Antibodies Specific to FcRn

The disclosure relates to antibodies specific to FcRn, formulations comprising the same, use of each in therapy, processes for expressing and optionally formulating said antibody, DNA encoding the antibodies and hosts comprising said DNA.

FcRn is a non-covalent complex of membrane protein FcRn α chain and β2 microglobulin (β2M). In adult mammals FcRn plays a key role in maintaining serum antibody levels by acting as a receptor that binds and salvages antibodies of the IgG isotype. IgG molecules are endocytosed by endothelial cells, and if they bind to FcRn, are recycled transcytosed out into, for example circulation. In contrast, IgG molecules that do not bind to FcRn enter the cells and are targeted to the lysosomal pathway where they are degraded. A variant IgG1 in which His435 is mutated to alanine results in the selective loss of FcRn binding and a significantly reduced serum half-life (Firan et al. 2001, International Immunology 13:993).

It is hypothesised that FcRn is a potential therapeutic target for certain autoimmune disorders caused at least in part by autoantibodies. The current treatment for certain such disorders includes plasmapheresis. Sometimes the plasmapheresis is employed along with immunosuppressive therapy for long-term management of the disease. Plasma exchange offers the quickest short-term answer to removing harmful autoantibodies. However, it may also be desirable to suppress the production of autoantibodies by the immune system, for example by the use of medications such as prednisone, cyclophosphamide, cyclosporine, mycophenolate mofetil, rituximab or a mixture of these.

Examples of diseases that can be treated with plasmapheresis include: Guillain-Barré syndrome; Chronic inflammatory demyelinating polyneuropathy; Goodpasture's syndrome; hyperviscosity syndromes; cryoglobulinemia; paraproteinemia; Waldenström macroglobulinemia; myasthenia gravis; thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome; Wegener's granulomatosis; Lambert-Eaton Syndrome; antiphospholipid antibody syndrome (APS or APLS); microscopic polyangiitis; recurrent focal and segmental glomerulosclerosis in the transplanted kidney; HELLP syndrome; PANDAS syndrome; Refsum disease; Behcet syndrome; HIV-related neuropathy; Graves' disease in infants and neonates; pemphigus vulgaris; multiple sclerosis, rhabdomyolysis and alloimune diseases.

Plasmapheresis is sometimes used as a rescue therapy for removal of Fc containing therapeutics, for example in emergencies to reduced serious side effects.

Though plasmapheresis is helpful in certain medical conditions there are potential risks and complications associated with the therapy. Insertion of a rather large intravenous catheter can lead to bleeding, lung puncture (depending on the site of catheter insertion), and, if the catheter is left in too long, it can lead to infection and/or damage to the veins giving limited opportunity to repeat the procedure.

The procedure has further complications associated with it, for example when a patient's blood is outside of the body passing through the plasmapheresis instrument, the blood has a tendency to clot. To reduce this tendency, in one common protocol, citrate is infused while the blood is running through the circuit. Citrate binds to calcium in the blood, calcium being essential for blood to clot. Citrate is very effective in preventing blood from clotting; however, its use can lead to life-threateningly low calcium levels. This can be detected using the Chvostek's sign or Trousseau's sign. To prevent this complication, calcium is infused intravenously while the patient is undergoing the plasmapheresis; in addition, calcium supplementation by mouth may also be given.

Other complications of the procedure include: hypotension; potential exposure to blood products, with risk of transfusion reactions or transfusion transmitted diseases, suppression of the patient's immune system and bleeding or hematoma from needle placement.

Additionally facilities that provide plasmapheresis are limited and the procedure is very expensive.

An alternative to plasmapheresis is intravenous immunoglobulin (IVIG), which is a blood product containing pooled polyclonal IgG extracted from the plasma of over one thousand blood donors. The therapy is administered intravenously and lasts in the region of 2 weeks to 3 months.

Complications of the IVIG treatment include headaches, dermatitis, viral infection from contamination of the therapeutic product, for example HIV or hepatitis, pulmonary edema, allergic reactions, acute renal failure, venous thrombosis and aseptic meningitis.

Thus there is a significant unmet need for therapies for autoimmune disorders which are less invasive and which expose the patients to less medical complications.

Thus there is a significant unmet need for therapies for immunological disorders and/or autoimmune disorders which are less invasive and which expose the patients to less medical complications.

Accordingly agents that block or reduce the binding of IgG to FcRn may be useful in the treatment or prevention of such autoimmune and inflammatory diseases. Anti-FcRn antibodies have been described previously in WO2009/131702, WO2007/087289 and WO2006/118772.

However, there remains a need for improved anti-FcRn antibodies.

Summary of the Disclosure

Thus in one aspect there is provided an anti-FcRn antibody or binding fragment thereof comprising a heavy chain or heavy chain fragment having a variable region, wherein said variable region comprises one, two or three CDRs independently selected from SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, for example wherein CDR H1 is SEQ ID NO: 1, CDR H2 is SEQ ID NO: 2 and/or CDR H3 is SEQ ID NO: 3.

Thus one embodiment CDR H1 is SEQ ID NO: 1 and CDR H2 is SEQ ID NO: 2, or CDR H1 is SEQ ID NO: 1 and CDR H3 is SEQ ID NO: 3, or CDR H2 is SEQ ID NO: 2 and CDR H3 is SEQ ID NO: 3.

In another aspect there is provided an antibody or fragment comprising a sequence or combinations of sequences as defined herein, for example a cognate pair variable region.

The antibodies of the disclosure block binding of IgG to FcRn and are thought to be useful in reducing one or more biological functions of FcRn, including reducing half-life of circulating antibodies. This may be beneficial in that it allows the patient to more rapidly clear antibodies, such as autoantibodies. Accordingly antibodies of the disclosure reduce binding of IgG to FcRn.

Importantly the antibodies of the present invention are able to bind human FcRn, for example at both pH6 and pH7.4 with comparable and high binding affinity. Advantageously therefore the antibodies are able to continue to bind FcRn even within the endosome, thereby maximising the blocking of FcRn binding to IgG.

In one embodiment the antibodies or binding fragments according to the present disclosure comprise a light chain or light chain fragment having a variable region, for example comprising one, two or three CDRs independently selected from SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 7 and SEQ ID NO: 6, in particular wherein CDR L1 is SEQ ID NO: 4, CDR L2 is SEQ ID NO: 5 or SEQ ID NO: 7 and CDR L3 is SEQ ID NO: 6.

Thus one embodiment CDR L1 is SEQ ID NO: 4 and CDR L2 is SEQ ID NO: 5 or SEQ ID NO: 7, or CDR L1 is SEQ ID NO: 1 and CDR L3 is SEQ ID NO: 6, or CDR L2 is SEQ ID NO: 5 or SEQ ID NO: 7 and CDR L3 is SEQ ID NO:6.

In one embodiment the antibodies or binding fragments according to the present disclosure comprise CDR sequences selected from SEQ ID NOs: 1 to 7, for example wherein CDR H1 is SEQ ID NO: 1, CDR H2 is SEQ ID NO: 2, CDR H3 is SEQ ID NO: 3, CDR L1 is SEQ ID NO: 4, CDR L2 is SEQ ID NO: 5 or SEQ ID NO: 7 and CDR L3 is SEQ ID NO: 6.

Also provided is an antibody or binding fragment that binds the same epitope as an antibody or binding fragment explicitly disclosed herein. Accordingly there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 of human FcRn extracellular domain (SEQ ID NO: 48) and wherein the anti-FcRn antibody or binding fragment thereof further binds one or more (such as all) residues selected from the group consisting of A81, G83, G84, K85, G86, P87, N113, L135, A136, and Q139 and optionally further binds one or more residues selected from the group consisting of L82, Y88, L112 and D130.

In one embodiment there is provided an antibody or binding fragment that cross-blocks an antibody or binding fragment explicitly disclosed herein to human FcRn, or is cross-blocked from binding human FcRn by said antibody.

In one embodiment antibodies and binding fragments of the present disclosure block binding of human IgG to human FcRn.

In one embodiment antibodies and binding fragments of the present disclosure do not bind β2 microglobulin.

In one embodiment antibodies and binding fragments of the present disclosure do not bind human β2 microglobulin

In one example antibodies and binding fragments of the present disclosure do not reduce circulating albumin levels by more than 50%, preferably by no more than 25%.

In one example antibodies and binding fragments of the present disclosure do not reduce circulating albumin levels.

The disclosure also extends to a polynucleotide, such as DNA, encoding an antibody or fragment as described herein, for example where the DNA is incorporated into a vector.

Also provided is a host cell comprising said polynucleotide.

Methods of expressing an antibody or fragment are provided herein as are methods of conjugating an antibody or fragment to a polymer, such as PEG.

The present disclosure also relates to pharmaceutical compositions comprising said antibodies and fragments.

In one embodiment there is provided a method of treatment comprising administering a therapeutically effective amount of an antibody, fragment or composition as described herein.

The present disclosure also extends to an antibody, fragment or composition according to the present disclosure for use in treatment, particularly in the treatment of an immunological and/or autoimmune disorder.

Thus the present disclosure provides antibodies, fragments thereof and methods for removal of pathogenic IgG, which is achieved by accelerating the body's natural mechanism for catabolising IgG.

In essence the antibodies and fragments according to the disclosure block the system that recycles IgG in the body.

The present therapy is likely to provide a replacement or supplement for certain diseases where plasmapheresis is a therapy or IVIg therapy, which is advantageous for patients.

Brief Description of the Figures

Figure 1 Shows % hIgG in transgenic mice determined by LC-MS/MS

Figure 1a shows the effect of 1638 IgG4P format on the concentration of human IVIg in serum of human FcRn-transgenic mice.

Figure 1b shows the effect of 1638 FabFv and Fab'PEG formats on the concentration of human IVIg in human FcRn-transgenic mice

Figure 1c shows the pharmacokinetics of 1638 IgG4P format in human FcRn-transgenic mice.

Figure 1d shows the pharmacokinetics of 1638 FabFv and Fab'PEG formats in human FcRn-transgenic mice

Figure 1e The effect of 1638 FabFv and Fab'PEG formats on the concentration of serum albumin in human FcRn-transgenic mice.

Figure 1f The effect of 1638 IgG4P format on the concentration of serum albumin in human FcRn-transgenic mice.

Figure 2 shows representative binding curves for CA170_1638.g49 IgG4. The mean K_D values (n = 3) were 0.20nM in neutral buffer, & 0.22nM in acidic buffer, respectively

Figure 3 shows CA170_1638.g49 IgG4 inhibits IgG recycling in MDCK II clone 15 cells

Figure 4 shows CA170_1638.g49 IgG4 inhibits IgG transcytosis in MDCK II clone 15 cells.

Figure 5 shows CA170_1638.g49 FabFv inhibits IgG transcytosis in MDCK II clone 15 cells.

Figure 6 shows representative binding curves for CA170_1638.g49 IgG4. The mean KD values (n = 3) were 0.3 in neutral buffer, and 0.43 in acidic buffer, respectively (see Table 2).

Figure 7 shows CA170_1638 CDR sequences

Figure 8 Antibody sequences according to the present disclosure

Figure 9a Humanisation of antibody 1638.g49

Figure 9b Humanisation of antibody 1638.g49

Details of the Disclosure

FcRn as employed herein refers to the non-covalent complex between the human IgG receptor alpha chain, also known as the neonatal Fc receptor, the amino acid sequence of which is in UniProt under number P55899, the extracellular domain of which is provided in Figure 8 (SEQ ID NO:48), together with human β2 microglobulin (β2M), the amino acid sequence of which is in UniProt under number P61769 (provided herein with signal peptide (SEQ ID NO:50), without signal peptide (SEQ ID NO:72)).

Antibody molecule as employed herein refers to an antibody or binding fragment thereof.

The term 'antibody' as used herein generally relates to intact (whole) antibodies i.e. comprising the elements of two heavy chains and two light chains. The antibody may comprise further additional binding domains, for example as per the molecule DVD-Ig as disclosed in WO 2007/024715, or the so-called $(FabFv)_2Fc$ described in WO2011/030107. Thus antibody as employed herein includes bi, tri or tetra-valent full length antibodies.

Binding fragments of antibodies include single chain antibodies (i.e. a full length heavy chain and light chain); Fab, modified Fab, Fab', modified Fab', F(ab')$_2$, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (e.g. VH or VL or VHH), scFv, dsscFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, tribodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). The Fab-Fv format was first disclosed in WO2009/040562 and the disulphide stabilised versions thereof, the Fab-dsFv was first disclosed in WO2010/035012. Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO92/22583 and WO05/113605). One such example of the latter is a Tri-Fab (or TFM) as described in WO92/22583.

In one embodiment there is provided a Fab fragment.

In one embodiment there is provided a Fab' fragment.

A typical Fab' molecule comprises a heavy and a light chain pair in which the heavy chain comprises a variable region V_H, a constant domain C_H1 and a natural or modified hinge region and the light chain comprises a variable region V_L and a constant domain C_L.

In one embodiment there is provided a dimer of a Fab' according to the present disclosure to create a F(ab')$_2$ for example dimerisation may be through the hinge.

In one embodiment the antibody or binding fragment thereof comprises a binding domain. A binding domain will generally comprises 6 CDRs, three from a heavy chain and three from a light chain. In one embodiment the CDRs are in a framework and together form a variable region. Thus in one embodiment an antibody or binding fragment comprises a binding domain specific for antigen comprising a light chain variable region and a heavy chain variable region.

It will be appreciated that one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the CDRs or other sequences (e.g variable domains) provided by the present invention without significantly altering the ability of the antibody to bind to FcRn. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described herein, in particular in the Examples, to determine FcRn binding/blocking.

In one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the framework region employed in the antibody or fragment provided by the present invention and wherein binding affinity to FcRn is retained or increased.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat *et al.* (*supra*)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

Antibodies and fragments of the present disclosure block FcRn and may thereby prevent it functioning in the recycling of IgG. Blocking as employed herein refers to physically blocking such as occluding the receptor but will also include where the antibody or fragments binds an epitope that causes, for example a conformational change which means that the natural ligand to the receptor no longer binds. Antibody molecules of the present invention bind to FcRn and thereby decrease or prevent (e.g. inhibit) FcRn binding to an IgG constant region.
In one embodiment the antibody or fragment thereof binds FcRn competitively with respect to IgG.

