

NEW STREPTOCOCCAL PROTEASES

Field of the invention

The invention relates to a new Streptococcal proteases which displays IgG degrading cysteine protease activity. The invention further relates to the treatment of and vaccination against streptococcal infections, the treatment of conditions mediated by pathogenic IgG antibodies, such as autoimmune diseases, and to the development of new tools for biotechnology.

Background to the invention

Pathogenic bacteria have evolved various strategies to colonize and invade their host and a wide variety of virulence factors are employed to promote growth and to mediate evasion from host immune responses. To avoid the obvious risk of extinction, pathogenic bacteria have to deal with both innate immune responses, but most importantly also with specific immunoglobulins. Specific Ig are central to the adaptive immune system by initiating the complement based and/or phagocyte based immune response. Ig consists of variable antigen-recognizing Fab regions that are linked through a flexible hinge region with the Fc effector part. The Fc region mediates contact with specific receptors on phagocytic cells or triggers the classical pathway of complement by binding C1q. Thus, the hinge region is target for several microbial proteases and examples include IdeS from *S. pyogenes* (von Pawel-Rammingen *et al.* 2002. *EMBO J.* 21, 1607-161), Gingipain K from *Porphyromonas gingivalis* (Vincent *et al.* 2011. *FASEB J.* 10, 3741-3750) and SspA from *Staphylococcus aureus* (Prokesová *et al.* 1992. *Immunol. Lett.* 31, 259-265).

Streptococcal infection are common in humans, as well as domestic animal, like swine, horses and cattle. Streptococcal infections vary in severity from relatively mild diseases to serious life threatening conditions. The provision of streptococcal antigens that can be used in human and veterinary vaccines in the prophylaxis, prevention and treatment of streptococcal infections is greatly needed.

Proteases with strict sequence specificity are useful as biotechnological tools. Proteases that degrade immunoglobulins can have medical use, e.g. can proteases that specifically degrade IgG be used for treatment or prevention of diseases or conditions mediated by IgG antibodies.

Summary of the invention

The present inventors have identified, purified and characterised a novel family of IgG degrading enzymes from streptococci. This family of enzymes, designated IgdE for Immunoglobulin G-degrading Enzyme, are cysteine proteases distinct from previous
 5 characterized streptococcal immunoglobulin degrading proteases of the IdeS family and mediates efficient cleavage of the hinge region of IgG with a high degree of specificity.

The enzyme has been identified in strains of *S. suis*, designated IgdE_{suis}; in strains of *S. agalactiae*, designated IgdE_{agalactiae}; in strains *S. porcinus*, designated IgdE_{porcinus}; in strains of *S. equi*, designated IgdE_{equi}; and in strains of *S. pseudoporcinus*, designated
 10 IgdE_{pseudoporcinus}.

The IgdE from *S.suis* is demonstrated to be highly specific for porcine IgG. Similar to the IgM cleaving protease IdeS_{suis}, the 1121 amino acid large protein carries the proteolytic active domain in the N-terminal part of the protein. A full size protein is not essential for IgG cleavage in vitro, as a truncated *S.suis* IgdE protein consisting of the N-
 15 terminal 470 amino acids retains IgG cleavage activity.

Cleavage of porcine IgG by *S.suis* IgdE occurred just N-terminal of the hinge region cysteine residues that are likely to form covalent disulphide bonds between the two IgG heavy chains. Thus, IgG cleavage results in the formation of a 64 kDa Fc fragment and two Fab fragments. This cleavage pattern is distinct from the IgG endopeptidase IdeS of
 20 *S. pyogenes* that hydrolyses IgG in the lower hinge region, thereby generating one F(ab')₂ fragment and two identical ½Fc fragments (von Pawel-Rammingen *ibid*) and from IdeS_{suis} that cleaves IgM C-terminal from the intra-chain disulphide bonds generating free ½Fc fragments (Seele *et al.* 2015. *J. Vet. Res.* 46, 45).

S. suis can e.g. cause meningitis in humans and swine. *S. agalactiae* can e.g. cause
 25 meningitis and sepsis in humans and cattle. *S. porcinus* can e.g. cause respiratory tract infections in swine, porcine strangles. *S. equi* can e.g. cause respiratory tract infections in horses, strangles.

Proteases of the IgdE family are of use in the prevention and treatment of Streptococcal infections, such as a vaccine for immunization against streptococcal
 30 infections. IgdE antibodies are further of use in passive immunization and treatment of conditions associated with streptococcal infections. IgdE proteases are also useful for developing new biotechnological tools, and for treating or preventing diseases or conditions mediated by IgG antibodies, such as autoimmune diseases, transplant rejection, post-operative treatment and acquired haemophilia.

In one aspect, the present invention provides an *in vitro* method for the cleavage of human IgG, comprising contacting human IgG with a polypeptide comprising: (a)

the amino acid sequence of SEQ ID NO: 3; (b) a variant thereof having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG cysteine protease activity; or (c) a fragment thereof of either (a) or (b), having IgG cysteine protease activity.

Disclosed is an isolated Igde polypeptide for use in generating an immune response in a subject.

The isolated Igde polypeptide is preferably an Igde_{suis}, Igde_{agalactiae}, Igde_{porcinus}, Igde_{equi}, or an Igde_{pseudoporcinus} polypeptide, or a variant or fragment of any thereof which retains cysteine protease activity and/or is capable of generating an immune response to a streptococcus in a subject. The variant may be an Igde polypeptide from another bacterium. The bacterium is preferably a Streptococcus.

In one example the disclosure provides an Igde polypeptide for use in generating an immune response in a subject comprising:

(a) the amino acid sequence of SEQ ID NO: 1;

(b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;

(c) a fragment of SEQ ID NO: 1, a variant of SEQ ID NO:1, or a fragment of a variant of SEQ ID NO: 1 which is capable of generating an immune response to a streptococcus, preferably a *S. suis* in a subject;

(d) the amino acid sequence of SEQ ID NO: 3;

(e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3, a variant of SEQ ID NO:3, or a fragment of a variant of SEQ ID NO: 3 which is capable of generating an immune response to a streptococcus, preferably a *S. agalactiae* in a subject;

(g) the amino acid sequence of SEQ ID NO: 5;

(h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;

(i) a fragment of SEQ ID NO: 5, a variant of SEQ ID NO:5, or a fragment of a variant of SEQ ID NO: 5 which is capable of generating an immune response to a *S. porcinus* in a subject;

(j) the amino acid sequence of SEQ ID NO: 7;

(k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity;

(l) a fragment of SEQ ID NO: 7, a variant of SEQ ID NO:7, or a fragment of a variant of SEQ ID NO: 7 which is capable of generating an immune response to a

5 streptococcus, preferably a *S. equi* in a subject; or

(m) the amino acid sequence of SEQ ID NO: 9;

(n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;

(o) a fragment of SEQ ID NO: 9, a variant of SEQ ID NO:9, or a fragment of a variant of SEQ ID NO: 9 which is capable of generating an immune response to a

5 streptococcus, preferably a *S. pseudoporcinus* in a subject;

Preferably the immune response is a protective immune response.

Preferably the immune response generates antibodies able to neutralize the IgG degrading cysteine protease activity of the Igde of an infectious streptococcus in the immunized subject.

10 In another example the disclosure provides a method for generating an immune response in a subject comprising administering an Igde polypeptide to said subject.

Also disclosed is an isolated polynucleotide encoding an Igde polypeptide for use in generating an immune response in a subject.

15 The Igde polypeptide is preferably an Igde_{suis}, Igde_{agalactiae}, Igde_{porcinus}, Igde_{equi} or an Igde_{pseudoporcinus} polypeptide, or a variant or fragment of any thereof which retains cysteine protease activity and/or is capable of generating an immune response to a streptococcus in a subject. The variant may be an Igde polypeptide from another bacterium. The bacterium is preferably a Streptococcus.

20 In one example the disclosure provides a polynucleotide encoding an Igde polypeptide for use in generating an immune response in a subject comprising:

(a) a sequence which encodes the polypeptide SEQ ID NO:1 or a variant or a fragment thereof as defined above;

25 (b) a sequence which encodes the polypeptide SEQ ID NO:3 or a variant or a fragment thereof as defined above;

(c) a sequence which encodes the polypeptide SEQ ID NO:5 or a variant or a fragment thereof as defined above;

(d) a sequence which encodes the polypeptide SEQ ID NO:7 or a variant or a fragment thereof as defined above; or

30 (e) a sequence which encodes the polypeptide SEQ ID NO:9 or a variant or a fragment thereof as defined above.

In another example the disclosure provides a polynucleotide encoding an IgdE polypeptide for use in generating an immune response in a subject comprising:

(a) SEQ ID NO: 2 or a complementary sequence thereto;

5 (b) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (a);

(c) a sequence which hybridises under stringent conditions to the sequence defined in (a) or (b);

(d) a sequence having at least 70% identity to a sequence as defined in (a) or (b);

10 (e) a fragments of any of the sequences (a), (b), (c) or (d), and which encodes a polypeptide having IgdE cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. suis* in a subject.

(f) SEQ ID NO: 4 or a complementary sequence thereto;

(g) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (f);

15 (h) a sequence which hybridises under stringent conditions to the sequence defined in (f) or (g);

(i) a sequence having at least 70% identity to a sequence as defined in (f) or (g);

20 (j) a fragments of any of the sequences (f), (g), (h) or (i), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. agalactiae* in a subject;

(k) SEQ ID NO: 6 or a complementary sequence thereto;

(l) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (k);

25 (m) a sequence which hybridises under stringent conditions to the sequence defined in (k) or (l);

(n) a sequence having at least 70% identity to a sequence as defined in (k) or (l);

30 (o) a fragments of any of the sequences (k), (l), (m) or (n), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. porcinus* in a subject;

(p) SEQ ID NO: 8 or a complementary sequence thereto;

(q) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (p);

(r) a sequence which hybridises under stringent conditions to the sequence defined in (p) or (q);

(s) a sequence having at least 70% identity to a sequence as defined in (p) or (q); or

(t) a fragments of any of the sequences (p), (q), (r) or (s), and which encodes a

5 polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. equi* in a subject;

(u) SEQ ID NO: 10 or a complementary sequence thereto;

(v) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (u);

10 (w) a sequence which hybridises under stringent conditions to the sequence defined in (u) or (v);

(x) a sequence having at least 70% identity to a sequence as defined in (u) or (v); or

(y) a fragments of any of the sequences (u), (v), (w) or (x), and which encodes a

15 polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. pseudoporcinus* in a subject..

Preferably the immune response is a protective immune response.

Preferably the immune response generates antibodies able to neutralize the IgG degrading cysteine protease activity of the IgGE of an infectious streptococcus in the

20 immunized subject.

In another example the disclosure provides a method for generating an immune response in a subject comprising administering a polynucleotide encoding an IgGE polypeptide to said subject.

25 Also disclosed is an isolated IgGE polypeptide for use in treatment or prevention of a disease or condition mediated by IgG antibodies.

The IgGE polypeptide is preferably an IgGE_{suis}, IgGE_{agalactiae}, IgGE_{porcinus}, IgGE_{equi} or an IgGE_{pseudoporcinus} polypeptide, or a variant or fragment of any thereof which retains cysteine protease activity. The variant may be an IgGE polypeptide from another

30 bacterium. The bacterium is preferably a Streptococcus.

In one example the disclosure provides an IgGE polypeptide for use in treatment or prevention of a disease or condition mediated by IgG antibodies comprising:

(a) the amino acid sequence of SEQ ID NO: 1;

(b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;

(c) a fragment of SEQ ID NO: 1 or a fragment of a variant of SEQ ID NO: 1 having IgG degrading cysteine protease activity;

5 (d) the amino acid sequence of SEQ ID NO: 3;

(e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity;

10 (g) the amino acid sequence of SEQ ID NO: 5;

(h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;

(i) a fragment of SEQ ID NO: 5 or a fragment of a variant of SEQ ID NO: 5 having IgG degrading cysteine protease activity;

15 (j) the amino acid sequence of SEQ ID NO: 7;

(k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity;

(l) a fragment of SEQ ID NO: 7 or a fragment of a variant of SEQ ID NO: 7 having IgG degrading cysteine protease activity;

20 (m) the amino acid sequence of SEQ ID NO: 9;

(n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;

(o) a fragment of SEQ ID NO: 9 or a fragment of a variant of SEQ ID NO: 9 having IgG degrading cysteine protease activity;

25 (p) the amino acid sequence of SEQ ID NO: 11;

(q) a variant of SEQ ID NO: 11 having at least 70% identity to the amino acid sequence of SEQ ID NO: 11 and having IgG degrading cysteine protease activity; or

(r) a fragment of SEQ ID NO: 11 or a fragment of a variant of SEQ ID NO: 11 having IgG degrading cysteine protease activity.

