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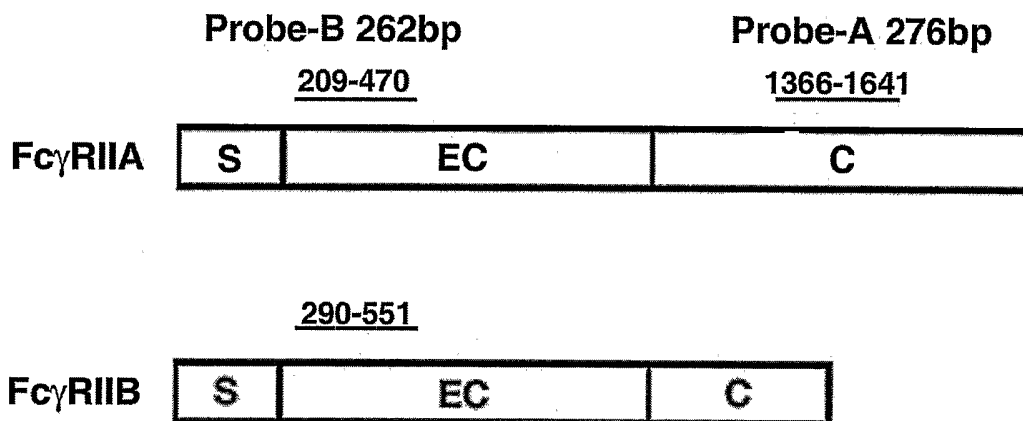
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(54) Title: FcγRIIA-SPECIFIC NUCLEIC ACID INTERFERENCE



(57) Abstract: The present invention provides methods and compositions for attenuating expression of FcγRIIA. In general, the described methodology involves the use of RNAi constructs that are targeted to a FcγRIIA mRNA sequence.

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Fc γ RIIA-SPECIFIC NUCLEIC ACID INTERFERENCE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional
5 Application No. 60/519,899, filed November 14, 2003, entitled "Inhibition of
phagocytosis with RNAi specific for cell surface receptors", and of U.S. Provisional
Application No. 60/564,459, filed April 22, 2004, entitled "Short interfacing (si)
RNA sequence(s) specific for the SH2 tyrosine kinase, Syk, inhibits phagocytosis by
human macrophages". The teachings of both of the above-mentioned applications
10 are incorporated by reference herein in their entirety.

BACKGROUND OF THE INVENTION

Immunoglobulins are typically composed of two fundamental domains, the
constant domain (Fc) and the variable domain. While the variable domain interacts
15 with target antigens, the constant domain mediates a variety of biological events by
interacting with other proteins of the host organism. Receptors for the Fc portion of
IgG , Fc γ receptors, play an essential role in the protection of the organism against
foreign antigens by removing antigen-antibody complexes from the circulation.
Receptors are present on monocytes, macrophages, neutrophils, natural killer (NK)
20 cells, platelets, and T and B lymphocytes, and they participate in diverse functions
such as phagocytosis of immune complexes, NK cell ADCC, platelet activation, and
modulation of antibody production by B cells.

Fc γ receptors also play a role in a number of diseases characterized by a
hyperactive immune system or other undesirable immunological activity. Fc γ
25 receptors participate in a number of autoimmune and inflammatory diseases. As an
example, Fc γ receptors are implicated in immune thrombocytopenia. The
pathogenic mechanism of immune thrombocytopenia involves antibody-mediated
destruction of platelets in the reticuloendothelial system through Fc γ receptors
(Fc γ Rs) expressed on tissue macrophages, particularly in the spleen and liver.

Fc γ Rs signal via immunoreceptor tyrosine-based activation motifs (ITAMs) that are located either in the cytosolic domains of the receptors themselves (Fc γ RIIA), or within associated γ (Fc γ RI and Fc γ RIIA) or ζ (Fc γ RIIA) subunits. Following clustering of the Fc γ Rs and their associated γ subunits by bound IgG ligands, tyrosine residues within the ITAMs become phosphorylated. The tyrosine-phosphorylated residues of the ITAMs serve as high affinity binding sites for Syk, a tyrosine kinase that contains tandem SH2 domains, which propagates intracellular signaling processes. In humans, Fc γ RIIA and Fc γ RIII are the primary activating receptors.

10 The Fc γ RIIB receptor has an inhibitory role. Fc γ RIIB recruits the SHIP kinase and abrogates signaling triggered by activating Fc γ receptors. The overall cellular response depends in part on the ratio of signaling mediated by inhibiting and activating receptors.

15 An object of the present disclosure is to provide nucleic acid agents that inhibit Fc γ RIIA expression and to provide methods of using such agents for therapeutic purposes.

SUMMARY OF THE INVENTION

20 The disclosure provides, in part, RNAi constructs that target Fc γ RIIA and decrease Fc γ RIIA expression. Such constructs may be used in essentially any method where it is desirable to decrease the level of Fc γ RIIA protein. In particular, the disclosed nucleic acids will be useful in treating various disorders related to immune system function, such as immune thrombocytopenia, heparin-induced thrombocytopenia and asthma.

25 In certain aspects, the disclosure provides RNAi constructs for inhibiting the expression of Fc γ RIIA. Such nucleic acids may comprise (a) an antisense polynucleotide strand that hybridizes to at least a portion of a Fc γ RIIA transcript and inhibits Fc γ RIIA expression; and (b) a sense polynucleotide that hybridizes to said antisense polynucleotide. The antisense and sense strands may be two separate

nucleic acid strands, or the strands may be joined by a linker or by a stretch of further nucleic acid. For example, the RNAi construct may be a single stranded nucleic acid containing two regions that are complementary, thereby forming a hairpin nucleic acid with a stretch of double-stranded helix. Such hairpin nucleic acids are often processed into an siRNA inside a cell. Optionally, the portion of the nucleic acid that forms a double helix is about 19 to about 23 base pairs in length. The antisense polynucleotide strand may be complementary to a sequence of the human Fc γ RIIA mRNA of SEQ ID NO:1, or that of another animal of interest. The RNAi construct may be designed so as to have little or no effect on Fc γ RIIB expression. For example, the antisense strand may be designed to have no more than 15 consecutive nucleotides that are complementary to an Fc γ RIIB mRNA sequence, and preferably the antisense strand has no more than 10, no more than 5, or no more than 3 consecutive nucleotides that are complementary to an Fc γ RIIB mRNA sequence. The antisense polynucleotide strand may be complementary to at least 5, 6, 7, 8, 10, 15, 20 or more nucleotides of a sequence selected from the group consisting of: SEQ ID Nos. 4 through 13. The antisense polynucleotide may consist of a sequence that is complementary to a sequence selected from the group consisting of: SEQ ID Nos. 4-13. The sense and antisense strands may be essentially any suitable nucleic acid, including RNA, DNA or other nucleic acid species that are not readily categorized as DNA or RNA. The sense and antisense strands need not be formed of the same nucleic acids. Either or both strands may include one or more modifications to the typical nucleic acid structure. For example, the antisense strand may comprise one or more of the following modifications: (a) a modification to the sugar-phosphate backbone; (b) a modification to a base portion of a nucleotide; and (c) a conjugated hydrophobic moiety. The sense strand may be similarly modified.

