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ANTIKÖRPERVARIANTEN

VARIANTES D'ANTICORPS

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(56) References cited:
WO-A1-2017/158092 WO-A1-2017/158426

- **R. DENG ET AL: "Pharmacokinetics of Humanized Monoclonal Anti-Tumor Necrosis Factor- Antibody and Its Neonatal Fc Receptor Variants in Mice and Cynomolgus Monkeys", DRUG METABOLISM AND DISPOSITION, vol. 38, no. 4, 1 April 2010 (2010-04-01), pages 600 - 605, XP055019071, ISSN: 0090-9556, DOI: 10.1124/dmd.109.031310**
- **YEUNG YIK ANDY ET AL: "Engineering Human IgG1 Affinity to Human Neonatal Fc Receptor: Impact of Affinity Improvement on Pharmacokinetics in Primates", THE JOURNAL OF IMMUNOLOGY, THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 182, no. 12, 1 June 2009 (2009-06-01), pages 7663 - 7671, XP002566420, ISSN: 0022-1767, DOI: 10.4049/JIMMUNOL.0804182**
- **TIMOTHY T. KUO ET AL: "Neonatal Fc receptor and IgG-based therapeutics", MABS, 1 September 2011 (2011-09-01), pages 422 - 430, XP055079703, DOI: 10.4161/mabs.3.5.16983**
- **T. SUZUKI ET AL: "Importance of Neonatal FcR in Regulating the Serum Half-Life of Therapeutic Proteins Containing the Fc Domain of Human IgG1: A Comparative Study of the Affinity of Monoclonal Antibodies and Fc-Fusion Proteins to Human Neonatal FcR", THE JOURNAL OF IMMUNOLOGY, vol. 184, no. 4, 15 February 2010 (2010-02-15), pages 1968 - 1976, XP055064717, ISSN: 0022-1767, DOI: 10.4049/jimmunol.0903296**
- **YASMINA NOUBIA ABDICHE ET AL: "The neonatal Fc receptor (FcRn) binds independently to both sites of the IgG homodimer with identical affinity", MABS, vol. 7, no. 2, 4 March 2015 (2015-03-04), US, pages 331 - 343, XP055331493, ISSN: 1942-0862, DOI: 10.1080/19420862.2015.1008353**

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- **WARD E SALLY ET AL: "Targeting FcRn for the modulation of antibody dynamics", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 67, no. 2, 9 March 2015 (2015-03-09), pages 131 - 141, XP029246896, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2015.02.007**
- **"Therapeutic Fc-Fusion Proteins", 19 February 2014, WILEY-VCH VERLAG GMBH & CO. KGAA, Weinheim, Germany, ISBN: 978-3-52-733317-2, article ARVIND RAJPAL ET AL: "Introduction: Antibody Structure and Function", pages: 1 - 44, XP055198075, DOI: 10.1002/9783527675272.ch01**

Remarks:

•The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

•The file contains technical information submitted after the application was filed and not included in this specification

Description

FIELD OF THE INVENTION

[0001] The present invention relates to modified antibodies having altered effector functions and/or altered pharmacokinetic properties. The antibodies are useful in the therapeutic treatment of various disorders, in particular of inflammatory conditions.

BACKGROUND

[0002] Monoclonal antibodies have gained increasing importance as therapeutic reagents in clinical medicine over the last 20 years. For many years, efforts to improve antibodies concentrated on reducing their potential immunogenicity, leading to humanized or even fully human antibodies. Another approach aims to optimize antibodies by improving their effector functions. While direct effects are mediated by the variable antigen binding region of the antibody, indirect effects are mediated by the constant Fc region. Efforts to improve effector functions mainly concentrate on modulating the Fc region. In addition, improving the serum half-life of therapeutic antibodies is desirable, which may reduce the amount of required antibodies, and may increase their convenience for patients by prolonging treatment intervals.

[0003] For therapeutic applications, immunoglobulin G (IgG) has been the preferred class of choice for several reasons; IgGs are easy to purify, are relatively stable on storage, can be administered intravenously, have extended biological half-life in vivo and are able to engage a range of biological effector functions such as activation of complement dependent cytotoxicity (CDC) and recruitment of effector cells through various Fc-receptor interactions (antibody-dependent cellular cytotoxicity; ADCC). Of the five immunoglobulin classes, IgG exhibits the longest biological half-life due to its unique interaction with the IgG recycling receptor, the neonatal Fc receptor (FcRn). One of the known functions of the receptor is to rescue IgG from catalytic degradation. A solved FcRn-Fc cocrystal structure has shown that the interaction with Fc occurs in the IgG hinge-C_H2-C_H3 region. This interaction occurs in a strictly pH-dependent manner at acidic pH of 6.0-6.5 in the endosomes. Bound IgG molecules are recycled back to the cell surface where they are released at physiological pH of 7.4 into the circulation, whereas noncomplexed IgG molecules are destined for lysosomal degradation. This recycling is the mechanism for the extended half-life of IgG; modulation of the FcRn-IgG interaction will therefore allow specific control of the serum half-lives of gamma immunoglobulins and Fc-fusion proteins.

[0004] Depending on the application it may be desirable to increase or reduce the serum residence time of IgG. For therapeutic application a longer half-life is desirable as smaller doses and fewer injections will be required. Several approaches to increase the half-life have been investigated including the use of polyethylene glycol (PEG), generation of albumin- or Fc-fusion proteins and strengthening the FcRn-IgG interaction. PEGylated pharmaceuticals have been in the clinic since 1990 and PEGylation is an established technology for extension of drug residence in the blood. Since human serum albumin (HSA) is also recycled by FcRn via a pH-dependent interaction, several albumin-fusion proteins to enhance stability and half-life have also been produced. Additionally, antibody fragments fused to albumin or albumin-binding domains have demonstrated prolonged serum residence time in preclinical studies. The generation of Fc-fusion proteins is another strategy that will endow proteins or peptides with properties similar to an intact antibody.

[0005] Modifications of the Fc region that have been investigated are summarized in Saxena (2016) Frontiers in Immunology, Vol. 7, Article 580.

[0006] Deng et al. (2010) Drug Metabolism and Disposition, vol. 38, no. 4, pages 600-605 investigated pharmacokinetics of a humanized monoclonal anti-tumor necrosis factor antibody and its neonatal Fc receptor variants in mice and cynomolgus monkeys.

[0007] Yeung et al. (2009) The Journal of Immunology, Vol. 182, No. 12, pages 7663-7671 describes engineering of human IgG1 affinity to human neonatal Fc receptors and investigates the impact of affinity improvement on pharmacokinetics in primates.

[0008] Kuo et al. (2011) MABS, Vol. 3, No. 5, pages 422-430 is a review on neonatal Fc receptors and IgG-based therapeutics.

[0009] Suzuki et al. (2010) The Journal of Immunology, Vol. 184, No. 4, pages 1968-1976 discusses the importance of neonatal FcR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1.

[0010] Yasmina Noubia Abdiche et al. (2015) found that the neonatal Fc receptor (FcRn) binds independently to both sites of the IgG homodimer with identical affinity (MABS, vol. 7, no. 2, pages 331-343).

[0011] Ward et al. (2015) Molecular Immunology, vol. 67, no. 2, pages 131-141 is a review on FcRn as a target for the modulation of antibody dynamics.

[0012] Rajpal et al. (2014) chapter 1, pages 1-44 in "Therapeutic Fc-fusion proteins", Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, gives an overview about antibody structure and function.

[0013] There is an ongoing need for antibodies having improved effector functions and/or pharmacokinetics.

SUMMARY OF THE INVENTION

[0014] The inventors of this application found that certain specific mutations in the Fc region of an antibody dramatically increase the antibody's affinity to FcRn at pH 6, whereas the affinity at pH 7.4 remains low. Antibodies having the mutations are expected to have improved pharmacokinetic properties. In addition, the antibodies exhibit superior effector functions as compared to known antibodies such as infliximab (IFX).

[0015] In a first aspect, the present invention relates to an IgG_1 antibody comprising a TNFα-binding domain and an FcRn-binding site, having a high affinity to human FcRn at pH 6, said high affinity being characterized by a dissociation equilibrium constant (K_D) determined by surface plasmon resonance of less than 100 nM, and further having no affinity or a low affinity to human FcRn at pH 7.4, said low affinity being characterized by a K_D determined by surface plasmon resonance, of greater than 10 μM, wherein the amino acid sequence of the antibody comprises the amino acids 311R (EU numbering), 428E (EU numbering) and 434W (EU numbering), and wherein the antibody comprises (i) a V_L domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:3, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:4, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:5, and (ii) a VH_H domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:6, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:7, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8.

[0016] In one embodiment, the antibody is a non-fucosylated antibody or an antibody having reduced fucosylation.

[0017] In another embodiment, the antibody comprises a heavy chain which comprises the amino acid sequence as shown in SEQ ID NO:13.

[0018] In yet another embodiment, the antibody comprises a V_H domain having the amino acid sequence as shown in SEQ ID NO:9 and a V_L domain having the amino acid sequence as shown in SEQ ID NO:10.

[0019] In yet another embodiment, the antibody comprises a light chain having the amino acid sequence as shown in SEQ ID NO:1 and a heavy chain having the amino acid sequence as shown in SEQ ID NO:11.

[0020] In a second aspect, the invention relates to the antibody of the first aspect for use in the treatment of an inflammatory condition.

[0021] In one embodiment of the second aspect, the inflammatory condition is an inflammatory disorder of the gastrointestinal tract.

[0022] In another embodiment of the second aspect, said treatment comprises orally administering an effective amount of said antibody.

[0023] In yet another embodiment of the second aspect, said antibody is applied topically.

[0024] In a third aspect, the invention relates to a pharmaceutical composition comprising the antibody of the first aspect.

[0025] The invention is defined by the appended claims.

[0026] Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy (or for diagnosis).

DESCRIPTION OF THE FIGURES

[0027]

Figure 1: Potency of anti-TNFa antibody variants to neutralize human TNFa in the L929 assay. Dose response curves for anti-TNFa antibody variants and the reference infliximab are shown.

Figure 2: Transport of anti-TNFa IgG variants across polarized T84 cells. The amounts of anti-TNFa antibody variants and Infliximab (IFX) from the apical to the basolateral reservoir at 4 hours post adding are shown. Presented as ng/cm^2. Error bars indicate SD of two to four individual monolayers.

Figure 3: Transport of anti-TNFa IgG variants across polarized T84 cells in the presence of excess amounts of myeloma IgG. The amounts of the anti-TNFa Ab variants and IFX transported from the apical to the basolateral reservoir in the presence of 10-fold excess of human myeloma IgG at 4 hours post adding are shown. Presented as ng/cm^2. Error bars indicate SD of three to four individual monolayers.

Figure 4: ADCC activity. Induction of ADCC by anti-TNFa antibody variants, wild-type antibody and IFX.

Figure 5: Binding to human C1q. Binding of IFX and anti-TNFa antibody variants to human C1q. Each concentration was assayed in duplicate. Error bars indicate SD.