30 Preferably the IgDE polypeptide can be an IgDE_{agalactiae} polypeptide comprising:

(d) the amino acid sequence of SEQ ID NO: 3;

(e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity;

In another example the disclosure provides a method for treatment, prophylaxis or prevention of a disease or condition mediated by IgG antibodies comprising administering a therapeutically effective amount of an IgdE polypeptide to a subject.

The disease or condition mediated by IgG antibodies, can be an autoimmune diseases, transplant rejection, post-operative treatment and acquired haemophilia.

Also disclosed is an *in vitro* method for the cleavage of IgG, comprising contacting IgG with an IgdE polypeptide having cysteine protease activity.

The IgdE polypeptide is preferably an IgdE_{suis}, IgdE_{agalactiae}, IgdE_{porcinus}, IgdE_{equi}, or an IgdE_{pseudoporcinus} polypeptide, or a variant or fragment of any thereof which retains IgG degrading cysteine protease activity. The variant may be an IgdE polypeptide from another bacterium. The bacterium is preferably a Streptococcus.

In one example the present disclosure provides an *in vitro* method for the cleavage of IgG, comprising contacting IgG with an IgdE polypeptide comprising:

(a) the amino acid sequence of SEQ ID NO: 1;

(b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;

(c) a fragment of SEQ ID NO: 1 or a fragment of a variant of SEQ ID NO: 1 having IgG degrading cysteine protease activity.

(d) the amino acid sequence of SEQ ID NO: 3;

(e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity;

(g) the amino acid sequence of SEQ ID NO: 5;

(h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;

(i) a fragment of SEQ ID NO: 5 or a fragment of a variant of SEQ ID NO: 5 having IgG degrading cysteine protease activity;

(j) the amino acid sequence of SEQ ID NO: 7;

(k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity;

5 (l) a fragment of SEQ ID NO: 7 or a fragment of a variant of SEQ ID NO: 7 having IgG degrading cysteine protease activity;

(m) the amino acid sequence of SEQ ID NO: 9;

(n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;

10 (o) a fragment of SEQ ID NO: 9 or a fragment of a variant of SEQ ID NO: 9 having IgG degrading cysteine protease activity;

(p) the amino acid sequence of SEQ ID NO: 11;

(q) a variant of SEQ ID NO: 11 having at least 70% identity to the amino acid sequence of SEQ ID NO: 11 and having IgG degrading cysteine protease activity; or

15 (r) a fragment of SEQ ID NO: 11 or a fragment of a variant of SEQ ID NO: 11 having IgG degrading cysteine protease activity.

Preferably the Igde polypeptide can be an Igde_{agalac} polypeptide comprising:

(d) the amino acid sequence of SEQ ID NO: 3 ;

20 (e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity; or

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity.

In another example the present disclosure provides an *in vitro* method for the generation of Fc or Fab fragments of IgG, comprising contacting IgG with an Igde
25 polypeptide having IgG degrading cysteine protease activity.

Also disclosed is a method for identifying a substance that activates or inhibits the IgG cysteine activity of an Igde polypeptide. The method can comprise;

30 a) contacting the Igde polypeptide and IgG with a candidate substance under conditions permitting IgG cysteine activity in the absence of the substance,

b) determining the amount of IgG digested in the presence of the candidate substance compared to in the absence of the said substance,

c) thereby determining whether the substance activates or inhibits the IgG cysteine activity of the Igde polypeptide.

Quantitative analysis of IgG degradation can be performed as set out in Example 1.

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Candidate compounds which may be tested in the methods according to the disclosure include simple organic molecules, commonly known as “small molecules”, for example those having a molecular weight of less than 2000 Daltons. The method may also be used to screen compound libraries such as peptide libraries, including synthetic peptide libraries and peptide phage libraries. Other suitable molecules include polynucleotide sequences and any other molecules which modulate the IgG degrading activity of Igde.

Brief description of the Figures

Figure 1. IgG degradation activity in culture supernatants of S. suis.

15 (A) Concentrated (20x) culture supernatants of *S. suis* strains 10 and 10 Δ ide_{Ssuis} were incubated with 2% porcine plasma for 16 h at 37 °C and analyzed by SDS-PAGE under reducing conditions. A degradation product (*) of approximately 32 kDa was observed. (B) Anti-IgG Western Blot analysis of culture supernatant of *S. suis* strain 10 fractionated by ammonium sulfate precipitation. IgG degradation products (*) were
20 obtained after incubation of 1% porcine plasma with 20-40% and 40-60% ammonium sulfate saturation fractions for 16 h at 37 °C. Lane 1 showing the protein size standard is a photographic image of the membrane before detection of the chemiluminescence signal. (C) Culture supernatants of *S. suis* strains of all tested serotypes cleaved IgG. Concentrated (10x) culture supernatants were incubated with 1% porcine plasma for 16 h at 37 °C and
25 analyzed by anti-IgG Western blot. IgG cleavage products (*) at approximately 32 kDa of varying intensity were observed in all lanes. Images of different western blots have been assembled into one figure.

Figure 2. Identification of the active site of S. suis Igde.

30 (A) Schematic illustration of Igde and the different rIgde constructs with potential active site residues substitutions and the C-terminal truncation variant. The secretion signal peptide (residues 1-37) is marked in light grey, the transglutaminase domain is boxed and the potential active site residues and substitutions are indicated. (B) 3.3 μ M porcine IgG

was incubated for 16 h at 37 °C with soluble fractions of *E. coli* cells expressing different rIgdE constructs. The reaction was analyzed SDS-PAGE under reducing conditions. IgG cleavage (*) occurred upon incubation with rIgdE, rIgdE^{D348A} and rIgdE^{ΔC} but not with rIgdE^{C302S} or rIgdE^{H333A}. The weak protein band of 37 kDa is a contaminant present in lysate preparation and not related to IgdE activity.

Figure 3. Time course and inhibitor profile of recombinant S. suis IgdE.

The time course of cleavage (A) was monitored by continuous sampling prior to Coomassie fluor orange stained SDS-PAGE under reducing conditions followed by densitometric quantification of the cleavage product. 1.67 μM porcine IgG was incubated with 0.2 μM purified rIgdE^{ΔC}. Overnight cleavage (16h) was set as 100% relative cleavage. For the inhibitor profile (B) initial cleavage in presence of 250 μM and 2.5 μM of each inhibitor was monitored. Initial cleavage activity of the uninhibited control was set as 100% relative activity. The DMSO control correlates to 250 μM Z-LVG-CHN2. Data are presented as mean±SEM of three experiments. Differences to the uninhibited control (*) or DMSO control (□) were analyzed by Dunnett's Multiple Comparison Test with significance set at P values of <0.05(*), <0.01(**) and <0.001(***) or □□□.

Figure 4. S. suis IgdE cleaves the heavy chain of porcine IgG in the hinge region.

The reaction was analyzed by non-reducing (A) and reducing (B) Coomassie blue stained SDS-PAGE. 3.3 μM IgG was incubated with (+) or without (-) 10 nM purified rIgdE for 16h at 37°C. (C) The observed cleavage pattern and cleavage site proposes a model where one IgG heavy chain is first hydrolyzed by IgdE resulting in one free Fab fragment (Fab) and single cleaved IgG (scIgG) and in a second step the other heavy chain is hydrolyzed resulting in one Fc fragment (Fc) and two Fab fragments.

Figure 5. IgdE is highly specific for porcine IgG.

(A) 2% porcine plasma was incubated with (+) or without (-) 10 nM purified rIgdE. The reaction was analyzed by anti-porcine IgG, IgM and IgA Western blots under reducing conditions. Only cleavage of IgG was observed (*). (B) 0.5 mg/ml IgG of different species was incubated with (+) or without (-) 10 nM purified rIgdE and analyzed by SDS-PAGE under reducing conditions. No cleavage of IgG derived from any other species than pig (*) was observed. The weak protein band of 37 kDa is a contaminant present in lysate

preparation (see lane 2) and not related to IgdE activity. Images of different gels have been assembled into one figure.

Figure 6. S. suis IgdE degradation of endogenous IgG

5 *S. suis* IgdE degrades endogenous IgG in all tested body fluids of healthy pigs and pigs with respective lesions. (size indicated with arrows). No other degradation products could be observed. Body fluids were incubated with (+) or without (-) 10 nM purified rIgdE for 16h at 37°C. The reactions were analyzed by SDS-PAGE (A) and anti-IgG Western blots (B) under reducing conditions. Lanes showing the protein size standard are a
10 photographic image of the membrane before detection of the chemiluminescence signal. Images of different gels have been assembled into one figure.

Figure 7. IgdE is necessary for IgG cleavage by S. suis and IgG cleavage is independent of Ide_{Ssuis}.

15 Concentrated (10x) supernatants of *S. suis* strains 10, 10 Δ igdE, 10 Δ ide_{Ssuis} and 10 Δ ide_{Ssuis} Δ igdE were incubated with 1% porcine plasma for 16h at 37°C. The reaction was analyzed by anti-IgG Western blot (A) or anti-IgM Western blot (B) under reducing conditions. IgG degradation (*) was only observed when incubated with supernatants of strains 10 and 10 Δ ide_{Ssuis}, meanwhile IgM degradation (*) only was observed upon
20 incubation with supernatants of strains 10 and 10 Δ igdE.

Figure 8. Inhibitor profile of IgG degradation activity detected in culture supernatants of S. suis.

25 Concentrated (10x) culture supernatant of *S. suis* strain 10 was incubated with porcine plasma in presence of class specific protease inhibitors. The reaction was analyzed by anti-IgG western blot under reducing conditions. A cleavage product of IgG can be observed when incubated with 0.1-1 mM AEBSF, 0.1-5 mM EDTA, 50 μ M E-64, without any inhibitor and 1/200 diluted complete inhibitor cocktail (marked with an asterisk), meanwhile no degradation product was observed when incubated with 5 mM AEBSF, 250
30 μ M E-64, without concentrated supernatant (-C), 0.1-5 mM Z-LVG-CHN2, 0.1-5 mM iodoacetamide and 1/50 diluted complete inhibitor cocktail.

Figure 9. Representative Coomassie blue stained SDS-PAGE gel monitoring rIgde^{ΔC} purification.

rIgde^{ΔC} was purified from lysate of IPTG induced *E. coli* carrying an expression plasmid. The elute of a Ni²⁺-affinity chromatography purification containing His-ZZ-tagged rIgde^{ΔC} was incubated with Tev-protease to remove the His-ZZ-tag prior to a second round of Ni²⁺-affinity chromatography purification. The flow through fractions 2 and 3 of this purification step containing untagged rIgde^{ΔC} were pooled, buffer ex-changed to PBS and used for further experiments. Two major bands around 50 kDa can be observed in these fractions, due to partial degradation of rIgde^{ΔC}.

Figure 10. rIgde_{Eagalactiae} specifically cleaves human IgG1.

(A) rIgde_{Eagalactiae} was incubated with polyclonal IgG of six different species for 18h at 37°C and analyzed by SDS-PAGE under reducing conditions. Degradation products are indicated by asterisks (*). Images of different gels have been used to create the figure. (B) rIgde_{Eagalactiae} was incubated with human or bovine serum for 18h in 37°C and analyzed by SDS-PAGE under reducing conditions. Degradation products are indicated by asterisks (*). (C) Western Blot with crude extracts of rIgde_{Eagalactiae} incubated with human and porcine serum for 18h in 37°C. Membrane was treated with antibodies against human and porcine polyclonal IgG respectively. Degradation products are indicated by asterisks (*). (D) rIgde_{Eagalactiae} was incubated with subclasses of human IgG as well as human IgA and IgM for 18h in 37°C and analyzed by SDS-PAGE under reducing conditions. Degradation products are indicated by asterisks (*). Images of different gels have been used to create the figure.

Figure 11. rIgde_{Eagalactiae} degrades human serum IgG1 completely to single cleaved IgG1.

Human serum and monoclonal human IgG1 were incubated in presence (+) or absence (-) of rIgde_{Eagalactiae} for 18h at 37°C and analyzed by Western blot under non-reducing conditions with a human IgG1-specific antibody.

