(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2008 (12.09.2008)

PCT

(10) International Publication Number
WO 2008/109237 A2

(51) International Patent Classification:
A61K 39/12 (2006.01)

(21) International Application Number:
PCT/US2008/053887

(22) International Filing Date:
13 February 2008 (13.02.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/889,583 13 February 2007 (13.02.2007) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) **Title:** MACROPHAGE CELL-LINES FOR PROPAGATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

FIGURE 1A

(57) **Abstract:** Particular aspects provide novel recombinant cells and cell lines (e.g., macrophage cell lines) that are permissive for propagation of porcine reproductive and respiratory syndrome virus (PRRSV) propagation *in vitro or in vivo*. In certain aspects, novel nucleic acid sequences encoding porcine sialoadhesin were transfected into existing macrophage cell-lines from other species, rendering them permissive to PRRSV infection, and suitable for propagation of PRRSV. Particular aspects provide exemplary recombinant cloned cell lines that support the replication of PRRSV, with an obtainable PRRSV titre of between $2x10^5$/ml and $2x10^6$/ml. Additional aspects provide novel nucleic acid sequences and polymorphisms thereof that encode for porcine sialoadhesin. Further aspects provide PRRSV propagation and preparation methods using the novel recombinant cell lines, and methods for PRRSV antigen and vaccine production using same. Yet further aspect provide transgenic, chimerical or engrafted animals having cell comprising nucleic acid sequences encoding porcine sialoadhesin.

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MACROPHAGE CELL-LINES FOR PROPAGATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to United States Provisional Patent Application Serial Number 60/889,583, filed 13 February 2007, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

Aspects of the present invention relate generally to porcine viruses (e.g., porcine reproductive and respiratory syndrome virus (PRRSV)), propagation of such viruses and viral antigen or vaccine production, and in particular aspects to novel nucleic acids encoding porcine sialoadhesin and to recombinant mouse cells (e.g., macrophage cell lines) transfected with said nucleic acids that are permissive for infection and propagation of PRRSV.

STATEMENT OF SPONSORED RESEARCH

This invention was made with support under Grant # 04-113 awarded by the National Pork Board.

BACKGROUND

Porcine Reproductive & Respiratory Syndrome Virus. Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-strand RNA virus that belongs to the Arteriviridae family. The PRRSV genomic RNA is approximately 15 kb comprising multiple open reading frames ("ORFs") encoding the RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7). Exemplary strains are the North American and European PRRSV strains represented by the prototype VR-2332 and Lelystad virus (LV) strains, respectively (Nelsen et al., *J. Virol.* 73:270-280, 1999).

Porcine reproductive and respiratory syndrome. PRRSV and porcine parvovirus (PPV) are the most common viral causes of porcine reproductive failure (Mengeling et al., *Anim. Reprod. Sci.* 60-61:199-210, 2000). PRRSV causes porcine reproductive and respiratory syndrome ("PRRS"; variously referred to as "mystery swine disease," "swine infertility and respiratory syndrome," "porcine epidemic abortion and respiratory syndrome," abortus blauw" and "blue ear disease"), which is a major problem to the swine industry worldwide (Meng, *Vet.*

Microbiol. 74:309-29, 2000). The respiratory form of the disease exhibits clinical signs which are most pronounced in piglets of 3-8 weeks in age, but are reported to occur in pigs of all ages in infected herds. The diseased piglets grow slowly, have roughened hair coats, respiratory distress (*e.g.*, respiratory dyspnea or "thumping") and increased mortality (up to about 80% pre-weaning mortality).

PRRS is associated with both gastrointestinal and systemic secondary infections (*e.g.*, interstitial pneumonia, diarrhea, *Salmonella choleraesuis, Streptococcus suis, Haemophilus parasuis, Actinobacillus pleuropneumoniae*) that often overwhelm the host in advanced stages.

PRRS is also associated with 'wasting syndrome.' Pigs surviving PRRSV infection are often afflicted by retarded growth rates (*i.e.*, "runt," or "wasting" syndrome) and require additional time on feed before becoming large enough (if ever) for slaughter, producing particularly marked and commercially devastating effects.

Epidemiologically, PRRSV infection is a communicable disease that is both *epidemic* and *endemic*. It can spread like an epidemic in naive swine populations, but appears to endemically linger in affected populations (Blaha, *Vet. Res.* 31:77-83, 2000). A typical epidemic of PRRSV-induced reproductive failure is presented as a broad spectrum of clinical features including infertility, anorexia, delayed return to estrus, abortions, pre-mature births, late-term dead fetuses, stillborn pigs, weak-born pigs, and in its most severe form, sow death. There may also be an increase in the number of mummified fetuses in the later stages of an PRRSV epidemic (Mengeling et al., *supra*). The initial infection of sows may go unnoticed, or may manifest itself by an impaired condition or general malaise lasting up to a few days. For example, the sows may go "off feed," and experience body temperatures either above or below normal. In the farrowing phase, the sows may exhibit depression, lethargy, pyrexia and occasional vomiting. In some affected herds, up to 75% of all piglets may be lost. The economic consequences of the disease are thus devastating.

PRRSV infects and replicates in porcine alveolar macrophages (PAMs) which are the cells of predilection in the natural host. Although, PAMs survive in culture for several days or even weeks, like other differentiated cells they eventually undergo senescence and die. Acquisition of unlimited growth *in vitro* is one of the characteristics that define cellular immortality.

The cell-line currently available for propagation of PRRSV *in vitro* is the green monkey kidney cell-line (and its derivatives), which has been patented (US 5,476,778; US 5,840,563; US 5,846,805; US 5,989,563; US 6,042,830; US 2001 21383 and patents and patent applications derived therefrom, both U.S. non-U.S.).

There is, therefore, a pronounced need in the art for the development of alternative cell culture systems for propagation (e.g., *in vitro* and *in vivo* propagation) of PRRSV that would provide for preparation of virus and development of efficacious vaccines based thereon, or based on epitopes thereof.

SUMMARY OF EXEMPLARY ASPECTS OF THE INVENTION

Particular aspects provide novel recombinant cells and cell lines (*e.g.*, macrophage cell lines) that are permissive for propagation of porcine reproductive and respiratory syndrome virus (PRRSV) propagation *in vitro* or *in vivo*. In certain aspects, novel nucleic acid sequences encoding porcine sialoadhesin were transfected into existing macrophage cell-lines from other species, rendering them permissive to PRRSV infection, and suitable for propagation of PRRSV. Particular aspects provide exemplary recombinant cloned cell lines that support the replication of PRRSV, with an obtainable PRRSV titre of between $2x10^5$/ml and $2x10^6$/ml. Additional aspects provide novel nucleic acid sequences and polymorphisms thereof that encode for porcine sialoadhesin. Further aspects provide PRRSV propagation and preparation methods using the novel recombinant cell lines, and methods for PRRSV antigen and vaccine production using same. Yet further aspect provide transgenic, chimerical or engrafted animals having cell comprising nucleic acid sequences encoding porcine sialoadhesin.

Particular aspects provide a recombinant cell (e.g., macrophage cell) comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor or portion thereof sufficient to provide for PRRSV binding, endocytosis, or susceptibility to PRRSV infection. In certain embodiments, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In particular implementations, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof. In particular embodiments, the transfected nucleic acid that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In certain aspects, the cell is that of a macrophage cell line. In particular embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1. and portions thereof. In particular embodiments, the transfected

nucleic acid comprises a cDNA and/or and expression vector. In particular embodiments, the macrophage cell line is heterologous, being other than porcine. In preferred embodiments, the macrophage cell line is murine.

Further aspects provide a method for propagating PRRSV, comprising: obtaining recombinant macrophage cells comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor or portion thereof sufficient to provide for PRRSV binding, endocystosis or susceptibility to PRRSV infection; and inoculating the cells with PRRSV, wherein PRRSV infection and propagation is afforded. In certain implementations, the method further comprises: isolating the propatated PRRSV; and preparing a PRRSV antigen or vaccine based on the isolated PRRSV, or on an epitope thereof. In certain embodiments of the methods, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In preferred aspects, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In particular embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof. In preferred aspects, the nucleic acid comprises SEQ ID NO:1 or a sequence having greater than 99% sequence identity with SEQ ID NO:1, or a portion thereof. In certain implementations, the transfected nucleic acid comprises a cDNA and/or an expression vector. In preferred aspects the macrophage cells are that of a macrophage cell line. In certain embodiments, the macrophage cell line is heterologous, being other than porcine. Preferably, the macrophage cell line is murine. In particular implementations, at least one of inoculating and propagating of PRRSV is *in vitro*. In alternate implementations, at least one of inoculating and propagating of PRRSV is *in vivo*, wherein at least one of inoculating and propagating of PRRSV comprises use of a transgenic animal or an engrafted animal comprising recombinant macrophage cells that encode a cell-surface porcine sialoadhesin receptor, the cell-surface receptor sufficient to provide for PRRSV binding, endocytosis or susceptibility to PRRSV infection. Preferably, the transgenic or engrafted animal is a mouse.