Figure 6: CDC activity. Percent cell death for the various anti-TNFa variants compared to IFX. Each sample point is the mean of 6 independent replicates.

Figure 7: Induction of $CD14^{+}CD206^{+}$ macrophages by each compound relative to the induction of IFX. Summarized data of 4 independent experiments. Bars represent mean, error bars represent SEM.

Figure 8: Suppression of T-cell proliferation by each compound relative to IFX. Summarized data of 3 independent experiments. Bars represent mean, error bars represent SEM.

Figure 9: Schematic presentation of site directed mutagenesis.

Figure 10: A schematic illustration of the dominating N-glycan forms attached to N297 of the anti-TNFa antibody variants. The two N-glycan profiles that dominated among the panel of tested anti-TNFa antibody variants were 4GlcNac-1Fuc-3Man and 4GlcNac-1Fuc-3Man-1Gal while for the IgG variants produced in the presence of 2FF the same bi-antennary structures occurred except that these lacked the fucose.

DETAILED DESCRIPTION

[0028] The present invention relates to an IgG1 antibody that is capable of binding to TNFa and comprises an FcRn-binding site. In accordance with this application, an antibody comprises an FcRn-binding site if it is capable of binding to FcRn, preferably to human FcRn, at pH 6. Binding to FcRn at pH 6 can be determined by SPR, e.g. as described in Example 4 of this application. If binding of an antibody to FcRn at pH 6 can be detected by SPR, such antibody has an FcRn binding site. The antibody of the invention has a high affinity to human FcRn at pH 6, characterized by a dissociation equilibrium constant (K_D) of less than 100 nM. The antibody further has a low affinity to human FcRn at pH 7.4, characterized by a K_D of greater than 10 μM. The amino acid sequence of the antibody comprises the amino acid tryptophan at position 434 (EU numbering).

[0029] Throughout the present specification and claims, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain and residues 1-113 of the heavy chain) (Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Unless stated otherwise herein, references to residues numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (see e.g., WO 2006/073941).

Antibody

[0030] In the context of the present application, the term "antibody" is used as a synonym for "immunoglobulin" (Ig), which is defined as a protein belonging to the class IgG1, and includes all conventionally known antibodies and functional fragments thereof. In the context of the present invention, a "functional fragment" of an antibody/immunoglobulin is defined as antigen-binding fragment or other derivative of a parental antibody that essentially maintains one or more of the properties of such parental antibody. An "antigen-binding fragment" or "antigen-binding domain" of an antibody/immunoglobulin is defined as fragment (*e.g.*, a variable region of an IgG1) that retains the antigen-binding region. An "antigen-binding region" of an antibody typically is found in one or more hypervariable region(s) of an antibody, i.e., the CDR-1, -2, and/or -3 regions. The antibodies of the present invention may be part of bi- or multifunctional constructs.

[0031] Preferably the antibody is a monoclonal antibody. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. (Harlow and Lane, "Antibodies, A Laboratory Manual" CSH Press 1988, Cold Spring Harbor N.Y.).

[0032] In another aspect of the disclosure, including embodiments relating to the *in vivo* use of the anti-TNFa antibodies in humans, chimeric, primatized, humanized, or human antibodies can be used. In a preferred embodiment, the antibody is a human antibody or a humanized antibody, more preferably a monoclonal human antibody or a monoclonal humanized antibody.

TNFα-binding *domain*

[0033] Preferably, the antibody of the invention specifically binds to TNFα. As used herein, an antibody "specifically recognizes", or "specifically binds to" human TNFα, when the antibody is able to discriminate between human TNFa and one or more reference molecule(s). Preferably, the IC_{50} value for binding to each of the reference molecules is at least 1,000 times greater than the IC_{50} value for binding to TNFα. In its most general form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between human TNFa and an unrelated biomolecule, as determined, for example, in accordance with specificity assay methods known in the art. Such methods comprise, but are not limited to, Western blots and ELISA tests. For example, a standard ELISA assay can be carried out. Typically, determination of binding specificity is performed by using not a single reference biomolecule, but a set of about three to five unrelated biomolecules, such as milk powder, BSA, transferrin or the like. In one embodiment, specific binding refers to the ability of the antibody to discriminate between human TNFa and human TNFβ.

[0034] The antibody of the invention comprises a V_L domain and a V_H domain. The V_L domain comprises a CDR1 region (CDRL1), a CDR2 region (CDRL2), a CDR3 region (CDRL3) and Framework regions. The V_H domain comprises a CDR1 region (CDRH1), a CDR2 region (CDRH2), a CDR3 region (CDRH3) and Framework regions.

[0035] The term "CDR" refers to one of the six hypervariable regions within the variable domains of an antibody that mainly contribute to antigen binding. One of the most commonly used definitions for the six CDRs was provided by Kabat E. A. et al., (1991) Sequences of proteins of immunological interest. NIH Publication 91-3242). As used herein, Kabat's definition of CDRs only apply for CDR1, CDR2 and CDR3 of the light chain variable domain (CDR L1, CDR L2, CDR L3, or L1, L2, L3), as well as for CDR2 and CDR3 of the heavy chain variable domain (CDR H2, CDR H3, or H2, H3). CDR1 of the heavy chain variable domain (CDR H1 or H1), however, as used herein is defined by the following residues (Kabat numbering): It starts with position 26 and ends prior to position 36.

[0036] The antibody of the invention comprises (i) a V_L domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:3, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:4, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:5, and (ii) a V_H domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:6, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:7, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8.

[0037] In a more preferred embodiment, the antibody of the invention of the invention comprises a V_H domain having the amino acid sequence as shown in SEQ ID NO:9. In another more preferred embodiment the antibody comprises a V_L domain having the amino acid sequence as shown in SEQ ID NO:10. Most preferably, the antibody of the invention comprises (i) a V_H domain having the amino acid sequence as shown in SEQ ID NO:9, and (ii) a V_L domain having the amino acid sequence as shown in SEQ ID NO:10.

[0038] The antibody of the invention has a high affinity to human TNFα. The term "K_D," refers to the dissociation equilibrium constant of a particular antibody-antigen interaction. Typically, the antibody of the invention binds to human TNFa with a dissociation equilibrium constant (K_D) determined using surface plasmon resonance (SPR) technology in a BIACORE instrument. In particular, the determination of the K_D is carried out as described in Example 1.

Modifications affecting affinity to FcRn

[0039] The antibody of the invention comprises an amino acid sequence which differs from that of a native sequence of a wild-type antibody by virtue of at least one "amino acid modification" as herein defined. The at least one amino acid modification affects the affinity of the antibody to human FcRn. Typically, the at least one amino acid modification increases the affinity of the antibody to human FcRn at pH 6. In one embodiment, the at least one amino acid modification increases the affinity of the antibody to human FcRn at pH 6, wherein it does not substantially change the affinity to human FcRn at pH 7.4. Preferably, the modified antibody has at least one amino acid substitution compared to the amino acid sequence of a wild-type antibody or of a parent antibody, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions. Preferably, the at least one amino acid modification is within the FcRn binding site of the antibody. The antibody may have one or more amino acid modifications outside the FcRn binding site of the antibody which affect FcRn binding, e.g. by structural changes. The amino acid modification(s) can be generated by methods that are known *per se*, e.g. by site-directed mutagenesis as described in "Antibody Engineering - Methods and Protocols", edited by Patrick Chames, 2nd ed., 2012, Chapter 31 (ISBN 978-1-61779-973-0).

[0040] The antibody of the invention comprises the amino acid tryptophan at position 434 (EU numbering). This is referred to as "434W" herein. The native amino acid at position 434 of non-modified human IgG antibodies is asparagine (N). Thus, the antibody of the invention can be obtained by introducing the mutation N434W into an antibody. Preferably, the antibody of the invention is obtainable or obtained by substituting tryptophan for asparagine at position 434.

[0041] Furthermore, the antibody of the invention comprises the amino acid glutamic acid at position 428 (EU numbering). This is referred to as "428E" herein. The native amino acid at position 428 of non-modified human IgG antibodies is methionine (M). Thus, the antibody of the invention can be obtained by introducing the mutation M428E into an

antibody. Preferably, the antibody of the invention is obtainable or obtained by substituting glutamic acid for methionine at position 428.

[0042] Moreover, the antibody of the present invention further comprises the amino acid arginine at position 311 (EU numbering). This is referred to as "311R" herein. The native amino acid at position 311 of non-modified human IgG antibodies is glutamine (Q). Thus, the antibody of the invention can be obtained by introducing the mutation Q311R into an antibody. Preferably, the antibody of the invention is obtainable or obtained by substituting glutamine for arginine at position 311.

[0043] The antibody of the present invention comprises the amino acids 434W, 428E and 311R. This antibody is obtainable by introducing the mutations Q311R, M428E and N434W into an antibody.

[0044] The remaining amino acid sequence of the constant domain may be identical to the native amino acid sequence of a typical human IgG. It is possible, however, that the amino acid sequence of the antibody comprises one or more additional mutations or substitutions to the native amino acid sequence of the Fc region of a native antibody, as long as the antibody still has TNFα-binding activity and effector functions.

[0045] In a preferred embodiment, the Fc region of the antibody of the invention, including the hinge region, comprises or consists of the amino acid sequence as shown in SEQ ID NO:13.

[0046] In a preferred embodiment, the heavy chain of the antibody of the invention has the amino acid sequence as shown in SEQ ID NO:11. Preferably, this antibody further comprises a light chain having the amino acid sequence as shown in SEQ ID NO:1.

[0047] In another aspect of the disclosure, which is not covered by the claimed invention, the heavy chain of the antibody of the invention has the amino acid sequence as shown in SEQ ID NO:12. Preferably, this antibody further comprises a light chain having the amino acid sequence as shown in SEQ ID NO:23 or SEQ ID NO:24.

[0048] In a preferred embodiment of the invention, the antibody of the invention is a non-fucosylated antibody or an antibody having reduced fucosylation.

[0049] The term "antibody having reduced fucosylation", as used herein, refers to an antibody in which less than 90% of the N-glycans of the antibody are fucosylated. Methods to determine the percentage of fucosylation are known in the art. Preferably, the percentage of fucosylation is determined as described in Example 11 of this application.

[0050] In another aspect of the disclosure, less than 75%, or less than 50%, or less than 25% of the N-glycans of the antibody are fucosylated. Most preferably, less than 15% of the N-glycans of the antibody are fucosylated. In a particular embodiment, the N-glycans of the antibody of the invention do not contain any fucose.

[0051] Preferably, less than 90% of the N-glycans at N297 (EU numbering) of the antibody are fucosylated. In another embodiment, less than 75%, or less than 50%, or less than 25% of the N-glycans at N297 (EU numbering) of the antibody are fucosylated. Most preferably, less than 15% of the N-glycans at N297 (EU numbering) of the antibody are fucosylated.

[0052] In another embodiment, the N-glycans at N297 of the antibody do not contain any fucose.