Figure 12. rIgde_{Eequi} cleaves exclusively equine IgG7.

0.6 μM recombinant equine IgG subtypes or serum IgG were incubated for 16h at 37°C with (+) or without (-) the soluble fraction of *E. coli* cells expressing rIgde_{Eequi}. Reactions were analyzed by SDS-PAGE under reducing conditions. IgG cleavage (*)

occurred upon incubation with IgG7 and serum IgG. SDS-PAGE was either stained with Coomassie Fluor Orange Protein Gel Stain (A) or subjected to Western blot (B) analyses with Rabbit Anti-Horse IgG H&L (HRP) ab6921.

5 **Detailed description of the invention**

Brief description of the sequences

SEQ ID NO: 1 is the amino acid sequence of the IgdE isolated from *S. suis* strain 10. Additional amino acid sequences of IgdEs from *S. suis* can be found e.g. in GenBank as accession nos WP_012027720.1, WP_044687717.1, WP_045002893.1, WP_044981166.1, WP_044770432.1, WP_043041527.1, WP_014917307.1, WP_044981141.1, WP_044766031.1, WP_044780628.1, WP_044980481.1, WP_044768304.1, WP_043033176.1, WP_044772573.1, ABQ42883.1, ABQ42882.1, ABQ42884.1, ABQ42885.1, WP_024402604.1, WP_044671803.1, WP_014639000.1, WP_014636499.1, WP_044475270.1, WP_044683049.1, WP_044682603.1, WP_044982674.1, WP_024406212.1, WP_024402376.1, WP_024382860.1, WP_044684005.1, WP_044688055.1, WP_044754153.1, WP_024412941.1, WP_029172805.1, AER15932.1, WP_024414493.1, WP_044762560.1, WP_024417771.1, WP_044675281.1, WP_044666819.1, WP_043032980.1, WP_044671755.1, WP_024416363.1, ABL84354.1, ABL84413.1, WP_044758899.1, WP_024393919.1, WP_014736321.1, WP_024383620.1, WP_044475488.1, WP_024386700.1, WP_024381951.1, WP_024390579.1, WP_023371419.1, WP_044772576.1, WP_044766774.1, WP_044672596.1, WP_043028752.1, WP_044771508.1, WP_024383851.1, WP_024388957.1, WP_024394959.1, WP_029174632.1, WP_044752120.1, WP_044678077.1, WP_002938262.1, WP_044769991.1, WP_044770681.1, WP_044749736.1, and WP_044771392.1.

SEQ ID NO: 2 is a nucleic acid sequence encoding IgdE isolated from *S. suis* strain 10.

SEQ ID NO: 3 is the amino acid sequence of an IgdE isolated from *S. agalactiae* strain CCUG4208. Additional amino acid sequences of IgdEs from *S. agalactiae* can be found e.g. in GenBank as accession nos EPU21342.1, EFV98161.1, CAD47554.1, EPU23939.1, EPW71972.1, EPT99261.1, EPV90329.1, EGS26825.1, EPV84888.1, CFQ52811.1, EPT84780.1, EPT99074.1, CCW42936.1, CCW40848.1, EAO70450.1, EPU74838.1, EPT51236.1, EPU77761.1, EPT36280.1, WP_011058381.1, EPT59860.1, EPT39170.1, EPT38734.1, CFQ25568.1, EPU40877.1, WP_025193619.1,

WP_000440329.1, WP_000440333.1, WP_000440330.1, WP_000440331.1,
 WP_047199154.1, WP_001901206.1, WP_025193273.1, WP_025194923.1,
 WP_000440334.1, WP_025195559.1, WP_047200043.1, WP_041165773.1, EPU33307.1,
 CFW66620.1, WP_041981191.1, WP_000440332.1, WP_047200261.1,
 5 WP_029692083.1, WP_017770870.1, WP_025197885.1, EAO75153.1, WP_001884472.1,
 EAO62452.1, WP_025195776.1, WP_047200280.1, CFW66618.1, and EAO75155.1.

SEQ ID NO: 4 is a nucleic acid sequence encoding IgdE isolated from *S. agalactiae* strain CCUG4208.

10 SEQ ID NO: 5 is the amino acid sequence of the IgdE isolated from *S. porcinus* strain DSM20725. The amino acid sequences of IgdE from *S. porcinus* can be found e.g. in GenBank as accession no WP_003085269.1

SEQ ID NO: 6 is a nucleic acid sequence encoding IgdE isolated from *S. porcinus* strain DSM20725.

15 SEQ ID NO: 7 is the amino acid sequence of the IgdE isolated from *S. equi* strain *ssp zooepidemicus* #1. Additional amino acid sequences of IgdEs from *S. equi* can be found e.g. in GenBank as accession nos. KDE01980.1, KIS07668.1, KIS08707.1, KIS19971.1, WP_043038795.1, WP_043036602.1, WP_037584076.1, KIS20896.1, AIA68804.1, WP_043029522.1, WP_012678259.1, WP_042670323.1, WP_043040324.1, and WP_014622546.1.

20 SEQ ID NO: 8 is a nucleic acid sequence encoding IgdE isolated from *S. equi* strain *ssp zooepidemicus* #1.

SEQ ID NO: 9 is the amino acid sequence of the IgdE isolated from *S. pseudoporcinus* strain ATCC® BAA-1381. The amino acid sequences of IgdE from *S. pseudoporcinus* can be found e.g. in GenBank as accession no WP_007895676.1.

25 SEQ ID NO: 10 is a nucleic acid sequence encoding IgdE isolated from *S. pseudoporcinus* strain ATCC BAA-1381.

SEQ ID NO: 11 is the amino acid sequence of a C-terminally truncated variant of IgdE isolated from *S. suis*.

30 IgdE Polypeptides

The IgdE polypeptide according to the present invention is preferably an IgdE_{suis}, IgdE_{agalactiae}, IgdE_{porcinus}, IgdE_{equi}, or an IgdE_{pseudoporcinus} polypeptide, or a variant or fragment of any thereof which retains cysteine protease activity and/or is capable to raise

an immune response to a streptococcus in a subject. The variant may be an IgDE polypeptide from another bacterium. The bacterium is preferably a Streptococcus.

The IgDE polypeptide may comprise the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11; or a variant of any one thereof having at least 70% identity to the amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, or 11 and having IgG degrading cysteine protease activity; or a fragment of SEQ ID NOs: 1, 3, 5, 7, 9, 11; or a variant of any one thereof having IgG degrading cysteine protease activity.

The IgDE polypeptide may comprise the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11; or a variant of any one thereof having at least 70% identity to the amino acid sequence of any of SEQ ID NOs: 1, 3, 5, 7, 9, or 11 and being capable to raise an immune response to a streptococcus in a subject; or a fragment of SEQ ID NOs: 1, 3, 5, 7, 9, 11; or a variant of any one thereof being capable to raise an immune response to a streptococcus in a subject.

The streptococcus can be *S. suis*, *S. agalactiae*, *S. porcinus*, *S. equi*, or *S. pseudoporcinus*.

Preferably, the polypeptide comprises, or consists of, any of the sequences of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

The polypeptide may additionally include a signal sequence or a N-terminal methionine.

Variant polypeptides are those for which the amino acid sequence varies from that in SEQ ID NOs: 1, 3, 5, 7, 9, or 11 respectively, but which retain the same essential character or basic functionality as IgDE. The variant polypeptides may therefore display IgG degrading cysteine protease activity, and/or be capable of raising an immune response to a streptococcus in a subject. Preferably the immune response is a protective immune response. Preferably the immune response generates antibodies able to neutralize the IgG degrading cysteine protease activity of the IgDE of an infectious streptococcus in the immunized subject. Notably, an IgDE variant lacking the IgG cysteine protease activity can still be able to raise an immune response generating antibodies being able to neutralize the IgG cysteine protease activity of the IgDE of an infectious streptococcus in the immunized subject. Such IgDE variants are included in the IgDE variants according to the invention.

Typically, polypeptides with more than about 70% identity, preferably at least 80%, at least 90% and particularly preferably at least 95%, at least 97% or at least 99% identity, with the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, or 11 are considered variants of the protein. Such variants may include allelic variants and the deletion, modification or

addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the basic IgD_E functionality. The identity of variants of SEQ ID NOs: 1, 3, 5, 7, 9, or 11 may be measured over a region of at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, at least 275, at least 300 or more

contiguous amino acids of the sequence shown in SEQ ID NOs: 1, 3, 5, 7, 9, or 11, or more preferably over the full length of SEQ ID NOs: 1, 3, 5, 7, 9, or 11. It will be appreciated that any of the above listed lower limits may be combined with any of the above listed upper limits to provide a range for the length of the polypeptide of the invention. For example, the polypeptide may be 50 to 250 amino acids in length, or 100 to 300 amino acids in length. The polypeptide may be 100 to 586 amino acids in length, 150 to 500 amino acids in length or 100 to 400 amino acids in length.

Amino acid identity may be calculated using any suitable algorithm. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al.* (1984) Nucleic Acids Research 12, 387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al.* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al.*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl.

Acad. Sci. USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5787.

One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The variant sequences typically differ by at least 1, 2, 5, 10, 20, 30, 50 or more amino acids positions (which maybe substitutions, deletions or insertions of amino acids). For example, from 1 to 50, 2 to 30, 3 to 20 or 5 to 10 amino acid substitutions, deletions or insertions may be made. The substitutions are preferably conservative substitutions, for example according to the following Table 1. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

Table 1.

ALIPHATIC	Non-polar	Gly Ala Pro
		Ile Leu Val
	Polar-uncharged	Cys Ser Thr Met
		Asn Gln
	Polar-charged	Asp Glu
		Lys Arg
AROMATIC		His Phe Trp Tyr

Unless otherwise specified, the modifications are preferably conservative amino acid substitutions. Conservative substitutions replace amino acids with other amino acids of similar chemical structure, similar chemical properties or similar side-chain volume. The amino acids introduced may have similar polarity, hydrophilicity, hydrophobicity, basicity, acidity, neutrality or charge to the amino acids they replace. Alternatively, the conservative substitution may introduce another amino acid that is aromatic or aliphatic in the place of a pre-existing aromatic or aliphatic amino acid. Conservative amino acid

changes are well-known in the art and may be selected in accordance with the properties of the 20 main amino acids as defined in Table A1 below. Where amino acids have similar polarity, this can be determined by reference to the hydropathy scale for amino acid side chains in Table A2.

5

Table A1 - Chemical properties of amino acids

Ala (A)	aliphatic, hydrophobic, neutral	Met (M)	hydrophobic, neutral
Cys (C)	polar, hydrophobic, neutral	Asn (N)	polar, hydrophilic, neutral
Asp (D)	polar, hydrophilic, charged (-)	Pro (P)	hydrophobic, neutral
Glu (E)	polar, hydrophilic, charged (-)	Gln (Q)	polar, hydrophilic, neutral
Phe (F)	aromatic, hydrophobic, neutral	Arg (R)	polar, hydrophilic, charged (+)
Gly (G)	aliphatic, neutral	Ser (S)	polar, hydrophilic, neutral
His (H)	aromatic, polar, hydrophilic, charged (+)	Thr (T)	polar, hydrophilic, neutral
Ile (I)	aliphatic, hydrophobic, neutral	Val (V)	aliphatic, hydrophobic, neutral
Lys (K)	polar, hydrophilic, charged(+)	Trp (W)	aromatic, hydrophobic, neutral
Leu (L)	aliphatic, hydrophobic, neutral	Tyr (Y)	aromatic, polar, hydrophobic

Table A2 - Hydropathy scale

	<u>Side Chain</u>	<u>Hydropathy</u>
10	Ile	4.5
	Val	4.2
	Leu	3.8
	Phe	2.8
	Cys	2.5
15	Met	1.9
	Ala	1.8
	Gly	-0.4
	Thr	-0.7
	Ser	-0.8
20	Trp	-0.9
	Tyr	-1.3
	Pro	-1.6
	His	-3.2
	Glu	-3.5
25	Gln	-3.5
	Asp	-3.5
	Asn	-3.5
	Lys	-3.9
	Arg	-4.5
30		

Preferably the polypeptides comprise a cysteine residue and a histidine residue at a spacing typically found in cysteine proteases. For example, in SEQ ID NO: 1, these

residues are found at position 302 and position 333. An aspartic acid residue completing the catalytic triad is found at position 348.