In one example the antibody or binding fragment thereof functions as a competitive inhibitor of human FcRn binding to human IgG. In one example the antibody or binding fragment thereof binds to the IgG binding site on FcRn. In one example the antibody blocks the IgG binding site. In one example the antibody or binding fragment thereof does not bind β2M.

Antibodies for use in the present disclosure may be obtained using any suitable method known in the art. The FcRn polypeptide/protein including fusion proteins, cells (recombinantly or naturally) expressing the polypeptide (such as activated T cells) can be used to produce antibodies which specifically recognise FcRn, alone or incombination with β2M. The

polypeptide may be the 'mature' polypeptide or a biologically active fragment or derivative thereof. The human protein is registered in Swiss-Prot under the number P55899. The extracellular domain of human FcRn alpha chain is provided in SEQ ID NO: 48. The sequence of mature human β2M is provided in SEQ ID NO: 72.

In one embodiment the antigen is a mutant form of FcRn which is engineered to present FcRn on the surface of a cell, such that there is little or no dynamic processing where the FcRn is internalised in the cell, for example this can be achieved by making a mutation in the cytoplasmic tail of the FcRn alpha chain, wherein di-leucine is mutated to di-alanine as described in Ober et al 2001 Int. Immunol. 13, 1551–1559.

Polypeptides, for use to immunize a host, may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The FcRn polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag or similar.

Antibodies generated against the FcRn polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.,* 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al.,* Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93(15):7843-7848l; WO92/02551; WO2004/051268 and International Patent Application number WO2004/106377.

Screening for antibodies can be performed using assays to measure binding to human FcRn and/or assays to measure the ability to block IgG binding to the receptor. An example of a binding assay is an ELISA, in particular, using a fusion protein of human FcRn and human Fc, which is immobilized on plates, and employing a secondary antibody to detect anti-FcRn antibody bound to the fusion protein. Examples of suitable antagonistic and blocking assays are described herein below.

Specific as employed herein is intended to refer to an antibody that only recognises the antigen to which it is specific or an antibody that has significantly higher binding affinity to the antigen to which it is specific compared to binding to antigens to which it is non-specific, for example at least 5, 6, 7, 8, 9, 10 times higher binding affinity. Binding affinity may be measured by techniques such as BIAcore as described herein below. In one example the antibody of the present invention does not bind β2 microglobulin (β2M). In one example the antibody of the present invention binds cynomolgus FcRn. In one example the antibody of the present invention does not bind rat or mouse FcRn.

The amino acid sequences and the polynucleotide sequences of certain antibodies according to the present disclosure are provided and form an aspect of the invention.

In one embodiment the antibodies or binding fragments according to the present disclosure are fully human, for example prepared from a phage library or similar.

In one example the antibodies are rodent, such as rat derived and comprise the light chain variable domain sequence given in SEQ ID NO:8 and the heavy chain variable domain sequence given in SEQ ID NO:12.

In one embodiment the antibody or fragments according to the disclosure are humanised.

Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule (see, *e.g.* US 5,585,089; WO91/09967). It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived. The latter are often referred to as donor residues.

Thus in one embodiment as used herein, the term 'humanised antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a non-human antibody such as a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody) optionally further comprising one or more framework residues derived from the non-human species from which the CDRs were derived (donor residues). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions.

Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided specifically herein. Thus, provided in one embodiment is blocking humanised antibody which binds human FcRn wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al., supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at http://www.imgt.org/

In a humanised antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

One such suitable framework region for the heavy chain of the humanised antibody of the present invention is derived from the human sub-group VH3 sequence IGHV3-7 together with JH3 (SEQ ID NO: 46 and 47).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2 and the sequence given in SEQ ID NO: 3 for CDRH3, wherein the heavy chain framework region is derived from the human subgroup VH3 sequence IGHV3-7 together with JH3.

The sequence of human JH3 is as follows: (DAFDV)WGQGTMVTVS (SEQ ID No: 69). The DAFDV (SEQ ID NO: 70) motif is part of CDR-H3 and is not part of framework 4 (Ravetch, JV. et al., 1981, Cell, 27, 583-591).

In one example the heavy chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 25 or 59, such as 25.

A suitable framework region for the light chain of the humanised antibody of the present invention is derived from the human sub-group VK1 sequence IGKV1-27 sequence together with JK4 (SEQ ID NO: 44 and 45).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 or SEQ ID NO: 7 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDRL3, wherein the light chain framework region is derived from the human sub-group VK1 sequence IGKV1-27 together with JK4.

The JK4 sequence is as follows: (LT)FGGGTKVEIK (Seq ID No: 71). The LT motif is part of CDR-L3 and is not part of framework 4 (Hieter, PA., et al., 1982, J. Biol. Chem., 257, 1516-1522).

In one example the light chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 16 or 51, such as 16.

In a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO91/09967.

Thus in one embodiment 1, 2, 3, 4, or 5 residues in the framework are replaced with an alternative amino acid residue.

Accordingly, in one example there is provided a humanised antibody, wherein at least the residues at each of positions 48 and 78 of the variable domain of the heavy chain (Kabat numbering) are donor residues, see for example the sequence given in SEQ ID NO: 25.

In one embodiment residue 48 of the heavy chain variable domain is replaced with an alternative amino acid, for example valine.

In one embodiment residue 78 of the heavy chain variable domain is replaced with an alternative amino acid, for example leucine.

In one embodiment residue 48 is valine and residue 78 is leucine in the humanised heavy chain variable region according to the present disclosure.

Accordingly, in one example there is provided a humanised antibody, wherein at least the residues at each of positions 70 and 71 of the variable domain of the light chain (Kabat numbering) are donor residues, see for example the sequence given in SEQ ID NO: 16.

In one embodiment residue 70 of the light chain variable domain is replaced with an alternative amino acid, for example aspartic acid.

In one embodiment residue 71 of the light chain variable domain is replaced with an alternative amino acid, for example phenylalanine.

In one embodiment residue 70 is aspartic acid and residue 71 is phenylalanine in the humanised light chain variable region according to the present disclosure.

In one embodiment the disclosure provides an antibody sequence which is 80% similar or identical to a sequence disclosed herein, for example 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% over part or whole of the relevant sequence, for example a variable domain sequence, a CDR sequence or a variable domain sequence, excluding the CDRs. In one

embodiment the relevant sequence is SEQ ID NO: 16 or 51. In one embodiment the relevant sequence is SEQ ID NO: 25 or 59.

In one embodiment, the present invention provides an antibody molecule which binds human FcRn comprising a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity or similarity to a sequence herein, for example the sequence given in SEQ ID NO: 25 or 59, such as 25.

In one embodiment, the present invention provides an antibody molecule which binds human FcRn comprising a light chain, wherein the variable domain of the light chain comprises a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% identity or similarity to the sequence given in SEQ ID NO: 16 or 51, such as 16.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a heavy chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to a sequence given herein, for example the sequence given in SEQ ID NO: 25 but wherein the antibody molecule has the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2 and the sequence given in SEQ ID NO: 3 for CDR-H3.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a light chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to a sequence given herein, for example the sequence in SEQ ID NO:16 but wherein the antibody molecule has the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 or SEQ ID NO: 7 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDR-L3.

In one embodiment the present invention provides an antibody molecule which binds human FcRn wherein the antibody has a heavy chain variable domain which is at least 90% , 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to a sequence given herein, for example the sequence given in SEQ ID NO: 25 and a light chain variable domain which is at least 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% similar or identical to a sequence given herein, for example the sequence given in SEQ ID NO:16 but wherein the antibody molecule has the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2, the sequence given in SEQ ID NO: 3 for CDR-H3, the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 or SEQ ID NO: 7 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDR-L3.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for

isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.,* 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.,* 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.,* 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

The antibody molecules of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', modified Fab', $F(ab')_2$, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, dsscFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g bispecific or may be monospecific (see for example WO 92/22853, WO05/113605, WO2009/040562 and WO2010/035012).

In one embodiment the antibody molecule of the present disclosure is an antibody Fab fragment comprising the variable regions shown in SEQ ID NOs: 16 and 25, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 20 and a heavy chain comprising the sequence given in SEQ ID NO: 29.

In one embodiment the antibody molecule of the present disclosure is an antibody Fab fragment comprising the variable regions shown in SEQ ID NOs: 51 and 59, for example for the light and heavy chain respectively.

In one embodiment the antibody molecule of the present disclosure is an antibody Fab or Fab' fragment comprising the variable regions shown in SEQ ID NOs: 16 and 25, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 20 and a heavy chain comprising the sequence given in SEQ ID NO: 29 (Fab) or SEQ ID NO: 33 (Fab').

In one embodiment the antibody molecule of the present disclosure is an antibody Fab' fragment comprising the variable regions shown in SEQ ID NOs: 51 and 59, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 55 and a heavy chain comprising the sequence given in SEQ ID NO: 63.

In one embodiment the antibody molecule of the present disclosure is a full length IgG1 antibody comprising the variable regions shown in SEQ ID NOs: 16 and 25, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 20 and a heavy chain comprising the sequence given in SEQ ID NO: 73.

In one embodiment the antibody molecule of the present disclosure is a full length IgG1 comprising the variable regions shown in SEQ ID NOs: 51 and 59.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4 format comprising the variable regions shown in SEQ ID NOs: 16 and 25, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 20 and a heavy chain comprising the variable region sequence given in SEQ ID NO: 25.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4 format comprising the variable regions shown in SEQ ID NOs: 51 and 59, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 55 and a heavy chain comprising the variable region sequence given in SEQ ID NO: 59.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4P format comprising the variable regions shown in SEQ ID NOs: 16 and 25, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 20 and a heavy chain comprising the sequence given in SEQ ID NO: 37 or SEQ ID NO: 39.

In one embodiment the antibody molecule of the present disclosure is a full length IgG4P format comprising the variable regions shown in SEQ ID NOs: 51 and 59, for example for the light and heavy chain respectively. In one embodiment the antibody molecule has a light chain comprising the sequence given in SEQ ID NO: 55 and a heavy chain comprising the variable region sequence given in SEQ ID NO: 59.

IgG4P as employed herein is a mutation of the wild-type IgG4 isotype where amino acid 241 is replaced by proline, see for example where serine at position 241 has been changed to proline as described in Angal *et al.*, Molecular Immunology, 1993, 30 (1), 105-108.

In one embodiment the antibody according to the present disclosure is provided as an FcRn binding antibody fusion protein which comprises an immunoglobulin moiety, for example a Fab or Fab' fragment, and one or two single domain antibodies (dAb) linked directly or indirectly thereto, for example as described in WO2009/040562, WO2010035012, WO2011/030107, WO2011/061492 and WO2011/086091 all incorporated herein by reference.

In one embodiment the fusion protein comprises two domain antibodies, for example as a variable heavy (VH) and variable light (VL) pairing, optionally linked by a disulphide bond.

In one embodiment the Fab or Fab' element of the fusion protein has the same or similar specificity to the single domain antibody or antibodies. In one embodiment the Fab or Fab' has a different specificity to the single domain antibody or antibodies, that is to say the fusion protein is multivalent. In one embodiment a multivalent fusion protein according to the present invention has an albumin binding site, for example a VH/VL pair therein provides an albumin binding site. In one such embodiment the heavy chain comprises the sequence given in SEQ ID NO: 42 and the light chain comprises the sequence given in SEQ ID NO: 40.

In one embodiment the Fab or Fab' according to the present disclosure is conjugated to a PEG molecule or human serum albumin.

CA170_01638g49 and 1638.g49 are employed inchangeably herein and are used to refer to a specific pair of antibody variable regions which may be used in a number of different formats. These variable regions are the heavy chain sequence given in SEQ ID NO: 25 and the light chain sequence given in SEQ ID NO: 16.

CA170_01638g28 and 1638.g28 are employed inchangeably herein and are used to refer to a specific pair of antibody variable regions which may be used in a number of different formats. These variable regions are the heavy chain sequence given in SEQ ID NO: 59 and the light chain sequence given in SEQ ID NO: 51.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.*, Molecular Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by

one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995). Accordingly, the C-terminal lysine of the antibody heavy chain may be absent.

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

In one embodiment the light chain has the sequence given in SEQ ID NO: 20 and the heavy chain has the sequence given in SEQ ID NO: 29.

In one embodiment the light chain has the sequence given in SEQ ID NO: 20 and the heavy chain has the sequence given in SEQ ID NO: 33.

In one embodiment the light chain has the sequence given in SEQ ID NO: 20 and the heavy chain has the sequence given in SEQ ID NO: 37.

In one embodiment the light chain has the sequence given in SEQ ID NO: 20 and the heavy chain has the sequence given in SEQ ID NO: 74.

In one embodiment a C-terminal amino acid from the antibody molecule is cleaved during post-translation modifications.

In one embodiment an N-terminal amino acid from the antibody molecule is cleaved during post-translation modifications.

Also provided by the present invention is a specific region or epitope of human FcRn which is bound by an antibody provided by the present invention, in particular an antibody comprising the heavy chain sequence gH33 (SEQ ID NO: 25) and/or the light chain sequence gL7 (SEQ ID NO: 16 or an antibody comprising the heavy chain sequence gH2 (SEQ ID NO: 59) and the light chain sequence gL2 (SEQ ID NO: 51).

This specific region or epitope of the human FcRn polypeptide can be identified by any suitable epitope mapping method known in the art in combination with any one of the antibodies provided by the present invention. Examples of such methods include screening peptides of varying lengths derived from FcRn for binding to the antibody of the present invention with the smallest fragment that can specifically bind to the antibody containing the sequence of the epitope recognised by the antibody. The FcRn peptides may be produced synthetically or by proteolytic digestion of the FcRn polypeptide. Peptides that bind the antibody can be identified by, for example, mass spectrometric analysis. In another example, NMR spectroscopy or X-ray crystallography can be used to identify the epitope bound by an antibody of the present invention. In one example where X-ray crystallography is used, the epitope is determined as those residues on the FcRn polypeptide which are within 4 Å of the antibody. In one example the epitope is

determined as those residues on the FcRn polypeptide which are within 5 Å of the antibody. Once identified, the epitopic fragment which binds an antibody of the present invention can be used, if required, as an immunogen to obtain additional antibodies which bind the same epitope.

