

HUMANIZED ANTI-C5A ANTIBODIES

FIELD OF THE INVENTION

5 The present invention relates to antibodies that specifically bind to a conformational epitope of human C5a. The invention particularly relates to humanized anti-C5a antibodies. The antibodies described herein are useful as active agents in pharmaceutical compositions. The antibodies and pharmaceutical compositions are especially useful for the treatment and prevention of diseases or disorders involving pathological C5a activity.

10 BACKGROUND OF THE INVENTION

C5a

C5a is a 74 amino acid spanning split product of its “mother molecule” C5 and represents one endpoint of the complement activation cascade. It can be generated through activation of at least three well-described pathways (the alternative, the classical and the MBL pathway). All pathways merge at the level of C3, forming the C5- or alternative C5 convertase leading to cleavage of C5 into C5a and C5b. The latter binds with C6, C7, C8 and multiple C9 molecules ultimately leading to formation of pores in e.g. bacterial membranes (terminal Membrane Attack Complex = MAC). C5a is generated when the complement system is activated in settings of inflammation and other immunological and inflammatory disorders / diseases.

20 Among the complement activation products, C5a is one of the most potent inflammatory peptides, with a broad spectrum of functions (Guo and Ward 2005). C5a exerts its effects through the high-affinity C5a receptors (C5aR and C5L2) (Ward 2009). C5aR belongs to the rhodopsin family of G-protein-coupled receptors with seven transmembrane segments; C5L2 has a similar structure but appears not to be G-protein-coupled. It is currently believed that C5a exerts its biological functions primarily through C5a-C5aR interaction, as few biological responses have been found for C5a-C5L2 interaction. However, latest reports demonstrate signaling also through C5L2 activation (Rittirsch et al. 2008).

30 C5aR is widely expressed on myeloid cells including neutrophils, eosinophils, basophils, and monocytes, and non-myeloid cells in many organs, especially in the lung and liver, indicative of the importance of C5a/C5aR signaling. Widespread up-regulation of C5aR expression occurs during the onset of sepsis, and blockade of C5a/C5aR interaction by anti-

C5a, or anti-C5aR antibodies, or C5aR antagonists renders highly protective effects in rodent models of sepsis (Czermak et al. 1999; Huber-Lang et al. 2001; Riedemann et al. 2002).

C5a has a variety of biological functions (Guo and Ward 2005). C5a is a strong chemoattractant for neutrophils and also has chemotactic activity for monocytes and macrophages. C5a causes an oxidative burst (O_2 consumption) in neutrophils and enhances phagocytosis and release of granular enzymes. C5a has also been found to be a vasodilator. C5a has been shown to be involved in modulation of cytokine expression from various cell types and to enhance expression of adhesion molecule expression on neutrophils. High doses of C5a can lead to nonspecific chemotactic “desensitization” of neutrophils, thereby causing broad dysfunction. Many inflammatory diseases are attributable to the effects of C5a, including sepsis, acute lung injury, inflammatory bowel disease, rheumatoid arthritis and others. In the experimental setting of sepsis, exposure of neutrophils to C5a can lead to neutrophil dysfunction and paralysis of signaling pathways, leading to defective assembly of NADPH oxidase, paralysis of MAPK signaling cascades, a great depression of oxidative burst, phagocytosis and chemotaxis (Guo et al. 2006; Huber-Lang et al. 2002). Thymocytes apoptosis and delayed neutrophil apoptosis are two important pathogenic events for sepsis development, which are dependent on the presence of C5a. During experimental sepsis, C5a up-regulates $\beta 2$ -integrin expression on neutrophils to promote cell migration into organs, one of the major causes for multi-organ failure (MOF). It is also found that C5a is attributable to the activation of the coagulation pathway that occurs in experimental sepsis. C5a stimulates the synthesis and release from human leukocytes of pro-inflammatory cytokines such as $TNF-\alpha$, $IL-1\beta$, $IL-6$, $IL-8$, and macrophage migration inhibitory factor (MIF). Given that complement activation is an event occurring during the onset of acute inflammation, C5a may come into play before emergence of most of the inflammatory “cytokine storm”. It appears that C5a plays a key role in orchestrating and amplifying the performance of the cytokine network and the formation of systemic inflammatory response syndrome (SIRS).

In the immunological regulatory network tailing to the adaptive immunity, C5a affects the crosstalk between dendritic cells (DC) and $\gamma\delta$ T cells, and this may result in a large production of inflammatory mediators such as $IL-17$ (Xu et al. 2010). An essential role for C5a has been established and defined in the generation of pathogenic Th17 responses in systemic lupus erythematosus (SLE) (Pawaria et al. 2014). In addition, it has been reported that C5a is a key regulator for Treg cells offering a powerful suppressive effect for Treg propagation and induction (Strainic et al. 2013). Given the fact that Treg and TH17 are the essential players in

the autoimmune disease setting, inhibition of C5a signaling would be expected to significantly reduce overactive immune status in the autoimmune diseases.

IFX-1

5 IFX-1 is a chimeric monoclonal IgG4 antibody which specifically binds to the soluble human complement split product C5a. IFX-1 is composed of 1328 amino acids and has an approximate molecular weight of 148,472 Daltons. The CDR and FR sequences of IFX-1 are disclosed in Table 3 of WO 2015/140304 A1, also published as US 2017/0137499 A1. The contents of WO 2015/140304 A1 and US 2017/0137499 A1 are hereby incorporated by
10 reference in their entirety.

IFX-1 is expressed in a mammalian CHO cell line as recombinant protein and finally formulated in a phosphate buffered saline solution (PBS + 0.05% polysorbate 80) for intravenous administration. The binding of this antibody to human C5a facilitates a highly effective blockade of C5a-induced biological effects by disabling C5a binding to and reacting
15 with its corresponding cell-bound receptors.

Various nonclinical studies were conducted to assess pharmacological and toxicological aspects of IFX-1, which can be divided into *in vitro* / *ex vivo* tests and *in vivo* studies including GLP toxicology studies in cynomolgus monkey (using IFX-1). None of the conducted nonclinical tests and studies revealed any toxicological or safety concerns for IFX-1. Human
20 Phase I trial indicated that safety laboratory parameters, vital signs and ECG parameters showed no clinically relevant time- or dose-related changes.

In vitro analysis of IFX-1 demonstrates a strong binding capacity to soluble human C5a as well as a high blocking activity of C5a-induced biological effects such as lysozyme release from human neutrophils or CD11b up-regulation in neutrophils in human whole blood. One
25 IFX-1 antibody reaches the capability of neutralizing the effects of 2 molecules C5a with close to 100% efficiency in experimental *in vitro* settings. Clinical trials with IFX-1 have been ongoing to test its clinical efficacy in several inflammatory diseases.

TECHNICAL PROBLEMS UNDERLYING THE PRESENT INVENTION

30 Antibodies that specifically bind to the C5a part but not to the C5b part of C5 have been described in the prior art (Klos et al. (1998) J. Immunol. Meth. 111: 241-252; WO 01/15731; WO 03/015819). Previously generated anti-C5a antibodies exhibited only moderate blocking activities on biological effects induced by C5a. In consequence, anti-C5a antibodies of the prior art were either not capable of achieving a complete blockade of C5a-induced biological effects

or had to be used in superstoichiometric amounts to reach a reasonably high blockage of C5a activity. The inventors succeeded in producing two monoclonal anti-C5a antibodies (named INab308 and INab708) that exhibit a strong blocking activity to C5a-induced biological effects, even when employed in stoichiometric amounts, i.e. 0.5 mole of a bivalent antibody per mole of C5a (see WO 2011/063980 A1, the content of which is incorporated herein by reference).
Based on monoclonal antibody INab308, the inventors developed chimeric antibody IFX-1, which exhibits the same strong blocking activity (see WO 2015/140304 A1, the content of which is incorporated herein by reference).

However, the two monoclonal antibodies INab308 and INab708 specifically described in WO 2011/063980 A1 are murine antibodies. Antibody IFX-1 described in WO 2015/140304 A1 is a chimeric antibody. Consequently, these antibodies may elicit unwanted immunological responses when administered to human beings.

Thus, in view of an intended clinical use in patients, there remained a need in the prior art for anti-C5a antibodies that more closely resemble human antibodies but still exhibit excellent blockage of C5a activity.

The present inventors have now succeeded in producing humanized anti-C5a antibodies that have greatly improved humanness as compared to the antibodies described in WO 2011/063980 A1 and WO 2015/140304 A1, while still maintaining the advantageous properties of the antibodies described in WO 2011/063980 A1 and WO 2015/140304 A1, namely exhibiting the same high blocking activity to C5a-induced biological effects without affecting the biological activities of C5b.

The above overview does not necessarily describe all problems solved by the present invention.

