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(54) Titre : ANTAGONISTES DU RECEPTEUR DU GLUCAGON
(54) Title: GLUCAGON RECEPTOR ANTAGONISTS

(57) Abrégé/Abstract:
The present invention relates to glucagon receptor polypeptide antagonists which inhibit the binding of the hormone glucagon to its receptor. More particularly, the present invention relates to high affinity glucagon receptor antibodies or Fab fragments thereof that inhibit binding of glucagon to its receptor and their use in the treatment or prevention of type 2 diabetes (NIDDM) and related disorders in mammalian species.

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(54) Title: GLUCAGON RECEPTOR ANTAGONISTS

(57) Abstract: The present invention relates to glucagon receptor polypeptide antagonists which inhibit the binding of the hormone glucagon to its receptor. More particularly, the present invention relates to high affinity glucagon receptor antibodies or Fab fragments thereof that inhibit binding of glucagon to its receptor and their use in the treatment or prevention of type 2 diabetes (NIDDM) and related disorders in mammalian species.

GLUCAGON RECEPTOR ANTAGONISTS

Field of the invention

The present invention relates to glucagon receptor polypeptide antagonists which inhibit the binding of the hormone glucagon to its receptor. More particularly, the present invention relates to high affinity glucagon receptor antibodies or Fab fragments thereof that inhibit binding of glucagon to its receptor and their use in the treatment or prevention of type 2 diabetes (NIDDM) and related disorders in mammalian species.

Background to the invention

Glucagon is 29 amino acid peptide hormone produced by pancreatic α-cells in response to low blood glucose levels. Glucagon binds to a membrane-associated glucagon receptor on the surface of hepatocytes, which triggers a G-protein signal transduction cascade, activating intracellular cyclic AMP and leading to release of glucose through denovo synthesis (gluconeogenesis) and glycogen breakdown (glycogenolysis).

Unson *et al*., disclose polyclonal antibodies raised against synthetic peptides corresponding to two extracellular portions of the rat receptor. In the assay disclosed, polyclonal antibodies raised against amino acid residues 126-137 and 206-219 were found to block binding of glucagon to the receptor in rat liver membranes (Unson *et al*., PNAS Vol. 93, pp. 310-315, Jan 1996).

Buggy *et al*., discloses the preparation of a monoclonal antibody that is said to compete with glucagon for the hormone binding site of the receptor in an *in vitro* assay (Buggy *et al.,* Horm. Metab. Res. 28 (1996) 215-219). In the assay disclosed the antibody, given the designation CIV395.7A, recognizes the human and rat glucagon receptors, but not mouse. In order to develop antibodies for human therapeutic treatments it is commonly necessary to perform pre-clinical efficacy and safety studies in validated rat and/or murine animal models. It would therefore greatly facilitate drug development of a therapeutic antibody and thus be highly desirable to provide a pre-clinical therapeutic antibody candidates that are able to bind rat, murine and human forms of the glucagon receptor.

Wright *et al*., disclose a monoclonal antibody designated hGR-2 F6 and the amino acid sequence of a Fab fragment thereof. This antibody has been raised in a mouse against

the human glucagon receptor and described in the disclosed assay as a competitive antagonist at this receptor (Wright *et al.,* Acta Cryst. (2000) D56, 573-580). The applicant has found that hGR-2 F6 binds to the rat and murine forms of the glucagon receptor with only low affinity, and no therapeutic efficacy has been found for hGR-2F6 in a diabetic rat *in vivo* model at high doses. In particular, this antibody was unable to reduce blood serum glucose in the rat model with any statistical significance (unpublished).

The applicant has identified a need to provide therapeutic monoclonal antibodies that will bind with high affinity to the glucagon receptor and thereby inhibit the binding of glucagon thereto, to provide effective treatments for diabetes, preferably type 2 diabetes and related disorders. Furthermore, in order to allow pre-clinical drug development of an antibody it is clearly desirable to provide monoclonal antibodies that can bind to the human, rat and murine forms of the glucagon receptor to allow obligatory pre-clinical safety and efficacy studies to be undertaken.

Summary of Invention

The present invention relates to novel monoclonal antibodies or Fab fragments thereof that are able to specifically bind with a high affinity to native glucagon receptors of human, rat and murine origin. Furthermore, the present inventors provide monoclonal antibodies or Fab fragments thereof that not only bind glucagon receptors of multiple origins, but for the first time provide glucagon receptor binding monoclonal antibodies that show *in vivo* efficacy in their ability to reduce blood serum glucose.

In a first aspect the present invention relates to a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, wherein said Fab fragment capable of binding to human, rat and murine glucagon receptors and inhibits glucagon binding to each receptor with a Ki of less than 50nM.

In a further unexpected finding the inventors for the first time provide antibodies or Fab fragments thereof capable of specifically binding to the glucagon receptor that are able to significantly increase *in vivo* serum concentrations of GLP-1. Increasing serum levels of GLP-1 is known in the art to enhance β-cell function, reduce glucagon secretion and delay gastric emptying and is recognized as being highly advantageous in the treatment of diabetes type 2 and associated conditions.

In a second aspect, the invention relates to a pharmaceutical composition comprising an effective amount of a Fab fragment or humanized monoclonal antibody according to the present invention and a pharmaceutically acceptable excipient.

In a third aspect, the invention provides a method of treating type 1 or type 2 diabetes and in the achievement of weight loss in a human, wherein said method comprises administrating an effective amount of a Fab fragment or humanized monoclonal antibody according to the present invention, to a patient in need thereof.

A fourth aspect of the invention comprises a Fab fragment or humanized monoclonal antibody according to the present invention for use as a medicament.

A fifth aspect of the invention of relates to the use of a Fab fragment or humanized monoclonal antibody according to the present invention in the manufacture of a medicament for the treatment or prevention of type 1 or type 2 diabetes or in the achievement of weight loss in a human.

Detailed Description

The present invention relates to a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, wherein said Fab fragment is capable of binding to human, rat and murine glucagon receptors and inhibits glucagon binding to each receptor with a Ki of less than 50nM.