The fragment of the Igde polypeptide used in the invention is typically at least 10, for example at least 15, 20, 25, 30, 40, 50 or more amino acids in length, up to 100, 150, 200, 250 or 300 amino acids in length, as long as it retains the IgG degrading cysteine protease activity of Igde and/or being capable of raising an immune response to a streptococcus in a subject.

The polypeptides used in the invention may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated, phosphorylated or comprise modified amino acid residues. They may be modified by the addition of histidine residues to assist their purification or by the addition of a signal sequence to promote insertion into the cell membrane. Such modified polypeptides fall within the scope of the term "polypeptide" used herein.

Typically, polypeptides for use in accordance with the invention display immunoglobulin cysteine protease activity, and in particular IgG degrading cysteine protease activity. Preferably, the polypeptide cleaves IgG in the hinge region and more particularly in the hinge region of the heavy chain. Preferably, cleavage results in production of Fc and Fab fragments of IgG. Preferably the activity is specific for IgG. The IgG degrading cysteine protease activity may be determined by means of a suitable assay. For example, a test polypeptide may be incubated with IgG at a suitable temperature, such as 37°C. The starting materials and the reaction products may then be analysed by SDS PAGE to determine whether the desired IgG cleavage product is present. Typically this cleavage product is a 32kDa fragment. Typically there is no further degradation of IgG after this first cleavage. The cleavage product may be subjected to N-terminal sequencing to verify that cleavage has occurred in the hinge region of IgG.

The cysteine protease activity of the polypeptides can be further characterised by inhibition studies. Preferably, the activity is inhibited by the peptide derivative Z- LVG-CHN₂, and/or by iodoacetic acid which are protease inhibitors.

The IgG degrading cysteine protease activity of the polypeptides is generally IgG-specific in that the polypeptides may not degrade other classes of Ig, namely IgM, and IgA, when incubated with these immunoglobulins under conditions that permit cleavage of IgG. Igde from *S. suis*, *S. porcinus*, and *S. pseudoporcinus* were found to be specific for porcine IgG. Igde from *S. agalactiae* was found to be specific for human IgG, and specifically

human IgG1. IgdE from *S. equi* was found to be specific for equine IgG, and specifically equine IgG7.

Polypeptides for use in the invention may be in a substantially isolated form. It will be understood that the polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and still be regarded as substantially isolated. A polypeptide for use in the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 50%, e.g. more than 80%, 90%, 95% or 99%, by weight of the polypeptide in the preparation is a polypeptide of the invention.

Polypeptides for use in the present invention may be isolated from any suitable organism that expresses an IgdE polypeptide. Typically, the IgdE polypeptide is isolated from suitable IgdE expressing streptococcal strains. Suitable organisms and strains may be identified by a number of techniques. For example, streptococcal strains may initially be tested for the presence an *igdE* gene. Polynucleotide primers or probes may be designed based on for example, SEQ ID NOs: 2, 4, 6, 8, or 10. Examples of suitable primers are set out in SEQ ID NOs: 12-22. The presence of the *igdE* gene can then be verified by PCR using the primers or by hybridisation of the probes to genomic DNA of the strain.

Polynucleotides

A polynucleotide according to the invention may comprise or consist of: (a) the coding sequence of SEQ ID NOs: 4, 5, 6, 8, or 10; (b) a sequence which is degenerate as a result of the genetic code to the sequence as defined in (a); (c) a sequence having at least 70% identity to a sequence as defined in (a) or (b) and which encodes a polypeptide having IgG degrading cysteine protease activity and/or be capable of raising an immune response to a streptococcus in a subject; or (d) a fragment of any one of the sequences as defined in (a), (b) or (c) which encodes a polypeptide having IgG degrading cysteine protease activity and/or be capable of raising an immune response to a streptococcus in a subject.

Typically the polynucleotide is DNA. However, the polynucleotide may be a RNA polynucleotide. The polynucleotide may be single or double stranded, and may include within it synthetic or modified nucleotides. A polynucleotide of the invention can typically hybridize to the coding sequence or the complement of the coding sequence of SEQ ID NOs: 2, 4, 6, 8, or 10 at a level significantly above background. Background hybridization may occur, for example, because of other DNAs present in a DNA library. The signal level generated by the interaction between a polynucleotide of the invention and the coding

sequence or complement of the coding sequence of SEQ ID NOs: 2, 4, 6, 8, or 10 is typically at least 10 fold, preferably at least 100 fold, as intense as interactions between other polynucleotides and the coding sequence of SEQ ID NOs: 2, 4, 6, 8, or 10. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P . Selective hybridisation may typically be achieved using conditions of medium to high stringency. However, such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 1989). For example, if high stringency is required suitable conditions include from 0.1 to 0.2 x SSC at 60°C up to 65°C. If lower stringency is required suitable conditions include 2 x SSC at 60°C.

The coding sequence of SEQ ID NOs: 2, 4, 6, 8, or 10 may be modified by nucleotide substitutions, for example from 1, 2 or 3 to 10, 25, 50 or 100 substitutions. The polynucleotide of SEQ ID NO: 2, 4, 6, 8, 10 may alternatively or additionally be modified by one or more insertions and/or deletions and/or by an extension at either or both ends.

Additional sequences such as signal sequences may also be included. The modified polynucleotide generally encodes a polypeptide which has IgDE specific cysteine protease activity and/or is capable of raising an immune response to a streptococcus in a subject. Degenerate substitutions may be made and/or substitutions may be made which would result in a conservative amino acid substitution when the modified sequence is translated, for example as shown in the Table 1 above.

A nucleotide sequence which is capable of selectively hybridizing to the complement of the DNA coding sequence of SEQ ID NOs: 2, 4, 6, 8, or 10 will generally have at least 70%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to the coding sequence of SEQ ID NO: 2, 4, 6, 8, or 10 respectively, over a region of at least 20, preferably at least 30, for instance at least 40, at least 60, more preferably at least 100 contiguous nucleotides or most preferably over the full length of SEQ ID NO: 2, 4, 6, 8, or 10. Sequence identity may be determined by any suitable method, for example as described above.

Any combination of the above mentioned degrees of sequence identity and minimum sizes may be used to define polynucleotides of the invention, with the more stringent combinations (i.e. higher sequence identity over longer lengths) being preferred. Thus, for example a polynucleotide which has at least 90% sequence identity over 20, preferably over 30 nucleotides forms one aspect of the invention, as does a polynucleotide which has at least 95% sequence identity over 40 nucleotides. Polynucleotide fragments

will preferably be at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 or at least 40 nucleotides in length. They will typically be up to 40, 50, 60, 70, 100 or 150 nucleotides in length. Fragments can be longer than 150 nucleotides in length, for example up to 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nucleotides in length, or even up to a few nucleotides, such as five, ten or fifteen nucleotides, short of the coding sequence of SEQ ID NO: 2, 4, 6, 8, or 10 respectively.

Polynucleotides for use in the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques. The polynucleotides are typically provided in isolated and/or purified form.

In general, short polynucleotides will be produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for example using PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15-30 nucleotides) to a region of the *igdE* gene which it is desired to clone, bringing the primers into contact with DNA obtained from a bacterial cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector. Suitable primers are for example, those in SEQ ID NOs: 12-22.

Such techniques may be used to obtain all or part of the *igdE* gene sequence described herein. Although in general the techniques mentioned herein are well known in the art, reference may be made in particular to Sambrook *et al.* (1989). *IgdE* polynucleotides as described herein have utility in production of the polypeptides for use in the present invention, which may take place *in vitro*, *in vivo* or *ex vivo*. The polynucleotides may be used as therapeutic agents in their own right or may be involved in recombinant protein synthesis.

The polynucleotides for use in the invention are typically incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Therefore, polynucleotides for use in the invention may be made by

introducing an IgdE polynucleotide into a replicable vector, introducing the vector into a compatible host cell and growing the host cell under conditions which bring about replication of the vector.

Preferably the vector is an expression vector comprising a nucleic acid sequence that encodes an IgdE polypeptide. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals, which may be necessary and which are positioned in the correct orientation in order to allow for protein expression. Other suitable vectors would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook *et al.* (1989).

Preferably, a polynucleotide for use in the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in such a way that expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

The vectors may be for example, plasmid, virus or phage vectors provided with a origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vector is typically adapted to be used in vivo.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. Mammalian promoters, such as β -actin promoters, may be used. Tissue-specific promoters are especially preferred. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, adenovirus, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR). Viral promoters are readily available in the art. The vector may further include sequences flanking the polynucleotide giving rise to polynucleotides which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences. This will allow the introduction of the polynucleotides of the

invention into the genome of eukaryotic cells by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral
 5 vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the polynucleotide into the host genome. Replication- defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

10

Diseases and Conditions

The IgDE polypeptide, or polynucleotide, may be used to treat or prevent diseases or conditions mediated by pathogenic IgG antibodies. It is well known in the art that pathogenic IgG antibodies are involved in the pathogenesis of a number of different
 15 diseases and conditions. Consequently, the effects of pathogenic IgG antibodies in such diseases can be inhibited using an IgDE polypeptide or polynucleotide.

The disease or condition can be an autoimmune disease. Such diseases include Addison's disease, alopecia areata, ankylosing spondylitis, antiphospho lipid syndrome, aplastic anaemia, autoimmune gastritis, autoimmune hearing loss, autoimmune haemolytic
 20 anaemias, autoimmune hepatitis, autoimmune hypoparathyroidism, autoimmune hypophysitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, autoimmune polyendocrinopathy, Bechet's disease, bullous pemphigoid, cardiomyopathy, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, coeliac disease,
 25 Crohn's disease, CREST syndrome, Degos disease, epidermolysis bullosa acquisita, essential mixed cryoglobulinaemia, giant cells arteritis, glomerulonephritis, Goodpasture's syndrome, Graves' disease, Guillan-Barre syndrome, Hashimoto's thyroiditis, idiopathic thrombocytopenic purpura, inflammatory bowel disease, Kawasaki's disease, Meniere's syndrome, mixed connective tissue disease, Mooren's ulcer, multiple sclerosis, myasthenia
 30 gravis, pemphigus foliaceus, pemphigus vulgaris, pernicious anaemia, polyarteritis nodosa, polyglandular autoimmune syndrome type 1 (PAS-I), polyglandular autoimmune syndrome type 2 (PAS-2), polyglandular autoimmune syndrome type 3 (PAS-3), polymyositis/dermatomyositis, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's syndrome, Reiter's syndrome, rheumatoid arthritis, sarcoidosis, scleroderma,

Sjogren's syndrome, subacute thyroiditis, sympathetic ophthalmia, systemic lupus erythematosus, Takayasu's arteritis, type 1 diabetes mellitus, vitiligo, Vogt-Koyanagi-Harada disease or Wegener's granulomatosis. Preferably the autoimmune disease is rheumatoid arthritis (RA).

5 The disease or condition can be asthma. The asthma can be acute or chronic asthma.

 IgG activates the classical pathway of the complement system. IgG polypeptides and polynucleotides can therefore be used to treat diseases and conditions where complement activation is detrimental to the patient. For example, the IgG polypeptides and polynucleotides can be used to treat transplantation-derived disorders, for example
10 transplant rejection (such as allograft and xenograft rejection) and graft-versus-host disease. The transplantation-derived disorder may occur due to the transplantation of a tissue or an organ in a patient.

 IgG polypeptides and polynucleotides are also of use in post-operative treatment,
15 for example in the treatment of patients who have undergone heart by-pass operations.

 Further, IgG polypeptides and polynucleotides can be used for the treatment of acquired haemophilia, i.e. to remove IgG in haemophilia patients who have developed autoantibodies against coagulation factors.

 The term "a subject" as used herein includes any mammalian subject such as a
20 human, a pig, a horse, and any cattle.

In vitro Methods

 The invention encompasses *in vitro* methods for the cleavage of IgG, comprising
25 contacting IgG with a polypeptide comprising:

- (a) the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 9 or 11;
- (b) a variant thereof having at least 70% identity to the amino acid sequence of SEQ ID NO: 1, 3, 5, 7, 9 or 11 and having IgG cysteine protease activity; or
- (c) a fragment thereof of either (a) or (b), having IgG cysteine protease activity.

30 In a preferred embodiment, the amino acid sequence is SEQ ID NO: 3. The *in vitro* method for the cleavage of IgG comprises contacting IgG with a polypeptide comprising:

- (a) the amino acid sequence of SEQ ID NO: 3;
- (b) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid
35 sequence of SEQ ID NO: 3 and having IgG cysteine protease activity;

(c) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3, having IgG cysteine protease activity.