SUMMARY OF THE INVENTION

In a first aspect the present invention relates to an antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain (VH) and a light chain variable domain (VL),

wherein said VH domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 10 (QVQLVQSGAE VKKPGASVKV SCKASGYSFT TFWMDWVRQA PGQGLEWIGR IDPSDSESRL DQRFKDRV TM TVDKSTSTVY MELSSLRSED TAVYYCARGN DGY YGFAYWG QGTLVTVSS, VH4) or

an amino acid sequence having at least 80% sequence identity with SEQ ID NO: 10, wherein said amino acid sequence having at least 80% sequence identity with SEQ ID NO: 10 comprises the CDR1H, CDR2H, and CDR3H sequences of SEQ ID NO: 20 to 22, respectively, and wherein said amino acid sequence having at least 80% sequence identity with SEQ ID NO: 10 comprises a V at amino acid position 5, an E at amino acid position 10, a K at amino acid position 12, a K at amino acid position 13, an A at amino acid position 16, an A at amino acid position 40, and/or a T at the amino acid position 76, and wherein said VL domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 16 (DIQMTQSPSS LSASVGDRVT ITCKASQSVD YDGDSYMKWY QQKPGKAPKL LIYAASNLQS GVPSRFSGSG SGTDFTLTIS SLQPEDFATY YCQQSNEDPY TFGQGTKLEI K, V_K4) or

an amino acid sequence having at least 80% sequence identity with SEQ ID NO: 16, wherein said amino acid sequence having at least 80% sequence identity with SEQ ID NO: 16 comprises the CDR1L, CDR2L, CDR3L sequences of SEQ ID NO: 23 to 25, respectively, and wherein said amino acid sequence having at least 80% sequence identity with SEQ ID NO: 16 comprises an A at amino acid position 13, a V at amino acid position 15, a D at amino acid position 17, a V at amino acid position 19, a T at amino acid position 22, a K at amino acid position 46, an A at amino acid position 47, an S at amino acid position 64, a T at amino acid position 78, an S at amino acid position 80, an S at amino acid position 81, an L at amino acid position 82, a Q at amino acid position 83, an F at amino acid position 87, and/or a Q at amino acid position 104.

In a second aspect the present invention relates to an antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain (VH) and a light chain variable domain (VL),

wherein said VH domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 17 (QVQLVQSGX⁹E X¹¹KKPGASVKX²⁰ SCKASGYSFT TFWMDWVX³⁸QA PGQGLEWX⁴⁸GR IDPSDSESRL DQX⁶³FKDRX⁶⁸TX⁷⁰ TVDKSTSTVY MX⁸²LSSX⁸⁶X⁸⁷SED X⁹¹AVYYCARGN DGYYGFAFWG QGTLTVVSS), wherein X⁹ is A or P, X¹¹ is L or V, X²⁰ is I or V, X³⁸ is K or R, X⁴⁸ is I or M, X⁶³ is K or R, X⁶⁸ is A or V, X⁷⁰ is L or M, X⁸² is E or Q, X⁸⁶ is L or P, X⁸⁷ is R or T, and X⁹¹ is S or T, or

an amino acid sequence according to SEQ ID NO: 17 having one, two or three amino acid substitutions, wherein said amino acid sequence having one, two or three amino acid

substitutions comprises the CDR1H, CDR2H, CDR3H sequences of SEQ ID NO: 20 to 22, respectively, and

wherein said VL domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 18 (DIX³X⁴TQSPX⁹S

5 LX¹²ASVGDRVT ITCKASQSVD YDGDSYMKWY QQKPGKAPKL LIYAASNLS
GX⁶²PSRFSGSG SGTDFTLTIS SLQX⁸⁴EDFATY YCQQSNEDPY TFGQGTKLEI K),
wherein X³ is V or Q, X⁴ is L or M, X⁹ is A or S, X¹² is A or S, X⁶² is I or V, and X⁸⁴ is E or
P, or

an amino acid sequence according to SEQ ID NO: 18 having one, two, three, four,
10 five, six, or seven amino acid substitutions, wherein said amino acid sequence having one,
two, three, four, five, six, or seven amino acid substitutions comprises the CDR1L, CDR2L,
CDR3L sequences of SEQ ID NO: 23 to 25, respectively.

In a third aspect the present invention relates to a pharmaceutical composition
15 comprising:

the antibody or antigen-binding fragment thereof according to the first aspect or the
antibody or antigen-binding fragment thereof according to the second aspect; and

further comprising one or more pharmaceutically acceptable carriers, diluents,
excipients, fillers, binders, lubricants, glidants, disintegrants, adsorbents, and/or preservatives.

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In a fourth aspect the present invention relates to the antibody or antigen-binding
fragment thereof according to the first aspect or the antibody or antigen-binding fragment
thereof according to the second aspect for use in medicine.

25 In a fifth aspect the present invention relates to the antibody or antigen-binding
fragment thereof according to the first aspect or the antibody or antigen-binding fragment
thereof according to the second aspect for use in the treatment or prevention of a disease or
disorder involving pathological C5a activity.

30 This summary of the invention does not necessarily describe all features of the present
invention. Other embodiments will become apparent from a review of the ensuing detailed
description.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Binding of IFX clones to rhC5a at different concentrations in a comparable dose-dependent manner. The parent molecule IFX-1 was marked as VH0Vk0.

Figure 2. Plotting of sample hemolysis (%) and plasma dilution factor (-fold) to calculate the CH50. The parent molecule IFX-1 was marked as VH0Vk0.

- 5 **Figure 3.** Anaphylatoxin rhC5a induced-CD11b upregulation and its individual blockade by 12 IFX-2 clones at the Ag:Ab molar ratio of 1:1 in comparison to that by IFX-1 (testAB-IgG4-004).

DETAILED DESCRIPTION OF THE INVENTION

10 Definitions

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of
15 the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and
20 Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

25 Several documents (for example: patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.) are cited throughout the text of this specification. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Some of the documents cited herein are characterized as being
30 "*incorporated by reference*". In the event of a conflict between the definitions or teachings of such incorporated references and definitions or teachings recited in the present specification, the text of the present specification takes precedence.

Sequences: All sequences referred to herein are disclosed in the attached sequence listing that, with its whole content and disclosure, is a part of this specification.

As used herein, “human C5a” refers to the following 74 amino acid peptide:

TLQKKIEEIA AKYKHSVVKK CCYDGACVNN DETCEQRAAR ISLGPRCIKA
FTECCVVASQ LRANISHKDM QLGR (SEQ ID NO: 1).

- 5 The amino acid sequence of human C5 can be found under the accession number UniProtKB P01031 (CO5_HUMAN). The terms “human C5a” and “hC5a” are used interchangeably herein.

- As used herein, a first compound (e.g. an antibody) is considered to “bind” to a second compound (e.g. an antigen, such as a target protein), if it has a dissociation constant K_d to said
10 second compound of 1 μM or less, preferably 900 nM or less, more preferably 800 nM or less, more preferably 700 nM or less, more preferably 600 nM or less, more preferably 500 nM or less, more preferably 400 nM or less, more preferably 300 nM or less, more preferably 200 nM or less, even more preferably 100 nM or less, even more preferably 90 nM or less, even more preferably 80 nM or less, even more preferably 70 nM or less, even more preferably 60 nM or less,
15 less, even more preferably 50 nM or less, even more preferably 40 nM or less, even more preferably 30 nM or less, even more preferably 20 nM or less, even more preferably 10 nM or less, even more preferably 5 nM or less, even more preferably 4 nM or less, even more preferably 3 nM or less, even more preferably 2 nM or less, and even more preferably 1 nM or less.

- 20 The term “binding” according to the invention preferably relates to a specific binding. “Specific binding” means that a binding moiety (e.g. an antibody) binds stronger to a target such as an epitope for which it is specific compared to the binding to another target. A binding moiety binds stronger to a first target compared to a second target if it binds to the first target with a dissociation constant (K_d) which is lower than the dissociation constant for the second
25 target. Preferably the dissociation constant (K_d) for the target to which the binding moiety binds specifically is more than 10-fold, preferably more than 20-fold, more preferably more than 50-fold, even more preferably more than 100-fold, 200-fold, 500-fold or 1000-fold lower than the dissociation constant (K_d) for the target to which the binding moiety does not bind specifically.

- As used herein, the term “ K_d ” (usually measured in “mol/L”, sometimes abbreviated as
30 “M”) is intended to refer to the dissociation equilibrium constant of the particular interaction between a binding moiety (e.g. an antibody or fragment thereof) and a target molecule (e.g. an antigen or epitope thereof).

Methods for determining binding affinities of compounds, i.e. for determining the dissociation constant K_d , are known to a person of ordinary skill in the art and can be selected

for instance from the following methods known in the art: Surface Plasmon Resonance (SPR) based technology, Bio-layer interferometry (BLI), enzyme-linked immunosorbent assay (ELISA), flow cytometry, isothermal titration calorimetry (ITC), analytical ultracentrifugation, radioimmunoassay (RIA or IRMA) and enhanced chemiluminescence (ECL). Typically, the dissociation constant K_d is determined at 20°C, 25°C, 30°C, or 37°C. If not specifically indicated otherwise, the K_d values recited herein are determined at 20°C by SPR.