RNAi constructs may be formulated for administration to an organism. A pharmaceutical preparation for delivery of an RNAi construct to an organism may comprise a pharmaceutically acceptable carrier and an RNAi construct that inhibits expression of Fc γ RIIA. The preparation may be suitable for any desirable mode of administration. In certain instances, such as for the treatment of asthma or other

disorders of the airway system, the preparation may be designed for administration by inhalation (e.g., aerosolized or intranasal).

In certain aspects, the disclosure provides methods for decreasing expression of Fc γ RIIA in a cell. Such methods may comprise contacting the cell with a composition comprising an RNAi construct that inhibits Fc γ RIIA expression.

In certain aspects, the disclosure provides methods for decreasing expression of Fc γ RIIA in one or more cells of an individual. Such methods may comprise administering to the individual a composition comprising a double-stranded nucleic acid that inhibits Fc γ RIIA expression. The individual may be diagnosed with a condition associated with excess Fc receptor activity or with any of a variety of disorders related to the immune system, such as asthma, immune thrombocytopenia or an autoimmune disease.

In an additional aspect, the disclosure provides improved assays for measuring phagocytosis of target material in phagocytic cells. The methods employ phagocytic cells from a cell line, particularly a cell of a hematopoietic lineage, such as a monoblastic cell line or other immune cell line. Desirable cells will generally express one or more Fc γ receptors that mediate phagocytosis of opsonized material, such as Fc γ RIIA. A method may comprise (a) exposing target material to antibody to generate prepared target material; (b) exposing the phagocytic cells to the prepared target material; and (c) selectively detecting the prepared target material that is internalized by the phagocytic cells. The target material will often be cells, particularly platelets. However, phagocytic cells are generally take up materials non-specifically, and therefore other materials, such as microbeads, may be used as a target material. Target material may be labeled in a variety of ways to facilitate detection, and target material may be pre-labeled or labeled upon exposure to antibody. The label may, for example, be a fluorescent label. In a preferred embodiment, the label is a fluorescent label, and selectively detecting the prepared target material comprises selectively quenching any extracellular label. Thus, detection of the fluorescent signal necessarily detects signal from unquenched fluorophore within the phagocytic cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A diagram comparing the sequences of human Fc γ RIIA and Fc γ RIIB, showing the location of probes for Northern blots that distinguish between the two transcripts.

- 5 Figure 2: Using probes designed to hybridize with Fc γ RIIA only, or designed to hybridize with both Fc γ RIIA and Fc γ RIIB, siRNA transfection of THP-1 cells was shown to allow the selective knockdown of Fc γ RIIA.

Figure 3: Fc γ RIIA-specific siRNA inhibited antibody mediated platelet phagocytosis.

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DETAILED DESCRIPTION OF THE INVENTION

I. RNAi Constructs

- In part, the disclosure provides RNA interference ("RNAi") constructs that are useful for inhibiting Fc γ RIIA expression. As used herein, the term "RNAi construct" is a generic term used to include any of the various nucleic acid reagents that can be used to achieve a decrease in the levels of a targeted protein. Such decrease, however achieved, shall be referred to herein as a decrease in "expression" of the protein. While not wishing to be bound to any particular mechanism, it is expected that such constructs will act by an RNA interference ("RNAi") mechanism.
- 15 Such constructs may be, for example, small interfering RNAs (siRNAs), hairpin RNAs, and other RNA species which can be cleaved *in vivo* to form siRNAs. As will be apparent from the preceding examples, an RNAi construct may include a single nucleic acid molecule, as in the case of hairpin RNAs, or two nucleic acid molecules, as in the case of siRNAs, or more nucleic acid molecules. Nonetheless,
- 20 an RNAi construct will generally include at least some portion in which, under physiological conditions, a double helix is formed, where such double helix may result from intramolecular hybridization or intermolecular hybridization. Although RNAi is a term that was initially applied only with respect to RNA molecules, it is now understood that the same effect may be accomplished with modified nucleic

acids that are not necessarily RNA, and also with molecules containing one or more DNA moieties. Constructs including any of the various nucleic acids are intended to be included in the term "RNAi construct".

As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The term should also be understood to include, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides. The "canonical" nucleotides are adenosine (A), guanosine (G), cytosine (C), thymidine (T), and uracil (U), and include a ribose-phosphate backbone, but the term nucleic acid is intended to include polynucleotides comprising only canonical nucleotides as well as polynucleotides including one or more modifications to the sugar phosphate backbone or the nucleoside. DNA and RNA are chemically different because of the absence or presence of a hydroxyl group at the 2' position on the ribose. Modified nucleic acids exist that cannot be readily termed DNA or RNA (e.g. in which an entirely different moiety is positioned at the 2' position), as do functional analogs, such as peptide nucleic acids (PNAs) in which the backbone is a peptide backbone, in which the backbone contains neither sugar nor phosphate. All such molecules are included in the term "nucleic acid". An "unmodified" nucleic acid is a nucleic acid that contains only canonical nucleotides and a DNA or RNA backbone.

RNA interference is a phenomenon describing double-stranded nucleic acid-dependent gene specific posttranscriptional silencing. Initial attempts to harness this phenomenon for experimental manipulation of mammalian cells were foiled by a robust and nonspecific antiviral defense mechanism activated in response to long dsRNA molecules. Gil et al. Apoptosis 2000, 5:107-114. The field was significantly advanced upon the demonstration that synthetic duplexes of 21 nucleotide RNAs could mediate gene specific RNAi in mammalian cells, without invoking generic antiviral defense mechanisms. Elbashir et al. Nature 2001, 411:494-498; Caplen et al. Proc Natl Acad Sci 2001, 98:9742-9747. As a result, small-interfering RNAs (siRNAs) have become powerful tools to dissect gene function, and data from

Soutscheck et al. Nature 2004, 432:173-178 has established that double-stranded RNA constructs can be delivered in vivo to modulate expression of the target gene.

The mRNA sequence for human Fc γ RIIA (also known as CD32A and FCGR2A) is represented below:

5 Human Fc γ RIIA mRNA (Genbank NM_021642; gi:50511935; SEQ ID NO:1)

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1  gtctcttaaa acccactgga cgttggcaca gtgctgggat gactatggag
   acccaaatgt
61  ctcagaatgt atgtcccaga aacctgtggc tgcttcaacc attgacagtt
   ttgctgctgc
10 121 tggcttctgc agacagtcaa gctgctcccc caaaggctgt gctgaaactt
   gagccccctg
   181 ggatcaacgt gctccaggag gactctgtga ctctgacatg ccagggggct
   cgcagccctg
   241 agagcgactc cattcagtgg ttccacaatg ggaatctcat tcccacccac
15  acgcagccca
   301 gctacagggt caaggccaac aacaatgaca gcggggagta cacgtgccag
   actggccaga
   361 ccagcctcag cgaccctgtg catctgactg tgctttccga atggctggtg
   ctccagaccc
20 421 ctcacctgga gttccaggag ggagaaacca tcatgctgag gtgccacagc
   tggaaggaca
   481 agcctctggt caaggtcaca ttcttcaga atggaaaatc ccagaaattc
   tccatttgg
   541 atcccacctt ctccatccca caagcaaacc acagtcacag tggtgattac
25  cactgcacag
   601 gaaacatagg ctacacgctg ttctcatcca agcctgtgac catcactgtc
   caagtgccca
   661 gcatgggcag ctcttcacca atggggatca ttgtggctgt ggtcattgcg
   actgctgtag
30 721 cagccattgt tgctgctgta gtggccttga tctactgcag gaaaaagcgg
   atttcagcca
   781 attccactga tcctgtgaag gctgcccatt ttgagccacc tggacgtcaa
   atgattgcca
   841 tcagaaagag acaacttgaa gaaaccaaca atgactatga aacagctgac
35  ggccggctaca
   901 tgactctgaa ccccagggca cctactgacg atgataaaaa catctacctg
   actcttcctc
   961 ccaacgacca tgtcaacagt aataactaaa gagtaacgtt atgccatgtg
   gtcatactct
40 1021 cagcttgctg agtggatgac aaaaagaggg gaattgttaa aggaaaattt
   aaatggagac
   1081 tggaaaaatc ctgagcaaac aaaaccacct ggcccttaga aatagcttta
   acttttgctta
   1141 aactacaaac acaagcaaaa cttcacgggg tcatactaca tacaagcata
45  agcaaaactt
   1201 aacttggatc atttctggta aatgcttatg ttagaaataa gacaaccca
   gccaatcaca
   1261 agcagcctac taacatataa ttaggtgact agggactttc taagaagata
   cctaccccca