[0053] Non-fucosylated antibodies, sometimes also referred to as afucosylated antibodies, can be generated by various methods. For example, the synergistic knockdown of the genes for α1,6-fucosyltransferase (*FUT8*) and GDP-mannose 4,6-dehydratase (*GMD*) in CHO cells can be used to produce monoclonal antibody variants that are fully afucosylated and ADCC-enhanced (see, e.g., Imai-Nishiya et al. (2007) BMC Biotechnol. 7, 84). A method using zinc-finger nucleases (ZFNs) cleaving the *FUT8* gene in a region encoding the catalytic core of the α1,6-fucosyltransferase and thus disrupting the corresponding enzymatic function in CHO cells can be used to produce monoclonal antibodies completely lacking core fucose (see, e.g., Malphettes et al. (2010) Biotechnol. Bioeng. 106, 774-783).

[0054] Antibodies having reduced fucosylation can be prepared by addition of a decoy substrate such as 2-deoxy-2-fluoro-2-fucose to the culture medium (see, e.g., Dekker et al. (2016) Sci Rep 6:36964), resulting in a reduced incorporation of fucose in the IgG-Fc glycans.

[0055] In another aspect of the disclosure, the antibody has a high sialic acid content. In increase in sialylation can be achieved, e.g. by simultaneous transfection of cytidine monophosphate-sialic acid synthase (CMP-SAS), cytidine monophosphate-sialic acid transporter (CMP-SAT), and α 2,3-sialyltransferases (see, e.g., Son et al. (2011) Glycobiology 21, 1019-1028).

Affinity to FcRn

[0056] The affinity at pH 6 to human FcRn of the antibody of the invention is high. The high affinity binding of the antibody to human FcRn at pH 6 is characterized by a K_D value of less than 100 nM.

[0057] The affinity of the antibody of the invention to human FcRn is preferably determined by surface plasma resonance (SPR), for example as described in Example 4 of this application.

[0058] The antibody of the present invention typically has a low affinity to human FcRn at pH 7.4. The low affinity is characterized by a K_D value of greater than 1 μM. Preferably, the low affinity to human FcRn at pH 7.4 is characterized by a K_D value of greater than 10 μM.

Functional properties of the antibody

[0059] The antibody of the invention is efficiently transported across a polarized cell monolayer from the apical side to the basolateral side. Typically, the transport across the polarized cell monolayer is in a greater amount than that of infliximab, wherein the amount of antibody in infliximab refers to the mass/cm^2 of the polarized cell monolayer. The amount of antibody transported across the polarized cell monolayer, relative to the amount of infliximab transported across the polarized cell monolayer, is at least 110%, preferably at least 125%, more preferably at least 150%, or at least 175%, or at least 200% (wherein the amount of transported infliximab is set to 100%).

[0060] Furthermore, the antibody is specifically transported across the polarized cell monolayer from the apical side to the basolateral side in the presence of an excess of competing immunoglobulins. This is referred to as specific transport herein.

[0061] The percentage of the total mass of immunoglobulins transported across the polarized cell monolayer is greater than the percentage of infliximab transported across the polarized cell monolayer from the apical side to the basolateral side in the presence of a 10-fold excess of competing immunoglobulins. The percentage of antibody of the invention transported across the polarized cell monolayer in the presence of a 10-fold excess of unrelated immunoglobulins, relative to the percentage of infliximab transported across the polarized cell monolayer in the presence of a 10-fold excess of unrelated antibodies, is at least 150%, or at least 200%, or at least 250%, or at least 300% (infliximab is set to be 100%).

[0062] Preferably, the polarized cell monolayer is a monolayer of polarized T84 cells. The transport assay mimicking process of transcytosis can be carried out as described in Example 5 of this application.

[0063] The antibody described herein binds to CD64, CD32a(H), CD32a(R), CD32b, CD16a(V), CD16a(F) and CD16b(NA2).

[0064] The antibody described herein typically binds to CD64 with a K_D of less than 100 nM, preferably less than 10 nM.

[0065] The antibody described herein typically binds to CD32a(H) with a K_D of less than 10 μM.

[0066] The antibody described herein typically binds to CD32a(R) with a K_D of less than 10 μM.

[0067] The antibody described herein typically binds to CD32b with a K_D of less than 10 μM.

[0068] The antibody described herein typically binds to CD16a(V), e.g. with a K_D of less than 500 nM, preferably less than 100 nM.

[0069] The antibody described herein typically binds to CD16a(F), e.g. with a K_D of less than 10 μM, preferably less than 1 μM.

[0070] The antibody described herein typically binds to CD16b(NA2), e.g. with a K_D of less than 10 μM, preferably less than 1 μM.

[0071] The antibody described herein further binds to human C1q. Preferably, this binding is stronger than the binding of infliximab to human C1q.

[0072] The antibody described herein further has complement-dependent cytotoxicity (CDC) of rabbit complement. This CDC of the antibody of the invention is preferably greater than that of infliximab, in terms of relative ECso and/or relative maximal death.

[0073] The antibody described herein is further capable of inducing $CD14^+CD206^+$ macrophages. The level of induction is preferably comparable to, equal to, or greater than that of infliximab.

[0074] The antibody described herein is further capable of suppressing T-cell proliferation. The degree of suppression of T-cell proliferation is preferably comparable to, equal to, or greater than that of infliximab.

Pharmaceutical Compositions and Treatment

[0075] Treatment of a disease encompasses the treatment of patients already diagnosed as having any form of the disease at any clinical stage or manifestation; the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of the disease; and/or preventing and/or reducing the severity of the disease.

[0076] A "subject" or "patient" to whom an anti-TNFa antibody is administered can be a mammal, such as a non-primate (e.g., cow, pig, horse, cat, dog, rat, etc.) or a primate (e.g., monkey or human). In certain aspects, the human is a pediatric patient. In other aspects, the human is an adult patient.

[0077] Compositions comprising an anti-TNFa antibody and, optionally one or more additional therapeutic agents, such as the second therapeutic agents described below, are described herein. The compositions typically are supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient).

[0078] The anti-TNFa antibodies can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intrathecally, topically or locally, e.g. mucosally. The most suitable route for administration in any given case will depend on the particular antibody, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, an anti-TNFa antibody will be administered

intravenously.

[0079] In a particularly preferred embodiment, the antibody of the invention is administered orally.

[0080] Described herein is an anti-TNFa antibody is present in a pharmaceutical composition at a concentration sufficient to permit intravenous administration at 0.5 mg/kg body weight to 20 mg/kg body weight. In some embodiments, the concentration of antibody suitable for use in the compositions and methods described herein includes, but is not limited to, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, or a concentration ranging between any of the foregoing values, e.g., 1 mg/kg to 10 mg/kg, 5 mg/kg to 15 mg/kg, or 10 mg/kg to 18 mg/kg.

[0081] The effective dose of an anti-TNFa antibody can range from about 0.001 to about 750 mg/kg per single (e.g., bolus) administration, multiple administrations or continuous administration, or to achieve a serum concentration of 0.01-5000 μg/ml per single (e.g., bolus) administration, multiple administrations or continuous administration, or any effective range or value therein depending on the condition being treated, the route of administration and the age, weight and condition of the subject. In case of oral administration, the serum concentration may be very low or even below the detection limit. In certain embodiments, each dose can range from about 0.5 mg to about 50 mg per kilogram of body weight or from about 3 mg to about 30 mg per kilogram body weight. The antibody can be formulated as an aqueous solution.

[0082] In a particularly preferred embodiment, the antibody of the invention is administered orally. If the antibody is administered orally, the daily dose of antibody is typically in the range of about 0.01 mg/kg to about 100 mg/kg of body weight, or about 0.05 mg/kg to about 50 mg/kg of body weight, or about 0.1 mg/kg to about 25 mg/kg of body weight, or about 0.15 mg/kg to about 10 mg/kg of body weight, or about 0.16 mg/kg to about 5 mg/kg of body weight, or about 0.2 mg/kg to about 2 mg/kg of body weight, or about 0.2 mg/kg to about 1 mg/kg of body weight, Generally, advantageous doses are doses of 1 to 200 mg per day, preferably 5 to 100 or 10 to 50 mg per day.

[0083] Pharmaceutical compositions can be conveniently presented in unit dose forms containing a predetermined amount of an anti-TNFa antibody per dose. Such a unit can contain 0.5 mg to 5 g, for example, but without limitation, 1 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 750 mg, 1000 mg, or any range between any two of the foregoing values, for example 10 mg to 1000 mg, 20 mg to 50 mg, or 30 mg to 300 mg. Pharmaceutically acceptable carriers can take a wide variety of forms depending, e.g., on the condition to be treated or route of administration.

[0084] Determination of the effective dosage, total number of doses, and length of treatment an anti-TNFa antibody thereof is well within the capabilities of those skilled in the art, and can be determined using a standard dose escalation study.

[0085] Therapeutic formulations of the anti-TNFa antibodies suitable in the methods described herein can be prepared for storage as lyophilized formulations or aqueous solutions by mixing the antibody having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), i.e., buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[0086] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They can present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), citrate-phosphate buffers, succinate buffers (e.g., succinic acid- monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid- disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyuconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0087] The pharmaceutical composition of the invention may further comprise at least one salt, e.g. sodium chloride. The salt concentration preferably ranges from 100 mM to 200 mM, e.g. about 150 mM.

[0088] Preservatives can be added to retard microbial growth, and can be added in amounts ranging from 0.2%- 1% (w/v). Suitable preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonifiers

sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccacharides such as raffinose; and polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[0089] Non-ionic surfactants or detergents (also known as "wetting agents") can be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.). Non-ionic surfactants can be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, or in a range of about 0.07 mg/ml to about 0.2 mg/ml.

[0090] Additional miscellaneous excipients include bulking agents (e.g., starch), chelating agents (e.g., EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), protease inhibitors and co-solvents.

[0091] The formulation herein can also contain a second therapeutic agent in addition to an anti-TNFa antibody thereof. Examples of suitable second therapeutic agents are provided below.

[0092] The dosing schedule can vary from once a month to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the patient's sensitivity to the anti-TNFa antibody. In specific embodiments, an anti-TNFa antibody thereof is administered daily, twice weekly, three times a week, every other day, every 5 days, once weekly, every 10 days, every two weeks, every three weeks, every four weeks or once a month, or in any range between any two of the foregoing values, for example from every four days to every month, from every 10 days to every two weeks, or from two to three times a week, etc.

[0093] The dosage of an anti-TNFa antibody to be administered will vary according to the particular antibody, the subject, and the nature and severity of the disease, the physical condition of the subject, the therapeutic regimen (e.g., whether a second therapeutic agent is used), and the selected route of administration; the appropriate dosage can be readily determined by a person skilled in the art.

[0094] It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an anti-TNFa antibody thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the age and condition of the particular subject being treated, and that a physician will ultimately determine appropriate dosages to be used. This dosage can be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice.

Disorders to be treated

[0095] The invention relates to an antibody according to the first aspect for use in a method of treating or preventing an inflammatory condition, in particular, an inflammatory disorder of the gastrointestinal tract.