An “epitope”, also known as antigenic determinant, is the part of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. As used herein, an “epitope” is the part of a macromolecule capable of binding to a compound (e.g. an antibody or antigen-binding fragment thereof) as described herein. In this context, the term “binding” preferably relates to a specific binding. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes can be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

As used herein, a “conformational epitope” refers to an epitope of a linear macromolecule (e.g. a polypeptide) that is formed by the three-dimensional structure of said macromolecule. In the context of the present application, a “conformational epitope” is a “discontinuous epitope”, i.e. the conformational epitope on the macromolecule (e.g. a polypeptide) which is formed from at least two separate regions in the primary sequence of the macromolecule (e.g. the amino acid sequence of a polypeptide). In other words, an epitope is considered to be a “conformational epitope” in the context of the present invention, if the epitope consists of at least two separate regions in the primary sequence to which an antibody of the invention (or an antigen-binding fragment thereof) binds simultaneously, wherein these at least two separate regions are interrupted by one or more regions in the primary sequence to which an antibody of the invention (or an antigen-binding fragment thereof) does not bind. Preferably, such a “conformational epitope” is present on a polypeptide, and the two separate regions in the primary sequence are two separate amino acid sequences to which an antibody of the invention (or an antigen-binding fragment thereof) binds, wherein these at least two separate amino acid sequences are interrupted by one more amino acid sequences in the primary sequence to which an antibody of the invention (or an antigen-binding fragment thereof) does not bind. Preferably, the interrupting amino acid sequence is a contiguous amino acid sequence comprising two or more amino acids to which the antibody (or the antigen-binding fragment thereof) does not bind. The at least two separate amino acid sequences to which an antibody of

the invention (or an antigen-binding fragment thereof) binds are not particularly limited with regard to their length. Such a separate amino acid sequence may consist of only one amino acid as long as the total number of amino acids within said at least two separate amino acid sequences is sufficiently large to effect specific binding between the antibody (or the antigen-binding fragment thereof) and the conformational epitope.

A “paratope” is the part of an antibody that binds to the epitope. In the context of the present invention, a “paratope” is the part of an anti-C5a antibody (or an antigen-binding fragment thereof) as described herein that binds to the epitope.

The term “antibody” typically refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. The term “antibody” also includes all recombinant forms of antibodies, in particular of the antibodies described herein, e.g. antibodies expressed in prokaryotes, unglycosylated antibodies, antibodies expressed in eukaryotes (e.g. CHO cells), glycosylated antibodies, and any antigen-binding antibody fragments and derivatives as described below.

Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H or V_H) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L or V_L) and a light chain constant region. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

The term “antigen-binding fragment” of an antibody (or simply “binding portion”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding portion” of an antibody include (i) Fab fragments, monovalent fragments consisting of the V_L , V_H , CL and CH domains; (ii) $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) F_d fragments consisting of the V_H and $CH1$ domains; (iv) F_v fragments consisting of the V_L and V_H domains of a single arm of an antibody, (v) dAb fragments (Ward et al., (1989) Nature

341: 544-546), which consist of a V_H domain; (vi) isolated complementarity determining regions (CDR), and (vii) combinations of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242: 423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85: 5879-5883). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding fragment” of an antibody. A further example is a binding-domain immunoglobulin fusion protein comprising (i) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain CH₂ constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain CH₃ constant region fused to the CH₂ constant region. The binding domain polypeptide can be a heavy chain variable region or a light chain variable region. The binding-domain immunoglobulin fusion proteins are further disclosed in US 2003/0118592 and US 2003/0133939. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Further examples of “antigen-binding fragments” are so-called microantibodies, which are derived from single CDRs. For example, Heap et al., 2005, describe a 17 amino acid residue microantibody derived from the heavy chain CDR3 of an antibody directed against the gp120 envelope glycoprotein of HIV-1 (Heap C.J. et al. (2005) *Analysis of a 17-amino acid residue, virus-neutralizing microantibody*. J. Gen. Virol. 86:1791-1800). Other examples include small antibody mimetics comprising two or more CDR regions that are fused to each other, preferably by cognate framework regions. Such a small antibody mimetic comprising V_H CDR1 and V_L CDR3 linked by the cognate V_H FR2 has been described by Qiu et al., 2007 (Qiu X.-Q. et al. (2007) *Small antibody mimetics comprising two complementary-determining regions and a framework region for tumor targeting*. Nature biotechnology 25(8):921-929).

Thus, the term “antibody or antigen-binding fragment thereof”, as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain an antigen-binding site that immunospecifically binds an antigen. In a broad sense, the term “antibody or antigen-binding fragment thereof” includes immunoglobulin molecules of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY). However, the preferred immunoglobulin molecules of the present invention are of the IgG type. Within the

IgG type, the preferred immunoglobulin molecules of the present invention can be from any class or subclass (e.g., IgG1; IgG2, preferably IgG2a and IgG2b; IgG3; or IgG4).

Antibodies and antigen-binding fragments thereof usable in the invention may be from any animal origin including birds and mammals. Thus, antibodies or fragments thereof can be
5 from human, chimpanzee, rodent (e.g. mouse, rat, guinea pig, or rabbit), chicken, turkey, pig, sheep, goat, camel, cow, horse, donkey, cat, or dog origin. For many applications, it is particularly preferred that antibodies are of human or murine origin. Antibodies suitable for use in the present invention include chimeric molecules in which an antibody constant region derived from one species, e.g. human, is combined with the antigen binding site derived from
10 another species, e.g. mouse. In the most preferred embodiments of the invention, antibodies and the antigen-binding fragments thereof include humanized molecules in which the antigen-binding sites of an antibody derived from a non-human species (e.g. from mouse) are combined with constant and framework regions of human origin.

As exemplified herein, antibodies of the invention can be obtained directly from
15 hybridomas which express the antibody, or can be cloned and recombinantly expressed in a host cell (e.g., a CHO cell, or a lymphocytic cell). Further examples of host cells are microorganisms, such as *E. coli*, and fungi, such as yeast. Alternatively, they can be produced recombinantly in a transgenic non-human animal or plant.

The term “chimeric antibody” refers to those antibodies wherein one portion of each of
20 the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chain is homologous to corresponding sequences in another species or class. Typically, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are
25 homologous to sequences of antibodies derived from another. One clear advantage to such chimeric forms is that the variable region can conveniently be derived from presently known sources using readily available B-cells or hybridomas from non-human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation and the specificity is not affected
30 by the source, the constant region being human is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non-human source. However, the definition is not limited to this particular example.

The term “humanized antibody” refers to a molecule having an antigen-binding site that is substantially derived from an immunoglobulin from a non-human species, wherein the

remaining immunoglobulin structure of the molecule is based upon the structure and/or sequence of a human immunoglobulin. The antigen-binding site may either comprise complete variable domains fused onto constant domains or only the complementarity determining regions (CDR) grafted onto appropriate framework regions in the variable domains. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions, e.g. modified to resemble human immunoglobulins more closely. Some forms of humanized antibodies preserve all CDR sequences (for example a humanized mouse antibody which contains all six CDRs from the mouse antibody). Other forms have one or more CDRs which are altered with respect to the original antibody.

Different methods for humanizing antibodies are known to the skilled person, as reviewed by Almagro & Fransson, 2008, *Frontiers in Bioscience*, 13:1619-1633, the content of which is herein incorporated by reference in its entirety. The review article by Almagro & Fransson is briefly summarized in US 2012/0231008 A1 which is the national stage entry of international patent application WO 2011/063980 A1. The contents of US 2012/0231008 A1 and WO 2011/063980 A1 are herein incorporated by reference in their entirety.

As used herein, "human antibodies" include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). Human antibodies of the invention include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati & Jakobovits.

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. Typically, monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a non-human animal, e.g. mouse, fused to an immortalized cell.

The term "recombinant antibody", as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal with respect to the immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g. from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created

or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

The term “transfectoma”, as used herein, includes recombinant eukaryotic host cells expressing an antibody, such as CHO cells, NS/0 cells, HEK293 cells, HEK293T cells, plant
5 cells, or fungi, including yeast cells.

As used herein, a “heterologous antibody” is defined in relation to a transgenic organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic organism, and being generally derived from a species other than the transgenic
10 organism.

As used herein, a “heterohybrid antibody” refers to an antibody having light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody.

Thus, “antibodies and antigen-binding fragments thereof” generally include, but are not
15 limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, recombinant, heterologous, heterohybrid, chimeric, humanized (in particular CDR-grafted), deimmunized, or human antibodies, Fab fragments, Fab' fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, Fd, Fv, disulfide-linked Fvs (dsFv), single chain antibodies (e.g. scFv), diabodies or tetrabodies (Holliger P. et al. (1993) Proc. Natl. Acad.
20 Sci. U.S.A. 90(14), 6444-6448), nanobodies (also known as single domain antibodies), anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above.

