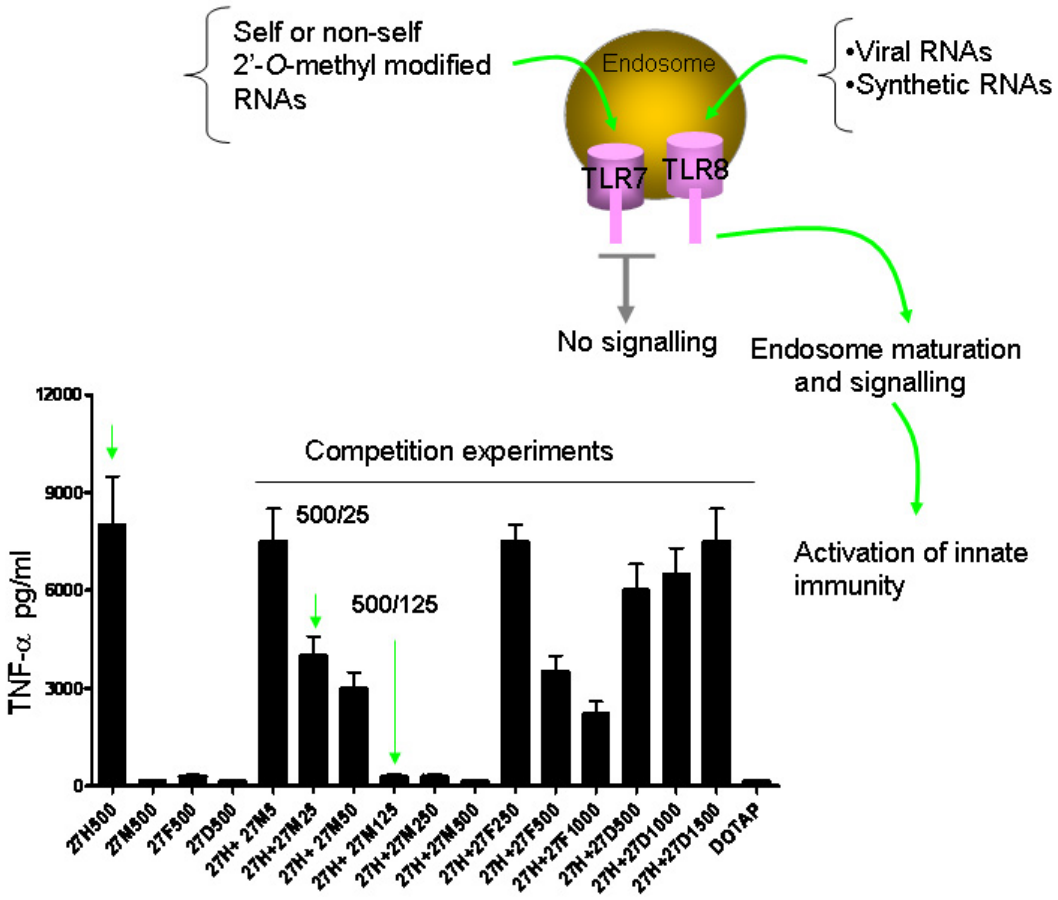

Figure 8. Discrimination between self and non-self RNAs at the signaling stage. A) Schematic representation of TLR7/8 recognition of RNAs. B) TNF-α production in human monocytes in response to unmodified ss siRNA 27 (27H) either alone or with combination with various concentration of 2'-*O*-methyl (27M), 2'-fluoro (27F) or 2'-deoxy (27D). The numbers indicate the concentration in ng/ml (47).

are not sensed by TLRs. Support of this view has been provided by Karikó and colleagues, who demonstrated that modifications that are frequently found in mammalian RNA (such as pseudouridine, 5'-methylcytidine, 2'-*O*-methyl) can interfere with the capacity of RNA to activate TLR-7 in human cells (45). Thus, unmodified RNAs corresponding to mammalian sequences would be expected to activate TLR7 more effectively than native RNAs provided they are delivered to the endosomes (35).

8. ENDOSOMAL ACIDIFICATION BLOCKS IMMUNE ACTIVATION BUT NOT GENE SILENCING

In light of the finding that endosomal maturation is a precondition for either double-stranded or single-stranded siRNA-based activation of the immune system (35), one could use inhibitors of endosome maturation/acidification to block immune activation (see Figure 4). Because chloroquine and bafilomycin A1 blocked the immunological activity of siRNAs (35), we have investigated whether the RNAi pathway is active in chloroquine-treated human cells. The data showed that the silencing activity of siRNAs was not inhibited, even enhanced, in the presence chloroquine (35). Similar results were obtained with bafilomycin A1. Therefore, the combination of low concentrations of chloroquine or bafilomycin A1 with siRNAs would facilitate basic and clinical applications of siRNAs, particularly the use of siRNA in functional genomics and drug-target validation (46).