In one embodiment the antibody of the present disclosure binds the human FcRn alpha chain extracellular sequence as shown below:

AESHLSLLYHLTAVSSPAPG TPAFWVSGWL GPQQYLSYNS LRGEAEPCGA WVWENQVSWY WEKETTDLRI KEKLFLEAFK **A*L*GGKGP**_Y_TL QGLLGCELGPDNTSVPTAKFA*L***N**G**E**_E_F**M**NF**D**LKQGTWGG***D*****WPEA**LA**I**S**Q**R WQQQDKAANK ELTFLLFSCP HRLREHLERG RGNLEWKEPPSMRLKARPSSPGFSVLTCSAFSFYPPELQL RFLRNGLAAG TGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELESPAKSS (SEQ ID NO: 48).

The residues underlined are those known to be critical for the interaction of human FcRn with the Fc region of human IgG. Those in bold are residues of the human FcRn polypeptide involved in binding the antibody comprising the heavy chain sequence given in SEQ ID NO: 25 and the light chain sequence given in SEQ ID NO:16, ie they are within 4Å of the antibody as determined by X-ray crystallography. Residues in italic are those involved in binding the same antibody at 5Å.

In one aspect of the invention there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 of human FcRn extracellular domain (SEQ ID NO: 48), and wherein the anti-FcRn antibody or binding fragment thereof further binds one or more residues, such as two, three, four, five, six, seven, eight, nine or ten residues selected from the group consisting of A81, G83, G84, K85, G86, P87, N113, L135, A136, and Q139 and optionally further binds one or more residues selected from the group consisting of L82, Y88, L112 and D130.

Accordingly in one example there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 and at least one residue, for example at least 2, 3, 4, 5, 6, 7, 8, 9, 10 residues selected from the group consisting of A81, G83, G84, K85, G86, P87, N113, L135, A136, and Q139 and wherein said anti-FcRn antibody or binding fragment thereof optionally further binds one or more residues, for example at least 2, 3 or 4 residues selected from the group consisting of L82, Y88, L112 and D130 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example an antibody according to this aspect of the invention does not bind V105, P106, T107, A108 and K109 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example an antibody according to this aspect of the invention does not bind E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128 and G129 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example an antibody according to this aspect of the invention does not bind V105, P106, T107, A108, K109, E116, F117, M118, N119, F120, D121, L122, K123, Q124, G128, and G129 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 and at least one residue, for example at least 2, 3, 4, 5, 6, 7 or 8 residues selected from the group consisting of A81, L82, G83, G84, K85, G86, P87 and Y88 of human FcRn extracellular domain (SEQ ID NO: 48)

In one example there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 and at least one residue, for example at least 2, 3, 4, 5 or 6 residues selected from the group consisting of L112, N113, D130, L135, A136, and Q139 of human FcRn extracellular domain (SEQ ID NO: 48)

In one example there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises one, two, three, or four amino acids selected from the group consisting of residues E115, W131, P132, and E133 and at least one residue selected from the group consisting of A81, L82, G83, G84, K85, G86, P87, Y88, L112, N113, D130, L135, A136, and Q139 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example there is provided an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises residues E115, W131, P132, and E133 and at least one residue selected from the group consisting of A81, L82, G83, G84, K85, G86, P87, Y88, L112, N113, D130, L135, A136, and Q139 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example the present invention provides an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises or consists of residues A81, G83, G84, K85, G86, P87, N113, E115, W131, P132, E133, L135, A136, and Q139 of human FcRn extracellular domain (SEQ ID NO: 48).

In one example the present invention provides an anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises or consists of residues A81, L82, G83, G84, K85, G86, P87, Y88, L112, N113, E115, D130, W131, P132, E133, L135, A136, and Q139 of human FcRn extracellular domain (SEQ ID NO: 48).

In one embodiment the antibodies which bind the epitope described herein above provided by the present invention are fully human. In one embodiment they are humanised. In one example they have an affinity for human FcRn of 150pM or less, typically 130pM or less.

Antibodies which cross-block the binding of an antibody molecule according to the present invention in particular, an antibody molecule comprising the heavy chain sequence given in SEQ ID NO: 25 and the light chain sequence given in SEQ ID NO:16 may be similarly useful in

blocking FcRn activity. Accordingly, the present invention also provides an anti-FcRn antibody molecule, which cross-blocks the binding of any one of the antibody molecules described herein above to human FcRn and/or is cross-blocked from binding human FcRn by any one of those antibodies. In one embodiment, such an antibody binds to the same epitope as an antibody described herein above. In another embodiment the cross-blocking neutralising antibody binds to an epitope which borders and/or overlaps with the epitope bound by an antibody described herein above.

Cross-blocking antibodies can be identified using any suitable method in the art, for example by using competition ELISA or BIAcore assays where binding of the cross blocking antibody to human FcRn prevents the binding of an antibody of the present invention or *vice versa*. Such cross blocking assays may use isolated natural or recombinant FcRn or a suitable fusion protein/polypeptide. In one example binding and cross-blocking is measured using recombinant human FcRn extracellular domain (SEQ ID NO: 48). In one example the recombinant human FcRn alpha chain extracellular domain is used in a complex with β2 microglobulin (β2M) (SEQ ID NO:72).

In one embodiment there is provided an anti-FcRn antibody molecule which blocks FcRn binding to IgG and which cross-blocks the binding of an antibody whose heavy chain comprises the sequence given in SEQ ID NO: 25 and whose light chain comprises the sequence given in SEQ ID NO: 16 to human FcRn. In one embodiment the cross-blocking antibodies provided by the present invention inhibit the binding of an antibody comprising the heavy chain sequence given in SEQ ID NO: 25 and the light chain sequence given in SEQ ID NO: 16 by greater than 80%, for example by greater than 85%, such as by greater than 90%, in particular by greater than 95% inhibition.

In one embodiment the cross-blocking antibodies provided by the present invention are fully human. In one embodiment the cross-blocking antibodies provided by the present invention are humanised. In one embodiment the cross-blocking antibodies provided by the present invention have an affinity for human FcRn of 150pM or less, 130pM or less or 100pM or less. In one embodiment the cross-blocking antibodies provided by the present invention have an affinity for human FcRn of 50pM or less. Affinity can be measured using the methods described herein below.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or solvent accessible surface thereof carries no net electrical charge. In one example, the FcRn antibody and fragments of the invention may be engineered to have an appropriate isoelectric point. This

may lead to antibodies and/or fragments with more robust properties, in particular suitable solubility and/or stability profiles and/or improved purification characteristics.

Thus in one aspect the invention provides a humanised FcRn antibody engineered to have an isoelectric point different to that of the originally identified antibody. The antibody may, for example be engineered by replacing an amino acid residue such as replacing an acidic amino acid residue with one or more basic amino acid residues. Alternatively, basic amino acid residues may be introduced or acidic amino acid residues can be removed. Alternatively, if the molecule has an unacceptably high pI value acidic residues may be introduced to lower the pI, as required. It is important that when manipulating the pI care must be taken to retain the desirable activity of the antibody or fragment. Thus in one embodiment the engineered antibody or fragment has the same or substantially the same activity as the "unmodified" antibody or fragment.

Programs such as ** ExPASY http://www.expasy.ch/tools/pi_tool.html, and http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/compo-p.html, may be used to predict the isoelectric point of the antibody or fragment. Alternatively or additionally, the pI can be measured using any suitable standard laboratory technique.

The antibody molecules of the present invention suitably have a high binding affinity, in particular in the nanomolar range. Affinity may be measured using any suitable method known in the art, including BIAcore, as described in the Examples herein, using isolated natural or recombinant FcRn or a suitable fusion protein/polypeptide. In one example affinity is measured using recombinant human FcRn extracellular domain as described in the Examples herein (SEQ ID NO: 48). In one example affinity is measured using the recombinant human FcRn alpha chain extracellular domain (SEQ ID NO: 48) in association with human β2 microglobulin (β2M) (SEQ ID NO: 72). Suitably the antibody molecules of the present invention have a binding affinity for isolated human FcRn of about 1nM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 500pM or lower (i.e. higher affinity). In one embodiment the antibody molecule of the present invention has a binding affinity of about 250pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 200pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity of about 150pM or lower. In one embodiment the present invention provides an anti-FcRn antibody with a binding affinity of about 100pM or lower. In one embodiment the present invention provides a humanised anti-FcRn antibody with a binding affinity of about 100pM or lower. In one embodiment the present invention provides an anti-FcRn antibody with a binding affinity of 50pM or lower.

In one embodiment the antibodies of the present invention are able to bind human FcRn at both pH6 or lower pH (in particular pH 6) and pH7.4 or higher pH (in particular pH7.4) with comparable binding affinity. Advantageously therefore the antibodies are able to continue to bind FcRn even within the endosome, thereby maximising the blocking of FcRn binding to IgG.

In one embodiment the antibodies of the present invention are able to bind human FcRn with a binding affinity of 150pM or lower when measured at pH6 and pH7.4. In one embodiment the antibodies of the present invention are able to bind human FcRn with a binding affinity of 130pM or lower when measured at pH6 and pH7.4. In one embodiment the antibodies of the present invention are able to bind human FcRn with a binding affinity of 130pM or lower when measured at pH6 and a binding affinity of 50pM or lower when measured at pH7.4.

The affinity of an antibody or binding fragment of the present invention, as well as the extent to which a binding agent (such as an antibody) inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard et al. (Ann. KY. Acad. Sci. 51:660-672 (1949)) or by surface plasmon resonance (SPR) using systems such as BIAcore. For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al, Cancer Res. 53:2560-65 (1993)).

In the present invention affinity of the test antibody molecule is typically determined using SPR as follows. The test antibody molecule is captured on the solid phase and human FcRn alpha chain extracellular domain in non-covalent complex with human β2M is run over the captured antibody in the mobile phase and affinity of the test antibody molecule for human FcRn determined. The test antibody molecule may be captured on the solid phase chip surface using any appropriate method, for example using an anti-Fc or anti Fab' specific capture agent. In one example the affinity is determined at pH6. In one example the affinity is determined at pH7.4.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for FcRn. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783, 1992), use of mutator strains of *E. coli* (Low *et al.*, J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten *et al.*, Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri *et al.*, Nature, 391, 288-291, 1998). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

In one embodiment the antibody molecules of the present invention block human FcRn activity. Assays suitable for determining the ability of an antibody to block FcRn are described

in the Examples herein. A suitable assay for determining the ability of an antibody molecule to block IgG recycling in vitro is described herein below.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982 , Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195, WO 89/01476 and WO 03/031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188}; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

In one embodiment a half-life provided by an effector molecule which is independent of FcRn is advantageous.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

In one embodiment the polymer is albumin or a fragment thereof, such as human serum albumin or a fragment thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971, WO2008/038024). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a

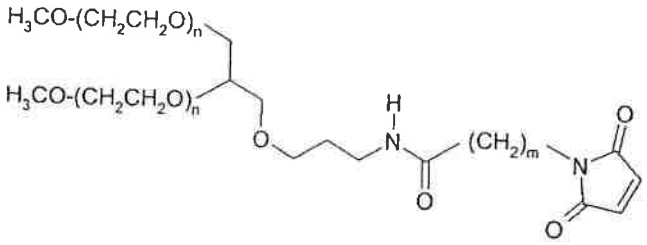
modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment, Fab' fragment or diFab which is PEGylated, *i.e.* has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.* according to the method disclosed in EP 0948544 or EP1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

Particular PEG molecules include 2-[3-(N-maleimido)propionamido]ethyl amide of N,N'-bis(methoxypoly(ethylene glycol) MW 20,000) modified lysine, also known as PEG2MAL40K (obtainable from Nektar, formerly Shearwater).

Alternative sources of PEG linkers include NOF who supply GL2-400MA3 (wherein m in the structure below is 5) and GL2-400MA (where m is 2) and n is approximately 450:

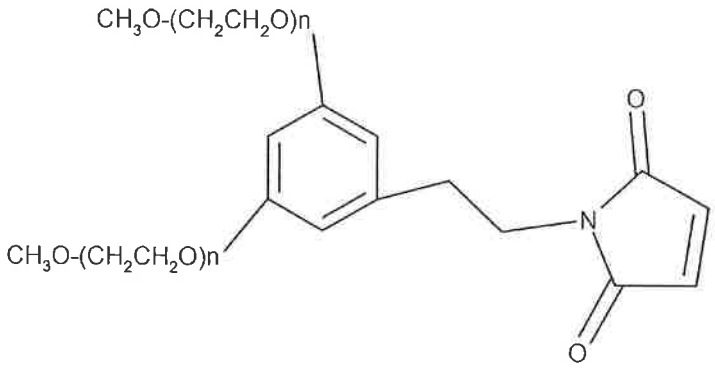


m is 2 or 5

That is to say each PEG is about 20,000Da.

Thus in one embodiment the PEG is 2,3-Bis(methylpolyoxyethylene-oxy)-1-{[3-(6-maleimido-1-oxohexyl)amino]propyloxy} hexane (the 2 arm branched PEG, -$CH_2)_3NHCO(CH_2)_5$-MAL, Mw 40,000 known as SUNBRIGHT GL2-400MA3.

Further alternative PEG effector molecules of the following type:



are available from Dr Reddy, NOF and Jenkem.

In one embodiment there is provided an antibody which is PEGylated (for example with a PEG described herein), attached through a cysteine amino acid residue at or about amino acid 232 in the chain, for example amino acid 232 of the heavy chain (by sequential numbering), for example amino acid 232 of SEQ ID NO: 33.

In one embodiment the present disclosure provides a Fab'PEG molecule comprising one or more PEG polymers, for example 1 or 2 polymers such as a 40kDa polymer or polymers.

Fab'-PEG molecules according to the present disclosure may be particularly advantageous in that they have a half-life independent of the Fc fragment. In one example the present invention provides a method treating a disease ameliorated by blocking human FcRn comprising administering a therapeutically effective amount of an anti-FcRn antibody or binding fragment thereof wherein the antibody or binding fragment thereof has a half life that is independent of Fc binding to FcRn.

In one embodiment there is provided a Fab' conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment there is provided a scFv conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment the antibody or fragment is conjugated to a starch molecule, for example to increase the half life. Methods of conjugating starch to a protein as described in US 8,017,739 incorporated herein by reference.