The antibodies described herein are preferably isolated. An “isolated antibody” as used
25 herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities; e.g., an isolated antibody that specifically binds to C5a is substantially free of antibodies that specifically bind antigens other than C5a. An isolated antibody that specifically binds to an epitope, isoform or variant of human C5a may, however, have cross-reactivity to other related antigens, e.g. from other species (e.g. C5a species homologs, such as rat C5a). Moreover, an isolated antibody may be substantially free of other
30 cellular material and/or chemicals. As used herein, a “combination of isolated antibodies” relates to antibodies having different specificities and being combined in a well-defined composition.

The term “naturally occurring”, as used herein, as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence

that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

“Conservative substitutions” may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. Amino acids can be grouped into the following six standard amino acid groups:

- (1) hydrophobic: Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro; and
- (6) aromatic: Trp, Tyr, Phe.

As used herein, “conservative substitutions” are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices. Some preferred conservative substitutions within the above six groups are exchanges within the following sub-groups: (i) Ala, Val, Leu and Ile; (ii) Ser and Thr; (iii) Asn and Gln; (iv) Lys and Arg; and (v) Tyr and Phe. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist can readily construct DNAs encoding the conservative amino acid variants.

As used herein, “non-conservative substitutions” or “non-conservative amino acid exchanges” are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5877), with hmalign (HMMER package) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673-80) or the CLUSTALW2 algorithm (Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948).

The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST protein searches are performed with the BLASTP program available e.g. on the web site:

5 http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome

Preferred algorithm parameters used are the default parameters as they are set on the indicated web site:

10 Expect threshold = 10, word size = 3, max matches in a query range = 0, matrix = BLOSUM62, gap costs = Existence: 11 Extension: 1, compositional adjustments = conditional compositional score matrix adjustment together with the database of non-redundant protein sequences (nr).

To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used.

15 Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1:I54-I62) or Markov random fields.

When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the indicated reference sequence, if
20 not specifically indicated otherwise. For example, the statement “*an amino acid sequence having at least 80% sequence identity to SEQ ID NO: XYZ*” means that the sequence identity percentage is calculated in relation to the total length of SEQ ID NO: XYZ.

A “biological activity” as used herein, refers to any activity a polypeptide may exhibit, including without limitation: enzymatic activity; binding activity to another compound (e.g.
25 binding to another polypeptide, in particular binding to a receptor, or binding to a nucleic acid); inhibitory activity (e.g. enzyme inhibitory activity); activating activity (e.g. enzyme-activating activity); or toxic effects. Regarding variants and derivatives of a polypeptide, it is not required that the variant or derivative exhibits such an activity to the same extent as the parent polypeptide. A variant is regarded as a variant within the context of the present application, if
30 it exhibits the relevant activity to a degree of at least 10% (e.g. at least 20%, at least 30%, at least 40%, or at least 50%) of the activity of the parent polypeptide. Likewise, a derivative is regarded as a derivative within the context of the present application, if it exhibits the relevant biological activity to a degree of at least 10% of the activity of the parent polypeptide. A particularly relevant “biological activity” in the context of the present invention is a binding

activity to the conformational epitope of human C5a formed by the amino acid sequences NDETCEQRA (SEQ ID NO: 2) and SHKDMQL (SEQ ID NO: 3). Preferably, the relevant “biological activity” in the context of the present invention is a binding activity to the conformational epitope of human C5a formed by the amino acid sequences DETCEQR (SEQ ID NO: 4) and HKDMQ (SEQ ID NO: 5). Even more preferably, the relevant “biological activity” in the context of the present invention is a binding activity to the conformational epitope of human C5a formed by the amino acid sequences DETCEQR (SEQ ID NO: 4) and KDM. Assays for determining binding activity are known to a person of ordinary skill in the art and include ELISA and surface plasmon resonance assays.

As used herein, a “patient” means any mammal or bird who may benefit from a treatment with an anti-C5a antibody described herein. Preferably, a “patient” is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, chicken, turkey, pig, sheep, goat, camel, cow, horse, donkey, cat, or dog), or primates including monkeys (e.g. African green monkeys, chimpanzees, bonobos, gorillas) and human beings. It is particularly preferred that the “patient” is a human being. The terms “patient” and “subject to be treated” (or in short: “subject”) are used interchangeably herein.

As used herein, “treat”, “treating” or “treatment” of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity and/or duration of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

As used herein, “prevent”, “preventing”, “prevention”, or “prophylaxis” of a disease or disorder means preventing that a disorder occurs in a subject for a certain amount of time. For example, if an antibody of the invention (or an antigen-binding fragment thereof) is administered to a subject with the aim of preventing a disease or disorder, said disease or disorder is prevented from occurring at least on the day of administration and preferably also on one or more days (e.g. on 1 to 30 days; or on 2 to 28 days; or on 3 to 21 days; or on 4 to 14 days; or on 5 to 10 days) following the day of administration.

As used herein, “administering” includes *in vivo* administration, as well as administration directly to tissue *ex vivo*, such as vein grafts.

A “pharmaceutical composition” according to the invention may be present in the form of a composition, wherein the different active ingredients and diluents and/or carriers are

admixed with each other, or may take the form of a combined preparation, where the active ingredients are present in partially or totally distinct form. An example for such a combination or combined preparation is a kit-of-parts.

5 An “effective amount” is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the subject to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

10 If the context does not state otherwise, the term “active agent” refers to the antibodies of the invention and to the antigen-binding fragments of the invention. The terms “active agent” and “therapeutic agent” are used interchangeably herein.

As used herein, the term “adjunctive therapy” refers to a combination therapy, in which at least two different drugs are administered to the patient. These at least two different drugs
15 can be formulated into one single pharmaceutical composition containing both drugs. Alternatively, each drug can be formulated into a separate pharmaceutical composition and the pharmaceutical compositions are separately administered (e.g. at different time-points and/or by different routes of administration) to the patient. In this latter alternative, the (at least) two different drugs can be provided in a kit-of-parts.

20 “Pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term “carrier”, as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic agent is administered. Such pharmaceutical carriers can be sterile
25 liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable
30 pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules,

powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc. Examples of suitable pharmaceutical carriers are described in "Remington: The Science and Practice of Pharmacy" 22nd edition, Loyd V. Allen Jr. et al. (eds.), Pharmaceutical Press, 2012. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

Generally known and practiced methods in the fields of molecular biology, cell biology, protein chemistry and antibody techniques are fully described in the continuously updated publications "Molecular Cloning: A Laboratory Manual", (Sambrook et al., Cold Spring Harbor); "Current Protocols in Molecular Biology" (F. M. Ausubel et al. Eds., Wiley & Sons); "Current Protocols in Protein Science" (J. E. Colligan et al. eds., Wiley & Sons); "Current Protocols in Cell Biology" (J. S. Bonifacino et al., Wiley & Sons) and "Current Protocols in Immunology" (J. E. Colligan et al., Eds., Wiley & Sons). Known techniques relating to cell culture and media are described in "Large Scale Mammalian Cell Culture (D. Hu et al., Curr. Opin. Biotechnol. 8:148-153, 1997); "Serum free Media" (K. Kitano, Biotechnol. 17:73-106, 1991); and "Suspension Culture of Mammalian Cells" (J.R. Birch et al. Bioprocess Technol. 10:251-270, 1990).

Embodiments of the Invention

The present invention will now be further described. In the following passages different aspects of the invention are defined in more detail. Each aspect defined below may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

In a first aspect the present invention is directed to an antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain (VH) and a light chain variable domain (VL),

wherein said VH domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 10 (QVQLVQSGAE
VKKPGASVKV SCKASGYSFT TFWMDWVRQA PGQGLEWIGR IDPSDSESRL
DQRFKDRVMT TVDKSTSTVY MELSSLRSED TAVYYCARGN DGYYGFAFWG
5 QGTLVTVSS, VH4) or

an amino acid sequence having at least 80% sequence identity (preferably at least 85%,
more preferably at least 90%, more preferably at least 95%, more preferably at least 96%, more
preferably at least 97%, even more preferably at least 98%, or most preferably at least 99%
sequence identity) with SEQ ID NO: 10, wherein said amino acid sequence having at least 80%
10 (at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least
99%) sequence identity with SEQ ID NO: 10 comprises the CDR1H, CDR2H, and CDR3H
sequences of SEQ ID NO: 20 to 22, respectively, and wherein said amino acid sequence having
at least 80% (at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%,
or at least 99%) sequence identity with SEQ ID NO: 10 comprises a V at amino acid position
15 5, an E at amino acid position 10, a K at amino acid position 12, a K at amino acid position 13,
an A at amino acid position 16, an A at amino acid position 40, and/or a T at the amino acid
position 76, and

wherein said VL domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 16 (DIQMTQSPSS LSASVGDRVT
20 ITCKASQSVD YDGDSYMKWY QQKPGKAPKL LIYAASNLSQ GVPSTRFSGSG
SGTDFTLTIS SLQPEDFATY YCQQSNEPDY TFGQGTKLEI K, Vκ4) or

an amino acid sequence having at least 80% sequence identity (preferably at least 85%,
more preferably at least 90%, more preferably at least 95%, more preferably at least 96%, more
preferably at least 97%, even more preferably at least 98%, or most preferably at least 99%
25 sequence identity) with SEQ ID NO: 16, wherein said amino acid sequence having at least 80%
(at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least
99%) sequence identity with SEQ ID NO: 16 comprises the CDR1L, CDR2L, CDR3L
sequences of SEQ ID NO: 23 to 25, respectively, and wherein said amino acid sequence having
at least 80% (at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%,
30 or at least 99%) sequence identity with SEQ ID NO: 16 comprises an A at amino acid position
13, a V at amino acid position 15, a D at amino acid position 17, a V at amino acid position 19,
a T at amino acid position 22, a K at amino acid position 46, an A at amino acid position 47, an
S at amino acid position 64, a T at amino acid position 78, an S at amino acid position 80, an S

at amino acid position 81, an L at amino acid position 82, a Q at amino acid position 83, an F at amino acid position 87, and/or a Q at amino acid position 104.

