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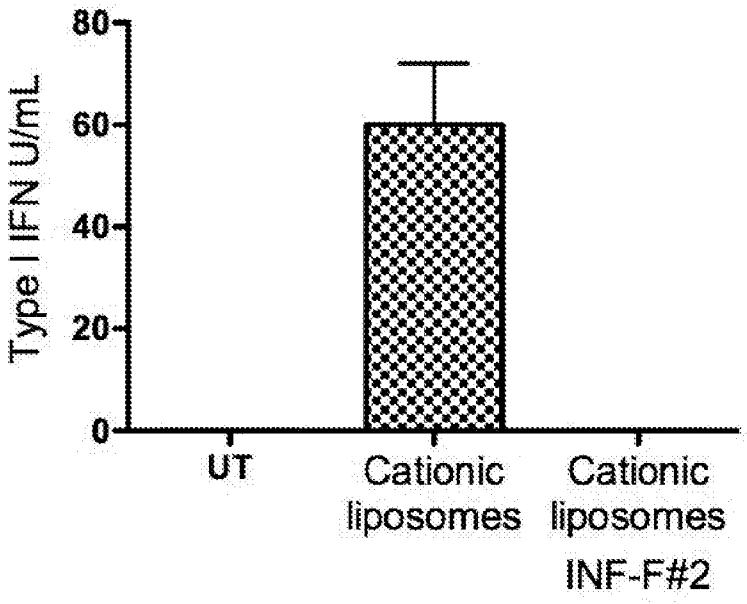
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(54) Title: PEPTIDES HAVING IMMUNE SUPPRESIVE DOMAINS FOR TRANSFECTION

(57) Abstract: The present invention relates to a use of a peptide, comprising an immune suppressive domain, for transfection. In particular, the present invention relates to the use of the peptide for immune suppression in a transfection mix, wherein the peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

Figure 1



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PEPTIDES HAVING IMMUNE SUPPRESIVE DOMAINS FOR TRANSFECTION

The present invention relates to a use of a peptide, comprising an immune suppressive domain, for introducing entities, such as foreign entities, to cells. The invention further relates to a use of a peptide, comprising an immune suppressive domain, for transfection. In particular, the present invention relates to the use of the peptide for immune suppression in a transfection mix.

Technical Background

Transfection is the process of deliberately introducing nucleic acids into cells. The term is used notably for non-viral methods in eukaryotic cells. Transfection is used for the introduction of DNA plasmids into live cells with the purpose of expressing either proteins or silencing / interfering RNA encoded in the plasmid. Transfection is also used to introduce immune-stimulatory DNA or RNA into cells with the purpose of studying aspects of immune reactions to DNA or RNA. Further, transfection can be used to introduce non-DNA non-RNA molecules into cells, which directly interfere with cell function.

The International patent application WO 98/40502 discloses transfection compositions in which a peptide is covalently linked to a transfection agent. The application purports that enhanced transfection efficiency is obtained.

The International patent application WO 2009/79635 discloses a delivery system for delivering interfering RNA molecules into a cell and methods for using the delivery system.

Membrane fusion and STING pathway

Membrane disturbance as induced by fusion induces type I interferon responses with expression of interferon-stimulated genes, *in vivo* recruitment of leukocytes and potentiation of signaling via Toll-like receptor 7 (TLR7) and TLR9. The fusion-dependent response is dependent on the stimulator of interferon genes STING. Membrane disturbance can be induced by fusion of cellular membranes with viral envelopes, cellular membranes of other cells, and fusogenic liposomes.

The molecule referred to as STING (stimulator of interferon genes) also known as MITA/MPYS/ERIS is also essential for cytosolic DNA-mediated type I IFNs induction. STING contains multi-putative transmembrane regions in the amino terminal region, and is found to associate with membranes. The existence of immune suppressive domains in the viral fusion proteins is expected to insert the immune suppressive activity partly through interference with this pathway either through direct or indirect interaction with STING. Hence an antagonist of this putative interaction will enhance the immune responses to proteins containing such immune suppressive domains and can be used as adjuvants.

Increased cellular production of type I IFNs and other cytokines in response to treatment with liposome based transfection reagent is a considerable problem. In the cases of introducing plasmids into live cells, the reaction to the transfection reagent itself (the increased production of type I IFN and cytokines) can result in undesirable responds to the introduced plasmid. Further, when studying immune responses to DNA or RNA, production of type I IFN and other cytokines are important measures for such responses. Therefore, for the transfection reagent itself to induce of type I IFN and other cytokines is undesirable. In some cases, the response to the transfection reagent itself overwhelms the response to the delivered DNA or RNA.

Typically, in viruses one or mores transmembrane glycoproteins, fusion proteins, undergo a conformational transition triggered by receptor recognition or low pH, leading to the insertion of a fusion peptide into the plasma membrane or the membrane of an endocytic vesicle. For some viruses, for example members of the paramyxovirus family, separate envelope proteins mediate attachment and fusion.

Membrane fusion can occur either at the plasma membrane or at an intracellular location following internalization of virus by receptor-mediated endocytosis. Fusion is mediated by viral transmembrane proteins known as fusion proteins. Upon appropriate triggering, the fusion protein interacts with the target membrane through a hydrophobic fusion peptide and undergoes a conformational change that drives the membrane fusion reaction. There are a variety of fusion triggers, including various combinations of receptor binding, receptor/coreceptor binding, and exposure to the mildly acidic pH within the endocytic pathway. Fusion proteins from different viruses have different names in spite of the common functionality.

Based on important structural features, many virus membrane fusion proteins are currently annotated to either the ''class I'' membrane fusion proteins exemplified by the influenza hemagglutinin (HA) or HIV-1 gp41, or the ''class II'' proteins of the alphaviruses and flaviviruses. The alphaviruses and flaviviruses are members of the Togaviridae and Flaviviridae families, respectively. These small enveloped positive-sense RNAviruses are composed of a capsid protein that assembles with the RNA into the nucleocapsid, and a lipid bilayer containing the viral transmembrane (TM) proteins.

Class I fusion proteins are synthesized as single chain precursors, which then assemble into trimers. The polypeptides are then cleaved by host proteases, which is an essential step in rendering the proteins fusion competent. This proteolytic event occurs late in the biosynthetic process because the fusion proteins, once cleaved are metastable and readily activated. Once activated, the protein refolds into a highly stable conformation. The timing of this latter event is of crucial importance in the fusion process. Maintenance of the intact precursor polypeptide during folding and assembly of the oligomeric structure is essential if the free energy that is released during the refolding event is to be available to overcome the inherent barriers to membrane fusion. The new amino-terminal region that is created by the cleavage event contains a hydrophobic sequence, which is known as the fusion peptide. The authentic carboxy-terminal region of the precursor polypeptide contains the transmembrane anchor. In the carboxy-terminal polypeptide, there are sequences known as the heptad repeat that are predicted to have an alpha helical structure and to form a coiled coil structure. These sequences participate in the formation of highly stable structure that characterizes the post-fusion conformation of the fusion protein.

The class II fusion proteins are elongated finger-like molecules with three globular domains composed almost entirely of ß-sheets. Domain I is a ß-barrel that contains the N-terminus and two long insertions that connect adjacent ß-strands and together form the elongated domain II. The first of these insertions contains the highly conserved fusion peptide loop at its tip, connecting the c and d ß-strands of domain II (termed the cd loop) and containing 4 conserved disulfide bonds including several that are located at the base of the fusion loop. The second insertion contains the ij loop at its tip, adjacent to the fusion loop, and one conserved disulfide bond at its base. A hinge region is located between domains I and II. A short linker region connects domain I to domain III, a ß-barrel with an immunoglobulin-like fold stabilized by three conserved disulfide bonds. In the full-length

molecule, domain III is followed by a stem region that connects the protein to the virus TM anchor. Fitting of the structure of alphavirus E1 to cryo-electron microscopy reconstructions of the virus particle reveals that E1 is located almost parallel to the virus membrane, and that E1–E1 interactions form the an icosahedral lattice.

Fusion peptides

Fusion peptides are moderately hydrophobic segments of viral and non-viral membrane fusion proteins that enable these proteins to disrupt and connect two closely apposed biological membranes. This process, which results in membrane fusion occurs in a well-controlled manner with a surprisingly small amount of leakage of the contents of the encapsulated volumes to the outside world. The sequences of fusion peptides are highly conserved within different groups of fusion proteins, for example within different virus families, but not between them. Most fusion peptides are located at the extreme N-termini of the transmembrane subunits of the fusion proteins. However, in a few cases such as the sperm protein fertilin-α, vesicular stomatitis virus G, baculovirus gp64, and Rous sarcoma virus gp37, internal fusion peptides have been found. Deletion of the fusion peptide and, in many cases, even relatively conservative single amino acid changes in the fusion peptide completely abolish the ability of fusion proteins to fuse membranes, while other structural and functional properties of these proteins may remain intact. Conversely, single amino acid changes in many other regions of these proteins are less deleterious to their function. Such mutagenesis experiments clearly point to a central role of the fusion peptides in membrane fusion. It has further been shown in a number of cases that even isolated fusion peptides alone can support membrane fusion in model systems. (Tamm and Han, Bioscience Reports, Vol. 20, No. 6, 2000).

Immune suppressive domains - Immunosuppressive properties of enveloped viruses

Fusion proteins of a subset of enveloped Type I viruses (retrovirus, lentivirus and filoviruses) have previously been shown to feature an immune suppressive activity. Inactivated retroviruses are able to inhibit proliferation of immune cells upon stimulation. Expression of these proteins is enough to enable allogenic cells to grow to a tumor in immune competent mice. In one study, introduction of ENV expressing construct into MCA205 murine tumor cells, which do not proliferate upon s.c. injection into an allogeneic host, or into CL8.1 murine tumor cells (which overexpress class I antigens and are rejected in a syngeneic host) resulted in tumor growth in both cases. Such immunosuppressive domains have been found in a variety of different viruses with type 1 fusion mechanism such as gamma-retroviruses like Mason pfeizer monkey virus (MPMV) and murine leukemia virus (MLV), lentiviruses such as HIV and in filoviruses such as Ebola and Marburg viruses.

This immune suppressive activity was in all cases located to a very well-defined structure within the class I fusion proteins, more precisely at the bend in the heptad repeat just N-terminale of the transmembrane structure in the fusion protein. The immunosuppressive effects range from significant inhibition of lymphocyte proliferation, cytokine skewing (up regulating IL-10; down regulating TNF-α, IL-12, IFN-γ) and inhibition of monocytic burst to cytotoxic T cell killing. Importantly, peptides spanning ISD in these assays must either be linked as dimers or coupled to a carrier (i.e. >monomeric) to be active. Such peptides derived from immune-suppressive domains are able to reduce or abolish immune responses such as cytokine secretion or proliferation of T-cells upon stimulation. The protection mediated by the immunosuppressive properties of the fusion protein from the immune system of the host is not limited to the fusion protein but covers all the viral

envelope proteins displayed at viral or cellular membranes in particular also the protein mediating attachment of the virus to the cell.

Co-location of the immunosuppression domain and the fusion domain

The immunosuppressive domains of viruses like but not limited to retro-, lenti-, Orthomyxo-, flavi- and filoviruses overlap structurally important parts of the fusion subunits of the surface glycoproteins. In several cases the primary structure (sequence) of the ISD can vary greatly from virus to virus, but the secondary structure, which is very well preserved among different virus families, is that of an alpha helix that bends in different ways during the fusion process This structure plays a crucial role during events that result in fusion of viral and cellular membranes. It is evident that the immunosuppressive domains of these (retroviral, lentiviral and filoviral) class I fusion proteins overlap with a very important protein structure needed for the fusion mechanistic function.

The energy needed for mediating the fusion of viral and cellular membranes is stored in the fusion proteins, which are thus found in a meta-stable conformation on the viral surface. Once the energy is released to drive the fusion event, the protein will find its most energetically stable conformation. In this regard fusion proteins can be compared with loaded springs that are ready to be sprung. This high energy conformation makes the viral fusion proteins very susceptible to modifications; Small changes in the primary structure of the protein often result in the protein to be folded in its stable post fusion conformation. The two conformations present very different tertiary structures of the same protein.

It has been shown in the case of simple retroviruses that small structural changes in the envelope protein are sufficient to remove the immune suppressive effect without changing structure and hence the antigenic profile.

The mutated non-immune suppressive envelope proteins are much better antigens for vaccination. The proteins can induce a 30-fold enhancement of anti-env antibody titers when used for vaccination and are much better at launching an effective CTL response. Furthermore, viruses that contain the non-immunosuppressive form of the friend murine leukemia virus envelope protein, although fully infectious in irradiated immunocompromised mice cannot establish an infection in immunocompetent animals. Interestingly in the latter group the non-immunosuppressive viruses induce both a higher cellular and humeral immune response, which fully protect the animals from subsequent challenge by wild type viruses.

Immunosuppressive domains in the fusion proteins (viral envelope proteins) from Retroviruses, lentiviruses and Filoviruses have been known since 1985 for retrovirus, since 1988 for lentivirus and since 1992 for filoviruses. These viruses, as mentioned above, all belong to enveloped RNA viruses with a type I fusion mechanism. The immunosuppressive domains of lentivirus, retroviruses and filoviruses show large structural similarity. Furthermore the immunosuppressive domain of these viruses are all located at the same position in the structure of the fusion protein, more precisely in the linker between the two heptad repeat structures just N-terminal of the transmembrane domain in the fusion protein. These heptad repeat regions constitute two alpha helices that play a critical role in the active mechanism of membrane fusion by these proteins. The immune suppressive domains can be located in relation to two well conserved cystein residues that are found in these structures. These cystein residues are between 4 and 6 amino acid residues from one another and in many cases are believed to form disulfide bridges that stabilize the fusion proteins. The immune

suppressive domains in all three cases include at least some of the first 22 amino acids that are located N-terminal to the first cysteine residue. Recently the immunosuppressive domains in the fusion protein of these viruses have been successfully altered in such a way that the fusogenic properties of the fusion protein have been preserved. Such mutated fusion proteins with decreased immunosuppressive properties have been shown to be superior antigens for vaccination purposes.

Other immunosuppressive domains are found in type II fusion proteins. Immunosuppressive domains have been identified at different positions in different groups of viruses. For example an immune suppressive domain might co-localize with the fusion peptide exemplified by the identification of an common immunosuppressive domain in the fusion peptide of Flavirius (Dengue virus, west Nile virus etc), or with the hydrophobic alpha helix N-terminal of the transmembrane domain in the fusion protein exemplified by the finding of an immunosuppressive domain in said helixes of all flaviridae e.g. Hepatitis C virus, Dengue, west nile etc.

The immune suppressive domains can also be located in the fusion peptide of the fusion protein among enveloped RNA viruses with type I fusion mechanism. For example HIV or influenza A and B types have an immune suppressive domain that co-localized with their fusion peptide.

immunosuppressive domains are identified among enveloped RNA viruses with type II fusion mechanism at different positions in different groups of viruses:

i. Co-localizing with the fusion peptide exemplified by the identification of an common immunosuppressive domain in the fusion peptide of Flavirius (Dengue virus, west Nile virus etc), and
ii. In the hydrophobic alpha helix N-terminal of the transmembrane domain in the fusion protein exemplified by the finding of an immunosuppressive domain in said helixes of all flaviridae e.g. Hepatitis C virus, Dengue, west nile etc.

2: Immunosuppressive domains have been identified in the fusion protein among enveloped RNA viruses with type I fusion mechanism. This position co-localizes with the fusion peptide of said fusion protein as demonstrated by the identification of a common immunosuppressive domain in the fusion peptide of all Influenza A and B types as well as HIV.

Functional homolog

The term "functional homologue" or "functional equivalent" refers to homologues of the molecules according to the present invention and is meant to comprise any molecule which is capable of mimicking the function of molecules as described herein. Thus, the terms refer to functional similarity or, interchangeably, functional identity, between two or more molecular entities. The term "functional homology" is further used herein to describe that one molecular entity are able to mimic the function of one or more molecular entities.

Functional homologues according to the present invention may comprise any molecule that can function as an antagonist of the immune suppressive activity exerted by an immune suppressive domains. Such a molecule when added to the composition containing said immune suppressive domains reduces the immune suppressive activity exerted by the latter in either an in vitro test

system (e.g. CTLL-2 or PBMC proliferation assays) or in vivo seen as an enhanced T- and/or B-cell responses.

Functional homologues according to the present invention may comprise polypeptides with an amino acid sequence, which are sharing at least some homology with the predetermined polypeptide sequences as outlined herein. For example such polypeptides are at least about 40 percent, such as at least about 50 percent homologous, for example at least about 60 percent homologous, such as at least about 70 percent homologous, for example at least about 75 percent homologous, such as at least about 80 percent homologous, for example at least about 85 percent homologous, such as at least about 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous with the predetermined polypeptide sequences as outlined herein above. The homology between amino acid sequences may be calculated using well known algorithms such as for example any one of BLOSUM 30, BLOSUM 40, BLOSUM 45, BLOSUM 50, BLOSUM 55, BLOSUM 60, BLOSUM 62, BLOSUM 65, BLOSUM 70, BLOSUM 75, BLOSUM 80, BLOSUM 85, and BLOSUM 90.

Functional homologues may comprise an amino acid sequence that comprises at least one substitution of one amino acid for any other amino acid. For example such a substitution may be a conservative amino acid substitution or it may be a non-conservative substitution. A conservative amino acid substitution is a substitution of one amino acid within a predetermined group of amino acids for another amino acid within the same group, wherein the amino acids within predetermined groups exhibit similar or substantially similar characteristics. Within the meaning of the term "conservative amino acid substitution" as applied herein, one amino acid may be substituted for another within groups of amino acids characterized by having

i) hydrophilic (polar) side chains (Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, Tyr, and Cys,)
ii) hydrophobic (non-polar) side chains (Gly, Ala, Val, Leu, Ile, Phe, Trp, Pro, and Met)
iii) aliphatic side chains (Gly, Ala Val, Leu, Ile)
iv) cyclic side chains (Phe, Tyr, Trp, His, Pro)
v) aromatic side chains (Phe, Tyr, Trp)
vi) acidic side chains (Asp, Glu)
vii) basic side chains (Lys, Arg, His)
viii) amide side chains (Asn, Gln)
ix) hydroxy side chains (Ser, Thr)
x) sulphor-containing side chains (Cys, Met), and
xi) amino acids being monoamino-dicarboxylic acids or monoamino-monocarboxylic-monoamidocarboxylic acids (Asp, Glu, Asn, Gln).

Non-conservative substitutions are any other substitutions. A non-conservative substitution leading to the formation of a functional homologue would for example i) differ substantially in

hydrophobicity, for example a hydrophobic residue (Val, Ile, Leu, Phe or Met) substituted for a hydrophilic residue such as Arg, Lys, Trp or Asn, or a hydrophilic residue such as Thr, Ser, His, Gln, Asn, Lys, Asp, Glu or Trp substituted for a hydrophobic residue; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Functional homologues according to the present invention may comprise more than one such substitution, such as e.g. two amino acid substitutions, for example three or four amino acid substitutions, such as five or six amino acid substitutions, for example seven or eight amino acid substitutions, such as from 10 to 15 amino acid substitutions, for example from 15 to 25 amino acid substitution, such as from 25 to 30 amino acid substitutions, for example from 30 to 40 amino acid substitution, such as from 40 to 50 amino acid substitutions, for example from 50 to 75 amino acid substitution, such as from 75 to 100 amino acid substitutions, for example more than 100 amino acid substitutions. The addition or deletion of an amino acid may be an addition or deletion of from 2 to 5 amino acids, such as from 5 to 10 amino acids, for example from 10 to 20 amino acids, such as from 20 to 50 amino acids. However, additions or deletions of more than 50 amino acids, such as additions from 50 to 200 amino acids, are also comprised within the present invention. The polypeptides according to the present invention, including any variants and functional homologues thereof, may in one embodiment comprise more than 5 amino acid residues, such as more than 10 amino acid residues, for example more than 20 amino acid residues, such as more than 25 amino acid residues, for example more than 50 amino acid residues, such as more than 75 amino acid residues, for example more than 100 amino acid residues, such as more than 150 amino acid residues, for example more than 200 amino acid residues.