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1321 aaaaacaatt atgtaattga aaaccaaccg attgccttta ttttgettcc
acattttccc
1381 aataaatact tgcctgtgac attttgccac tggaacacta aacttcatga
attgcgcctc
5 1441 agatttttcc tttaacatct tttttttttt tgacagagtc tcaatctgtt
accagggctg
1501 gagtgcagtg gtgctatctt ggctcactgc aaacccgcct cccagggtta
agcgattctc
1561 atgcctcagc ctcccagtag ctgggattag aggcattgtgc catcatatccc
10 agctaattttt
1621 tgtattttttt atttttttttt tttagtagag acaggggtttc gcaatgttgg
ccaggccgat
1681 ctcgaaacttc tggcctctag cgatctgccc gcctcggcct cccaaagtgc
tgggatgacc
15 1741 agcatcagcc ccaatgtcca gcctctttaa catcttcttt cctatgccct
ctctgtggat
1801 ccctactgct ggtttctgcc ttctccatgc tgagaacaaa atcacctatt
cactgcttat
1861 gcagtccgaa gctccagaag aacaaagagc ccaattacca gaaccacatt
20 aagtctccat
1921 tgttttgcct tgggatttga gaagagaatt agagaggtga ggatctggta
tttcctggac
1981 taaattcccc ttggggaaga cgaagggatg ctgcagttcc aaaagagaag
gactcttcca
25 2041 gagtcatcta cctgagtccc aaagctccct gtcctgaaag ccacagacaa
tatgggtcca
2101 aatgactgac tgcaccttct gtgcctcagc cgttcttgac atcaagaatc
ttctgttcca
2161 catccacaca gccaatacaa ttagtcaaac cactgttatt aacagatgta
30 gcaacatgag
2221 aaacgcttat gttacagggtt acatgagagc aatcatgtaa gtctatatga
cttcagaaat
2281 gttaaaatag actaacctct aacaacaaat taaaagtgat tgtttcaagg
tgatgcaatt
35 2341 attgatgacc tattttattt ttctataatg atcatatatt acctttgtaa
taaaacatta
2401 taaccaaaaac a

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Suitable nucleic acid constructs may be designed by selecting a target region
 40 of a Fc γ RIIA mRNA sequence, such as that of the human, listed above or other
 animal, and preparing a construct having a polynucleotide strand that hybridizes to
 the selected region of the mRNA (the "antisense strand") and a polynucleotide that
 hybridizes to the antisense strand (the "sense strand"). Constructs designed at
 random are unlikely to inhibit Fc γ RIIA expression. While it is possible to screen
 45 through large numbers of such constructs to identify those that are effective, it is
 generally desirable to follow a procedure for selecting target regions that have an
 improved likelihood of success.

For example, one may employ the algorithm of Elbashir et al. (EMBO J 20: 6877-6888, 2001) to select an appropriate target region and generate a construct with a higher chance of being effective. Briefly, this approach involves identifying nucleotide sequences in the target mRNA that begin with an AA dinucleotide and
5 have a length of 21 nucleotides. siRNAs with other 3' terminal dinucleotide overhangs have been shown to effectively induce RNAi, although overhang sequences containing a GG dinucleotide may be cleaved by RNase.

Double-stranded nucleic acid constructs may be evaluated for effectiveness by administering the construct to a cell and evaluating the effect on FcγRIIA protein
10 levels, kinase activity or another feature that is correlated with FcγRIIA expression. Different nucleic acid constructs will tend to affect FcγRIIA expression to different degrees, and it will not always be apparent what percent inhibition of FcγRIIA expression will achieve the desired effects. Accordingly, double-stranded nucleic acid constructs may also be evaluated for effectiveness in one or more bioassays.
15 For example, FcγRIIA -targeted constructs may be tested for effects on antibody mediated platelet phagocytosis to assess the effect on Fcγ-mediated events. Examples of such assays are provided below.

The FcγRIIA mRNA sequence is closely related to that of the FcγRIIB mRNA sequence. However, while FcγRIIA is an activating receptor, FcγRIIB is an
20 inhibiting receptor. Accordingly, it is desirable to selectively interfere with FcγRIIA, while leaving FcγRIIB unaffected. RNAi constructs disclosed herein may be designed to have this type of selectivity. Notably, such selectivity has not, to our knowledge, been achieved previously by antibody-based inhibition or by inhibition using traditional antisense techniques. The 3' end of the FcγRIIA transcript is
25 particularly divergent from that of FcγRIIB, as shown schematically in Figure 1. An RNAi construct selective for FcγRIIA may be designed to have few or no consecutive nucleic acids in the antisense strand that are complementary to FcγRIIB transcript. For example, the antisense strand may be designed to have no more than 15 consecutive nucleotides that are complementary to an FcγRIIB mRNA sequence,
30 and preferably the antisense strand has no more than 10, no more than 5, or no more

than 3 consecutive nucleotides that are complementary to an Fc γ RIIB mRNA sequence. However, a small number of complementary nucleic acids may be included and yet have no significant effect on Fc γ RIIB transcript. Such effects may be evaluated empirically, by, for example, transcript specific Northern blotting or
 5 RT-PCR. Examples of human Fc γ RIIB mRNA sequences for comparison may be found in Genbank entries NM_001002273; NM_004001; NM_001002275; NM_001002274.

The table below lists the target sequences (sense DNA) for a series of RNA interference constructs designed to inhibit Fc γ RIIA expression.