9. SUPPRESSIVE 2'-MODIFIED RNAs

The finding that unmodified, but not 2'-modified RNA, are potent triggers of RNAi raised questions about the differences in their structures that might be relevant to binding to TLRs. So, which steps are affected by 2'-modifications, and why can't 2'-modified RNAs trigger immune activation? One way to address the first question is to assess whether 2'-modified RNAs antagonize with immunostimulatory RNAs to trigger TLR7/8 signaling. Studies of transfected human monocytes show that 2'-*O*-methyl modified RNAs abrogates the activation of TLRs by immunostimulatory RNAs (46). Of considerable interest, is that 2'-*O*-methyl modified RNAs suppressed immune activation at very low concentrations (47), Figure 8). In

addition, we have shown that they can effectively inhibit immune activation by a variety of immunostimulatory RNA sequences, including bacterial and mitochondrial RNAs (47). Interestingly, chemically modified RNA can antagonize with immunostimulatory RNAs to activate IDO, an immunosuppressive factor, in human monocytes (48). In accordance with our data, Robbins and colleagues have reported that 2'-modified immunostimulatory RNAs can function as TLR7/8 antagonist by inhibiting TLR7 activation by imunostimulatory RNA and loxoribine in both murine and human cells (49). Also, 2'-modified RNA inhibited R848-induced TNF production in human monocytes and PBMC (Furset & Sioud, unpublished data). Altogether, these studies would indicate that natural 2'-modifications in host RNAs not only evade immune activation as suggested by Karikó and colleagues (39), but also can suppress the activation of TLRs triggered by other immunostimulatory RNAs derived from host cells (Figure 8). Suppressive 2'-modified RNAs should represent a new class of TLR antagonists that may be useful in the treatment of inflammatory diseases. Further analysis of our microarray data using pathway analysis tools gave insights into several pathways that are activated by TLR7/8 signaling in human PBMC and purified monocytes. These networks were enriched in genes involved in endosome maturation, apoptosis, and anti-bacterial and antiviral immune responses. Interestingly, re-stimulation of human PBMC with immunostimulatory RNAs after a 24 hours stimulation period induced the expression a set of genes that are involved in apoptosis and negative regulation of TLR signaling, bacterial and viral replication, whereas the genes encoding for proinflammatory cytokines (e.g. TNF-α and IL-6) were less induced than during the first stimulation. This observation indicates that during persistent infections or repeated vaccination, gene expression might not be equally primed during the infection or vaccination schedule (Sioud M, unpublished data). Understanding the nature of mechanisms that regulate this differential innate immune response will be important for the application of immunostimulatory RNAs as a vaccine adjuvants.

10. STRUCTURAL BASIS OF siRNA AND miRNA EVASION OF RIG-I, A RNA HELICASE

In addition to the demonstration that endosomal TLR are required for siRNA-sensing by the immune system, our data indicate that PKR and RIG-I are neither necessary nor sufficient for siRNA-induced immune activation. Indeed, cytoplasmic delivery of either Single- or double-stranded siRNAs through electroporation did not induce cytokine production, however; when the same sequences were delivered to the endosomes thought lipid they did (35, Figure 3). Despite our understanding of self and non-self discrimination, it remains unknown how unmodified synthetic RNAs and endogenously expressed microRNAs (miRNAs) evade immune recognition by PKR and RIG.I. Notably, endogenous long ds RNA can derive from various sources such as simultaneous sense and antisense transcription of specific genomic loci or viral replication intermediates. However, the predominant form of naturally occurring ds RNA in mammalian cells is derived from endogenously expressed miRNAs that constitute a large class of noncoding small RNAs involved in gene regulation in a variety of organisms ranging from plants to mammalians (50). Presently, more than 1000 potential human miRNAs have been identified and numerous have been experimentally validated. Usually miRNAs are transcribed from endogenous genes by RNA polymerase II as long RNA precursor called a primary miRNA (pri-miRNA), containing one or more distinct miRNAs. In the nucleus the RNA precursors are processed by Drosha to 60-80 nt RNA hairpin intermediate, bearing 2nt 3' overhang, called a pre-miRNA. Interestingly, the Drosha cleavage site was shown to be 11 base pairs from the stem single-stranded RNA junction (51). Processed pre-miRNAs are then transported from the nucleus to the cytoplasm by exportin-5, where its 2 nt 3'-overhang is recognized by Dicer, which generates the mature miRNA of approximate 22 nt length (50).

Recently, long ds RNA (27-30 nt) have shown to induce RNAi more efficiently than 21 nt siRNAs because they enter the RNAi pathway in a Dicer-dependent fashion (52). However, it was not clear why these dsRNAs did not activate innate immunity, in particular RIG-I. A Further study showed that siRNAs with blunt ends activated interferon response genes whereas siRNAs with 2 base 3'-overhangs did not (53). The authors showed that RIG-I can recognize siRNAs with or without 2-base 3'-overhangs, but only those with blunt ends could trigger its ATPase activity and subsequent downstream signaling pathways (53) Similarly, endogenously expressed shRNAs (substrate for Dicer) are not recognized by the cytoplasmic sensors of RNAs (54). The reported data support the notion that miRNA and shRNAs are protected by the presence of 2 base 3' overhangs (55).