Yet additional aspects, provide an isolated nucleic acid comprising a sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and

PRRSV-binding portions thereof. In certain embodiments, the isolated nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof.

Yet further aspects provide a recombinant expression system, comprising an expression vector into which is inserted a nucleic acid that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In certain embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof. In particular aspects of the recombinant expression system, the nucleic acid molecule is heterologous to the expression vector. In particular aspects of the recombinant expression system, the nucleic acid molecule is inserted into said vector in proper sense orientation and correct reading frame.

Further aspects provide a non-human transgenic or chimeric animal, harboring a nucleic acid transgene that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In certain embodiments, the nucleic acid transgene encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. Preferably, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof. Preferably, the animal is a transgenic rodent, and more preferably a transgenic mouse. In certain aspects, the transgenic mouse is homozygous for the transgene, or is heterozygous for the transgene. In certain preferred embodiments of the non-human transgenic or chimeric animal, the transgene expression is driven by a promoter suitable to promote transgene expression in macrophages and/or alveolar macrophages. Preferably the animal is a transgenic mouse, harboring a porcine sialoadhesin transgene expressed in macrophages and/or alveolar macrophages

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B and 1C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone AA9 is permissive for reproductive and respiratory syndromne virus (PRRSV) infection. Figure 1A shows transfectant

clone AA9 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 1B shows transfectant clone AA9 mock-infected with no PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 1C shows transfectant clone AA9 infected with PRRSV followed by staining with control MAb.

Figures 2A, 2B and 2C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone BG10 is permissive for PRRSV infection. Figure 2A shows transfectant clone BG10 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 2B shows transfectant clone BG10 mock-infected with no PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 2C shows transfectant clone BG10 infected with PRRSV followed by staining with control MAb.

Figures 3A, 3B and 3C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone BD4 is permissive for PRRSV infection. Figure 3A shows transfectant clone BD4 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 3B shows transfectant clone BD4 mock-infected with no PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 3C shows transfectant clone BD4 infected with PRRSV followed by staining with control MAb.

Figures 4A, 4B and 4C shows, according to particular exemplary aspects of the present invention, that the exemplary porcine sialoadhesin transfectant cell line AA9 is permissive for PRRSV propagation. MARC145 cells were infected with AA9-propagated PRRSV, and the virus produced in the MARC145 cells was stained with the anti-nucleocapsid MAb SDOW17, followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 4A shows PRRSV infected MARC145 cells stained with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 4B shows mock-infected MARC145 cells stained with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 4C shows PRRSV infected MARC145 cells stained with control MAb.

DETAILED DESCRIPTION

According to particular aspects, transfection of porcine sialoadhesin into cell lines (e.g., macrophage cell-lines) from other species renders them susceptible to reproductive and respiratory syndromne virus (PRRSV) infection.

In certain aspects, the cDNA for porcine sialoadhesin was cloned and sequenced (GenBank accession no. DQ176853 (SEQ ID NO:1)), and has been identified as a receptor for PRRSV (SEQ ID NO:2). Comparison of the instant sequence data with a previously published sequence (GenBank accession no. NM_214346 (SEQ ID NOS:3 and 4)) revealed 15 amino acid differences, which, according to additional aspects, represent novel polymorphisms (see TABLE 3). Particular exemplary aspects provide novel macrophage cell-lines (*e.g.*, mouse) that are permissive for PRRSV infection and/or propagation (*e.g.*, *in vitro* or *in vivo*). In particular aspects, a transfection technique was utilized to render existing macrophage cell-lines from other species, permissive to PRRSV infection. In exemplary embodiments, the mouse macrophage cell-line J774A.1 was transfected with a novel cDNA for porcine sialoadhesin, the transfectants labeled with a monoclonal antibody specific to sialoadhesin and subjected to fluorescence-activated cell sorting. A total of 51 single-cell clones were obtained, which expressed cell-surface porcine sialoadhesin. Seventeen of these clone demonstrated persistent cell-surface expression of porcine sialoadhesin to varying degrees, and three of these clones (AA9, BG10 and BD4) were determined to be susceptible to PRRSV infection. The clone AA9 was further tested and was determined to efficiently support PRRSV replication, with an obtainable virus titre of between $2x10^5$ $TCID_{50}$/ml and $2x10^6$/$TCID_{50}$/ml.

Definitions

PRRSV, as used herein, refers to porcine reproductive and respiratory syndrome virus (PRRSV), which is a positive-strand RNA virus that belongs to the Arteriviridae family. The PRRSV genomic RNA is approximately 15 kb comprising multiple open reading frames ("ORFs") encoding the RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7). Exemplary strains are the North American and European PRRSV strains represented by the prototype VR-2332 and Lelystad virus (LV) strains, respectively (Nelsen et al., *J. Virol.* 73:270-280, 1999).

As used herein, a composition refers to any mixture. It can be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

As used herein, a combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two

collections, can be a mixture thereof, such as a single mixture of the two or more items, or any variation thereof.

As used herein, a pharmaceutical effect refers to an effect observed upon administration of an agent intended for treatment of a disease or disorder or for amelioration of the symptoms thereof.

As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease or other indication, are ameliorated or otherwise beneficially altered.

As used herein therapeutic effect means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition. A therapeutically effective amount refers to the amount of a composition, molecule or compound which results in a therapeutic effect following administration to a subject.

In particular aspects, a therapeutic effect may also encompass prophylaxis of symptoms of a condition.

As used herein, the term "subject" refers to animals, particularly mammals.

As used herein, the phrase "associated with" or "characterized by" refers to certain biological aspects such as expression of a receptor or signaling by a receptor that occurs in the context of a disease or condition. Such biological aspects may or may not be causative or integral to the disease or condition but merely an aspect of the disease or condition.

The term "epitope" refers herein, as is known in the art, to an antigenic determinant of a protein of polypeptide. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids. An epitope of a polypeptide or protein antigen can be formed by contiguous or noncontinguous amino acid sequences of the antigen. A single viral protein, for example, may contain many epitopes. Additionally, a polypeptide fragment of a viral protein may contain multiple epitopes. The present invention encompasses epitopes and/or polypeptides recognized by antibodies of the present invention, along with conservative substitutions thereof, which are still recognized by the antibodies. Further truncation of these epitopes may be possible.

Novel sialoadhesin nucleic acids

As described herein under Example 2 below, novel porcine sialoadhesin polymorphisms were identified. Table 3 shows a comparison of the instant sequence data SEQ ID NOS:1 and 2 (accession No. DQ176853) with previously published sequences SEQ ID NOS:3 and 4 (accession no. NM_214346), showing 15 amino acid differences, which, according to additional

aspects, represent novel polymorphisms (see TABLE 3). According to additional aspects, novel sialoadhesin polymorphic sequences having at least one polymorphic residue substitution of SEQ ID NO:4 selected from the group consisting of substitution with Arg at position 547 (SEQ ID NO:7), Leu at position 552 (SEQ ID NO:8), Ser at position 579 (SEQ ID NO:9), Ala at position 583 (SEQ ID NO:10), Leu at position 839 (SEQ ID NO:11), His at position 846 (SEQ ID NO:12), Arg at position 864 (SEQ ID NO:13), Thr at position 1275 (SEQ ID NO:14), Arg at position 1381 (SEQ ID NO:15), Ala at position 1392 (SEQ ID NO:16), Ile at position 1425 (SEQ ID NO:17), Ala at position 1428 (SEQ ID NO:18), Phe at position 1468 (SEQ ID NO: 19), Met at position 1475 (SEQ ID NO:20) and His at position 1672 (SEQ ID NO:21) are provided.