In the preferred embodiment, the polypeptide is specific for human IgG, and specifically human IgG1. Furthermore, the polypeptide cleaves IgG1 as shown in Figure 4, irrespective of whether the light chain is kappa or lambda type.

The polypeptide is an IgG cysteine protease, which may typically comprise a catalytic site including a cysteine residue, a histidine residue and an aspartic acid residue in positions which correspond to positions 265, 296 and 311 of SEQ ID NO: 1. For example, in SEQ ID NO:3 a cysteine residue, histidine residue and an aspartic acid residue are found at positions 262, 293 and 308. The variants and fragments of the polypeptide typically retain these residues.

The method of the present invention comprises incubating the polypeptide with a sample containing IgG under conditions which permit specific cysteine protease activity to occur. The method further comprises identification and/or isolation of the cleavage products. The analysis may be by any suitable method known in the art. In some instances, the analysis is by gel electrophoresis (*e.g.* SDS-PAGE) or mass spectrometry.

In another embodiment of the present invention, the *in vitro* method is used to generate Fc or Fab fragments of IgG. The method comprises contacting IgG with a polypeptide of the invention.

In a further embodiment of the invention, the method of the invention is used to detect IgG. This may comprise (i) contacting a sample with a polypeptide under conditions that permit the IgG specific cysteine protease activity of the polypeptide; and (ii) monitoring for the presence of IgG specific cleavage fragments; wherein the presence of the specific cleavage fragments is indicative of IgG in the sample.

EXAMPLES

Example 1. Identification of IgG from *S. suis*

Bacterial strains and growth conditions

S. suis strain 10 is a virulent serotype 2 strain that has been used in several studies for mutagenesis and experimental infections of pigs (Smith *et al.* 1999. *Infect. Immun.* 67, 1750-1756). Strain 10M7 was kindly provided by Hilde Smith (AWG, Lelystad, Netherlands) (Smith *et al.* 1996. *Infect. Immun.* 64, 4409-4412). Streptococci were grown

on Columbia agar plates with 6% sheep blood or in Bacto Todd-Hewitt broth (THB) under anaerobic conditions at 37°C. *Escherichia coli* strains were cultured in Luria Broth (LB). When appropriate, antibiotics were added at 50 µg/ml for kanamycin and 20 µg/ml for gentamycin.

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Materials from animals

Samples from experimentally infected piglets were drawn within a previous study. The protocol for this animal experiment was approved by the Committee on Animal Experiments of the Lower Saxonian State Office for Consumer Protection and Food Safety in Germany (permit no. 33.9-42502-04-07/1243). Collection of blood from conventional piglets for bactericidal assays was registered under N19/14 at the regional office in Saxonia, Germany. The animal studies were performed in strict accordance with the principles and recommendations outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Treaty Series, no. 123: <http://www.conventions.coe.int/Treaty/en/Treaties/Html/123.htm>) and the German Animal Protection Law.

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Identification of the IgG degrading activity

S.suis strain 10M7 cultures were harvested at approximately OD₆₀₀ of ~0.6 and culture supernatants were sterile-filtrated through a 0.22 µm Express PLUS membrane filter (Millipore) prior to fractionating the culture supernatant with ammonium sulfate to 30% saturation. The resulting precipitate was discarded and ammonium sulfate was added to the remaining supernatant to a final concentration of 50% saturation. The second precipitate was resuspended in 1/100 of the starting volume with 20 mM Bis-Tris pH 6.8 and buffer exchange against the same buffer was performed by HiPrep 26/10 Desalting column (GE Healthcare). The material was further fractionated by FPLC on a HiTrap Q HP column (GE Healthcare). Proteins were eluted by a linear NaCl gradient, and fractions eluted at ~0.2 M NaCl were found to contain the IgG degrading activity. The active fractions were subjected to size exclusion chromatography (HiPrep 16/60 Sephacryl S-100 HR, GE Healthcare) and there the IgG degrading activity was eluted at ~37 ml elution volume. Active fractions were analyzed by SDS-PAGE and protein bands were subjected to Mass spectrometry analysis.

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Mass spectroscopy

MALDI-TOF mass spectroscopy was performed by Umeå Protein Analysis Facility (Umeå University). Peptides for MS analysis were prepared by in-gel digestion using trypsin (sequencing grade modified, Promega) and analyzed by ESI LC-MS/MS using an HCT ultra ETD II iontrap instrument (Bruker) linked to an Easy nano LC system (Proxeon). Processing, deconvolution and compound detection for the LC-MS/MS datasets was performed using the DataAnalysis software (4.0 SP4, Bruker). Database searches using the peaklists files of the processed datasets were performed using the Mascot search engine (Matrixscience) in the bacterial sequences of the NCBI database. The search parameters permitted a mass error of 0.3 Da for both the MS and the MS/MS mode and variable modifications of methionine by oxidation, of cysteine by propionamide derivation and by N-terminal acetylation.

Screening of S. suis strains for IgG degrading capacity

Culture supernatants of 15 different *S. suis* strains (Table 2) were isolated at OD₆₀₀ of ~0.6 and concentrated by addition of saturated ammonium sulfate solution to a final saturation of 50%. The precipitate was re-suspended in 1/20 of the starting volume in PBS following buffer exchange against PBS by Zeba Spin Desalting Columns 7K MWCO (Thermo Scientific). The 50% ammonium sulfate precipitation fraction of *S. suis* culture supernatants were used for IgG degradation analysis.

Table 2. *S. suis* strains used in this study

Strain	Capsule type	Source (Reference)
10	cps 2	Smith <i>et al.</i> 1999. <i>Infect. Immun.</i> 67, 1750-1756
A1731/94	cps 1	Allgaier <i>et al.</i> 2001. <i>J Clin Microbiol.</i> 39, 445-453
P1/7	cps 2	Jacobs <i>et al.</i> 1994. <i>Infect. Immun.</i> 62, 1742-1748
T15	cps 2	Smith <i>et al.</i> 1999. <i>Infect. Immun.</i> 67, 1750-1756
I9841/1	cps 2	Allgaier <i>ibid</i>
199	cps 2 (human)	Baums <i>et al.</i> 2007. <i>Appl. Environ. Microbiol.</i> 73, 711-717
MAC724	cps 2 (human)	Baums <i>ibid</i>
B2795/96	cps 7	Allgaier <i>ibid</i>
B2441/96	cps 2	Allgaier <i>ibid</i>
A5505/93	untypeable	Allgaier <i>ibid</i>
V2569/1	cps 5	Baums, unpublished
#451	cps 7	Unterweger <i>et al.</i> 2014. <i>Berl. Münch Tierarztl. Wochenschr.</i> 127, 194-201
V3667/1	cps 7	Baums, unpublished
A5683/94	cps 9	Allgaier <i>ibid</i>
A3286/94	cps 9	Allgaier <i>ibid</i>

5223	cps 14	Wisselink <i>et al.</i> 2002. <i>J. Clin. Microbiol.</i> 40, 2922-2929
10M7	cps 2	Smith <i>et al.</i> 1996. <i>Infect. Immun.</i> 64, 4409-4412
10 Δ ideSsuis	cps 2	Seele <i>et al.</i> 2013 <i>J. Bacteriol.</i> 195, 930-940
10 Δ igdE	cps 2	this application
10 Δ ideSsuis Δ igdE	cps 2	this application
5223	cps 14	Wisselink <i>ibid</i>

Generation of a Δ igdE deletion strain

In frame deletion of *igdE* was principally conducted as in Seele *et al.*, 2013 (*J. Bacteriol.* 195, 930-940) with *S. suis* strain 10. For construction of the thermosensitive vector pSET5_ Δ igdE a 618 bp 5'-*igdE* amplicon was generated with primers preProIgdEPstI (TCACTGCAGTTTTGGGGAGTAGG, SEQ ID NO: 12) and postSSIgdEBamHI (ATGGATCCCAGTTCAGAACCTC, SEQ ID NO: 13) and a 612 bp 3'-*igdE* amplicon was generated with primers preEndIgdEBamHI (CGGGATCCAGAGAAAAAAGAGATCC, SEQ ID NO:14) and postEndIgdEEcoRI (AGGAATTCACCGTTATTGTAGCG, SEQ ID NO:15). These amplicons were cloned into pSET5 (18) with the restriction enzymes indicated in the names of the primers to generate pSET5_ Δ igdE. Deletion clones were confirmed by selective PCR analysis and sequencing of genomic amplicons using primers IgdE-seq_fr (ATTGTATTTGGTGGAGGAG, SEQ ID NO:16) and IgdE-seq_rev (TTTAGCAGCTAAGTTGATACC, SEQ ID NO:17).

Sequence analysis

Sequence analyses were performed using the SIB Bioinformatics Resource Portal Expasy (www.expasy.ch). SignalP 4.0 was run to identify a putative signal peptide and the respective cleavage sites (www.cbs.dtu.dk/services/SignalP/). *In silico* modelling of *S. suis* IgdE to identify the potential active site residues was performed using SWISS-MODEL (<http://swissmodel.expasy.org/>). A putative transmembrane region was identified using the consensus prediction web server TOPCONS (<http://topcons.cbr.su.se/>) last accession 2015-11-06.

DNA techniques and primer sequences

Primers were designed based on gene SSU_RS08150 in the genome *S. suis* P1/7. All PCRs were conducted with Phusion Master Mix HF (Thermo Scientific). All obtained plasmids were checked by restriction analyses, PCR and sequencing.

Cloning of S. suis igdE and generation of IgdE mutants

The *S. suis igdE* gene lacking the signal peptide coding sequences (encoding amino acid 38-1121) was amplified from chromosomal DNA of *S. suis* strain 10 as template using
 5 primers IgdE-frw_NcoI (GTTTCCATGGATGAAAACCTCACATTACAAATCG, SEQ ID NO:18) and IgdE-rev_NotI (ACGTGCGGCCCGCATAAGCTTCGTAC, SEQ ID NO:19) and cloned into pET_ZZ_1a after digestion with *NcoI* and *NotI* (Thermo Scientific). The entire insert was sequenced to verify the cloning and the sequence of *S. suis igdE*. The obtained sequence of *S. suis* strain 10 *igdE* was identical to the one of *S. suis* 05ZYH33
 10 and contained an insertion of 32 amino acids compared to SSU_RS08150 of *S. suis* P1/7.

Directed mutagenesis of the putative active site residues C302 to S, H333 to A, and D348 to A were performed with QuickChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent Technologies) and primers IgdE-C302S (AACGTCAGAAAGCGATGAGTGTAGGTTTCAGCACT, SEQ ID NO:20), IgdE-
 15 H333A (CAGAAGGTGTCCCGGCTGCTACAGCGCGTG, SEQ ID NO:21) and IgdE-D348A (TAAAAAGTGGCACACCATTGCCGGTACAGGTTTTATTACAG, SEQ ID NO:22) according to the manufacturer's instructions.

IgdE^{ΔC}, consisting of the N-terminal 470 amino acids of *S. suis* IgdE was created by digesting full-length *igdE* in pZZ1a with restriction endonucleases *XhoI* (Thermo Scientific).
 20 The digested plasmids were purified, re-ligated and transformed into *E. coli*.

Expression and purification of recombinant S. suis IgdE

E. coli ArcticExpress (DE3)_RIL (Agilent Technologies) isolates carrying pET_ZZ_1a *igdE*, *igdE*^{C302S}, *igdE*^{H302A}, *igdE*^{D302A} or *igdE*^{ΔC} were grown to OD₆₀₀ 0.6 at
 25 30°C. Protein expression was induced with 1 mM IPTG at 12°C and incubation was continued for an additional 22 h.

Cells were lysed for crude extracts by BugBuster HT Protein Extraction Reagent (Novagen) or for purification by Stansted high pressure cell disrupter (Stansted Fluid Power) in 50 mM Bis-Tris pH 7, 0.5 M NaCl, 5% glycerol, 40 μM imidazole. The His-ZZ-
 30 tagged protein was purified on HisTrap FF (GE healthcare) using standard protocols. The tag was removed by enzymatic cleavage by Tev-protease for 20h at 5°C followed by a second round of purification on HisTrap FT. The flow through, containing untagged rIgdE, was collected. Recombinant IgdE^{ΔC} was purified as described above with higher yields and purity compared to full-length rIgdE.