In a preferred embodiment of the first aspect, the amino acid sequence having at least 80% (preferably at least 85%, more preferably at least 90%, more preferably at least 95%,
5 more preferably at least 96%, more preferably at least 97%, even more preferably at least 98%, or most preferably at least 99%) sequence identity with SEQ ID NO: 10 comprises at least four (preferably at least 5, more preferably at least 6, most preferably 7) of the following seven amino acids at the indicated positions:

- a V at amino acid position 5,
- 10 - an E at amino acid position 10,
- a K at amino acid position 12,
- a K at amino acid position 13,
- an A at amino acid position 16,
- an A at amino acid position 40, or
- 15 - a T at the amino acid position 76.

In a preferred embodiment of the first aspect, the amino acid sequence having at least 80% (preferably at least 85%, more preferably at least 90%, more preferably at least 95%,
more preferably at least 96%, more preferably at least 97%, even more preferably at least 98%, or most preferably at least 99%) sequence identity with SEQ ID NO: 16 comprises at
20 least eight (preferably at least 9, more preferably at least 10, more preferably at least 11, even more preferably at least 12, even more preferably at least 13, even more preferably at least 14, most preferably 15) of the following fifteen amino acids at the indicated positions:

- an A at amino acid position 13,
- a V at amino acid position 15,
- 25 - a D at amino acid position 17,
- a V at amino acid position 19,
- a T at amino acid position 22,
- a K at amino acid position 46,
- an A at amino acid position 47,
- 30 - an S at amino acid position 64,
- a T at amino acid position 78,
- an S at amino acid position 80,
- an S at amino acid position 81,
- an L at amino acid position 82,

- a Q at amino acid position 83,
- an F at amino acid position 87, or
- a Q at amino acid position 104.

In a preferred embodiment of the first aspect, the amino acid sequence having at least
5 80% (preferably at least 85%, more preferably at least 90%, more preferably at least 95%,
more preferably at least 96%, more preferably at least 97%, even more preferably at least
98%, or most preferably at least 99%) sequence identity with SEQ ID NO: 10 comprises at
least four (preferably at least 5, more preferably at least 6, most preferably 7) of the following
seven amino acids at the indicated positions:

- 10 - a V at amino acid position 5,
- an E at amino acid position 10,
- a K at amino acid position 12,
- a K at amino acid position 13,
- an A at amino acid position 16,
- 15 - an A at amino acid position 40, or
- a T at the amino acid position 76;

and optionally comprises 1, 2, or 3 amino acid substitutions, preferably conservative
substitutions, located at amino acid positions 1 to 4 or 6 to 9 of SEQ ID NO: 10;

and

20 the amino acid sequence having at least 80% (preferably at least 85%, more preferably at least
90%, more preferably at least 95%, more preferably at least 96%, more preferably at least
97%, even more preferably at least 98%, or most preferably at least 99%) sequence identity
with SEQ ID NO: 16 comprises at least eight (preferably at least 9, more preferably at least
10, more preferably at least 11, even more preferably at least 12, even more preferably at least
25 13, even more preferably at least 14, most preferably 15) of the following fifteen amino acids
at the indicated positions:

- an A at amino acid position 13,
- a V at amino acid position 15,
- a D at amino acid position 17,
- 30 - a V at amino acid position 19,
- a T at amino acid position 22,
- a K at amino acid position 46,
- an A at amino acid position 47,
- an S at amino acid position 64,

- a T at amino acid position 78,
- an S at amino acid position 80,
- an S at amino acid position 81,
- an L at amino acid position 82,
- 5 - a Q at amino acid position 83,
- an F at amino acid position 87, or
- a Q at amino acid position 104;

and optionally comprises 1, 2, or 3 amino acid substitutions, preferably conservative substitutions, located at amino acid positions 1 to 10 of SEQ ID NO: 16.

10

In a second aspect the present invention is directed to an antibody or an antigen-binding fragment thereof comprising a heavy chain variable domain (VH) and a light chain variable domain (VL),

wherein said VH domain comprises, essentially consists of, or consists of

- 15 an amino acid sequence according to SEQ ID NO: 17 (QVQLVQSGX⁹E
X¹¹KKPGASVKX²⁰ SCKASGYSFT TFWMDWVX³⁸QA PGQGLEWX⁴⁸GR IDPSDSERL
DQX⁶³FKDRX⁶⁸TX⁷⁰ TVDKSTSTVY MX⁸²LSSX⁸⁶X⁸⁷SED X⁹¹AVYYCARGN
DGYYGFAFWG QGTLTVSS), wherein X⁹ is A or P, X¹¹ is L or V, X²⁰ is I or V, X³⁸ is K
or R, X⁴⁸ is I or M, X⁶³ is K or R, X⁶⁸ is A or V, X⁷⁰ is L or M, X⁸² is E or Q, X⁸⁶ is L or P,
20 X⁸⁷ is R or T, and X⁹¹ is S or T, or

an amino acid sequence according to SEQ ID NO: 17 having one, two or three amino acid substitutions, wherein said amino acid sequence having one, two or three amino acid substitutions comprises the CDR1H, CDR2H, CDR3H sequences of SEQ ID NO: 20 to 22, respectively, and

- 25 wherein said VL domain comprises, essentially consists of, or consists of

an amino acid sequence according to SEQ ID NO: 18 (DIX³X⁴TQSPX⁹S
LX¹²ASVGDRVT ITCKASQSVD YDGDSYMKWY QKPGKAPKL LIYAASNLS
GX⁶²PSRFSGSG SGTDFLTIS SLQX⁸⁴EDFATY YCQQSNEDPY TFGQGTKLEI K),
wherein X³ is V or Q, X⁴ is L or M, X⁹ is A or S, X¹² is A or S, X⁶² is I or V, and X⁸⁴ is E or
30 P, or

an amino acid sequence according to SEQ ID NO: 18 having one, two, three, four, five, six, or seven amino acid substitutions, wherein said amino acid sequence having one, two, three, four, five, six, or seven amino acid substitutions comprises the CDR1L, CDR2L, CDR3L sequences of SEQ ID NO: 23 to 25, respectively.

In an embodiment of the first or second aspect, the VH domain comprises, essentially consists of, or consists of an amino acid sequence selected from the group consisting of VH1 to VH5 (SEQ ID NO: 7 to 11); and/or the VL domain comprises, essentially consists of, or consists of an amino acid sequence selected from the group consisting of Vκ1 to Vκ4 (SEQ ID NO: 13 to 16).

In an embodiment of the first or second aspect, the antibody or the antigen-binding fragment thereof comprises:

- a) a VH domain comprising, essentially consisting of, or consisting of an amino acid sequence of SEQ ID NO: 10 (VH4) and a VL domain comprising, essentially consisting of, or consisting of an amino acid sequence according to SEQ ID NO: 16 (Vκ4), or
- b) a VH domain comprising, essentially consisting of, or consisting of an amino acid sequence of SEQ ID NO: 11 (VH5) and a VL domain comprising, essentially consisting of, or consisting of an amino acid sequence according to SEQ ID NO: 15 (Vκ3).

In an embodiment of the first or second aspect, the antibody is a humanized antibody.

In an embodiment of the first or second aspect, the antibody or the antigen-binding fragment thereof further comprises a constant domain. In some embodiments of the first or second aspect, the constant domain comprises, essentially consists of, or consists of the amino acid sequence according to SEQ ID NO: 19 (IgG4 WT constant), wherein said amino acid sequence according to SEQ ID NO: 19 optionally comprises one or more of the following amino acid exchanges:

- an amino acid exchange S108P;
- the amino acid exchanges T130Q and M308L;
- the amino acid exchanges M132Y, S134T, and T136E.