Table 3. Novel porcine sialoadhesin polymorphisms

Amino Acid Position No.	SEQ ID NO. 2 (Accession No. DQ176853)	SEQ ID NO. 4 (Accession No. NM_214346)
547	Arg	Leu
552	Leu	Ile
579	Ser	Asn
583	Ala	Thr
839	Leu	Ser
846	His	Arg
864	Arg	His
1275	Thr	Ala
1381	Arg	His
1392	Ala	Val
1425	Ile	Val
1428	Ala	Asp
1468	Phe	Leu
1475	Met	Ile
1672	His	Tyr

Polynucleotides and expression vectors. Particular embodiments provide an isolated nucleic acid with a sequence comprising a transcriptional initiation region and a sequence encoding a porcine sialoadhesin (e.g., nucleic acid that encodes a polypeptide comprising SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, or PRRSV-binding portions thereof, and a recombinant vector comprising this polynucleotide (*e.g.*, expression vector). Preferably, the nucleic acid sequence encodes a porcine sialoadhesin comprising SEQ ID NO:2, or a sequence having greater than 99% sequence identity with SEQ ID NO:2, or a PRRSV-binding portion thereof. Preferably, the

nucleic acid comprises SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, or a portion thereof. Preferably, the transcriptional initiation region is a strong constitutively expressed mammalian pol III-or pol II-specific promoter, or a viral promoter. An exemplary expression vector used for cloning the novel porcine sialoadhesin nucleic acids disclosed herein is the pcDNA3.1D/V5-His-TOPO mammalian expression vector (Invitrogen), to yield applicants' expression vector pWL/PS constructs.

A variety of mammalian expression vectors may be used to express recombinant porcine sialoadhesin in mammalian cells. Expression vectors provide DNA sequences that are required for the transcription of cloned DNA and, in particular instances the translation of their mRNAs in an appropriate host. Such vectors can be used to express DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Certain vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. Particular expression vectors may contain at least one of the following: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant recombinant porcine sialoadhesin expression, include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLUMUS29, pLITMUS38 and pLITMUS39 (New England Bioloabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMCIneo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and 1ZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant porcine sialoadhesin in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant porcine sialoadhesin expression include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia).

In particular aspects, the term "macrophage promoter" and promoter specific or suitable for cells of macrophage derived refers to a promoter or a gene that encodes a protein endogenously produced by macrophage cells and/or cells of macrophage derived lineage. Examples of such promoters include promoters for any proteins expressed in macrophages and

cells of macrophage derived lineage including CD3, CD4, the CDl l antigens (such as CDI I A, Cl 11 B and CD 11 C), CD12, CD13, CD14, CD15, CD16, CD17, CD21, CD23, CD25, CD26, CD30, CD31, CD32, CD33, CD36, CD39, CD40, CD45RO, CD45RA, CD45RB, CD49A, CD49B, CD49D, CD49E, CD49F, CD50, CD57, CD60, CD61, CD62L, CD63, CD64, CD65, CD68, CD69, CD70, CD74, CD80, CD84, CD85, CD86, CD87, CD88, CD89, CD91, CD92, CD93, CD97, CD101, CD102, CD105, CD114, CD115 (MCSFreceptor), CD119, CD121B, CD127, CD135, CD148, CD155, CD156, CD157, CD 163, proteins involved in the maintenance of homeostasis in the cell, proteins involved in cell motion including actin, cellular adhesion molecules, chemokines (RANTES, MIP 1 a, MIP1p, MDC, TARK), and molecules involved in the immune system (MHC-I, MHC-11, etc.). Macrophage promoters also includes promoters of any genes encoding for any proteins expressed specifically in macrophages and/or cells of macrophage derived lineage including catalase, CD 156, M- CSFR, p73, and FcgRI. The term "macrophage-specific promoter" and/or and promoter specific for cells of macrophage derived lineage refers, in particular aspects to a promoter of a gene that encodes a protein endogenously produced exclusively by macrophage cells and/or cells of macrophage derived lineage. Examples of such promoters include promoters for proteins expressed specifically in macrophages such as catalase, CD156, M-CSFR, p73, and FcgRI. However, any promoter that provides for expression in macrophages may be used. Preferably, the promoter is a macrophage-specific promoter.

Preferred exemplary embodiments

Particular aspects provide a recombinant macrophage cell comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor or portion thereof sufficient to provide for porcine reproductive and respiratory syndrome virus (PRRSV) binding, endocytosis, or susceptibility to PRRSV infection. In certain embodiments, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In particular implementations, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof. Preferably, where a portion of the porcine sialoadhesin receptor is used, where the portion is sufficient to provide for porcine reproductive and respiratory syndrome virus (PRRSV) binding, endocytosis, or susceptibility to PRRSV infection, the portion compirses at least 50, at least 75, at least 100,

at least 200, at least 500, or at least 1000 contiguous amino acids (see, US 2004 0248124, incorporated herein in its entirety).

Additional aspects, provide a recombinant cell comprising a transfected nucleic acid that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In certain aspects, the cell is that of a macrophage cell line.

In certain embodiments of the above methods, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1. and portions thereof. In particular embodiments, the transfected nucleic acid comprises a cDNA and/or and expression vector. In particular embodiments, the macrophage cell line is heterologous, being other than porcine. In preferred embodiments, the macrophage cell line is murine.

Further aspects provide a method for propagating PRRSV, comprising: obtaining recombinant macrophage cells comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor or portion thereof sufficient to provide for PRRSV binding, endocystosis or susceptibility to PRRSV infection; and inoculating the cells with PRRSV, wherein PRRSV infection and propagation is afforded. In certain implementations, the method further comprises: isolating the propatated PRRSV; and preparing a PRRSV antigen or vaccine based on the isolated PRRSV, or on an epitope thereof. In certain embodiments of the methods, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In preferred aspects, the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In particular embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof. In preferred aspects, the nucleic acid comprises SEQ ID NO:1 or a sequence having greater than 99% sequence identity with SEQ ID NO:1, or a portion thereof. In certain implementations, the transfected nucleic acid comprises a cDNA and/or an expression vector. In preferred aspects the macrophage cells are that of a macrophage cell line. In certain embodiments, the macrophage cell line is heterologous, being other than porcine. Preferably, the macrophage cell line is murine. In particular implementations, at least one of inoculating and propagating of PRRSV is

in vitro. In alternate implementations, at least one of inoculating and propagating of PRRSV is *in vivo*, wherein at least one of inoculating and propagating of PRRSV comprises use of a transgenic animal or an engrafted animal comprising recombinant macrophage cells that encode a cell-surface porcine sialoadhesin receptor, the cell-surface receptor sufficient to provide for PRRSV binding, endocytosis or susceptibility to PRRSV infection. Preferably, the transgenic or engrafted animal is a mouse.

Yet additional aspects, provide an isolated nucleic acid comprising a sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In certain embodiments, the isolated nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof.

Yet further aspects provide a recombinant expression system, comprising an expression vector into which is inserted a nucleic acid that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. In certain embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof. In particular aspects of the recombinant expression system, the nucleic acid molecule is heterologous to the expression vector. In particular aspects of the recombinant expression system, the nucleic acid molecule is inserted into said vector in proper sense orientation and correct reading frame.

Particular exemplary aspects provide a non-human transgenic or chimeric animal, harboring a nucleic acid transgene that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In certain embodiments, the nucleic acid transgene encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. Preferably, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof. Preferably, the animal is a transgenic rodent, and more preferably a transgenic mouse. In certain aspects, the transgenic mouse is homozygous for the transgene, or is heterozygous for the transgene. In certain preferred embodiments of the non-

human transgenic or chimeric animal, the transgene expression is driven by a promoter suitable to promote transgene expression in macrophages and/or alveolar macrophages. Preferably the animal is a transgenic mouse, harboring a porcine sialoadhesin transgene expressed in macrophages and/or alveolar macrophages

Biologically active variants of sialoadhesin nucleic acids and proteins

As used herein, a "biological activity" refers to a function of a polypeptide including but not limited to complexation, dimerization, multimerization, receptor-associated ligand binding and/or endocytosis, receptor-associated protease activity, phosphorylation, dephosphorylation, autophosphorylation, ability to form complexes with other molecules, ligand binding, catalytic or enzymatic activity, activation including auto-activation and activation of other polypeptides, inhibition or modulation of another molecule's function, stimulation or inhibition of signal transduction and/or cellular responses such as cell proliferation, migration, differentiation, and growth, degradation, membrane localization, and membrane binding. A biological activity can be assessed by assays described herein and by any suitable assays known to those of skill in the art, including, but not limited to *in vitro* assays, including cell-based assays, *in vivo* assays, including assays in animal models for particular diseases.