The invention also provides monoclonal antibodies, which in addition to the glucagon receptors of human, rat and murine origin, are also able to bind with a high affinity to the glucagon receptor of a cynomologous monkey. Preferably, the Fab fragment or humanized monoclonal antibody comprising said Fab fragment therefore also inhibits glucagon binding to a cynomologous monkey glucagon receptor with a Ki of less than 50nM. Preferably the Fab fragment or humanized monoclonal antibody comprising said Fab fragment has a Ki at each of the named glucagon receptors of less than 30nM, more preferably less than 20nM, further preferred less than 10nM. Further preferred the Fab fragment has an *in vitro* Ki at the rat, murine and cyno receptors of less than 20nM and *in vitro* Ki at the human receptor of less than 5nM. More preferably the Ki of the Fab fragment, and in particular at the human glucagon receptor, is from 0.1nM to 15nM, most preferably from 1 to 10nM.

A Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to the present invention preferably has a functional binding affinity (Kb) at the human and rat glucagon receptors of at least 100nM. Further preferred the Fab fragment or humanized monoclonal antibody comprising said Fab fragment has a functional binding affinity at these receptors of at least 50nM, more preferably at least 10nM. In a most preferred embodiment the Fab fragment or humanized monoclonal antibody comprising said Fab fragment has a functional binding affinity at the human and rat glucagon receptor of 1 to 10nM.

In a particularly unexpected finding, the applicant has noted a very rapid rate at which serum GLP-1 is increased and serum blood glucose decreased on *in vivo* exposure to the Fab fragment or humanized monoclonal antibody comprising said Fab fragment having the binding properties identified in accordance with this invention. In order to maximize this favourable effect it is preferable that the Fab fragment or humanized monoclonal antibody comprising said Fab fragment does not appreciably bind to the GLP receptor i.e. Ki greater than 5000nM.

Description of the Sequences:

SEQ ID NOS 1 to 22 refer to the light and heavy chain CDRs of Table 1:

SEQ ID NOS 23 to 30 refer to preferred human framework regions described herein:

SEQ ID NOS 31 and 32 are amino acid and cDNA sequences of the human GluR:

SEQ ID NOS 33 and 34 are amino acid and cDNA sequences of the rat GluR:

SEQ ID NOS 35 and 36 are amino acid and cDNA sequences of the murine GluR:

SEQ ID NOS 37 and 38 are amino acid and cDNA sequences of the cyno GluR:

SEQ ID NOS 39 and 40 are the variable region amino acid sequences of example 1 (Ab1)

SEQ ID NOS 41 and 42 are the variable region amino acid sequences of example 2 (Ab2)

SEQ ID NOS 43 and 44 are the variable region amino acid sequences of example 3 (Ab3)

SEQ ID NOS 45 and 46 are the variable region amino acid sequences of example 4 (Ab4)

SEQ ID NOS 45 and 47 are the variable region amino acid sequences of example 5 (Ab5)

SEQ ID NOS 45 and 48 are the variable region amino acid sequences of example 6 (Ab6)

SEQ ID NOS 45 and 49 are the variable region amino acid sequences of example 7 (Ab7)

SEQ ID NO: 50 refers to a preferred kappa light chain IgG4 constant region.

SEQ ID NO: 51 refers to a preferred heavy chain CH1 constant domain.

SEQ ID NO: 52 refers to a preferred modified human IgG4 Fc region.

SEQ ID NOS 53 to 55 refer to preferred light chain CDRs.

SEQ ID NOS 56 to 68 refer to preferred heavy chain CDRs.

SEQ ID NOS 59 and 60 are light and heavy chain protein sequences of example 1 (Ab1).

SEQ ID NOS 61 and 62 are light and heavy chain protein sequences of example 2 (Ab2).

SEQ ID NOS 63 and 64 are light and heavy chain protein sequences of example 3 (Ab3).

SEQ ID NOS 65 and 66 are light and heavy chain protein sequences of example 4 (Ab4).

SEQ ID NOS 65 and 67 are light and heavy chain protein sequences of example 5 (Ab5).

SEQ ID NOS 65 and 68 are light and heavy chain protein sequences of example 6 (Ab6).

SEQ ID NOS 65 and 69 are light and heavy chain protein sequences of example 7 (Ab7).

SEQ ID NOS 70 and 71 are light and heavy chain DNA sequences of example 1 (Ab1).

SEQ ID NOS 72 and 73 are light and heavy chain DNA sequences of example 2 (Ab2).

SEQ ID NOS 74 and 75 are light and heavy chain DNA sequences of example 3 (Ab3).

SEQ ID NOS 76 and 77 are light and heavy chain DNA sequences of example 4 (Ab4).

SEQ ID NOS 76 and 78 are light and heavy chain DNA sequences of example 5 (Ab5).

SEQ ID NOS 76 and 79 are light and heavy chain DNA sequences of example 6 (Ab6).

SEQ ID NOS 76 and 80 are light and heavy chain DNA sequences of example 7 (Ab7).

Definitions:

The "glucagon receptor" also referred to herein as "GluR" belongs to the G protein-coupled receptor class 2 family consisting of a long amino terminal extracellular domain, seven transmembrane segments, and an intracellular C-terminal domain. Glucagon receptors are notably expressed on the surface of hepatocytes where they bind to glucagon and transduce the signal provided thereby into the cell. DNA sequences encoding glucagon receptors of rat and human origin have been isolated and disclosed in the art (EP0658200B1). The murine and cynomologous monkey homologues have also been isolated and sequenced (Burcelin, *et al.*, Gene 164 (1995) 305-310); McNally *et al.*, Peptides 25 (2004) 1171-1178).

The term "inhibits" as used herein with respect to an activity of an antibody or Fab fragment thereof of the invention means the ability to substantially antagonize the biological activity of the glucagon receptor. This ability is reflected in the Ki values calculated from the [^{125}I] glucagon binding assay described herein.

The term "humanized" as used in reference to a monoclonal antibody of the invention refers to an antibody with at least human frameworks and constant regions (CL, CH domains (e.g., CH1, CH2, CH3), and hinge), and CDRs derived from glucagon receptor binding antibodies. Human frameworks comprise frameworks that correspond to human germline sequences as well as sequences with somatic mutations. Human frameworks and constant regions may be fully human or may vary from the native sequences by one or more amino acid substitutions, terminal and intermediate additions and deletions, and the like. CDRs may be derived from one or more CDRs that bind to the glucagon specific receptors in this application in the context of any antibody framework. For example, the CDRs of the humanized antibody of the present invention may be derived from CDRs that bind glucagon receptors in the context of a mouse antibody framework and then are engineered to bind glucagon receptors in the context of a human framework.