In one embodiment there is provided an anti-FcRn binding molecule (i.e an antibody or binding fragment thereof) which:

- Causes 50-85% reduction, such as a 70% reduction of plasma IgG concentration,
- With not more than 25% or 20% reduction of plasma albumin concentration, and/or
- With the possibility of repeat dosing to achieve long-term maintenance of low plasma IgG concentration.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule of the present invention. Suitably, the DNA sequence encodes the heavy or the light chain of an antibody molecule of the present invention. The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Examples of suitable DNA sequences are provided in herein.

Examples of suitable DNA sequences encoding the 1638.g49 light chain variable region are provided in SEQ ID NO: 17, SEQ ID NO: 19 and SEQ ID NO: 21.

Examples of suitable DNA sequences encoding the 1638.g28 light chain variable region are provided in SEQ ID NO: 52 and SEQ ID NO: 54.

Examples of suitable DNA sequences encoding the 1638.g49 heavy chain variable region are provided in SEQ ID NO: 26 and SEQ ID NO: 28.

Examples of suitable DNA sequences encoding the 1638.g28 heavy chain variable region are provided in SEQ ID NO: 60 and 62.

Examples of suitable DNA sequences encoding the 1638.g49 light chain (variable and constant) are provided in SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 24, and for

1638.g28 light chain the sequence given in SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO:58 and 1638.g28 heavy chain the sequence given in SEQ ID NO: 64 or SEQ ID NO: 66.

Examples of suitable DNA sequences encoding the 1638.g49 heavy chain (variable and constant, depending on format) are provided in SEQ ID NO: 30 (Fab), SEQ ID NO: 34 or 36 (Fab'), SEQ ID NO: 38 (IgG4P), SEQ ID NO: 43 (FabFv) and SEQ ID NO:74 (IgG1).

Accordingly in one example the present invention provides an isolated DNA sequence encoding the heavy chain of an antibody Fab or Fab' fragment of the present invention which comprises the sequence given in SEQ ID NO: 30, 32, 34, 36, 64 or 66. Also provided is an isolated DNA sequence encoding the light chain of an antibody Fab or Fab' fragment of the present invention which comprises the sequence given in SEQ ID NO: 21, 22 or 56.

In one example the present invention provides an isolated DNA sequence encoding the heavy chain and the light chain of an IgG4(P) antibody of the present invention in which the DNA encoding the heavy chain comprises the sequence given in SEQ ID NO: 38 and the DNA encoding the light chain comprises the sequence given in SEQ ID NO: 22.

In one example the present invention provides an isolated DNA sequence encoding the heavy chain and the light chain of an IgG1 antibody of the present invention in which the DNA encoding the heavy chain comprises the sequence given in SEQ ID NO: 74 and the DNA encoding the light chain comprises the sequence given in SEQ ID NO: 22.

In one example the present invention provides an isolated DNA sequence encoding the heavy chain and the light chain of a Fab-dsFv antibody of the present invention in which the DNA encoding the heavy chain comprises the sequence given in SEQ ID NO: 43 and the DNA encoding the light chain comprises the sequence given in SEQ ID NO: 41.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody of the present invention. Suitably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively and suitable signal sequences. In one example the vector comprises an intergenic sequence between the heavy and the light chains (see WO03/048208).

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Accordingly the present invention also provides a host cell for expression of an antibody according to to the invention comprising:

i) a DNA sequence encoding the heavy chain of said antibody, and

ii) a DNA sequence encoding the light chain of said antibody

wherein the DNA sequences are provided in one or more cloning or expression vectors.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used (especially for expressing antibody fragments or eukaryotic, for example mammalian, host cell expression systems may also be used (especially for expressing full-length antibodies). Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

Suitable types of Chinese Hamster Ovary (CHO cells) for use in the present invention may include CHO and CHO-K1 cells including dhfr- CHO cells, such as CHO-DG44 cells and CHO-DXB11 cells, which may be used with a DHFR selectable marker or CHOK1-SV cells which may be used with a glutamine synthetase selectable marker. Other cell types of use in expressing antibodies include lymphocytic cell lines, e.g., NSO myeloma cells and SP2 cells, COS cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector or vectors of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The antibodies and fragments according to the present disclosure are expressed at good levels from host cells. Thus the properties of the antibodies and/or fragments are conducive to commercial processing.

Thus there is a provided a process for culturing a host cell and expressing an antibody or fragment thereof, isolating the latter and optionally purifying the same to provide an isolated antibody or fragment. In one embodiment the process further comprises the step of conjugating an effector molecule to the isolated antibody or fragment, for example conjugating to a PEG polymer in particular as described herein.

In one embodiment there is provided a process for purifiying an antibody (in particular an antibody or fragment according to the invention) comprising the steps: performing anion exchange chromatography in non-binding mode such that the impurities are retained on the column and the antibody is eluted.

In one embodiment the purification employs affinity capture on an FcRn column.

In one embodiment the purification employs cibacron blue or similar for purification of albumin fusion or conjugate molecules.

Suitable ion exchange resins for use in the process include Q.FF resin (supplied by GE-Healthcare). The step may, for example be performed at a pH about 8.

The process may further comprise an intial capture step employing cation exchange chromatography, performed for example at a pH of about 4 to 5, such as 4.5. The cation exchange chromatography may, for example employ a resin such as CaptoS resin or SP sepharose FF (supplied by GE-Healthcare). The antibody or fragment can then be eluted from the resin employing an ionic salt solution such as sodium chloride, for example at a concentration of 200mM.

Thus the chromatograph step or steps may include one or more washing steps, as appropriate.

The purification process may also comprise one or more filtration steps, such as a diafiltration step.

Thus in one embodiment there is provided a purified anti-FcRn antibody or fragment, for example a humanised antibody or fragment, in particular an antibody or fragment according to the invention, in substantially purified from, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

Purified form as used *supra* is intended to refer to at least 90% purity, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% w/w or more pure.

Substantially free of endotoxin is generally intended to refer to an endotoxin content of 1 EU per mg antibody product or less such as 0.5 or 0.1 EU per mg product.

Substantially free of host cell protein or DNA is generally intended to refer to host cell protein and/or DNA content 400μg per mg of antibody product or less such as 100μg per mg or less, in particular 20μg per mg, as appropriate.

The antibody molecules of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving FcRn.

As the antibodies of the present invention are useful in the treatment and/or prophylaxis of a pathological condition, the present invention also provides a pharmaceutical or diagnostic composition comprising an antibody molecule of the present invention in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody molecule of the invention for the manufacture of a medicament. The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable excipient.

The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody molecule of the present

invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients or non-antibody ingredients such as steroids or other drug molecules, in particular drug molecules whose half-life is independent of FcRn binding.

The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100mg/Kg.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Therapeutic doses of the antibodies according to the present disclosure show no apparent toxicology effects *in vivo*.

In one embodiment of an antibody or fragment according to the invention a single dose may provide up to a 70% reduction in circulating IgG levels. In one example of an antibody or fragment according to the invention a single dose may provide up to a 80% reduction in circulating IgG levels. In one example of an antibody or fragment according to the invention a single dose may provide a greater than 80% reduction in circulating IgG levels.

The maximal therapeutic reduction in circulating IgG may be observed about 1 week after administration of the relevant therapeutic dose. The levels of IgG may recover over the weeks following dosing if further therapeutic doses are not delivered. Recover as employed herein refers to levels returning to levels similar to those observed before initial dosing commenced.

Advantageously, the levels of IgG *in vivo* may be maintained at an appropriately low level by administration of sequential doses of the antibody or fragments according to the disclosure.

Compositions may be administered individually to a patient or may be administered in combination (*e.g.* simultaneously, sequentially or separately) with other agents, drugs or hormones.

Agents as employed herein refers to an entity which when administered has a physiological affect.

Drug as employed herein refers to a chemical entity which at a therapeutic dose has an appropriate physiological affect.

In one embodiment the antibodies or fragments according to the present disclosure are employed with an immunosuppressant therapy, such as a steroid, in particular prednisone.

In one embodiment the antibodies or fragments according to the present disclosure are employed with Rituximab or other B cell therapies.

In one embodiment the antibodies or fragments according to the present disclosure are employed with any B cell or T cell modulating agent or immunomodulator. Examples include methotrexate, microphenyolate and azathioprine.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the extent of the inflammation present and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dosing will depend on the half life of the antibody, its target-mediated disposition, the duration of its effect, and the presence of anti-drug antibodies. If the antibody has a short half life (a few hours) or a limited activity, and/or if it is desirable to deliver small volumes of drug (e.g. for subcutaneous injection), it may be necessary to dose frequently, as frequently as once or more per day. Alternatively, if the antibody has a long half life, has long duration of activity, or can be dosed in large volumes (such as by infusion) dosing may be infrequent, once per day, or every few days, weeks or months. In one embodiment, sufficient time is allowed between doses to allow anti-drug antibody levels to decline.

Half life as employed herein is intended to refer to the duration of the molecule in circulation, for example in serum/plasma.

Pharmacodynamics as employed herein refers to the profile and in particular duration of the biological action of the molecule according the present disclosure.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pI of the protein is in the range 8-9 or above then a formulation pH of 7 may be appropriate. Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one example the pharmaceutical formulation at a pH in the range of 4.0 to 7.0 comprises: 1 to 200mg/mL of an antibody molecule according to the present disclosure, 1 to 100mM of a buffer, 0.001 to 1% of a surfactant, a) 10 to 500mM of a stabiliser, b) 10 to 500mM of a stabiliser and 5 to 500 mM of a tonicity agent, or c) 5 to 500 mM of a tonicity agent.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial

space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 1 to 5 μm. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellent gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellent gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellent gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellent-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for

example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The antibody of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Examples of buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulizable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solutionbuffer.

The antibodies disclosed herein may be suitable for delivery via nebulisation.

It is also envisaged that the antibody of the present invention may be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

The present invention also provides an antibody molecule (or compositions comprising same) for use in the control of autoimmune diseases, for example Acute Disseminated Encephalomyelitis (ADEM), Acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, ANCA-associated vasculitis,

Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome (APS), Autoimmune angiocdema, Autoimmune aplastic anemia, Autoimmune dysautonomia, Autoimmune hepatitis, Autoimmune hyperlipidemia, Autoimmune immunodeficiency , Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune thrombocytopenic purpura (ATP), Autoimmune thyroid disease, Autoimmune urticarial, Axonal & nal neuropathies, Balo disease, Behcet's disease, Bullous pemphigoid, Cardiomyopathy, Castleman disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, Cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogans syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST disease, Essential mixed cryoglobulinemia, Demyelinating neuropathies, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Dilated cardiomyopathy, Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic angiocentric fibrosis, Eosinophilic fasciitis, Erythema nodosum, Experimental allergic encephalomyelitis, Evans syndrome, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis (GPA) see Wegener's, Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura, Herpes gestationis, Hypogammaglobulinemia, Idiopathic hypocomplementemic tubulointestitial nephritis, Idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related disease, IgG4-related sclerosing disease, Immunoregulatory lipoproteins, Inflammatory aortic aneurysm, Inflammatory pseudotumour, Inclusion body myositis, Insulin-dependent diabetes (type1), Interstitial cystitis, Juvenile arthritis, Juvenile diabetes, Kawasaki syndrome, Kuttner's tumour, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus (SLE), Lyme disease, chronic, Mediastinal fibrosis, Meniere's disease, Microscopic polyangiitis, Mikulicz's syndrome, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal fibrosclerosis, Multiple sclerosis, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (Devic's), Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Ormond's disease (retroperitoneal fibrosis), Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), Paraneoplastic cerebellar degeneration, Paraproteinemic polyneuropathies, Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis (peripheral uveitis), Pemphigus vulgaris, Periaortitis, Periarteritis, Peripheral neuropathy, Perivenous encephalomyclitis, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Type I, II, & III autoimmune polyglandular syndromes, Polymyalgia rheumatic, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Progesterone dermatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis, Psoriasis, Psoriatic arthritis, Idiopathic pulmonary

fibrosis, Pyoderma gangrenosum, Pure red cell aplasia, Raynauds phenomenon, Reflex sympathetic dystrophy, Reiter's syndrome, Relapsing polychondritis, Restless legs syndrome, Retroperitoneal fibrosis (Ormond's disease), Rheumatic fever, Rheumatoid arthritis, Riedel's thyroiditis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjogren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome, Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombotic, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, Transverse myelitis, Ulcerative colitis, Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vesiculobullous dermatosis, Vitiligo, Waldenstrom Macroglobulinaemia, Warm idiopathic haemolytic anaemia and Wegener's granulomatosis (now termed Granulomatosis with Polyangiitis (GPA).

Additional indications may also include hyperviscosity syndromes; cryoglobulinemia; recurrent focal and segmental glomerulosclerosis in the transplanted kidney; HELLP syndrome; Refsum disease; HIV-related neuropathy; rhabdomyolysis and alloimune diseases.

In one embodiment the antibodies or fragments according to the disclosure are employed in the treatment or prophylaxis of epilepsy or seizures.

In one embodiment the antibodies or fragments according to the disclosure are employed in the treatment or prophylaxis of multiple sclerosis.

In embodiment the antibodies and fragments of the disclosure are employed in alloimmune disease/indications which includes:

- Transplantation donor mismatch due to anti-HLA antibodies
- Foetal and neonatal alloimmune thrombocytopenia, FNAIT (or neonatal alloimmune thrombocytopenia, NAITP or NAIT or NAT, or foeto-maternal alloimmune thrombocytopenia, FMAITP or FMAIT).

Additional indications include: rapid clearance of Fc-containing biopharmaceutical drugs from human patients and combination of anti-FcRn therapy with other therapies – IVIg, Rituxan, plasmapheresis. For example anti-FcRn therapy may be employed following Rituxan therapy. In addition anti-FcRn therapy may be used to rapidly clear imaging agents such as radiolabelled antibodies used in imaging tumors.

In embodiment the antibodies and fragments of the disclosure are employed in a neurology disorder such as:

- Chronic inflammatory demyelinating polyneuropathy (CIDP)
- Guillain-Barre syndrome
- Paraproteinemic polyneuropathies
- Neuromyelitis optica (NMO, NMO spectrum disorders or NMO spectrum diseases), and
- Myasthenia gravis.