10 Table 1: Human Fc γ RIIA siRNA Target Sequences

	CD32IIA-A	AAACTTGAGCCCCCGTGGATC	(SEQ ID NO:2)
	CD32IIA-B	AATTTGAGCCACCTGGACGTC	(SEQ ID NO:3)
	CD32IIA-C	AAAGAGACAACCTGAAGAAAC	(SEQ ID NO:4)
	CD32IIA-D	AAACCATCATGCTGAGGTGCC	(SEQ ID NO:5)
15	CD32IIA-E	AACCATCATGCTGAGGTGCCA	(SEQ ID NO:6)
	CD32IIA-F	AAATTCTCCCGTTTGGATCCC	(SEQ ID NO:7)
	CD32IIA-G	AATTCTCCCGTTTGGATCCCA	(SEQ ID NO:8)
	CD32IIA-H	AAACCCGCCTCCCAGGTTTAA	(SEQ ID NO:9)
	CD32IIA-I	AACTTCTGGCCTCTAGCGATC	(SEQ ID NO:10)
20	CD32IIA-J	AAGTGCTGGGATGACCAGCAT	(SEQ ID NO:11)
	CD32IIA-K	AATGTCCAGCCTCTTTAACAT	(SEQ ID NO:12)
	CD32IIA-L	AACATCTTCTTTCCTATGCCC	(SEQ ID NO:13)

As demonstrated herein, RNAi constructs containing an antisense strand that
 25 hybridizes to a sequence of any of SEQ ID Nos. 4-13 are effective in reducing Fc γ RIIA transcript levels. See Example 1, Table 2. RNAi constructs containing an antisense strand that hybridizes to a sequence of any of SEQ ID Nos. 10-13 are effective in reducing Fc γ RIIA transcript levels by greater than 30%, while those containing an antisense strand that hybridizes to a sequence of any of SEQ ID Nos.
 30 6, 7, and 9 are effective in reducing Fc γ RIIA protein levels by greater than 60%. RNAi constructs containing an antisense strand that hybridizes to a sequence of any of SEQ ID Nos. 4, 6, 7, 8 and 9 are effective in reducing phagocytosis of opsonized platelets, while those containing an antisense strand that hybridizes to a sequence of any of SEQ ID Nos. 8 and 9 are effective in reducing phagocytosis of opsonized
 35 platelets by greater than 30%. Where an RNAi construct is to be used for a purpose

related to reducing phagocytosis by immune cells, it may be preferable to use an RNAi construct that produces an effect in a phagocytosis bioassay, such as an RNAi construct having an antisense strand that hybridizes to a sequence of any of SEQ ID Nos. 4, 6, 7, 8 and 9. Where an RNAi construct is to be used for a purpose not
5 related to reducing phagocytosis in immune cells, it may be preferable to use an RNAi construct that causes a measurable decrease in Fc γ RIIA levels, such as an RNAi construct containing an antisense strand that hybridizes to a sequence of any of SEQ ID Nos. 4-13. RNAi constructs containing an antisense strand that hybridizes to a sequence of SEQ ID No. 9 are particularly notable for having a
10 strong effect on Fc γ RIIA levels and on platelet phagocytosis.

RNAi constructs may be designed to contain a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the mRNA transcript for the gene to be inhibited (i.e., the "target" gene) and is sufficient for decreasing the expression of the protein encoded by the
15 target gene. The nucleotide sequence of the RNAi construct may hybridize to coding or non-coding portions of a transcript, including, for example, the 3' and 5' untranslated regions ("UTRs"). The RNAi construct need only be sufficiently similar to natural RNA that it has the ability to be effective. Thus, sequence variations that might be expected due to genetic mutation, strain polymorphism or
20 evolutionary divergence may be tolerated. In certain cases, the number of tolerated nucleotide mismatches between the target sequence and the RNAi construct sequence is no more than 1 in 5 basepairs, or 1 in 10 basepairs, or 1 in 20 basepairs, or 1 in 50 basepairs. Mismatches in the center of the siRNA duplex are most critical and may essentially abolish cleavage of the target RNA. In contrast, nucleotides at
25 the 3' end of the siRNA antisense strand do not significantly contribute to specificity of the target recognition.

Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent
30 difference between the nucleotide sequences by, for example, the Smith-Waterman

algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 90% sequence identity, or even 100% sequence identity, between the interfering RNA and the portion of the target gene is preferred. Alternatively, the duplex region
5 of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 °C or 70 °C hybridization for 12-16 hours; followed by washing).

Production of RNAi constructs can be carried out by chemical synthetic
10 methods or by recombinant nucleic acid techniques. Endogenous RNA polymerase of the treated cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vitro*.

One or both strands of an RNAi construct may include modifications to the phosphate-sugar backbone and/or the nucleoside. In general, the sense strand does
15 not directly participate in the formation of a silencing complex (RISC) and is therefore subject to few constraints in the number and type of modifications that may be introduced. The sense strand need only retain the ability to hybridize with the antisense strand, and, in the case of longer nucleic acids, should not interfere with the activity of RNAses, such as Dicer, that participate in cleaving longer
20 double-stranded constructs to yield smaller, active siRNAs. The antisense strand should retain the ability to hybridize with both the sense strand and the target transcript, and the ability to form an RNAi induced silencing complex (RISC). Optionally, the sense strand comprises at least 20%, 30%, 50%, 70%, 90% and 100% modified nucleic acids. Optionally, the antisense strand comprises no more
25 than 0%, 10%, 20%, 30%, 40% or 50% modified nucleic acids. Modifications may be useful for, e.g., reduced susceptibility to nucleases, improved bioavailability, improved formulation characteristics, and changed pharmacokinetic properties. Many nucleases are progressive enzymes that initiate degradation at one end of a nucleic acid. Therefore, resistance to nucleases may be conferred by modification
30 of, for example, one, two, three or more nucleotides at either the 5' end, the 3' end or

both ends of a sense or antisense strand. For example, one, two or three phosphorothioate linkages may be positioned at either the 5'end, the 3'end or both ends of a sense or antisense strand. As another example, one, two or three 2' O-methyl modified nucleotides may be positioned at either the 5'end, the 3'end or both
 5 ends of a sense or antisense strand. For a demonstration of the effectiveness of such modifications in improving the in vivo performance of RNAi constructs see, for example, Soutscheck et al. Nature 2004, 432:173-178. Nucleotides in the sense strand may also be DNA.

Methods of chemically modifying RNA molecules can be adapted for
 10 modifying RNAi constructs (see, for example, Heidenreich et al. (1997) Nucleic Acids Res. 25:776-780; Wilson et al. (1994) J Mol Recog 7:89-98; Chen et al. (1995) Nucleic Acids Res 23:2661-2668; Hirschbein et al. (1997) Antisense Nucleic Acid Drug Dev 7:55-61). Merely to illustrate, the backbone of an RNAi construct can be modified with phosphorothioates, phosphoramidate, phosphodithioates,
 15 chimeric methylphosphonate-phosphodiester, peptide nucleic acids, 5-propynyl-pyrimidine containing oligomers or sugar modifications (e.g., 2'-substituted ribonucleosides, α -configuration). Additional modified nucleotides are as follows (this list contains forms that are modified on either the backbone or the nucleoside or both, and is not intended to be all-inclusive): 2'-O-Methyl-2-aminoadenosine; 2'-O-Methyl-5-methyluridine; 2'-O-Methyladenosine; 2'-O-Methylcytidine; 2'-O-Methylguanosine; 2'-O-Methyluridine; 2-Amino-2'-deoxyadenosine; 2-Aminoadenosine; 2-Aminopurine-2'-deoxyriboside; 4-Thiothymidine; 4-Thiouridine; 5-Methyl-2'-deoxycytidine; 5-Methylcytidine; 5-Methyluridine; 5-Propynyl-2'-deoxycytidine; 5-Propynyl-2'-deoxyuridine; N1-Methyladenosine; N1-Methylguanosine; N2-Methyl-2'-deoxyguanosine; N6-Methyl-2'-deoxyadenosine; N6-Methyladenosine; O6-Methyl-2'-deoxyguanosine; and O6-Methylguanosine.
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The double-stranded structure may be formed by a single self-complementary nucleic acid strand or by two complementary nucleic acid strands. A double-stranded nucleic acid disclosed herein may include portions of single-stranded nucleic acid. Duplex formation may be initiated either inside or outside the
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cell. The RNAi construct may be introduced in an amount that allows delivery of at least one copy per cell. Higher doses (e.g., at least 5, 10, 100, 500 or 1000 copies per cell) of double-stranded material may yield more effective inhibition, while lower doses may also be useful for specific applications. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition.