During our studies we have also found that synthetic single stranded RNAs (21 nt) do not activate innate immunity when delivered to the cytoplasm via electroporation (Figure 3). These single-stranded RNAs do not contain 2 base 3' overhang. Thus, why chemically made single-stranded RNAs are not sensed RIG-I and other cytoplasmic RNA sensors? To examine the potential contribution of RIG-1 in sensing viral RNAs, we have transfected adherent PBMCs with either T7-transcribed or chemically synthesized single stranded RNAs (56). Interestingly, the inhibition of endosome maturation by chloroquine abrogated the immunostimulatory activity of chemically made RNAs, but not the T-7 made RNAs. In addition, the immunostimulatory effect of the T7-made RNAs was not inhibited with 2-aminopurine, a specific inhibitor of PKR (56). So, which cytoplasmic protein(s) sense *in vitro* transcribed RNA that is characterized by the presence 5'-triphosphate? Recent studies indicate that RIG-I senses single-stranded RNA-bearing 5'-triphosphate (57). Therefore, the cytoplasmic presence of RNA containing accessible 5'-triphosphate constitutes a viral "pathogen-associated molecular pattern" responsible for immune discrimination between self- and viral RNA. In contrast to viral RNAs, most self-RNAs undergo several modifications to eliminate or protect the 5'-triphosphate from being recognized by RIG-I. However, the reported data do not

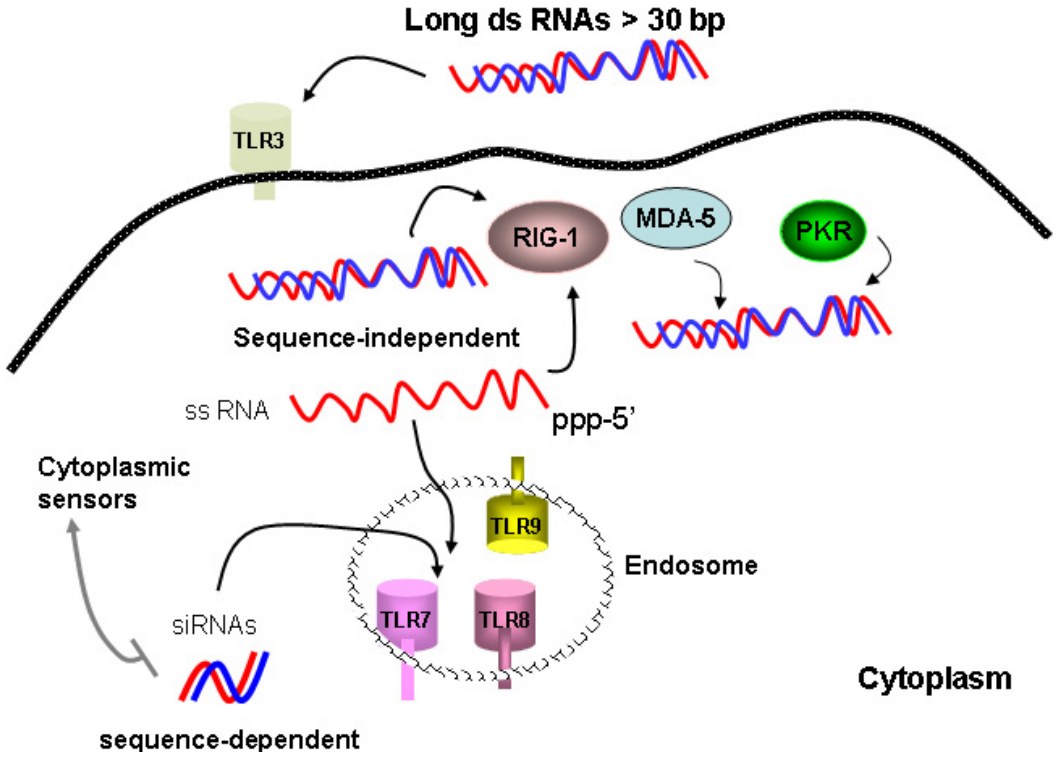

Figure 9. An overview of immune sensing of single- and double-stranded RNAs in human cells. For detail see the text.

explain why certain endogenous RNA with 5'-triphosphate escape RIG-I recognition. The naturally occurring 2'-ribose modifications might block RIG-1 activity. It should be noted that MDA5, the most closely related protein of RIG-I, is also an IFN-inducible protein that are involved in sensing ds RNA structures. On the basis of the recent studies dealing with immune sensing of RNAs, we can conclude that viral dsRNA and single stranded RNAs are mainly recognized by RIG-1 helicase. Viral ss RNAs are also recognized by TLR7/8, but in a sequence-independent manner. In contrast, synthetic small RNAs such as siRNAs are recognized by TLR7/8 in a sequence-dependent manner. Exogenous viral ds RNA derived from infected cells are sensed by TLR3 that is localized on the cell surface as well as the endosomes (Figure 9). It should be noted that RIG-I and MDA5 recognize distinct sets of RNA viruses and their function might be cell-dependent.