Preferably, the porcine sialoadhesin, or polymorphic variant thereof (e.g., SEQ ID NOS:7-21) comprises an amino acid sequence of SEQ ID NO:2 (or of SEQ ID NO:2 having from 1, to about 3, to about 5, to about 10, or to about 20 conservative amino acid substitutions), or a fragment of a sequence of SEQ ID NO:2 (or of SEQ ID NO:2 having from 1, to about 3, to about 5, to about 10, or to about 20 conservative amino acid substitutions), and wherein the polypeptide is sufficient to provide for PRRSV binding, endocystosis or susceptibility to PRRSV infection. Preferably, porcine sialoadhesin, or variant thereof, comprises a sequence of SEQ ID NO:2, or a conservative amino acid substitution variant thereof.

Functional porcine sialoadhesin, functional porcine sialoadhesin variants are those proteins that display one or more of the biological activities of porcine sialoadhesin, including but not limited to ligand binding, receptor-mediated endocytosis, receptor-meidated signal transduction, receptor activation, receptor down-regulation, etc.

Variants of porcine sialoadhesin have utility for aspects of the present invention. Variants can be naturally or non-naturally occurring. Naturally occurring variants (*e.g.*, polymorphisms) are found in various species and comprise amino acid sequences which are substantially identical to the amino acid sequence shown in SEQ ID NO:2. Species homologs of the protein can be obtained using subgenomic polynucleotides of the invention, as described

below, to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, yeast, or bacteria, identifying cDNAs which encode homologs of the protein, and expressing the cDNAs as is known in the art.

Non-naturally occurring variants which retain substantially the same biological activities as naturally occurring protein variants are also included here. Preferably, naturally or non-naturally occurring variants have amino acid sequences which are at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater than 99% identical to the amino acid sequence shown in SEQ ID NOS:2. More preferably, the molecules are at least 98%, 99% or greater than 99% identical. Percent identity is determined using any method known in the art. A non-limiting example is the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1. The Smith-Waterman homology search algorithm is taught in Smith and Waterman, *Adv. Appl. Math.* 2:482-489, 1981.

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are generally in the "L" isomeric form. Residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in J. Biol. Chem., 243:3552-59 (1969) and adopted at 37 C.F.R. §§ 1.821 - 1.822, abbreviations for amino acid residues are shown in Table 2:

TABLE 2 – Table of Correspondence

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
Y	Tyr	Tyrosine
G	Gly	Glycine
F	Phe	Phenylalanine
M	Met	Methionine
A	Ala	Alanine
S	Ser	Serine
I	Ile	Isoleucine
L	Leu	Leucine

SYMBOL		
1-Letter	**3-Letter**	**AMINO ACID**
T	Thr	Threonine
V	Val	Valine
P	Pro	Praline
K	Lys	Lysine
H	His	Histidine
Q	Gln	Glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	Tryptophan
R	Arg	Arginine
D	Asp	aspartic acid
N	Asn	Asparagines
B	Asx	Asn and/or Asp
C	Cys	Cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by a formula have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred to in 37 C.F.R., §§ 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH_2 or to a carboxyl-terminal group such as COOH.

Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR software. Preferably, amino acid changes in the protein variants disclosed herein are conservative amino acid changes, *i.e.,* substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline,

phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. Preferably, amino acid changes in the porcine sialoadhesin polypeptide variants are conservative amino acid changes, *i.e.,* substitutions of similarly charged or uncharged amino acids.

It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological properties of the resulting variant. Properties and functions of porcine sialoadhesin polypeptide protein or polypeptide variants are of the same type as a protein comprising the amino acid sequence encoded by the nucleotide sequence shown in SEQ ID NO:1, although the properties and functions of variants can differ in degree.

Variants of the porcine sialoadhesin polypeptide disclosed herein include glycosylated forms, aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties (*e.g.*, pegylated molecules). Covalent variants can be prepared by linking functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art. Variants also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect functional activity of the proteins are also variants. Covalent variants can be prepared by linking functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art.

A subset of mutants, called muteins, is a group of polypeptides in which neutral amino acids, such as serines, are substituted for cysteine residues which do not participate in disulfide bonds. These mutants may be stable over a broader temperature range than native secreted proteins (see, *e.g.*, Mark *et al.,* United States Patent No. 4,959,314).

It will be recognized in the art that some amino acid sequences of the porcine sialoadhesin polypeptides of the invention can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there are critical areas on the protein which determine activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein. The replacement of amino acids can also change the selectivity of ligand binding to cell surface receptors (Ostade et al., *Nature 361*:266-268, 1993). Thus, the porcine sialoadhesin polypeptides of the present

invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

Amino acids in the porcine sialoadhesin polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as binding to a natural or synthetic binding partner. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J. Mol. Biol. 224*:899-904 (1992) and de Vos et al. *Science 255*:306-312 (1992)).

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein. Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of substitutions for any given porcine sialoadhesin polypeptide will not be more than 50, 40, 30, 25, 20, 15, 10, 5 or 3.

Fusion Proteins

Fusion proteins comprising proteins or polypeptide fragments of porcine sialoadhesin polypeptide can also be constructed. Fusion proteins are useful for generating antibodies against amino acid sequences and for use in various targeting and assay systems. For example, fusion proteins can be used to identify proteins which interact with a porcine sialoadhesin polypeptide of the invention or which interfere with its biological function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can also be used for this purpose. Such methods are well known in the art and can also be used as drug screens. Fusion proteins comprising a signal sequence can be used.

A fusion protein comprises two protein segments fused together by means of a peptide bond. Amino acid sequences for use in fusion proteins of the invention can be utilize the amino acid sequence shown in SEQ ID NO: 2 or can be prepared from biologically active variants of SEQ ID NO: 2, such as those described above. The first protein segment can include of a full-length porcine sialoadhesin polypeptide. Other first protein segments can consist of about functional portions of SEQ ID NO:2.

The second protein segment can be a full-length protein or a polypeptide fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags can be used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and virus protein fusions.

These fusions can be made, for example, by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises a coding region for the protein sequence of SEQ ID NO:2 in proper reading frame with a nucleotide encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies that supply research labs with tools for experiments, including, for example, Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), Clontech (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Transfected macrophage cell lines expressing porcine sialoadhesin

As described herein under Example 3 below, the novel cDNA (SEQ ID NO:1) for sialoadhesin was transfected (e.g., in the context of an exeprssion vector such as the pcDNA3.1D/V5-His-TOPO mammalian expression vector (Invitrogen) to yield the expression vector pWL/PS) into the mouse macrophage cell-line, J774A.1. The transfectants were labeled with an antibody specific for porcine sialoadhesin, followed by sorting in a fluorescence-activated cell sorter. Fifty-one (51) single clones were obtained. Of these, 17 clones continued to express porcine sialoadhesin to varying degrees (Table 1).

According to additional aspects, other macrophage lines including but not limited to mammalian, rodent (mouse, rat, hamster, etc.), bovine, ovine, equine, porcine, human, primate, simian, etc., may be used. Preferably a suitable expression vector with a promoter appropriate to the cell type is used to generate the recombinant cell lines.

Identification of transfectant macrophage cell-lines permissive for PRRSV infection

As described herein under Example 4 below, transfectant cell-lines that are permissive for PRRSV infection were identified. Immunofluorescence assay of transfectant cells following infection with PRRSV revealed that three of the porcine sialoadhesin transfectant cell-lines were susceptible to PRRSV infection (FIGURES 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C; see also Table 1).

Identification of transfectant macrophage cell-lines efficient for PRRSV propagation

As described herein under Example 5 below, efficient propagation of PRRSV in AA9 cells was demonstrated. The AA9 cell-line cultured in tissue culture flasks was infected with PRRSV. Taken together, the results indicate that AA9 cells support the replication of PRRSV. These cells can be successfully used for propagation of PRRSV. Virus titers ranging from $2x10^5$/ml to $2x10^6$/ml can be obtained from AA9 cells. This titer is only slightly less than that obtainable from MARC145 cells.

Use of recombinant vectors and transfectant macrophage cell-lines for vaccine production

As described herein under Example 5 below, PRRSV produced using the novel recombinant cells has utilitiy for vaccine production. Vaccination of pigs against PRRSV is the most prudent and logical way of controlling this economically important disease. Production of vaccines requires large scale propagation of PRRSV in the laboratories of pharmaceutical companies. The cell-line that is currently available for propagation of PRRSV *in vitro* is the green monkey kidney cell-line (and its derivatives). According to additional aspects, the availability of cell-lines that are permissive for PRRSV propagation for propagation of PRRSV provides for production of efficacious vaccines against PRRSV.