In certain embodiments, the subject nucleic acid constructs are “small interfering RNAs” or “siRNAs.” These nucleic acids include an antisense RNA strand that is from about 19 to about 30 nucleotides in length, and possibly from about 21 to 23 nucleotides in length, e.g., corresponding in length to the fragments generated by nuclease “dicing” of long double-stranded RNAs. siRNAs may include a sense strand that is RNA, DNA or other modified nucleic acids. The siRNAs are understood to recruit nuclease complexes and guide the complexes to the target mRNA by pairing to the specific sequences. As a result, the target mRNA is degraded by the nucleases in the protein complex.

siRNA molecules can be obtained using a number of techniques known to those of skill in the art. For example, the siRNA can be chemically synthesized or recombinantly produced using methods known in the art. For example, short sense and antisense oligomers can be synthesized and annealed to form double-stranded structures with 2-nucleotide overhangs at each end (Caplen, et al. (2001) Proc Natl Acad Sci USA, 98:9742-9747; Elbashir, et al. (2001) EMBO J, 20:6877-88). These double-stranded siRNA structures can then be introduced into cells, either by passive uptake or a delivery system of choice, such as described below.

In certain embodiments, the siRNA constructs can be generated by the processing of longer double-stranded RNAs, for example, in the presence of the enzyme dicer. In one embodiment, the *Drosophila in vitro* system is used. In this embodiment, dsRNA is combined with a soluble extract derived from *Drosophila* embryo, thereby producing a combination. The combination is maintained under conditions in which the dsRNA is processed to RNA molecules of about 21 to about

23 nucleotides. In this embodiment, modifications should be selected so as to not interfere with the activity of the RNase.

The siRNA molecules can be purified using a number of techniques known to those of skill in the art. For example, gel electrophoresis can be used to purify
5 siRNAs. Alternatively, non-denaturing methods, such as non-denaturing column chromatography, can be used to purify the siRNA. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, affinity purification with antibody can be used to purify siRNAs.

In certain embodiments, at least one strand of an siRNA has a 3' overhang
10 consisting of from about 1 to about 6 nucleotides. The overhang may consist of from about 2 to about 4 nucleotides. Typically, the 3' overhangs consist of about 1 to about 3 nucleotides. In certain embodiments, one strand has a 3' overhang and the other strand is either blunt-ended or also has a 3' overhang. The length of the overhangs may be the same or different for each strand. In order to further enhance
15 the stability of the siRNA, the 3' overhangs can be stabilized against degradation. In one embodiment, the RNA antisense strand is stabilized by including purine nucleotides, such as adenosine or guanosine nucleotides. Alternatively, substitution of pyrimidine nucleotides by modified analogues, e.g., substitution of uridine nucleotide 3' overhangs by 2'-deoxythymidine is tolerated and does not affect the
20 efficiency of RNAi. The absence of a 2' hydroxyl significantly enhances the nuclease resistance of the overhang in tissue culture medium and may be beneficial *in vivo*.

A sense or antisense strand may be conjugated to one or more additional moieties to improve properties such as stability, cellular uptake or pharmacokinetics.
25 Hydrophobic moieties, such as lipids and sterols, such as cholesterol, may be conjugated to a sense or antisense strand. See for example, Lorenz et al. Bioorg. Med. Chem. Lett. 14, 4975-4977 (2004) and Soutscheck et al. Nature 2004, 432:173-178. A sense or antisense strand may include one, two, three or more such hydrophobic moieties.

In certain embodiments, an RNAi construct is in the form of a hairpin structure. The hairpin can be synthesized exogenously or can be formed by transcribing from RNA polymerase III promoters *in vivo*. Examples of making and using such hairpin RNAs for gene silencing in mammalian cells are described in, for example, Paddison et al., Genes Dev, 2002, 16:948-58; McCaffrey et al., Nature, 2002, 418:38-9; McManus et al., RNA, 2002, 8:842-50; Yu et al., Proc Natl Acad Sci U S A, 2002, 99:6047-52). Preferably, such hairpin RNAs are engineered in cells or in an animal to ensure continuous and stable suppression of a desired gene. It is known in the art that siRNAs can be produced by the processing of a hairpin RNA in the cell. In such an embodiment, the single strand portion connecting the sense and antisense portions should be designed so as to be cleavable by nucleases *in vivo*, and any duplex portion should be susceptible to processing by nucleases such as Dicer.

II. Methods for Using Fc γ RIIA -Targeted Interfering Nucleic Acids

RNAi acid constructs disclosed herein may be used in essentially any *in vivo* or *in vitro* setting where inhibition of Fc γ RIIA expression is desirable.

In human patients and other mammals, Fc γ RIIA -targeted nucleic acid constructs may be used to treat a variety of diseases related to the immune system. For example, Fc γ RIIA inhibition may be useful in the treatment of autoimmune diseases, particularly those characterized by interactions of immune complexes (eg, IgG-containing immune complexes) with Fc receptors (for example, those present on the surface of macrophages). Diseases such as immune cytopenias (e.g. immune thrombocytopenia, immune hemolytic anemia, or immune neutropenia), Guillain-Barre syndrome, myasthenia gravis, anti-Factor VIII immune disease, dermatomyositis, vasculitis, uveitis, rheumatoid arthritis and systemic lupus erythematosus may be treated with RNAi constructs disclosed herein. See, e.g., Samuelsson et al. 2001 Science 291:484.

Fc γ RIIA inhibition may also be useful in the treatment of allergic disorders and asthma. Agents that block the activity of Fc γ RIIA kinase may be used in

treating inflammatory and allergic disorders including asthma, chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), ulcerative colitis, Crohn's disease, bronchitis, conjunctivitis, psoriasis, scleroderma, urticaria, dermatitis and allergic rhinitis.

- 5 Fc γ RIIA is expressed in platelets and mediates activation in platelets. Chacko et al. J. Biol. Chem. 271(18):10775-81, 1996. Thus inhibition of Fc γ RIIA may be useful in patients at risk for thrombosis, and particularly thrombosis mediated by Fc γ R signaling. For example, Fc γ RIIA inhibitors may be administered to heparinized patients to reduce the risk of heparin induced thrombocytopenia.
- 10 Fc γ RIIA inhibitors may also be used in diabetic patients to reduce the risk of arterial thrombosis in these patients. Calverley et al. Br. J. Haemat. 121(1):139-42, 2003.

III. Formulations

- The RNAi constructs disclosed herein may be admixed, encapsulated,
- 15 conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, polymers, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption of the constructs. The subject RNAi constructs can be provided in formulations also including penetration enhancers, carrier compounds
- 20 and/or transfection agents.