In an embodiment of the first or second aspect, the antigen-binding fragment of an antibody is selected from the group consisting of Fab fragments, Fab' fragments, F(ab')₂ fragments, Fd fragments, Fv fragments, disulfide-linked Fvs (dsFv), single domain antibodies and single chain Fv (scFv) antibodies.

In an embodiment of the first or second aspect, the antibody or the antigen-binding fragment thereof exhibits one or more of the following properties:

- said antibody or said antigen-binding fragment thereof has a binding constant to C5a with a K_d value of 10 nM or less (e.g. 9 nM or less; 8 nM or less, 7 nM or less, 6 nM or less, 5 nM or less, 4 nM or less, 3 nM or less, or 2 nM or less);
- said antibody or said antigen-binding fragment thereof exhibits at least 75% blocking activity for biological effects induced by one molecule C5a;
- said antibody or said antigen-binding fragment thereof does not inhibit CH50 activity in human plasma;
- said antibody or said antigen-binding fragment thereof is capable of reducing *E. coli* induced IL-8 production in human whole blood.
- said antibody or said antigen-binding fragment thereof has a reduced immunogenicity as compared to IFX-1.

A method for determining relative immunogenicity is shown in the examples below, i.e. an ADA screening assay. The abbreviation ADA refers to anti-drug antibodies.

In an embodiment of the first or second aspect, the antibody or antigen-binding fragment thereof binds to a conformational epitope formed by amino acid sequences NDETCEQRA (SEQ ID NO: 2) and SHKDMQL (SEQ ID NO: 3) of C5a,

wherein the antibody or antigen-binding fragment thereof binds to at least one amino acid within the amino acid sequence according to SEQ ID NO: 2 and to at least one amino acid within the amino acid sequence according to SEQ ID NO: 3.

In a further embodiment of the first or second aspect, the antibody or antigen-binding fragment thereof binds to a conformational epitope formed by amino acid sequences DETCEQR (SEQ ID NO: 4) and HKDMQ (SEQ ID NO: 5) of C5a,

wherein the antibody or antigen-binding fragment thereof binds to at least one amino acid within the amino acid sequence according to SEQ ID NO: 4 and to at least one amino acid within the amino acid sequence according to SEQ ID NO: 5.

In a further embodiment of the first or second aspect, the antibody or antigen-binding fragment thereof binds to a conformational epitope formed by amino acid sequences DETCEQR (SEQ ID NO: 4) and KDM of C5a,

wherein the antibody or antigen-binding fragment thereof binds to at least one amino acid within the amino acid sequence according to SEQ ID NO: 4 and to at least one amino acid within the amino acid sequence according to KDM.

In a third aspect the present invention is directed to a pharmaceutical composition comprising:

the antibody or antigen-binding fragment thereof according to the first aspect or the antibody or antigen-binding fragment thereof according to the second aspect; and

5 further comprising one or more pharmaceutically acceptable carriers, diluents, excipients, fillers, binders, lubricants, glidants, disintegrants, adsorbents, and/or preservatives.

In a fourth aspect the present invention is directed to the antibody or antigen-binding fragment thereof according to the first aspect or the antibody or antigen-binding fragment
10 thereof according to the second aspect for use in medicine.

In a fifth aspect the present invention is directed to the antibody or antigen-binding fragment thereof according to the first aspect or the antibody or antigen-binding fragment thereof according to the second aspect for use in the treatment or prevention of a disease or
15 disorder involving pathological C5a activity.

In an embodiment of the fifth aspect, the disease or disorder is selected from the group consisting of

- autoimmune disorders,
- 20 - inflammatory disorders, auto-inflammatory disorders, or related conditions,
- cardiovascular or cerebrovascular disorders,
- bacterial or viral infections,
- neurodegenerative disorders or related diseases, and
- cancers or precancerous conditions.

25 In some embodiments of the fifth aspect, the viral infection is HIV or AIDS.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the compounds,
30 compositions and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used but some experimental errors and deviations should be accounted for. Unless indicated otherwise, molecular weight is average molecular weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

Example 1: Generation of Fully Humanized Anti-C5a Antibodies (IFX-2 Clones)**Derived from IFX-1**

5 This example details work in which the V region gene sequences encoding the chimeric antibody IFX-1 (VH0/Vκ0) were used to construct a series of fully humanized antibodies using Composite Human Antibody™ technology. The designed variable region genes of heavy chain and light chain were respectively cloned into vectors encoding a human IgG4 heavy chain constant domain (see SEQ ID NO: 19 but with an S108P amino acid exchange) and a human kappa light chain constant domain. In other publications, the S108P amino exchange is sometimes referred to as an S241P amino acid exchange (using Kabat numbering of a full-length human IgG4). Chimeric and humanized antibodies were transiently expressed in HEK EBNA cells and Protein A purified.

METHODS AND RESULTS

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Design of Composite Human Antibody™ Variable Region Sequences

Structural models of the IFX-1 antibody V regions were produced using Swiss PDB and analyzed in order to identify important “constraining” amino acids in the V regions that were likely to be essential for the binding properties of the antibody. Most residues contained within the CDRs together with a number of framework residues were considered to be important. The VH and Vκ sequences of IFX-1 contain typical framework residues and the CDR 1, 2 and 3 motifs are comparable to many murine antibodies.

From the above analysis, it was considered that composite human sequences of IFX-1 could be created with a wide latitude for alternative residues outside of the CDRs but with only a narrow menu of possible residues within the CDR sequences. Preliminary analysis indicated that corresponding sequence segments from several human antibodies could be combined to create CDRs similar or identical to those in the murine sequences. For regions outside of, and flanking the CDRs, a wide selection of human sequence segments were identified as possible components of the novel humanized V regions.

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CD4+ T Cell Epitope Avoidance

Based upon the structural analysis, a large preliminary set of sequence segments that could be used to create IFX-1 humanized variants were selected and analyzed using iTope™ technology for *in silico* analysis of peptide binding to human MHC class II alleles (Perry *et al*
35 *New Approaches to Prediction of Immune Responses to Therapeutic Proteins during*

Preclinical Development (2008). *Drugs R D* 9 (6): 385-396), and using the TCED™ of known antibody sequence-related T cell epitopes (Bryson *et al Prediction of Immunogenicity of Therapeutic Proteins* (2010). *Biodrugs* 24 (1):1-8). Sequence segments that were identified as significant non-human germline binders to human MHC class II or that scored significant hits against the TCED™ were discarded. This resulted in a reduced set of segments, and combinations of these were again analyzed, as above, to ensure that the junctions between segments did not contain potential T cell epitopes. Selected sequence segments were assembled into complete V region sequences that were devoid of significant T cell epitopes. Five heavy chain (VH1 to VH5) and four light chain (Vκ1 to Vκ4) sequences were then chosen for gene synthesis and expression in mammalian cells. The amino acid sequences of VH1 to VH5 are shown in the sequence listing as SEQ ID NOs: 7 to 11, respectively. The amino acid sequences of Vκ1 to Vκ4 are shown in the sequence listing as SEQ ID NOs: 13 to 16, respectively.

15 Construction of Chimeric Antibody and Humanised Variants

The VH and Vκ sequences of IFX-1 (VH0 (SEQ ID NO: 6) and Vκ0 (SEQ ID NO: 12)) and its humanized variants were synthesized with flanking restriction enzyme sites for cloning into Abzena's pANT expression vector system for human IgG4 (S241P) heavy chain and kappa light chain, respectively. The VH regions were cloned between the Mlu I and Hind III restriction sites, and the Vκ regions were cloned between the BssH II and BamH I restriction sites. All constructs were confirmed by sequencing.

Expression and Purification of Antibodies

Chimeric IFX-1 (VH0/Vκ0), two control antibodies (VH0/Vκ1, VH1/Vκ0) and combinations of composite IgG4 (S241P) VH and Vκ chains (a total of 23 pairings, **Table 1**) were transiently transfected into HEK EBNA adherent cells (LGC Standards, Teddington, UK) using a PEI transfection method and incubated for seven days post-transfection. Supernatant antibody titres were determined by ELISA, and it was observed that VH1 containing variants showed consistently poorer expression when compared to other variants (data not shown).

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Table 1. Summary table of the antibodies produced by transient transfection (crossed boxes) including a chimeric (VH0/Vκ0), two control humanized variants (VH1/Vκ0 and VH0/Vκ1) and 20 IFX-1 Composite Human Antibody™ variants.

	VH0	VH1	VH2	VH3	VH4	VH5
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Vκ0	X	X				
Vκ1	X	X	X	X	X	X
Vκ2		X	X	X	X	X
Vκ3		X	X	X	X	X
Vκ4		X	X	X	X	X

Antibodies (excluding poorly expressing VH1 variants) were purified from cell culture supernatants on Protein A sepharose columns (GE Healthcare, Little Chalfont, UK), buffer exchanged into 1x PBS pH 7.2 and quantified by OD_{280nm} using an extinction coefficient (E_{c(0.1%)}) based on the predicted amino acid sequence. Antibodies were analyzed using SDS-PAGE by loading 1 µg of each antibody on the gel and bands corresponding to the profile of a typical antibody were observed (data not shown).