11. EFFECTS OF CHEMICAL MODIFICATIONS ON SiRNA OFF-TARGET EFFECTS

siRNA-mediated mRNA downregulation was initially reported to be highly specific (7). However, recent studies have shown that siRNAs with only partial complementarily to mRNAs can also cause a reduction in the RNA levels of a large number of transcripts. Another potential source of siRNA toxicity is therefore the destruction of cellular mRNAs that share partial homology to the siRNA sequences (17). Because the cellular pathways activated by miRNAs and siRNAs are similar, it is more likely that each siRNA sequence will exhibit a miRNA activity (50). The most commonly used strategy to ensure siRNA target specificity is the basic local alignment search tool BLAST. However, short sequence stretches may not be detected by BLAST program. In addition, the identification of such sequences does not necessarily indicate the occurrence of off-target effects. Similarly, the absence of short homologies will not rule out off-target effects. The best way to deal with this problem is to analyze global gene expression, specifically when siRNAs are going to be used in functional genomics or to develop therapeutics. During our studies with siRNAs, we have found that 2'-uridine modifications of siRNAs not only evade immune activation but they can reduce ss siRNAs and ds siRNA off-target effects (43). Although the evading mechanism remains to be investigated, it is probable that the interaction of ss siRNAs or ds siRNAs with unintended cellular mRNAs is affected by chemical modifications. In accordance with our observations, Jackson and colleagues found that the incorporation of 2'-*O*-methyl group at the second position of the guide strand reduced most off-target-gene silencing without significantly affecting silencing of the intended targets (58). Collectively, these studies offer a simple strategy for reducing off-target effects.

12. IMMUNE MODULATION OF DC FUNCTION BY BIFUNCTIONAL siRNAs

Among the innate immune sensors that link innate and adaptive immunity, dendritic cells (DCs) play a crucial role in immune responses and are the only cell type capable of initiating adaptive immune responses by activating naïve T cells (59, 60). DCs can be divided into two broad types: those that reside in the peripheral tissues