The term "monoclonal antibody" refers to an antibody that is derived from a single copy or clone, including *e.g.,* any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Preferably a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

The term "*in vivo* efficacy" as used herein with respect to an antibody of the invention means the ability of the antibody to impart a positive biological effect in a human or animal model. Preferably *in vivo* efficacy refers to a glucose normalization effect on an animal showing elevated blood glucose in response to an antibody of the present invention as compared to a control response. A diabetic Zucker diabetic fatty rat (ZDF) model (Horm Metab Res. 2005 Feb;37(2):79-83) may be appropriately used to assess *in vivo* efficacy, wherein *in vivo* efficacy preferably denotes 100% blood glucose normalization on exposure of the animal to ≤30mg/kg dosage of humanized antibody according to the present invention. More preferably *in vivo* efficacy denotes 100% blood glucose normalization on exposure of the animal to a dosage of ≤15mg/kg of antibody, further preferred at a dosage of ≤10mg/kg, more preferably 0.1 to 5 mg/kg. In a most preferred embodiment *in vivo* efficacy is used to denote 100% blood glucose normalization in a diabetic ZDF rat model on exposure of the animal to 1 to 3mg/kg dosage of humanized antibody according to the present invention.

The term "glucose normalization" refers to mean plasma glucose values in a ZDF rat model of less than 120mg/dL, preferably in the range of 110 to 120 mg/dL. Plasma glucose may be determined in accordance with Etgen *et al*., (Metabolism 2000; 49(5): 684-688) or calculated from a conversion of whole blood glucose concentration in accordance with D'Orazio *et al*., (Clin. Chem. Lab. Med. 2006; 44(12): 1486-1490).

As used herein, "Fab fragment" refers to that portion of an antibody molecule, within the variable region, which contains the amino acid residues of the light and heavy chain CDR and framework sequences in addition to CL and CH1 domain.

The 3 CDRs of the heavy chain are herein referred to as "CDRH1, CDRH2, and CDRH3" and the 3 CDRs of the light chain are referred to as "CDRL1, CDRL2 and CDRL3". Assignment of amino acids to each domain is in accordance with a well-known convention (Kabat, *et al*., *Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat, *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)). The antigen-binding domain, or the CDRs of the antigen-binding domain, can be derived from other non-human species including, but not limited to, rabbit, mouse, rat or hamster.

The present inventors have identified heavy and light chain CDR sequences which may be used in combination to prepare antibody Fab fragments which demonstrate particularly high affinity for glucagon receptors of murine, rat, cynomologous monkey and human origin. Fab fragments preferably comprise;

(i)	a light chain CDRL1:	S X S S S V S Y X_1 H	SEQ ID NO: 53
(ii)	a light chain CDRL2:	T T S X_2 L A H	SEQ ID NO: 54
(iii)	a light chain CDRL3:	X_3 X_4 R S T X_5 P P T	SEQ ID NO: 55
(iv)	a heavy chain CDRH1:	G D D I T S G Y X_6 X_7	SEQ ID NO: 56
(v)	a heavy chain CDRH2:	Y I S Y S G S T X_8Y X_9 P S L K S	SEQ ID NO: 57
(vi)	a heavy chain CDRH3:	P P X_{10} Y Y G F G P Y A X_{11} D Y	SEQ ID NO: 58

wherein:

X	= Y or A	X_6	= W or H
X_1	= M or I	X_7	= N, D or E
X_2	= N or Y	X_8	= Y, Q, S or V

X_3 = Q or L
X_4 = Q or W
X_5 = L or I
X_9 = N, S or I
X_{10} = G or A
X_{11} = M or L

More preferably, X is Y or A; X_1 is I; X_2 is Y; X_3 is Q or L; X_4 is Q or W; X_5 is L or I; X_6 is W or H; X_7 is D or E; X_8 is Y, Q, S or V; X_9 is S; X_{10} is G or A; X_{11} is L. Further preferred, X is A; X_1 is I; X_2 is Y; X_3 is Q; X_4 is Q; X_5 is L; X_6 is H; X_7 is D or E; X_8 is Y, Q or S; X_9 is S; X_{10} is G or A; X_{11} is L.

Preferably a Fab fragment or humanized monoclonal antibody comprising said Fab fragment of the present invention comprises the CDR sequences:

CDRL1	1	2	3	4	5	6	7	8	9	10						
	S	A	S	S	S	V	S	Y	I	H						
CDRL2	1	2	3	4	5	6	7									
	T	T	S	Y	L	A	H									
CDRL3	1	2	3	4	5	6	7	8	9							
	Q	Q	R	S	T	L	P	P	T							
CDRH1	1	2	3	4	5	6	7	8	9	10						
	G	D	D	I	T	S	G	Y	H	D						
CDRH2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Y	I	S	Y	S	G	S	T	Y	Y	S	P	S	L	K	S
CDRH3	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
	P	P	G	Y	Y	G	F	G	P	Y	A	L	D	Y		

wherein said Fab fragment has one, two or three amino acid substitutions selected from the group consisting of:

CDRL1: A2Y, I9M;
CDRL2: Y4N;
CDRL3: Q1L, Q2W, L6I;
CDRH1: H9W, D10E, D10N;
CDRH2: Y9Q, Y9S, Y9V, S11N, S11I;
CDRH3: G3A, L12M.

More preferably said Fab fragment comprises one, two or three amino acid substitutions selected from: CDRH1: D10E; CDRH2: Y9Q, Y9S; CDRH3: G3A.

A Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to the present invention preferably comprises CDR sequences selected from:

(i) a light chain with a CDRL1 of SEQ ID NO 2; CDRL2 of SEQ ID NO: 4; CDRL3 of SEQ ID NO: 7; and a heavy chain with a CDRH1 of SEQ ID NO: 11; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 21;

(ii) a light chain with a CDRL1 of SEQ ID NO 1; CDRL2 of SEQ ID NO: 4; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 10; CDRH2 of SEQ ID NO: 16; CDRH3 of SEQ ID NO: 20;

(iii) a light chain with a CDRL1 of SEQ ID NO: 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 8; and a heavy chain with a CDRH1 of SEQ ID NO: 11; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 21;

(iv) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 21;

(v) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 17; CDRH3 of SEQ ID NO: 21;

(vi) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 22; and

(vii) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 13; CDRH2 of SEQ ID NO: 18; CDRH3 of SEQ ID NO: 22.