Example 2: Comparison of different IFX-2 clones to parent antibody IFX-1

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MATERIALS AND REAGENTS

For CD11b assay:

- ACD, Sigma Aldrich (Taufkirchen, Germany), Cat. No. C3821-50ML
- reagents for flow cytometer (all BD Bioscience, NJ, USA)
 - FACS Flow Sheat Fluid, Cat. No. 342003
 - FACS Shutdown solution, Cat. No. 334224
 - FACS Clean solution, Cat. No. 340345
 - rat anti-mouse CD11b:FITC, Cat. No. 553310, 0.5 mg/mL
 - 10× lysing solution, BD Bioscience (NJ, USA), Cat. No. 349202 → 1:10 diluted in AnalaR water
- HBSS, Life Technologies GmbH (Darmstadt, Germany), Cat. No. 14025-050
- FBS, Invitrogen (CA, USA), Cat. No. 10099133 → heat-inactivation: 56°C, 30 min
- sodium azide, Merck (Darmstadt, Germany), Cat. No. 1.06688.0250
- recombinant human C5a (rhC5a), Sigma Aldrich (Taufkirchen, Germany), Cat. No. C5788-1MG, expressed in *E. coli*, purity: ~ 95%, dissolved in sterile AnalaR water
- staining buffer (SB-buffer): 1% heat-inactivated FBS + 0.1% sodium azide in 1× PBS
- human blood (used immediately) from healthy donor containing 12% ACD

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For C5a-base PK ELISA:

- AnalAR water, VWR International (Darmstadt, Germany), Cat. No. 102923C
- recombinant human C5a (rhC5a), Hycult Biotech (Uden, The Netherlands), Cat. No. HC2101, expressed in *E. coli*, dissolved in sterile AnalAR water
- rhC5a desarginine derivative (rhC5aDArg), Hycult Biotech (Uden, The Netherlands), Cat. No. HC2102, expressed in *E. coli*, dissolved in sterile AnalAR water
- IFX-1, anti-human C5a antibody applied as control, InflaRx (Jena, Germany), 10 mg/mL in PBS + 0.05% Tween80
- mouse anti-human IgG4:HRP, AbD Serotec (Puchheim, Germany), Cat. No. MCA2098P, 1 mg/mL
- Na₂CO₃, Sigma-Aldrich (Taufkirchen, Germany), Cat. No. 71350 (Sigma, WI, USA, Cat. No. 31432)
- NaHCO₃, VWR International (Darmstadt, Germany), Cat. No. L1730
- NaN₃, VWR International, (Darmstadt, Germany), Cat. No. 1.06688.0100
- PBS powder, Sigma-Aldrich (Taufkirchen, Germany), Cat. No. P5368-10PAK → 1 package dissolved in 1000 mL deionised water
- Tween 20, Sigma-Aldrich, (Taufkirchen, Germany), Cat. No. P1379-25ML
- BSA, Sigma-Aldrich (Taufkirchen, Germany), Cat. No. A7030-100G
- coating buffer: 1.59 g Na₂CO₃ + 2.93 g NaHCO₃ + 0.2 g NaN₃ in 1000 mL AnalAR water, pH 9.6
- washing buffer: 1x PBS + 0.05% Tween 20
- assay diluent: 3% BSA in 1x PBS + 0.05% Tween 20 (3% BSA/PBST)
- TMB substrate (substrate solution C), BioLegend/Biozol (Heidelberg, Germany), Cat. No. BLD-78105
- stop solution: sulfuric acid (Sigma-Aldrich, Cat. No. 258105-100ML) 1:10 diluted in AnalAR water

30 For Plasma Hemolytic Activity (CH50):

- AnalAR water, VWR International (Darmstadt, Germany), Cat. No. 102923C
- Complement CH50 assay, Haemoscan (Groningen, Netherlands), Cat. No. K002
- Healthy human plasma pool, huPP-013, in-house preparation

METHODS

rhC5a-based ELISA

For the ELISA, 100 μ L of rhC5a (1 μ g/mL) was coated on a 96-well ELISA plate overnight at 4°C. Plate was washed (5 $\times$) and blocked with 200 μ L of blocking buffer for 1 h at 37°C. Following the washing steps (5 $\times$), IFX clones in 2-fold serial dilution from 250 ng/mL – 3.91 ng/mL were added to the C5a-coated wells. After the 2-h incubation at 37°C and washing steps (5 $\times$), 0.04 μ g/mL HRP-labeled anti-human IgG4 antibody was applied for 1 h at 37°C to detect the bound IFX clones. For color development, 100 μ L of TMB substrate was added after wash (5 $\times$) and incubated for 5 min at RT. Color reaction was stopped with 100 μ L stop solution. Absorbance readout at 450 nm was performed within 30 min after color reaction using the plate reader. Blank reduction was performed with the raw data.

Plasma Hemolytic Activity (CH50)

The total hemolytic complement titer (CH50) is a conventional method to determine the activation of classical complement pathway. In brief, sheep red blood cells (sRBC) were prepared from fresh sheep whole blood by centrifugation and sensitized with an anti-sRBC antibody. Plasma samples from healthy volunteers containing IFX clones were serially diluted and incubated with the sensitized sRBC at 37°C for 30 min. After incubation, the mixture was centrifuged, and the degree of hemolysis was quantified by measuring the absorbance of the hemoglobin released into the supernatant at 450 nm. The amount of complement activity was determined by examining the capacity of various dilutions of test plasma sample to lyse the sensitized sRBC.

CD11b potency assay

Human whole blood was stimulated with 16.7 nM rhC5a. To test the blocking activity of IFX-1 and IFX-2 clones on rhC5a-induced CD11b upregulation, the antibodies were diluted to the final concentration with Ag:Ab ratio of 1:1. Blood incubated with buffer alone (HBSS control) served as the non-stimulated condition to assess the baseline CD11b expression. Blood with IFX antibody alone was used to exclude the inhibitory or stimulatory effect of the antibody on human blood. The mixed samples were incubated at 37°C for 20 min to induce C5a-mediated CD11b upregulation. Afterwards, a FITC-conjugated anti-mouse CD11b antibody was further incubated with the samples for 30 min on ice to stain the CD11b on the cell surface of granulocytes. The fluorescence signal was captured through Flow Cytometer.

Statistical analysis

Graphs and statistical analysis were performed with GraphPad PRISM® V7.05.

RESULTS

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IFX-2 clones bound equally well to C5a in comparison to IFX-1

To characterize the binding of IFX-2 clones to C5a, the rhC5a-based ELISA platform was employed. 12× IFX-2 and 1× IFX-1 (VH0Vk0) clones in range of 3.91 ng/mL – 250 ng/mL were applied as samples to the 1 µg/mL rhC5a-coated 96-well plate. The C5a-captured IFX clones were then detected with 0.04 µg/mL of HRP-conjugated anti-human IgG4 mAb. The more IFX antibody bound to C5a, the stronger chemiluminescent signal would appear. As shown in **Figure 1**, all tested antibodies in the applied concentration range bound to the coated rhC5a in a comparable dose-dependent manner.

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A similar ELISA-based assay was performed in ABZENA to quantify the EC₅₀ of IFX clones on C5a binding. In the range of 0 – 100 ng/mL, all IFX clones showed a very close binding behavior to C5a. In comparison to the parent molecule IFX-1, the relative EC₅₀s of all IFX-2 clones were within the 2-fold range, the median value was 1.04 (Table 2).

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Table 2. The relative EC₅₀ of IFX-2 to IFX-1 (VH0Vk0) on C5a binding affinity.

Sample ID	Relative EC ₅₀	Sample ID	Relative EC ₅₀
VH0Vk0 (IFX-1)	1.00	VH4Vk3	0.91
VH2Vk3	1.09	VH4Vk4	1.03
VH2Vk4	0.99	VH5Vk1	1.28
VH3Vk2	1.05	VH5Vk2	1.05
VH3Vk4	0.86	VH5Vk3	1.14
VH4Vk1	1.00	VH5Vk4	1.53
VH4Vk2	0.86	Median of IFX-2 clones	1.04

IFX-2 antibodies showed no inhibition on the formation of membrane attack complex (MAC)

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The parent IFX-1 (VH0Vk0) is known to neutralize C5a without interfering with the C5 cleavage to C5a and C5b. C5b is the starting material of membrane attack complex C5b-9,

which is the terminal product of classical complement pathway and forms pores in membranes. Total hemolytic complement titer (CH50) was used to index the activation of classical complement pathway via MAC-mediated hemolysis of sensitized sheep red blood cells (SRBC). Pooled human plasma with an initial concentration of 100 µg/mL of respective IFX clones (12× IFX-2 and 1× IFX-1 (VH0Vk0)) was serially diluted to 4-, 8-, 16-, 32-, 64-, and 128-fold, and incubated with the erythrocyte suspension. The degree of hemolysis was quantified by measuring the absorbance of the hemoglobin released into the supernatant at 450 nm.