[0096] Most preferably, the antibody of the invention is used to treat an inflammatory disorder of the gastrointestinal tract, in particular Crohn's disease, ulcerative colitis or microscopic colitis. The Crohn's disease may be ileal, colonic, ileocolonic or isolated upper Crohn's disease (gastric, duodenal and/or jejunal) including non-stricturing/non-penetrating, stricturing, penetrating and perianal disease behavior, allowing any combination of localization and disease behavior of any of the above mentioned. The ulcerative colitis may be ulcerative proctitis, proctosigmoiditis, left-sided colitis, pan-ulcerative colitis and pouchitis.

Combination Therapy and other aspects

[0097] Preferably, the patient being treated with an anti-TNFa antibody is also treated with another conventional medicament. For example, a patient suffering from inflammatory bowel disease, especially if having moderate to severe disease is typically also being treated with mesalazine or derivatives or prodrugs thereof, corticosteroids, e.g. budesonide or prednisolone (oral or i.v.), immunosuppressants, e.g. azathioprine/6-mercaptopurine (6-MP) or methotrexate, cy-

closporine or tacrolimus. Other medicaments which can be co-administered to the patient include other anti-TNFa antibodies (e.g. infliximab, adalimumab, etanercept, certolizumab pegol, golimumab), integrin antagonists (e.g. natalizumab, vedolizumab), anti-IL-23 antibodies (e.g. MEDI2070), anti-β7 antibodies (e.g. etrolizumab), JAK inhibitors in the JAK/STAT pathway (e.g. tofacitinib), and others. Further medicaments which can be co-administered to the patient include immunosupressants (e.g. azathioprine/6-MP or methotrexate or oral cyclosporine) in order to maintain stable and longer remission. Yet another aspect of the invention is the use of an anti-TNFa antibody as defined hereinabove for reducing inflammation.

[0098] Yet another aspect of the invention is an anti-TNFa antibody as defined hereinabove for use in reducing inflammation in a patient suffering from an inflammatory condition.

[0099] A further aspect of this invention is an anti-TNFa antibody as defined hereinabove for use in a method of treating an inflammatory condition, comprising administering to a patient in need thereof an effective amount of said anti-TNFa antibody as defined hereinabove. The inflammatory condition is preferably one of the conditions described above.

[0100] A further aspect of this invention is an anti-TNFa antibody as defined hereinabove for use in a method of preventing an inflammatory condition, comprising administering to a patient in need thereof an effective amount of said anti-TNFa antibody as defined hereinabove.

[0101] Further disclosed herein is a method for improving the transcytosis of an antibody directed against TNFα, comprising introducing the substitutions Q311R, M428E and N434W in the amino acid sequence of the antibody so as to obtain a modified antibody having improved transcytosis. The modified antibody is preferably an antibody as described hereinabove.

[0102] Further disclosed herein is a method for extending the plasma half-life of an antibody directed against TNFα, comprising introducing the substitutions Q311R, M428E and N434W in the amino acid sequence of the antibody so as to obtain a modified antibody having an extended plasma half-life. The modified antibody is preferably an antibody as described hereinabove. The plasma half-life may be increased by at least 10%, or least 20%, or least 30%, or least 40%, or least 50%, relative to the plasma half-life of the non-modified antibody (i.e., the parent antibody lacking the substitutions Q311R, M428E and N434W).

Table 1. Overview of the sequences of the sequence listing.

SEQ ID NO:	Description of the amino acid sequence
1	Light chain of Ab-wt, the parent antibody of the modified antibodies used in the examples
2	Heavy chain of Ab-wt, the parent antibody of the modified antibodies used in the examples
3	CDR L1 of clone 16-22-H05
4	CDR L2 of clone 16-22-H05
5	CDR L3 of clone 16-22-H05
6	CDR H1 of clone 16-22-H05
7	CDR H2 of clone 16-22-H05
8	CDR H3 of clone 16-22-H05
9	V_H of humanized IgG of clone 16-22-H05
10	V_L of humanized IgG of clone 16-22-H05
11	Heavy chain of Ab-REW (based on clone 16-22-H05)
12	Heavy chain of Ab-REW (based on clone 17-22-B03)
13	Fc region of Ab-REW (including hinge region)
14	CDR L1 of clone 17-22-B03
15	CDR L2 of clone 17-22-B03
16	CDR L3 of clone 17-22-B03
17	CDR H1 of clone 17-22-B03
18	CDR H2 of clone 17-22-B03
19	CDR H3 of clone 17-22-B03
20	V_H of humanized IgG of clone 17-22-B03

(continued)

SEQ ID NO:	Description of the amino acid sequence
21	V_L of humanized IgG of clone 17-22-B03 (sc08)
22	V_L of humanized IgG of clone 17-22-B03 (sc02)
23	Light chain of humanized IgG of clone 17-22-B03 (sc08)
24	Light chain of humanized IgG of clone 17-22-B03 (sc02)

Examples

Antibody variants

[0103] Several variants of an anti-TNFa antibody (hereinafter referred to as "parent antibody" or "Ab-wt") were generated by introducing substitutions in the Fc region of the antibody amino acid sequence. The light chain of Ab-wt has the amino acid sequence as shown in SEQ ID NO:1, and the heavy chain of Ab-wt has the amino acid sequence as shown in SEQ ID NO:2. The mutations were introduced by site-directed mutagenesis by established methods. Briefly, mutations were introduced by PCR. The forward primer was designed to contain the intended mutation while the reverse primer was designed so that the 5' ends of the two primers anneal back-to-back (but do not overlap) (Figure 9). PCR was run for 25 cycles (98°C for 10 s, 64°C for 30s, 72°C for 3 min). Before running the PCR product on an agarose gel, the non-mutated PCR template was removed from the pool of PCR products using the restriction enzyme DpnI. Following gel purification of the PCR product the blunt ends were ligated to obtain a circularized plasmid which was transformed into competent E.coli cells. Following overnight incubation several colonies were picked, the plasmid DNA isolated and sequenced to confirm that the mutation had been incorporated.

[0104] The non-fucosylated variants were generated by addition of 0.15 mM of the decoy substrate 2-deoxy-2-fluoro-2-fucose to the culture medium (Dekkers et al. (2016) Sci Rep 6:36964). This resulted in a significantly reduced incorporation of fucose in the IgG-Fc glyan, as shown in Example 11 hereinbelow.

Table 2: Generated antibody variants of an anti-TNFa antibody (EU numbering)

Designation	Mutations relative to parent antibody
Ab-wt*	None (=parent antibody)
Ab-REW**	Q311 R/M428E/N434W
Ab-REW-2FF**	non-fucosylated variant of Ab-REW
* antibody not according to the invention; **antibody according to the invention	

[0105] Antibodies Ab-REW and Ab-REW-2FF are antibodies in accordance with the present invention.

Example 1. Affinity to TNFα

Method:

[0106] Affinity to TNFa was measured by Biacore. A CM5 chip was prepared using standard amine immobilisation Biacore procedures. Upon insertion of a CM5 chip the system was primed and then normalised with BIA-normalising solution (Biacore Preventative Maintenance Kit 2). The chip was added to the system with PBS-T running buffer; prior to immobilisation the chip surface was primed with three injections of 50 mM NaOH. Protein A was immobilised on the chip surface. For this, the protein was diluted to 5 μg/mL into 10 mM acetate buffer at pH 4.5 and injected so to generate a bound response of -1000 RU's in all 4 flow cells. To remove non-covalently bound material from all the chip flow cells, three 15 second 50 mM NaOH washes were performed. On the Protein A chip, antibody was captured in flow cells 2 and 4, with flow cells 1 and 3 used for reference subtraction. The trial antibodies were diluted in PBS-T to 10 nM and 2.5-7.5 uL injected to obtain 120 RU of captured antibody. The analyte TNFα was prepared at 500 μg/mL in water as directed by the supplier and further diluted into the running buffer PBS-T. Single cycle kinetics was used to estimate the steady state affinity. For each single cycle analysis cycle a titration of 5 analyte concentrations were injected over the ligand and then the dissociation of the complex was measured. The surface was regenerated using glycine pH 1.7. A double referencing method was employed in which data from the ligand bound capture surface (fc 2 and 4) were

subtracted from the references surfaces where no ligand was captured (fc 1 and 3 respectively). Blank injections of buffer were run every 3-4 cycles and then subtracted from analyte injection cycles, to correct for small changes in the ligand capture surface. Repeat injections of analyte at the start and end of each analytical run were used to check for sample degradation, or changes in the instrument performance. All analysis was performed at 25°C and the sample rack was incubated at 10°C during experimental runs. Each experiment was run at least three times. A 1-to-1 binding model was used to fit the resulting kinetic data.

Results:

[0107] All antibodies displayed similar binding kinetics to TNFa indicating that any introduced modification had not led to significant changes in the antigen binding region. For antibody Ab-REW-2FF the dissociation rate could not be measured, therefore affinity (K_D) could not be determined. However, the association rate was comparable to the other antibodies indicating that the introduction of the mutation does not affect the binding significantly.

Table 3: Binding kinetics of human IgG1 variants to TNFa as determined by SPR

	k_a (10^6/Ms)	k_d (10^{-5}/s)	K_D (pM)
Ab-wt	8.37 ± 0.11	3.45 ± 0.20	4.13 ± 0.19
Ab-REW	6.22 ± 0.91	2.04 ± 0.52	3.30 ± 0.74
Ab-REW-2FF	5.80 ± 0.58	nd*	nd*

*Dissociation rate (kd) could not be determined.

Example 2. Potency

Method:

[0108] L929 cells were incubated with 0.25 ng/mL of TNFa and 1 μg/well of actinomycin D in the presence of serial dilutions of anti-TNFa antibody variants. Following incubation for 20 h at 37°C/5% CO_2, the proliferative responses were measured using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and an electron coupling reagent (phenazine ethosulfate, PES). MTS was converted into formazan product by dehydrogenase enzymes present in metabolically active cells. The quantity of formazan product as measured by absorbance at 492 nm was directly proportional to the number of living cells in culture.

Results:

[0109] The results are shown in Figure 1. The introduction of mutations into the Fc region of the anti-TNFa antibody did not affect the potency.

Example 3. Affinity to Fcγ receptors (CD64, CD32a, CD32b, CD16a, CD16b)

Method:

[0110] Affinity to FcγRs was measured by Biacore. A CM5 chip was prepared using standard amine immobilisation Biacore procedures. Upon insertion of a CM5 chip the system was primed and then normalised with BIA-normalizing solution (Biacore Preventative Maintenance Kit 2). The chip was added to the system with Phosphate Buffered Saline Tween-20 (PBS-T) running buffer; prior to immobilisation the chip surface was primed with three injections of 50 mM NaOH. FcγRs were immobilised on the chip surface using a His-tag capture system. The anti-His tag chip was prepared according to the Biacore kit instructions, with -12000 RU's of the antibody deposited on all 4 flow cells. To remove non-covalently bound material from all the chip flow cells, three 30 second 10 mM glycine pH 1.5 washes were performed. The Fcγ receptors were diluted in PBS-T to a range of 0.5-2 μg/mL, with 2.5-5.0 μL injected onto the chip generating capture levels between 60 and 200 RU's. Antibodies were diluted into PBS-T prior to analysis. Single cycle kinetics were used to estimate the steady state affinity. For each single cycle analysis cycle a titration of 5 antibody concentrations were injected over the FcγR ligand and then the dissociation of the complex was measured. The surface was regenerated using the recommended solution, 10 mM glycine pH 1.5 for the anti-His capture surface. A double referencing method was employed in which data from the ligand bound capture surface (fc 2 and 4) were subtracted from the references surfaces where no ligand was captured (fc 1 and 3 respectively). Blank injections of buffer were run for every antibody

titration cycle and then subtracted from analyte injection cycles, to correct for small changes in the ligand capture surface. All analysis was performed at 25°C and the sample rack was incubated at 10°C during experimental runs. Each experiment was run at least three times.