"Vaccine," as used herein and in the art, refers to any type of biological agent in an administratable form capable of stimulating an immune response in an animal inoculated with the vaccine. For purposes of preferred embodiments of this invention, an inventive vaccine may comprise as the viral agent, one or more immunogenic (antigenic) components of the virus, and including polypeptide-based vaccines.

In particular embodiments, the PRRSV polypeptides provide vaccines, based on the use of one or more PRRSV antigens in vaccine compositions. Such peptide-based vaccines are well known in the art, and may contain additional antigenic and adjuvant elements. Peptide-based vaccine are advantageous over traditional vaccines for several reasons: they are substantially

safer; they have a relatively long shelf-life; they have the ability to target the immune response towards specific epitopes that are not suppressive nor hazardous for the host; and they offer the possibility of preparing multi-component and multi-pathogen vaccines.

The efficacy of inventive vaccines and peptide-based vaccines is enhanced by adequate presentation of the epitopes to the immune system. Therefore, in preferred aspects, the PRRSV antigens/epitopes are coupled to, or are expressed (*e.g*, hydrid-gene expression) as part of, a carrier that may also offer an adjuvant function. Additional adjuvants may or may not be included in the immunization.

In particular aspects, immunizations are performed with one or more PRRSV protein or polypeptide antigens selected from the group consisting of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7), and epitope-bearing fragments of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7).

Antibodies. In particular embodiments, porcine sialoadhesin polypeptides, as described above, have utility as antigens or epitopes for developing respective antibodies (*e.g.*, monoclonal antibodies), and compositions comprising such antibodies.

In particular embodiments, the porcine sialoadhesin protein or polypeptide antigen is selected from the group consisting of SEQ ID NOS:2, 7-21, and epitope-bearing fragments of SEQ ID NOS:2 and 7-21. In particular embodiments, immunologic assay may be used in connection with the antibodies, for example, selected from the group consisting of ELISA, immunoprecipitation, immunocytochemistry, immunoelectrophoresis, immunochemical methods, Western analysis, antigen-capture assays, two-antibody sandwich assays, binder-ligand assays, agglutination assays, complement assays, and combinations thereof. In particular embodiments, the antibody is selected from the group consisting of a single-chain antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, and a Fab fragment. In particular embodiments, a plurality of antibodies, or eptitope-binding portions thereof, are used, in each case specific for an PRRSV protein or polypeptide antigen or epitope.

Therapeutic agents. Additionally, because of the nature of the relevant specific binding interactions, antibodies and antibody-containing compositions of the present invention have *therapeutic utility* for treatment or prevention of PRRSV infections. The inventive antibodies and antibody compositions have utility for treating an infection, for alleviating symptoms of an infection, and/or to prevent pathogen infection. Preferably, the antibodies and antibody

compositions are directed against PRRSV virus, or PRRSV proteins or polypeptides, and can be used to treat or prevent PRRSV virus infection by administration to subjects in need thereof.

Specifically, particular embodiments of the present invention provide an antibody directed against a PRRSV protein or polypeptide antigen selected from the group consisting of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7), and epitope-bearing fragments of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7).

In particular embodiments, the antibody is a monoclonal antibody, or antigen-binding portion thereof. In particular embodiments, the monoclonal antibody, or antigen-binding portion thereof, is a single-chain antibody, chimeric antibody, humanized antibody or Fab fragment.

Additional aspects provide a composition, comprising at least one of the above-described antibodies. Preferably, at least one of the antibodies forms specific immunocomplexes with PRRSV whole virions, or proteins or polypeptides associated with PRRSV virions. Preferably, the composition comprises a monoclonal antibody specific for a PRRSV protein or polypeptide antigen selected from the group consisting of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7), and epitope-bearing fragments of RNA replicase (ORF1a and ORF1b), the glycoproteins GP2 to GP5, the integral membrane protein M, and the nucleocapsid protein N (ORFs 2 to 7).

Yet further aspects provide a *pharmaceutical* composition, comprising at least one of the above-described antibodies of, along with a pharmaceutically acceptable diluent, carrier or excipient. Preferably, the composition is administered to a subject, whereby the composition prevents or inhibits PRRSV infection. In particular embodiments, the composition is administered to a subject, whereby the composition ameliorates symptoms of PRRSV infection. In particular embodiments, at least one of the antibodies of the composition forms specific immunocomplexes with PRRSV whole virions, or proteins or polypeptides associated with PRRSV virions.

Yet further aspects provide methods of treating, or of preventing PRRSV virus infection, comprising administering to a subject in need thereof, a therapeutically effective amount of at least one of the above-described antibodies, or of a pharmaceutical composition comprising at least one of the antibodies. In particular embodiments, the immunoglobulin sequences are, or substantially are, porcine immunoglobulin sequences.

Antigen and vaccine production

As described in detail herein under Example 6 below, vaccination of pigs against PRRSV is the most prudent and logical way of controlling this economically important disease. Production of vaccines requires large scale propagation of PRRSV in the laboratories of pharmaceutical companies. The cell-line that is currently available for propagation of PRRSV *in vitro* is the green monkey kidney cell-line (and its derivatives). According to additional aspects, the availability of cell-lines that are permissive for PRRSV propagation for propagation of PRRSV provides for production of efficacious vaccines against PRRSV.

Transgenic, chimeric or engrafted animals

As described in detail herein under Example 7 below, in particular embodiments, there is provided a transgenic or chimeric mouse whose genome comprises a porcine sialoadhesin receptor (or portion thereof) transgene. Engrafted animals, such murine xenografts are also within the scope of the present invention, and can be produced using the disclosed recombinant cell lines using art-recognized methods.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the claimed invention in any way.

EXAMPLE 1
(*Materials and Methods*)

Materials and Methods

Isolation of Porcine alveolar macrophages. Porcine alveolar macrophages were obtained from the lungs of 1-2 month-old piglets following euthanasia with sodium pentabarbitol. The PAMs were obtained by infusing the lungs with 200 ml of cold sterile phosphate-buffered saline (PBS), and subjected to Ficoll-Paque density gradient centrifugation. PAMs were collected from the band at the PBS–Ficoll interface, and washed twice with ice-cold PBS.

Cloning of porcine sialoadhesin. Total RNA from PAMs was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The cDNA was obtained by reverse transcription and PCR. Total RNA from PAMs was reverse-transcribed to generate the

first strand cDNA with M-MLV reverse transcriptase (Promega) following manufacturer's recommendations. This cDNA was subsequently used in PCR reactions to obtain the full length Sialoadhesin coding sequence (CDS) using the following primers: forward primer 5' CACCATGGACTTCCTGCTCCTGCTCCTC (SEQ ID NO:5) and reverse primer 5′ CTTGGGGTTTGAAGCTAGGTCATAA (SEQ ID NO:6). PCR reactions were carried out in a total volume of 50 µl consisting of 2.5 µl of cDNA, 300 pM each of forward and reverse primers, 0.2 mM dNTPs, 1× Pfu buffer, 0.25 mM $MgSO_4$ and 5 U Pfu Turbo Hotstart™ Polymerase (Stratagene). DNA was denatured at 95 °C for 2 min, followed by 35 amplification cycles of 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 5 min. An additional extension at 72°C for 10 min followed. The single-band PCR amplicons were cloned into the pcDNA3.1D/V5-His-TOPO mammalian expression vector (Invitrogen), yielding the expression vector pWL/PS. Following transformation of TOP10 chemically competent cells (Invitrogen) with pWL/PS, positive clones were selected on LB-ampicillin plates, screened by PCR, and confirmed by restriction enzyme digest analysis. Four independent clones were sequenced in both directions using BigDye™ Terminator Chemistries and an ABI Prism™ 377 DNA sequencer (Applied Biosystems). The porcine PRRSV receptor sialoadhesin cDNA sequence was deposited by applicants at GenBank (accession no. DQ176853).

Alignment of nucleotide and amino acid sequences and similarity analyses were performed with ClustalW (http:// dot.imgen.bcm.tmc.edu:9331 / multi-align / Options / clustalw.html), GeneDoc v.2.6.002 (Nicholas et al., 1997), and the Alignx™ module of Vector NTI Advance™ 9.1 (Invitrogen). Primer design was performed using Primer 3 (Rozen and Skaletsky, 2000) and Vector NTI (Invitrogen). DNA sequence analysis, fragment assembly, and amino acid sequence prediction were performed with the ContigExpress™ module of Vector NTI Advance™ 9.1 (Invitrogen) and Sequencher 4.5 (Gene Codes Corporation). SignalP v.2.0.b2 and NetNGlyc v.1.0 provided peptide signal and *N*-glycosylation sites prediction, respectively.