Qualitative IgG degradation analyses

To monitor IgG cleavage activity in concentrated and fractionated culture supernatants, samples were incubated with 1% porcine plasma in PBS for 16h at 37°C prior to Western blot analyses.

To identify the catalytic type of the IgG protease, the IgG degradation reaction was performed in the presence of 0.1-5 mM AEBSF (Sigma), 0.1-5 mM EDTA, 50-250 µM E-64 (Sigma), 0.1-5 mM Z-LVG-CHN₂ (Bachem), 0.1-5 mM Iodoacetamide (Sigma) or 1/200 to 1/50 dilution of complete protease inhibitor cocktail (Roche).

0.5 mg/ml of porcine IgG (Sigma) in PBS or of other species (human, goat, cow, horse and mouse; all Sigma) were incubated with either crude extracts of induced *E. coli* carrying *S. suis* IgGE constructs mentioned above or 10 nM purified rIgGE for 16 h at 37°C prior subjection to SDS-PAGE under either reducing or non-reducing conditions.

To analyze degradation of endogenous IgG in porcine body fluids following dilutions were used: 1/10 for heart sac fluid, abdominal cavity fluid and joint fluid; 1/50 for serum; undiluted for cerebrospinal fluid. Cerebrospinal and joint fluids were either from piglets with fibrino-suppurative meningitis and synovialitis caused by *S. suis* infection or from piglets with no lesions. Otherwise IgG degradation analyses were conducted as mentioned above with purified rIgGE and analyzed by both SDS-PAGE and western blot analyses. Experiments were repeated at least three times and representative analyses are shown.

Quantitative IgG degradation analyses

For time course analyses and inhibitor profiles the IgG degradation reaction was performed with 0.25 mg/ml porcine IgG and 2 µM purified IgGE^{AC} at 37°C in PBS uninhibited or in the presence of 2.5 µM or 250 µM AEBSF (Sigma), EDTA, E-64 (Sigma), Iodoacetamide (Sigma), or Z-LVG-CHN₂ (Bachem). DMSO (Sigma) at 0.275%, corresponding to the DMSO content in 250 µM Z-LVG-CHN₂, was used as a solvent control. The reactions were continuously sampled and subjected to SDS-PAGE under reducing conditions. Protein bands were detected by staining with Coomassie FluorTM Orange Protein Gel Stain (Invitrogen). The IgG degradation product was densitometrical quantified by imaging with LAS4000 imaging system (Fujifilm) and analyzed with Image Studio Version 3.1 (LI-COR Biosciences) software. Uninhibited over-night cleavage was

set as 100% relative cleavage for the time course experiment. For the inhibitor profile the initial cleavage rate was calculated from the initial increase of cleavage product (0-25% relative cleavage) and the initial cleavage rate of the uninhibited reaction was set as 100% relative activity. Experiments were at least performed in triplicates. Statistical analyses

5 were performed using GraphPad Prism Version 5.0.

SDS-PAGE and Western blot analyses

Samples for SDS-PAGE were prepared with either reducing or non-reducing sample buffer and heated to 95°C for 5 min. 12% SDS-PAGE was either stained with

10 Coomassie blue (Sigma), Coomassie Fluor™ Orange Protein Gel Stain (Invitrogen) or blotted to Hybond-P PVDF membrane (GE Healthcare) for western blot analyses.

Membranes were blocked with 5% dry milk powder in 0.1% PBS-Tween, followed by incubation with horse-radish peroxidase conjugated antibodies. Goat anti-pig IgG-HRP (Thermo Scientific) and goat anti-porcine IgM-HRP (Thermo Scientific) was diluted

15 1:25'000 and goat anti-porcine IgA-HRP (Thermo Scientific) was diluted 1:12'500.

Membranes were thoroughly washed with 0.1% PBS-Tween prior to development with Amersham ECL Select Western blotting detection reagent (GE Healthcare) according to manufactures instruction and pictured by LAS4000 imaging system (Fujifilm).

20 *N-terminal Edman sequencing*

S. suis IgGE processed porcine IgG was separated by SDS-PAGE as previously explained and transferred by semi-dry blotting on Hybond-P PVDF membrane (GE Healthcare) with 50 mM sodium-borate / 20% MeOH as blotting buffer. The membrane was stained with Ponceau S (Sigma) and after drying the ~32 kDa degradation product

25 was tightly cut out. N-terminal Edman sequencing of the degradation product was performed by Proteome Factory (Berlin, Germany) and the sequence W/C PICPACE was obtained. The first position sequence determination was complicated due to a likely contamination yielding a strong tryptophan peak and only a minor peak at the cysteine position. However, in BLAST homology searches the sequence indubitable scored porcine

30 IgG hinge region sequences containing a cysteine residue in the corresponding position. Cysteines were identified by signals of propionamide modified cysteine, nevertheless cysteine is not part of the calibration standard.

ELISA

For detection of IgG titers against *S. suis* IgG E Maxisorb[®] plates (Nunc) were coated with 0.6 µg rIgD protein using carbonate buffer. After coating the plates were washed three times with PBS plus 0.1% Tween 20 (PBST) and blocked with 5% milk powder in PBS for 2 h at 37°C. Every sample and the controls were measured in a duplicate series of four (positive reference serum: six) twofold dilutions in PBST starting with a dilution of 1:100. For the detection of *S. suis* IgD specific IgG antibodies the plates were incubated with goat anti-pig IgG-HRP (1 mg/ml, Bethyl, A100-105P) at a dilution of 1:10000 for 1 h at 37°C. All incubation steps at 37°C were performed on a shaker and after each incubation step the plates were washed with PBST. The plates were developed with 2,2-azino-di-[3-ethylbenzothiazoline sulphonate] (ABTS) and 0.003% H₂O₂ as substrate. Absorbance was measured at 405 nm in a microplate reader (Synergy H1, BioTek Instruments GmbH). Optical densities were converted to antibody concentrations through log linear regression analysis after background subtraction. The ELISA units for each sample were defined as the mean of the calculated units for each of the four dilutions of the two series. ELISA values obtained from a serum sample drawn 20 days after experimental infection with *S. suis* strain 10 was arbitrary set to 100 ELISA units, while ELISA values from serum samples derived from colostrum deprived piglets were used as negative control.

RESULTS

S. suis secretes an IgG-cleaving enzyme

Proteolytic activities of extracellular enzymes of *S. suis* were analyzed by incubating concentrated supernatants of *S. suis* strain 10 and the isogenic IgM protease mutant 10Δ*ide_{Ssuis}* with 2% porcine plasma as putative substrate. After 3 hours of incubation, the reaction was analyzed by reducing SDS-PAGE. Interestingly, the plasma protein band patterns obtained from bacterial culture supernatants of both wild type strain 10 and the isogenic IgM protease mutant 10Δ*ide_{Ssuis}* contained an additional protein band of approximately 32 kDa, which was absent in the porcine plasma control (Fig. 1A). The 32 kDa band was excised, subjected to MALDI-TOF mass spectroscopy and identified as an IgG degradation product showing the presence of an IgG proteolytic activity in *S. suis*. To confirm this finding and to sustain that the proteolytic activity is due to a secreted enzyme of *S. suis*, growth supernatant of bacterial cultures was fractionated by adding increasing amounts of ammonium sulfate (0 to 80% saturation). Fractions were tested for

IgG cleaving activity with porcine plasma and IgG degradation fragments were detected by Western blot using specific polyclonal anti- porcine IgG antibodies. Precipitates of 20 to 60% ammonium sulfate saturation clearly exhibited IgG-cleaving activity, demonstrating that the observed IgG proteolytic activity is distinct from the recently described IgM protease Ide_{Ssuis} (Seele *supra*) and due to a secreted protein in the culture supernatant of *S. suis* (Fig. 1B).

Culture supernatant of 15 different *S. suis* strains, including several strains of the clinical relevant serotype 2; two human isolates of serotype 2, and a set of strains representing additional serotypes (Table 2) were analyzed by Western blot as described above. For all strains an IgG cleavage product of approximately 32 kDa could be detected by Western blot analysis (Fig. 1C), indicating that IgG degradation activity is conserved among different *S. suis* strains.

Purification and sequence characteristics of Igde, a novel protease of S. suis

Prior to a purification trial, protease enriched ammonium sulfate precipitates were tested for IgG cleaving activity in the presence of class specific protease inhibitors to preliminary classify the putative IgG protease. Metallo protease inhibitor EDTA did not affect IgG cleavage, while serine protease inhibitor AEBSF interfered moderately with IgG cleavage. Cysteine protease inhibitors E-64, Z-LVG-CHN₂ and iodoacetamide, however, all appeared to inhibit IgG degrading activity (Fig. 8). Thus, since active site cysteine residues can be inhibited by AEBSF (24, 25), the overall inhibitor profile is most consistent with the assumption that the IgG cleaving protease belongs to the class of cysteine proteases. For purification, strain *S. suis* 10M7, an isogenic mutant of strain 10, was used to avoid masking of low expressed proteins by the in culture supernatants highly abundant muraminidase-released protein (MRP). Bacterial culture supernatant was fractionated by ammonium sulfate precipitation and precipitates were subjected to anion-exchange chromatography and size exclusion chromatography (SEC). Samples showing IgG cleaving activity were separated by reducing SDS-PAGE. Protein bands were identified by MALDI-TOF mass spectrometry and similarity searches against NCBI databases using a pBLAST algorithm. Since the initial characterization of the IgG proteolytic enzyme indicated that the protease is most likely a secreted cysteine protease, sequences of identified proteins were screened for i) the presence of a cysteine residue within the core of the protein and ii) the presence of a secretion signal peptide. Beside Ide_{Ssuis} that previously has been shown to be IgM specific two (out of ten) identified

proteins contained a putative catalytic cysteine residue, but only one contained both a signal peptide sequence predicted by the SignalP algorithm and a core cysteine residue. This protein is annotated as a putative exported protein in the genomic sequences database of *S. suis* serotype 2 strains (www.sanger.ac.uk) and designated SSU_RS08150 in strain P1/7. SSU_RS08150 encodes a 1121 amino acid protein (GenBank accession no WP_012027720) with a putative transglutaminase core sequence motif located in the N-terminal half of the protein within amino acids 188 to 265. The TOPCONS consensus prediction of membrane protein topology identified a putative transmembrane helix in the C-terminal approximately between amino acids 1059-1080. The predicted size of 118kDa (without signal sequence) is somewhat less than the estimated size of the initially purified protein band after SDS-PAGE (data not shown), which is due to the slightly slower migration of proteins with low pI (pI 4.66) on SDS-PAGE. The full-length putative protein sequence was used in similarity searches against NCBI databases using a pBLAST algorithm. This search revealed no similarities of the full-length protein to any known protease of the MEROPS peptidase database (Rawlings *et al.* 2014. *Nucleic Acids Res.* 42, 503-509); no similarities to any eukaryotic protein, but some similarity of the N-terminal half of the protease, containing the transglutaminase core motif, to hypothetical proteins of *Streptococcus porcinus* and *Streptococcus pseudoporcinus* (54% identity in a region of 480 - 492 amino acid residues) and a hypothetical protein of *Streptococcus equi* (up to 40% identity in a region of 264 to 406 amino acid residues). The N-terminal part of the protease shows also some similarity to hypothetical proteins of *Streptococcus agalactiae* and *Streptococcus merionis* (approximately 32% identity). The putative protein is in some databases denoted as ribonuclease or ribonucleases G and E, but due to the absence of an experimentally confirmed function and based on the enzymatic activity against porcine IgG, the protein was denoted IgdE for Immunoglobulin G degrading ezyme of *Streptococcus suis*.

IgdE is a novel cysteine protease

The *S. suis* IgdE sequence reveals the presence of a single cysteine residue in position 302 that, due to the inhibitor profile, is assumed to represent the catalytic site cysteine. *In silico* 3D-modelling (<http://swissmodel.expasy.org>) of *S. suis* IgdE, using known transglutaminase domain structures as template, revealed a putative active site cleft in the N-terminal part of the protein containing a potential catalytic triad consisting of cysteine 302, histidine 333 and aspartic acid 348.