It is particularly desirable that an antibody in accordance with the present invention shows *in vivo* efficacy at a low plasma concentration. *In vivo* efficacy should be observed at a dosage of 30 mg/kg in a ZDF rat model, preferably at a dosage of less than 15mg/kg, more preferably less than 5mg/kg, further preferred with the range 0.1 to 5mg/kg. Most preferably an antibody in accordance with the present invention achieves 100% glucose normalization in a ZDF rat model at a dosage of about 1 to 3mg/kg. It has

been found that particularly preferred antibodies in accordance with the present invention are able to show 100% glucose normalization in an *in vivo* ZDF rat model at the low dosage of 3mg/kg. The present invention therefore preferably comprises a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, wherein said Fab fragment comprises:

(i) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 21;

(ii) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 17; CDRH3 of SEQ ID NO: 21;

(iii) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 15; CDRH3 of SEQ ID NO: 22; or

(iv) a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 13; CDRH2 of SEQ ID NO: 18; CDRH3 of SEQ ID NO: 22.

In a further preferred embodiment the present invention relates to a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, wherein said Fab fragment comprises a light chain with a CDRL1 of SEQ ID NO 3; CDRL2 of SEQ ID NO: 5; CDRL3 of SEQ ID NO: 6; and a heavy chain with a CDRH1 of SEQ ID NO: 12; CDRH2 of SEQ ID NO: 17; CDRH3 of SEQ ID NO: 21. It has been found that an antibody comprising Fab fragments in accordance with this embodiment has a particularly advantageous property of maintaining *in vivo* efficacy over an extended period as compared to other similar antibodies within the genus being described.

A Fab fragment or humanized monoclonal antibody comprising said Fab fragment of the present invention, preferably comprises light and heavy variable chain framework regions of human origin. Moreover, a variety of different human framework sequences may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. Preferably, the framework regions of the Fab fragment or humanized monoclonal antibody of the invention are of human origin or substantially of human origin (at least 95%, 97% or 99% of human origin). The sequences of framework

regions of human origin may be obtained from ImMunoGenetics (IMGT) via their website http://imgt.cines.fr/textes/IMGTindex/FR.html or from The immunoglobulin Factsbook, by Marie-Paule Lefranc, Gerard Lefranc, Academic Press 2001, ISBN 012441351. For example, germline light chain frameworks may be selected from the group consisting of: Al1, A17, A18, A19, A20, A27, A30, LI, L1I, L12, L2, L5, L15 L6, L8, O12, O2, and O8 and germline heavy chain framework regions may be selected from the group consisting of: VH2-5, VH2-26, VH2-70, VH3-20, VH3-72, VHI-46, VH3-9, VH3-66, VH3-74, VH4-31, VH I - 18, VH I -69, VI-13-7, VH3-11, VH3-15, VH3-21, VH3-23, VH3-30, VH3-48, VH4-39, VH4-59, and VH5-5I.

The specific antibodies disclosed herein can be used as a template or parent antibody to make additional antibodies of the invention. In one approach the parent antibody CDRs are grafted into a human framework that has a high sequence identity with the parent antibody framework. The sequence identity of the new framework will generally be at least 80%, at least 85%, or at least 90% with the corresponding framework in the parent antibody. This grafting may result in a reduction in binding affinity compared to the parent antibody. If this is the case, the framework can be back-mutated to the parent framework at certain positions based on specific criteria published by Queen et al., [Queen, *et al.*, Proc. Natl. Acad. Sci. USA 88, 2869 (1991)]. Further methods that may be used include, for example, Jones *et al.*, Nature, 321:522 (1986); Riechmann *et al.*, Nature , 332:323-327 (1988); Verhoeyen *et al.*, Science, 239:1534 (1988).

Most preferably the Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to the present invention comprises the following light chain framework (FR) sequences: FR1 SEQ ID NO: 23; FR2 SEQ ID NO: 24; FR3 SEQ ID NO: 25; FR4 SEQ ID NO: 26; and heavy variable chain frameworks sequences: FR5 SEQ ID NO: 27; FR6 SEQ ID NO: 28; FR7 SEQ ID NO: 29; FR8 SEQ ID NO: 30; wherein these are arranged as light chain variable sequence FR1 - CDRL1 - FR2 - CDRL2 - FR3 - CDRL3 - FR4 and heavy chain variable sequence FR5 - CDRH1 - FR6 - CDRH2 - FR7 - CDRH3 - FR8.

The applicant has surprisingly determined that when predicting the *in vivo* efficacy of a glucagon receptor antagonism antibody through the use of an *in vitro* competitive glucagon binding assay, that it is the affinity of the Fab fragment that is positively correlated with *in vivo* efficacy. Conversely, the binding affinity (Ki) of a full

antibody to the glucagon receptor is not necessarily a valid predictor of *in vivo* efficacy. A process of preparing monoclonal antibodies having the favourable properties sought herein, therefore preferably comprises selecting a Fab fragment that binds to each glucagon receptor with a Ki of less than 50nM in an *in vitro* competitive glucagon binding assay using heterologously expressed glucagon receptor gene. More preferably said process comprises selecting a Fab fragment that has an *in vitro* Ki at each of the glucagon receptors of less than 30nM, more preferably less than 20nM, further preferred less than 10nM. More preferably, the Fab fragment is selected by an *in vitro* Ki at the rat, murine and cyno receptors of less than 20nM and *in vitro* Ki at the human receptor of less than 5nM. More preferably the Ki of the selected Fab fragment, and in particular at the human glucagon receptor, is from 0.1nM to 15nM, most preferably from 1 to 10nM. Fab fragments identified by this process may then be suitably expressed as full antibodies for therapeutic use by techniques commonly known in the art.

It will be appreciated that applying the teaching of the present invention the person skilled in the art may use common techniques e.g. site directed mutagenesis, to substitute amino acids within the specific CDR and framework sequences herein disclosed and in so doing generate further variable region amino acid sequences derived from the sequences herein provided. Up to all 20 alternative naturally occurring amino acids may be introduced at a specific substitution site. The *in vitro* selection process defined here above may then be suitably used to screen these additional variable region amino acid sequences for Fab fragments having the claimed cross reactivity and *in vitro* Ki that has been found by the present applicants to be indicative of *in vivo* efficacy. In this way further Fab fragments are identified that are suitable for preparing a humanized antibody in accordance with the present invention. Preferably the amino acid substitution within the frameworks is restricted to one, two or three positions within one or each of the framework sequences disclosed herein. Preferably amino acid substitution within the CDRs is restricted to one to three positions within one or each CDR, more preferably substitution at one or two amino acid positions within one or each CDR is performed. Further preferred, amino acid substitution is performed at one or two amino acid positions in the CDRs of the heavy chain variable region. Most preferably amino acid substitution is performed at one or two amino acid positions within CDRH2.