In embodiment the antibodies and fragments of the disclosure are employed in a dermatology disorder such as:

- Bullous pemphigoid
- Pemphigus vulgaris
- ANCA-associated vasculitis
- Dilated cardiomyopathy

In embodiment the antibodies and fragments of the disclosure are employed in an Immunology, haematology disorder such as:

- Idiopathic thrombocytopenic purpura (ITP)
- Thrombotic thrombocytopenic purpura (TTP)
- Warm idiopathic haemolytic anaemia
- Goodpasture's syndrome
- Transplantation donor mismatch due to anti-HLA antibodies

In one embodiment the disorder is selected from Myasthenia Gravis, Neuro- myelitis Optica, CIDP, Guillaume-Barre Syndrome, Para-proteinemic Poly neuropathy, Refractory Epilepsy, ITP/TTP, Hemolytic Anemia, Goodpasture's Syndrome, ABO mismatch, Lupus nephritis, Renal Vasculitis, Sclero-derma, Fibrosing alveolitis, Dilated cardio-myopathy, Grave's Disease, Type 1 diabetes, Auto-immune diabetes, Pemphigus, Sclero-derma, Lupus, ANCA vasculitis, Dermato-myositis, Sjogren's Disease and Rheumatoid Arthritis.

In one embodiment the disorder is selected from autoimmune polyendocrine syndrome types 1 (APECED or Whitaker's Syndrome) and 2 (Schmidt's Syndrome); alopecia universalis; myasthenic crisis; thyroid crisis; thyroid associated eye disease; thyroid ophthalmopathy; autoimmune diabetes; autoantibody associated encephalitis and/or encephalopathy; pemphigus foliaceus; epidermolysis bullosa; dermatitis herpetiformis; Sydenham's chorea; acute motor axonal neuropathy (AMAN); Miller-Fisher syndrome; multifocal motor neuropathy (MMN); opsoclonus; inflammatory myopathy; Isaac's syndrome (autoimmune neuromyotonia), Paraneoplastic syndromes and Limbic encephalitis.

The antibodies and fragments according to the present disclosure may be employed in treatment or prophylaxis.

The present invention also provides a method of reducing the concentration of undesired antibodies in an individual comprising the steps of administering to an individual a therapeutically effective dose of an anti-FcRn antibody or binding fragment thereof described herein.

The present invention further provides the use of an antibody molecule according to the present invention in the manufacture of a medicament for the treatment and/or prophylaxis of a pathological disorder described herein such as an autoimmune disease.

In one embodiment the present disclosure comprises use of antibodies or fragments thereof as a reagent for diagnosis, for example conjugated to a reporter molecule. Thus there is provided antibody or fragment according to the disclosure which is labelled. In one aspect there is provided a column comprising an antibody or fragment according to the disclosure.

Thus there is provided an anti-FcRn antibody or binding fragment for use as a reagent for such uses as:

1) purification of FcRn protein (or fragments thereof) – being conjugated to a matrix and used as an affinity column, or (as a modified form of anti-FcRn) as a precipitating agent (e.g. as a form modified with a domain recognised by another molecule, which may be modified by addition of an Fc (or produced as full length IgG), which is optionally precipitated by an anti-Fc reagent)
2) detection and/or quantification of FcRn on cells or in cells, live or fixed (cells *in vitro* or *in vivo* in tissue or cell sections). Uses for this may include quantification of FcRn as a biomarker, to follow the effect of anti-FcRn treatment. For these purposes, the candidate might be used in a modified form (e.g. by addition of an Fc domain, as in full length IgG, or some other moiety, as a genetic fusion protein or chemical conjugate, such as addition of a fluorescent tag used for the purposes of detection).
3) purification or sorting of FcRn-bearing cells labeled by binding to candidate modified by ways exemplified in (1) and (2).

Also provided by the present invention is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block FcRn activity and in particular the ability of the cells to recycle IgG. Such an assay may be useful for identifying inhibitors of FcRn activity, such as antibody molecules or small molecules and as such may also be useful as a batch release assay in the production of such an inhibitor.

In one aspect there is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:

a) coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),
b) contacting the cells under mildly acidic conditions such as about pH5.9 with a test molecule and an IgG to be recycled by the cell for a period of time sufficient to allow binding of both the test molecule and the IgG to FcRn, optionally adding the test molecule before the IgG to be recycled and incubating for a period of time sufficient to allow binding of the test molecule to FcRn.
c) washing with a slightly acidic buffer, and
d) detecting the amount of IgG internalised and/or recycled by the cells.

In one aspect there is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:

a) coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),

b) contacting the cells under mildly acidic conditions such as about pH5.9 with a test antibody molecule and an IgG to be recycled by the cell for a period of time sufficient to allow binding of both the test antibody molecule and the IgG to FcRn, optionally adding the test antibody molecule before the IgG to be recycled and incubating for a period of time sufficient to allow binding of the test antibody molecule to FcRn.

c) washing with a slightly acidic buffer to remove unbound IgG and test antibody molecule, and

d) detecting the amount of IgG recycled by the cells.

In one aspect there is provided an assay suitable for assessing the ability of a test molecule such as an antibody molecule to block human FcRn activity and in particular the ability of human FcRn to recycle IgG, wherein the method comprises the steps of:

a) coating onto a surface non-human mammalian cells recombinantly expressing human FcRn alpha chain and human β2 microglobulin (β2M),

b) contacting the cells under mildly acidic conditions such as about pH5.9 with a test antibody molecule and an IgG to be recycled by the cell for a period of time sufficient to allow binding of both the test antibody molecule and IgG to FcRn, optionally adding the test antibody molecule before the IgG to be recycled and incubating for a period of time sufficient to allow binding of the test antibody molecule to FcRn.

c) washing with a slightly acidic buffer to remove unbound IgG and test antibody molecule,

d) incubating the cells in a neutral buffer such as about pH 7.2

e) detecting the amount of IgG recycled by the cells by determining the amount of IgG released into the supernatant.

Suitable cells include Madin-Darby Canine Kidney (MDCK) II cells. Transfection of MDCKII cells with human FcRn alpha chain and human β2 microglobulin (β2M) has previously been described by Claypool *et al.*, 2002, Journal of Biological Chemistry, 277, 31, 28038-28050. This paper also describes recycling of IgG by these transfected cells.

Media for supporting the cells during testing includes complete media comprising MEM (Gibco #21090-022), 1 x non-essential amino acids (Gibco 11140-035), 1 x sodium pyruvate (Gibco #11360-039), and L-glutamine (Gibco # 25030-024).

Acidic wash can be prepared by taking HBSS+ (PAA #H15-008) and adding 1M MES until a pH 5.9 +/- 0.5 is reached. BSA about 1% may also be added (Sigma # A9647).

A neutral wash can be prepared by taking HBSS+ (PAA #H15-008) and adding 10M Hepes pH 7.2 +/- 0.5 is reached. BSA about 1% may also be added (Sigma # A9647).

Washing the cells with acidic buffer removes the unbound test antibody and unbound IgG and allows further analysis to be performed. Acidic conditions used in step (b) encourage the binding of the IgG to FcRn and internalisation and recycling of the same.

The amount of test antibody or fragment and IgG on only the surface of the cells may be determined by washing the cells with neutral wash and analysing the supernatant/washings to

detect the quantity of test antibody or IgG. Importantly a lysis buffer is not employed. To determine the amount of IgG internalised by the cells the antibody may first be removed from the surface of the cell with a neutral wash and the cells lysed by a lysis buffer and then the internal contents analysed. To determine the amount of IgG recycled by the cells the cells are incubated under neutral conditions for a suitable period of time and the surrounding buffer analysed for IgG content. If the surface and internal antibody content of the cell is required then the cell can be washed with acid wash to maintain the antibody presence on the cell surface, followed by cell lysis and analysis of the combined material.

Where it is desired to measure both internalisation and recycling of the IgG samples are run in duplicate and testing for internalisation and recycling conducted separately.

A suitable lysis buffer includes 150mM NaCl, 20mM Tris, pH 7.5, 1mM EDTA, 1mM EGTA, 1% Triton-X 100, for each 10ml add protease inhibitors/phosphate inhibitors as described in manufacturer's guidelines.

Typically the IgG to be recycled is labelled, in one example a biotinylated human IgG may be used. The IgG can then be detected employing, for example a streptavidin sulfo-tag detection antibody (such as MSD # r32ad-5) 25mL at 0.2ug/mL of MSD blocking buffer. Blocking buffer may comprise 500mM Tris, pH7.5. 1.5M NaCl and 0.2% Tween-20 and 1.5% BSA.

Alternatively the IgG may be pre-labelled with a fluorophore or similar label.

In one embodiment a suitable surface is a plastic plate or well such as a 96 well plate or similar, a glass slide or a membrane. In one example cells are coated onto the surface at a density that results in the formation of a monolayer.

In one embodiment the assay described herein is not a measurement of transcytosis of an antibody top to bottom across a membrane with a pH gradient there-across, for example acid conditions one side of the membrane and neutral conditions on the underside of the membrane.

In one example the test antibody or fragment and IgG may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding.

In one example the test antibody or fragment may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding before addition of the IgG to be recycled. Subsequently the IgG to be recycled by the cell may be incubated with the cells in step (b) for about 1 hour for example at ambient temperature under acidic conditions to allow binding.

Neutral conditions facilitate release of the IgG into the supernatant.

Comprising in the context of the present specification is intended to meaning including.

Where technically appropriate embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Technical references such as patents and applications are incorporated herein by reference.

The present invention is further described by way of illustration only in the following examples, which refer to the accompanying Figures, in which:

Figure 1 Shows % hIgG in transgenic mice determined by LC-MS/MS

Figure 1a shows the effect of 1638 IgG4P format on the concentration of human IVIg in serum of human FcRn-transgenic mice.

Figure 1b shows the effect of 1638 FabFv and Fab'PEG formats on the concentration of human IVIg in human FcRn-transgenic mice

Figure 1c shows the pharmacokinctics of 1638 IgG4P format in human FcRn-transgenic mice.

Figure 1d shows the pharmacokinetics of 1638 FabFv and Fab'PEG formats in human FcRn-transgenic mice

Figure 1e The effect of 1638 FabFv and Fab'PEG formats on the concentration of serum albumin in human FcRn-transgenic mice.

Figure 1f The effect of 1638 IgG4P format on the concentration of serum albumin in human FcRn-transgenic mice.

Figure 2 shows representative binding curves for CA170_1638.g49 IgG4. The mean K_D values (n = 3) were 0.20nM in neutral buffer, & 0.22nM in acidic buffer, respectively

Figure 3 shows CA170_1638.g49 IgG4 inhibits IgG recycling in MDCK II clone 15 cells

Figure 4 shows CA170_1638.g49 IgG4 inhibits IgG transcytosis in MDCK II clone 15 cells.

Figure 5 shows CA170_1638.g49 FabFv inhibits IgG transcytosis in MDCK II clone 15 cells.

Figure 6 shows representative binding curves for CA170_1638.g49 IgG4. The mean KD values (n = 3) were 0.3 in neutral buffer, and 0.43 in acidic buffer, respectively (see Table 2).

Figure 7 shows CA170_1638 CDR sequences

Figure 8 Antibody sequences according to the present disclosure

Figure 9a Humanisation of antibody 1638.g49

Figure 9b Humanisation of antibody 1638.g49

EXAMPLES

Abbreviations

°C temperature, degrees centigrade.

ATR FTIR	Attenuated Total Reflectance Fourier Transform Infra-Red Spectroscopy
CH2	constant heavy chain region 2
cIEF	capillary isoelectric focusing
DSC	differential scanning calorimetry
G0F	fucosylated aglactosyl biantennary glycan
H chain	Heavy chain
HPLC	high performance liquid chromatography
IgG	immunoglobulin G
L chain	Light chain
nLCMS	nano-liquid chromatography mass spectrometry
PBS	phosphate-buffered saline buffer
pI	isoelectric point
SD	standard deviation
SEC	size exclusion chromatography
ToF	time of flight
T_m	melting temperature
TCEP	tris(2-carboxyethyl)phosphine
THP	Tris(hydroxypropyl)phosphine
Tris	tris(hydroxymethyl)aminomethane

The following immunizations were performed in order to generate material for B cell culture and antibody screening:

Sprague Dawley rats were immunized with three shots of NIH3T3 mouse fibroblasts co-expressing mutant human FcRn (L320A; L321A) (Ober et al., 2001 Int. Immunol. 13, 1551–1559) and mouse β2M with a fourth final boost of human FcRn extracellular domain.

Sera were monitored for both binding to mutant FcRn on HEK-293 cells and for its ability to prevent binding of Alexafluor 488-labelled human IgG. Both methods were performed by flow cytometry. For binding, phycoerythrin (PE)-labelled anti mouse or rat Fc specific secondary reagents were used to reveal binding of IgG in sera.

B cell cultures were prepared using a method similar to that described by Zubler *et al.* (1985). Briefly, B cells at a density of approximately 5000 cells per well were cultured in bar-coded 96-well tissue culture plates with 200 µl/well RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS (PAA laboratories ltd), 2% HEPES (Sigma Aldrich), 1% L-Glutamine (Gibco BRL), 1% penicillin/streptomycin solution (Gibco BRL), 0.1% β-mercaptoethanol (Gibco BRL), 2-5% activated rabbit splenocyte culture supernatant and gamma-irradiated EL-4-B5 murine thymoma cells ($5x10^4$/well) for seven days at 37°C in an atmosphere of 5% CO_2.

The presence of FcRn-specific antibodies in B cell culture supernatants was determined using a homogeneous fluorescence-based binding assay using HEK-293 cells transiently transfected with mutant FcRn (surface-stabilised) as a source of target antigen. 10 ul of supernatant was

transferred from barcoded 96-well tissue culture plates into barcoded 384-well black-walled assay plates containing 5000 transfected HEK-293 cells per well using a Matrix Platemate liquid handler. Binding was revealed with a goat anti-rat or mouse IgG Fcγ-specific Cy-5 conjugate (Jackson). Plates were read on an Applied Biosystems 8200 cellular detection system. From 3800 x 96-well culture plates, representing 38 different immunized animals, 9800 anti-human FcRn binders were identified. It was estimated that this represented the screening of approximately 2.5 billion B cells.