For identification of the putative protease domain and catalytic site residues of *S. suis* IgdE several recombinant IgdE constructs were created (Fig. 2A). All three putative catalytic site residues were replaced by site directed mutagenesis generating mutant proteins, IgdE^{C302S}, IgdE^{H333A} and IgdE^{D348A} respectively. In addition, a construct lacking the C-terminal part of *S. suis* IgdE was created by an XhoI restriction enzyme cut-back. Crude soluble fractions of *E. coli* expressing these constructs were incubated with porcine IgG and analyzed by SDS-PAGE. In the presence of recombinant *S. suis* IgdE, the 32kDa IgG-derived band appeared, demonstrating that the recombinant protein contains IgG proteolytic activity (Fig. 2B, lane 3). The IgG cleaving activity could be assigned to the N-terminal part of the protein, as a recombinant protein lacking amino acids 471 to 1121 is sufficient for IgG cleaving activity (Fig. 2B, lane 7). SDS-PAGE analysis of porcine IgG incubated with the mutant proteins (Fig. 2B, lane 4-6), revealed that neither rIgdE^{C302S} nor rIgdE^{H333A} exhibited IgG cleaving activity, while rIgdE^{D348A} showed somewhat reduced IgG cleaving activity. Altogether, these data strongly indicate that these three residues are part of the catalytic site of *S. suis* IgdE.

The inhibitor screen was repeated with purified recombinant IgdEΔC and class specific protease inhibitors. In contrast to classical transglutaminase enzymes, IgdE is not calcium dependent, as the protease is fully active in the presence of EDTA, while cysteine class specific inhibitors iodoacetamide and Z-LVG-CHN2 efficiently interfered with IgG proteolytic activity as no cleavage product at all could be detected at 125fold molar excess of inhibitor even after 18h incubation (Fig. 3). The serine protease inhibitor AEBSF inhibited the IgdEΔC moderately at 125 fold molar excess. Interestingly, E-64 did not inhibit purified IgdEΔC in this experimental setting, which might be due to a narrow active site similar as described for other streptococcal Ig- proteases, like IdeS and IdeS_{suis}. However, at very high concentrations, E-64 interfered with IgG cleavage activity of *S. suis* culture supernatants (Fig. 8). Z-LVG-CHN2 a cysteine protease specific inhibitor structurally based on the inhibitory reactive site of cystatin C (Green and Shaw. 1981. *J. Biol. Chem.* 256, 1923-1928) completely inhibited IgdEΔC at 125 fold molar excess. The inhibition of enzymatic activity with iodoacetamide and Z-LVG-CHN2 as well as the lack of enzymatic activity of the IgdEC302S and IgdEH333A mutant suggest that IgdE is a novel and so far unique member of the cysteine protease family.

S. suis IgdE cleaves porcine IgG with high specificity in the hinge region

Porcine IgG degradation products were analyzed by reducing and non-reducing SDS-PAGE. The observed band pattern under non-reducing conditions is consistent with a cleavage site located in the hinge region of the heavy chain just N-terminal of the interconnecting disulfide bonds (Fig. 4A). IgG degradation products obtained under reducing conditions (Fig. 4B) were subjected to N-terminal Edman sequencing and the obtained sequence C*PICPACE was found in the hinge region sequences of porcine IgG2ab, IgG4ab, and IgG6ab subtypes. Similar sequences with CPICPGCE as common motif are present in the hinge region of IgG1ab and IgG5ab subtypes, while IgG3 exhibited a CPxxxxC sequence in the hinge region (Table3).

Table 3. N-terminal Edman sequencing of porcine IgG degradation products

Sub-types	Hinge (corresponding to aa 99-121 of IgG1a)
IgG1a	G T K T K P P ↓ <u>C P I C</u> P G C E V A G
IgG1b	G I H Q P Q T ↓ <u>C P I C</u> P G C E V A G
IgG2a	G T K T K P P ↓ <u>C P I C</u> P A C E S P G
IgG2b	G T K T K P P ↓ <u>C P I C</u> P A C E A P G
IgG3	D I E P P T P I ↓ <u>C P E I C</u> S C P A A E V L G A
IgG4a	G T K T K P P ↓ <u>C P I C</u> P A C E G P G
IgG4b	G I H Q P Q T ↓ <u>C P I C</u> P A C E G P A
IgG5a	G R P ↓ <u>C P I C</u> P G C E V A G
IgG5b	G K K T K P R ↓ <u>C P I C</u> P G C E V A G
IgG6a	G R P ↓ <u>C P I C</u> P A C E G P G
IgG6b	G R P ↓ <u>C P I C</u> P A C E G N G

Hinge region sequences of all porcine IgG sub-types. Cysteine residues believed to be involved in S-S covalent bonds (underlined) and the potential cleavage site (↓) are marked in the table.

The observed cleavage patterns on reducing and non-reducing SDS-PAGE; in addition to the identified cleavage site, are consistent with a cleavage reaction in which one IgG heavy chain is hydrolyzed just N-terminal of the homo-dimer disulfide bonds, before a second step hydrolysis cleaves the second heavy chain (Fig. 4C). Interestingly, sequences similar to the IgG hinge region could not be found in the heavy chain sequences of porcine IgA and IgM. Therefore *S. suis* IgdE specificity was further investigated employing specific antibodies against porcine IgG, IgM and IgA. Porcine plasma incubated with

recombinant *S. suis* IgdE was analyzed by Western blot demonstrating that the protease targets IgG in porcine plasma, but does not cleave IgA or IgM (Fig. 5A).

The host specificity of the protease was investigated by incubation of purified *S. suis* IgdE with IgG preparations of humans, goat, bovine, horse and mouse (Fig. 5B).

5 Interestingly, only pig IgG was found to be a substrate for this novel IgG protease. Thus, IgdE shows a pronounced species specificity and targets only porcine IgG.

To further evaluate the specificity of *S. suis* IgdE *ex vivo*, body fluids of healthy or diseased piglets were used. Fluids from heart sac, abdominal cavity, and joint, as well as cerebrospinal fluid and serum were treated with recombinant *S. suis* IgdE and analyzed by SDS-PAGE (Fig. 6A) and by Western blot using porcine IgG specific antibodies (Fig. 6B). No apparent change in the protein band pattern, except for the appearance of the diagnostic IgG cleavage product could be observed in the presence of rIgdE in heart sac, abdominal cavity and serum samples. Also no IgG, i.e. no cleavage products, were present in joint fluid and cerebrospinal fluids of healthy pigs. In contrast, all body fluids obtained from piglets with clinical signs, e.g. suffering from meningitis and synovialitis, contained IgG as consequence of an inflammatory response and again a single diagnostic 32 kDa band could be observed in all samples treated with rIgdE (Fig. 6). The specificity of *S. suis* IgdE is emphasized by the observation that no additional degradation products could be identified on SDS-PAGE following incubation of porcine body fluids with the protease. Thus, although it cannot be excluded that additional substrates exist, these results indicate that *S. suis* IgdE is a highly specific IgG protease, complementing the previously described IgA and IgM degrading proteases of *S. suis* (Seele *supra*; Zhang *et al.* 2010. Vet. Microbiol. 140, 171-175).

25 *IgdE is the sole IgG cleaving enzyme expressed by S. suis*

Strain 10 and strain 10 Δ *ide*_{*Ssuis*}, the latter lacking IgM cleaving activity, were used to generate *S. suis* *igdE* in-frame deletion mutants. IgG and IgM cleaving activities in growth supernatant from wild type strain 10 were determined by Western blot using specific polyclonal anti-porcine IgG or IgM antibodies (Fig. 7). As expected, wild type strain 10 exhibited IgG and IgM cleaving activity (Fig. 7A and B, lane 3), while supernatant of the isogenic mutant strain 10 Δ *igdE* only contained IgM cleaving activity and neither IgG or IgM degradation was detectable in supernatant of double mutant strain 10 Δ *ide*_{*Ssuis*} Δ *igdE* (Fig. 7A and B, lane 5) Thus, it appears that *S. suis* IgdE is required and

sufficient to cleave porcine IgG and that no other IgG cleaving activity is released under these experimental conditions.

In vivo expression of S. suis IgdE

5 Weaning piglets are initially protected from bacterial infection through colostrum derived maternal antibodies. Levels of maternal antibodies decrease over time and active IgG mediated immunity develops at earliest after two weeks. Accordingly, levels of IgG antibodies in serum of colostrum-deprived piglets (SCDP) do not exceed background values in ELISA measurements. An ELISA was conducted with rIgdE as antigen to
10 investigate different serum samples from piglets for the presence of antibodies directed against this IgG protease. A serum sample drawn 20 days after experimental infection with *S. suis* strain 10 contained very high titers against rIgdE (defined as 100 ELISA units, see Material and Methods). In contrast to SCDP that was used as negative control, significant amounts of specific antibodies against *S. suis* IgdE were detectable in seven out of nine 5-6
15 weeks old conventional weaning piglets (ELISA units ranging from 36 and 92). These results demonstrated that *S. suis* IgdE is an immunogenic antigen expressed by *S. suis in vivo*.

Example 2. Identification IgdE from other Streptococcus species

20 The coding sequences of all available Streptococcus genomes were downloaded from NCBI [<ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/> on Aug-21-2015] and from PATRIC [<ftp://ftp.patricbrc.org/patric2/> on Aug-25-2015].

As reference sequence for IgdE we used the RefSeq sequence WP_014636499.1. The N-terminal signal peptide and the C-terminal region only present in sequences from *S.*
25 *suis* were removed, leaving amino acids 38-520, hereafter called "IgdE_domain".

IgdE_domain was used as query sequence in blastp searches (E-value cutoff 1 to keep all possible proteases) against the NCBI sequences as well as the PATRIC sequences. The obtained hits were in turn used as query sequences against the same databases, using the same parameters. From the list of matched sequences we chose those that in the second
30 round had a match that overlap with the region matched in the first round, when IgdE was used as query. Sequences not containing the 'C' in the catalytic site were removed from further consideration, and in case of identical sequences only one copy was kept.

Many of the sequences found are annotated as S-layer proteins or as containing an S-layer homology domain W. These are often present in two or more copies in the same

genome, and have a SxC or GxC motif in the catalytic site instead the AxC motif found in the original Igde sequences. When searching all the sequences against Pfam, these sequences fit well to the Pfam model 'Transglut_core'. In order to remove these sequences, that are not members of the Igde family, all sequences matching to the Transglut_core model with an E-value of at most 1e-6 were removed, as well as sequences lacking the AxC motif. The remaining sequences were cut at both ends to contain only the parts matching the Igde_domain sequence. In cases this resulted in identical "domain" sequences only one copy was kept.

Proteases belonging to the Igde family were identified in *S. porcinus*, *S. agalactiae*, *S. equi*, and *S. pseudoporcinus*. The protease were cloned, expressed and characterized.

Example 3. Properties of Igde isolated from *S. porcinus*

- Recombinant Igde_{porcinus} cleaved purified polyclonal porcine IgG resulting in a 32 kDa degradation product detected by reducing SDS-PAGE.
- No degradation product was observed when recombinant Igde_{porcinus} was incubated with purified polyclonal IgG from cow, horse, human, goat or mouse.
- Recombinant Igde_{porcinus} cleaved IgG in porcine serum, but not IgM or IgA detected by anti-IgG, IgM and IgA Western blot analyses.
- The N-terminal sequence of the 32 kDa degradation product of porcine IgG is CPICPACE as shown by N-terminal Edman sequencing indicating that that this protein has the same cleavage site as Igde from *S. suis* within porcine IgG.
- The recombinant Igde_{porcinus} was easily over-expressed and purified to great purity.
- All tested *S. porcinus* strains showed the same porcine IgG cleaving phenotype in their growth culture supernatants as the recombinant protein.

Example 4. Properties Igde isolated from *S. agalactiae*

- Recombinant Igde_{agalactiae} cleaved purified polyclonal human IgG resulting in a 32 kDa degradation product detected by reducing SDS-PAGE (Fig 10).
- Recombinant Igde_{agalactiae} cleaved purified monoclonal human IgG1 kappa and IgG1 lambda resulting in a 32 kDa degradation product detected by reducing SDS-PAGE (Fig 10D).

- No degradation product was observed when recombinant IgdE_{agalactiae} was incubated with monoclonal human IgG2, IgG3 or IgG4 (Fig 10D).
- No degradation product was observed when recombinant IgdE_{agalactiae} was incubated with purified polyclonal IgG from cow, horse, pig, goat and mouse (Fig10A).
- Recombinant IgdE_{agalactiae} cleaved IgG in human serum, but not IgM or IgA detected by anti-IgG, IgM and IgA Western blot analyses
- Recombinant IgdE_{agalactiae} degrades human serum IgG1 completely to single cleaved IgG1 (Fig 11).
- The N-terminal sequence of the 32 kDa degradation product of human IgG is H(or G or S)TCPPCPAPE as shown by N-terminal Edman sequencing indicating that that this protein has the same cleavage site as papain in IgG1 in the hinge region N-terminal of the cysteine residues believed to be involved in H-H covalent bonds between the heavy chains.
- Recombinant IgdE_{agalactiae} was easily over-expressed and purified.