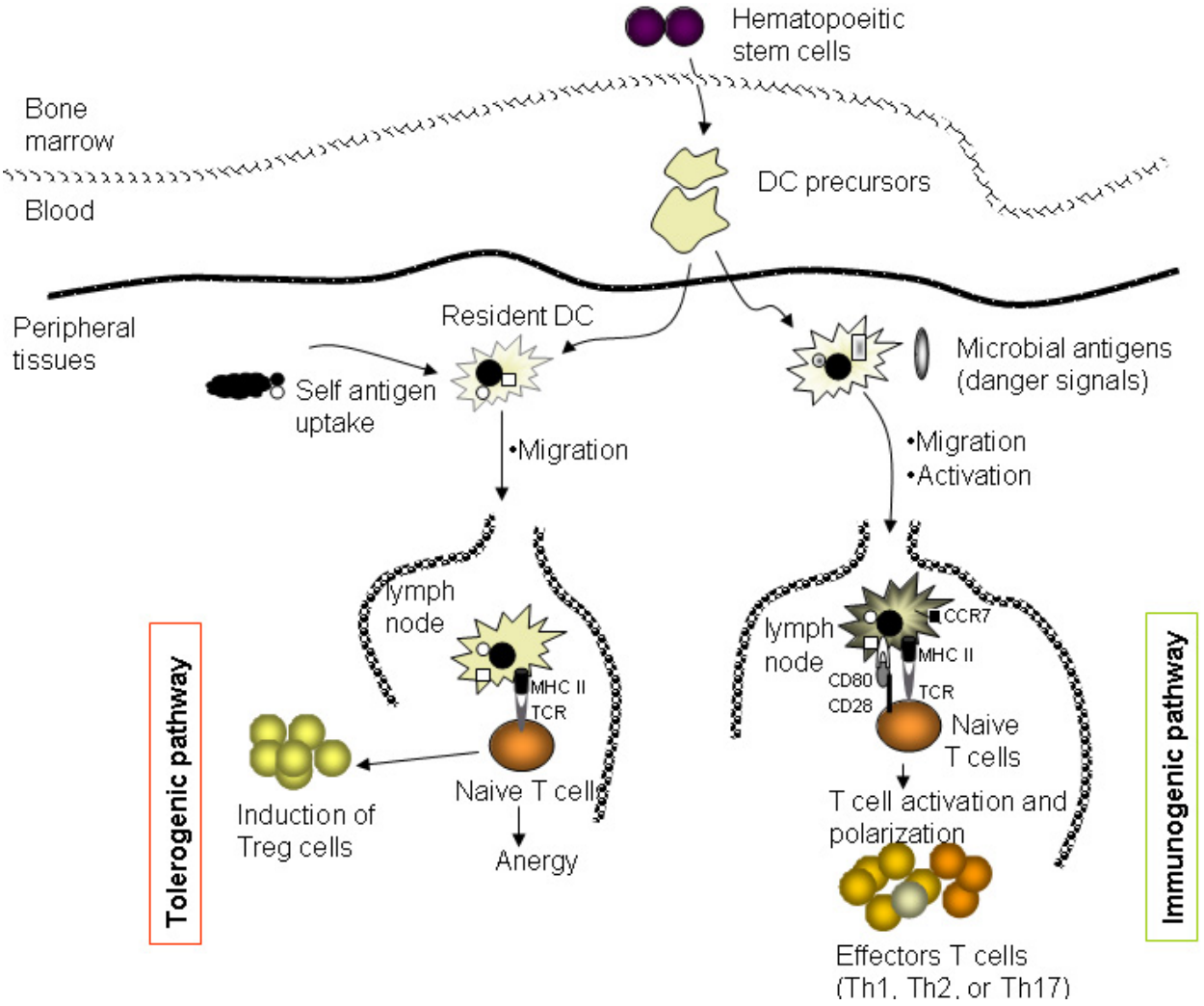

Figure 10. Schematic representation of dendritic cell activation. Interaction of DCs with microbes (danger signals) leads to their maturation and homing to the lymph nodes where they activate naïve T cells (immunogenic pathway). In the absence of danger signals, DCs induced tolerance to self-antigens (telorogenic pathway).

such as mucosa, skin, and internal organs (tissue DCs) and those that reside in the blood and lymphoid tissues (blood DCs). Both immunity and tolerance are controlled by DCs (Figure 10). In the absence of "danger signals" immature DCs mediate peripheral tolerance, leading to T cell anergy or deletion due to the absence of appropriate costimulation by CD80/CD86 molecules (60). TLR signaling induces the maturation of DCs, a process that entails upregulation of major histocompatibility complex class I and class II molecules, costimulatory molecules such as CD40, CD80 and CD86, and the production of IL12. Unlike pathogens, tumors do not induce an effective inflammatory response leading to DC activation. DC maturation with cytokines without endosomal maturation may induce tolerance rather than T cell priming. Furthermore, tumor microenvironment can protect tumor cells from immune destruction. Indeed, soluble immunosuppressive factors, and membrane-bound molecules such as transforming growth factor β, interleukin IL10, prostaglandin E2, and CTLA-4 represent a barrier for antitumour immunity (61). Interfering with the expression of these immunosuppressive factors might potentiate antitumour T cell effector function *in vivo* (52).

As indicated above, pathogen-mediated maturation of DCs is mediated mainly through the TLRs that are expressed on immature DCs. Optimal DC maturation might therefore require a combination of both cytokines and TLR ligands. Previously, we have shown that stimulation of DC with immunostimulatory siRNAs induce their maturation to secrete cytokines, including IL6 and IL12 (35). While IL12 is required for Th-1 type response, IL6 renders CD4+ effector T cells refractory to T reg cell-mediated suppression. To extend these findings, we have tested the possibility of designing bifunctional siRNAs capable of triggering TLR signaling and simultaneously blocking the expression of immunosuppressive factors (56). The inappropriate expression of immunosuppressive cytokines and other negative regulators is expected to

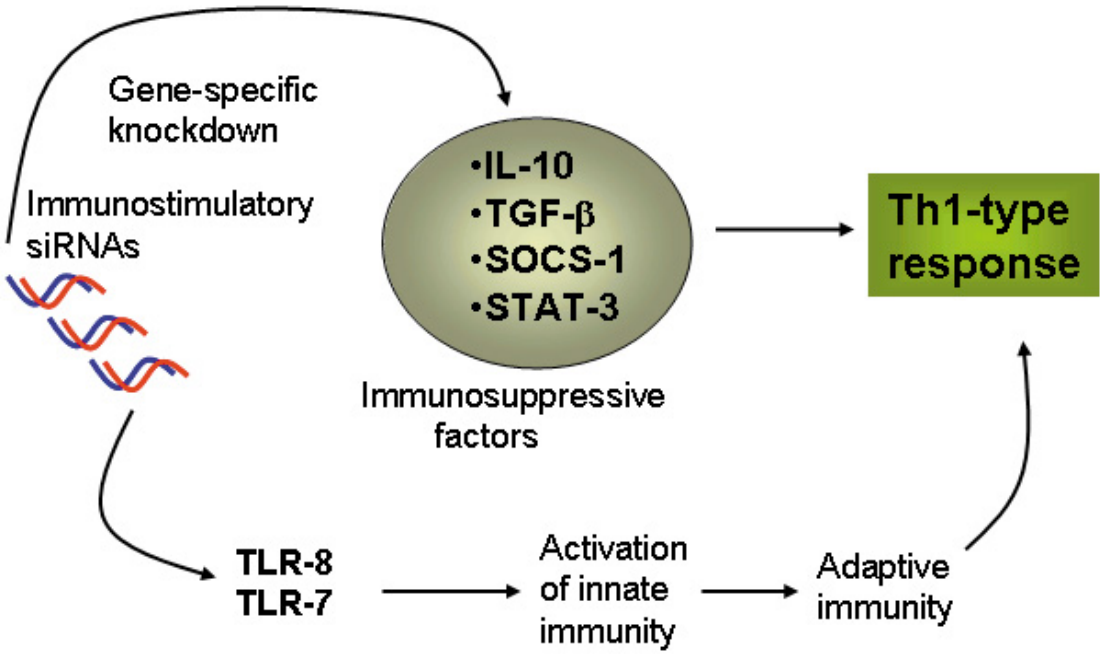

Figure 11. Potential effects of bifunctional siRNAs on the immune system. By targeting factors involved in immune suppression such as IL10, TGF-β, and SOCS-1 by immunostimulatory siRNAs, one may improve immune response to cancer cells by activating innate immunity that initiate the activation of adaptive immunity

