molecule than the second chain (see schematic representation in Figure 18), particularly when the IgG is an IgG2 isotype. A polypeptide of the invention may be more effective at cleaving IgG1 than IgG2. The polypeptide of the invention is typically less immunogenic than IdeS and may preferably be no more immunogenic than IdeZ or IdeS/Z.

Unless otherwise stated, all references to numbering of amino acid positions in the polypeptides disclosed herein is based on the numbering of the corresponding positions in SEQ ID NO: 3, starting from the N terminus. Thus, since SEQ ID NOs: 4 and 5 lack the N terminal methionine and 33 amino acid signal sequence of SEQ ID NO: 3, the aspartic acid (D) residue at the N terminus of SEQ ID NOs: 4 and 5 is referred to as position 35 as this the corresponding position in SEQ ID NO: 3. Applying this numbering scheme, the most critical residue for IgG cysteine protease activity of IdeS is the cysteine (C) at position 102 (68^{th} residue from the N terminus of SEQ ID NOs: 4 and 5). Other residues likely to be important for IgG cysteine protease activity are the lysine (K) at position 92, the histidine (H) at position 272, and the aspartic acid (D) at each of positions 294 and 296 of SEQ ID NO: 3. These are the 58^{th}, 238^{th}, 260^{th} and 262^{nd} residues from the N terminus of SEQ ID NO: 4 and the 58^{th}, 236^{th}, 258^{th} and 260^{th} from the N terminus of SEQ ID NO: 5, respectively.

In accordance with the present invention, there is thus provided a polypeptide having IgG cysteine protease activity and comprising a variant of the sequence of SEQ ID NO:4 or 5, which variant:

(a) is at least 80% identical to SEQ ID NO: 4 or 5;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and optionally

(c) has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively;

wherein said polypeptide is more effective at cleaving human IgG than IdeZ and/or is at least as effective at cleaving human IgG as IdeS.

Preferably, said variant of SEQ ID NO: 4 or 5:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(2) has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(3) does not include the contiguous sequence DDYQRNATEA YAKEVPHQIT; and/or

(4) has at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3;

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

The at least one modification of (4) is typically selected from options i. to vii. above. A polypeptide of the invention may comprise a variant of the amino acid sequence of SEQ ID NO: 4 or 5, which variant has at least two, three, four, five, six or all seven of the modifications of options i. to vii.

The invention also provides a polynucleotide, an expression vector or a host cell encoding or expressing a polypeptide of the invention.

Also described herein is a method of treating or preventing a disease or condition mediated by IgG antibodies in a subject, the method comprising administering to the subject a therapeutically or prophylactically effective amount of a polypeptide as described herein. The method may typically comprise multiple administrations of said polypeptide to the subject. In another aspect, the present invention refers to a polypeptide according to the present invention for use in the prevention or treatment of a disease or condition in a subject.

The invention also provides a method of treating, *ex vivo*, blood taken from a patient, typically a patient suffering from a disease or condition mediated by IgG antibodies, which method comprises contacting the blood with a polypeptide of the disclosure.

Also described herein is a method for improving the benefit to a subject of a therapy or therapeutic agent, the method comprising (a) administering to the subject a polypeptide of the disclosure; and (b) subsequently administering said therapy or said therapeutic agent to the subject; wherein:

- said therapy is an organ transplant or said therapeutic agent is an antibody, a gene therapy such as a viral vector, a replacement for a defective endogenous factor such as an enzyme, a growth or a clotting factor, or a cell therapy;
- the amount of said polypeptide administered is sufficient to cleave substantially all IgG molecules present in the plasma of the subject; and
- steps (a) and (b) are separated by a time interval which is sufficient to cleave substantially all IgG molecules present in the plasma of the subject.

The disclosure also provides a method of generating Fc, Fab or $F(ab')_2$ fragments of IgG comprising contacting IgG with a polypeptide of the disclosure, preferably ex vivo.

Also provided are kits for carrying out the methods according to the disclosure.

Brief Description of the Figures

Figures 1 and 2 show the results of a representative assay to determine the potency (efficacy at cleavage of IgG) of polypeptides of the invention as compared to controls.

