IdeZ is an IgG cysteine protease produced by *Streptococcus equi ssp. zooepidemicus*, a bacterium predominantly found in horses. IdeZ has approximately 66% identity to IdeS. Since *Streptococcus equi ssp. zooepidemicus* is not a human pathogen, IdeZ was considered to be an alternative to IdeS-based therapies because humans may have fewer or no antibodies (anti-drug antibodies, ADA) against IdeZ. However, IdeZ has a level of IgG cysteine protease activity against human IgG which is considerably lower than that of IdeS, in particular when cleaving IgG2.

Thus, there remains a need for cysteine proteases derived from IdeZ having high activity (preferably higher than the wild type IdeZ and even more preferably higher than IdeS) against human IgG. In particular, there remains a need for cysteine proteases derived from IdeZ having high activity (preferably higher than the wild type IdeZ and even more preferably higher than IdeS) against human IgG1 and IgG2.

Summary

The full sequence of IdeS is publically available as NCBI Reference Sequence no. WP_010922160.1 and is provided herein as SEQ ID NO: 6. This sequence includes an N terminal methionine followed by a 28 amino acid secretion signal sequence. The N terminal methionine and the signal sequence (a total of 29 amino acids at the N terminus) are typically removed to form the mature IdeS protein, the sequence of which is publically available as Genbank accession no. ADF13949.1 and is provided herein as SEQ ID NO: 4.

The full sequence of IdeZ is publically available as NCBI Reference Sequence no WP_014622780.1 and is provided herein as SEQ ID NO: 5. This sequence includes an N terminal methionine followed by a 33 amino acid secretion signal sequence. The N terminal methionine and the signal sequence (a total of 34 amino acids at the N terminus) are typically removed to form the mature IdeZ protein, the sequence of which is provided herein as SEQ ID NO: 3.

The present inventors have been able to identify specific positions within the sequence of IdeZ which, when modified as described herein, lead to novel polypeptides which have increased IgG cysteine protease activity against human IgG relative to IdeZ. The IgG cysteine protease activity against human IgG (e.g. at cleaving human IgG) of a polypeptide of the disclosure is preferably at least as high as the IgG cysteine protease activity against human IgG of IdeS. A polypeptide of the disclosure may be more effective at cleaving human IgG than the IgG cysteine protease of IdeS, particularly when the IgG is an IgG1 or IgG2 isotype. A polypeptide of the disclosure may be more effective at cleaving

human IgG than the IgG cysteine protease IdeS, particularly when measured with the cleavage of the second chain of IgG1. A polypeptide of the disclosure may be more effective at cleaving IgG1 than IgG2. The polypeptide of the disclosure is typically less immunogenic than IdeS and may preferably be no more immunogenic than IdeZ.

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: 5, starting from the N terminus. Thus, since SEQ ID NO: 1 lacks the N terminal methionine and 33 amino acid signal sequence of SEQ ID NO: 5, the aspartic acid (D) residue at the N terminus of SEQ ID NO: 1 is referred to as position 35 as this is the corresponding position in SEQ ID NO: 5. Applying this numbering scheme, the most critical residue for IgG cysteine protease activity of IdeS is the cysteine (C) at position 102 corresponding to SEQ ID NO: 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 two aspartic acids (D) at positions 294 and 296 corresponding to SEQ ID NO: 5. It has also been found that deleting the first twenty residues at the N terminus of SEQ ID NO: 1 may enhance the potency of a polypeptide which incorporates this change and/or may reduce immunogenicity without adversely affecting potency. The first twenty residues at the N terminus of SEQ ID NO: 1 consists of the contiguous sequence DDYQRNATEAYAKEVPHQIT. Thus, a polypeptide according to the present disclosure may comprise the amino acid sequence of SEQ ID NO: 2 (which does not include the contiguous sequence DDYQRNATEAYAKEVPHQIT). The first twenty residues of SEQ ID NO: 1 correspond to positions 35-54 of SEQ ID NO: 5. This particular modification may be identified herein by the term "D35_T54del". Thus, since SEQ ID NO: 2 lacks the N terminal methionine, the 33 amino acid signal sequence of SEQ ID NO: 5, and further has a deletion of the sequence DDYQRNATEAYAKEVPHQIT corresponding to positions 35-54 of SEQ ID NO: 5, the serine (S) residue at the N terminus of SEQ ID NO: 2 is referred to as position 55 as this is the corresponding position in SEQ ID NO: 5

Thus, in one aspect, the present disclosure provides a polypeptide having IgG cysteine protease activity and comprising or consisting of an amino acid sequence which is:

(i) SEQ ID NO: 1; or

(ii) SEQ ID NO: 2; or

(iii) a variant of SEQ ID NO: 1 or SEQ ID NO: 2, which has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modification(s) relative to SEQ ID NO: 1 or SEQ ID NO: 2 respectively, provided that the sequence retains: (a) an asparagine (N) at the position which

corresponds to position 95 of SEQ ID NO: 5, (b) an aspartic acid (D) at the position which corresponds to position 99 of SEQ ID NO: 5 and (c) an asparagine (N) at the position which corresponds to position 226 of SEQ ID NO: 5, and provided that the polypeptide is at least as effective at cleaving human IgG as the polypeptide consisting of the amino acid sequence of SEQ ID NOs: 1 or 2 respectively, when measured in the same assay.