Results:

[0111] Binding to CD64 was not affected for the engineered anti-TNFa antibodies. The introduction of the mutations did not affect the affinity to CD32a(H), CD32a(R) and CD32b. However, Ab-REW-2FF showed a 5.5-fold increase in affinity to CD16a(V). The non-fucosylated antibody Ab-REW-2FF had also an improved binding to the low affinity CD16a receptor and to CD16b.

Table 4: Affinity to Fcγ receptors CD64, CD32a(H), CD32a(R) and CD32b as determined by SPR. The mean and standard deviation affinity calculated from two or more independent experiments is shown.

	Affinity (K_D)			
	CD64 (nM)	**CD32a(H)** (μM)	**CD32a(R)** (μM)	**CD32b** (μM)
Ab-wt	2.92 ± 0.07	0.67 ± 0.04	nd	3.14 ± 0.80
Ab-REW	2.80 ± 0.24	0.78 ± 0.05	1.60 ± 0.01	1.21 ± 0.32
Ab-REW-2FF	2.93 ± 0.16	1.87 ± 0.37	1.45 ± 0.01	1.13 ± 0.25

Table 5: Affinity to Fcγ receptors CD16a(V), CD16a(F) and CD16b as determined by SPR. The mean and standard deviation affinity calculated from two or more independent experiments is shown.

	Affinity (K_D)		
	CD16a(V) (nM)	**CD16a(F)** (μM)	**CD16b(NA2)** (μM)
Ab-wt	184 ± 31.9	nd	> 3.00
Ab-REW	280 ± 36.1	2.41 ± 0.68	1.65 ± 0.36
Ab-REW-2FF	33.5 ± 0.99	0.15 ± 0.01	0.40 ± 0.07

Example 4. Affinity to FcRn

Method:

[0112] SPR was performed using a Biacore 3000 instrument with CM5 sensor chips coupled with anti-TNFα IgG1 antibodies (-500 resonance units (RU)) using amine-coupling chemistry as described by the manufacturer. The coupling was performed by injecting 2.0 ug/mL of each protein in 10 mM sodium acetate, pH 4.5, using the amine-coupling kit (GE Healthcare). HBS-P buffer pH 7.4 (10 mM HEPES, 150 mM NaCl, 0.005% surfactant P20) or phosphate buffer pH 6.0 (67 nM phosphate buffer, 150 mM NaCl, 0.005% Tween 20) were used as running and dilution buffer. Binding kinetics were determined by injecting titrated amounts (1000 - 31.2 nM) of monomeric His-tagged human FcRn (hFcRn) over immobilised antibodies at pH 7.4 or pH 6.0. All SPR experiments were conducted at 25°C with a flow rate of 40 ul/min. Binding data were zero-adjusted, and reference cell value subtracted. The Langmuir 1:1 ligand binding model provided by the BIAevaluation software (version 4.1) was used to determine the binding kinetics.

Results:

[0113] The results showed that the wild-type antibody Ab-wt bound strictly pH dependently to hFcRn. All engineered antibody variants had a higher affinity to FcRn at pH 6.0, but kept their pH dependency and did not bind to the receptor at pH 7.4. Surprisingly, the REW containing variants showed > 160-fold stronger binding at acidic pH and still no detectable binding under conditions tested at neutral pH. The antibody variants showed improved binding to FcRn compared to infliximab which contains a wildtype IgG1 Fc region.

Table 6: Affinity of anti-TNFα antibody variants to FcRn at pH 6.0 and pH 7.4 as determined by SPR

	pH 6.0			pH 7.4
	K_D (nM)	Fold change from wt	Fold change from IFX	K_D (nM)
Ab-wt	1000			NA
Ab-REW	5.61	178	75.8	NA
Ab-REW-2FF	6.24	160	68.1	NA
IFX	425			NA
NA: not acquired due to weak binding.				

Example 5. Transcytosis

Method:

[0114] Transwell filters (1.12 cm^2) with collagen coated polytetrafluoroethylene (PTFE) membranes with 0.4 μm pore size were incubated O/N in complete growth medium followed by seeding of 1.0×10^6 T84 cells per well. Transepithelial electrical resistance (TEER) was monitored daily using a MILLICELL-ERS-2 volt-ohm meter. The cultures were grown for 4 - 5 days before reaching confluence with a TEER value of ~1000 - 1300 $\Omega \times cm^2$. Prior to experiments the monolayers were starved for 1 h in Hank's Balanced Salt Solution (HBSS). Then, 400 nM of the antibody variants or IFX alone or together with 4000 nM human myeloma IgG with irrelevant specificity were added to the apical Transwell chamber. Samples were collected from the basolateral reservoir at 0 and 4 h post adding. Antibody concentrations in the basolateral reservoir were determined by ELISA. Briefly, 96-well Maxisorp plates were coated O/N with either recombinant TNFa or an anti-human Fc specific antibody from goat, both diluted to 1 μg/ml in PBS. Subsequently, the plates were blocked with PBS containing 4% skimmed milk for 2 h at RT followed by washing 4 times with PBS containing 0.05% Tween 20. Samples collected during the transcytosis experiments were added to the wells and incubated for 2 h at RT before washing as above. Captured antibody variants, IFX or total IgG were detected using an alkaline phosphatase (ALP)-conjugated anti-human Fc specific antibody from goat. Binding was visualized by addition of 100 μl ALP-substrate and the 405 nm absorption spectrum was recorded. The amount of antibody variants, IFX and total IgG transported were calculated from standard curves of each of the individual antibody variants.

Transcytosis of antibody variants across polarized human epithelial cells

Results:

[0115] The engineered anti-TNFα antibody variants were tested for transcytosis across a cell monolayer and compared to the wt antibody or IFX as another human IgG1 anti-TNFα antibody. The results are depicted in Figure 2. The wt anti-TNFα antibody was transported from the apical to the basolateral reservoir. Compared to IFX, another IgG1 antibody with a wt Fc region, 2.8-fold more Ab-REW was transported which was also the case for Ab-REW-2FF.

Transcytosis of antibody variants across polarized human epithelial cells in the presence of competing IgG

Results:

[0116] The total amount of immunoglobulin transported across a polarized T84 cell monolayer from the apical to the basolateral reservoir when the anti-TNFα antibody variants were incubated with a 10-fold excess of human myeloma IgG at 4 hours post adding was comparable for all antibodies. However, an increased affinity to FcRn at pH 6.0 resulted in a significantly higher percentage of specific anti-TNFα transport across the cell monolayer also in the presence of an excess of competing human IgG with irrelevant specificity. The results are depicted in Figure 3.

Example 6. ADCC

Method:

[0117] An ADCC reporter bioassay core kit from Promega was used. Briefly, mTNFα CHO-K1 target cells at 1×10^5/mL were seeded on white (clear bottom) tissue culture plates, 100 μL per well. The plates were incubated O/N at

37°C/5% CO_2. On day 2, 95 μL of assay medium was removed and replaced with 25 μL of engineered Jurkat effector cells at 3×10^6/mL. The plates were then incubated for 6 h at 37°C/5% CO_2. The BioGlo™ reagent was prepared towards the end of the incubation. Plates were equilibrated to RT for 10 - 20 min before adding 75 μL of BioGlo™ reagent per well. After 5 - 10 min of incubation in the dark, luminescence was measured. A 4-PL model was used to fit the data.

Results:

[0118] The results (see Figure 4) showed that all of the anti-TNFα antibodies induced ADCC but with distinct strengths. Compared to the wildtype antibody Ab-wt, the antibody variants showed increased ADCC. Specifically the non-fucosylated antibody variant Ab-REW-2FF had significantly improved ADCC.

Example 7. C1q binding

Method:

[0119] ELISA was performed using 96-well MaxiSorp plates where the wells were coated with human TNFa diluted to 1 μg/mL in PBS. After incubation O/N at 4°C, the plates were blocked with PBS containing 4% skimmed milk for 1 h and washed four times with PBS containing 0.05% Tween-20 (PBS-T). Then, titrated amounts of the anti-TNFα IgG antibodies were diluted in PBS-T, added and incubated for 1h at RT. After washing using PBS-T, human C1q (0.5 μg/mL) was diluted in 0.1 M Veronal buffer (0.25 mM $CaCl_2$ and 0.8 mM $MgCl_2$ pH 7.2), added to the wells and incubated for 1 h. Subsequently, the wells were washed as above before rabbit anti-human C1q diluted 1:5000 in PBS-T was added to the wells and incubated for 1 h. After washing, an HRP-conjugated anti-rabbit IgG from donkey diluted 1:5000 in PBS-T was added. Subsequently, the wells were washed and 100 μL 3,3',5,5'-Tetramethylbenzidine substrate was added to each well. The absorbance was measured at 620 nm using a Sunrise spectrophotometer.

Results:

[0120] The anti-TNFα antibody variants were captured on human TNFα before human C1q was added. The results (see Figure 5) showed that the antibodies bound C1q but with distinct binding strengths. Specifically, Ab-REW and Ab-REW-2FF bound somewhat stronger than IFX. The presence of fucose at the bi-antennary N-glucan attached to N297 had no or only a minor influence on binding. The binding hierarchy from strongest to weakest was as follows: Ab-REW > Ab-REW-2FF > IFX.

Example 8. CDC

Method:

[0121] The anti-TNFα CDC assay measured antibody dependent cytotoxicity of rabbit complement. Target cells expressing mTNFα were seeded into microplates in the presence of anti-TNFα antibodies that promoted the cytotoxic potential of complement. Six independent replicates of samples (anti-TNFα antibody variants) and 4 replicates of reference standard (IFX) were prepared at 60 μg/mL, serially diluted (1.3-fold dilution steps) and the dilution plates sealed until use. Target cells containing a LUC viability reporter were prepared to 1.5×10^5/mL and stored in a water bath at 37°C. Rabbit complement was diluted to 3-fold final assay concentration in DMEM High Glucose. Immediately after preparation, complement was combined with target cells in a 1:1 ratio (v/v). Forty μL of the complement/target cell preparation were transferred to each well of the assay plate. The antibodies prepared in the dilution plate were transferred to the cells (20 μL/well) and the plate was then incubated at 36°C/1% CO_2 for 3.5 h. Assay plates were equilibrated to RT in the dark for 35 min. Steady Glo which was equilibrated to ambient temperature for 120 min before use, was added to the assay plate (20 μL/well) and stored at RT in the dark for 35 min before luminescence was measured. Percent cell death was then calculated for each concentration of each sample and a 4-PL model was used to fit the data.