Genetic code

The genetic code is the set of rules by which information encoded within genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences) by living cells. Biological decoding is accomplished by the ribosome, which links amino acids in an order specified by mRNA, using transfer RNA (tRNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time. The genetic code is highly similar among all organisms, and can be expressed in a simple table with 64 entries.

The code defines how sequences of these nucleotide triplets, called codons, specify which amino acid will be added next during protein synthesis. With some exceptions a three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. Because the vast majority of genes are encoded with exactly the same code (see the RNA codon table), this particular code is often referred to as the canonical or standard genetic code, or simply the genetic code, though in fact some variant codes have evolved. For example, protein synthesis in human mitochondria relies on a genetic code that differs from the standard genetic code.

Not all genetic information is stored using the genetic code. All organisms' DNA contains regulatory sequences, intergenic segments, chromosomal structural areas, and other non-coding DNA that can

contribute greatly to phenotype. Those elements operate under sets of rules that are distinct from the codon-to-amino acid paradigm underlying the genetic code.

Genetically encoded amino acids are as described below. Any other amino acid except for the 20 described below is considered a non-genetically encoded amio acid.

Amino acid	Codons	Compressed	Amino acid	Codons	Compressed
Ala/A	GCU, GCC, GCA, GCG	GCN	**Leu/L**	UUA, UUG, CUU, CUC, CUA, CUG	YUR, CUN
Arg/R	CGU, CGC, CGA, CGG, AGA, AGG	CGN, MGR	**Lys/K**	AAA, AAG	AAR
Asn/N	AAU, AAC	AAY	**Met/M**	AUG	
Asp/D	GAU, GAC	GAY	**Phe/F**	UUU, UUC	UUY
Cys/C	UGU, UGC	UGY	**Pro/P**	CCU, CCC, CCA, CCG	CCN
Gln/Q	CAA, CAG	CAR	**Ser/S**	UCU, UCC, UCA, UCG, AGU, AGC	UCN, AGY
Glu/E	GAA, GAG	GAR	**Thr/T**	ACU, ACC, ACA, ACG	ACN
Gly/G	GGU, GGC, GGA, GGG	GGN	**Trp/W**	UGG	
His/H	CAU, CAC	CAY	**Tyr/Y**	UAU, UAC	UAY
Ile/I	AUU, AUC, AUA	AUH	**Val/V**	GUU, GUC, GUA, GUG	GUN

D- and L-Amino acids

Of the standard α-amino acids, all but glycine can exist in either of two enantiomers, called L or D amino acids, which are mirror images of each other. While L-amino acids represent all of the amino acids found in proteins during translation in the ribosome, D-amino acids are found in some proteins produced by enzyme posttranslational modifications after translation and translocation to the endoplasmic reticulum, as in exotic sea-dwelling organisms such as cone snails. They are also abundant components of the peptidoglycan cell walls of bacteria, and D-serine may act as a neurotransmitter in the brain. The L and D convention for amino acid configuration refers not to the optical activity of the amino acid itself, but rather to the optical activity of the isomer of glyceraldehyde from which that amino acid can, in theory, be synthesized (D-glyceraldehyde is dextrorotary; L-glyceraldehyde is levorotatory).

Lipids

Fatty acids

Fatty acids, or fatty acid residues when they form part of a lipid, are a diverse group of molecules synthesized by chain-elongation of an acetyl-CoA primer with malonyl-CoA or methylmalonyl-CoA

groups in a process called fatty acid synthesis. They are made of a hydrocarbon chain that terminates with a carboxylic acid group; this arrangement confers the molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water. The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building-block of more structurally complex lipids. The carbon chain, typically between four and 24 carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen, and sulfur. Where a double bond exists, there is the possibility of either a cis or trans geometric isomerism, which significantly affects the molecule's configuration. Cis-double bonds cause the fatty acid chain to bend, an effect that is compounded with more double bonds in the chain. This in turn plays an important role in the structure and function of cell membranes. Most naturally occurring fatty acids are of the cis configuration, although the trans form does exist in some natural and partially hydrogenated fats and oils.

Examples of biologically important fatty acids are the eicosanoids, derived primarily from arachidonic acid and eicosapentaenoic acid, that include prostaglandins, leukotrienes, and thromboxanes. Docosahexaenoic acid is also important in biological systems, particularly with respect to sight. Other major lipid classes in the fatty acid category are the fatty esters and fatty amides. Fatty esters include important biochemical intermediates such as wax esters, fatty acid thioester coenzyme A derivatives, fatty acid thioester ACP derivatives and fatty acid carnitines. The fatty amides include N-acyl ethanolamines, such as the cannabinoid neurotransmitter anandamide.

Glycerolipids

Glycerolipids are composed mainly of mono-, di-, and tri-substituted glycerols, the most well-known being the fatty acid triesters of glycerol, called triglycerides. The word "triacylglycerol" is sometimes used synonymously with "triglyceride", though the latter lipid contains no hydroxyl group. In these compounds, the three hydroxyl groups of glycerol are each esterified, typically by different fatty acids. Because they function as an energy store, these lipids comprise the bulk of storage fat in animal tissues. The hydrolysis of the ester bonds of triglycerides and the release of glycerol and fatty acids from adipose tissue are the initial steps in metabolising fat.

Additional subclasses of glycerolipids are represented by glycosylglycerols, which are characterized by the presence of one or more sugar residues attached to glycerol via a glycosidic linkage. Examples of structures in this category are the digalactosyldiacylglycerols found in plant membranes and seminolipid from mammalian sperm cells.

Glycerophospholipids

Glycerophospholipids, usually referred to as phospholipids, are ubiquitous in nature and are key components of the lipid bilayer of cells, as well as being involved in metabolism and cell signaling. Neural tissue (including the brain) contains relatively high amounts of glycerophospholipids, and alterations in their composition has been implicated in various neurological disorders. Glycerophospholipids may be subdivided into distinct classes, based on the nature of the polar headgroup at the sn-3 position of the glycerol backbone in eukaryotes and eubacteria, or the sn-1 position in the case of archaebacteria.

Examples of glycerophospholipids found in biological membranes are phosphatidylcholine (also known as PC, GPCho or lecithin), phosphatidylethanolamine (PE or GPEtn) and phosphatidylserine (PS or GPSer). In addition to serving as a primary component of cellular membranes and binding sites for intra- and intercellular proteins, some glycerophospholipids in eukaryotic cells, such as phosphatidylinositols and phosphatidic acids are either precursors of or, themselves, membrane-derived second messengers. Typically, one or both of these hydroxyl groups are acylated with long-chain fatty acids, but there are also alkyl-linked and 1Z-alkenyl-linked (plasmalogen) glycerophospholipids, as well as dialkylether variants in archaebacteria.

Sphingolipids

Sphingolipids are a complicated family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized de novo from the amino acid serine and a long-chain fatty acyl CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids and other compounds. The major sphingoid base of mammals is commonly referred to as sphingosine. Ceramides (N-acyl-sphingoid bases) are a major subclass of sphingoid base derivatives with an amide-linked fatty acid. The fatty acids are typically saturated or mono-unsaturated with chain lengths from 16 to 26 carbon atoms.

The major phosphosphingolipids of mammals are sphingomyelins (ceramide phosphocholines), whereas insects contain mainly ceramide phosphoethanolamines and fungi have phytoceramide phosphoinositols and mannose-containing headgroups. The glycosphingolipids are a diverse family of molecules composed of one or more sugar residues linked via a glycosidic bond to the sphingoid base. Examples of these are the simple and complex glycosphingolipids such as cerebrosides and gangliosides.

Sterol lipids

Sterol lipids, such as cholesterol and its derivatives, are an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins. The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signaling molecules. The eighteen-carbon (C18) steroids include the estrogen family whereas the C19 steroids comprise the androgens such as testosterone and androsterone. The C21 subclass includes the progestogens as well as the glucocorticoids and mineralocorticoids. The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure. Other examples of sterols are the bile acids and their conjugates, which in mammals are oxidized derivatives of cholesterol and are synthesized in the liver. The plant equivalents are the phytosterols, such as β-sitosterol, stigmasterol, and brassicasterol; the latter compound is also used as a biomarker for algal growth. The predominant sterol in fungal cell membranes is ergosterol.

Prenol lipids

Prenol lipids are synthesized from the five-carbon-unit precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid (MVA) pathway. The simple isoprenoids (linear alcohols, diphosphates, etc.) are formed by the successive addition of C5 units, and are classified according to number of these terpene units. Structures containing greater

than 40 carbons are known as polyterpenes. Carotenoids are important simple isoprenoids that function as antioxidants and as precursors of vitamin A. Another biologically important class of molecules is exemplified by the quinones and hydroquinones, which contain an isoprenoid tail attached to a quinonoid core of non-isoprenoid origin. Vitamin E and vitamin K, as well as the ubiquinones, are examples of this class. Prokaryotes synthesize polyprenols (called bactoprenols) in which the terminal isoprenoid unit attached to oxygen remains unsaturated, whereas in animal polyprenols (dolichols) the terminal isoprenoid is reduced.

Saccharolipids

Saccharolipids describe compounds in which fatty acids are linked directly to a sugar backbone, forming structures that are compatible with membrane bilayers. In the saccharolipids, a monosaccharide substitutes for the glycerol backbone present in glycerolipids and glycerophospholipids. The most familiar saccharolipids are the acylated glucosamine precursors of the Lipid A component of the lipopolysaccharides in Gram-negative bacteria. Typical lipid A molecules are disaccharides of glucosamine, which are derivatized with as many as seven fatty-acyl chains. The minimal lipopolysaccharide required for growth in E. coli is Kdo2-Lipid A, a hexa-acylated disaccharide of glucosamine that is glycosylated with two 3-deoxy-D-manno-octulosonic acid (Kdo) residues.

Polyketides

Polyketides are synthesized by polymerization of acetyl and propionyl subunits by classic enzymes as well as iterative and multimodular enzymes that share mechanistic features with the fatty acid synthases. They comprise a large number of secondary metabolites and natural products from animal, plant, bacterial, fungal and marine sources, and have great structural diversity. Many polyketides are cyclic molecules whose backbones are often further modified by glycosylation, methylation, hydroxylation, oxidation, and/or other processes. Many commonly used anti-microbial, anti-parasitic, and anti-cancer agents are polyketides or polyketide derivatives, such as erythromycins, tetracyclines, avermectins, and antitumor epothilones.

Biological functions in membranes

Eukaryotic cells are compartmentalized into membrane-bound organelles that carry out different biological functions. The glycerophospholipids are the main structural component of biological membranes, such as the cellular plasma membrane and the intracellular membranes of organelles; in animal cells the plasma membrane physically separates the intracellular components from the extracellular environment. The glycerophospholipids are amphipathic molecules (containing both hydrophobic and hydrophilic regions) that contain a glycerol core linked to two fatty acid-derived "tails" by ester linkages and to one "head" group by a phosphate ester linkage. While glycerophospholipids are the major component of biological membranes, other non-glyceride lipid components such as sphingomyelin and sterols (mainly cholesterol in animal cell membranes) are also found in biological membranes. In plants and algae, the galactosyldiacylglycerols, and sulfoquinovosyldiacylglycerol, which lack a phosphate group, are important components of membranes of chloroplasts and related organelles and are the most abundant lipids in photosynthetic tissues, including those of higher plants, algae and certain bacteria.

Bilayers have been found to exhibit high levels of birefringence, which can be used to probe the degree of order (or disruption) within the bilayer using techniques such as dual polarization interferometry and Circular dichroism.

A biological membrane is a form of lipid bilayer. The formation of lipid bilayers is an energetically preferred process when the glycerophospholipids described above are in an aqueous environment. This is known as the hydrophobic effect. In an aqueous system, the polar heads of lipids align towards the polar, aqueous environment, while the hydrophobic tails minimize their contact with water and tend to cluster together, forming a vesicle; depending on the concentration of the lipid, this biophysical interaction may result in the formation of micelles, liposomes, or lipid bilayers. Other aggregations are also observed and form part of the polymorphism of amphiphile (lipid) behavior. Phase behavior is an area of study within biophysics and is the subject of current academic research. Micelles and bilayers form in the polar medium by a process known as the hydrophobic effect. When dissolving a lipophilic or amphiphilic substance in a polar environment, the polar molecules (i.e., water in an aqueous solution) become more ordered around the dissolved lipophilic substance, since the polar molecules cannot form hydrogen bonds to the lipophilic areas of the amphiphile. So in an aqueous environment, the water molecules form an ordered "clathrate" cage around the dissolved lipophilic molecule.

Summary of the invention

According to an aspect, the present invention concerns a use of a peptide, comprising an immune suppressive domain, for transfection.

According to another aspect, the invention concerns a use of a peptide comprising an immune suppressive domain for transfection, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, subject to the proviso that a monomeric peptide should comprise at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a use of a peptide comprising an immune suppressive domain in a transfection mix, subject to the proviso that said peptide is a dimer or multimer or comprises at least two immune suppressive domain motifs.

It will be clear for the person skilled in the art that the word “or” is not necessarily an exclusive expression. Thus, a dimer or multimer may comprise more than one immune suppressive domain motif.

According to an aspect, the invention concerns a use of a peptide comprising an immune suppressive domain for transfection of a cell, said peptide providing immune suppression of the transfected cell.

According to an aspect, the invention concerns a use of a molecule, which comprises at least two parts, each part comprising an immune suppressive domain, for transfection.

According to an aspect, the invention concerns a kit-of-parts or composition comprising:

a. a transfection agent; and

b. a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a transfection reagent comprising a lipid based molecule coupled covalentely to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a nano particle coupled covalentely to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a hydrophobic vehicle for delivery of hydrophobic molecules coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a drug coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a use of a peptide, composition, a kit-of-parts, a transfection agent, a nano particle, a hydrophobic vehicle or a drug according to the invention for inhibiting immune response.

According to an aspect, the invention concerns a method for transfecting a cell, said method comprising:

a. Providing a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences;

b. Incubating the cell with said peptide;

c. Providing a transfection agent;

d. Providing a material to be transfected into the cell; and

e. Further incubating the cell with said transfection agent and said material.

According to an aspect, the invention concerns a cell obtainable with said method for transfecting a cell.

According to an aspect, the invention concerns a cell obtainable by incubating a cell with a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a cell comprising a peptide, said peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an aspect, the invention concerns a use of a cell according to the invention, for diagnostics, prophylaxis or therapy.

Detailed Disclosure

According to an embodiment, the present invention concerns a use of a peptide, comprising an immune suppressive domain, for transfection.

Transfection is the process of deliberately introducing nucleic acids into cells. The term "for transfection" is meant to encompass that the peptide is used as part of the transfection process, for example together with at least one transfection agent, and/or for preparing a cell for additional steps of the transfection process.

An immune suppressive peptide exhibits immune suppressive activity. The term "immune suppressive activity" means that it can inhibit proliferation of CTLL-2 or PBMCs in assays as described in the examples, i.e. by more than 20% or interfere with type I IFN production in response to lipid based formulations.

According to an embodiment, the invention concerns a use of a peptide comprising an immune suppressive domain for transfection, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, subject to the proviso that a monomeric peptide should comprise at least two immune suppressive domain sequences.

The term "peptide" as used here, comprises shorter amino sequences as well as longer sequences such as proteins, and monomers as well as dimers and multimers.

The immune suppressive peptides do not need to be covalently bound to form a dimer or multimer. If they contain multimerization domains, the monomeric peptides may associate together through non-covalent interactions and form dimers or multimers that will also be functional.

According to an embodiment, the invention concerns the use of a peptide of the invention, wherein said peptide is a dimer.

According to an embodiment, the invention concerns a use of a peptide comprising an immune suppressive domain in a transfection mix, subject to the proviso that said peptide is a dimer or multimer or comprises at least two immune suppressive domain motifs.

It will be clear for the person skilled in the art that the word “or” is not necessarily an exclusive expression. Thus, a dimer or multimer may comprise more than one immune suppressive domain motif, and still be within the scope of the present invention.

According to an embodiment, the invention concerns the use of the invention, wherein said at least two immune suppressive domain motifs are two identical or different motifs.

According to an embodiment, the invention concerns a use of a peptide comprising an immune suppressive domain for transfection of a cell, said peptide providing immune suppression of the transfected cell.

According to an embodiment, the invention concerns the use of a peptide according to the invention, which is soluble in water. The peptide is preferably sufficiently soluble to provide immune suppressive activity in an aqueous solution.

According to an embodiment, the invention concerns the use of a peptide according to the invention, wherein said immune suppressive domain is part of a virus.

According to an embodiment, the invention concerns the use of a peptide of the invention, wherein said virus is an influenza virus.

According to an embodiment, the invention concerns the use of a peptide according to the invention, wherein said peptide forms part of a protein.

According to an embodiment, the invention concerns a use of a molecule, which comprises at least two parts, each comprising an immune suppressive domain, for transfection.

According to an embodiment, the invention concerns a kit-of-parts or composition comprising:

a. a transfection agent; and

b. a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an embodiment, the invention concerns the kit-of-parts or composition of the invention, wherein said transfection agent is coupled covalentely to said peptide.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said transfection agent is a lipid based transfection agent.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said lipid based transfection agent is selected among an anionic and a cationic transfection agent.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said transfection agent is a lipid based liposome or virosome or viral vector.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, comprising a nano particle.

A nanoparticle may e.g. be used for drug delivery, it may have an inherent therapeutic effect, or it may be fluorescent and used for tests.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, further comprising a hydrophobic vehicle for delivery of hydrophobic molecules.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, comprising a drug.

The immune suppressive domain may suppress an unwanted immune response induced by the drug, which e.g. may comprise a carrier oil, known to induce an immune response.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is an immune suppressive peptide.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide forms part of a protein of a pathogen.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said pathogen is a virus.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide forms part of a protein on the surface of a pathogen.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide forms part of a virus surface glycoprotein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said immune suppressive peptide forms part of an enveloped virus surface glycoprotein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said immune suppressive peptide has a length of at least 6, preferably 7, more preferred 8, preferably 9, more preferred 10, preferably 11, more preferred 12, preferably 13, more preferred 14, preferably 15, more preferred 16, preferably 17, more preferred 18, preferably 19, more preferred 20, preferably 21 more preferred 22, preferably 23, more preferred 24, preferably 25 amino acids.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide has a length selected among 5 – 200, preferably 10 - 100, more preferred 20 – 50, preferably 30 – 40 amino acids.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, further comprising a fusion peptide from a fusion protein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said fusion peptide is from the fusion protein of an enveloped virus.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said fusion peptide is from a type I fusion protein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, comprising a fusion peptide from a type II fusion protein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said fusion peptide has 1, 2, 3 or 4 mutations, deletions or insertions with respect to the wild type.

The term "mutation" is used with a number about this number of point mutation(s), i.e. 3 mutations mean 3 point mutations. The term "deletion" is used with a number about the deletion of this number of amino acid(s), i.e. 2 deletions means the deletion of 2 amino acids. The term "insertion" is used with a number about insertion of this number of amino acid(s), i.e. 1 insertion means the insertion of 1 amino acid.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide, or a functional homologue thereof, binds either directly or indirectly to a cellular protein complex containing the protein STING encoded by the gene Tmem173.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide, or a functional homologue thereof, affects type I interferon responses.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide, or a functional homologue thereof, affects type I interferon responses induced by membrane fusion.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, comprising a peptide from selected among the group consisting of the lists of Table 1 and among the sequences with Seq. Id. 1 - 287.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, comprising a peptide from an influenza virus or a Flu peptide.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide has immune suppressive activity as dimer or mulitimer or when coupled to carrier proteins.

The coupling of peptides to larger proteins is one of the known methods for producing mulitimeric peptides or antigens. Proteins such as Bovine serum albumin (BSA) or Keyhole limpet hemocyanin (KLH) can be used as carrier proteins.

KLH is used extensively as a carrier protein in the production of antibodies for research, biotechnology and therapeutic applications. KLH is an effective carrier protein for several reasons. Its large size and numerous epitopes generate a substantial immune response, and abundance of lysine residues used as coupling sites.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide comprises at least one non-genetically encoded amino acid residue. The non-genetically encoded amino acid residues are amino acid residues, which are not genetically encoded.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide comprises at least one D-amino acid.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide comprises at least one D-amino acid residue.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is coupled to any other molecule.

Two peptides may be joined via another molecule, and each of the two peptides may comprise an immune suppressive domain sequence.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is attached to lipids.