- Therapeutic applications of the disclosed nucleic acids can be performed with a variety of compositions and methods of administration. In view of the teachings herein, methods of administration to cells and organisms will be available to persons skilled in the art. Dosing regimens, for example, are known to depend on
- 25 the severity and degree of responsiveness of the disease or disorder to be treated, with a course of treatment spanning from days to months, or until the desired effect on the disorder or disease state is achieved. Chronic administration of the agent may be required in certain cases for lasting desired effects with some diseases or disorders. Suitable dosing regimens can be determined by, for example,

administering varying amounts of one or more RNAi constructs in a pharmaceutically acceptable carrier or diluent, by a pharmaceutically acceptable delivery route, and amount of agent accumulated in the body of the recipient organism can be determined at various times following administration. Similarly, the
5 desired effect (for example, degree of suppression of transcription or expression or activity of a gene product or gene activity) can be measured at various times following administration of the RNAi construct, and this data can be correlated with other pharmacokinetic data, such as body or organ accumulation. Those of ordinary skill can determine optimum dosages, dosing regimens, and the like. Those of
10 ordinary skill may employ EC₅₀ data from in vivo and in vitro animal models as guides for human studies.

The RNAi constructs disclosed herein also encompass any pharmaceutically acceptable salts, esters or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or
15 indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to RNAi constructs and pharmaceutically acceptable salts of such RNAi constructs, and other bioequivalents. A pharmaceutical preparation will generally be free of substantial amounts of pyrogenic impurities.

20 Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N1-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine,
25 and procaine (see, for example, Berge et al., "Pharmaceutical Salts," J. of Pharma Sci., 1977, 66,1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the
30 conventional manner. The free acid forms differ from their respective salt forms

somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids.

For polynucleotides, examples of pharmaceutically acceptable salts include, but are not limited to, (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalene disulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

Further, the polynucleotides can be prepared for topical, oral, local or parenteral administration. Parenteral administration, includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl (or similar) modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Electroporation may be used as a further means to introduce polynucleotides into cells. Specific approaches to delivering

RNAi constructs are described in the following references: Sorensen et al. J. Mol. Biol. 327:761-766, 2003 (intravenous injection of cationic liposomes); Sioud et al. BBRC 312, 1220-1225, 2003 (intraperitoneal injection of cationic liposomes); Tompkins et al. Proc. Natl. Acad. Sci. 101:8682-8686, 2004.

5 Another aspect of the disclosure provides aerosols for the delivery of RNAi constructs to the respiratory tract. The respiratory tract includes the upper airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by bifurcations into the bronchi and bronchioli. The upper and lower airways are called the conductive airways. The terminal bronchioli then divide
10 into respiratory bronchioli which then lead to the ultimate respiratory zone, the alveoli, or deep lung.

Administration may be accomplished by oral or nasal inhalation. Examples of pharmaceutical devices for aerosol delivery include metered dose inhalers (MDIs), dry powder inhalers (DPIs), and air-jet nebulizers. Exemplary nucleic acid
15 delivery systems by inhalation which can be readily adapted for delivery of the subject nucleic acid constructs are described in, for example, U.S. patents 5,756,353; 5,858,784; and PCT applications WO98/31346; WO98/10796; WO00/27359; WO01/54664; WO02/060412. Other aerosol formulations that may be used for delivering the double-stranded RNAs are described in U.S. Patents 6,294,153;
20 6,344,194; 6,071,497, and PCT applications WO02/066078; WO02/053190; WO01/60420; WO00/66206. Further, methods for delivering nucleic acid constructs can be adapted from those used in delivering other oligonucleotides (e.g., an antisense oligonucleotide) by inhalation, such as described in Templin et al., Antisense Nucleic Acid Drug Dev, 2000, 10:359-68; Sandrasagra et al., Expert Opin
25 Biol Ther, 2001, 1:979-83; Sandrasagra et al., Antisense Nucleic Acid Drug Dev, 2002, 12:177-81. Formulations of cationic liposomes may be used for administration by inhalation, particular after aerosolization. Nanospheres of biodegradable polymers may also be used. The deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic delivery of nucleic acid
30 constructs.

In certain embodiments, particularly where systemic dosing with the nucleic acid construct is desired, the aerosoled RNAi constructs are formulated as microparticles. Microparticles having a diameter of between 0.5 and ten microns can penetrate the lungs, passing through most of the natural barriers. A diameter of less than ten microns is required to bypass the throat; a diameter of 0.5 microns or greater is required to avoid being exhaled.

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1. Antibody-mediated platelet phagocytosis by human macrophages is inhibited by siRNA specific for sequences in Fc γ RIIA.

Study Design:

Cell lines, platelets, and other reagents

The human monoblastic cell line, THP-1 was purchased from ATCC (Manassas, VA). Anti-HPA-1a allo-antiserum was obtained from the mother of an infant with neonatal alloimmune thrombocytopenia due to HPA-1a incompatibility. Human platelets were derived from a blood group "O" Rh⁺ donor with the approval of the Institutional Review Board of Yale University School of Medicine.

SiRNA and transfection

According to the Elbashir's criteria ¹¹, siRNAs were chosen for the present study (Table 1). SiRNAs were selected so as to either hybridize only to Fc γ RIIA or so as to hybridize to both Fc γ RIIA and Fc γ RIIB. Sense and anti-sense RNAs were synthesized. Double strand RNAs (dsRNAs) were made

followed by transfection with TransMessenger reagent according to annealing instructions from Qiagen-Xeragon, Germantown, MD. For the transfection of dsRNA, 6×10^5 THP-1 cells were plated into 6-well plates 24 hours prior to the transfection.

5

Analysis of protein expression

The THP-1 cellular proteins were isolated 48 hours after siRNA transfection. Northern blotting was performed to detect and quantify the levels of Fc γ RIIA and Fc γ RIIB transcripts.

10

Phagocytosis *in vitro* assay

Platelets at 10^8 cells/ml in EDTA buffer (2mM EDTA, 0.1 % BSA in PBS) were incubated in 10 μ m CFDA at 20°C for 30 minutes, washed and incubated in 1:5 (V:V) test serum for 30 minutes. The platelets were washed and resuspended in M199 at 3×10^8 /ml before exposure to THP-1 cells in a ratio of 300:1 platelets:THP-1 for 2 hours. After washing 3 times with EDTA buffer, the cells were re-suspended in 1% paraformaldehyde in EDTA buffer and analyzed by flow cytometry (FACScan, BD Biosciences, San Jose, (CA). Immediately before the FACS analysis, the surface bound fluorescence on the cells were quenched by adding 0.2% Trypan Blue^{16; 17}

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Results and discussion

Cell-based platelet phagocytosis

Phagocytosis of antibody-coated platelets by human granulocytes *in vitro* was first reported by Handin *et al*¹⁸. To quantify the uptake of platelets by neutrophils, serum-treated platelets were labeled with ⁵¹Cr, and the radioactivity was measured in the washed leukocyte pellet after incubation. A phagocytosis assay of antibody sensitized platelets by primary monocytes was

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recently developed by using fluorescein diacetate-labeled platelets^{19; 20}. However, false positive results were frequent due to non-specific binding. In this study, we employed the THP-1 cells to establish a cell-based platelet phagocytosis assay, because THP-1 cells have the ability to undergo phagocytosis and constitutively express Fc γ R I and IIA and IIB^{21; 22}. When THP-1 cells were co-incubated with anti-HPA-1a antibody coated, CFDA-labeled platelets at 37°C, the positive population was 46%. After quenching the surface fluorescence with Trypan Blue immediately prior to analysis, the positive population was still 33%. In the control group, the co-incubation of THP-1 with CFDA-labeled, normal serum coated platelets at 37°C in our experiments showed only 6.6% non-specific bright population, though aged platelets can be cleared by macrophage cells via a scavenger receptor²³. This indicated that non-specific platelet phagocytosis by THP-1 cells was minimal in this assay system. Fluorescent microscopy confirmed that CFDA-labeled platelets were located in the intracellular space of cells in the sorted positive THP-1 cells. The result was reproducible in five separate experiments. Thus, this assay is reliable for quantitation of antibody-mediated platelet phagocytosis. This assay provides a useful model to study the intracellular pathway of Fc-receptor mediated platelet phagocytosis. It can also be used for clinically evaluating patients with pathologic anti-platelet antibodies as well as those with other immune diseases involving phagocytosis in the pathogenic mechanism.