In another aspect, the present disclosure provides a polynucleotide encoding a polypeptide as described herein. In yet another aspect, the present disclosure provides an expression vector comprising the polynucleotide as described herein. In yet another aspect, the present disclosure provides a host cell comprising the polynucleotide as described herein or expression vector as described herein, which is preferably a bacterial cell, most preferably a cell of *E. coli.*. In yet another aspect, the present disclosure provides a composition comprising a polypeptide as described herein and at least one pharmaceutically acceptable carrier or diluent. The disclosure also provides a polynucleotide, an expression vector or a host cell encoding or expressing a polypeptide of the disclosure.

In a further aspect, the present disclosure provides a polypeptide as described herein for use in therapy. In a further aspect, the present disclosure provides a use of a polypeptide as described herein or the composition as described herein in the manufacture of a medicament for the prevention or treatment of a disease or condition in a subject, wherein said disease or condition is a disease or condition mediated in whole or in part by pathogenic IgG antibodies, preferably wherein said disease or condition is listed in Table D. The disclosure also provides 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 of the disclosure. The method may typically comprise multiple administrations of said polypeptide to the subject.

In a further aspect, the present disclosure provides an *ex vivo* method for the cleavage of IgG, the method comprising contacting a sample containing IgG with a polypeptide as described herein under conditions which permit IgG cysteine protease activity to occur. The disclosure 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.

The disclosure also provides 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

Figure 1 shows SDS-PAGE analysis of the expression and purification of pCART239 (SEQ ID NO: 1 with N-terminal Met and C-terminal His tag). (A) Overexpression of pCART239 – lanes 1 and 2 show lysates obtained from cells harvested 1 hour after induction with IPTG; lane 3 shows the pooled lysates. (B) Purification of pCART239 – lane 1 shows the flow

CLAIMS

1. A polypeptide having IgG cysteine protease activity and comprising or consisting of an amino acid sequence which is:

(i) SEQ ID NO: 1; or

(ii) SEQ ID NO: 2; or

(iii) a variant of SEQ ID NO: 1 or SEQ ID NO: 2, which has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modification(s) relative to SEQ ID NO: 1 or SEQ ID NO: 2 respectively, provided that the sequence retains: (a) an asparagine (N) at the position which corresponds to position 95 of SEQ ID NO: 5, (b) an aspartic acid (D) at the position which corresponds to position 99 of SEQ ID NO: 5 and (c) an asparagine (N) at the position which corresponds to position 226 of SEQ ID NO: 5, and provided that the polypeptide is at least as effective at cleaving human IgG as the polypeptide consisting of the amino acid sequence of SEQ ID NOs: 1 or 2 respectively, when measured in the same assay.

2. The polypeptide according to claim 1, wherein at least one of the modifications in (iii) does not result in the same amino acid as is present in the corresponding position in the polypeptide sequence of SEQ ID NO: 3, preferably wherein all of the modifications in (iii) do not result in the same amino acid as is present in the corresponding position in the polypeptide sequence of SEQ ID NO: 3.

3. A polypeptide according to claim 1 or 2, wherein the polypeptide further comprises an additional methionine at the N terminus and/or an additional histidine tag at the C terminus.

4. A polypeptide according to any one of the preceding claims, wherein the polypeptide is more effective at cleaving human IgG than an IdeZ polypeptide and/or is at least as effective at cleaving human IgG as an IdeS polypeptide, when measured in the same assay.

5. A polypeptide according to any one of the preceding claims, wherein the polypeptide is more effective at cleaving human IgG than an IdeS polypeptide when measured in the same assay, optionally wherein efficacy is measured *in vitro* in a blood or serum sample taken from a human subject.

6. A polypeptide according to claim 5, wherein the polypeptide is at least 1.2 fold, preferably 1.3 fold, most preferably 1.4 fold more effective at cleaving human IgG than an IdeS polypeptide when measured in the same assay.

7. A polypeptide according to any one of the preceding claims, wherein said polypeptide is less immunogenic than an IdeS polypeptide and/or is not more immunogenic than an IdeZ polypeptide, when measured in the same assay.

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

9. A polynucleotide encoding a polypeptide according to any one of the preceding claims.

10. An expression vector comprising the polynucleotide according to claim 9.

11. A host cell comprising the polynucleotide according to claim 9 or expression vector according to claim 10, which is preferably a bacterial cell, most preferably a cell of *E. coli*.

12. A composition comprising a polypeptide according to any one of claims 1 to 8 and at least one pharmaceutically acceptable carrier or diluent.

13. A polypeptide according to any one of claims 1 to 8 for use in therapy.

14. Use of a polypeptide according to any one of claims 1 to 8 or the composition of claim 12 in the manufacture of a medicament for the prevention or treatment of a disease or condition in a subject, wherein said disease or condition is a disease or condition mediated in whole or in part by pathogenic IgG antibodies, preferably wherein said disease or condition is listed in Table D.

15. An *ex vivo* method for the cleavage of IgG, the method comprising contacting a sample containing IgG with a polypeptide according to any one of claims 1 to 8 under conditions which permit IgG cysteine protease activity to occur.

16. A method according to claim 15 which is conducted to generate Fc, Fab and/or $F(ab')_2$ fragments.

17. A method according to claim 15 or 16, wherein the sample is a blood sample taken from a subject suffering from a disease or condition mediated in whole or in part by pathogenic IgG antibodies, preferably wherein said disease or condition is listed in Table D.