Transfection of cDNA for porcine sialoadhesin into already existing macrophage cell-lines from other species. The mouse macrophage cell line J774A.1 was used as an exemplary heterologous cell line for transfection with pWL/PS by using transfect amine 2000 (Invitrogen). Transfectants that continued to grow in the selection medium containing Geneticin were subjected to flow cytometric analysis with the monoclonal antibody, 41D3, specific for porcine sialoadhesin (kindly provided by Dr. Hans Nauwynck, Ghent University, Belgium and Michael Murtaugh, University of Minnesota, USA), followed by FITC-conjugated goat antibodies to murine Ig. Transfectants expressing sialoadhesin on their surface were sorted with a fluorescence-

activated cell sorter (FACSVantage™ SE), and the sorted cells were directly transferred into 96-well microtiter culture plates at a cell concentration of one cell per well. Single clones were picked up from the individual wells of the culture plates and cultured to obtain the cell-lines.

Identification of cell-lines that are permissive for PRRSV infection. The transfectant cell-lines were tested for susceptibility to PRRSV infection. The cells cultured on chamber slides were infected with PRRSV at an moi of 5. The infectivity was determined by immunofluorescence assays with a monoclonal antibody specific for the nucleocapsid protein (SDOW17). Briefly, 48 hours post-infection, cells were fixed and permeabilized using methanol and acetone (50:50 vol/vol) at -20^{0}C for 10 min. The permeabilized cells were incubated with the anti-nucleocapsid mAb, SDOW17, followed by FITC-conjugated secondary antibody (goat anti-mouse Ig). The cells were then subjected to fluorescence microscopy.

Propagation of PRRSV in the transfectant cell-line AA9. AA9 cells were seeded into T25cm^2 tissue culture flasks and incubated until the cells showed >70% confluence. On the day of virus infection, the spent culture medium was discarded and the cells were washed twice with DMEM medium. Virus was diluted in DMEM medium (without serum) and inoculated onto the cells at an moi of 1. Following 2 hours of incubation with intermittent rocking of the flask, complete culture medium (DMEM containing 5% fetal bovine serum, 2mM glutamine and 20 ug/ml gentamicin) was added. The flasks were incubated at 37°C. Three days post-infection, the infected cells showed changes in morphology, and subsequently began to die. The un-infected (mock-infected) cells did not show these changes. On day 5 post-infection, the virus was harvested by subjecting the flask to repeated freeze/thaw cycles followed by centrifugation (600xg for 5 min) of the culture supernatant fluid, and stored at -80°C until used later. This experiment was repeated 3 times.

The titer of AA9-propagated PRRSV was determined by two methods:

1. *Immunofluorescence Assay.* The AA9-propagated virus was diluted ten-fold in serum-free DMEM medium. The virus was inoculated at dilutions ranging from 1x10^{-2} to 1x10^{-7}onto MARC145 cells grown on chamber slides. After 72 hrs, the cells were fixed in methanol:acetone (50:50) solution at -20^0 C for 10 minutes and stained with anti-nucleocapsid antibody, SDOW-17 (1:200 dilution), followed by GAM-FITC (1:200 dilution), or an isotyped-matched control antibody. The stained cells were observed under a fluorescence microscope. This assay was repeated two times.

2. *Reed and Muench Method.* MARC145 and AA9 cells were seeded into the wells of 96 well tissue culture plates, and incubated until a confluent monolayer of cells was observed.

Ten fold dilutions ($1x10^{-2}$ to $1x10^{-8}$) of the virus in DMEM medium (without serum) were prepared. The spent culture medium was removed from the wells, and cells were washed twice with DMEM medium. 50 µl of diluted virus was added to each well (each dilution had 5 replicates). After, two hours of incubation at 37°C with intermittent rocking of the plates, 100 µl of complete growth medium were added to all the wells. The plates were incubated until death of cells was observed. The cells showed plaques 4 to 5 days post-inoculation of the virus. The number of wells totally affected (in each dilution) was counted, and $TCID_{50}$ was calculated. This assay was repeated three times.

EXAMPLE 2

(Novel porcine sialoadhesin polymorphisms were identified)

Table 3 shows a comparison of the instant sequence data SEQ ID NO:2 with a previously published sequence (GenBank accession no. NM_214346 (SEQ ID NO:3)), showing 15 amino acid differences, which, according to additional aspects, represent novel polymorphisms (see TABLE 3).

Table 3. Porcine Sialoadhesin Polymorphisms

Amino Acid Position No.	SEQ ID NO. 2 (Accession No. DQ176853)	SEQ ID NO. 4 (Accession No. DQ176853)
547	Arg	Leu
552	Leu	Ile
579	Ser	Asn
583	Ala	Thr
839	Leu	Ser
846	His	Arg
864	Arg	His
1275	Thr	Ala
1381	Arg	His
1392	Ala	Val
1425	Ile	Val
1428	Ala	Asp
1468	Phe	Leu
1475	Met	Ile
1672	His	Tyr

EXAMPLE 3

(*The porcine sialoadhesin gene was stably transfected into existing macrophage cell-lines of other species (non-porcine)*)

A novel gene, comprising novel polymorhphic amino acid positions and residues, and coding for porcine sialoadhesin was cloned as described herein. A porcine sialoadhesin sequence (SEQ ID NOS:3 and 4) was preciously as a receptor for PRRSV on PAMs. Comparison of the instant sequence data with that published in Europe revealed 15 polymmorphic amino acid differences (see above Table 3). The cDNA (SEQ ID NO:1) for sialoadhesin was transfected into the mouse macrophage cell-line, J774A.1. The transfectants were labeled with an antibody specific for porcine sialoadhesin, followed by sorting in a fluorescence-activated cell sorter. Fifty-one (51) single clones were obtained. Of these, 17 clones continued to express porcine sialoadhesin to varying degrees (Table 1).

Table 1. Sialoadhesin expression and PRRSV susceptibility of sialoadhesin transfectants:

Clone Designation	Sialoadhesin Expression	PRRSV Susceptibility	Clone Designation	Sialoadhesin Expression	PRRSV Susceptibility
AA9	+	+	**CE9**	-	
AB5	-		**CE10**	-	
AB8	-		**CE11**	-	
AB10	+	-	**CF6**	-	
AC6	-		**CF8**	-	
AD3	-		**CG2**	-	
AF4	+	-	**CG5**	-	
AF10	-		**CG6**	+	-
AG8	+	-	**CG11**	-	
AG11	+	-	**CH7**	-	
AH12	-		**DA2**	-	
BA3	+	-	**DA4**	+	-
BA9	+	-	**DA7**	-	
BC9	-		**DB1**	-	
BD4	+	+	**DB2**	-	
BD10	-		**DB7**	+	-
BE6	+	-	**DC12**	-	
BE7	-		**DD6**	+	-
BF7	-		**DE5**	-	
BG10	+	+	**DF2**	-	

Clone Designation	Sialoadhesin Expression	PRRSV Susceptibility	Clone Designation	Sialoadhesin Expression	PRRSV Susceptibility
CB3	-		DF5	-	
CB6	-		DF8	+	-
CB9	-		DF9	+	-
CC1	-		DF10	-	
CC3	-		DH6	-	
CD6	+	-			

EXAMPLE 4

(*Transfectant cell-lines that are permissive for PRRSV infection were identified*)

Immunofluorescence assay of transfectant cells following infection with PRRSV revealed that three of the porcine sialoadhesin transfectant cell-lines were susceptible to PRRSV infection (FIGURES 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B and 3C; see also Table 1).

Figures 1A, 1B and 1C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone AA9 is permissive for PRRSV infection. Figure 1A shows transfectant clone AA9 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 1B shows transfectant clone AA9 mock-infected with no PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 1C shows transfectant clone AA9 infected with PRRSV followed by staining with control MAb.

Figures 2A, 2B and 2C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone BG10 is permissive for PRRSV infection. Figure 2A shows transfectant clone BG10 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 2B shows transfectant clone BG10 mock-infected with no PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 2C shows transfectant clone BG10 infected with PRRSV followed by staining with control MAb.

Figures 3A, 3B and 3C show, according to particular exemplary aspects of the present invention, that the inventive porcine sialoadhesin transfectant clone BD4 is permissive for PRRSV infection. Figure 3A shows transfectant clone BD4 infected with PRRSV followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 3B shows transfectant clone BD4 mock-infected with no PRRSV

followed by staining with the anti-nucleocapsid MAb SDOW17 followed by FITC-conjugated goat anti-mouse Ig antibodies. Figure 3C shows transfectant clone BD4 infected with PRRSV followed by staining with control MAb.