Results:

[0122] The results are shown in Figure 6 and Table 7. The relative EC_{50} and relative maximal % death results for test sample performance in the CDC assay provided a clear activity ranking that was consistent across both activity measures. Ab-REW showed greater CDC activity than IFX, irrespective of fucose content. In a direct comparison between fucose variant samples, to better understand the impact of fucose content on CDC activity, Ab-REW-2FF in comparison to Ab-REW, provided similar CDC activity responses. The comparison showed a relative EC_{50} response of 97.1% and a relative maximal % death response of 100.4%.

Table 7: CDC activity of anti-TNFα antibody variants in terms of relative EC_{50} and relative % death performance in comparison to IFX

Sample ID	Relative EC50 (%)	Relative Maximal Death (%)
IFX	100	100
Ab-REW	123.7	113.8
Ab-REW-2FF	120.1	114.2

Example 9. Induction of regulatory macrophages

Method:

[0123] Peripheral blood mononuclear cells (PBMC) were isolated from healthy buffy coats. Cells were isolated through Ficoll gradient centrifugation. Cells of two individual donors were mixed in equal numbers and 2×10^5 cells of the mixture were plated in 96 well plates in a total volume of 100 μL/well. Cells were incubated for 48 h at 37°C/5% CO_2. After 48 h, anti-TNFa antibody variants or IFX were added to reach a final concentration of 10 μg/mL. Each compound was added in replicates of five or six. Final volume was 150 μL/well. Human serum IgG1 (Sigma #I5154) was used as control. After addition of the compounds, mixed lymphocyte reactions (MLRs) were cultured for another 4 days at 37°C/5% CO_2. Afterwards, plates were washed using PBS/5 mM EDTA (PBS/EDTA) and incubated with 50 μL/well PBS/ EDTA for 20 min at RT. Plates were centrifuged and liquid was flicked out. Antibody was diluted in PBS/EDTA (anti-CD14-PE, anti-CD206-APC, both diluted 1:10). Cells were resuspended in 50 μL of antibody solution and incubated for 20 min at RT. Afterwards, cells were washed with PBS/EDTA and resuspended in 50 μL PBS/EDTA. Stained samples were analysed on a FACS Fortessa using FACSDiva software. Analysis was performed using FlowJo software.

Results:

[0124] Induction of regulatory macrophages was analysed in four independent MLRs and was successful in all experiments (comparing IFX to IgG control). The results are shown in Figure 7. The levels of induction by IFX can differ between experiments due to the fact that each experiment was performed using different donors with inter-individual variation. All tested anti-TNFα antibody variants induced $CD14^+CD206^+$ regulatory macrophages with slight variation between the compounds. Ab-REW and Ab-REW-2FF induced slightly more regulatory macrophages than IFX, however, only in case of Ab-REW-2FF the increase was significant.

Example 10. Inhibition of T-cell proliferation

Method:

[0125] PBMC were isolated from healthy buffy coats. Cells were isolated through Ficoll gradient centrifugation. Cells of two individual donors were mixed in equal numbers and 2×10^5 cells of the mixture were plated in 96 well plates in a total volume of 100 μL/well. Cells were incubated for 48 h at 37°C/5% CO_2. After 48 h, anti-TNFα antibody variants or IFX were added to reach a final concentration of 10 μg/mL. Each compound was added in replicates of five or six. Final volume was 150 μL/well. Human serum IgG1 (Sigma #15154) was used as control. After addition of the compounds, mixed lymphocyte reactions (MLRs) were cultured for another 2 days at 37°C/5% CO_2. Afterwards, tritiated thymidine (^{3}H thymidine, 0.5 microCurie/well) was added to the cultures. Cultures were further incubated for 18 h at 37°C/5% CO_2. Samples were harvested using a Microbeta Filtermat 96 cell harvester and analysed using a Microbeta MicroplateCounter equipped with a single detector. Samples were counted for 10 seconds/well and converted to counts per minute (cpm).

Results:

[0126] Inhibition of T-cell proliferation was measured in three independent MLRs and was defined as successful if IFX as positive control induced suppression. The levels of suppression by IFX in individual experiments can differ presumably due to the variance in regulatory macrophage induction. In each experiment, the potential of the anti-TNFα antibody variants to suppress T-cell proliferation was calculated relative to the positive control IFX. Antibody Ab-REW-2FF showed significantly enhanced suppression compared to IFX while suppression by Ab-REWwas comparable to IFX (see Figure 8).

Example 11. Analysis of N-glycans

Method:

[0127] 50 μl of each IgG variant (1 mg/ml) was spun down for 10 min at 13,000×g before 1 μg trypsin dissolved in 100 μl 50 mM ammonium bicarbonate (pH 7.8) was added and incubated overnight at 37°C. The centrifugal devices were spun down at 13,000×g for 10 min, and the flow-through was transferred to an Eppendorf tube and dried in a SpeedVac (Heto Maxi dry). Dried samples were dissolved in 20 μl 1% formic acid, sonicated for 30 s, and centrifuged for 10 min at 16,100×g. Subsequently, each sample was transferred to new vials, and reverse phase (C18) nano online liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of proteolytic peptides was performed using a Dionex Ultimate 3000 UHPLC systems (Thermo Fisher Scientific, USA). 5 μl of peptide solution was injected into the extraction column and peptides were eluted in the back-flush mode from the extraction column onto the analytical column. The mobile phase consists of acetonitrile and mass spectrometry grade water, both containing 0.1 % formic acid. Chromatographic separation was achieved using a binary gradient from 3 to 50 % of acetonitrile in water for 60 minutes with a flow rate of 0.3 μl/min. The LC system was coupled via a nanoelectrospray ion source to a Q exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific, USA). Peptide samples were analyzed with a high energy collisional dissociation (HCD) fragmentation method with normalized collision energy at 20, acquiring one Orbitrap survey scan in the mass range of m/z 300-2000 followed by MS/MS of the ten most intense ions in the Orbitrap.

[0128] Data analysis was performed on Xcalibur v2.0. MS/MS spectra for all N-glyco-peptides were extracted by oxonium ion search; 204.086 (N-acetylhexosamine) and 366.1388 (N-acetylhexosamine-hexose). By using HCD fragmentation with normalized collision energy at 20 the glycan structure and the peptide mass for IgG were detected. Extracted ion chromatograms for target glycol-peptides (EEQYNSTYR for IgG1) were extracted with 10 ppm accuracy and the corresponding MS/MS spectra were manually verified. HCD fragmentation with normalized collision energy at 35 was used to detect the peptide sequence and to verify that the peptide mass corresponded to the correct peptide sequence. The area under the curve for all extracted glycol-peptides was calculated and the percentage ratio for each glycoform was determined.

Results:

[0129] For Ab-REW two N-glycan forms dominated and corresponded to > 90% of the total N-glycan pool, namely 4GlcNac-1 Fuc-3Man and 4GlcNac-1 Fuc-3Man-1Gal. Both the N-glycan forms that dominated contained a core fucose. To produce the "non-fucosylated" version of Ab-REW (Ab-REW-2FF), the decoy substrate 2-deoxy-2-fluoro-1-fucose (2FF) was used. MS mapping of this antibody revealed that this strategy successfully resulted in greatly reduced incorporation of fucose, as a drop from > 90% to 13% was detected. The dominating N-glycan forms after treatment were the same as the variants produced in the absence of 2FF, except that these structures lacked fucose (see also Figure 10).

Table 8: Percentage of N-glycan forms attached to N297 of anti-TNFα IgG antibodies

***N*-glycan structure**	**Ab-REW (%)**	**Ab-REW-2FF (%)**
4GlcNac-1Fuc-3Man	69.1	8.5
4GlcNac-1Fuc-3Man-1Gal	21.7	4.5
4GlcNac-3Man	0.7	64.3
4GlcNac-3Man-1Gal	0	17.3
Other glycosylation patterns	8.5	5.4

Claims

1. An IgG_1 antibody comprising a TNFα-binding domain and an FcRn-binding site, having a high affinity to human FcRn at pH 6, said high affinity being **characterized by** a dissociation equilibrium constant (K_D) determined by surface plasmon resonance of less than 100 nM, and further having no affinity or a low affinity to human FcRn at pH 7.4, said low affinity being **characterized by** a K_D determined by surface plasmon resonance, of greater than 10 μM, wherein the amino acid sequence of the antibody comprises the amino acids 311R, 428E and 434W as defined by EU numbering, and wherein the antibody comprises (i) a V_L domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:3, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:4, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:5, and (ii) a V_H domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:6, a CDR2 region having the

amino acid sequence as shown in SEQ ID NO:7, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8.

2. The antibody of claim 1, which is a non-fucosylated antibody or an antibody having reduced fucosylation.

3. The antibody of claim 1 or 2, comprising a heavy chain which comprises the amino acid sequence as shown in SEQ ID NO:13.

4. The antibody of any one of the preceding claims, comprising a V_H domain having the amino acid sequence as shown in SEQ ID NO:9 and a V_L domain having the amino acid sequence as shown in SEQ ID NO:10.

5. The antibody of any one of the preceding claims, comprising a light chain having the amino acid sequence as shown in SEQ ID NO:1 and a heavy chain having the amino acid sequence as shown in SEQ ID NO:11.

6. The antibody of any one of the preceding claims for use in the treatment of an inflammatory condition.

7. The antibody for use according to claim 6, wherein the inflammatory condition is an inflammatory disorder of the gastrointestinal tract.

8. The antibody for use according to claim 6 or 7, wherein said treatment comprises orally administering an effective amount of said antibody.

9. The antibody for use according to claim 6 or 7, wherein said antibody is applied topically.

10. A pharmaceutical composition comprising the antibody of any one of claims 1 or 5.

Patentansprüche

1. IgG1-Antikörper, umfassend eine TNFα-bindende Domäne und eine FcRn-Bindungsstelle, der eine hohe Affinität für humanen FcRn bei pH 6 aufweist, wobei die hohe Affinität durch eine Dissoziationsgleichgewichtskonstante (K_D), bestimmt durch Oberflächen-Plasmonresonanz, von weniger als 100 nM gekennzeichnet ist, und ferner keine Affinität oder eine geringe Affinität für humanen FcRn bei pH 7,4 aufweist, wobei die geringe Affinität durch eine K_D, bestimmt durch Oberflächen-Plasmonresonanz, von mehr als 10 μM gekennzeichnet ist, wobei die Aminosäuresequenz des Antikörpers die Aminosäuren 311R, 428E und 434W, definiert durch EU-Nummerierung, umfasst und wobei der Antikörper (i) eine VL-Domäne, umfassend eine CDR1-Region mit der in SEQ ID NO:3 gezeigten Aminosäuresequenz, eine CDR2-Region mit der in SEQ ID NO:4 gezeigten Aminosäuresequenz und eine CDR3-Region mit der in SEQ ID NO:5 gezeigten Aminosäuresequenz, und (ii) eine VH-Domäne, umfassend eine CDR1-Region mit der in SEQ ID NO:6 gezeigten Aminosäuresequenz, eine CDR2-Region mit der in SEQ ID NO:7 gezeigten Aminosäuresequenz und eine CDR3-Region mit der in SEQ ID NO:8 gezeigten Aminosäuresequenz umfasst.