Lipids are here defined as cationic, anionic or neutrally charged fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as sterol-containing metabolites such as cholesterol).

Liposomes may be used as a delivery system, and vesicles may be at least partly covered by immune suppressive domains, thereby suppressing a potential immune system response to the delivery system.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is coupled to a molecule through a peptide bond.

The molecule may e.g. be a ligand of a receptor, thereby targeting the peptide, or it may e.g. be a molecule providing different solubility characteristics of the combination of the peptide and the molecule as compared to the peptide alone, or the molecule may be a nanoparticle. The peptide may further form part of a protein, which may provide advantages such as easy production, as the protein may be derived from natural sources.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is coupled to a protein.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is a circular peptide.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is attached to at least one biological membrane.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide is modified in a way in which one of the peptide bonds is replaced by a non-peptide bond.

According to an embodiment, the invention concerns the kit-of-parts or composition according to the invention, wherein said peptide interferes with an interferon response induced by the transfection agent upon addition to a cell.

According to an embodiment, the invention concerns the kit-of-parts or composition comprising a functional homologue of a peptide according to the invention.

A functional homologue may be replaced by an altered peptide, obtained from a peptide of the invention, by making 1, 2, 3, or 4 mutations, deletions or insertions of the immune suppressive domain. An alternated peptide may have a %identity vis-a-vis the unaltered form of at least 60%, preferably at least 70%, more preferred at least 80%, preferably at least 90%, more preferred at least 95%, preferably at least 98%, more preferred at least 99%.

According to an embodiment, the invention concerns a transfection reagent comprising a lipid based molecule coupled covalentely to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an embodiment, the invention concerns a nano particle coupled covalentely to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an embodiment, the invention concerns a hydrophobic vehicle for delivery of hydrophobic molecules coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an embodiment, the invention concerns a drug coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

According to an embodiment, the invention concerns a use of a peptide, composition, a kit-of-parts, a transfection agent, a nano particle, a hydrophobic vehicle or a drug according to the invention for inhibiting immune response.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for introducing a molecule into a cell.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention, for introducing a DNA or RNA molecule into a cell.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention, for introducing a pharmaceutical molecule into a cell.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention, for introducing a pharmaceutical molecule into a tissue.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention, for introducing a gene-therapeutic pharmaceutical molecule into a cell.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for initiating expression of proteins via transfected plasmids.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for targeting active genes by microRNA silencing.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for examining the immune response to DNA or RNA.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for gene therapy.

According to an embodiment, the invention concerns the use of a peptide, composition, or a kit of parts according to the invention for delivery of any molecule using liposomes.

According to an embodiment, the invention concerns a method for transfecting a cell, said method comprising:

a. Providing a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences;

b. Incubating the cell with said peptide;

c. Providing a transfection agent;

d. Providing a material to be transfected into the cell; and

e. Further incubating the cell with said transfection agent and said material.

According to an embodiment, the invention concerns a cell obtainable with said method for transfecting a cell.

The cells obtainable by this process produce less or no cytokines, in particular interferons, compared to cells obtained without the use of the peptides of the invention. I.e. when compared to untreated cells, cells treated with the INF-F#2 peptide produce less or no cytokines, in particular interferons, but appear to retain the ability to react to other and stronger stimuli.

According to an embodiment, the invention concerns a cell obtainable by incubating a cell with a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences. Such a cell may be used e.g. as an intermediate product for producing a transfected cell with suppressed immune activity.

According to an embodiment, the invention concerns a cell comprising a peptide, said peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences. The peptide may e.g. be bound to the surface of the cell, internally or externally, or may be present inside of the cell.

According to an embodiment, the invention concerns a use of a cell according to the invention, for diagnostics, prophylaxis or therapy.

According to an embodiment, the invention concerns the use according to the invention, for cell therapy and/or gene therapy.

Gene therapy and cell therapy are overlapping fields of biomedical research with similar therapeutic goals. Gene therapy can be defined as the use of genetic material (usually deoxyribonucleic acid - DNA) to manipulate a patient's cells for the treatment of an inherited or acquired disease. Cell Therapy can be defined as the infusion or transplantation of whole cells into a patient for the treatment of an inherited or acquired disease.

According to an embodiment, the invention concerns the use according to the invention, for stem cell transplantation.

The co-pending patent application PCT/DK2012/050381 provides a number of immunosuppressive domains and motifs.

Table 1 is provided below. Table 1 likewise provides a number of immunosuppressive domains and motifs.

TABLE 1

Family	Genus	Species (group)	Species (Strain)	Putative ISU as identified using the criteria described in this application for identification of immunosuppressive domains	Peptides from domains from fusion proteins exhibiting immunosuppressive activity (ISU)	Name of envelope attachment/ fusion protein	IU group and fusion type
Flavi-viridae	Flavi-virus	Aroa virus	Bussuquara virus	seqid85 NRGWNNGCGLFGKG ********** seqid7 GDAAWDFGSVGGVFNSLGK **o****o******oo*o**		Envelope protein prME Fusion protein E	Group 1 Type II Fusion mechanism
			guape virus				
			Naranjal virus				
		Dengue virus	*Dengue 1*	seqid8 GGTAWDFGSIGGVFTSVGK *o*********************	seqid2 DRGWGNGCGLFGKG ********** seqid172 KGSSIGKMFESTYRGAKRMAIL G		
			Dengue 2	seqid9 GDTAWDFGSLGGVFTSVGK **********o** seqid173 KGSSIGKMFEATARGARRMAILG	seqid2 DRGWGNGCGLFGKG **********		
			Dengue 3	seqid174 KGSSIGQMFETTMRGAKRMAILG	seqid2 DRGWGNGCGLFGKG ********** seqid204 GDTAWDFGSVGGVLNSLGK		

				**********
	Dengue 4	seqid10 GETAWDFGSVGGLLTSLGK **********oo***** seqid173 KGSSIGKMFEATARGARRMAILG	seqid2 DRGWGNGCGLFGKG **********	
Japanese encephalitis virus group	*Japanese encephalitis virus*	seqid11 LGDTAWDFGSIGGVFNSIG ***o********** seqid12 LGDTAWDFGSVGGIFTSLG	seqid2 DRGWGNGCGLFGKG ********** seqid2 DRGWGNGCGLFGKG **********	
	Koutango virus			
	Murray Valley encephalitis virus	seqid13 LGDTAWDFGSVGGVFNSIG	seqid2 DRGWGNGCGLFGKG **********	
	St. Louis encephalitis virus	seqid11 LGDTAWDFGSIGGVFNSIG **********	seqid2 DRGWGNGCGLFGKG **********	
	Usutu virus	seqid14 LGDTAWDFGSVGGIFNSVG **********	seqid2 DRGWGNGCGLFGKG **********	
	West Nile virus	seqid15 LGDTAWDFGSVGGVFTSVG **********o*********	seqid2 DRGWGNGCGLFGKG **********	
Kokobera virus group	*Kokobera virus unclassified* *Kokobera virus group*	seqid16 IGDDAWDFGSVGGILNSVG	seqid2 DRGWGNGCGLFGKG	
Modoc virus group	*Modoc virus*	seqid17 VGSAFWNSDQRFSAINLMD seqid18 DRGWGNGCALFGKG		
	Cowbone Ridge virus *Jutiapa virus* *Sal Vieja virus* *San Perlita virus*			
mosquito-borne viruses	*Ilheus virus*	seqid84 LGDTAWDFGSVGGIFNSIG	seqid2 DRGWGNGCGLFGKG	
	Sepik virus	seqid19 TGEHSWDFGSTGGFFASVG	seqid2 DRGWGNGCGLFGKG	
Ntaya virus group	Bagaza virus	seqid20 LGDTAWDFGSVGGFFTSLG	seqid2 DRGWGNGCGLFGKG	
	Tembusu virus	seqid83 LGDTAWDFGSVGGVLTSIG	seqid2 DRGWGNGCGLFGKG	
	Yokose virus	seqid21 IGDDAWDFGSTGGIFNTIG	seqid2 DRGWGNGCGLFGKG	

Rio Bravo virus group	Apoi virus	seqid22 SSAFWNSDEPFHFSNLISII	seqid2 DRGWGNGCGLFGKG	
	Entebbe bat virus	seqid23 GDDAWDFGSTGGIFNTIGKA	seqid2 DRGWGNGCGLFGKG	
	Rio Bravo virus	seqid24 SSAYWSSSEPFTSAGIMRIL	seqid2 DRGWGNGCGLFGKG	
	Saboya virus	seqid18 DRGWGNGCALFGKG seqid25 GSSSWDFSSAGGFFGSIGKA		
Seaborne tick-borne virus group	Meaban virus	seqid26 GDAAWDFGSVGGFMTSIGRA seqid27 DRGWGNHCGLFGKG		
	Saumarez Reef virus	seqid28 GETAWDFGSAGGFFTSVGRG seqid27 DRGWGNHCGLFGKG		
	Tyuleniy virus	seqid29 GEAAWDFGSAGGFFQSVGRG seqid27 DRGWGNHCGLFGKG		
Spondweni virus group	Zika virus	seqid30 LGDTAWDFGSVGGVFNSLGK **********oo***o**	seqid2 DRGWGNGCGLFGKG	
	Kyasanur forest disease virus	seqid31 VGEHAWDFGSVGGMLSSVG **********o***** seqid27 DRGWGNHCGLFGKG		
	Langat virus	seqid32 VLGEHAWDFGSVGGVMTSIG seqid27 DRGWGNHCGLFGKG		
	Louping ill virus	seqid33 IGEHAWDFGSAGGFFSSIG **********o***oo*o* seqid27 DRGWGNHCGLFGKG		
	Omsk hemorrhagic fever virus	seqid34 LGEHAWDFGSTGGFLSSIG seqid27 DRGWGNHCGLFGKG		
	Powassan virus	seqid35 VGEHAWDFGSVGGILSSVG		

			**********o***** seqid36 DRGWGNHCGFFGKG **********	
		Royal Farm virus	seqid27 DRGWGNHCGLFGKG	
		Tick-borne encephalitis virus	seqid37 IGEHAWDFGSAGGFLSSIG seqid38 IGEHAWDFGSTGGFLTSVG seqid39 IGEHAWDFGSTGGFLASVG seqid27 DRGWGNHCGLFGKG	
	Yaounde virus		seqid40 LGDTAWDFGSIGGVFTSLG	seqid2 DRGWGNGCGLFGKG
	Yellow fever virus group	Banzi virus	seqid41 VGSSSWDFSSTSGFFSSVG	seqid2 DRGWGNGCGLFGKG
		Bouboui virus	seqid42 VGRSSWDFSSAGGFFSSVG	seqid2 DRGWGNGCGLFGKG
		Edge Hill virus Uganda S virus Wesselsbron virus		
		Yellow fever virus	seqid43 MGDTAWDFSSAGGFFTSVG ***o**********	seqid2 DRGWGNGCGLFGKG
	unclassified Flavivirus	Batu Cave virus Cacipacore virus Calbertado virus Cell fusing agent virus Chaoyang virus Chimeric Tick-borne encephalitis virus/Dengue virus 4 Culex theileri flavivirus Donggang virus Duck hemorrhagic ovaritis virus Flavivirus Aedes/MO-Ac/ITA/2009 Flavivirus Anopheles/PV-Am/ITA/2009 Flavivirus CbaAr4001 Flavivirus FSME Flavivirus Phlebotomine/76/Arrabida/2007 Gadgets Gully virus Greek goat encephalitis virus	seqid44 NRGWGTGCFKWGIG seqid45 NRGWGTGCFEWGLG	seqid2 DRGWGNGCGLFGKG

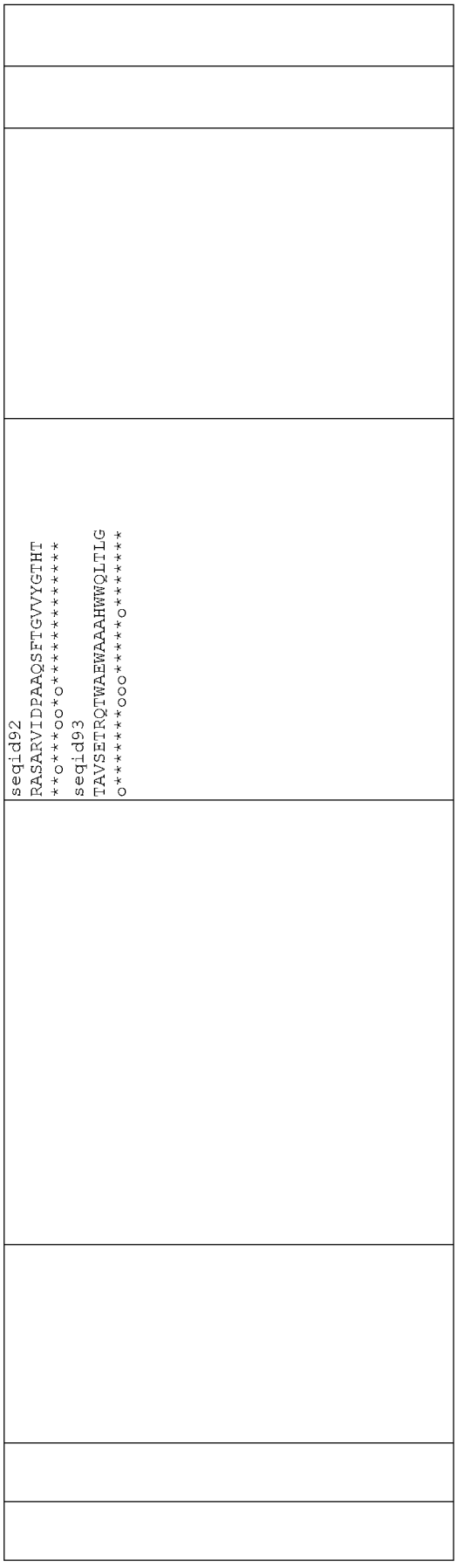
		Jugra virus Kadam virus Kamiti River virus Kedougou virus Montana myotis leukoencephalitis virus Mosquito flavivirus Ngoye virus Nounane virus Phlebotomus flavivirus Alg_F19 Phlebotomus flavivirus Alg_F8 Quang Binh virus Russian Spring-Summer encephalitis virus Sokoluk virus Spanish sheep encephalitis virus T'Ho virus Tai forest virus B31 Tamana bat virus Tick-borne flavivirus Wang Thong virus Flavivirus sp.	Aedes flavivirus	seqid45 NRGWGTGCFEWGLG seqid46 HVAGRYSKHGMAGIGSVWEDLVR	
			Culex flavivirus	seqid44 NRGWGTGCFKWGIG seqid47 VDKYRRFGTAGVGG	
	Hepacivirus	Hepatitis C virus genotype 1 a		seqid3 GLIHLHQNIVDVQYLYG seqid175 PALSTGLIHLHQNIVDVQ	E1/E2
		Hepatitis C virus genotype 1b		seqid48 GLIHLHRNIVDVQYLYG seqid176 PALSTGLIHLHRNIVDVQ	
		Hepatitis C virus genotype 2		seqid49 GLIHLHQNIVDVQYMYG seqid175 PALSTGLIHLHQNIVDVQ	
		Hepatitis C virus genotype 3		seqid175 PALSTGLIHLHQNIVDVQ	seqid3 GLIHLHQNIVDVQYLYG
		Hepatitis C virus genotype 4		seqid175	seqid3

			PALSTGLIHLHQNIVDVQ	GLIHLHQNIVDVQYLYG	
		Hepatitis C virus genotype 5	seqid50 GLIHLHQNIVDTQYLYG seqid177 PALSTGLIHLHQNIVDTQ		
		Hepatitis C virus genotype 6	seqid175 PALSTGLIHLHQNIVDVQ	seqid3 GLIHLHQNIVDVQYLYG	
		All Hepatitis C virus		seqid3 GLIHLHQNIVDVQYLYG	
Pestivirus	Border disease virus	Border disease virus - Border disease virus - X818 Border disease virus 1 Border disease virus 2 Border disease virus 3 Border disease virus isolates	seqid51 NTTLLNGSAFQLICPYGWVGRVEC seqid52 SYFQQYMLKGQYQYWFDLE		E1/E2
	Bovine viral diarrhea virus 1	Bovine viral diarrhea virus 1-CP7 Bovine viral diarrhea virus 1-NADL Bovine viral diarrhea virus 1-Osloss Bovine viral diarrhea virus 1-SD1 Bovine viral diarrhea virus isolates and strains Bovine viral diarrhea virus type 1a Bovine viral diarrhea virus type 1b Pestivirus isolate 97-360 Pestivirus isolate Hay 87/2210 Pestivirus strain mousedeer Pestivirus type 1 isolates	seqid53 NTTLLNGPAFQMVCPLGWTGTVSC seqid54 SYFQQYMLKGEYQYWFDLE		
	Bovine viral diarrhea virus 2 (BVDV-2)	Bovine viral diarrhea virus 2 Pestivirus sp. strain 178003 Pestivirus sp. strain 5250Giessen-3 Bovine viral diarrhea virus-2 isolate SCP	seqid55 SLLNGPAFQMVCPQGWTGTIEC seqid56 DRYFQQYMLKGKWQYWFDLD		
	Classical swine fever virus	Classical swine fever virus Hog cholera virus strain Zoelen	seqid57 TLLNGSAFYLVCPIGWTGVIEC seqid58 SYFQQYMLKGEYQYWFDLD		
	unclassified Pestivirus	Bovine viral diarrhea virus 3	seqid59 TLLNGPAFQLVCPYGWTGTIEC seqid60 DNYFQQYMLKGKYQYWFDLEATD		

						E2/E1
	Chamois pestivirus 1		seqid61 TLLNGSAFQMVCPFGWTGQVEC seqid62 DSYFQQYMLKGEYQYWFDLDAKD			
	Porcine pestivirus isolate		seqid205			
	Bungowannah		TLLNGPAFQLVCPYGWTGTIECDSYYQQY IIKSGYQYWFDLTAKD			
Unclassified Flaviviridae	Barkedji virus					
	Canine hepacivirus AAK-2011					
	GB virus A					
	Douroucouli hepatitis GB virus A					
	GBV-A-like agents					
	GB virus D					
	GBV-C/HGV group					
	GB virus C					
	Hepatitis GB virus C-like virus					
	Hepatitis GB virus B					
	Lammi virus					
	Marmoset hepatitis GB virus A					
	Nakiwogo virus					
	Turkey meningoencephalitis virus		seqid63 GVYPFMWGGAYCFCDTENTQVS **********o****o**o*o* seqid64 APFGCEIYTNPIRAENCAVGSIP *****o*ooo*o**oo*oo*oo* seqid65 SDFGGIATVKYSASKSGKCAVH o***oooooo*ooooo*o*oo* seqid66 FSTANIHPEFRLQICTSYVTCKGDCHPP *oooooooo*oooo*ooooo*ooo*o****			
Togaviridae	*Alpha-virus*	*Aura virus*				
		Barmah Forest virus				
		Middelburg virus				
		Ndumu virus				
		Salmon pancreas disease virus				
		Getah virus				
		Mayaro virus				
		Trocara virus				
		EEEV complex				
		WEEV complex	Fort Morgan virus Highlands J virus Sindbis virus Western equine encephalomyelitis virus Whataroa virus			
		VEEV complex	Cabassou virus Mucambo virus Pixuna virus			

		Venezuelan equine encephalitis virus	seqid67 GVYPFMWGGAYCFCD ********** seqid68 GDCHPPKDHIVTHPQYHAQ **********o**o*o* seqid69 AVSKTAWTWLTS **********oo*		
		SFV complex	Bebaru virus O'nyong-nyong virus Ross River virus Semliki forest virus Una virus	seqid63 GVYPFMWGGAYCFCDTENTQVS **********o****o**o*o* seqid64 APFGCEIYTNPIRAENCAVGSIP *****o*ooo*o**oo*oo*oo* seqid65 SDFGGIATVKYSASKSGKCAVH o***oooooo*ooooo*o*oo* seqid66 FSTANIHPEFRLQICTSYVTCKGDCHPP *oooooooo*oooo*ooooo*ooo*o**		
			Chikungunya virus	seqid67 GVYPFMWGGAYCFCD ********** seqid70 VHCAAECHPPKDHIVNY oo*o*o**o********* seqid71 PASHTTLGVQDISATAMSWV o*****oo*****o*******		