The AA9 was selected for further characterization (see Example 4 below).

EXAMPLE 5

(Efficient propagation of PRRSV in AA9 cells was demonstrated)

The AA9 cell-line cultured in tissue culture flasks was infected with PRRSV. On day 5 post-infection, the virus was harvested and titered by two methods.

Immunofluorescence assay. The immunofluorescence assay of AA9-propagated PRRSV on MARC145 cells grown on chamber slides revealed that the MARC145 cells were infected by the AA9-propagated PRRSV up to the dilution of $1x10^{-5}$ (FIGURE 4A, 4B and 4C). These results indicate that the titer of the PRRSV propagated in AA9 cells grown in tissue culture flasks was $1x10^{5}$ per ml.

Reed and Muench Method. AA9-propagated PRRSV was also titered by the Reed and Muench Method, as described under Materials and Methods. The titering was performed on both MARC145 cells and AA9 cells. For comparison, PRRSV propagated in MARC145 cells was also titered on MARC145 cells and AA9 cells (Table 2).

Table 2. Determination of the titer of PRRSV propagated in AA9 cells. One experiment representative of 5 experiments is shown.

Virus dilution	# of infected wells (out of 5)	# of uninfected wells	Accumulative		Ratio infected	% infected
			Infected	Not infected		
10^{-2}	5	0	17	0	17/17	100
10^{-3}	5	0	12	0	12/12	100
10^{-4}	5	0	7	0	7/7	100
10^{-5}	2	3	2	3	2/5	40
10^{-6}	0	5	0	8	0/8	0
10^{-7}	0	5	0	13	0/13	0
10^{-8}	0	5	0	18	0/18	0

The proportionate distance (PD) between the 2 dilutions (10^{-4} and 10^{-5}), where the 50% end point lies:

$$= \frac{\text{\% infection above 50\% - 50\%}}{\text{\% infection above 50\% - \% infection below 50\%}}.$$

$$= \frac{100-50}{100-40} = \frac{50}{60} = 0.833$$

Exponential of dilution (ED) of exactly 50% infectivity
= PD X [ED next below 50% - ED next above 50%] + ED next above 50%
= 0.833 X [(-5) – (-4)] + (-4)
= (-0.833) + (-4) = -4.833

Titer of the virus = $10^{4.833}$ $TCID_{50}$/ 50ul, or 2 X $10^{5.833}$ $TCID_{50}$/ml

The titer of the virus propagated in AA9 cells and titered on MARC145 cells in three different experiments was $2x10^{5.8}$/ml, $2x10^{6}$/ml, $2x10^{5.6}$/ml, respectively.

The titer of the virus propagated in MARC145 cells and titered on MARC145 cells was $2x10^{6.4}$/ml.

The titer of the virus propagated in AA9 cells and titered on AA9 cells was $2x10^{6}$/ml.

The titer of the virus previously propagated in MARC145 cells and titered on AA9 cells was $2x10^{6}$/ml.

Taken together, the above results clearly indicate that AA9 cells support the replication of PRRSV. These cells can be successfully used for propagation of PRRSV. Virus titers ranging from $2x10^{5}$/ml to $2x10^{6}$/ml can be obtained from AA9 cells. This titer is only slightly less than that obtainable from MARC145 cells.

EXAMPLE 6

(*PRRSV produced using the novel recombinant cells has utility for vaccine production*)

Vaccination of pigs against PRRSV is the most prudent and logical way of controlling this economically important disease. Production of vaccines requires large scale propagation of PRRSV in the laboratories of pharmaceutical companies. The cell-line that is currently available for propagation of PRRSV *in vitro* is the green monkey kidney cell-line (and its derivatives). According to additional aspects, the availability of cell-lines that are permissive for PRRSV propagation for propagation of PRRSV provides for production of efficacious vaccines against PRRSV.

EXAMPLE 7

(Transgenic and/or chimeric animals harboring)

A "transgenic animal" is an animal containing one or more cells bearing genetic information received, directly or indirectly, by deliberate genetic manipulation at a subcellular level, such as by microinjection or infection with recombinant virus. This introduced DNA molecule can be integrated within a chromosome, or it can be extra-chromosomally replicating DNA. Unless otherwise noted or understood from the context of the description of an animal, the term "transgenic animal" as used herein refers to a transgenic animal in which the genetic information was introduced into a germ line cell, thereby conferring the ability to transfer the information to offspring. If offspring in fact possess some or all of the genetic information, then they, too, are transgenic animals. The genetic information is typically provided in the form of a transgene carried by the transgenic animal.

In particular embodiments, there is provided a transgenic mouse whose genome comprises a transgene comprising a transcriptional control region operably linked to a nucleic acid (e.g., cDNA) encoding a porcine sialoadhesin receptor (or portion thereof) polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof, or preferably comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof, wherein said control region comprises a promoter wherein expression of the porcine sialoadhesin receptor (or portion thereof) polypeptide at the cell surface is sufficient to provide for PRRSV binding, endocytosis, or susceptibility to PRRSV infection. In other embodiments, the nucleic acid sequence within the transgene may include sequence variations, polymorphisms, mutations and deletions, which do not abrogate the sufficiency to provide for PRRSV binding, endocytosis, or susceptibility to PRRSV infection. In certain embodiments, there is provided a transgene comprising a transcriptional control region operably linked to a nucleic acid (e.g., cDNA) encoding a porcine sialoadhesin receptor (or portion thereof) polypeptide as defined above wherein said control region comprises a promoter suitable to provide for macrophage expression of the porcine sialoadhesin receptor (or portion thereof). In particular some embodiments, the transgene may include a suitable enhancer.

In another aspect of the invention, there is provided a method for producing a transgenic mouse whose genome comprises a transgene comprising a transcriptional control region operably linked to a nucleic acid (e.g., cDNA) encoding a porcine sialoadhesin receptor (or

portion thereof) polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof, or preferably comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof, the method comprising: introducing into a fertilized mouse egg a transgene comprising a transcriptional control region operably linked to the nucleic acid as described above, wherein said control region comprises a promoter; transplanting the injected egg in a foster parent female mouse; and selecting a mouse derived from an injected egg whose genome comprises the transgene. As will be appreciated by one of skill in the art, the transgene may be introduced into the mouse egg by any of a number of suitable methods known in the art. Alternatively, the transgene may be introduced into embryonic stem cells (ES cells), and chimeric animals produded using the transgenic ES cells, using art recognized methods. The resulting chimeric animals are then crossed to produce the desired transgenic mice.

Engrafted animals, such murine xenografts are also within the scope of the present invention, and can be produced using the disclosed recombinant cell lines using art-recognized methods.

Particular exemplary aspects provide a non-human transgenic or chimeric animal, harboring a nucleic acid transgene that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof. In certain embodiments, the nucleic acid transgene encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof. Preferably, the animal is a transgenic rodent, and more preferably a transgenic mouse. In certain aspects, the transgenic mouse is homozygous for the transgene, or is heterozygous for the transgene. In certain preferred embodiments of the non-human transgenic or chimeric animal, the transgene expression is driven by a promoter suitable to promote transgene expression in macrophages and/or alveolar macrophages. Preferably the animal is a transgenic mouse, harboring a porcine sialoadhesin transgene expressed in macrophages and/or alveolar macrophages.

According to additional aspects, such transgenic or engrafted animals provide novel model systems and/or tools for studying PRRSV-mediated infection, viral propagation and disease.

Applicants have, therefore, cloned a novel polymorphic cDNA (SEQ ID NO:1) that encodes for a novel sialoadhesin polypeptide (SEQ ID NO:2), that functions, as demonstrated herein, as a PRRSV receptor. The porcine sialoadhesin cDNA sequence (SEQ ID NO:1) was transfected into a murine macrophage cell-line rendering it permissive for PRRSV propagation. Fifty-one (51) clones of transfectants were isolated and, of these, 17 clones continued to express sialoadhesin to varying degrees. Three (3) transfectant clones were identified that were susceptible to PRRSV. One of these exemplary clones, AA9, was further characterized and was found to support the replication of PRRSV. The titer of PRRSV obtainable in AA9 cells was determined to be between $2x10^5$/ml and $2x10^6$/ml.

These cell-lines have substantial utility as cell-lines for large scale production of PRRSV for development of vaccines against PRRSV. This present cloned gene sequences, and the transfectants expressing the sialoadhesin will also be useful for determining the mechanism by which PRRSV gets into the PAMs and causes the disease in pigs.

