In one aspect, the present invention provides 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: 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 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 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.

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.

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.

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;

(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

(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;

(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);

(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);

(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 *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);

(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 *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 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);

(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..

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.

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

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

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. 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 treatment or prevention of a disease or condition mediated by IgG antibodies comprising:

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

(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;

(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.

Preferably the IgdE polypeptide can be an $IgdE_{agalac}$ 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; 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 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;

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.

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.

(A) Concentrated (20x) culture supernatants of *S. suis* strains 10 and 10Δide_{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 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 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.

(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 μM porcine IgG

CLAIMS

1. 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: 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.

2. A method according to claim 1, wherein the amino acid sequence is SEQ ID NO: 3.

3. A method according to claim 1 or 2, wherein the method comprises incubating the polypeptide with a sample containing IgG under conditions which permit specific cysteine protease activity to occur.

4. A method according to claim 2, wherein the IgG is human IgG1.

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 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.

8. A method according to any one of claims 1 to 7, wherein the method is used to detect IgG.

9. A method according to claim 8, comprising:

(i) contacting a sample with a polypeptide as defined in claim 1 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.

5