Rubivirus					
	Rubella virus	Rubella virus (strain BRD1)	seqid72		
		Rubella virus (strain BRDII)	ACTFWAVNAYSSGGYAQLASYFNPGGSYYK		
		Rubella virus (strain Cendehill)	***o*o*****o**oo****o**o*o******o		
		Rubella virus (strain M33)	seqid73		
		Rubella virus (strain RN-UK86)	QYHPTACEVEPAFGHSDAACWGFPTDT		
		Rubella virus (strain THERIEN)	***o*o*o*o*****o*********o***		
		Rubella virus (strain TO-336	seqid74		
		vaccine)	MSVFALASYVQHPHKTVRVKFHT		
		Rubella virus (strain TO-336)	***oo*****o**o**o*******		
		Rubella virus (vaccine strain	seqid159		
		RA27/3)	ETRTVWQLSVAGVSC		
			o*o**********oo*		
			seqid76		
			NVTTEHPFCNMPHGQLEVQVPP		
			o*o*o**oo*o*o*****o*oo*		
			seqid77		
			DPGDLVEYIMNYTGNQQSRW		
			****o********o*o******		
			seqid78		
			GSPNCHGPDWASPVCQRHSPDCS		
			****o***o**********************		
			seqid79		
			RLVGATPERPRLRLV		
			o***o**o**o****		
			seqid80		
			DADDPLLRTAPGP		
			*oo**********		
			seqid81		
			GEVWVTPVIGSQARKCGL		
			oo*o**o**o*****o**		
			seqid86		
			HIRAGPYGHATVEM		
			oo************o		
			seqid87		
			PEWIHAHTTSDPWHP		
			o**oooo*o***o*o		
			seqid88		
			PGPLGLKFKTVRPVALPR		
			****o***o**o*oo***		
			seqid89		
			ALAPPRNVRVTGCYQCGTPAL		
			oooo**o*o*o**o********		
			seqid90		
			EGLAPGGGNCHLTVNGEDVG		
			o**o**oo*o*oo*		
			seqid207		
			LLNTPPPYQVSCGG		
			******o*o*o***		


seqid92
RASARVIDPAAQSETGVVYGTHT
o**oo*o**********
seqid93
TAVSETRQTWAEWAAAHWWQLTLG
o********ooo*****o********

Bunya-viridae					Gn(G2)/Gc(G1)
Hanta-virus (continued on next page)	Amur virus		seqid94		
	Bayou virus		NPPDCPGVGTGCTACGVYLD		
	Black Creek Canal virus		**o****o********o***		
			seqid95		
	Cano Delgadito virus		RKVCIQLGTEQTCKTIDSNDC		
			*oo*o*o*o*oo**oo*o***		
	Calabazo virus		seqid96		
	Catacamas virus		DTLLFLGPLEEGGMIFKQWCTTTCQFGDP		
	Choclo virus		GDIM		
	Dobrava-Belgrade virus		seqid97		
			GSFRKKCSFATLPSCQYDGNTVSG		
	El Moro Canyon virus		*o****o*o****o*o*ooo**oo**		
			seqid98		
	Hantaan virus		ATKDSFQSFNITEPH		
	Isla Vista virus		**o*****o**oooo*		
			seqid99		
	Khabarovsk virus		GSGVGFNLVCSVSLTEC		
			*******o*o*ooo****		
	Laguna Negra virus		seqid100		
			KACDSAMCYGSSTANLVRGQNT		
	Limestone Canyon virus		****o*o****ooooo*o**o**		
			seqid101		
	Monongahela virus		GKGGHSGSKFMCCHDKKCSATGLVAAAPH L		
	Muleshoe virus		*********o*o***ooo*ooo**o*oo**		
	Muju virus		*		
	New York virus		seqid102		
	Oran virus		DDGAPQCGVHCWFKKSGEW		
	Playa de Oro virus		***o*o*ooo****oo*****		
	Prospect Hill virus				
	Puumala virus				

	Rio Mamore virus Rio Segundo virus Saaremaa virus Seoul virus Sin Nombre virus Soochong virus Thailand virus Thottapalayam virus Topografov virus Tula virus				

Ortho-bunya-virus	Anopheles A virus Anopheles B virus Bakau virus Batama virus Bwamba virus Caraparu virus Kaeng Khoi virus Kairi virus Madrid virus Main Drain virus Marituba virus Nyando virus Oriboca virus Oropouche virus Sathuperi virus Shamonda virus Shuni virus Simbu virus Tacaiuma virus Tete virus Turlock virus unclassified Orthobunyavirus		seqid103 KHDELCTGPCPVNINHQTGWLT *o*o***o**ooooooo*o*o seqid104 WGCEEFGCLAVSDGCVFGSCQD **o*oo**o*ooo**oo***** seqid105 GNGVPRFDYLCHLASRKEVIVRKC *o*ooo*ooo*oooo*ooooo*o* seqid106 SCAGCINCFQNIHC *o**ooooooooo*		
	Akabane virus	Sabo virus Tinaroo virus Yaba-7 virus			

	Bunyamwera virus	Batai virus Birao virus Bozo virus Cache Valley virus Fort Sherman virus Germiston virus Guaroa virus Iaco virus Ilesha virus Lokern virus Maguari virus Mboke virus Ngari virus Northway virus Playas virus Potosi virus Shokwe virus Tensaw virus Tlacotalpan virus Xingu virus			
	California Encephalitis virus	California encephalitis serogroup virus LEIV California encephalitis virus - BFS-283 Chatanga virus Inkoo virus Jamestown Canyon virus Jamestown Canyon-like virus Jerry Slough virus Keystone virus La Crosse virus Lumbo virus Melao virus Morro Bay virus San Angelo virus Serra do Navio virus Snowshoe hare virus South River virus Tahyna virus Trivittatus virus			

	Phlebovirus	Bujaru virus Candiruvirus Chilibre virus Frijoles virus Punta Tor□Salehabad virus Sandflyfever Naples virus Ukuniemi virus viruso virus						
	Caraparu virus	Manzanilla virus	Marituba virus	Wyeomyia virus	Apeu virus Bruconha virus Ossa virus Vinces virus	Buttonwillow virus Ingwavuma virus Mermet virus	Gumbo Limbo virus Murutucu virus Nepuyo virus Restan virus	Anhembi virus BeAr328208 virus Macaua virus Sororoca virus Taiassui virus

	Rift Valley fever virus		seqid107 KTVSSELSCREGQSYWT **oo**oo*o***o*o** seqid108 GSFSPKCLSSRRC *******ooooo seqid109 ENKCFEQCGGWGCGCFNVNPSCLFVHT **o*o***o*oo*oo***ooo***o***o seqid110 WGSVSLSLDAEGISGSNSFSF **ooo*o***o*o*o*o*oo** seqid111 RQGFLGEIRCNSE *o******o**oo* seqid112 AHESCLRAPNLVSYKPMIDQLEC *oo**oo**oooo*o*oo*ooo* seqid113 DPFVVFERGSLPQTR **ooo*oo*o***o* seqid114 QAFSKGSVQADLTLMFD **ooo*ooo*oooooo* seqid115 CDAAFLNLTGCYSCNAG *o*o*o*oo******oo* seqid116 CQILHFTVPEVEEEFMYSC *ooo*ooo*ooooooo*o* seqid117 STVVNPKSGSWN *o*o**oooooo seqid118 FFDWFSGLMSWFGGPLK *o***oo*o**oooooo	

		Anhanga virus			
		Arumowot virus			
		Chagres virus			
		Corfou virus			
		Gabek Forest virus			
		Itaporanga virus			
		Phlebovirus Adria/ALB1/2005			
		Phlebovirus Adria/ALB5/2005			
		Phlebovirus AH12			
		Phlebovirus AH12/China/2010			
		Phlebovirus AH15/China/2010			
		Phlebovirus B105-05			
		Phlebovirus B151-04			
		Phlebovirus B43-02			
		Phlebovirus B68-03			
		Phlebovirus B79-02			
		Phlebovirus Chios-A			
		Phlebovirus Cyprus			
		Phlebovirus HB29/China/2010			
		Phlebovirus HN13/China/2010			
		Phlebovirus HN6/China/2010			
		Phlebovirus Hu/Xinyang1/China/2010			
		Phlebovirus Hu/Xinyang2/China/2010			
		Phlebovirus IB13-04			
		Phlebovirus JS2007-01			
		Phlebovirus JS24			
		Phlebovirus JS26			
		Phlebovirus JS3/China/2010			
		Phlebovirus JS4/China/2010			
		Phlebovirus JS6			
		Phlebovirus JSD1			
		Phlebovirus LN2/China/2010			
		Phlebovirus LN3/China/2010			
		unclassified Phlebovirus (continued on next page)			

Orthomyxoviridae	Influenzavirus A	Influenza A virus	Phlebovirus sandflies/Gr29/Spain/2004 Phlebovirus sandflies/Gr36/Spain/2004 Phlebovirus sandflies/Gr44/Spain/2004 Phlebovirus sandflies/Gr49/Spain/2004 Phlebovirus sandflies/Gr52/Spain/2004 Phlebovirus sandflies/Gr65/Spain/2004 Phlebovirus sandflies/Gr98/Spain/2004 Phlebovirus SD24/China/2010 Phlebovirus SD4/China/2010 Phlebovirus tick/XCQ-2011 Phlebovirus XLL/China/2009 Rio Grande virus Salobo virus Sandfly fever sicilian virus Sandfly Sicilian Turkey virus Utique virus Phlebovirus sp. Phlebovirus sp. Be An 24262 Phlebovirus sp. Be An 356637 Phlebovirus sp. Be An 416992 Phlebovirus sp. Be An 578142 Phlebovirus sp. Be Ar 371637 Phlebovirus sp. Co Ar 170255 Phlebovirus sp. Co Ar 171616 Phlebovirus sp. GML 902878 Phlebovirus sp. Pa Ar 2381 Phlebovirus sp. PAN 479603 Phlebovirus sp. PAN 483391 Phlebovirus sp. VP-161A Phlebovirus sp. VP-334K Phlebovirus sp. VP-366G	INFA H1	seqid119 GLFGAIAGFIEGGWTG seqid178 WTYNAELLVLLENERTLD seqid179 NAELLVLLENERTLDYHD		INF F#2 DELTA6: seqid201	HA (HA1/HA2)
				INFA H2	seqid120			

		GLFGAIAGFIEGGWQG seqid180 WTYNAELLVLMENERTLD seqid181 NAELLVLMENERTLDYHD	
	INFA_H3	seqid121 GIFGAIAGFIENGWEG seqid182 WSYNAELLVALENQHTID seqid183 NAELLVALENQHTIDLTD	seqid4 GLFGAIAGFIENGWEG
	INFA_H4	seqid122 GLFGAIAGFIENGWQG seqid182 WSYNAELLVALENQHTID seqid184 NAELLVALENQHTIDVTD	
	INFA_H5	seqid120 GLFGAIAGFIEGGWQG seqid180 WTYNAELLVLMENERTLD seqid185 NAELLVLMENERTLDFHD	
	INFA_H6	seqid123 GIFGAIAGFIEGGWTG seqid119 GLFGAIAGFIEGGWTG seqid178 WTYNAELLVLLENERTLD seqid186 NAELLVLLENERTLDMHD	
	INFA_H7	seqid187 WSYNAELLVAMENQHTID seqid208 WSYNAELLVAMENQHLAD	seqid4 GLFGAIAGFIENGWEG
	INFA_H8	seqid124 GLFGAIAGFIEGGWSG seqid189 WAYNAELLVLLENQKTLD seqid190 NAELLVLLENQKTLDEHD	
	INFA_H9	seqid125 GLFGAIAGFIEGGWPG seqid124 GLFGAIAGFIEGGWSG	

		seqid189 WAYNAELLVLLENQKTLD seqid190 NAELLVLLENQKTLDEHD		
	INFA_H10	seqid191 WTYQAELLVAMENQHTID seqid192 QAELLVAMENQHTIDMAD	seqid4 GLFGAIAGFIENGWEG	
	INFA_H11	seqid125 GLFGAIAGFIEGGWPG seqid193 WSYNAQLLVLLENEKTLD seqid194 NAQLLVLLENEKTLDLHD		
	INFA_H12	seqid125 GLFGAIAGFIEGGWPG seqid189 WAYNAELLVLLENQKTLD seqid190 NAELLVLLENQKTLDEHD		
	INFA_H13	seqid125 GLFGAIAGFIEGGWPG seqid195 WSYNAKLLVLLENDKTLD seqid196 NAKLLVLLENDKTLDMHD		
	INFA_H14	seqid122 GLFGAIAGFIENGWQG seqid182 WSYNAELLVALENQHTID seqid184 NAELLVALENQHTIDVTD		
	INFA_H15	seqid187 WSYNAELLVAMENQHTID seqid188 NAELLVAMENQHTIDLAD	seqid4 GLFGAIAGFIENGWEG	
	INFA_H16	seqid125 GLFGAIAGFIEGGWPG seqid197 WSYNAKLLVLIENDRTLD seqid198 NAKLLVLIENDRTLDLHD		

						F0 (F2/F1)
Paramyxoviridae						
Paramyxovirinae						
Influenzavirus C	Influenza C virus					
		Avulavirus	Avian paramyxovirus 2 Yucaipa virus	seqid127 GAIALGVATAAAVTAG oooo*o*oo*o*oo**		
			Avian paramyxovirus 3			
			Avian paramyxovirus 3b			
			Avian paramyxovirus 4			
			Avian paramyxovirus 5			
			Avian paramyxovirus 6			
			Avian paramyxovirus 7			
			Avian paramyxovirus 8			
			Avian paramyxovirus 9			
			Newcastle disease virus			
			Pigeon paramyxovirus 1			
			unclassified Avulavirus			
			Avian paramyxovirus 10_Avian paramyxovirus duck/Miyagi/885/05			
			Avian paramyxovirus penguin/Falkland Islands/324/2007			
			Goosramyxovirus HZ			
			Goose paramyxovirus JS/1/97/Go			
			Goose paramyxovirus SF02			
		Henipavirus	Hendra virus Hendra virus horse/Australia/Hendra/1994			
			Nipah virus unclassified Henipavirus			
			Bat paramyxovirus Eid.hel/GH45/2008			
Influenzavirus B	Influenza B virus	All strains	seqid126 GFFGAIAGFLEGGWEG seqid199 ISSQIELAVLLSNEGIIN seqid200 QIELAVLLSNEGIINSED			

Morbillivirus	Canine distemper virus Cetacean morbillivirus_Dolphin morbillivirus_Pilot whale morbillivirus Porpoise morbillivirus Measles virus Peste-des-petits-ruminants virus Phocine distemper virus Phocine distemper virus 1 Phocine distemper virus-2 Rinderpest virus				
Respirovirus	Bovine parainfluenza virus 3 Porcine paramyxovirus strain Frost Porcine paramyxovirus strain Texas Human parainfluenza virus 1 Human parainfluenza virus 3 Simian Agent 10 Sendai virus unclassified Respirovirus Atlantic salmon respirovirus Guinea pig parainfluenza virus TS-9 Pacific salmon paramyxovirus Trask River 1983 Swine parainfluenza virus 3 Tursiops truncatus parainfluenza virus 1				

Rubulavirus	unclassified Paramyxovirinae	Atlantic salmon paramyxovirus Beilong virus Canine parainfluenza virus Chimeric human parainfluenza virus rPIV3-2 Fer-de-lance virus J-virus Menangle virus Mossman virus Murayama virus Ovine parainfluenza virus 3 Pacific salmon paramyxovirus Paramyxovirus GonoGER85 Recombinant PIV3/PIV1 virus Reptilian paramyxovirus Salem virus Salmo salar paramyxovirus Snake ATCC-VR-1408 paramyxovirus Snake ATCC-VR-1409 paramyxovirus Tioman virus Tupaia paramyxovirus	Human parainfluenza virus 2 Human parainfluenza virus 2 (strain Greer) Human parainfluenza virus 2 (strain Toshiba) Human parainfluenza virus 4 Human parainfluenza virus 4a Human parainfluenza virus 4b Mapuera virus Mumps virus Parainfluenza virus 5 Porcine rubulavirus Simian virus 41 unclassified Rubulavirus Porcine parainfluenza virus Tuhoko virus 1 Tuhoko virus 2 Tuhoko virus 3			

Pneumovirus	Human respiratory syncytial virus	Human respiratory syncytial virus A	seqid128 FLGLILGLGAAVTAGVA		
		Human respiratory syncytial virus (strain RSB1734)	***oo**o*o*ooo*o* seqid129		
		Human respiratory syncytial virus (strain RSB5857)	TNEAVVSLTNGMSVL **o*****o**o***		
		Human respiratory syncytial virus (strain RSB6190)	seqid130 VIRFQQLNKRLLE		
		Human respiratory syncytial virus (strain RSB6256)	**o***o*o**** seqid131		
		Human respiratory syncytial virus (strain RSB642)	REFSSNAGLT ****o****o*		
		Human respiratory syncytial virus (strain RSB6614)	seqid132 MLTDRELTSIVGGM		
		Human respiratory syncytial virus A strain Long LinkOut	***oo***o*oooo* seqid133		
		Human respiratory syncytial virus A2	YVIQLPLFGVMDTDCW *oo***oo**o**o**		
		Human respiratory syncytial virus B	seqid134 CLARADNGWYCHNAGSLSYFP		
		Human respiratory syncytial virus (subgroup B / strain 18537)	**ooo*o***o*o****o*o** seqid135		
		Human respiratory syncytial virus (subgroup B / strain 8/60)	DTLKSLTVPVTSRECN **oo****o*ooooo**		
		Human Respiratory syncytial virus 9320	seqid136 YDCKISTSKTYVSTAVLTTMG		
		Human respiratory syncytial virus B1	*o*o*o***ooo*oo*o*oo* seqid137		
		Human respiratory syncytial virus S2	VSCYGHNSCTVIN *****ooo**oo*		
		Human respiratory syncytial virus strain RSS-2	Seqid75 GIIRTLPDGCHYISNKGVDRVQVGNTVYY		
		unclassified Human respiratory syncytial virus	LSKEVGK ***o*ooo**o*o**o*o*o****o** oo*oo** seqid139 PLSFPDDKFDVAIRDVEHSINQTRTFLKA SDQLL **o***o*o*ooo*oo*ooo***ooo*ooo **o** seqid140 KIMTSKTDISSSVITSIGAIVSCYG o*o***ooo*oo*o*oo*oo*****		

Coronaviridae		Bovine respiratory syncytial virus	All strains	seqid128 FLGLILGLGAAVTAGVA ***oo**o*o*ooo*o*			
	Metapneumovirus	Avian metapneumo-virus	All strains	seqid134 CLARADNGWYCHNAGSLSYFP **ooo*o**o*o****o*o**			
		Human metapneumo-virus	All strains	seqid133 YVIQLPLFGVMDTDCW *oo***oo**o**o**			
	Coronavirinae	Alphacorona-virus	Alphacoronavirus 1	seqid141 RSAIEDLLFDKVKLSDVG **oo****oo**ooo*o*	seqid135 VLSQNQKLIANAFNNALHAIQ **oo****o*ooo*oo*ooo**		S (S1/S2)
			Coronavirus group 1b	seqid142 VPFYLNVQYRINGLGVT o**ooooo**o**o***			
			Human coronavirus 229E	seqid143 TNSALVKIQAVVNANA *oo**o*o*o***oo*			
			Human coronavirus NL63	seqid144 AEAQIDRLINGRLTALNAYVSQQL *oo******o***oo*oo*oo***			
			Miniopterus bat coronavirus 1	seqid145 SAAQAMEKVNECVKSQSSRINFCGNGNHI IS			
			Miniopterus bat coronavirus HKU8	seqid146 o*oo*oo*oo***oo*oo*oo***o*o*o o*			
			Porcine epidemic diarrhea virus	seqid147 APYGLYFTHFNYVP **o*oo*o*oo*o*			
			Rhinolophus bat coronavirus HKU2	seqid148 LQEAIKVLNHSYINLKDIGTYEYYVKWPW YVW			
			Scotophilus bat coronavirus 512				
			unclassified Alphacoronavirus				
		Betacorona-virus	Betacoronavirus 1				
			Coronavirus group 2b				
			Coronavirus group 2c				
			Human coronavirus HKU1				
			Murine coronavirus				
			Pipistrellus bat coronavirus HKU5				
			Rousettus bat coronavirus HKU9				
			Severe acute respiratory syndrome-related coronavirus recombinant				
			SARSr-CoV				
			SARS coronavirus				
			Tylonycteris bat coronavirus HKU4				
			unclassified Betacoronavirus				
		Gammacorona-virus	Avian coronavirus				
			Beluga Whale coronavirus SW1				