CLAIMS

1. A recombinant macrophage cell comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor or portion thereof sufficient to provide for porcine reproductive and respiratory syndrome virus (PRRSV) binding, endocytosis, or susceptibility to PRRSV infection.

2. The recombinant macrophage cell of claim 1, wherein the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof.

3. The recombinant macrophage cell of claim 1, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof.

4. A recombinant cell comprising a transfected nucleic acid that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof.

5. The recombinant cell of any one of claims 1, 2, 3 and 4, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1. and portions thereof.

6. The recombinant cell of any one of claims 1, 2, 3 and 4, wherein the transfected nucleic acid comprises a cDNA.

7. The recombinant cell of any one of claims c and 4, wherein the transfected nucleic acid comprises an expression vector.

8. The recombinant cell of claim 4, wherein the cell is that of a macrophage cell line.

9. The recombinant cell of any one of claims 1, 2, 3 and 8, wherein the macrophage cell line is heterologous, being other than porcine.

10. The recombinant cell of claim 9, wherein the macrophage cell line is murine.

11. A method for propagating porcine reproductive and respiratory syndrome virus (PRRSV), comprising:

obtaining recombinant macrophage cells comprising a transfected nucleic acid that encodes a cell-surface porcine sialoadhesin receptor or portion thereof, the cell-surface receptor

or portion thereof sufficient to provide for PRRSV binding, endocystosis or susceptibility to PRRSV infection; and

inoculating the cells with PRRSV, wherein PRRSV infection and propagation is afforded.

12. The method of claim 11, further comprising:

isolating the propatated PRRSV; and

preparing a PRRSV antigen or vaccine based on the isolated PRRSV, or on an epitope thereof.

13. The method of any one of claims 11 and 12, wherein the wherein the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof.

14. The method of any one of claims 13, wherein the cell-surface porcine sialoadhesin receptor comprises a sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof.

15. The method of any one of claims 11 and 12, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:1 or 3, and portions thereof.

16. The method of claim 15, wherein the nucleic acid comprises SEQ ID NO:1 or a sequence having greater than 99% sequence identity with SEQ ID NO:1, or a portion thereof.

17. The method of any one of claims 11 and 12, wherein the transfected nucleic acid comprises a cDNA .

18. The method of any one of claims 11 and 12, wherein the transfected nucleic acid comprises an expression vector.

19. The method of any one of claims 11 and 12, wherein the cells are that of a macrophage cell line.

20. The method of claim 19, wherein the macrophage cell line is heterologous, being other than porcine.

21. The method of claim 20, wherein the macrophage cell line is murine.

22. The method of any one of claims 11 and 12, wherein at least one of inoculating and propagating of PRRSV is *in vitro*.

23. The method of any one of claims 11 and 12, wherein at least one of inoculating and propagating of PRRSV is *in vivo*.

24. The method of claim 26, wherein at least one of inoculating and propagating of PRRSV comprises use of a transgenic animal or an engrafted animal comprising recombinant macrophage cells that encode a cell-surface porcine sialoadhesin receptor, the cell-surface receptor sufficient to provide for PRRSV binding, endocytosis or susceptibility to PRRSV infection.

25. The method of claim 24, wherein the transgenic or engrafted animal is a mouse.

26. An isolated nucleic acid comprising a sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof.

27. The isolated nucleic acid of claim 26, wherein the isolated nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof.

28. A recombinant expression system comprising an expression vector into which is inserted a nucleic acid that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof.

29. The recombinant expression system of claim 28, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof.

30. The recombinant expression system according to any one of claims 27 and 28, wherein the nucleic acid molecule is heterologous to the expression vector.

31. The recombinant expression system according to any one of claims 27 and 28, wherein the nucleic acid molecule is inserted into said vector in proper sense orientation and correct reading frame.

32. A non-human transgenic or chimeric animal, harboring a nucleic acid transgene that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, a sequence having at least 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NOS:2 or 4, and PRRSV-binding portions thereof.

33. The non-human transgenic or chimeric animal of claim 37, wherein the nucleic acid transgene encodes a polypeptide comprising an amino acid sequence selected from the

group consisting of SEQ ID NO:2, a sequence having greater than 99% sequence identity with SEQ ID NO:2, and PRRSV-binding portions thereof.

34. The non-human transgenic or chimeric animal of claim 37, wherein the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, a sequence having greater than 99% sequence identity with SEQ ID NO:1, and portions thereof

35. The animal of claim 32, wherein the animal is a transgenic rodent.

36. The transgenic rodent of claim 33, wherein the transgenic rodent is a transgenic mouse.

37. The transgenic mouse of claim 34, wherein the mouse is homozygous for the transgene.

38. The transgenic mouse of claim 34, wherein the mouse is heterozygous for the transgene.

39. The non-human transgenic or chimeric animal of claim 37, wherein transgene expression is driven by a promoter suitable to promote transgene expression in macrophages or alveolar macrophages.

FIGURE 1A

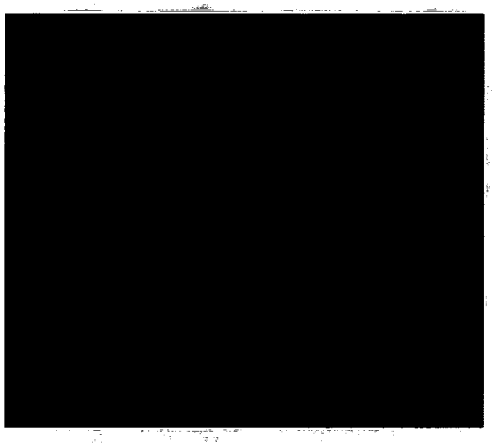

FIGURE 1B

FIGURE 1C

FIGURE 2A

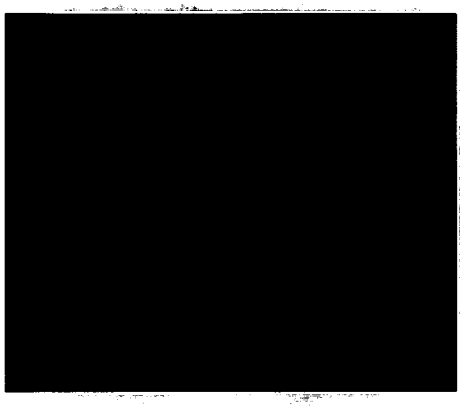

FIGURE 2B

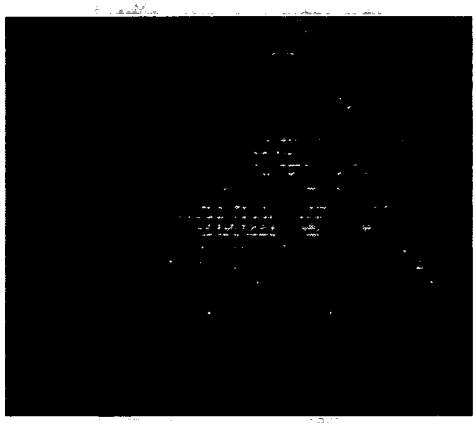

FIGURE 2C

FIGURE 3A

FIGURE 3B

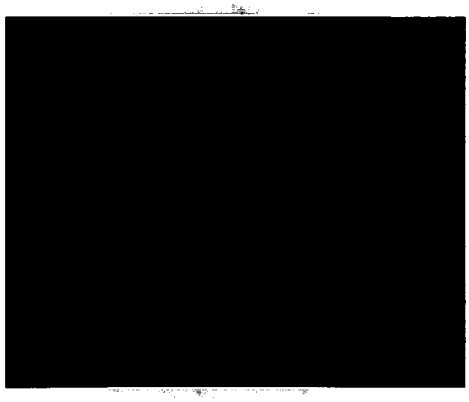

FIGURE 3C

FIGURE 4A

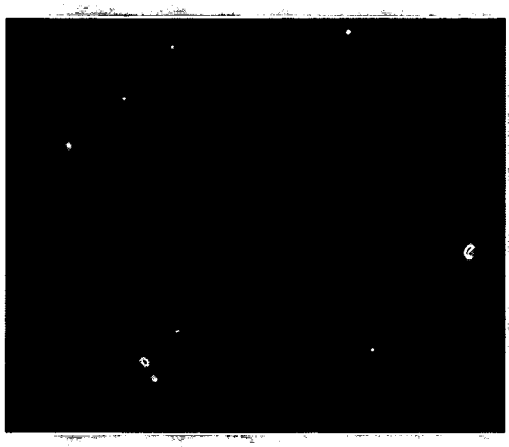

FIGURE 4B

FIGURE 4C