Figure 3 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG1 with polypeptides of the invention or controls.

Figure 4 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figure 5 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG1 with further polypeptides of the invention or controls.

Figure 6 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IgG2 with polypeptides of the invention or controls.

Figure 7 shows the results of a representative SDS-PAGE gel used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figures 8 and 9 show the results of representative competition assays to determine the level of recognition of polypeptides of the invention by IdeS-specific antibodies, as compared to controls.

Figures 10 and 11 show the results of representative titration assays to determine the level of recognition of polypeptides of the invention by IdeS-specific antibodies, as compared to controls.

Figure 12 shows representative titration curves for cleavage of IgG1 by different IgG cysteine protease polypeptides.

CLAIMS

1. A polypeptide having IgG cysteine protease activity and comprising a variant of the sequence of SEQ ID NO:4 or 5, which variant:

(a) is at least 80% identical to SEQ ID NO: 4 or 5;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 102 of SEQ ID NO: 3; and optionally

(c) has, at the positions in said variant sequence which correspond to positions 92, 272, 294 and 296 of SEQ ID NO: 3, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively;

wherein said polypeptide is more effective at cleaving human IgG than IdeZ and/or is at least as effective at cleaving human IgG as IdeS.

2. A polypeptide according to claim 1, wherein said variant of the sequence of SEQ ID NO: 4 or 5:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 138 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(2) has a positively charged amino acid at the position in said variant which corresponds to position 139 of SEQ ID NO: 3, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

(3) does not include the contiguous sequence DDYQRNATEA YAKEVPHQIT; and/or

(4) has at least one of the following modifications:

i. a deletion of the leucine (L) and threonine (T) residues at the positions in said variant which correspond to positions 64 and 65 of SEQ ID NO: 3;

ii. a threonine (T) in place of the arginine (R) at the position in said variant which corresponds to position 70 of SEQ ID NO: 3;

iii. a deletion of the tyrosine (Y) at the position in said variant which corresponds to position 71 of SEQ ID NO: 3;

iv. a glutamine (Q) in place of the asparagine (N) at the position in said variant which corresponds to position 72 of SEQ ID NO: 3;

v. a glycine (G) in place of the asparagine (N) at the position in said variant which corresponds to position 73 of SEQ ID NO: 3;

vi. a alanine (A) in place of the glutamic acid (E) at the position in said variant which corresponds to position 67 of SEQ ID NO: 3;

vii. a asparagine (N) in place of the glutamine (Q) at the position in said variant which corresponds to position 68 of SEQ ID NO: 3.

3. A polypeptide according to claim 1 or 2, wherein said variant of the sequence of SEQ ID NO: 4 or 5 is at least 90%, 95% or 99% identical to SEQ ID NO: 4 or 5, respectively, and/or wherein said polypeptide is less immunogenic than IdeS and is preferably no more immunogenic than IdeZ or IdeS/Z, when measured in the same assay.

4. A polypeptide according to any one of the preceding claims, which comprises or consists of the sequence of any one of SEQ ID NOs: 6 to 25, optionally wherein said sequence includes an additional methionine at the N terminus and/or a histidine tag at the C terminus.

5. A polypeptide according to any one of the preceding claims, wherein said polypeptide is at least 2.0 fold more effective than IdeZ at cleaving human IgG, when measured in the same assay.

6. A polypeptide according to any one of the preceding claims which is less immunogenic than IdeS, wherein preferably the immunogenicity of said polypeptide is no more than 85% of the immunogenicity of IdeS when measured in the same assay.

7. A polynucleotide or expression vector which comprises a nucleic acid sequence encoding a polypeptide of any one of the preceding claims.

8. A host cell comprising the polynucleotide or expression vector of claim 7, which is preferably bacterial cell, most preferable a cell of *E. coli.*

9. A composition comprising a polypeptide according to any one of claims 1 to 6 and at least one pharmaceutically acceptable carrier or diluent.

10. A polypeptide according to any one of claims 1 to 6 for use in the treatment of the human or animal body.