hamper immunity against tumors and virus-infected cells (62). Therefore, the development of agents that stimulate DCs and subsequently suppress the expression of negative regulators, such as IL10, TGF-β and SOCS proteins would facilitate the development of effective cancer vaccines. In a recent study, we have assessed the possibility of combining gene-silencing and immunostimulation in one siRNA molecule (Figure 11). Immature monocyte-derived dendritic cells incubated with anti-IL10 siRNAs produced cytokines (e.g. IL6, TNF-α, IL12), upregulated the expression of the costimulatory (e.g. CD80, CD86), MHC class II molecules, and the chemokine receptors CCR7. Also, IL10 siRNAs enhanced the ability of DCs to activate T cells in MLR assays (60). Thus, the possibility of triggering endogenous IL12 and IL6 production through deliberate activation of TLR7 or TLR8 pathway via IL-10 siRNAs is of considerable interest. In addition to IL10, bifunctional siRNAs against other key factors involved in immune suppression were developed. These include SOCS1, STAT-3, and TGF-β.

The SOCS proteins have been identified as inhibitors of cytokines signaling and shown to function in a classical feedback loop (63). These proteins can be induced by cytokines (e.g. IL6, Il-2, TNF-α, IL-1) and by innate immune stimulatory factors (e.g. LPS). They regulate signaling via the Jak/Stat pathway and have been shown to modulate DC function by switching off IFN-γ and/or IL12 signaling during immune response. By targeting SOCS1 with conventional siRNAs in DCs, Chen and colleagues demonstrated that antigen-specific antitumor immunity can be enhanced (64). A major challenge in developing dendritic cell-based cancer vaccines is the *in vitro* generation of immunopotent dendritic cells, namely dendritic cells that, when loaded with tumor antigens and injected into patients, will home to the draining lymph node and activate cognate T cells. Bifunctional siRNAs are expected to enhance DC function.

13. ACKNOWLEDGMENTS.

I thank Drs Anne Dybwad and Sébastien Wälchli for critical reading of the manuscript.

14. RERERENCES

1. Jorgensen, R.: Altered gene expression in plants due to trans interactions between homologous genes. *Trends Biotechnol*, 8, 340-4 (1990)
2. Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver & C. C. Mello: Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*, 391, 806-11 (1998)
3. Svoboda, P., P. Stein, H. Hayashi & R. M. Schultz: Selective reduction of dormant maternal mRNAs in mouse oocytes by RNA interference. *Development*, 127, 4147-56 (2000)
4. Sen, G. C.: Viruses and interferons. *Annu Rev Microbiol*, 55, 255-81 (2001)
5. Zamore, P. D., T. Tuschl, P. A. Sharp & D. P. Bartel: RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell*, 101, 25-33 (2000)
6. Elbashir, S. M., W. Lendeckel & T. Tuschl: RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev*, 15, 188-200 (2001)