Following primary screening, positive supernatants were consolidated on 96-well bar-coded master plates using an Aviso Onyx hit-picking robot and B cells in cell culture plates frozen at -80C. Master plates were then screened in a Biacore assay in order to identify wells containing antibodies of high affinity and those which inhibited the binding of human IgG to FcRn (see below).

Biomolecular interaction analysis using surface plasmon resonance technology (SPR) was performed on a BIAcore T200 system (GE Healthcare). Goat anti-rat IgG, Fc gamma (Chemicon International Inc.) in 10mM NaAc, pH 5 buffer was immobilized on a CM5 Sensor Chip via amine coupling chemistry to a capture level of approx. 19500 response units (RU) using HBS-EP$^+$ as the running buffer. 50mM Phosphate, pH6 + 150mM NaCl was used as the running buffer for the affinity and blocking assay. B cell culture supernatants were diluted 1 in 5 in 200mM Phosphate, pH6 +150mM NaCl. A 600s injection of diluted B cell supernatant at 5µl/min was used for capture by the immobilized anti-rat IgG,Fc. Human FcRn at 100nM was injected over the captured B cell culture supernatant for 180s at 30µl/min followed by 360s dissociation. Human IgG (Jackson ImmunoResearch) was injected over for 60s with 180s dissociation at 30µl/min.

The data was analysed using T200 evaluation software (version 1.0) to determine affinity constants (K_D) of antibodies and determine those which blocked IgG binding.

As an alternative assay, master plate supernatants were also screened in a cell-based human IgG blocking assay. 25 ul of B cell culture supernatant from master plates were added to 96 well U-bottomed polypropylene plate. Mutant hFcRn-transfected HEK-293 cells (50,000 cells per well in 25 ul PBS pH6/1% FCS) were then added to each well and incubated for 1 hour at 4°C. Cells were washed twice with 150 ul of PBS media. Cells were then resuspended in 50 ul/well PBS/FCS media containing human IgG labelled with Alexafluor 488 or 649 at 7.5ug/ml and incubated 1 hour at 4°C. Cells were then washed twice with 150 ul of media and then resuspended in 35 ul / well of PBS/FCS media containing 1% formaldehyde as fixative. Plates were then read on a FACS Canto 2 flow cytometer.

To allow recovery of antibody variable region genes from a selection of wells of interest, a deconvolution step had to be performed to enable identification of the antigen-specific B cells in a given well that contained a heterogeneous population of B cells. This was achieved using the Fluorescent foci method. Briefly, Immunoglobulin-secreting B cells from a positive well were

mixed with streptavidin beads (New England Biolabs) coated with biotinylated human FcRn and a 1:1200 final dilution of a goat anti-rat or mouse Fcγ fragment-specific FITC conjugate (Jackson). After static incubation at 37°C for 1 hour, antigen-specific B cells could be identified due to the presence of a fluorescent halo surrounding that B cell. These individual B cells, identified using an Olympus microscope, were then picked with an Eppendorf micromanipulator and deposited into a PCR tube. Fluorescent foci were generated from 268 selected wells.

Antibody variable region genes were recovered from single cells by reverse transcription polymerase chain reaction (RT)-PCR using heavy and light chain variable region-specific primers. Two rounds of PCR were performed on an Aviso Onyx liquid handling robot, with the nested 2° PCR incorporating restriction sites at the 3' and 5' ends allowing cloning of the variable regions into a mouse γ1 IgG (VH) or mouse kappa (VL) mammalian expression vector. Paired heavy and light chain constructs were co-transfected into HEK-293 cells using Fectin 293 (Invitrogen) and cultured in 48-well plates in a volume of 1 ml. After 5-7 days expression, supernatants were harvested and antibody subjected to further screening.

PCR successfully recovered heavy and light chain cognate pairs from single B cells from 156 of the selected wells. DNA sequence analysis of the cloned variable region genes identified a number of unique families of recombinant antibody. Following expression, transient supernatants were interrogated in both human IgG FACS blocking (described above) and IgG recycling assays. In some cases, purified mouse γ1 IgG was produced and tested (data labeled accordingly).

The recycling assay used MDCK II cells (clone as described in Examples 5, 6 and 7 below) over-expressing human FcRn and beta 2 microglobulin plated out at 25,000 cells per well of a 96 well plate. These were incubated overnight at 37°C, 5% CO_2. The cells were washed with HBSS+ Ca/Mg pH 7.2+1% BSA and then incubated with 50µl of varying concentrations of HEK-293 transient supernatant or purified antibody for 1 hour at 37°C, 5% CO_2. The supernatant was removed and 500ng/ml of biotinylated human IgG (Jackson) in 50µl of HBSS+ Ca/Mg pH 5.9 +1%BSA was added to the cells and incubated for 1 hour at 37°C, 5% CO_2. The cells were then washed three times in HBSS+ Ca/Mg pH 5.9 and 100µl of HBSS+ Ca/Mg pH 7.2 added to the cells and incubated at 37°C, 5% CO_2 for 2 hours. The supernatant was removed from the cells and analysed for total IgG using an MSD assay with an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD). The inhibition curve was analysed by non-linear regression to determine IC50 values.

Based on performance in these assays a family of antibodies was selected comprising the six CDRs given in SEQ ID NOs 1 to 6. Antibody CA170_01638 had the best activity and was selected for humanisation.

Example 1 Humanisation Method

Antibody CA170_01638 was humanised by grafting the CDRs from the rat antibody V-regions onto human germline antibody V-region frameworks. In order to recover the activity of the antibody, a number of framework residues from the rat V-regions were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair et al. (1991) (Humanised antibodies WO91/09967). Alignments of the rat antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figures 9A and B, together with the designed humanised sequences. The CDRs grafted from the donor to the acceptor sequence are as defined by Kabat (Kabat et al., 1987), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair et al., 1991 Humanised antibodies. WO91/09967). Human V-region IGKV1-27 plus JK4 J-region (http://www.imgt.org/) was chosen as the acceptor for the light chain CDRs. Human V-region IGHV3-7 plus JH3 J-region (http://www.imgt.org/) was chosen as the acceptor for the heavy chain CDRs.

Genes encoding a number of variant heavy and light chain V-region sequences were designed and constructed by an automated synthesis approach by Entelechon GmbH. Further variants of both heavy and light chain V-regions were created by modifying the VH and VK genes by oligonucleotide-directed mutagenesis. These genes were cloned into a number of vectors to enable expression of humanised 1638 Fab or IgG4 antibody in *E. coli* and mammalian cells, respectively. The variant chains, and combinations thereof, were assessed for their potency relative to the parent antibody, their biophysical properties and suitability for downstream processing, leading to the selection of the gL7 light chain graft and gH33 heavy chain graft. The final selected gL7 and gH33 graft sequences are shown in Figures 9A and B, respectively. This V-region pairing was named 1638.g49.

The light chain framework residues in graft gL7 are all from the human germline gene, with the exception of residues 70 and 71 (Kabat numbering), where the donor residues Histidine (H70) and Tyrosine (T71) were retained, respectively. Retention of these two residues was important for full potency of the humanised antibody or Fab. Residue 56 in CDRL2 of the gL7 graft was mutated from an Aspartic acid (D56) to a Glutamic acid (E56) residue, thus removing a potential Aspartic acid isomerization site from the gL7 sequence. The heavy chain framework residues in graft gH33 are all from the human germline gene, with the exception of residues 48 and 78 (Kabat numbering), where the donor residues Leucine (L48) and Alanine (A78) were retained, respectively. Retention of these two residues was essential for full potency of the humanised antibody or Fab.

For expression of 1638.g49 Fab in *E. coli*, the humanised heavy and light chain V-region genes were cloned into the UCB expression vector pTTOD, which contains DNA encoding the human C-kappa constant region (K1m3 allotype) and the human gamma-1 CH1 region (with or without hinge region) (G1m17 allotype).

For expression of 1638.g49 IgG4 in mammalian cells, the humanised light chain V-region gene was joined to a DNA sequence encoding the human C-kappa constant region (K1m3 allotype), to create a contiguous light chain gene. The humanised heavy chain V-region gene was joined to a DNA sequence encoding the human gamma-4 heavy chain constant region with the hinge stabilising mutation S241P (Angal et al., Mol Immunol. 1993, 30(1):105-8), to create a contiguous heavy chain gene. The heavy and light chain genes were cloned into a mammalian expression vector.

Another earlier graft, 1638.g28 was used in Example 8A described herein below and this contained more donor residues in the heavy chain (gH2) than the 1638.g49 graft (F24, L48, K71, T73, A78 and V93). Also the light chain of this antibody (gL2) contains the unmodified CDRL2 given in SEQ ID NO: 5 rather than the modified CDRL2 of SEQ ID NO: 7 which is used in 1638.g49. Sequences of both sets of antibodies are given in Figure 8. Antibody 1638.g28 was expressed as a Fab' fragment as described above for 1638.g49.

Example 2 Preparation of 1638.g49 Fab'-PEG conjugate

Fab' expressed in the periplasm of *E.coli* was extracted from cells by heat extraction. Fab' purified by Protein G affinity purification with an acid elution. Fab' reduced and PEGylated with 40kDa PEG (SUNBRIGHT GL2-400MA3). PEG is covalently linked via a maleimide group to one or more thiol groups in the antibody fragment. PEGylation efficiency was confirmed by SE-HPLC. Fab'PEG was separated from un-PEGylated Fab' and diFab' by cation exchange chromatography. Fractions analyzed by SE-HPLC and SDS-PAGE. Pooling carried out to minimize levels of impurities. Final sample concentrated and diafiltered into desired buffer.

Example 3 Affinity for hFcRn binding

Biomolecular interaction analysis using surface plasmon resonance technology (SPR) was performed on a Biacore T200 system (GE Healthcare) and binding to human FcRn extracellular domain determined. Human FcRn extracellular domain was provided as a non-covalent complex between the human FcRn alpha chain extracellular domain (SEQ ID NO: 48) and β2 microglobulin (β2M) (SEQ ID NO: 72). Affinipure $F(ab')_2$ fragment goat anti-human IgG, Fc fragment specific (for IgG4 capture) (Jackson ImmunoResearch Lab, Inc.) at 50μg/ml in 10mM NaAc, pH 5 buffer was immobilized on a CM5 Sensor Chip via amine coupling chemistry to a capture level between 5000 - 6000 response units (RU) using HBS-EP$^+$ (GE Healthcare) as the running buffer.

50mM Phosphate, pH6 + 150mM NaCl + 0.05%P20 or HBS-P$^+$, pH7.4 (GE Healthcare) was used as the running buffer for the affinity assay. The antibody, 1638.g49 IgG4P was diluted to 1μg/ml in running buffer. A 60s injection of IgG4 at 10μl/min was used for capture by the immobilized anti-human IgG, Fc. Human FcRn extracellular domain was titrated from 20nM to

1.25nM over the captured anti-FcRn antibody (IgG4) for 300s at 30µl/min followed by 1200s dissociation. The surface was regenerated by 2 x 60s 50mM HCl at 10µl/min for the running buffer at pH6 or by 60s 40mM HCl and 30s 10mM NaOH for the running buffer at pH7.4. The data was analysed using T200 evaluation software (version 1.0) using the 1:1 binding model with local Rmax.

Table 1 Affinity data for anti-hFcRn 1638.g49 IgG4P at pH6.0 and pH7.4

pH6.0	Human FcRn		
1638.g49 IgG4P	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**KD (M)**
1	1.10E+06	1.43E-04	1.29E-10
2	1.10E+06	1.39E-04	1.26E-10
3	1.11E+06	1.40E-04	1.27E-10
Mean	**1.10E+06**	**1.41E-04**	**1.27E-10**

pH7.4	Human FcRn		
1638.g49 IgG4P	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**KD (M)**
1	9.75E+05	2.51E-05	2.57E-11
2	9.62E+05	3.19E-05	3.32E-11
3	9.62E+05	2.82E-05	2.93E-11
Mean	**9.67E+05**	**2.84E-05**	**2.94E-11**

The affinity of 1638.49g IgG4 was therefore determined to be 127pM at pH 6.0 and 29pM at pH7.4.

Example 4

An IgG4P full length molecule and Fab-dsFv molecule where the 1638.g49 variable region was incorporated into the Fab domain of each format were analysed for biochemical integrity and biophysical stability.

Methods and Results

1. Sequence Confirmation.

i) Protein sequencing (Edman chemical method)

The N-terminal amino acid sequence of both IgG4 and Fab-dsFv samples was obtained using an Applied Biosystems Procise 494 instrument. This was operated as recommended by the instrument manufacturer. Approximately 100pmoles of each sample was applied to discs of polyvinylidene difluoride (Prosorb, used as per manufacturer's recommendations) and subjected

to 18 cycles which included two blank runs and a standard hence resulting in the analysis of the first 15 amino acid residues of the heavy and light chains. Analysis was performed using SequencePro Data Analysis Application V2.0.

For each sample, the observed sequence was a mixture of two, approximately equally-abundant sequences, EVQLVESGGGLVQPG (SEQ ID NO: 67) and DIQMTQSPSSLSASV (SEQ ID NO: 68) consistent with the N-terminal sequences expected from the heavy and light chain gene sequences respectively. The approximately equal abundance suggested equal molar amounts of the 2 chains, with little to no significant N-terminal blockage.

ii) Mass Spectrometry Analysis

a) Intact Mass analysis

Intact mass spectrometry analysis was performed on two batches of the IgG4 and the Fab- dsFv molecule after reduction with 20mM TCEP for one hour. Masses were measured on an Agilent 6510 mass spectrometer equipped with a chip cube interface and a C8 chip (43mm Zorbax 300A C8 column + 43nL trap). All samples were diluted to 0.1mg/ml in 98% water / 2% methanol /0.3% formic acid (solvent A) prior to injection and 0.3µL was loaded onto the system. Proteins were eluted from the chip into the mass spectrometer using a gradient to 40% acetonitrile / 0.1% formic at 350nL/min. ToF-MS data were collected in positive-ion mode between 500 and 5000m/z and processed using Agilent MassHunter software.

The observed masses for both the light chain and the heavy chain for both formats is shown below (Table 2).