Example 5. Properties of IgdE isolated from *S. equi*

- Lysate of *E. coli* expressing recombinant IgdE_{equi} cleaved purified polyclonal horse IgG resulting in a 32 kDa degradation product detected by reducing SDS-PAGE.
- No degradation product was observed when recombinant IgdE_{equi} was incubated with purified polyclonal IgG from cow, human, pig, goat, rat, rabbit and mouse.
- Recombinant IgdE_{equi} cleaved IgG in equine serum, but not IgM or IgA detected by anti-IgG, IgM and IgA Western blot analyses
- Recombinant IgdE_{equi} cleaved purified monoclonal recombinant equine IgG7 resulting in a 32 kDa degradation product detected by reducing SDS-PAGE (Fig 12).
- No degradation product was observed when recombinant IgdE_{equi} was incubated with recombinant monoclonal equine IgG1, IgG2, IgG3, IgG4, IgG5 or IgG6.
- The N-terminal sequence of the 32 kDa degradation product of equine IgG as shown by N-terminal Edman sequencing is (G)PTCPECXGV. This sequence is found in the hinge region of equine IgG7.

Example 6. Properties of IgdE isolated from *S. pseudoporcinus*

- *S. pseudoporcinus* culture supernatant possessed porcine IgG cleaving phenotype, but not human IgG cleaving phenotype.
- Recombinant IgdE_{pseudoporcinus} cleaved purified polyclonal human and porcine IgG resulting in a 32 kDa degradation product as detected by reducing SDS-PAGE.
- No degradation product was observed when recombinant IgdE_{pseudoporcinus} was incubated with purified polyclonal IgG from cow, horse, goat, mouse, or rat.
- Recombinant IgdE_{pseudoporcinus} cleaved IgG in human and porcine serum, but not IgM or IgA in porcine serum as detected by anti-IgG, IgM and IgA Western blot analyses.
- The N-terminal sequence of the 32 kDa degradation product of porcine IgG is CPICPACE as shown by N-terminal Edman sequencing indicating that that this protein has the same cleavage site as IgdE within porcine IgG.
- The recombinant IgdE_{pseudoporcinus} was easily over-expressed in *E. coli* BL21 pLysS.

The invention described herein also relates to the following aspects:

1. A polypeptide for use in generating an immune response in a subject comprising:
 - (a) the amino acid sequence of SEQ ID NO: 1;
 - 5 (b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;
 - (c) a fragment of SEQ ID NO: 1, a variant of SEQ ID NO:1, or a fragment of a variant of SEQ ID NO: 1 which is capable of generating an immune response to a streptococcus, preferably a *S. suis* in a subject;
 - 10 (d) the amino acid sequence of SEQ ID NO: 3;
 - (e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;
 - (f) a fragment of SEQ ID NO: 3, a variant of SEQ ID NO:3, or a fragment of a variant of SEQ ID NO: 3 which is capable of generating an immune response to a streptococcus, preferably a *S. agalactiae* in a subject;
 - 15 (g) the amino acid sequence of SEQ ID NO: 5;
 - (h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;
 - (i) a fragment of SEQ ID NO: 5, a variant of SEQ ID NO:5, or a fragment of a variant of SEQ ID NO: 5 which is capable of generating an immune response to a streptococcus, preferably a *S. porcinus* in a subject;
 - 20 (j) the amino acid sequence of SEQ ID NO: 7;
 - (k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity ;
 - 25 (l) a fragment of SEQ ID NO: 7, a variant of SEQ ID NO:7, or a fragment of a variant of SEQ ID NO: 7 which is capable of generating an immune response to a streptococcus, preferably a *S. equi* in a subject;
 - (m) the amino acid sequence of SEQ ID NO: 9;
 - (n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;
 - 30 (o) a fragment of SEQ ID NO: 9, a variant of SEQ ID NO:9, or a fragment of a variant of SEQ ID NO: 9 which is capable of generating an immune response to a streptococcus, preferably a *S. pseudoporcinus* in a subject;

2. A polynucleotide encoding a polypeptide according to aspect 1 for use in generating an immune response in a subject.

5 3. A polynucleotide for use in generating an immune response in a subject comprising:

(a) SEQ ID NO: 2 or a complementary sequence thereto;

(b) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (a);

10 (c) a sequence which hybridises under stringent conditions to the sequence defined in (a) or (b);

(d) a sequence having at least 70% identity to a sequence as defined in (a) or (b);

(e) a fragments of any of the sequences (a), (b), (c) or (d), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of

15 generating an immune response against a streptococcus, preferably a *S. suis* in a subject.

(f) SEQ ID NO: 4 or a complementary sequence thereto;

(g) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (f);

20 (h) a sequence which hybridises under stringent conditions to the sequence defined in (f) or (g);

(i) a sequence having at least 70% identity to a sequence as defined in (f) or (g);

(j) a fragments of any of the sequences (f), (g), (h) or (i), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably a *S. agalactiae* in a
25 subject;

(k) SEQ ID NO: 6 or a complementary sequence thereto;

(l) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (k);

30 (m) a sequence which hybridises under stringent conditions to the sequence defined in (k) or (l);

(n) a sequence having at least 70% identity to a sequence as defined in (k) or (l);

(o) a fragments of any of the sequences (k), (l), (m) or (n), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of

generating an immune response against a streptococcus, preferably a *S. porcinus* in a subject;

(p) SEQ ID NO: 8 or a complementary sequence thereto;

(q) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (a);

(r) a sequence which hybridises under stringent conditions to the sequence defined in (p) or (q);

(s) a sequence having at least 70% identity to a sequence as defined in (p) or (q);

(t) a fragments of any of the sequences (p), (q), (r) or (s), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably a *S. equi* in a subject;

(u) SEQ ID NO: 10 or a complementary sequence thereto;

(v) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (u);

(w) a sequence which hybridises under stringent conditions to the sequence defined in (u) or (v);

(x) a sequence having at least 70% identity to a sequence as defined in (u) or (v); or

(y) a fragments of any of the sequences (u), (v), (w) or (x), and which encodes a polypeptide having IgG degrading cysteine protease activity and/or is capable of generating an immune response against a streptococcus, preferably *S. pseudoporcinus* in a subject.

4. A polypeptide for use in treatment or prevention of a disease or condition mediated by IgG antibodies comprising:

(a) the amino acid sequence of SEQ ID NO: 1;

(b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;

(c) a fragment of SEQ ID NO: 1 or a fragment of a variant of SEQ ID NO: 1 having IgG degrading cysteine protease activity.

(d) the amino acid sequence of SEQ ID NO: 3;

(e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity;

- (g) the amino acid sequence of SEQ ID NO: 5;
- (h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;
- (i) a fragment of SEQ ID NO: 5 or a fragment of a variant of SEQ ID NO: 5 having
5 IgG degrading cysteine protease activity;
- (j) the amino acid sequence of SEQ ID NO: 7;
- (k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity; or
- (l) a fragment of SEQ ID NO: 7 or a fragment of a variant of SEQ ID NO: 7 having
10 IgG degrading cysteine protease activity; or
- (m) the amino acid sequence of SEQ ID NO: 9;
- (n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;
- (o) a fragment of SEQ ID NO: 9 or a fragment of a variant of SEQ ID NO: 9 having
15 IgG degrading cysteine protease activity;
- (p) the amino acid sequence of SEQ ID NO: 11;
- (q) a variant of SEQ ID NO: 11 having at least 70% identity to the amino acid sequence of SEQ ID NO: 11 and having IgG degrading cysteine protease activity; or
- (r) a fragment of SEQ ID NO: 11 or a fragment of a variant of SEQ ID NO: 11
20 having IgG degrading cysteine protease activity.

5. A polynucleotide encoding a polypeptide according to aspect 4 for use in treatment or prevention of a disease or condition mediated by IgG antibodies.

- 25 6. An *in vitro* method for the cleavage of IgG, comprising contacting IgG with a polypeptide comprising:
 - (a) the amino acid sequence of SEQ ID NO: 1;
 - (b) a variant of SEQ ID NO: 1 having at least 70% identity to the amino acid sequence of SEQ ID NO: 1 and having IgG degrading cysteine protease activity;
 - 30 (c) a fragment of SEQ ID NO: 1 or a fragment of a variant of SEQ ID NO: 1 having IgG degrading cysteine protease activity.
 - (d) the amino acid sequence of SEQ ID NO: 3;
 - (e) a variant of SEQ ID NO: 3 having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG degrading cysteine protease activity;

(f) a fragment of SEQ ID NO: 3 or a fragment of a variant of SEQ ID NO: 3 having IgG degrading cysteine protease activity;

(g) the amino acid sequence of SEQ ID NO: 5;

(h) a variant of SEQ ID NO: 5 having at least 70% identity to the amino acid sequence of SEQ ID NO: 5 and having IgG degrading cysteine protease activity;

(i) a fragment of SEQ ID NO: 5 or a fragment of a variant of SEQ ID NO: 5 having IgG degrading cysteine protease activity;

(j) the amino acid sequence of SEQ ID NO: 7;

(k) a variant of SEQ ID NO: 7 having at least 70% identity to the amino acid sequence of SEQ ID NO: 7 and having IgG degrading cysteine protease activity;

(l) a fragment of SEQ ID NO: 7 or a fragment of a variant of SEQ ID NO: 7 having IgG degrading cysteine protease activity;

(m) the amino acid sequence of SEQ ID NO: 9;

(n) a variant of SEQ ID NO: 9 having at least 70% identity to the amino acid sequence of SEQ ID NO: 9 and having IgG degrading cysteine protease activity;

(o) a fragment of SEQ ID NO: 9 or a fragment of a variant of SEQ ID NO: 9 having IgG degrading cysteine protease activity;

(p) the amino acid sequence of SEQ ID NO: 11;

(q) a variant of SEQ ID NO: 11 having at least 70% identity to the amino acid sequence of SEQ ID NO: 11 and having IgG degrading cysteine protease activity; or

(r) a fragment of SEQ ID NO: 11 or a fragment of a variant of SEQ ID NO: 11 having IgG degrading cysteine protease activity.

7. A method for identifying a substance that activates or inhibits the IgG cysteine activity of an IgGE polypeptide comprising the steps;

a) contacting the IgGE polypeptide and IgG with a candidate substance under conditions permitting IgG cysteine activity in the absence of the substance,

b) determining the amount of IgG digested in the presence of the candidate substance compared to in the absence of said substance,

c) based on the results obtain in b) determining whether the substance activates or inhibits the IgG cysteine activity of the IgGE polypeptide.

CLAIMS

- 5 1. An *in vitro* method for the cleavage of human IgG, comprising contacting human IgG with a polypeptide comprising:
- (a) the amino acid sequence of SEQ ID NO: 3;
- (b) a variant thereof having at least 70% identity to the amino acid sequence of SEQ ID NO: 3 and having IgG cysteine protease activity; or
- 10 (c) a fragment thereof of either (a) or (b), having IgG cysteine protease activity.
2. A method according to claim 1, wherein the amino acid sequence is SEQ ID NO: 3.
- 15 3. A method according to claim 1 or 2, wherein the method comprises incubating the polypeptide with a sample containing human IgG under conditions which permit specific cysteine protease activity to occur.
4. A method according to claim 2, wherein the human IgG is human IgG1.
- 20 5. A method according to any one of the preceding claims, which further comprises identification and/or isolation of the cleavage products.
6. A method according to claim 5, wherein said identification and/or isolation
- 25 comprises analysis by gel electrophoresis or mass spectrometry.
7. A method according to any one of the preceding claims, wherein the method is used to generate Fc and Fab fragments.
- 30 8. A method according to any one of claims 1 to 7, wherein the method is used to detect human IgG.
9. A method according to claim 8, comprising:
- (i) contacting a sample with a polypeptide as defined in claim 1
- 35 under conditions that permit the IgG specific cysteine protease activity of the polypeptide; and

(ii) monitoring for the presence of IgG specific cleavage fragments;
wherein the presence of the specific cleavage fragments is indicative of human IgG in the sample.