RNAi down-regulation of Fc γ RIIA protein expression

We transfected designed Fc γ RIIA-targeted siRNAs into THP-1 cells to test if such siRNAs can down-regulate the Fc γ RIIA protein expression, and also to test whether siRNAs could selectively downregulate Fc γ RIIA while leaving Fc γ RIIB relatively unaffected. Results shown in Table 2 indicate the Fc γ RIIA siRNA can specifically knockdown Fc γ RIIA transcript expression *in vitro*.

Inhibition of anti-platelet antibody-mediated platelet phagocytosis by THP-1 cells by Fc γ RIIA dsRNAs

Based on above results, we then tested whether or not Fc γ RIIA dsRNAs could interfere with the phagocytotic function of THP-1 cells. Figure 3 shows that Fc γ RIIA dsRNA treated THP-1 cells decreased the ability to ingest antibody-coated platelets substantially. These results indicate that Fc γ RIIA is important for antibody-mediated platelet phagocytosis. Targeting Fc γ RIIA is expected to be a potential pharmacologic intervention for immune thrombocytopenia. Targeting Fc γ RIIA with siRNA may also be applied to other disease condition of antibody-mediated phagocytosis. Moreover, knockdown of a given gene with siRNAs combining a cell-based assay system offers an important strategy for human functional genomics, like the studies of gene function in gene knockout animals. In conclusion, we report that Fc γ RIIA siRNA specifically inhibits antibody-mediated platelet phagocytosis in human system *in vitro*.

ATTY. DKT. No. YU-PWO-029

Table 2: FcγRIIA Target Sequences and Effects on Phagocytosis

Probe:	Sequence	SEQ ID NO:	Effect on mRNA	Effect on phagocytosis
5	CD32IIA-A	AAACTTGAGCCCCCGTGGATC	0	0
	CD32IIA-B	AAATTGAGCCACCTGGACGTC	0	0
	CD32IIA-C	AAAGAGACAACCTTGAAGAAAC	+	+
	CD32IIA-D	AAACCATCATGCTGAGGTGCC	+	0
	CD32IIA-E	AACCATCATGCTGAGGTGCCA	+++	+
	CD32IIA-F	AAATTCTCCCGTTTGGATCCC	+++	+
10	CD32IIA-G	AAATCTCCCGTTTGGATCCCA	+	++
	CD32IIA-H	AAACCCGCCCTCCCAGGTTAA	+++	++
	CD32IIA-I	AACTTCTGGCCTCTAGCGATC	++	nt
	CD32IIA-J	AAGTGCTGGGATGACCAAGCAT	++	nt
15	CD32IIA-K	AATGTCAGCCCTCTTTAACAT	++	nt
	CD32IIA-L	AACATCTTCTTTCCTATGCCC	++	nt

Key:
“0” = <10% inhibition
“+” = 10-30% inhibition
“++” = 31-60% inhibition
“+++” = >60% inhibition
“nt” = not tested

Reference List for Example 1:

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What is claimed is:

1. An RNAi construct for inhibiting the expression of Fc γ RIIA, comprising
 - (a) an antisense polynucleotide strand that hybridizes to at least a portion of a Fc γ RIIA transcript and inhibits Fc γ RIIA expression; and
 - 5 (b) a sense polynucleotide that hybridizes to said antisense polynucleotide.
2. The RNAi construct of claim 1, wherein the double-stranded nucleic acid is a hairpin nucleic acid.
3. The RNAi construct of claim 1, wherein the antisense strand and the sense strand form a double helix of about 19 to about 30 base pairs in length.
- 10 4. The RNAi construct of claim 1, wherein the antisense polynucleotide strand is complementary to a sequence of the human Fc γ RIIA mRNA of SEQ ID NO:1.
5. The RNAi construct of claim 4, wherein the RNA antisense polynucleotide strand is complementary to at least 7 nucleotides of a sequence selected from the group consisting of: SEQ ID Nos. 4-13.
- 15 6. The RNAi construct of claim 4, wherein the RNA antisense polynucleotide strand consists of a sequence that is complementary to a sequence selected from the group consisting of: SEQ ID Nos. 4-13.
7. The RNAi construct of claim 4, wherein greater than 50% of the nucleotides of the antisense strand are RNA.
- 20 8. The RNAi construct of claim 4, wherein the antisense strand comprises one or more modifications selected from the group consisting of:
 - (a) a modification to the sugar-phosphate backbone;
 - (b) a modification to a base portion of a nucleotide; and
 - (c) a conjugated hydrophobic moiety.
- 25 9. The double-stranded nucleic acid of claim 4, wherein the sense strand comprises one or more modifications selected from the group consisting of:
 - (a) a modification to the sugar-phosphate backbone;

(b) a modification to a base portion of a nucleotide; and

(c) a conjugated hydrophobic moiety.

10. A pharmaceutical preparation for delivery of an RNAi construct to an organism, the composition comprising a pharmaceutically acceptable carrier and an
5 RNAi construct that inhibits expression of Fc γ R1IA.

11. The pharmaceutical preparation of claim 10, wherein the RNAi construct comprises:

(a) an antisense polynucleotide strand that hybridizes to at least a portion of a Fc γ R1IA transcript and inhibits Fc γ R1IA expression; and

10 (b) a sense polynucleotide that hybridizes to said antisense polynucleotide.

12. The pharmaceutical preparation of claim 11, wherein the antisense polynucleotide strand is complementary to at least 7 nucleotides of a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

13. The pharmaceutical preparation of claim 11, wherein the antisense
15 polynucleotide strand consists of a sequence that is complementary to a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

14. The pharmaceutical preparation of claim 11, , wherein greater than 50% of the nucleotides of the antisense strand are RNA.

15. The pharmaceutical preparation of claim 11, wherein the antisense strand
20 comprises one or more modifications selected from the group consisting of:

(a) a modification to the sugar-phosphate backbone;

(b) a modification to a base portion of a nucleotide; and

(c) a conjugated hydrophobic moiety.

16. The pharmaceutical preparation of claim 11, wherein the sense strand
25 comprises one or more modifications selected from the group consisting of:

(a) a modification to the sugar-phosphate backbone;

(b) a modification to a base portion of a nucleotide; and

(c) a conjugated hydrophobic moiety.

17. The pharmaceutical preparation of claim 10, wherein the preparation is suitable for administration by inhalation.

18. A method for decreasing expression of Fc γ R1IA in a cell, the method
5 comprising contacting the cell with an RNAi construct which comprises:

(a) an antisense polynucleotide strand that hybridizes to at least a portion of a Fc γ R1IA transcript and inhibits Fc γ R1IA expression; and

(b) a sense polynucleotide that hybridizes to said antisense polynucleotide.