						GpC (Gp1/Gp2)
Arena-viridae	Arena-virus	LCMV-Lassa virus (Old World) complex	unclassified coronaviruses	Alpaca coronavirus CA08-1/2008	*o*	
				Bat coronavirus	seqid209	
				Bird droppings coronavirus	EVFAQVKQMYKTPTLKYFGGFNFSQIL	
				Bovine respiratory coronavirus	seqid210	
				Chicken enteric coronavirus	EVFAQVKQMYKTPAIKDFGGFNFSQIL	
				Coronavirus Anas	Seqid211	
				Coronavirus	SFIEDLLFNKVTLADAGF	
				oystercatcher/p17/2006/GBR	Seqid212	
				Coronavirus red knot/p60/2006/GBR	SAIEDLLFNKVRLSDVGF	
				Ferret enteric coronavirus 1202	Seqid213	
				Ferret systemic coronavirus MSU-S	SLLEDLLFNKVKLSDVGF	
				Ferret systemic coronavirus WADL	Seqid214	
				Guangxi coronaviridae	SAIEDLLFSKVKLADVGF	
				Human coronavirus NO	Seqid215	
				Human enteric coronavirus strain 4408	SAIEDLLFDKVKLSDVGF	
				Kenya bat coronavirus	seqid149	
				Mink coronavirus strain WD1133	NALINDQLIMKNHLRDIMGIPYC	
				Parrot coronavirus AV71/99	*o**o****o*o***o*o**o**o***	
				Quail coronavirus Italy/Elvia/2005	seqid150	
				Tai Forest coronavirus	FTWTLSDSEGKDTPGGYCLT	
				unidentified coronavirus	oo*ooo*oo*ooo***o**o	
				unidentified human coronavirus	seqid151	
					KCFGNTAIAKCNQKHDEEFCDMLRLFDFN	
					o*ooo**oo*oo****ooo*ooo*	
					seqid152	
					MLQKEYMERQGKTPLGLVDLFVFS	
					*ooo*oo**oo**oo*o*o*oooo*o	

Hepadnaviridae	Genus Orthohepadnavirus	Tacaribe virus (New World) complex	Amapari virus		seqid150	
			Chapare virus		FTWTLSDSEGKDTPGGYCLT	
			Flexal virus		oo*ooo*oo*ooo***o**o	
			Guanarito virus		seqid151	
			Junin virus		KCFGNTAIAKCNQKHDEEFCDMLRLFDFN	
			Latino virus		***o*ooo*****oo*oo*****ooo*ooo*	
			Machupo virus		seqid152	
			Oliveros virus		MLQKEYMERQGKTPLGLVDLFVFS	
			Paraná virus		*ooo*oo**oo**oo*o*oooo*o	
			Pichinde virus			
			Pirital virus			
			Sabiá virus			
			Tacaribe virus			
			Tamiami virus			
			Whitewater Arroyo virus			
		Hepatitis B virus	HBV genotype A		seqid153	
			HBV genotype B		FNPLGFFPSHQLDPLF	
			HBV genotype C		o***o*o*o*o*o*o*	
			HBV genotype D		seqid154	
			HBV genotype E		ADWDKNPNKDPWP	
			HBV genotype F		o*o*o*oo*oooo	
			HBV genotype G		seqid155	
			HBV genotype H		MESITSGFLGPLLVLQAVFF	
			Hepatitis B virus alpha1		oooooooo*ooooo**oooo	
					seqid156	
				Hepatitis B virus LSH/chimpanzee	LLTRILTIPQSLDSWWTSLNFLGGA	
				Hepatitis B virus strain cpz	oooooo*oooo*oooo***o*o*oo	
				Hepatitis B virus subtype adr	seqid157	
				Hepatitis B virus subtype adw	CPPTCPGYRWMC	
				Hepatitis B virus subtype adyw	oo*o*****o*o	
				Hepatitis B virus subtype ayw	seqid158	
					LFTLLLCLIFLLVLLDYQ	
					*oo*ooo*oo*oo*oooo	
						L and M and S

Rhabdoviridae					Glycoprotein G
Dimarhabdovirus					
Ephemerovirus	Bovine ephemeral fever virus	seqid160 LDGYLCRKQKWEVTCTETWYFVTD *o*oo****o*ooo*o*****o*o seqid161 KYQIIEVIPTENEC o***o***o*oooo* seqid162 LKGEYIPPYYPPTNCVWNAIDTQE oo*oo*******oo*o**oooo** seqid163 IEDPVTMTLMDSKFTKPC ooo*oooooo***o*oo** seqid164 LHCQIKSWECIPV o**oo*o*****o* seqid165 SHRNMMEALYLESPD *oo*oo*o*oo*o** seqid166 LTFCGYNGILLDNGEWWSIY o****oo**oooo****** seqid167 ELEHEKCLGTLEKLQNGE *****o***o*oo*oo*o* seqid168 LDLSYLSPSNPGKHYAY **o****o*oo**oo*** seqid169 IRAVCYYHTFSMNLD o***o*o*oo*oooo*		
Vesiculovirus	Carajas virus Chandipura virus Cocal virus Isfahan virus Maraba virus Piry virus recombinant Vesiculovirus Spring viraemia of carp virus Vesicular stomatitis Alagoas virus Vesicular stomatitis Indiana virus Vesicular stomatitis New Jersey virus	seqid170 EWKTTCDYRWYGPQYITHSI o*o*****o******o*o*o* seqid171 LGFPPQSCGWASVTT o****oo**oooooo seqid1 VQVTPHHVLVDEYTGEWVDSQFINGKC ooooo*o*oooo*o*o*o*ooooooo		

Lyssavirus	Aravan virus Australian bat lyssavirus Duvenhage virus European bat lyssavirus 1 European bat lyssavirus 2 Irkut virus Khujand virus Lagos bat virus Mokola virus West Caucasian bat virus	Rabies virus	Rabies virus AB21 Rabies virus AB22 Rabies virus AVO1 Rabies virus BNG4 Rabies virus BNG5 Rabies virus China/DRV Rabies virus China/MRV Rabies virus CVS-11 Rabies virus ERA Rabies virus Eth2003 Rabies virus HEP-FLURY Rabies virus India Rabies virus Nishigahara RCEH Rabies virus Ontario fox Rabies virus Ontario skunk Rabies virus PM Rabies virus red fox/08RS-1981/Udine/2008 Rabies virus SAD B19 Rabies virus silver-haired bat-associated SHBRV Rabies virus strain Pasteur vaccin Rabies virus strain Street Rabies virus vnukovo-32 Thailand genotype 1 dog lyssavirus	seqid5 GFTCTGVVTEAETYTNFVGYVT *o****o**o*oo*oooo*** seqid6 SLHNPYPDYRWLRTVKTT *ooooooooooo***o* Seqid138 ESLVIISPSVADLDPYDRSLHS *ooo***oooo*o**ooo Seqid91 CKLKLCGVLGLRLMDGT *ooo****oooo*ooo* Seqid206 ILGPDGNVLIPEMQSS o**o*ooo*******o seqid82 QHMELLESSVIPLVHPL *ooo**o*ooo**oo**		

	unclassified Lyssavirus	Bokeloh bat lyssavirus European bat lyssavirus Lyssavirus Ozernoe Shimoni bat virus				
Novirhabdovirus	Hirame rhabdovirus Infectious hematopoietic necrosis virus Snakehead rhabdovirus Viral hemorrhagic septicemia virus					
unassigned Rhabdoviridae	Bangoran virus Bimbo virus Bivens Arm virus Flanders virus Garba virus Klamath virus Malpais Spring virus Nasoule virus Ngaingan virus Ouango virus Sigma virus Tupaia virus Wongabel virus					
		Lloviu virus (LLOV) Bundibugyo virus (BDBV; previously BEBOV) Reston virus (RESTV; previously REBOV) Sudan virus (SUDV; previously SEBOV) Taï Forest virus (TAFV; previously CIEBOV) Ebola virus (EBOV; previously ZEBOV) Marburg virus (MARV) Ravn virus (RAVV)	Seqid216 GAAIGLAWIPYFGPAAE oo*o*oo***o***ooo seqid217 GAAVGLAWIPYFGPAAE Seqid218 GAAAGLAWIPYFGPAAE Seqid219 DLAAGLSWIPFFGPGIE Seqid220 HNAAGIAWIPYFGPGAE		
Filoviridae						

Lentivirisae	Hiv1		Seqid221 AVGLGALFLGFLGAAGSTMGAAS oooo**ooo*o*oo******o**o seqid222 LTLTGQARQLLS o***o*o*o*oo seqid223 GTVQQQSNLLQAIEAQQ o*****o***o*****o seqid224 GIGAMFLGFLGAAGSTMGAASLTLTVQAR QLLS Seqid225 GIGAMFLGLLSAAGSTMSAAAITLTVQTR QLLS Seqid226 GIGAMFLGLLSAAGSTMGAAAITLTVQTR QLLS Seqid227 GIGAVFLGFLGAAGSTMGAASITLTVQAR QLLS Seqid228 GVGALFLGFLSAAGSTMGAASITLTVQAR QLLS Seqid229 GIGAMILGFLGAAGSTMGAASITLTVQAR QLLS Seqid230 GLGAMFLGFLGAAGSTMGAASITLTVQAR QLLS Seqid231 GFGAMFLGFLGAAGSTMGAASITLTVQAR QLLS Seqid232 TLGAMFLGFLGAAGSTMGAASMTLTVQAR QLLS Seqid233 GLGAVFLGFLGAAGSTMGAASITLTVQAR QLLS Seqid234 TIGAMFLGFLGAAGSTMGAASITLTVQAR RLLS Seqid235 TIGAMFLGFLGAAGSTMGAASMTLTVQAR LLLS

				Seqid236 TLGAMFLGFLGAAGSTMGAASMTLTVQAR LLFS Seqid237 TLGAMFLGFLGAAGSTMGAASLTLTVQAR LLLS Seqid238 GVGAMFLGFLGAAGSTMGAASLTLTVQAR QLLS Seqid239 GLGAMFLGFLGAAGSTMGAASITLTVQAR LLLS Seqid240 TLGAVFLGFLGAAGSTMGAASLTLTVQAR LLLS Seqid241 GIGAVFLGFLGAAGSTMGAASITLTVQAR KLLS Seqid242 GIGALFLGFLGAAGSTMGAASVTLTVQAR QLLS Seqid243 GLGALFLGFLGAAGSTMGAASVTLTVQAR QLLS Seqid244 GIGAMFLGFLGAAGSTMGAASITLTVQAR LLLS Seqid245 GIGAMFLGFLGAAGSTMGAASVTLTVQAR LLLS Seqid246 AIGALFLGFLGAAGSTMGAASVTLTVQAR LLLS Seqid247 TLGAMFLGFLGAAGSTMGAASLTLTVQAR QLLS Seqid248 GIGALFLGFLGAAGSTMGAASMTLTVQAR QLLS Seqid249 GIGAMFLGFLGAAGSTMGAASLTLTVQAR QLLS Seqid250 GIGAVFLGFLGAAGSTMGAASMTLTVQAR LLLS			

Seqid251 GIGALFLGFLGAAGSTMGAASLTLTVQAR QLLS Seqid252 GIGAVFLGILGAAGSTMGAASITLTVQAR QLLS Seqid253 GIGAVFLGFLGAAGSTMGAASVTLTVQAR QLLF Seqid254 GLGAMFFGFLGAAGSTMGAASVTLTVQAR QLLS Seqid255 GIGALFLGFLGAAGSTMGAASITLTVQAR LLLS Seqid256 GLGALFVGFLGAAGSTMGAASITLTVQAR QLLS Seqid257 GIGALFLGFLGTAGSTMGAASVTLTVQAR QLLS Seqid258 GIGAMIFGFLGAAGSTMGAASITLTVQAR QLLS Seqid259 GLGAVLLGFLGTAGSTMGAASLTLTVQVR QLLS Seqid260 GIGAVLFGFLGAAGSTMGAASITLTVQVR QLLS Seqid261 GLGALFLGFLGAAGSTMGAASLTLTGQAR QLLS oo**ooo*o*oo*****o**oo***o*o* o*oo Seqid262 GTLGAMFLGFLGAAGSTMGAASMTLTVQA RQLL Seqid263 GTIGAMFLGFLGAAGSTMGAASITLTVQA RRLL						

			Seqid264 GTIGAMFLGFLGAAGSTMGAAASMTLTVQA RLLL Seqid265 IGALFLGFLGAAGSTMGAASVTLTVQARL LLSG		
		Bovine lentivirus group	Seqid266 AVGMVIFLLVLAIMAMTASVTAA ***oo**********o*o*oo** Seqid267		
		Equine lentivirus group	FGISAIVAAIVAATAIAASA **o*ooo**********o*o Seqid268		
		Feline lentivirus group	TLALVTATTAGLIGTTTGTSA Seqid269		
		Ovine/caprine lentivirus group	HVMLALATVLSMAGAGTGATA Seqid270 GIGLVIMLVTMAIVAAAGAS *o****oo*oo***o*o***o		
		Human immunodeficiency virus 2	Seqid271 GVMVLGFLGFLAMAGSAMGA		
		Simian immunodeficiency virus	ooo***o**oooo*oooooo Seqid272		
		Simian immunodeficiency virus others	GVFVLGFLGFLATAGSAMGA oooo**oo*o*oo**ooooo Seqid273		
		Ovine lentivirus	GAIVLGLLGFLGLAGSAMG *ooooooo*o*ooo**ooo Seqid274		
			GIGLVIVLAIMAIIAAAGAGLGVANAVQ		

	Arteriviridae	Porcint Reproductions og Respirations Syndrome (PRRS)	PRRS Type I	seqid275 SRKLGRSLIPHSCFWWLFLLC seqid276 GNGNSSTYQYIYNLTIC seqid277 GTAWLSTHFSWAVETFVLYHILSL seqid278 GFLTTSHFFDTLGLGAVSITGFC seqid279 RYAHTRFTNFIVDDRGRIHRW	
			PRRS Type II	seqid280 SNNNSSHIQLIYNLTLC seqid281 GTDWLAQKFDWAVETFVIFPVLTH seqid282 GALTTSHFLDTVGLATVSTAGYY seqid283 IYAVCALAALICFVIRLAKNC seqid284 VSTAGYYHGRYVLSSIYAVCALAALICFV IRL		

Figures

In all the figure texts, the INF-F#2 peptide has the sequence GLFGAIAGFIENGWEGCGGEKEKEK [Seq id 287] and is dimerized through a disulfide bridge between the cystein residues.

Fig. 1 shows type I IFN production in cells either untreated (UT) or treated with cationic liposomes. Cells were either pre-treated with INF-F#2 or not pre-treated with any substance. In figure 1 it can be seen that treatment with cationic liposomes induce the production of type I IFN and that this production was inhibited when cells were pre-treated with INF-F#2. The cells used were bonemarrow derived dendritic cells (BMDCs) from mice of the laboratory strain C57BL. The peptide INF-F#2 was used at a concentration of 10µM and initiated 15minutes before treatment with cationic liposomes. Cells were then left to incubate 16 hours at 5% CO_2 and 37.5 degrees Celcius. After 16 hours of incubation the supernatants were harvested. Type I IFN activity was then measured using a type I IFN bioassay.

Cationic liposomes were prepared using a mix of the lipids DOTAP/DOPE/LR (Lissamine Rhodamine)-DOPE in a w/w/w of 1/1/0.1 dissolved in chloroform. The mix was purchased from the company Avanti Polar Lipids, inc. Chloroform was evaporated using rotation evaporation using a dry nitrogen stream. The lipids film was then dried in vaccum for 4 hours. To created liposomes, the lipid film was hydrated in phosphate buffered saline solution at pH 7.4 at ambient temperature. Liposomes were then size restricted using a 0.2µm filter and an Avanti Mini-extruder purchased form Avanti Polar Lipids.

Fig. 2 shows type I IFN production in cells either untreated (UT) or treated with the transfection reagent Lipofectamine2000 (Lipofect) according to the manufactures instructions. In this case Lipofectamine2000 was used alone and not with the purpose of transfecting DNA or RNA into the cells. The type I IFN produces therefore represent the cellular response to the transfection reagent Lipofectamine2000 itself. Cells were either pre-treated with INF-F#2 or not pre-treated with any substance. In figure 2 it can be seen that treatment with Lipofectamine2000 induced the production of type I IFN and that this production was inhibited when cells were pre-treated with INF-F#2. Cells used were BMDCs as described in the Figure 1 text.

Fig. 3 shows type I IFN production in cells either untreated (UT) or transfected with double stranded DNA (dsDNA) using the reagent Lipofectamine2000 according to the manufactures instructions. Cells were either pre-treated with INF-F#2 or not pre-treated with any substance. In figure 3 it can be seen that transfection with dsDNA induces the production of type I IFN and that this production was NOT inhibited when cells were pre-treated with INF-F#2. Therefore, pretreatment does not interfere with type I IFN levels induced by the transfected dsDNA but only with the type I IFN induced by the transfection reagent itself. Cells used were BMDCs as described in the Figure 1 text.

Fig. 4 shows co-precipitation of endogenous STING protein with biotinylated INF-F#2 but not the with the INF-F#2 mutant DI6. In this experiment, the human monocytic cell line THP-1 was lysed using a native lysis buffer. Cell lysates were cleared by centrifugation at 300xg for 5 min. Supernatants were then incubated with a biotinylated form of INF-F#2 (**B**), with a biotinylated form of the peptide mutant DI6 (**C**) or with no peptide (**A**) for 30min on ice. Cells lysates were then mixed with biotin binding streptavidin beads and mixed for 30 min on ice. Beads were then collected by centrifugation at 2000xg for 2min. Beads were then washed x3 in PBS at pH 7.4. Beads were then headed to 95

deg. Celcius in reducing denaturating loading buffer for 5 min. yielding the pull-down lysate. This was then loaded and run on a SDS-PAGE for 45 min at 120V. Proteins were then transferred to protein PVDF membrane and blotted for STING and Giantin. The protein Giantin is a golgi-resident transmembrane protein unrelated to STING function. As depicted in figure 4, the INF-F#2 but not beads alone or the peptide DI6 was able to precipitate STING. Further, INF-F#2 was unable to precipitate other transmembrane proteins such as Giantin.

The peptide INF-F#2 therefore blocks STING dependent type I IFN production in response to membrane disturbance as introduced by liposome based transfection agents. This is possibly through direct or indirect interaction with STING itself as INF-F#2 binds either directly to STING or to a complex which contains STING. This occurs without significant interference with type I IFN production with response to transfected immuno-stimulatory DNA itself (Fig. 3)

Fig. 5, (left panel): shows the effect of INF-F#2 (INF ISD) on STING dimerization in response to cationic liposomes. Cells of the human moncytic cell line THP-1 were either culture in the presence or absence of INF-F#2 (INF ISD) as depicted. Cells were then either treated with or left untreated with cationic liposomes (as in Figure 1) for 3.5 hours. Cells were then lysed using RIPA lysis buffer with 0.1% SDS. Lysates were then run on a SDS-PAGE at 120V for 45min, transferred to a PVDF membrane, and blottet using anti-STING antibodies. As seen in figure 5, cationic liposomes were able to induce STING dimerization, which is a necessary step in STING dependent induction of type I IFN. Pretreatment of cells with INF-F#2 resultet in an inhibition of the formation of STING dimers. (Right panel): Lysates from cells treated with cationic liposomes either treated with the reducing agent DTT or left untreated for 5min.

Therefore, INF-F#2 binds either directly to STING or to a STING containing complex (Figure 4), and interferes with STING dimerization in response to cellular treatment with cationic liposomes.

Figure 6 shows inflammation-related enzyme and transcription factor gene expression kinetics of THP-1 monocytes stimulated with 1µg/ml LPS. Gene expression was expressed as relative gene expression towards RPL13a-expression and non-stimulated cells at time zero (ΔΔCt). Data shown are means + standard deviation from two independent biological replications.

Figure 7 shows effects of INF-F#2 peptide on expression of NF-kappaB mRNA in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM, 60µM INF-F#2 peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the medians ± standard deviation from two independent biological replications.