2. Antikörper nach Anspruch 1, der ein nicht-fucosylierter Antikörper oder ein Antikörper mit reduzierter Fucosylierung ist.

3. Antikörper nach Anspruch 1 oder 2, umfassend eine schwere Kette, die die in SEQ ID NO:13 gezeigte Aminosäuresequenz umfasst.

4. Antikörper nach einem der vorstehenden Ansprüche, umfassend eine VH-Domäne mit der in SEQ ID NO:9 gezeigten Aminosäuresequenz und eine VL-Domäne mit der in SEQ ID NO:10 gezeigten Aminosäuresequenz.

5. Antikörper nach einem der vorstehenden Ansprüche, umfassend eine leichte Kette mit der in SEQ ID NO:1 gezeigten Aminosäuresequenz und eine schwere Kette mit der in SEQ ID NO:11 gezeigten Aminosäuresequenz.

6. Antikörper nach einem der vorstehenden Ansprüche zur Verwendung bei der Behandlung eines Entzündungsleidens.

7. Antikörper zur Verwendung nach Anspruch 6, wobei das Entzündungsleiden eine Entzündungsstörung des Magen-Darm-Traktes ist.

8. Antikörper zur Verwendung nach Anspruch 6 oder 7, wobei die Behandlung das orale Verabreichen einer wirksamen Menge des Antikörpers umfasst.

9. Antikörper zur Verwendung nach Anspruch 6 oder 7, wobei der Antikörper topisch angewandt wird.

10. Pharmazeutische Zusammensetzung, umfassend den Antikörper nach einem der Ansprüche 1 oder 5.

Revendications

1. Anticorps IgG_1 comprenant un domaine de liaison au TNFa et un site de liaison au FcRn, ayant une forte affinité avec le FcRn humain à pH 6, ladite forte affinité étant **caractérisée par** une constante d'équilibre de dissociation (KD) déterminée par résonance plasmonique de surface inférieure à 100 nM, et n'ayant en outre aucune affinité ou une faible affinité avec le FcRn humain à pH 7,4, ladite faible affinité étant **caractérisée par** une K_D déterminée par résonance plasmonique de surface, supérieure à 10 μM, dans lequel la séquence d'acides aminés de l'anticorps comprend les acides aminés 311R, 428E et 434W tels que définis par la numérotation UE, et dans lequel l'anticorps comprend (i) un domaine V_L comprenant une région CDR1 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 3, une région CDR2 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 4, et une région CDR3 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 5, et (ii) un domaine V_H comprenant une région CDR1 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 6, une région CDR2 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 7, et une région CDR3 ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 8.

2. Anticorps de la revendication 1 qui est un anticorps non fucosylé ou un anticorps ayant une fucosylation réduite.

3. Anticorps de la revendication 1 ou 2, comprenant une chaîne lourde qui comprend la séquence d'acides aminés indiquée dans SEQ ID NO : 13.

4. Anticorps de l'une quelconque des revendications précédentes, comprenant un domaine V_H ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 9 et un domaine V_L ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 10.

5. Anticorps de l'une quelconque des revendications précédentes, comprenant une chaîne légère ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 1 et une chaîne lourde ayant la séquence d'acides aminés indiquée dans SEQ ID NO : 11.

6. Anticorps de l'une quelconque des revendications précédentes pour une utilisation dans le traitement d'une condition inflammatoire.

7. Anticorps utilisé selon la revendication 6, dans lequel la condition inflammatoire est un trouble inflammatoire du tractus gastro-intestinal.

8. Anticorps à utiliser selon la revendication 6 ou 7, dans lequel ledit traitement comprend l'administration orale d'une quantité efficace dudit anticorps.

9. Anticorps à utiliser selon la revendication 6 ou 7, dans lequel ledit anticorps est appliqué par voie topique.

10. Composition pharmaceutique comprenant l'anticorps de l'une quelconque des revendications 1 ou 5.

Figure 1

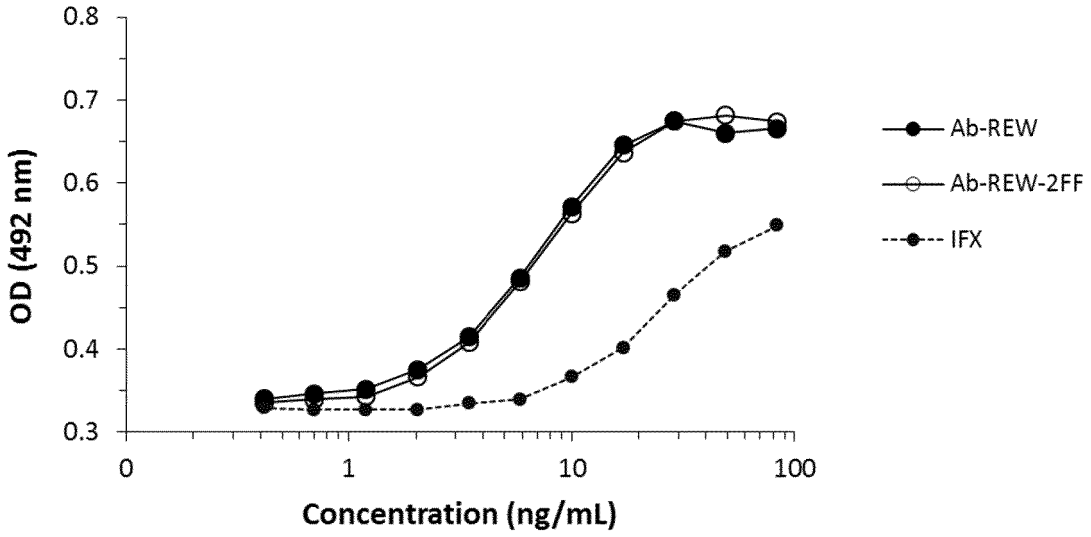


Figure 2

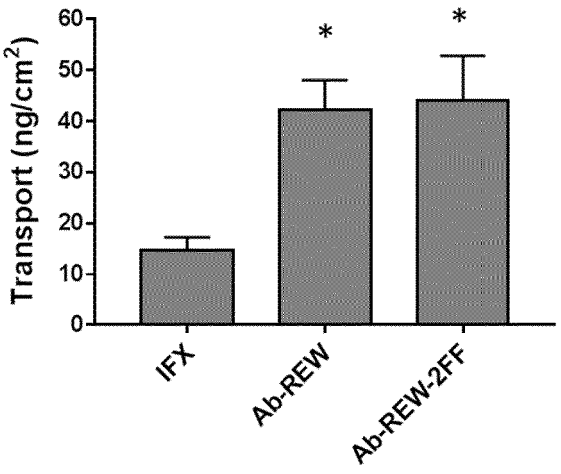


Figure 3

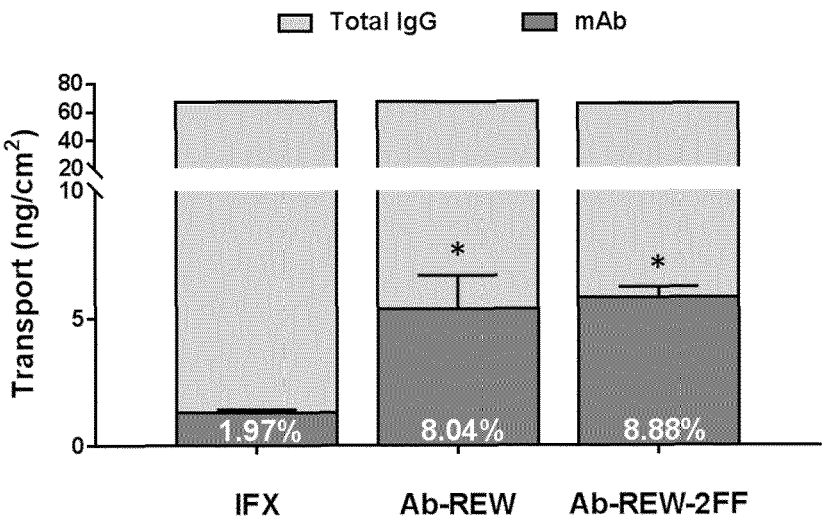


Figure 4

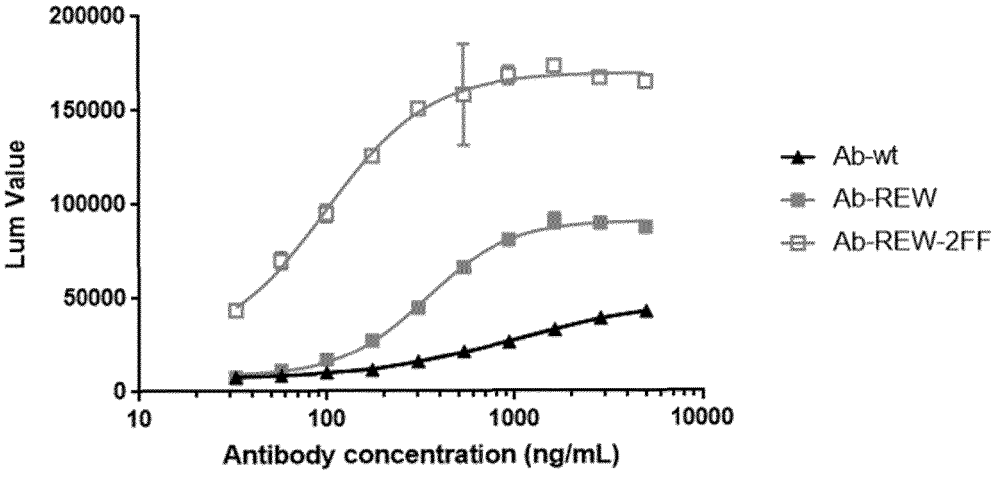


Figure 5

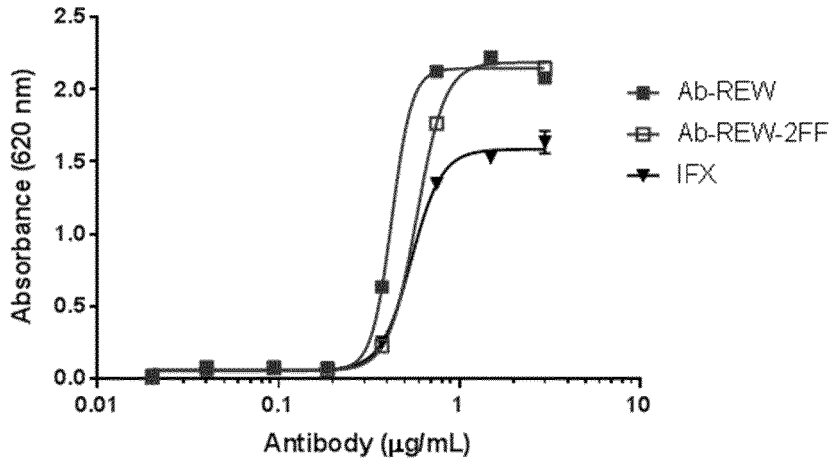
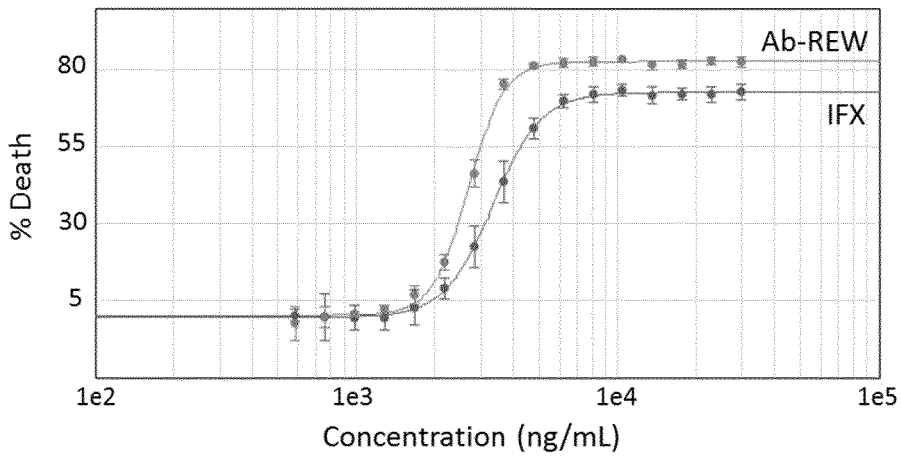


