The present inventors have adopted an entirely different approach. The inventors have identified specific positions within the sequence of IdeS which, when modified as described herein, lead to novel polypeptides for which the problems associated with immunogenicity are reduced as compared to IdeS. Some modifications may increase the efficacy at cleaving IgG of the polypeptide of the disclosure relative to IdeS, thereby indirectly reducing immunogenicity by permitting the use of a lower dose or concentration to achieve the same effect. Alternatively, or in addition, other modifications may directly reduce immunogenicity by reducing the ability of IdeS-specific antibodies to recognise the polypeptide of the disclosure relative to IdeS.

The full sequence of IdeS is publically available as NCBI Reference Sequence no WP_010922160.1 and is provided herein as SEQ ID NO: 1. 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: 2.

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: 1, starting from the N terminus. Thus, since SEQ ID NO: 2 lacks the N terminal methionine and 28 amino acid signal sequence of SEQ ID NO: 1, the aspartic acid (D) residue at the N terminus of SEQ ID NO: 2 is referred to as position 30 as this the corresponding position in SEQ ID NO: 1. Applying this numbering scheme, the most critical residue for IgG cysteine protease activity of IdeS is the cysteine (C) at position 94 (65th residue from the N terminus of SEQ ID NO: 2). Other residues likely to be important for IgG cysteine protease activity are the lysine (K) at position 84, the histidine (H) at position 262, and the aspartic acid (D) at each of positions 284 and 286. These are the 55th, 233rd, 255th and 257th residues from the N terminus of SEQ ID NO: 2, respectively.

In accordance with the present invention, in one aspect, there is thus provided a polypeptide having IgG cysteine protease activity and comprising a variant of the sequence of SEQ ID NO:2, which variant:

(a) is at least 50% identical to SEQ ID NO: 2;

(b) has a cysteine (C) at the position in said variant sequence which corresponds to position 94 of SEQ ID NO: 1; and optionally

(c) has, at the positions in said variant sequence which correspond to positions 84, 262, 284 and 286 of SEQ ID NO: 1, a lysine (K), a histidine (H), an aspartic acid (D) and an aspartic acid (D), respectively;

wherein said polypeptide is more effective at cleaving IgG than IdeS and/or is less immunogenic than IdeS. The polypeptide of the disclosure may be more effective at cleaving IgG1 than IgG2.

Preferably said variant of SEQ ID NO: 2:

(1) has a positively charged amino acid at the position in said variant which corresponds to position 130 of SEQ ID NO: 1, 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 131 of SEQ ID NO: 1, optionally wherein said positively charged amino acid is arginine (R) or lysine (K); and/or

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

(4) does not include the contiguous sequence DSFSANQEIR YSEVTPYHVT.

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

In another aspect, the present invention provides a polynucleotide or expression vector which comprises a nucleic acid sequence encoding a polypeptide as disclosed herein.

In yet another aspect, the present invention provides a host cell comprising the polynucleotide or expression vector as disclosed herein, which is preferably bacterial cell, most preferable a cell of *E. coli*.

In yet another aspect, the present invention provides a composition comprising a polypeptide as disclosed herein and at least one pharmaceutically acceptable carrier or diluent.

In yet another aspect, the present invention provides a polypeptide as disclosed herein for use in the treatment of the human or animal body.

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.

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.

In yet another aspect, the present invention provides a polypeptide as disclosed herein for use in the prevention or treatment of a disease or condition in a subject, wherein the polypeptide is to be administered to the subject in a prophylactically or therapeutically effective amount.

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.

In yet another aspect, the present invention provides a method for the cleavage of IgG, the method comprising contacting a sample containing IgG with a polypeptide as disclosed herein under conditions which permit IgG cysteine protease activity to occur.

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

Brief Description of the Figures

Figure 1 shows 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 2 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 3 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 4 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.

Figures 5 and 6 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.

Figure 7 shows representative titration curves for cleavage of IgG1 by different IgG cysteine protease polypeptides.

Figure 8 shows representative titration curves for cleavage of IgG2 by different IgG cysteine protease polypeptides.

Figure 9 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IgG with polypeptides of the invention or controls.

Figure 10 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IgG with polypeptides of the invention or controls.

Figure 11 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.

Figure 12 shows the results of a representative SDS-PAGE used to visualize the cleavage products produced by incubation of IVIg with polypeptides of the invention or controls.
Figure 13 Schematic representation of the cleavage of immunoglobulins by polypeptides of the invention.

5. A polypeptide according to any one of the preceding claims, wherein said polypeptide is at least 1.5 fold, 2.0 fold, 2.5 fold, 3.0 fold, 4.0 fold, 4.5 fold, 5.0 fold, 6.0 fold, 7.0 fold or 7.5 fold greater more effective than IdeS at cleaving 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.

11. A polypeptide according to any one of claims 1 to 6 for use in the prevention or treatment of a disease or condition in a subject, wherein the polypeptide is to be administered to the subject in a prophylactically or therapeutically effective amount.

12. A polypeptide according to claim 11, 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.

13. A 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 6 under conditions which permit IgG cysteine protease activity to occur.