Table 3. CH50 (plasma dilution factor) of each test IFX clone.

Sample ID	CH50 (DF, -fold)	Sample ID	CH50 (DF, -fold)
VH0Vk0 (IFX-1)	74.25	VH4Vk3	70.47
VH2Vk3	72.62	VH4Vk4	75.31
VH2Vk4	74.19	VH5Vk1	71.20
VH3Vk2	76.71	VH5Vk2	74.21
VH3Vk4	75.46	VH5Vk3	73.24
VH4Vk1	75.69	VH5Vk4	76.87
VH4Vk2	72.04	Median of IFX-2 clones	74.20

As shown in **Figure 2** and Table 3, a 70- to 77-fold (median 74-fold) dilution of the IFX-2 clones or a 74-fold dilution of the parent molecule IFX-1 were required to achieve the 50% hemolysis (CH50), which is fully comparable. It demonstrated that the mutations on IFX-2 clones did not impair the activation of classical complement pathway.

IFX-2 antibodies effectively blocked the rhC5a-driven CD11b upregulation

CD11b upregulation on the surface of human granulocytes is detectable within minutes after addition of rhC5a to human whole blood. CD11b levels were determined using fluorescence labeled anti-CD11b antibody and indicated as the mean fluorescence intensity (MFI). Blocking of the rhC5a-driven CD11b up-regulation was evaluated with 12× IFX-2 antibodies in comparison to IFX-1 in two CD11b potency assays. In both assays, 16.7 nM rhC5a was used as the stimulus, which respectively induced 3.65- and 5.89-fold upregulation of CD11b expression. All IFX clones were tested in a 1:1 molar ratio for the blockade on CD11b upregulation. The blocking activity of IFX clones were calculated according to the respective fluorescence intensity change to the rhC5a-stimulated condition. The activity of parent

molecule IFX-1 was set to be 100%. At the rhC5a:IFX clone ratio of 1:1, IFX-2 clones showed 94%-104% of relative blocking activity (see **Figure 3**). The median relative blocking activities of IFX-2 clones was 100.68%.

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Example 3: ADA analysis in non-human primate after IFX-1/IFX-2 treatment

MATERIALS AND REAGENTS

10 **Table 4.** Reagents and their suppliers.

precursors	Supplier	Product-Number
AnalaR water	VWR International	102923C
Avidin-HRP	Biolegend	405103
Solution C (TMB)	Biolegend	78105
Rabbit anti-CaCP29-IgG4 pAB	Charles River	n/a
IFX-1	InflaRx	n/a
IFX-2	InflaRx	n/a
Blocking Buffer I	AppliChem	A7099.0500
Cross Down Buffer	AppliChem	A6485.0500
Monkey serum	LPT Hamburg	n.a.
PBS powder	Sigma-Aldrich	P5368-10PAK
Sodium azide NaN_3	VWR International	1.06688.0100
Sodium bicarbonate NaHCO_3	VWR International	L1703
Sodium carbonate Na_2CO_3	Honeywell	31432-250G
Sulfuric acid H_2SO_4	VWR International	258105-100ML
Tween20	Sigma-Aldrich	P1379-25ML

METHOD

Anti-drug antibodies are determined in a two-step approach. Serum samples are screened for anti-drug antibodies within the ADA screening ELISA. A fixed assay cut-point based on optical density (OD) is 0.102 (according to ADA screening ELISA validation with human plasma for detection of IFX-1 ADA), and should result in 9% false positive samples. Therefore, samples obtained positive in the ADA screening ELISA must be analyzed within

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the ADA confirmatory ELISA to confirm or reject the results and to determine the ADA concentration, if applicable.

The ADA screening ELISA is a bridging ELISA employing the drug IFX-1 (anti-human C5a antibody) as capture antibody (non-labeled) and detection antibody (biotin-labeled). The anti-drug antibodies used for the preparation of calibration (CAL) curve and spiking of quality control (QC) samples were purified from rabbit serum after immunization of the rabbit with purified IFX-1. As the anti-drug antibodies have two binding sites, they can bind (bridge) both, the capture IFX-1 as well as the labeled IFX-1 used for detection. All plasma samples screened ADA-positive are re-evaluated in the ADA confirmatory ELISA which is based on the ADA screening ELISA setup. Additional free unlabeled drug (IFX-1) is added to the samples to compete the binding of potential anti-drug antibodies to the captured IFX-1 on the plate. If there are anti-drug antibodies available, they would bind to the added free unlabeled IFX-1 and would therefore be undetectable. If a drug-specific ADA inhibition is observed and the screening result is confirmed, the sample is considered to be confirmed positive.

ADA screening ELISA and ADA confirmatory ELISA were carried out as described in the following:

Briefly, 100 µl/well capture antibody (IFX-1, 0.5 µg/ml) is incubated over night at $5 \pm 3^{\circ}\text{C}$ on high binding plates. After a washing step, 200 µl blocking buffer is pipetted into each well and the plate is incubated for 2 hours at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After another washing step, 100 µl/well CAL samples, QC samples and samples of interest (test samples) are pipetted into the wells and incubated for 2 hours at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Following a washing step, 100 µl of detection antibody (biotinylated IFX-1, 0.08 µg/ml) is pipetted into each well for an incubation of 60 minutes at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The plates are washed thereafter and 100 µl diluted Avidin-HRP (1:3000) is added to each well and incubated for 30 minutes at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After a final washing step, 100 µl/well TMB (substrate solution) is added, incubated for 5 minutes at RT protected from light, and the reaction is stopped by adding 100 µl/well of diluted sulfuric acid. The color intensity is analyzed at 450 nm with the Tecan Infinite M200 reader using magellan™ 6.5 software.

Plasma samples with unknown ADA concentrations are diluted 1:2 in CrossDown buffer before analyzed in ADA screening ELISA. For re-evaluation in the ADA confirmatory ELISA, samples are diluted 1:2 once in CrossDown buffer and once in drug-spiked CrossDown buffer (IFX-1 concentration 100 µg/ml).

Samples with an OD value below 0.102 in the ADA screening assay are classified as “negative”. Samples with a signal blocking of more than 50% in the ADA confirmatory assay are classified as “confirmed positive”. Results are shown in Table 5:

5 RESULT

The IFX-2 treated animals did not develop detectable ADA 8 weeks after administration.

Table 5. Summary of ADA screening and confirmatory ELISA

IFX-1 treated animals			IFX-2 treated animals		
animal ID (timepoint)	ADA screening	ADA confirmatory	animal ID (timepoint)	ADA screening	ADA confirmatory
#1 (pre)	negative	not applicable	#13 (pre)	negative	not applicable
#1 (TD 57)	negative	not applicable	#13 (TD 57)	negative	not applicable
#2 (pre)	negative	not applicable	#14 (pre)	negative	not applicable
#2 (TD 57)	potential positive	confirmed positive	#14 (TD 57)	negative	not applicable
#3 (pre)	negative	not applicable	#15 (pre)	negative	not applicable
#3 (TD 57)	negative	not applicable	#15 (TD 57)	negative	not applicable
#4 (pre)	negative	not applicable	#16 (pre)	negative	not applicable
#4 (TD 57)	potential positive	confirmed positive	#16 (TD 57)	negative	not applicable
#5 (pre)	negative	not applicable	#17 (pre)	negative	not applicable
#5 (TD 57)	negative	not applicable	#17 (TD 57)	negative	not applicable
#6 (pre)	negative	not applicable	#18 (pre)	negative	not applicable
#6 (TD 57)	negative	not applicable	#18 (TD 57)	negative	not applicable

SEQUENCE LISTING FREE TEXT INFORMATION

	SEQ ID NO: 6	VH0
	SEQ ID NO: 7	VH1
	SEQ ID NO: 8	VH2
5	SEQ ID NO: 9	VH3
	SEQ ID NO: 10	VH4
	SEQ ID NO: 11	VH5
	SEQ ID NO: 12	V_kappa_0
	SEQ ID NO: 13	V_kappa_1
10	SEQ ID NO: 14	V_kappa_2
	SEQ ID NO: 15	V_kappa_3
	SEQ ID NO: 16	V_kappa_4
	SEQ ID NO: 17	Consensus sequence formed from the combination of VH1 to VH5
	SEQ ID NO: 18	Consensus sequence formed from the combination of V_kappa_1 to
15	V_kappa_4	
	SEQ ID NO: 20	IFX-1 CDR1 heavy chain
	SEQ ID NO: 21	IFX-1 CDR2 heavy chain
	SEQ ID NO: 22	IFX-1 CDR3 heavy chain
20	SEQ ID NO: 23	IFX-1 CDR1 light chain
	SEQ ID NO: 24	IFX-1 CDR2 light chain
	SEQ ID NO: 25	IFX-1 CDR3 light chain