A suitable methodology for combining CDR and framework substitutions to prepare alternative antibodies according to the present invention, using an antibody described herein as a parent antibody, is provided in Wu *et al.*, J. Mol. Biol., 294:151-162.

As used herein, the Fc portion of an immunoglobulin refers to the constant region of an antibody from both heavy chains, which associate through non-covalent interactions and disulfide bonds. The Fc portion can include the hinge regions and extend through the CH2 and CH3 domains to the C-terminus of the antibody. The Fc portion can further include one or more glycosylation sites. Monoclonal antibodies of the present invention may have a heavy chain constant region selected from any of the immunoglobulin classes IgA, IgD, IgG, IgM and IgE. Preferably antibodies of the invention contain an Fc portion which is derived from human IgG4 Fc region because of its reduced ability to bind FcγR and complement factors as compared to other IgG sub-types. More preferably, the IgG4 Fc region of an antibody of the present invention contains substitutions that further reduce effector function [Issacs et al., (1996) Clin. Exp. Immunol. 106:427-433]. These may be selected from one or more of the group comprising proline for glutamate at residue 233, alanine or valine for phenylalanine at residue 234 and alanine or glutamate for leucine at residue 235 (EU numbering, Kabat, E.A. et al. (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. U.S. Dept. of Health and Human Services, Bethesda, MD, NIH Publication no. 91-3242). These residues corresponds to positions 15, 16 and 17 in SEQ ID NO: 52 and positions 235, 236 and 237 of SEQ ID NO: 67. Further, removing the N-linked glycosylation site in the IgG4 Fc region by substituting Ala for Asn at residue 297 (EU numbering) which corresponds to position 79 of SEQ ID NO:52 is another way to ensure that residual effector activity is eliminated in the context of a humanized antibody.

In addition, the IgG4 Fc portion for use with a humanized monoclonal antibody of present invention preferably contains a substitution that stabilizes heavy chain dimer formation and prevents the formation of half-IgG4 Fc chains. This construct consists of serine at position at 228 (EU numbering) being substituted by proline (amino acid residue 10 in SEQ ID NO:52). The C-terminal lysine residue present in the native molecule may also be deleted in the IgG4 derivative Fc portion of the antibodies discussed herein

(position 229 of SEQ ID NO:52; deleted lysine referred to as des-K). A most preferred IgG4 Fc portion is provided by amino acids 221 to 448 of SEQ ID NO: 67.

The invention is further directed to an isolated nucleic acid sequence encoding an antibody of the invention; a vector (or vectors) comprising that nucleic acid, optionally operably linked to control sequences recognized by a host cell transformed with the vector; a host cell comprising that vector; a process for producing an antibody or Fab fragment thereof according to the invention comprising culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture medium.

In another embodiment, the invention provides a pharmaceutical composition comprising the Fab fragment or humanized monoclonal antibody of the invention. The pharmaceutical composition of the invention may further comprise a pharmaceutically acceptable carrier. In said pharmaceutical composition, the Fab fragment or humanized monoclonal antibody of the invention is the active ingredient. Preferably the pharmaceutical composition comprises a homogeneous or substantially homogeneous population of the Fab fragment or humanized monoclonal antibody of the invention. The composition for therapeutic use is sterile and may be lyophilized, optionally supplied with an appropriate diluent.

A further embodiment of the present invention comprises a host cell or cell culture that is a recipient of any isolated polynucleotide of the invention or any recombinant vector(s) comprising a sequence encoding a HCVR, LCVR, monoclonal antibody or Fab fragment of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transformed, transduced or infected in vivo or in vitro with one or more a recombinant vectors or a polynucleotide expressing a monoclonal antibody of the invention or a light chain or heavy chain thereof. A host cell which comprises a recombinant vector of the invention (either stably incorporated into the host chromosome or not) may also be referred to as a "recombinant host cell". Preferred host cells for use in the invention are CHO cells, NS0 cells, HeLa, SP2/0 cells or COS cells. Additional host cells for use in the invention include plant cells, yeast cells, other mammalian cells and prokaryotic cells.

The invention embodies an article of manufacture comprising a packaging material and a Fab fragment or humanized monoclonal antibody of the invention contained within said packaging material and wherein the packaging material comprises a package insert which indicates that the Fab fragment or humanized monoclonal antibody neutralizes a GluR or decreases the level of GluR activity in the patient.

Biological Assays:

Glucagon Receptor (GlucR) Membrane Preparations

Membrane preparations for binding studies are prepared from 293HEK cells expressing cloned human, mouse, cynomologous monkey or rat glucagon receptor. Each clonal cell line is first grown as a suspension culture and the frozen cell pellet is resuspended in membrane prep buffer consisting of 25 mM Tris, pH 7.5, 1 mM $MgCl_2$, Complete^R EDTA-free protease inhibitor tablets (Roche Applied Science), and 20 U/ml DNase I (Sigma Chemical Company) at 4°C. The cells are homogenized with a motor-driven Teflon-glass Potter-Elvehjem homogenizer using 25 strokes, followed by centrifugation at 1800 x g for 15 minutes at 4°C. The supernatant is collected and the pellet resuspended in membrane prep buffer, rehomogenized and centrifuged. The second supernatant is combined with the first supernatant and recentrifuged at 1800 x g for 15 mins to clarify. The clarified supernatant is transferred to high speed tubes and centrifuged at 25000 x g for 30 minutes at 4°C. The membrane pellet (P2) is resuspended in the membrane prep buffer (without DNAase), aliquoted, quick frozen on dry ice and stored at -80°C until needed.

[^{125}I]Glucagon Binding by Scintillation Proximity Assay (SPA).