19. The method of claim 18, wherein the antisense polynucleotide strand is
10 complementary to a sequence of the human Fc γ R1IA mRNA of SEQ ID NO:1.

20. The method of claim 18, wherein the antisense polynucleotide strand is complementary to at least 7 nucleotides of a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

21. The method of claim 18, wherein the antisense polynucleotide strand consists
15 of a sequence that is complementary to a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

22. A method for decreasing expression of Fc γ R1IA in one or more cells of an individual, the method comprising administering to the individual a composition comprising an RNAi construct which comprises:

20 (a) an antisense polynucleotide strand that hybridizes to at least a portion of a Fc γ R1IA transcript and inhibits Fc γ R1IA expression; and

(b) a sense polynucleotide that hybridizes to said antisense polynucleotide.

23. The method of claim 22, wherein the antisense polynucleotide strand is complementary to a sequence of the human Fc γ R1IA mRNA of SEQ ID NO:1.

24. The method of claim 22, wherein the antisense polynucleotide strand is
25 complementary to at least 7 nucleotides of a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

25. The method of claim 22, wherein the antisense polynucleotide strand consists of a sequence that is complementary to a sequence selected from the group consisting of: SEQ ID Nos. 4-13.

26. The method of claim 22, wherein the individual has a condition associated
5 with excess Fc receptor activity.

27. The method of claim 22, wherein the individual has asthma or experiences symptoms associated with asthma or allergic rhinitis.

28. The method of claim 27, wherein the composition comprising the RNAi construct is administered by a mode selected from the group consisting of:
10 inhalation, topical and intravenous.

29. The method of claim 22, wherein the individual has an immune cytopenia or a heparin induced thrombocytopenia.

30. The RNAi construct of claim 1, wherein the construct does not substantially inhibit Fc γ RIIB expression.

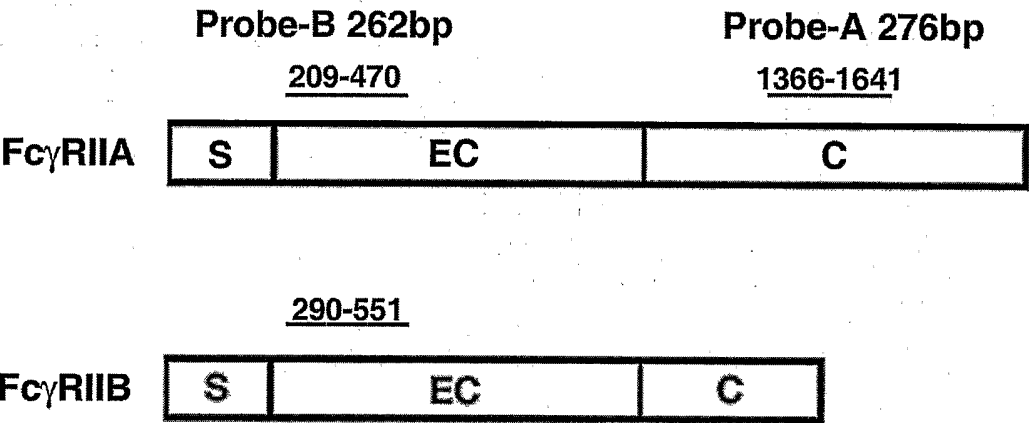


Figure 1

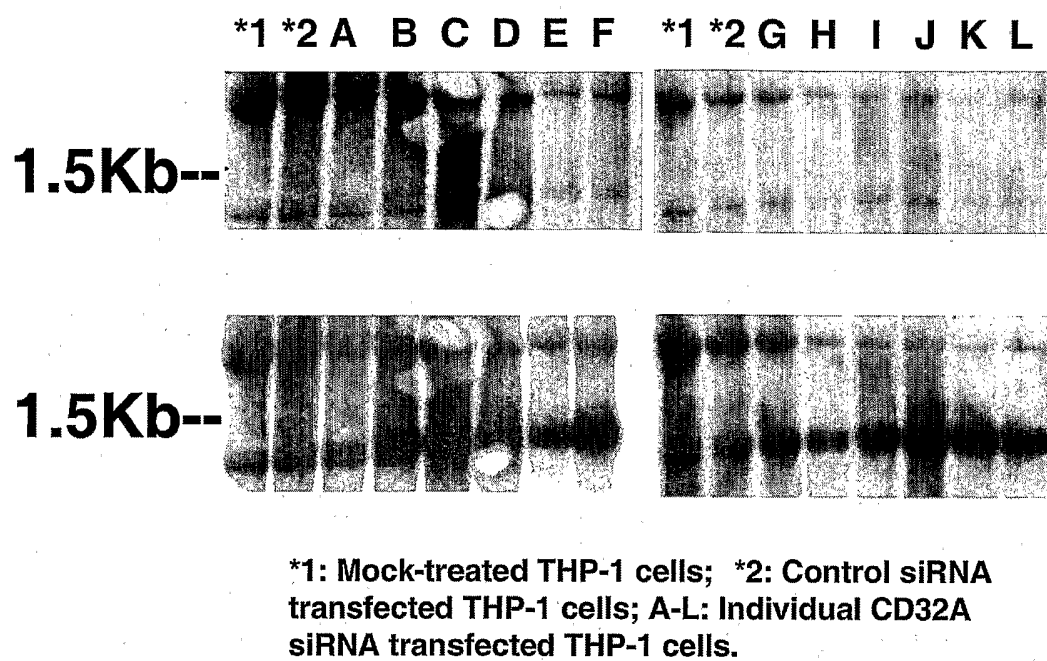
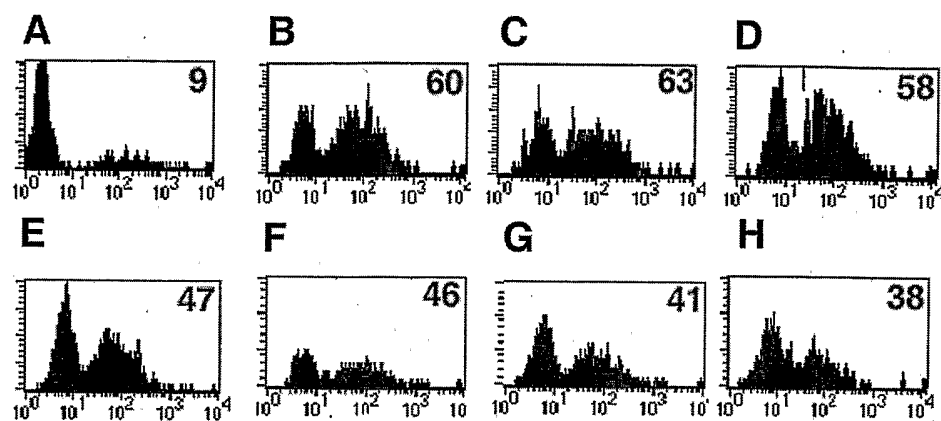


Figure 2



A: Control without anti-platelet antibody; B: THP-1 with antibody-coated platelet; C: Mock-treated THP-1 with antibody-coated platelets; D: Control-siRNA treated THP-1 with antibody-coated platelets; E, F, G: Individual CD32A-siRNA (e,f,h) treated THP-1 with antibody-coated platelets; H: Syk-siRNA treated THP-1 with antibody-coated platelets.

Figure 3