7. Elbashir, S. M., J. Harborth, W. Lendeckel, A. Yalcin, K. Weber & T. Tuschl: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, 411, 494-8 (2001)
8. Sioud, M: Therapeutic siRNAs. *Trends Pharmacol Sci.* 25, 22-28 (2004)
9. Hannon, G. J. & J. J. Rossi: Unlocking the potential of the human genome with RNA interference. *Nature*, 431, 371-8 (2004)
10. Liu, J., M. A. Carmell, F. V. Rivas, C. G. Marsden, J. M. Thomson, J. J. Song, S. M. Hammond, L. Joshua-Tor & G. J. Hannon: Argonaute2 is the catalytic engine of mammalian RNAi. *Science*, 305, 1437-41 (2004)
11. Song, J. J., S. K. Smith, G. J. Hannon & L. Joshua-Tor: Crystal structure of Argonaute and its implications for RISC slicer activity. *Science*, 305, 1434-7 (2004)
12. Matranga, C., Y. Tomari, C. Shin, D. P. Bartel & P. D. Zamore: Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell*, 123, 607-20 (2005)
13. Rand, T. A., S. Petersen, F. Du & X. Wang: Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell*, 123, 621-9 (2005)
14. Ma, J. B., Y. R. Yuan, G. Meister, Y. Pei, T. Tuschl & D. J. Patel: Structural basis for 5'-end-specific recognition of guide RNA by the A. fulgidus Piwi protein. *Nature*, 434, 666-70 (2005)
15. Sioud, M. & D. R. Sorensen: Cationic liposome-mediated delivery of siRNAs in adult mice. *Biochem Biophys Res Commun*, 312, 1220-5 (2003)
16. Jackson, A. L., S. R. Bartz, J. Schelter, S. V. Kobayashi, J. Burchard, M. Mao, B. Li, G. Cavet & P. S. Linsley: Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol*, 21, 635-7 (2003)
17. Semizarov, D., L. Frost, A. Sarthy, P. Kroeger, D. N. Halbert & S. W. Fesik: Specificity of short interfering RNA determined through gene expression signatures. *Proc Natl Acad Sci U S A*, 100, 6347-52 (2003)
18. Sledz, C. A., M. Holko, M. J. de Veer, R. H. Silverman & B. R. Williams: Activation of the interferon system by short-interfering RNAs. *Nat Cell Biol*, 5, 834-9 (2003)
19. Parkin, J. & B. Cohen: An overview of the immune system. *Lancet*, 357, 1777-89 (2001)
20. Janeway, C. A., Jr. & R. Medzhitov: Innate immune recognition. *Annu Rev Immunol*, 20, 197-216 (2002)
21. Takeda, K. & S. Akira: Toll-like receptors in innate immunity. *Int Immunol*, 17, 1-14 (2005)
22. Sioud, M.: Innate sensing of self and non-self RNAs by Toll-like receptors. *Trends Mol Med*, 12, 167-76 (2006)
23. Brennan, C. A. & K. V. Anderson: Drosophila: the genetics of innate immune recognition and response. *Annu Rev Immunol*, 22, 457-83 (2004)
24. Lemaitre, B., E. Nicolas, L. Michaut, J. M. Reichhart & J. A. Hoffmann: The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell*, 86, 973-83 (1996)
25. Akira, S. & K. Takeda: Toll-like receptor signalling. *Nat Rev Immunol*, 4, 499-511 (2004)
26. Li, S., G. A. Peters, K. Ding, X. Zhang, J. Qin & G. C. Sen: Molecular basis for PKR activation by PACT or dsRNA. *Proc Natl Acad Sci U S A*, 103, 10005-10 (2006)
27. Samuel, C. E.: Antiviral actions of interferons. *Clin Microbiol Rev*, 14, 778-809, table of contents (2001)
28. Kato, H., O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K. J. Ishii, O. Yamaguchi, K. Otsu, T. Tsujimura, C. S. Koh, C. Reis e Sousa, Y. Matsuura, T. Fujita & S. Akira: Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*, 441, 101-5 (2006)
29. Meylan, E., J. Tschopp & M. Karin: Intracellular pattern recognition receptors in the host response. *Nature*, 442, 39-44 (2006)
30. Alexopoulou, L., A. C. Holt, R. Medzhitov & R. A. Flavell: Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*, 413, 732-8 (2001)
31. Heil, F., H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner & S. Bauer: Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*, 303, 1526-9 (2004)
32. Krieg, A. M.: CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol*, 20, 709-60 (2002)
33. Brentano, F., D. Kyburz, O. Schorr, R. Gay & S. Gay: The role of Toll-like receptor signalling in the pathogenesis of arthritis. *Cell Immunol*, 233, 90-6 (2005)
34. Cao, W. & Y. J. Liu: Innate immune functions of plasmacytoid dendritic cells. *Curr Opin Immunol*, 19, 24-30 (2007)
35. Sioud, M.: Induction of inflammatory cytokines and interferon responses by double-stranded and single-stranded siRNAs is sequence-dependent and requires endosomal localization. *J Mol Biol*, 348, 1079-90 (2005)
36. Hornung, V., M. Guenthner-Biller, C. Bourquin, A. Ablasser, M. Schlee, S. Uematsu, A. Noronha, M. Manoharan, S. Akira, A. de Fougerolles, S. Endres & G. Hartmann: Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nat Med*, 11, 263-70 (2005)
37. Judge, A. D., V. Sood, J. R. Shaw, D. Fang, K. McClintock & I. MacLachlan: Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. *Nat Biotechnol*, 23, 457-62 (2005)
38. Sioud, M.: Single-stranded small interfering RNA are more immunostimulatory than their double-stranded counterparts: a central role for 2'-hydroxyl uridines in immune responses. *Eur J Immunol*, 36, 1222-30 (2006)
39. Kariko, K., P. Bhuyan, J. Capodici & D. Weissman: Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. *J Immunol*, 172, 6545-9 (2004)
40. Song, E., P. Zhu, S. K. Lee, D. Chowdhury, S. Kussman, D. M. Dykxhoorn, Y. Feng, D. Palliser, D. B. Weiner, P. Shankar, W. A. Marasco & J. Lieberman: Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nat Biotechnol*, 23, 709-17 (2005)
41. Sioud, M.: On the delivery of small interfering RNAs into mammalian cells. *Expert Opin Drug Deliv*, 2, 639-51 (2005)
42. Morrissey, D. V., J. A. Lockridge, L. Shaw, K. Blanchard, K. Jensen, W. Breen, K. Hartsough, L. Machemer, S. Radka, V. Jadhav, N. Vaish, S. Zinnen, C.