A competitive receptor/ligand binding experiment is adapted to a scintillation proximity assay (SPA) format. Incubations are performed in clear bottom, opaque 96-well microplates. Compound is serially diluted 3-fold in binding buffer consisting of 25 mM Hepes, pH 7.4, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% fatty acid free BSA, 0.003% tween-20 and Complete^R EDTA-free protease inhibitor tablets. P2 membranes (prepared above) are diluted in binding buffer from each receptor preparation, then added to the diluted compound followed by addition of 0.15 mgs of wheat germ agglutinin (WGA) SPA beads (GE Healthcare) previously blocked with 1% fatty acid free BSA, and 0.15

nM [^{125}I]-Glucagon (Perkin-Elmer). Plates are sealed with adhesive sealing tape, mixed end over end, and incubated at room temperature for 12 hours. The radioactivity bound to the receptor (in close proximity to the WGA SPA bead) is quantified on a PE Life and Analytical Sciences Trilux Microbeta plate scintillation counter and expressed as counts per minute (CPM). Total binding is determined in the absence of added compound and nonspecific binding is determined by adding 1 uM glucagon (Lilly Research Labs). The final concentration of 1 uM of unlabeled glucagon is capable of completely inhibiting [^{125}I]-glucagon binding to background levels.

[^{125}I]Glucagon Binding Data Analysis.

Raw CPM data for concentration curves of compound are converted to percent inhibition by subtracting nonspecific binding from the individual CPM values and dividing by the total binding signal, also corrected for nonspecific binding. Data is analyzed using four-parameter (curve maximum, curve minimum, IC_{50}, Hill slope) nonlinear regression routines (XLFit version 3.0: Activity Base, IDBS). The equilibrium dissociation constant determined by competitor inhibited radioligand binding, Ki, is calculated from the absolute IC_{50} value based upon the equation [$Ki = IC_{50}/(1 + D/Kd)$] where D equals the concentration of radioligand used in the experiment and Kd equals the equilibrium binding affinity constant of [^{125}I]glucagon, in the assay for each individual receptor species.

Glucagon-Stimulated cAMP Functional Antagonist Assay.

The functional antagonist activity is determined from the dose-dependent inhibition of increases in intracellular cAMP with a sub-maximal dose of glucagon using the same clonal rat, mouse, cynomologous, and human glucagon receptor-293HEK cell lines. Quantitation of the intracellular cAMP level is done with an Amplified Luminescent Proximity Homogeneous Assay, (Alpha Screen) from Perkin Elmer (6760625R). Briefly, cAMP generated within the cell competes for binding of a biotinylated cAMP-streptavidin coated Donor bead and a coated anti-cAMP antibody Acceptor bead. As the cAMP level within the cell increases, a disruption of the Acceptor bead-biotinlyated cAMP –Donor bead complex occurs. The functional assay is performed in 10 mM Hepes, pH 7.4, with 0.25 mM IBMX in HBSS containing Mg+2 and

Ca+2. The clonal glucagon receptor-293HEK cells are suspended at 2500 cells per well and 1 unit/well of biotinylated cAMP from the kit in a total volume of 20 uls. The cells are pre-incubated for 30 minutes at room temperature with 20 uls of either 3-fold serially diluted compounds or of 3-fold serially diluted cAMP for use as a standard curve. The reaction is started by the addition of 20 uls of 300 pM glucagon (3X), a dose sufficient to produce 90% of the maximal intracellular cAMP. After 60 minutes at room temperature in the dark, the reaction is stopped by the addition of 30 uls of lysis buffer made of 1% IGEPAL CA630 (Sigma) and 0.1% fatty-acid free BSA (Gibco) in 10 mM Hepes, pH 7.4 containing 1 unit each of the kit Donor and Acceptor beads per well. The plates are wrapped in foil to protect the Donor and Acceptor beads from light and mixed on Titertek shaker medium speed for 30 secs. After incubation overnight at room temperature, the plates are read on a Packard Fusion™-α Instrument.

Data Analysis for Functional cAMP Activity.

The alpha screen units are converted to pmoles cAMP generated per well based upon the cAMP standard curve. The pmoles cAMP produced in the presence of compound are converted to % of a maximal response with the submaximal dose of glucagon alone. Within each experiment, the concentration of glucagon needed to produce a 50% response in pmoles cAMP is determined. This EC50 concentration is used to normalize results between runs to a Kb where Kb = (EC50 compound)/ [1 + (pM glucagon used/ EC50 in pM for glucagon dose response)]. The data is analyzed using four-parameter (curve maximum, curve minimum, IC_{50}, Hill slope) nonlinear regression routines (XLFit version 3.0: Activity Base, IDBS).

Examples:

Antibody examples Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6 and Ab-7 are made and purified as known in the art. An appropriate host cell, such as HEK 293 EBNA or CHO, is either transiently or stably transfected with an expression system for secreting antibodies using an optimal predetermined light chain to heavy chain vector ratio or a single vector system encoding both a light chain (set out in SEQ ID NOS: 70, 72, 74, 76) and a heavy chain (set out in SEQ ID NOS: 71, 73, 75, 77, 78, 79, 80). Clarified medium into which the antibody has been secreted is purified using any of many commonly-used

techniques. For example, the medium may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient (such as 0.1 M sodium phosphate buffer pH 6.8 to 0.1 M sodium citrate buffer pH 2.5). Antibody fractions are detected, such as by SDS-PAGE, and then are pooled. Further purification is optional, depending on the intended use. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The purity of the antibody after these chromatography steps is greater than 99%. The product may be immediately frozen at -70°C or may be lyophilized.

Fab expression is achieved in E. coli wherein the Fab molecules are secreted into the periplasmic space. The cell wall is disrupted by osmotic shock, and the Fab containing a His tag is purified on an IMAC column.

Table 1 sets out the CDR combinations used in the antibody examples according to the present invention. The full antibody light chain combines the light chain framework sequences interspaced by three light chain CDRs; Framework 1 (SEQ ID NO: 23) - CDRL1 - Framework 2 (SEQ ID NO: 24) - CDRL2 - Framework 3 (SEQ ID NO: 25) - CDRL3 - Framework 4 (SEQ ID NO: 26) and the light chain constant region (SEQ ID NO: 53). The heavy chain framework sequences are interspaced by three heavy chain CDRs Framework 5 (SEQ ID NO: 27) - CDRH1 - Framework 6 (SEQ ID NO: 28) - CDRH2 - Framework 7 (SEQ ID NO: 29) - CDRH3 - Framework 8 (SEQ ID NO: 30) and then the heavy chain CH1 constant region (SEQ ID NO: 51) followed by Fc domain for the Ab, absent in Fab fragment (SEQ ID NO: 52, wherein X_{10}=P; X_{15}=E; X_{16}=A; X_{17}=A; X_{79}=N; X_{229}= is absent).