Figure 8 shows effects of INF-F#2 peptide on expression of SP-1 mRNA in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM, 60µM INF-F#2 peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the medians ± standard deviation from two independent biological replications.

Figure 9 shows effects of INF-F#2 peptide on protein secretion of IL-8 in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM or 60µM INF-F#2 peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the median ± standard deviation from three independent experiments performed in duplicates.

Figure 10 shows effects of INF-F#2 peptide on protein secretion of IL-10 in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM or 60µM INF-F#2 peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the median ± standard deviation from three independent experiments performed in duplicates.

Figure 11 shows effect of different stimulus on the secretion of IFN-gamma in PBMCs. PBMCs were incubated either with 1µg/ml or 50ng/ml PMA and 1µg/ml ionomycin or 10ng/ml SEB for indicated time periods. Data shown are the medians ± standard deviation from three independent technical replicates.

Figure 12 shows expression kinetics of IFN gamma expression in response to PMA/ionomycin treatment. Gene expression was expressed as relative gene expression towards RPL13a expression and non-stimulated cells at time zero (ΔΔ Ct). Data shown are the medians ± standard deviation from three independent technical replicates.

Figure 13 shows effect of INF-F#2 peptide on secretion of protein of IFN-gamma in PMA/ionomycin stimulated PBMCs. PBMCs were incubated with either medium alone, 30µM or 60µM INF-F#2 peptide or 30µM or 60µM control peptide, and stimulated with 50ng/ml PMA and 1µg/ml ionomycin. Data shown are the medians ± standard deviation from three independent experiments performed in duplicates.

Figure 14 shows effects of SARS ([Seq id 285] AEVQIDRLITGRLQSLQTYVCGGEKEKEK) or Filo ISD ([Seq id 286] GAAIGLAWIPYFGPAAECGGEKEKEK) on expression of TNF-alpha mRNA in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM, 60µM SARS or Filo ISD peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the medians ± standard deviation from two independent biological replications.

Figure 15 shows effects of SARS or Filo ISD on expression of IL-1 β mRNA in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM, 60µM SARS or Filo ISD peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the medians ± standard deviation from two independent biological replications.

Figure 16 shows effects of SARS or Filo ISD on expression of IL-1 β mRNA in LPS-stimulated THP-1 cells. THP-1 cells were incubated with either medium alone, 30µM, 60µM SARS or Filo ISD peptide or 30µM, 60µM control peptide, and stimulated with 1µg/ml LPS. Data shown are the medians ± standard deviation from two independent biological replications.

Figure 17 shows interactions between INF-F#2 peptide and STING depends on distinct STING domains. To investigate further the interaction between STING and INF-F#2 peptide the C-terminal domian of STING was expressed with a HA-tag in HEK293 cells. STING was either in a wt form or with deletions. Lysates from tansfected cells were used for pulldown using biotinylated INF-F#2 peptide and streptavidin coated beads. The bead eluate was then immunoblotted using antibodies against HA-tag. As seen in the figure wt STING and the deletion mutant DN5 (162-N) was readily pulled down using INF-F#2 peptide whereas the deletion mutants DN6 (172-N) was not. These data indicate that amino acids 162-172 are necessary for interactions between INF-F#2 peptide and STING.

Figure 18: Bonemarrow derived dendritic cells (BMDCs) were pretreated with indicated peptides at 5µM. After 30min cells were then treated with cationic liposomes (Lipo) or iwht lmw(low molecular

weight) dsRNA (Poly I:C). After 18 hours supernatants were analyzed for type I IFN using a bioassay based on vesicular stomatitis virus (VSV) and L929 cells.

INF wt is the INF-F#2 peptide.

INF D4-6 and INF DI6 are deletion mutants of the INF ISD (negative controls) INF mono is the monomeric form of the peptide that does not have any effect.

The data show that the INF-F#2 peptide inhibits interferon production induced by liposom fusion with the cells.

Figure 19: a+b) BMDCs were pretreated with indicated peptides at 5µM. After 30min cells were then treated with dsDNA (2µg/mL) by transfection using lipofectamine 2000. After 18hours supernatants were analyzed for type I IFN using a bioassay based on vesicular stomatitis virus (VSV) and L929 cells. c-e) Human monocyte derived macrophages were treated with virus like particles (Vlp) or cationic liposomes. Cells were either not pretreated or pretreated with INF-F#2 peptide (pFlu). After 4 hours cells were fixed, stained for STING (green) and with DAPI (blue). The quantity of large STING foci were then quantified by confocal microscopy (d+e). f) Left panel: Cells of the human monocytic cell line THP-1 were stimulated with cationic liposomes. Cells were either not pretreated or pretreated with INF-F#2 peptide (pFlu). STING protein was then assessed using immunoblotting after non-reducing SDS-PAGE. Right panel: Lysate from liposome treated cells were either analyzed under non-reducing conditions or under reducing conditions (DTT).

Influenza peptide or pFlu are identical to INF ISD peptide which is identical to INF-F#2 peptide.

Figure 20: BMDCs were treated with lipofectamine2000 according to manufactures directions for usage of lipofectamine2000 for transfection with DNA (but in this instance without DNA). After 18 hours supernatants were harvested and analyzed for type I IFN by bioassay. Before lipofectamin2000 treatment cells received either no pretreatment or pretreatment for 30 minutes with INF-F#2 peptide (product). The data shows that the INF-F#2 peptide prevents production of IFN caused by lipofectamine transfection.

All cited references are incorporated by reference.

The accompanying Figures and Examples are provided to explain rather than limit the present invention. It will be clear to the person skilled in the art that aspects, embodiments and claims of the present invention may be combined.

Examples

INF-F#2, a peptide from Influenza HA2, blocks type I IFN production induced by membrane fusion.

We wanted to investigate the ability of a conserved peptide (INF-F#2) from Influenza HA2 to interfere with type I IFN induced by membrane fusion. To do this we used bone marrow -derived dendritic cells (BMDCs), which were either left untreated or pretreated with a soluble form of INF-F#2. The cells were then stimulated with highly fusogenic cationic liposomes (Holm et al., 2012) (**Figure 1**) and incubated overnight. Supernatants were then analyzed for type I IFN. Interestingly,

INF-F#2 pretreatment completely blocked type I IFN production. Many commercially available transfection reagents (such as Lipofectamine2000 from Invitrogen) are based on cationic liposomes and function because such liposomes can transport charged molecules such as DNA into living cells. In many cases the reagent itself is harmful to the treated cells and induce these to produce cytokines such as type I IFN. To test whether INF-F#2 could also inhibit the response to Lipofectamine2000 we pretreated BMDCs with INF-F#2 for 30min and then treated the cells with Lipofectamine2000 according to instructions by Invitrogen. As seen in Figure 2, INF-F#2 completely blocked the type I IFN response to Lipofectamine. We have previously shown that type I IFN production in response to membrane fusion depends on STING (Holm et al., 2012). Therefore, we were interested in clarifying whether INF-F#2 also inhibited type I IFN production in response to intracellular dsDNA, which also depends on STING. To do so, we again pretreated BMDCs with INF-F#2 and then treated the cells with dsDNA delivered using Lipofectamine2000 according to Invitrogens instructions. As seen in Figure 3, pre-treatment with INF-F#2 did not interfere with dsDNA induced type I IFN (**Figure 3**). These data show that INF-F#2 treatment specifically inhibits STING dependent type I IFN induction in response to membrane fusion as induced in BMDCs by lipid based transfection reagents such as Lipofectamin2000.

pFlu co-precipitates with STING

To investigate further how INF-F#2 inhibits signalling in response to membrane fusion fusion, we used biotinylated INF-F#2 and DI6 peptides. To find proteins interacting with pFlu, non-denatured, non-reduced lysates from THP-1 cells were mixed with either of the biotinylated peptides and precipitated with streptavidin immobilized on resin beads. Precipitated proteins were separated using reducing SDS-PAGE with subsequent probing with anti-STING antibodies. Interestingly, STING was detectable in the bio-pFlu preparations but not in the bio-DI6 or no-peptide preparations (**Figure 4**). This did not seem to be due to an unspecific pull-down of membrane fragments caused by membrane associating properties of pFlu since the abundant transmembrane golgi resident protein giantin was not pulled down by pFlu. Instead, these data suggest that STING specifically co-precipitates with pFlu. The interaction between STING and pFlu was mapped further by using expression of C-terminal domain of CTD of STING in HEK293 cells. Either wt STING or deletion mutants with stepwise deletions of STING amino acids were used. By this approach it was possible to identify aminoacids 162-172 as being necessary for pFLu and STING interactions (Figure 17).

INF-F#2 blocks STING signalling in response to cationic liposomes

It has recently been shown that STING forms redox-sensitive homodimers in response to stimulation with cyclic di-nucleotides. We wanted to examine whether this was also the case when STING was liposome induced membrane fusion, and further whether homodimer formation was affected by IFN-F#2 pre-treatment. Here we found that membrane fusion indeed did induce homodimer formation and that this was abrogated by pre-treatment with INF-F#2 (**Figure 5**).

Claims

1. A use of a peptide comprising an immune suppressive domain for transfection.

2. The use of a peptide according to claim 1, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, subject to the proviso that a monomeric peptide should comprise at least two immune suppressive domain sequences.

3. The use of a peptide according to claim 1 or 2, wherein said peptide is a dimer.

4. A use of a peptide comprising an immune suppressive domain in a transfection mix, subject to the proviso that said peptide is a dimer or multimer or comprises at least two immune suppressive domain motifs.

5. The use according to any of the preceding claims, wherein said at least two immune suppressive domain motifs are two identical or different motifs.

6. A use of a peptide comprising an immune suppressive domain for transfection of a cell, said peptide providing immune suppression of the transfected cell.

7. The use of a peptide according to any of the preceding claims, which is soluble in water.

8. The use of a peptide according to any of the preceding claims, wherein said immune suppressive domain is part of a virus.

9. The use of a peptide according to claim 8, wherein said virus is an influenza virus.

10. The use of a peptide according to any of the preceding claims, wherein said peptide forms part of a protein.

11. A use of a molecule, which comprises at least two parts, each part comprising an immune suppressive domain, for transfection.

12. A kit-of-parts or composition comprising:
    a. a transfection agent; and
    b. a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

13. The kit-of-parts or composition according to claim 12, wherein said transfection agent is coupled covalently to said peptide.

14. The kit-of-parts or composition according to any of the claims 12 - 13, wherein said transfection agent is a lipid based transfection agent.

15. The kit-of-parts or composition according to claim 14, wherein said lipid based transfection agent is selected among an anionic and a cationic transfection agent.

16. The kit-of-parts or composition according to any of the claims 12 - 15, wherein said transfection agent is a lipid based liposome or virosome or viral vector.

17. The kit-of-parts or composition according to any of the claims 12 - 16, comprising a nano particle.

18. The kit-of-parts or composition according to any of the claims 12 - 17, further comprising a hydrophobic vehicle for delivery of hydrophobic molecules.

19. The kit-of-parts or composition according to any of the claims 12 - 18, comprising a drug.

20. The kit-of-parts or composition according to any of the claims 12 - 19, wherein said peptide is an immune suppressive peptide.

21. The kit-of-parts or composition according to any of the claims 12 - 20, wherein said peptide forms part of a protein of a pathogen.

22. The kit-of-parts or composition according to claim 21, wherein said pathogen is a virus.

23. The kit-of-parts or composition according to claim 22, wherein said peptide forms part of a protein on the surface of a pathogen.

24. The kit-of-parts or composition according to claim 23, wherein said peptide forms part of a virus surface glycoprotein.

25. The kit-of-parts or composition according to claim 24, wherein said immune suppressive peptide forms part of an enveloped virus surface glycoprotein.

26. The kit-of-parts or composition according to any of the claims 12 - 25, wherein said immune suppressive peptide has a length of at least 6, preferably 7, more preferred 8, preferably 9, more preferred 10, preferably 11, more preferred 12, preferably 13, more preferred 14, preferably 15, more preferred 16, preferably 17, more preferred 18, preferably 19, more preferred 20, preferably 21 more preferred 22, preferably 23, more preferred 24, preferably 25 amino acids.

27. The kit-of-parts or composition according to any of the claims 12 - 26, wherein said peptide has a length selected among 5 – 200, preferably 10 - 100, more preferred 20 – 50, preferably 30 – 40 amino acids.

28. The kit-of-parts or composition according to any of the claims 12 - 27, further comprising a fusion peptide from a fusion protein.

29. The kit-of-parts or composition according to claim 28, wherein said fusion peptide is from the fusion protein of an enveloped virus.

30. The kit-of-parts or composition according to claim 29, wherein said fusion peptide is from a type I fusion protein.

31. The kit-of-parts or composition according to claim 29, comprising a fusion peptide from a type II fusion protein.

32. The kit-of-parts or composition according to any of the claims 28 - 31, wherein said fusion peptide has 1, 2, 3 or 4 mutations, deletions or insertions with respect to the wild type.

33. The kit-of-parts or composition according to any of the claims 12 - 32, wherein said peptide, or a functional homologue thereof, binds either directly or indirectly to a cellular protein complex containing the protein STING encoded by the gene *Tmem173*.

34. The kit-of-parts or composition according to any of the claims 12 - 33, wherein said peptide, or a functional homologue thereof, affects type I interferon responses.

35. The kit-of-parts or composition according to any of the claims 12 - 34, wherein said peptide, or a functional homologue thereof, affects type I interferon responses induced by membrane fusion.

36. The kit-of-parts or composition according to any of the claims 12 - 35, comprising a peptide selected among the group consisting of the lists of Table 1 and the sequences with Seq. Id. 1 – 287, preferably Seq. Id. 287.

37. The kit-of-parts or composition according to any of the claims 12 - 36, comprising a peptide from an influenza virus or a Flu peptide.

38. The kit-of-parts or composition according to any of the claims 12 - 37, wherein said peptide has immune suppressive activity as dimer or mulitimer or when coupled to carrier proteins.

39. The kit-of-parts or composition according to any of the claims 12 - 38, wherein said peptide comprises at least one non-genetically encoded amino acid residue.

40. The kit-of-parts or composition according to any of the claims 12 - 39, wherein said peptide comprises at least one D-amino acid.

41. The kit-of-parts or composition according to any of the claims 12 - 40, wherein said peptide comprises at least one D-amino acid residue.

42. The kit-of-parts or composition according to any of the claims 12 - 41, wherein said peptide is coupled to any other molecule.

43. The kit-of-parts or composition according to any of the claims 12 - 42, wherein said peptide is attached to lipids.

44. The kit-of-parts or composition according to any of the claims 12 - 43, wherein said peptide is coupled to a molecule through a peptide bond.

45. The kit-of-parts or composition according to any of the claims 12 - 44, wherein said peptide is coupled to a protein.

46. The kit-of-parts or composition according to any of the claims 12 - 45, wherein said peptide is a circular peptide.

47. The kit-of-parts or composition according to any of the claims 12 - 46, wherein said peptide is attached to at least one biological membrane.

48. The kit-of-parts or composition according to any of the claims 12 - 47, wherein said peptide is modified in a way in which one of the peptide bonds is replaced by a non-peptide bond.

49. The kit-of-parts or composition according to any of the claims 12 - 48, wherein said peptide interferes with an interferon response induced by the transfection agent upon addition to a cell.

50. The kit-of-parts or composition comprising a functional homologue of a peptide according to the any of the claims 12 - 49.

51. A transfection reagent comprising a lipid based molecule coupled covalentely to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

52. A nano particle coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

53. A hydrophobic vehicle for delivery of hydrophobic molecules coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

54. A drug coupled covalently to a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

55. A use of a peptide, composition, a kit-of-parts, a transfection agent, a nano particle, a hydrophobic vehicle or a drug according to any of the preceding claims for inhibiting immune response.

56. The use of a peptide, composition, or a kit of parts according to any preceding claims for introducing a molecule into a cell.

57. The use of a peptide, composition, or a kit of parts according to claim 56, for introducing a DNA or RNA molecule into a cell.

58. The use of a peptide, composition, or a kit of parts according to claim 56, for introducing a pharmaceutical molecule into a cell.

59. The use of a peptide, composition, or a kit of parts according to claim 56, for introducing a pharmaceutical molecule into a tissue.

60. The use of a peptide, composition, or a kit of parts according to claim 59, for introducing a gene-therapeutic pharmaceutical molecule into a cell.

61. The use of a peptide, composition, or a kit of parts according to any preceding claims for initiating expression of proteins via transfected plasmids.

62. The use of a peptide, composition, or a kit of parts according to any preceding claims for targeting active genes by microRNA silencing.

63. The use of a peptide, composition, or a kit of parts according to any preceding claims for examining the immune response to DNA or RNA.

64. The use of a peptide, composition, or a kit of parts according to any preceding claims for gene therapy.

65. The use of a peptide, composition, or a kit of parts according to any preceding claims for delivery of any molecule using liposomes.

66. A method for transfecting a cell, said method comprising:
    a. Providing a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences;
    b. Incubating the cell with said peptide;
    c. Providing a transfection agent;
    d. Providing a material to be transfected into the cell; and
    e. Further incubating the cell with said transfection agent and said material.

67. A cell obtainable with the method according to claim 66.

68. A cell obtainable by incubating a cell with a peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

69. A cell comprising a peptide, said peptide comprising an immune suppressive domain, wherein said peptide is selected among a dimer, a multimer, and a monomeric peptide, which comprises at least two immune suppressive domain sequences.