Table 2.Observed mass table of two IgG4 batches and Fab-dsFv

	L-chain			**H-chain**		
	Expected[1]	**Observed**	**ppm**	**Expected[1]**	**Observed**	**ppm**
IgG4 Batch#1	23503.3	23505.8	106	50764.7	50768.9	83
IgG4 Batch#2		23505.7	102		50768.9	83
FabFv	36384.4	[L1] 36385.4 [L2] 36387.6	27 88	38298.8	[H1] 38301.1 [H2] 38302.6	60 99

[1] Expected mass calculated from the amino acid sequence with the addition of

IgG4: 2 L- and 4 H- intrachain disulphides, G0F glycosylation and clipping of C-terminal Lys from the

H-chain

FabFv: 3 L- and 3 H- intrachain disulphides.

The intact mass analysis of the TCEP reduced IgG4 was consistent with the expected sequences with predominantly G0F glycosylation and clipped C-terminal lysine (approximately 90%) on the H-chain which is typical of recombinant IgG.

Similarly the intact mass spectra of the Fab- dsFv chains was consistent with the sequence mass and expected number of disulphides. There was heterogeneity in the observed mass of both chains presumably due to partial reduction of the intra-chain disulphides by TCEP.

b) Disulphide mapping was performed on IgG4 only.

IgG4 (50ug) were treated with 0.15% Rapigest in Tris-HCl pH7.5 at 50°C for 15 minutes and any free cysteines alkylated with iodoacetamide. Trypsin (1:25w/w) was added and proteins were hydrolysed overnight at room temperature and then the reaction quenched by the addition of formic acid (5%v/v) and any precipitate was removed by centrifugation. Samples were stored at -20°C and diluted 1:1 with water before loading on the LC-MS system. Aliquots (~3-5ug) were loaded onto a 2.1 x 150mm C18 column (Waters BEH1.7u) equilibrated with water containing 0.2% formic acid and eluted with a gradient of acetonitrile/1-propanol into a Waters Xevo mass spectrometer operated in +ve-ion MS^E mode. Data was analysed with MassLynx and BioPharmaLynx software.

The results indicated that all the expected disulphide-linked peptides were observed except the inter H-H-chain peptide T19-SS-T19 species which was only observed with a single disulphide bond and at low intensity. There was no evidence for any scrambled disulphide species or carbamidomethylated cysteine residues.

2. Biochemical Analysis

Size Exclusion Chromatography HPLC (SEC HPLC)

Size exclusion chromatography allowed analysis of monomeric and oligomeric material. It was performed using a TSK G3000SW (7.7mm I.Dx30.0cm L) column connected to an Agilent 1100 system. The samples (25 µl/25 µg injection) were eluted isocratically in 0.2 M sodium phosphate , pH 7 at 1.0 ml/min for 30 minutes, 30°C. Elution was monitored by absorption at 280 nm.

The elution profiles showed that the IgG4 and Fab-dsFv were homogeneous and eluted at expected retention times as judged by SEC standards (BioRad 151-1901).

3. Molecular Charge.

Capillary isoelectric focusing (cIEF) was conducted to estimate pI and acidic species content.

IgG4 and Fab-dsFv samples were diluted to 1mg/ml in HPLC grade water for analysis (non- reduced condition) . The samples were also subjected to reduction (2mM THP/30 minutes) and alkylation (20mM iodoacetamide/80 minutes) to analyse for cysteine adducts.

Samples were prepared by mixing the following: 30 µl protein sample, 0.35% methylcellulose, 4% pH3-10 ampholytes (Pharmalyte), 1 µl of each synthetic pI marker (4.65 and 9.77) and HPLC grade water to make up the final volume to 100 µl. The mixture was then analysed using iCE280 IEF analyser (Convergent Biosciences), pre-focusing at 1500 V for 1 minute followed by focusing at 3000 V for 6 minutes. The calibrated electropherograms were then integrated using Empower software (from Waters).

The pI was taken to be that of the main species (largest peak).

For the IgG4 format, the main species had a pI of 7.3. This was assumed to be the clipped parent molecule (removal of the C terminal lysine, corroborated by mass spectrum analysis) which is not atypical for IgG molecules. The clipped molecule would be more acidic that the parent molecule (basic peak at 7.4). There was no change to the pI profile pre- and post-reduction and alkylation, indicating that there were no cysteine adducts.

For the Fab-dsFv format, the pI was taken to be that of the main species (largest peak) which was 9.0. A more acidic species (pI 8.8) was also evident which was less prominent post reduction/alkylation indicating the presence of a reducible adduct.

For both formats, minor peaks were present being either acidic (to the left of the main peak) or basic (to the right of the main peak). These species were presumed to be derivatives of the main species, but were not characterised further.

4. **Thermal Stability (T_m)**

When heated, a protein will tend to unfold, and the more stably-folded a protein structure is, the more heat is required to unfold it. Therefore, thermal stability (measured as melting temperature, T_m) is a measure of the stability of folding of a protein, or resistance of a molecule to unfolding (denaturation), which may be a prerequisite to aggregate formation. In a temperature gradient, in defined conditions, the temperature at which 50% of molecules are unfolded is T_m.

T_m estimations were made by two independent methods

i) Thermofluor Assay , measurement of 50% unfolding by binding of a fluorescent dye (Sypro Orange) to exposed hydrophobic surfaces that become exposed upon heat induced unfolding and

ii) Differential Scanning Calorimetry (DSC).

Results from the two techniques generally correlate, differing slightly in absolute value because methods employed are different.

i) Thermofluor Assay

Samples were prepared as follows: 5 µl of 30x sypro orange was placed in a 96 well V-bottomed plate. Then, 45 µl of protein sample at 0.1 mg/ml was then added. This mix was pipetted, in 10 µl quadruplicates, into a 384 well plate. The format of the 384 well plate was: sample 1: wells A1, B1, A2, B2; sample 2: wells C1, D1, C2, D2. An inter-assay control was included, being an irrelevant IgG4. This control, at 0.1 mg/ml (in PBS pH 7.4) was added to 5 µl of 30x concentrated dye, 10 µl of this master mix being placed into the 384 well in quadruplicate. The plates were placed in a 7900HT fast real-time PCR system and heated from 20°C to 99°C using a

ramp rate of 1.1°C/min; a CCD device simultaneously monitors the fluorescence changes in the wells. A modified XE template (IDBS) is used to process the intensity data and take into account multiple transitions.

Two unfolding transitions were evident for both the IgG4 and the Fab-dsFv molecules. The T_m 2 value for both molecules represented the Fab unfolding domain and was shown to be slightly lower for the IgG4 format. The T_m1 value represented the CH2 (constant heavy chain) domain and the dsFv domain of the IgG4 and Fab-dsFv molecule respectively. The Fab-dsFv format was shown to be more thermally stable than the IgG format in PBS, pH 7.4.

Sample	T_m 1 Mean (°C)	T_m 1 SD	T_m 2 Mean (°C)	T_m 2 SD
IgG4	65.4	0.1	81.1	0.4
Fab-dsFv	73.6	0.4	83.1	0.4

ii) DSC method

DSC analysis was performed on the Fab-dsFv molecule only for corroboration of the Thermofluor data and to determine the effect of two different buffer types (PBS pH7.4 and 50mM sodium acetate/125mM sodium chloride, pH 5.0) on the thermal stability.

Samples at 1 mg/ml in PBS pH7.4 and 50mM sodium acetate/125mM sodium chloride, pH 5.0 with respective reference buffers were loaded onto the MicroCal VP Capillary DSC instrument in triplicate. The system settings included temperature scan from 20°C to 110°C and a scan rate of 60°C/hr. The final thermograms were processed using Origin software according to the manufacturer's instructions. The Tm was determined using software's automated T_m detection algorithm (for the main transition) and manually peak picked for any other transitions that was not automatically detected by the software.

Two distinct transitions could be observed in the two buffers tested.

The lower infolding transition (T_m 1) represented the dsFv domain of the Fab-dsFv molecule and the higher transition temperature (T_m 2) represented the Fab domain.

The DSC data was in good agreement with the data obtained from the Thermofluor assay. This technique was capable of being able to discriminate between the two unfolding domains more easily than the Thermofluor assay.

The Fab-dsFv molecule showed a slight increase in thermal stability in the 50mM sodium acetate/125mM sodium chloride, pH 5.

Buffer	**T_{m1} mean (°C)**	**SD**	**T_{m2} mean (°C)**	**SD**
Fab-dsFv (50mM NaOAc/125mM NaCl, pH 5)	86.1	0	73.6	0.15
Fab-dsFv (PBS, pH 7.4)	84.1	0.1	71.2	0.06

5. Molecular Structure: Attenuated Total Reflectance Fourier Transform Infra-Red Spectroscopy (ATR FTIR)

This technique was used to compare the extent of interaction between β-sheets within the molecule (intra-β-sheet) and between separate molecules (inter-β-sheet).

The analysis was performed using the Bruker Tensor 27 FTIR spectrometer and the BIOATR II cell sampling accessory using a resolution of $4cm^{-1}$; 120 scans; aperture setting 6mm and 20µL sample volume at 20 °C where the following procedure was performed for the analysis of the Fab-dsFv only.

1. Five air background spectra were measured using the method BIOATR 10 06 10. xpm .
2. 20µL of sigma PBS pH7.4 was added to the cell and then removed
3. 20µL of sigma PBS pH7.4 was added to the cell and a spectrum was taken, the buffer was removed and fresh buffer was added and a spectrum taken (in duplicate).
4. 20µL of sample was added to the cell and a spectrum was taken, the sample was then removed from the cell.
5. 20µL of sigma PBS pH7.4 was added to the cell and removed
6. 20µL of sample was added to the cell and a spectrum was taken, the sample was then removed from the cell. (in duplicate)
7. The cell was then cleaned following procedure below:
    a. 20µL 1% SDS added to cell + cleaned with Q-tip
    b. 20µL 1% SDS added to cell and removed
    c. 5 times 20µL H2O added to cell and removed
    d. 20µL buffer added to cell and removed
8. The data was analysed to produce the final data format in the following way.
    a. Buffer spectrum 1 was subtracted from the Fab-dsFv spectrum 1 and then repeated with buffer spectrum 2 and Fab-ds Fv spectrum 2.
    b. The data was cut to 2200cm-1 to 1000cm-1
    c. The duplicate spectra were averaged.
    d. A second derivative was then taken with a 25 point smoothing. This was the final data format shown.

The results of the analysis showed that the Fab-dsFv had the intra-beta sheet characteristics typical of antibody molecules.

Example 5 Cell-based potency

Cell-based assays were performed using Madin-Darby Canine Kidney (MDCK) II cells which had been stably transfected with a human FcRn and human B2M double gene vector with a Geneticin selection marker. A stable cell clone was selected that was able to recycle and transcytose human IgG and this was used for all subsequent studies. It will be referred to as MDCK II clone 15.

Cell based Affinity of CA170_1638.g49 IgG4 for human FcRn

Quantitative flow cytometry experiments were performed using MDCK II clone 15 cells and AlexaFluor 488-labelled CA170_1638.g49 IgG4. Specific binding of antibody to FcRn across a range of antibody concentrations was used to determine K_D. The analyses were performed in both neutral and acidic buffers to determine whether environmental pH comparable to that found in blood plasma (pH7.4) or endosomes (pH6) had any effect on the antibody binding.

Figure 2 shows representative binding curves for CA170_1638.g49 IgG4 The mean K_D values (n = 3) were 0.20 in neutral buffer, and 0.22 in acidic buffer, respectively (see Table 4).

Table 4 - Mean K_D values (nM) for CA170_1638.g49 IgG4 on MDCK II clone 15 cells.

Antibody format	Human FcRnpH 7.4	Human FcRnpH 6.0
1638.g49 IgG4	0.20	0.22

Figure 2 shows CA170_1638.g49 IgG4 binding on MDCK II clone 15 cells in acidic and neutral pH.

MDCK II clone 15 cells were incubated in Facs buffer (PBS with 0.2% w/v BSA, 0.09% w/v NaN3) for 30 mins prior to the addition of Alexa-fluor 488-labelled CA170_1638.g49 IgG4 for 1 hour in Facs buffer at either pH 7.4 or pH 6. The final antibody concentrations ranged from 400nM to 0.003nM. The cells were washed in ice cold Facs buffer then analysed by flow cytometry using a Guava flow cytometer (Millipore, UK). Titration data sets were also produced for isotype control antibodies for each antibody format to determine non-specific binding. The number of moles of bound antibody was calculated using interpolated values from a standard curve generated from beads comprised of differing amounts of fluorescent dye. Geometric mean fluorescence values were determined in the flow cytometric analyses of cells and beads. Non-specific binding was subtracted from the anti-FcRn antibody values and the specific binding curve generated was analysed by non-linear regression using a one-site binding equation (Graphpad Prism®) to determine the K_D. Data is representative of 3 experiments.

CA170_1638.g49 IgG4 can bind human FcRn expressed on cells at both acidic and neutral pH

Example 6 Functional cell based assays

FcRn expression is primarily intracellular (Borvak J et al. 1998, Int. Immunol., 10 (9) 1289-98 and Cauza K et al. 2005, J. Invest. Dermatol., 124 (1), 132-139), and associated with endosomal and lysosomal membranes. The Fc portion of IgG binds to FcRn at acidic pH (<6.5), but not at a neutral physiological pH (7.4) (Rhagavan M et al. 1995) and this pH-dependency facilitates the recycling of IgG.

Once it is taken up by pinocytosis and enters the acidic endosome, IgG bound to FcRn will be recycled along with the FcRn to the cell surface, whereas at the physiologically neutral pH the

IgG will be released. (Ober RJ et al. 2004, The Journal of Immunology, 172, 2021-2029). Any IgG not bound to FcRn will enter the lysosomal degradative pathway.

An *in vitro* assay was established to examine the ability of CA170_1638.g49 IgG4 to inhibit the IgG recycling capabilities of FcRn. Briefly, MDCK II clone 15 cells were incubated with biotinylated human IgG, in the presence and absence of 1638 IgG4in an acidic buffer (pH 5.9) to allow binding to FcRn. All excess antibody was removed and the cells incubated in a neutral pH buffer (pH 7.2) which allows release of surface-exposed, bound and internalised IgG into the supernatant. The inhibition of FcRn was followed using an MSD assay to detect the amount of IgG recycled and thus released into the supernatant.