Table 1:

Example	CDRL 1 SEQ ID	CDRL 2 SEQ ID	CDRL 3 SEQ ID	CDRH 1 SEQ ID	CDRH 2 SEQ ID	CDRH 3 SEQ ID
Ab - 1	2	4	7	11	15	21
Ab - 2	1	4	6	10	16	20
Ab - 3	3	5	8	11	15	21

Ab - 4	3	5	6	12	15	21
Ab - 5	3	5	6	12	17	21
Ab - 6	3	5	6	12	15	22
Ab - 7	3	5	6	13	18	22

Antibody examples 1 through 7 and Fab fragments thereof all inhibit glucagon binding to human, mouse, cynomologous monkey and rat glucagon receptors in the above glucagon receptor binding assay with a Ki of less than 50nM.

***In vitro* activity of antibody of Example 4 (Ab4)**

Table 2. Functional cAMP Antagonism

Compound	rat GluR		human GluR		
	Kb (nM)	n	Kb (nM)	Stdev	n
Ab4	6.85	1	6.49	0.34	2

This assay demonstrates that at nanomolar concentrations an Ab4 binding to the GluR can block down stream activities of the rat or human glucagon receptor cell line, and reduce cAMP production by the cells.

Table 3. *In vitro* Ki (nM), for full antibody of Example 4 and respective Fab

Compound	human GlucR	mouse GlucR	rat GlucR	cyno GlucR	human GLP-1 R
Glucagon	3.13	2.72	12.77	5.78	
Ab4	1.34	0.83	1.24	6.00	>5000
Fab4	2.90	2.43	3.30	9.42	ND

This assay demonstrates that in an *in vitro* glucagon competition binding assay Ab4 binds with high affinity (Ki) to glucagon receptor of human, mouse, rat, cynomologous monkey origin and low affinity to the human GLP-1 receptor.

***In vivo* activity of antibody of Example 4 (Ab4)**

ZDF rats approximately 8 weeks of age and approximately 400 g in weight are dosed with a single subcutaneous injection of antibody according to example 4 (i.e. Ab4) or a human IgG (hIgG4) control. Each treatment group consists of 6 animals. Blood samples are taken for glucose measurements pre-dose and daily for 13 days following a

single subcutaneous 3 or 15 mg/kg dose of the Ab4 or 15 mg/kg of a hIgG control. Blood samples for GLP-1 analysis are taken pre-dose and at 2, 4, 6 and 8 days following the 3 or 15 mg/kg subcutaneous dose of the Ab4 or 15 mg/kg of the hIgG control.

Table 4. Blood glucose levels following a single 3 or 15 mg/kg subcutaneous dose of Ab4 or 15 mg/kg negative control to ZDF rats.

Time (days)	Ab4, 3mg/kg		Ab4, 15mg/kg		Control (15 mg/kg hIgG)	
	Mean glucose conc. (mg/dL)	S.D.	Mean glucose conc. (mg/dL)	S.D.	Mean glucose conc. (mg/dL)	S.D.
0	345.3	37.8	359	29.2	366.5	22.9
1	225.7	53.5	143.8	27.2	374.3	22.4
2	97.5	4.3	85.7	5.7	389.3	22.3
3	92.0	7.8	89.8	9.6	361	51.5
4	103.7	20.6	90.0	14.2	381.7	69.0
5	105.3	8.9	97.3	5.2	400.8	62.2
6	108.3	13.8	99.3	10.0	401.2	61.3
7	113.7	7.8	97.5	12.2	451.7	45.5
8	135.8	15.5	104.3	11.4	452.8*	33.6
9	151.0	21.1	109.2	7.0	394.3	40.8
10	149.0	9.2	103.0	15.3	418.3	33.3
11	159.3	28.9	106.3	10.3	410.0	34.4
12	151.7	29.9	108.2	9.5	417.7	58.8
13	218.5	88.1	114.8	11.5	443.0	48.9

There were 6 rats per group except for one value (*) where one animal measured AQL therefore n=5.

Table 5. Plasma GLP-1 levels following a single 3 or 15 mg/kg subcutaneous dose of Ab4 or 15 mg/kg negative control to ZDF rats.

Time (days)	Ab4, 3 mg/kg			Ab4, 15 mg/kg			Control (15 mg/kg hIgG)		
	Mean GLP-1 (pM)	SD	n	Mean GLP-1 (pM)	SD	n	Mean GLP-1 (pM)	SD	n
0	< 6	N.D.	0	<6	N.D.	0	< 6	N.D.	0
2	< 6	N.D.	0	10	3	2	< 6	N.D.	0
4	15	N.D.	1	13	3	5	< 6	N.D.	0
6	21	2	3	29	2	5	< 6	N.D.	0
8	36	5	3	112	54	6	< 6	N.D.	0

The average and sd are determined only from those rats that had quantifiable GLP-1 levels and the n denotes the number of animals per group that had quantifiable GLP-1 levels. If no animals had quantifiable GLP-1 levels the result is listed as < 6 pM. N.D. signifies the value was not determined.

CLAIMS

1. A Fab fragment or humanized monoclonal antibody comprising said Fab fragment, wherein said Fab fragment is capable of binding to human, rat and murine glucagon receptors and inhibits glucagon binding to each receptor with a Ki of less than 50nM.

2. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1, wherein said Fab fragment inhibits glucagon binding to a cynomologous monkey glucagon receptor with a Ki of less than 50nM.

3. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises;

(i)	a light chain CDRL1:	S X S S S V S Y X_1 H	SEQ ID NO: 53
(ii)	a light chain CDRL2:	T T S X_2 L A H	SEQ ID NO: 54
(iii)	a light chain CDRL3:	X_3 X_4 R S T X_5 P P T	SEQ ID NO: 55
(iv)	a heavy chain CDRH1:	G D D I T S G Y X_6 X_7	SEQ ID NO: 56
(v)	a heavy chain CDRH2:	Y I S Y S G S T X_8Y X_9 P S L K S	SEQ ID NO: 57
(vi)	a heavy chain CDRH3:	P P X_{10} Y Y G F G P Y A X_{11} D Y	SEQ ID NO: 58

wherein:

X	= Y or A	X_6	= W or H
X_1	= M or I	X_7	= N, D or E
X_2	= N or Y	X_8	= Y, Q, S or V
X_3	= Q or L	X_9	= N, S or I
X_4	= Q or W	X_{10}	= G or A
X_5	= L or I	X_{11}	= M or L

4. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 3, wherein

X	= A	X_6	= H
X_1	= I	X_7	= D or E
X_2	= Y	X_8	= Y, Q or S
X_3	= Q	X_9	= S
X_4	= Q	X_{10}	= G or A
X_5	= L	X_{11}	= L

5. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises the CDR sequences:

CDRL1		1	2	3	4	5	6	7	8	9	10					
		S	A	S	S	S	V	S	Y	I	H					
CDRL2		1	2	3	4	5	6	7								
		T	T	S	Y	L	A	H								
CDRL3		1	2	3	4	5	6	7	8	9						
		Q	Q	R	S	T	L	P	P	T						
CDRH1		1	2	3	4	5	6	7	8	9	10					
		G	D	D	I	T	S	G	Y	H	D					
CDRH2		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Y	I	S	Y	S	G	S	T	Y	Y	S	P	S	L	K	S
CDRH3		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
		P	P	G	Y	Y	G	F	G	P	Y	A	L	D	Y		

wherein said Fab fragment has one, two or three amino acid substitutions selected from the group consisting of:

CDRL1:	A2Y, I9M;	
CDRL2:	Y4N;	
CDRL3:	Q1L, Q2W, L6I;	
CDRH1:	H9W, D10E, D10N;	

CDRH2: Y9Q, Y9S, Y9V, S11N, S11I;

CDRH3: G3A, L12M.

6. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises

(i)	a light chain CDRL1:	S A S S S V S Y I H	(SEQ ID NO: 3)
(ii)	a light chain CDRL2:	T T S Y L A H	(SEQ ID NO: 5)
(iii)	a light chain CDRL3:	Q Q R S T L P P T	(SEQ ID NO: 6)
(iv)	a heavy chain CDRH1:	G D D I T S G Y H D	(SEQ ID NO: 12)
(v)	a heavy chain CDRH2:	Y I S Y S G S T Y Y S P S L K S	(SEQ ID NO: 15)
(vi)	a heavy chain CDRH3:	P P G Y Y G F G P Y A L D Y	(SEQ ID NO: 21)

7. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises

(i)	a light chain CDRL1:	S A S S S V S Y I H	(SEQ ID NO: 3)
(ii)	a light chain CDRL2:	T T S Y L A H	(SEQ ID NO: 5)
(iii)	a light chain CDRL3:	Q Q R S T L P P T	(SEQ ID NO: 6)
(iv)	a heavy chain CDRH1:	G D D I T S G Y H D	(SEQ ID NO: 12)
(v)	a heavy chain CDRH2:	Y I S Y S G S T Q Y S P S L K S	(SEQ ID NO: 17)
(vi)	a heavy chain CDRH3:	P P G Y Y G F G P Y A L D Y	(SEQ ID NO: 21)

8. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises

(i)	a light chain CDRL1:	S A S S S V S Y I H	(SEQ ID NO: 3)
(ii)	a light chain CDRL2:	T T S Y L A H	(SEQ ID NO: 5)
(iii)	a light chain CDRL3:	Q Q R S T L P P T	(SEQ ID NO: 6)
(iv)	a heavy chain CDRH1:	G D D I T S G Y H D	(SEQ ID NO: 12)
(v)	a heavy chain CDRH2:	Y I S Y S G S T Y Y S P S L K S	(SEQ ID NO: 15)
(vi)	a heavy chain CDRH3:	P P A Y Y G F G P Y A L D Y	(SEQ ID NO: 22)

9. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to claim 1 or claim 2, wherein said Fab fragment comprises

(i) a light chain CDRL1: S A S S S V S Y I H (SEQ ID NO: 3)
(ii) a light chain CDRL2: T T S Y L A H (SEQ ID NO: 5)
(iii) a light chain CDRL3: Q Q R S T L P P T (SEQ ID NO: 6)
(iv) a heavy chain CDRH1: G D D I T S G Y H E (SEQ ID NO: 13)
(v) a heavy chain CDRH2: Y I S Y S G S T S Y S P S L K S (SEQ ID NO: 18)
(vi) a heavy chain CDRH3: P P A Y Y G F G P Y A L D Y (SEQ ID NO: 22)

10. The Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to any one of claims 1 to 9, wherein said Fab fragment comprises light variable chain framework region: Framework 1 SEQ ID NO: 23; Framework 2 SEQ ID NO: 24; Framework 3 SEQ ID NO: 25; Framework 4 SEQ ID NO: 26; and heavy variable chain frameworks region: Framework 5 SEQ ID NO: 27; Framework 6 SEQ ID NO: 28; Framework 7 SEQ ID NO: 29; Framework 8 SEQ ID NO: 30.

11. A humanized monoclonal antibody comprising:
(i) two light chains of SEQ ID NO: 65 and two heavy chains of SEQ ID NO: 66;
(ii) two light chains of SEQ ID NO: 65 and two heavy chains of SEQ ID NO: 67;
(iii) two light chains of SEQ ID NO: 65 and two heavy chains of SEQ ID NO: 68; or
(iv) two light chains of SEQ ID NO: 65 and two heavy chains of SEQ ID NO: 69.

12. A vector comprising a nucleic acid sequence encoding a Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to any one of claims 1 to 11.

13. A pharmaceutical composition comprising an effective amount of a Fab fragment or humanized monoclonal antibody comprising said Fab fragment according to any one of claims 1 to 11 and a pharmaceutically acceptable excipient.

14. A Fab fragment or humanized monoclonal antibody comprising said Fab fragment, according to any one of claims 1 to 11 for use as a medicament.

15. A Fab fragment or humanized monoclonal antibody comprising said Fab fragment, according to any one of claims 1 to 11 for use in the treatment of type 1 or type 2 diabetes or for achieving weight loss in a human.

16. Use of a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, according to any one of claims 1 to 11, in the manufacture of a medicament for the treatment or prevention of type 1 or type 2 diabetes or for achieving weight loss in a human.

17. Use of a Fab fragment or humanized monoclonal antibody comprising said Fab fragment, according to any one of claims 1 to 11, for the treatment or prevention of type 1 or type 2 diabetes or for achieving weight loss in a human.