70. A use of a cell according to any of the claims 67 to 69, for diagnostics, prophylaxis or therapy.

71. The use according to claim 70, for cell therapy and/or gene therapy.

72. The use according to claim 70 or 71, for stem cell transplantation.

Figure 1

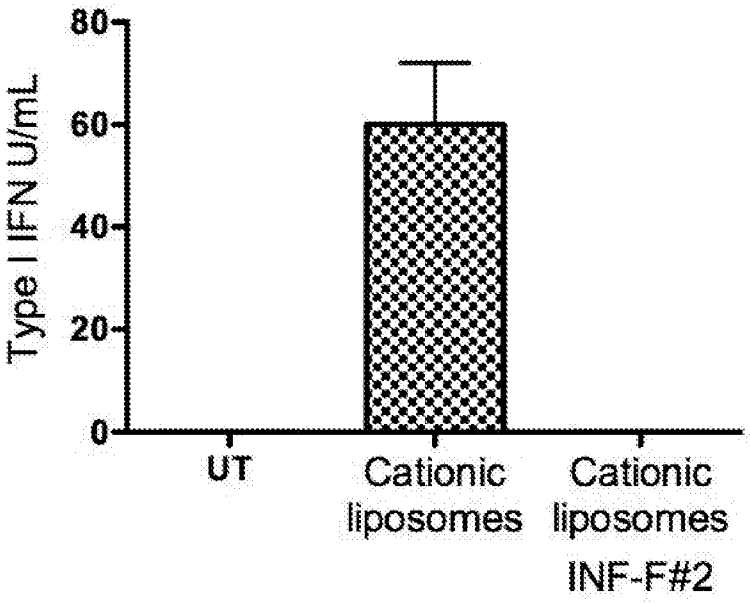


Figure 2

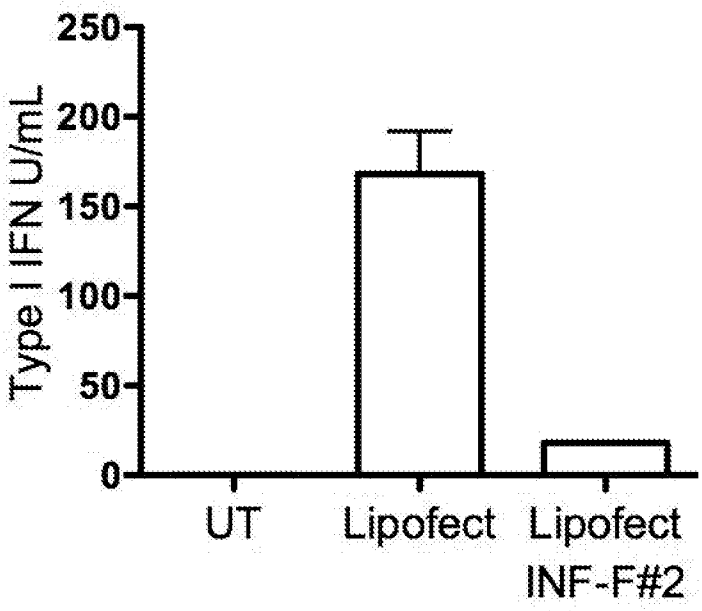


Figure 3

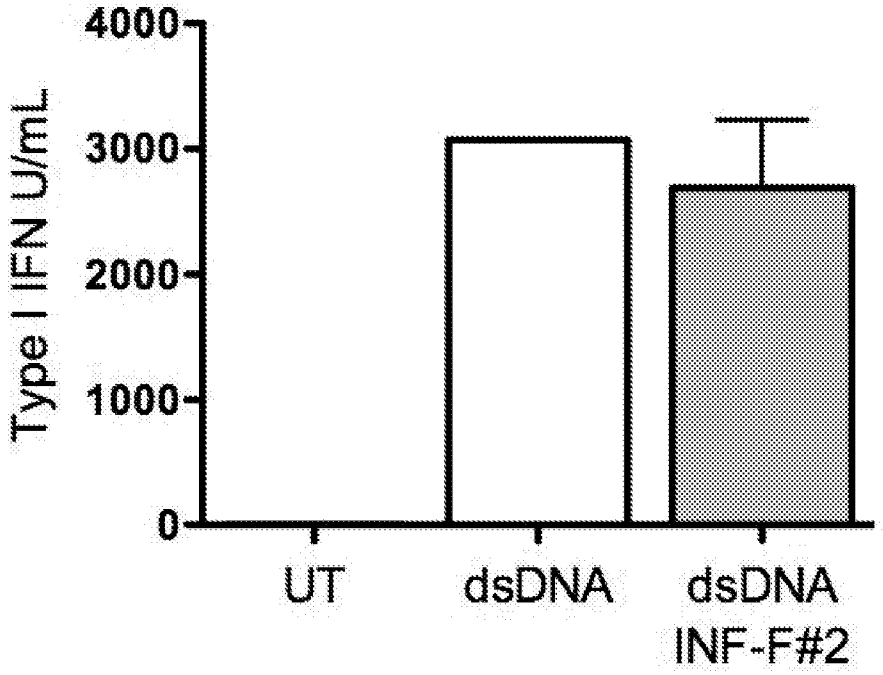


Figure 4

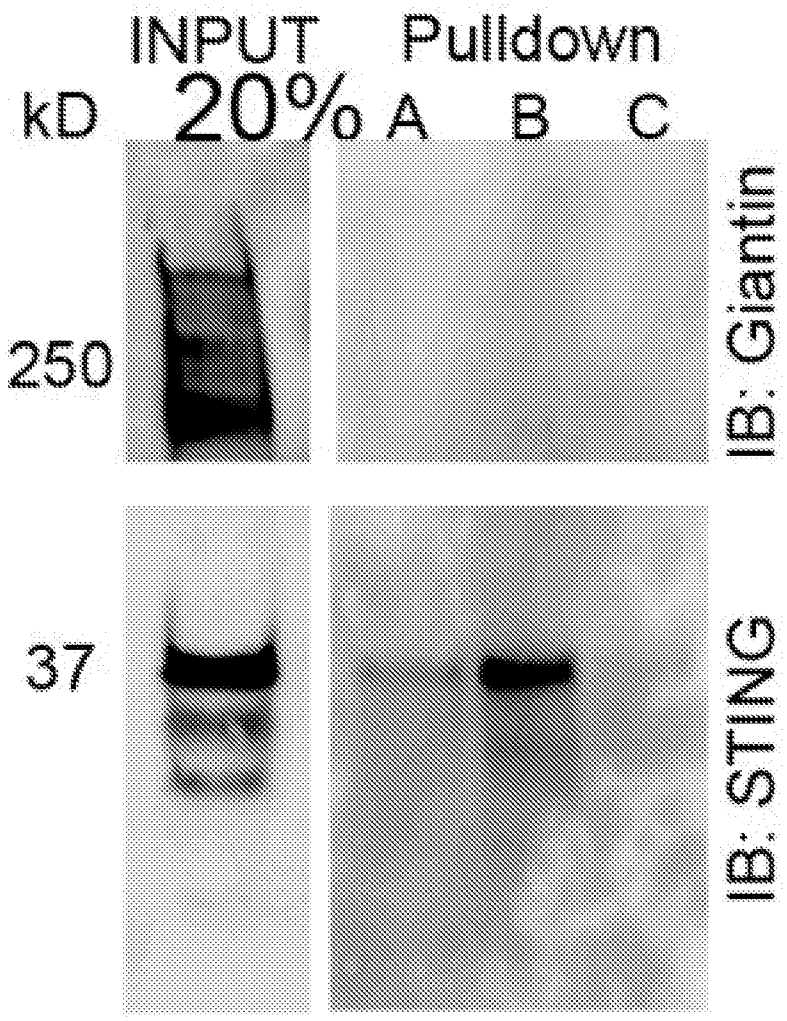
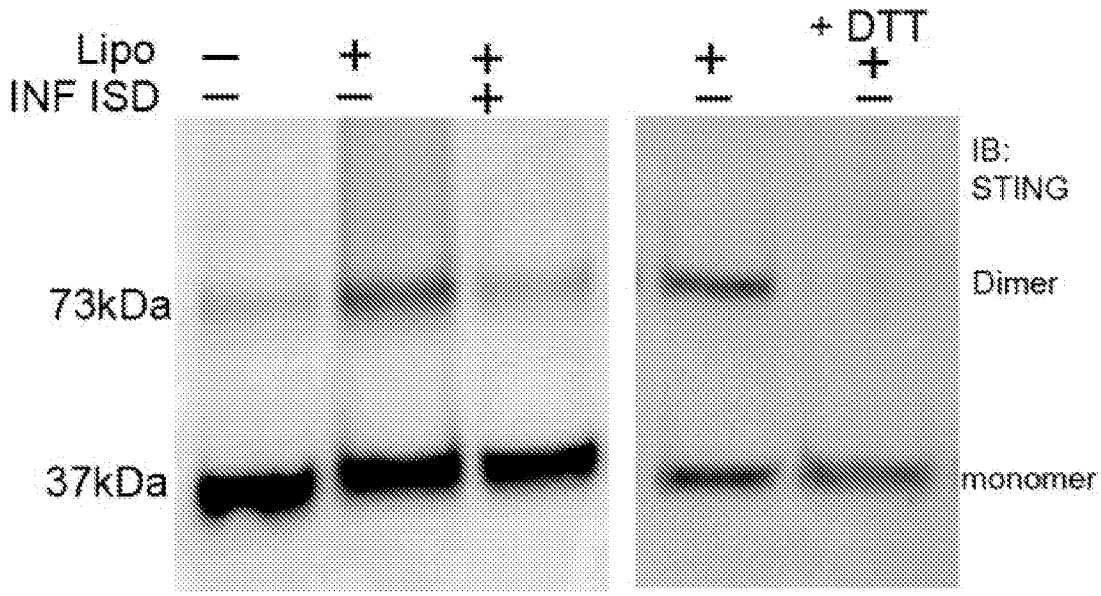


Figure 5



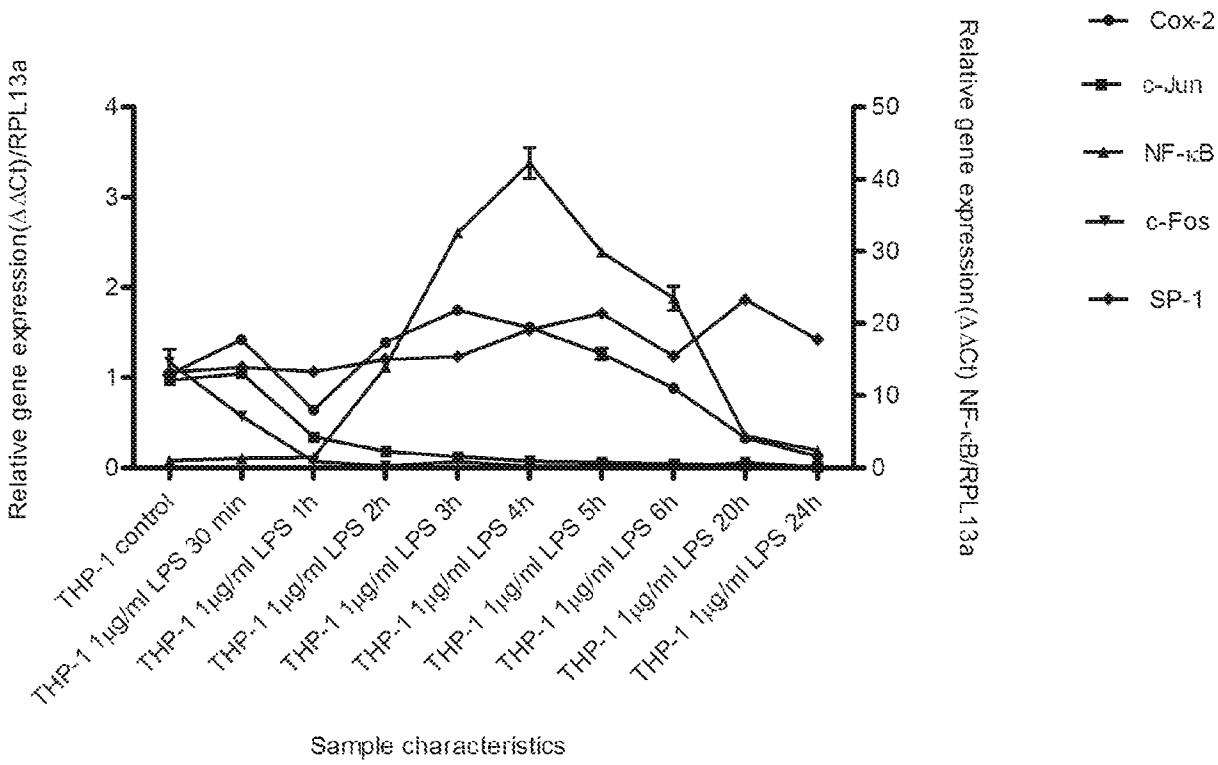


Fig. 6

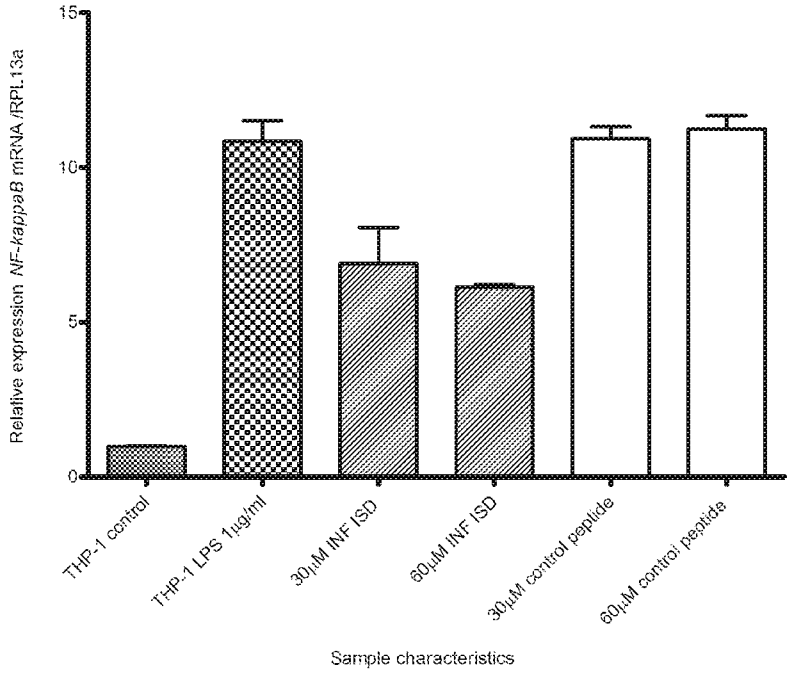

Relative expression NF-kappaB mRNA /RPL13a
15
10
5
0
THP-1 control
THP-1 LPS 1µg/ml
30µM INF ISD
60µM INF ISD
30µM control peptide
60µM control peptide
Sample characteristics

Fig. 7

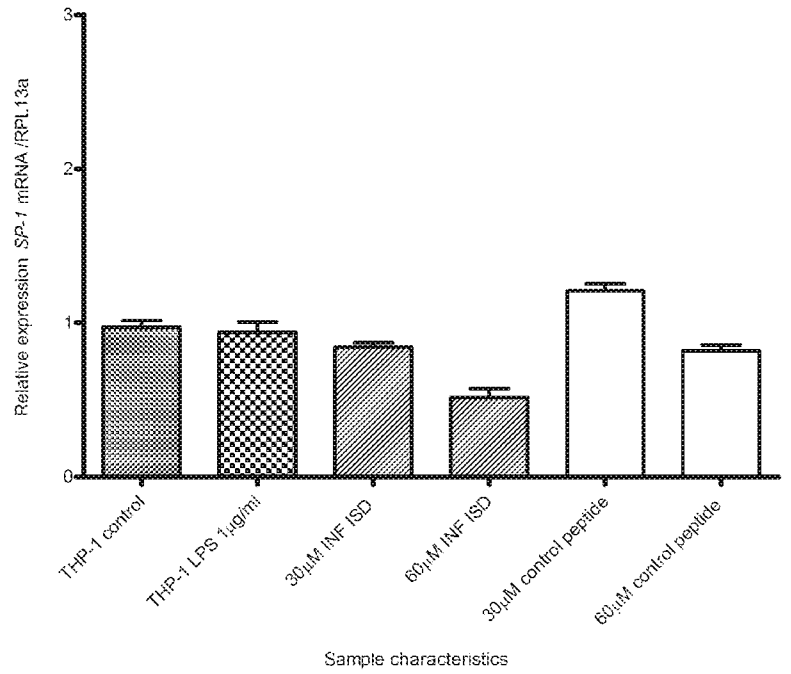

Relative expression SP-1 mRNA /RPL13a
3
2
1
0
THP-1 control
THP-1 LPS 1µg/ml
30µM INF ISD
60µM INF ISD
30µM control peptide
60µM control peptide
Sample characteristics

Fig. 8

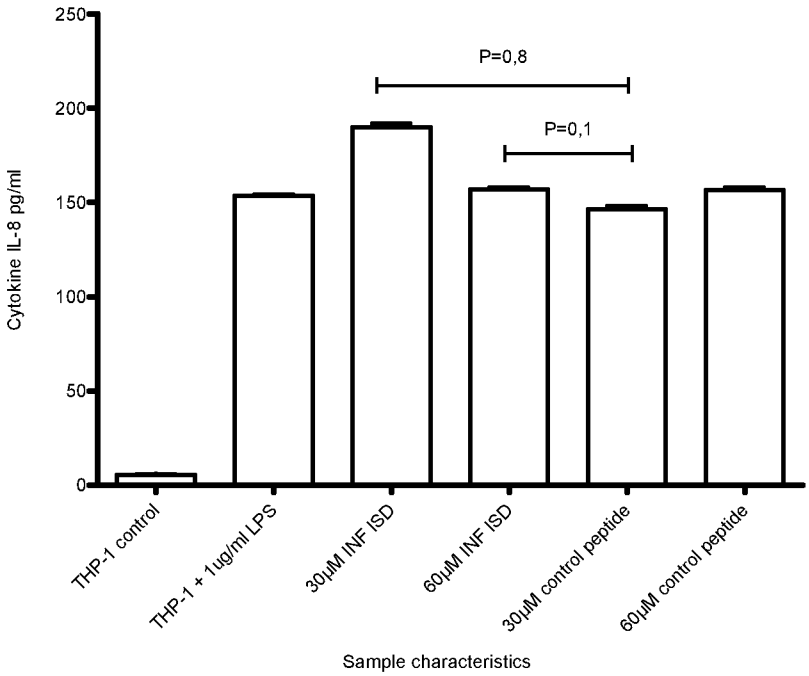

250
200
150
100
50
0
Cytokine IL-8 pg/ml
P=0,8
P=0,1
THP-1 control
THP-1 + 1ug/ml LPS
30µM INF ISD
60µM INF ISD
30µM control peptide
60µM control peptide
Sample characteristics

Fig. 9

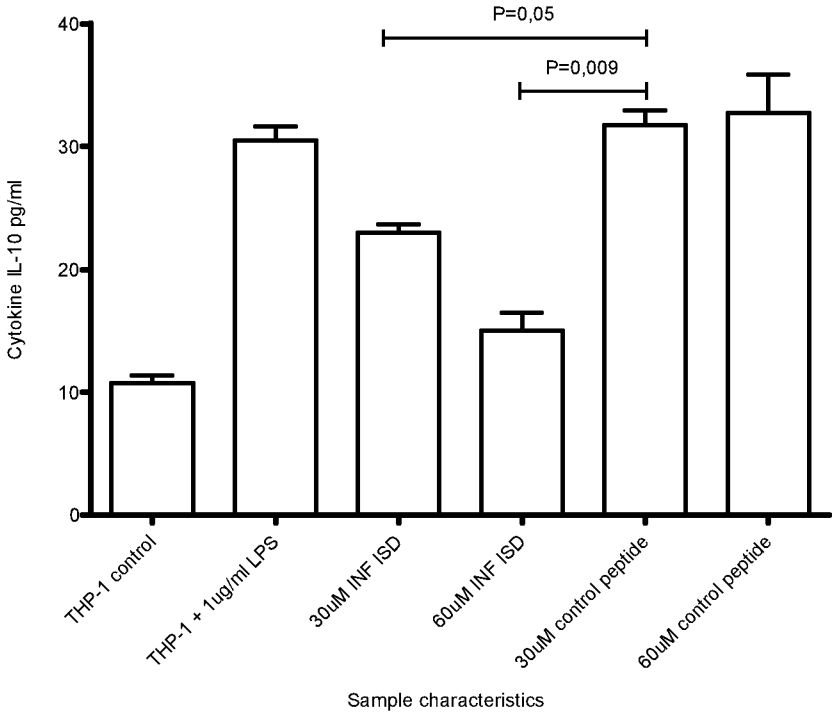

40
30
20
10
0
Cytokine IL-10 pg/ml
P=0,05
P=0,009
THP-1 control
THP-1 + 1ug/ml LPS
30uM INF ISD
60uM INF ISD
30uM control peptide
60uM control peptide
Sample characteristics

Fig. 10

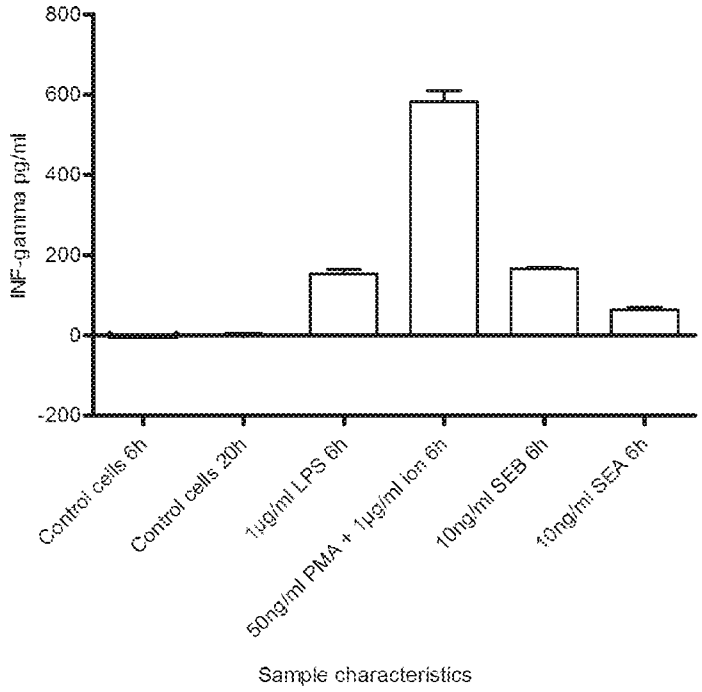

800
600
400
200
0
-200
INF-gamma pg/ml
Control cells 6h
Control cells 20h
1µg/ml LPS 6h
50ng/ml PMA + 1µg/ml ion 6h
10ng/ml SEB 6h
10ng/ml SEA 6h
Sample characteristics