Figure 6



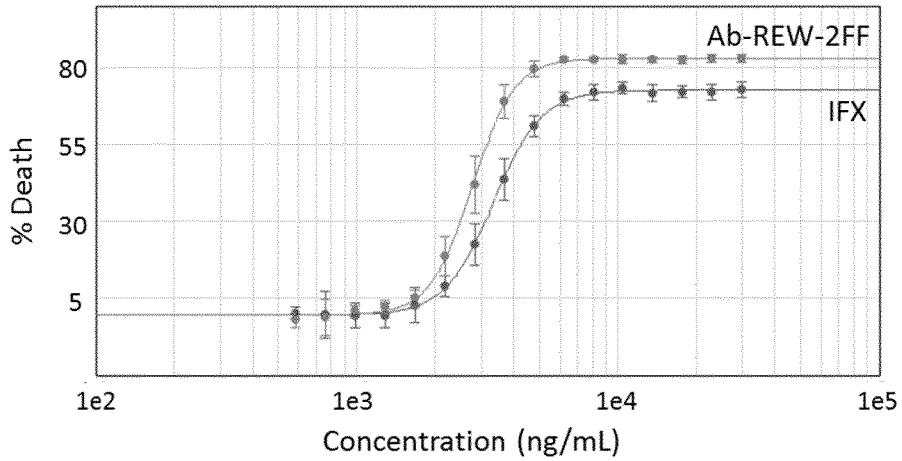


Figure 7

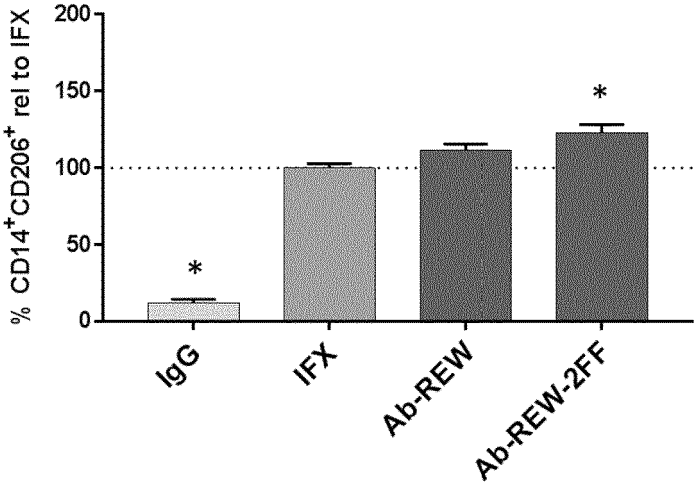


Figure 8

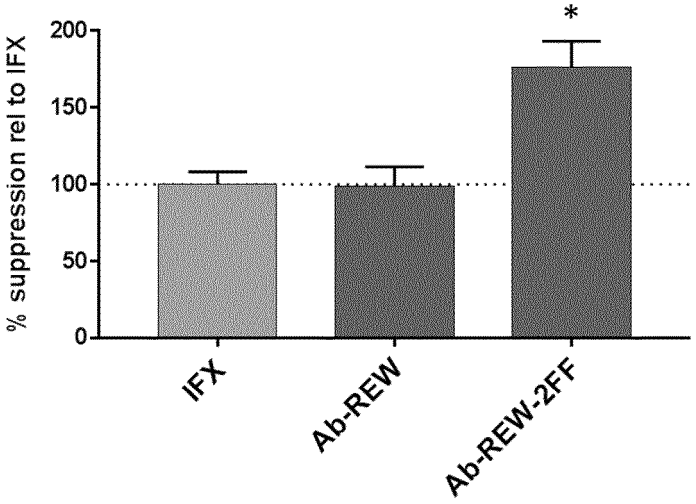


Figure 9

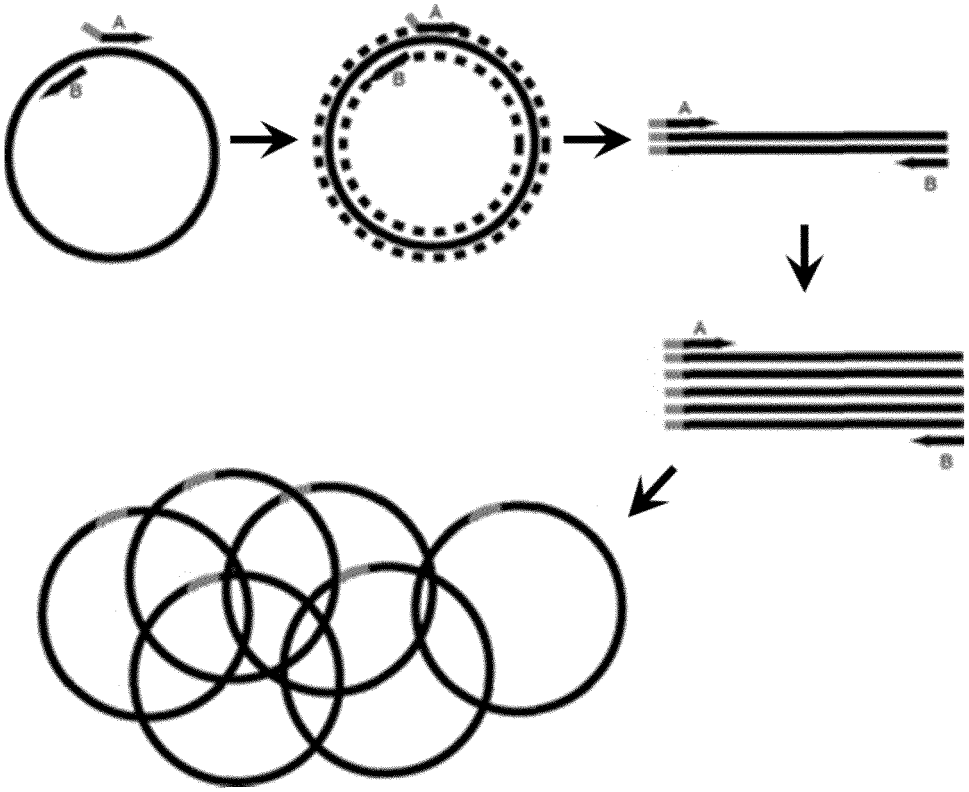
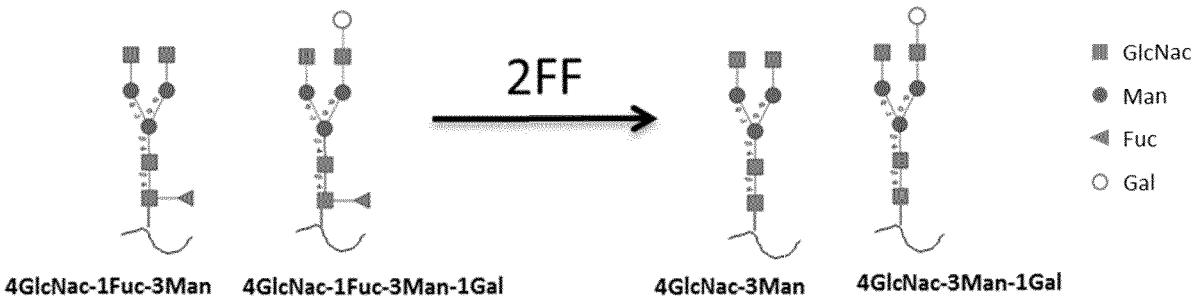


Figure 10



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2006073941 A **[0029]**

Non-patent literature cited in the description

- **SAXENA.** *Frontiers in Immunology,* 2016, vol. 7 **[0005]**
- **DENG et al.** *Drug Metabolism and Disposition,* 2010, vol. 38 (4), 600-605 **[0006]**
- **YEUNG et al.** *The Journal of Immunology,* 2009, vol. 182 (12), 7663-7671 **[0007]**
- **KUO et al.** *MABS,* 2011, vol. 3 (5), 422-430 **[0008]**
- **SUZUKI et al.** *The Journal of Immunology,* 2010, vol. 184 (4), 1968-1976 **[0009]**
- **YASMINA NOUBIA ABDICHE et al.** found that the neonatal Fc receptor (FcRn) binds independently to both sites of the IgG homodimer with identical affinity. *MABS,* 2015, vol. 7 (2), 331-343 **[0010]**
- **WARD et al.** *Molecular Immunology,* 2015, vol. 67 (2), 131-141 **[0011]**
- **RAJPAL et al.** Therapeutic Fc-fusion proteins. Wiley-VCH Verlag GmbH & Co. KGaA, 2014, 1-44 **[0012]**
- **KABAT et al.** Sequences of Immunological Interest. Public Health Service, 1991 **[0029]**
- **KABAT et al.** Sequences of Proteins of Immunological Interest. Public Health Service, National Institutes of Health, Bethesda, 1991 **[0029]**
- **HARLOW ; LANE.** Antibodies, A Laboratory Manual. CSH Press, 1988 **[0031]**
- **KABAT E. A. et al.** Sequences of proteins of immunological interest. 1991 **[0035]**
- Antibody Engineering - Methods and Protocols. 2012 **[0039]**
- **MALPHETTES et al.** *Biotechnol. Bioeng.,* 2010, vol. 106, 774-783 **[0053]**
- **DEKKER et al.** *Sci Rep,* 2016, vol. 6, 36964 **[0054]**
- **SON et al.** *Glycobiology,* 2011, vol. 21, 1019-1028 **[0055]**
- Remington's Pharmaceutical Sciences. 1980 **[0085]**
- **DEKKERS et al.** *Sci Rep,* 2016, vol. 6, 36964 **[0104]**