Vargeese, K. Bowman, C. S. Shaffer, L. B. Jeffs, A. Judge, I. MacLachlan & B. Polisky: Potent and persistent *in vivo* anti-HBV activity of chemically modified siRNAs. *Nat Biotechnol*, 23, 1002-7 (2005)
43. Cekaite, L., G. Furset, E. Hovig & M. Sioud: Gene expression analysis in blood cells in response to unmodified and 2'-modified siRNAs reveals TLR-dependent and independent effects. *J Mol Biol*, 365, 90-108 (2007)
44. Judge, A. D., G. Bola, A. C. Lee & I. MacLachlan: Design of noninflammatory synthetic siRNA mediating potent gene silencing *in vivo*. *Mol Ther*, 13, 494-505 (2006)
45. Kariko, K., M. Buckstein, H. Ni & D. Weissman: Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity*, 23, 165-75 (2005)
46. Sioud, M.: RNA interference and innate immunity. *Adv Drug Deliv Rev*, 59, 153-63 (2007)
47. Sioud, M., G. Furset & L. Cekaite: Suppression of immunostimulatory siRNA-driven innate immune activation by 2'-modified RNAs. *Biochem Biophys Res Commun*, 361, 122-6 (2007)
48. Furset, G., Y. Floisand & M. Sioud: Impaired expression of indoleamine 2, 3-dioxygenase in monocyte-derived dendritic cells in response to Toll-like receptor-7/8 ligands. *Immunology* (2007)
49. Robbins, M., A. Judge, L. Liang, K. McClintock, E. Yaworski & I. MacLachlan: 2'-O-methyl-modified RNAs act as TLR7 antagonists. *Mol Ther*, 15, 1663-9 (2007)
50. Bartel, D. P.: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116, 281-97 (2004)
51. Han, J., Y. Lee, K. H. Yeom, J. W. Nam, I. Heo, J. K. Rhee, S. Y. Sohn, Y. Cho, B. T. Zhang & V. N. Kim: Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell*, 125, 887-901 (2006)
52. Kim, D. H., M. A. Behlke, S. D. Rose, M. S. Chang, S. Choi & J. J. Rossi: Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. *Nat Biotechnol*, 23, 222-6 (2005)
53. Marques, J. T., T. Devosse, D. Wang, M. Zamanian-Daryoush, P. Serbinowski, R. Hartmann, T. Fujita, M. A. Behlke & B. R. Williams: A structural basis for discriminating between self and nonself double-stranded RNAs in mammalian cells. *Nat Biotechnol*, 24, 559-65 (2006)
54. Robbins, M. A., M. Li, I. Leung, H. Li, D. V. Boyer, Y. Song, M. A. Behlke & J. J. Rossi: Stable expression of shRNAs in human CD34+ progenitor cells can avoid induction of interferon responses to siRNAs *in vitro*. *Nat Biotechnol*, 24, 566-71 (2006)
55. Sioud, M.: RNA interference below the immune radar. *Nat Biotechnol*, 24, 521-2 (2006)
56. Furset, G. & M. Sioud: Design of bifunctional siRNAs: combining immunostimulation and gene-silencing in one single siRNA molecule. *Biochem Biophys Res Commun*, 352, 642-9 (2007)
57. Hornung, V., J. Ellegast, S. Kim, K. Brzozka, A. Jung, H. Kato, H. Poeck, S. Akira, K. K. Conzelmann, M. Schlee, S. Endres & G. Hartmann: 5'-Triphosphate RNA is the ligand for RIG-I. *Science*, 314, 994-7 (2006)
58. Jackson, A. L., J. Burchard, D. Leake, A. Reynolds, J. Schelter, J. Guo, J. M. Johnson, L. Lim, J. Karpilow, K. Nichols, W. Marshall, A. Khvorova & P. S. Linsley: Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *Rna*, 12, 1197-205 (2006)
59. Banchereau, J. & R. M. Steinman: Dendritic cells and the control of immunity. *Nature*, 392, 245-52 (1998)
60. Rossi, M. & J. W. Young: Human dendritic cells: potent antigen-presenting cells at the crossroads of innate and adaptive immunity. *J Immunol*, 175, 1373-81 (2005)
61. Pardoll, D.: Does the immune system see tumors as foreign or self? *Annu Rev Immunol*, 21, 807-39 (2003)
62. Grutz, G.: New insights into the molecular mechanism of interleukin-10-mediated immunosuppression. *J Leukoc Biol*, 77, 3-15 (2005)
63. Alexander, W. S. & D. J. Hilton: The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. *Annu Rev Immunol*, 22, 503-29 (2004)
64. Shen, L., K. Evel-Kabler, R. Strube & S. Y. Chen: Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. *Nat Biotechnol*, 22, 1546-53 (2004)

Abbreviations: RNAi: RNA interference, siRNA: small interfering RNAs, TLR: Toll-like receptor, RISC: RNA-induced gene silencing complex, miRNA: microRNA, ss: single-stranded RNA, ds: double-stranded RNAs, DC: dendritic cells, RIG-1: retinoid acid-inducible gene, MDA5: melanoma differentiation associated gene 5, TNF: tumor necrosis factor, TRAF: TNF receptor associated factor, IL: Interleukin, IRAK-1: IL-1-associated kinase

Key Words: RNA interference, Small interfering RNAs, Innate immunity; Toll-like receptors, 2'-ribose modifications, TLR Antagonisst, Off-Target Effects, Gene Expression Profiling, Review

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