Fig. 11

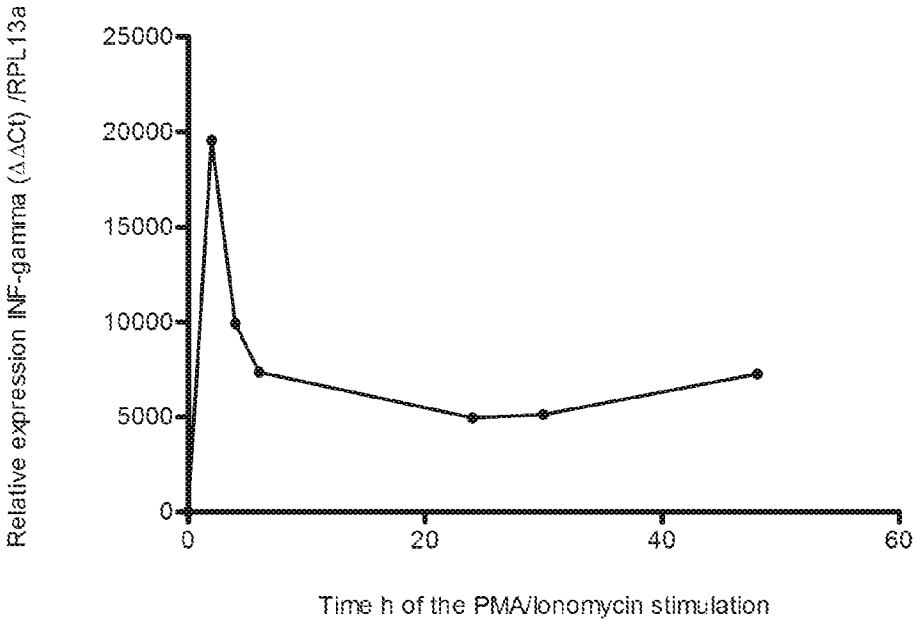

25000
20000
15000
10000
5000
0
Relative expression INF-gamma (ΔΔCt) /RPL13a
0
20
40
60
Time h of the PMA/Ionomycin stimulation

Fig. 12

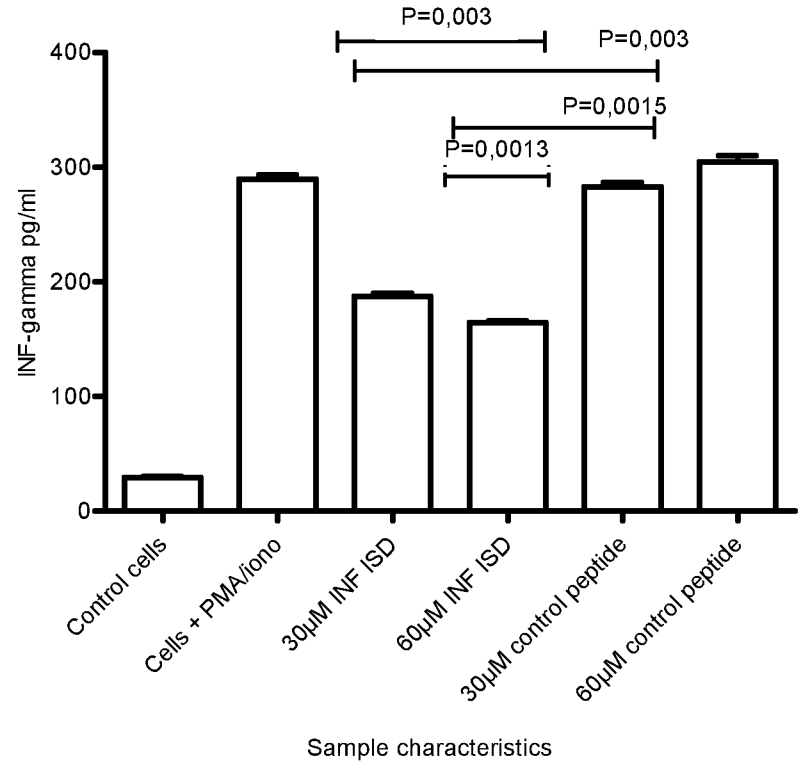

P=0,003
P=0,003
P=0,0015
P=0,0013
400
300
200
100
0
INF-gamma pg/ml
Control cells
Cells + PMA/iono
30µM INF ISD
60µM INF ISD
30µM control peptide
60µM control peptide
Sample characteristics

Fig. 13

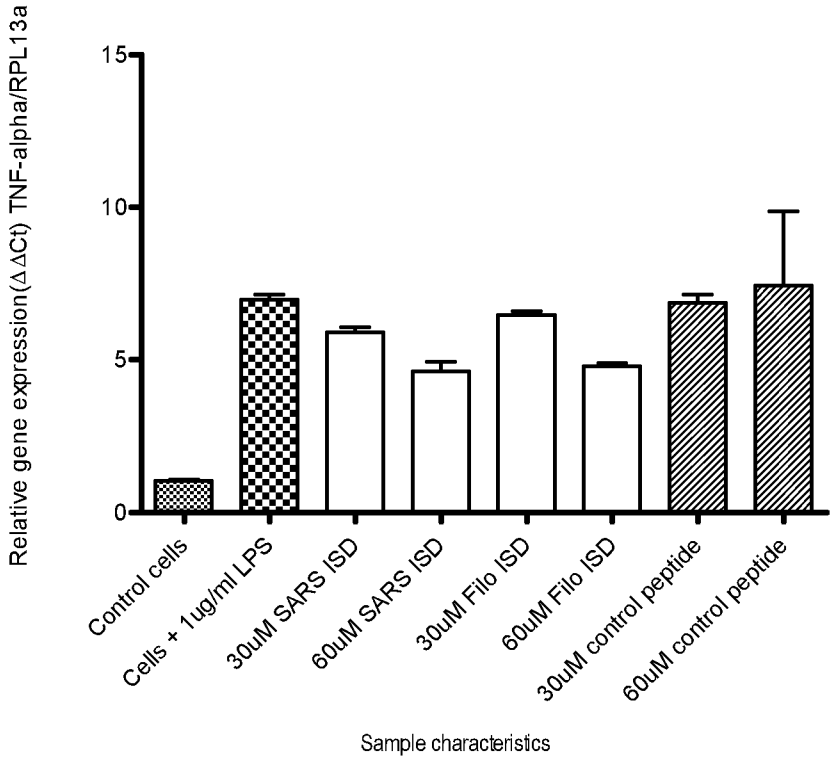

15
10
5
0
Relative gene expression(ΔΔCt) TNF-alpha/RPL13a
Control cells
Cells + 1ug/ml LPS
30uM SARS ISD
60uM SARS ISD
30uM Filo ISD
60uM Filo ISD
30uM control peptide
60uM control peptide
Sample characteristics

Fig. 14

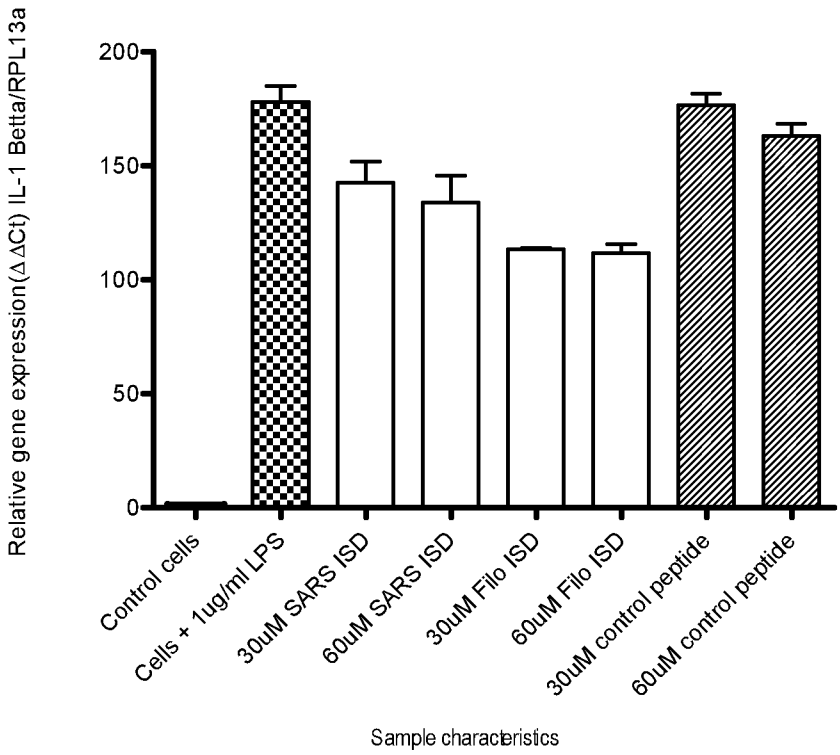

Relative gene expression(ΔΔCt) IL-1 Betta/RPL13a
200
150
100
50
0
Control cells
Cells + 1ug/ml LPS
30uM SARS ISD
60uM SARS ISD
30uM Filo ISD
60uM Filo ISD
30uM control peptide
60uM control peptide
Sample characteristics

Fig. 15

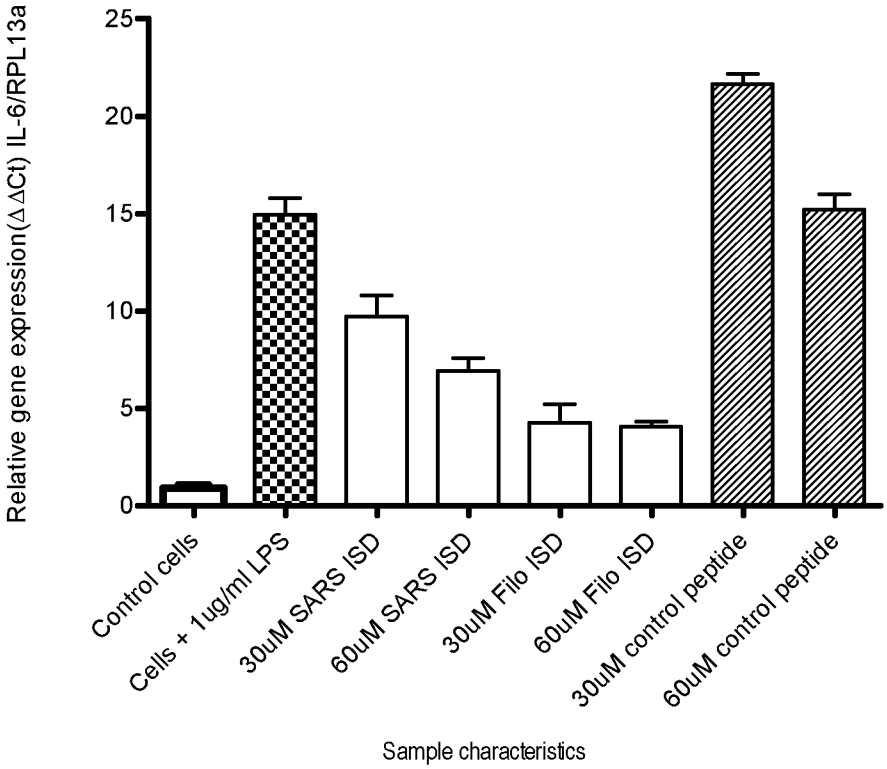

Relative gene expression(ΔΔCt) IL-6/RPL13a
25
20
15
10
5
0
Control cells
Cells + 1ug/ml LPS
30uM SARS ISD
60uM SARS ISD
30uM Filo ISD
60uM Filo ISD
30uM control peptide
60uM control peptide
Sample characteristics

Fig. 16

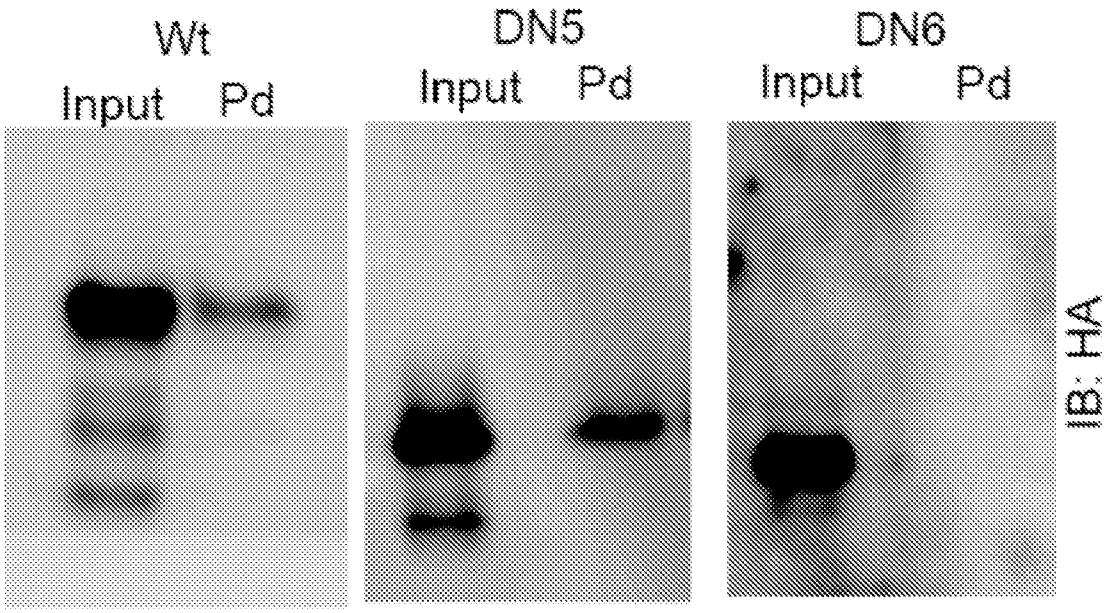


Fig. 17

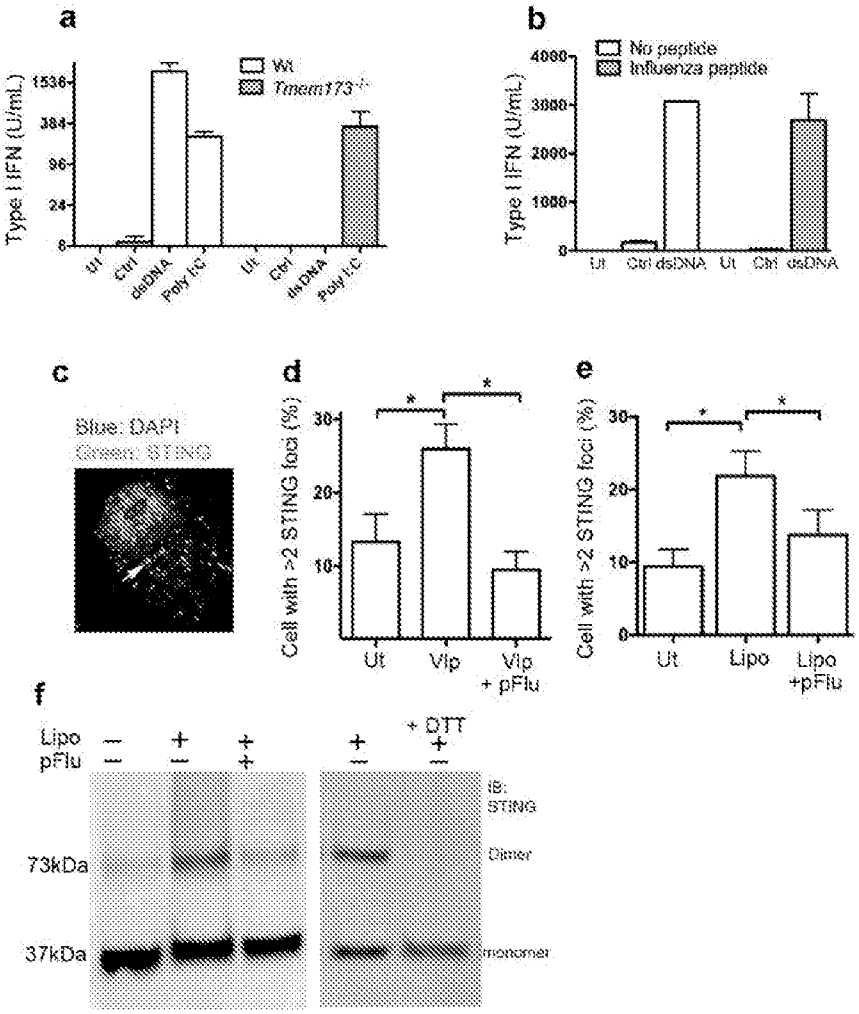

a
Type I IFN (U/mL)
1536
384
96
24
6
Wt
Tmem173-/-
Ut
Ctrl
dsDNA
Poly I:C
b
Type I IFN (U/mL)
4000
3000
2000
1000
0
No peptide
Influenza peptide
Ut
Ctrl
dsDNA
c
Blue: DAPI
Green: STING
d
Cell with >2 STING foci (%)
30
20
10
Ut
Vlp
Vlp + pFlu
e
Cell with >2 STING foci (%)
Ut
Lipo
Lipo +pFlu
f
Lipo
pFlu
+ DTT
IB: STING
73kDa
37kDa
Dimer
monomer

Fig. 18

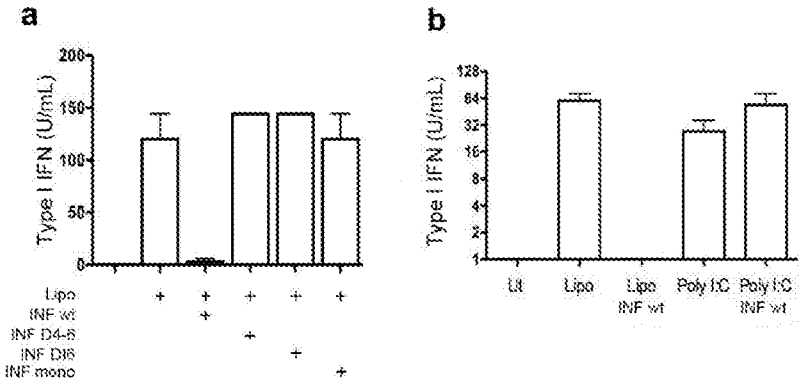

a
Type I IFN (U/mL)
200
150
100
50
0
Lipo
INF wt
INF D4-6
INF D6
INF mono
b
Type I IFN (U/mL)
128
64
32
16
8
4
2
1
Ut
Lipo
Lipo INF wt
Poly I:C
Poly I:C INF wt

Fig. 19

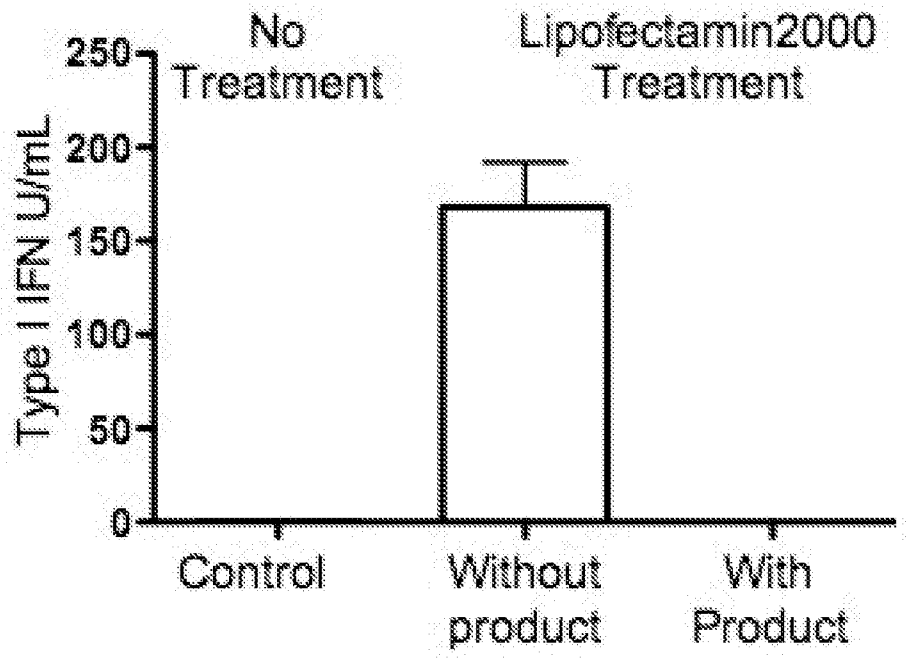

No Treatment
Lipofectamin2000 Treatment
Type I IFN U/mL
250
200
150
100
50
0
Control
Without product
With Product

Fig. 20