Figure 3 shows CA170_1638.g49 IgG4 inhibits IgG recycling in MDCK II clone 15 cells. MDCK II clone 15 cells were plated at 15,000 cells per well in a 96 well plate and incubated overnight at 37°C, 5% CO_2. The cells were incubated with 1 ug/ml of biotinylated human IgG (Jackson) in the presence and absence of CA170_1638.g49 IgG4 in $HBSS^+$ (Ca/Mg) pH 5.9 + 1% BSA for 1 hour at 37°C, 5% CO_2. The cells were washed with $HBSS^+$ pH 5.9 then incubated at 37°C, 5% CO_2 for 2 hours in $HBSS^+$ pH 7.2. The supernatant was removed from the cells and analysed for total IgG using an MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC_{50}. The graph represents combined data from 3 experiments. As shown in **Figure 3** CA170_1638.g49 IgG4 inhibits IgG recycling in a concentration dependent manner with a mean EC_{50} value (n= 3) of 0.31nM.

CA170_1638.g49 IgG4 and FabFv inhibits the transcytosis of human IgG

FcRn can traffic IgG across polarised epithelial cell layers in both the apical to basolateral and basolateral to apical directions and thus plays an important role in permitting IgG to move between the circulation and lumen at mucosal barriers (Claypool et al. 2004 Mol Biol Cell 15(4):1746-59).

FcRn can traffic IgG across polarised epithelial cell layers in both the apical to basolateral and basolateral to apical directions and thus plays an important role in permitting IgG to move between the circulation and lumen at mucosal barriers (Claypool et al. 2004 Mol Biol Cell 15(4):1746-59).

An *in vitro* assay was established to examine the ability of CA170_1638.g49 IgG4 and FabFv to inhibit FcRn dependent IgG transcytosis. Briefly, MDCK II clone 15 cells were plated in a 24 well transwell plate and allowed to form monolayers over 3 days. The cells were then incubated with biotinylated human IgG in an acidic buffer which facilitates binding to FcRn, on the apical side, in the presence and absence of CA170_1638.g49 IgG4 or FabFv. The human IgG is transcytosed through the cells from the apical to basolateral side and released into a neutral

buffer in the lower chamber. Levels of IgG on the basolateral side were then measured using an MSD assay.

Figures 4 and 5 shows CA170_1638.g49 IgG4 and FabFv inhibits apical to basolateral IgG transcytosis in MDCK II clone 15 cells. MDCK II clone 15 cells were plated at 500,000 cells per well of a 24 well transwell plate and incubated for 3 days at 37°C, 5% CO_2 until monolayers were formed. The pH of the apical compartment was adjusted to 5.9 and the basolateral side to 7.2 in a $HBSS^{+}$(Ca/Mg) buffer + 1% BSA. Cells on the apical compartment were incubated with 1µg/ml biotinylated human IgG (Jackson) in the presence and absence of CA170_1638.g49 IgG4 or FabFv at the indicated concentrations for 4 hours at 37°C, 5% CO_2. The basolateral medium was then collected and total IgG measured by MSD assay (using an anti-human IgG capture antibody (Jackson) and a streptavidin-sulpho tag reveal antibody (MSD)). The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the EC_{50}. The graph represents combined data from 3 experiments.

In summary Figure 4 and 5 shows that CA170_1638.g49 IgG4 and FabFv can inhibit the apical to basolateral transcytosis of human IgG in a concentration dependent manner with an EC_{50} value of 2.4 and 0.42 nM respectively (n=3).

Summary of *in vitro* effects of CA170_1638.g49 IgG4 and FabFv

CA170_1638.g49 IgG4 and FabFv inhibit both IgG recycling and transcytosis. The EC_{50} of 0.31nM achieved in the IgG recycling assay is comparable to the cell affinity binding data in which K_D values of 0.2nM in neutral buffer and 0.22nM in acidic buffer were obtained. In the IgG transcytosis assay, an EC_{50} of 2.4nM and 0.42nM was obtained for CA170_1638.g49 IgG4 and FabFv respectively, demonstrating a slight reduction in potency between the IgG4 and the FabFv. However, the data in this section have clearly shown that CA170_1638.g49 IgG4 and FabFv can inhibit human FcRn function.

Example 7 Cross reactivity of CA170_1638.g49 IgG4 with non-human primate FcRn.

To validate the use of CA170_1638.g49 IgG4 in a non-human primate PK/PD study and pre-clinical toxicology, its relative affinity with cynomolgus macaque FcRn was examined. MDCK II cells stably transfected with cynomolgus macaque FcRn and B2M (MDCKII (Clone 40) was used in a cell based assay, alongside the previously described MDCK II cells stably transfected with human FcRn and B2M (MDCK II clone 15).

Figure 6 shows CA170_1638.g49 IgG4 IgG4 binding on MDCK II clone 40 cells in acidic and neutral pH. Specific binding of antibody to FcRn across a range of antibody concentrations was used to determine K_D. The analyses were performed in both neutral and acidic buffers to determine whether environmental pH comparable to that found in blood plasma (pH7.4) or endosomes (pH6) had any effect on the antibody binding.

Figure 6 shows representative binding curves for CA170_1638.g49 IgG4. The mean K_D values (n = 3) were 0.3 in neutral buffer, and 0.43 in acidic buffer, respectively (see Table 5).

Table 5 - Mean K_D values (nM) for CA170_1638.g49 IgG4 on MDCK II clone 40 cells.

Antibody format	Cyno FcRnpH 7.4	Cyno FcRnpH 6.0
1638 IgG4	0.30	0.43

Example 8A Anti-FcRn treatment enhances the clearance of hIgG *in vivo* in hFcRn transgenic mice

The effect of anti-FcRn molecules (CA170_01519.g57 Fab'PEG (described in WO2014/019727) and CA170_01638.g28 Fab'PEG) on the clearance of human IVIG was determined in human FcRn transgenic mice (B6.Cg-*Fcgrt*^{tm1Dcr} Tg(FCGRT)32Dcr/DcrJ, JAX Mice). Mice were infused intravenously with 500mg/kg human IgG (Human IgI 10% Gamunex-c, Talecris Biotherapeutics). 24 hours later animals were dosed with vehicle control (PBS) or anti-FcRn intravenously as a single dose (100mg/kg). Serial tail tip blood samples were taken at -24, 8, 24, 48, 72, 96, 144 and 192 hours relative to anti-FcRn treatment. Serum levels of human IgG in hFcRn mice were determined by LC-MS/MS. Data presented in Figure 1 are mean ± SEM with 5-6 mice per treatment group. Blocking of hFcRn by each of the anti-FcRn molecules tested resulted in accelerated clearance of hIVIG and lower concentrations of total IgG were observed compared to control mice.

Example 8B. Anti-FcRn treatment enhances the clearance of hIgG in vivo in hFcRn transgenic mice

The anti-human FcRn antibody discovered bound to and inhibited the binding of human IgG to human FcRn, but did not bind or inhibit murine FcRn. Consequently, the effect of anti-FcRn molecules in IgG4P format (1638.g49), Fab'PEG format (1638.g28), and FabFv format on the clearance of human IVIg was determined in human FcRn transgenic mice (B6.Cg-Fcgrttm1Dcr Tg(FCGRT)32Dcr/DcrJ, JAX Mice). Mice were infused intravenously with 500mg/kg human IgG (Human IgI 10% Gamunex-c, Talecris Biotherapeutics). 24 hours later animals were dosed with vehicle control (PBS) or anti-FcRn intravenously as a single dose. Doses, sampling times and replicate numbers were as indicated in the Figures 1a to 1e. Samples were serial tail tip blood samples. Serum levels of human IgG, endogenous mouse albumin and the anti-FcRn molecule itself were determined by LC-MS/MS, with detection and quantification of peptide sequences unique to each of those analytes. Data presented in Figures 1a to 1e are each the Geometric mean and 95% confidence interval.

Blockade of hFcRn by each of the three anti-FcRn molecules tested resulted in clearance of hIVIg that was accelerated compared to that in control mice that were treated with vehicle only, or with a control Fab'PEG (A33, not anti-FcRn, conjugated to 40 kDa PEG, as was 1638

Fab'PEG) – see Figs, 1a and 1b. The effect was dose-related – larger doses gave more prolonged periods during which free anti-FcRn could be detected in serum (Fig. 1 c and 1d), this leading to a more prolonged, and more profound clearance of human IVIg from the mice. The 1638 Fab'PEG showed shorter pharmacokinetics (disappeared more rapidly from free solution in serum) than the control A33 Fab'PEG did, suggesting that the 1638 Fab'PEG had undergone target-mediated disposition – disppearing from free solution by binding to FcRn target. Although mouse IgG did not bind to the human FcRn present in these transgenic mice, endogenous mouse albumin did bind and was recycled by the human FcRn. Although binding of anti-human FcRn to human FcRn did not block binding of albumin to the FcRn in in vitro assay, if such inhibition occurred in vivo, it might have led to accelerated clearance of endogenous mouse albumin. Data are shown in Fig. 1e. Since albumin concentration in serum was somewhat variable (from 16.6 to 59.9 mg/mL in a group of 30 mice, prior to injection of anti-FcRn drug), to allow easier comparison of group results, albumin data were normalised and given as a percentage of the serum albumin concentration at time zero in Fig 1e. A recoverable effect on plasma albumin concentration might have occurred after dosing with Fab'PEG or FabFv formats. Analysis of variance (ANOVA) was carried out for repeated measurements, looking at the treatment differences and the time differences simultaneously. Each measurement of Fab'PEG or FabFv-treated animal compared to the control in the same experiment at the same time point, the controls being irrelevant (non-FcRn-binding) Fab'PEG or vehicle only, respectively. These two formats showed a lowering of albumin concentrations (at 5% level in the ANOVA analysis of data) at around 48 to 72 hours post injection of drug, with levels recovering to pre-dose levels thereafter. The maximum reduction of plasma albumin concentration was about 10% after 100 mg/kg of the Fab'PEG format (at 48 hours), or about 25% after 250 mg/kg FabFv at 144 hours. A similar ANOVA analysis was carried out on data showing the effect of 1638 IgG4P on plasma albumin levels (shown in Fig 1f). There was no significant difference between treated and control animals, suggesting that treatment with the IgG4P format of 1638 did not affect plasma albumin concentration.

Example 9 Crystal Structure and Analysis of 1638.g49 Fab : FcRn complex

The 1638.g49 Fab was co-crystalised with hFcRn alpha chain ECD region (SEQ ID NO: 48) and human beta 2 microglobulin (SEQ ID NO: 72). The proteins were in 50mM Sodium Acetate, 125mM NaCl pH6.0 and a crystallisation conditions were 0.1M Tris pH8.5, 40% PEG400 and 0.2M $LiSO_4.H_2O$ at a protein concentration of 10mg/mL and a drop volume ratio of 0.4µL protein to 0.4µL reservoir in a sitting drop, vapour diffusion experiment. Crystalls were allowed to grow for 8-21 days, followed by harvestingfrom the drop, transfer to well buffer (since it already contained 40% PEG400) and flash-frozen in liquid nitrogen (-180°C) within 10 seconds. X-ray data was collected at SOLEIL, using the oscillation method. The cell dimensions of the crystals were a=101.49 Å, b=210.4 Å, c=101.49 Å; alpha = 90 degrees, beta =90 degrees and

gamma=90 degrees. The space group was determined to be $P2_12_12$. The molecular packing was determined using Phaser, and refinement was carried out with Refmac, using data between 30 and 2.7 Å, to give a final R factor of 21.8% and Rfree of 27.2%. The results are shown below:

The residues interacting with 1638.49 Fab'were all in the FcRn α chain (not β2M) and are indicated below in bold in the FcRn extracellular domain sequence

AESHLSLLYHLTAVSSPAPG TPAFWVSGWL GPQQYLSYNS LRGEAEPCGA WVWENQVSWY WEKETTDLRI KEKLFLEAFK **A*L*GGKGP*Y***TL QGLLGCELGPDNTSVPTAKFA***L*****N**GEEFMNFDLKQGTWGG***D*** **WPEALA**ISQR WQQQDKAANK ELTFLLFSCP HRLREHLERG RGNLEWKEPPSMRLKARPSSPGFSVLTCSA FSFYPPELQL RFLRNGLAAG TGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELESPAKSS (SEQ ID NO: 48).

The residues underlined are those known to be critical for the interaction of human FcRn with the Fc region of human IgG. Those in bold are residues involved in binding the 1638.49 Fab' antibody at 4Å. Residues in italic are those involved in binding the same antibody at 5Å.

The epitope defined by antibody residues closer than 4Å was: A81, G83, G84, K85, G86, P87, N113, E115, W131, P132, E133, L135, A136, Q139.

The epitope defined by antibody residues closer than 5Å wass: A81, G83, G84, K85, G86, P87, N113, E115, W131, P132, E133, L135, A136, Q139, L82, Y88, L112, D130.

Claims:

1. An anti-FcRn antibody or binding fragment thereof comprising (i) a heavy chain or heavy chain fragment having a variable region, wherein said variable region comprises three CDRs, wherein CDR H1 has the sequence given in SEQ ID NO: 1, CDR H2 has the sequence given in SEQ ID NO: 2, and CDR H3 has the sequence given in SEQ ID NO: 3 and (ii) a light chain or fragment thereof having a variable region comprising three CDRs, wherein CDR L1 has the sequence given in SEQ ID NO: 4, CDR L2 has the sequence given in SEQ ID NO: 5 or SEQ ID NO: 7 and CDR L3 has the sequence given in SEQ ID NO: 6.

2. An anti-FcRn antibody or binding fragment thereof according to claim 1 having a heavy chain comprising the sequence given in SEQ ID NO:12 and a light chain comprising the sequence given in SEQ ID NO: 8.

3. An anti-FcRn antibody or binding fragment thereof according to claim 1, wherein the antibody is humanized.

4. An anti-FcRn antibody or binding fragment thereof according to claim 1 having a heavy chain comprising the sequence given in SEQ ID NO: 25 and a light chain comprising the sequence given in SEQ ID NO: 16.

5. An anti-FcRn antibody or binding fragment thereof according to claim 1 having a heavy chain comprising the sequence given in SEQ ID NO: 59 and a light chain comprising the sequence given in SEQ ID NO: 51.

6. An anti-FcRn antibody or binding fragment thereof which binds human FcRn comprising a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 90% identity to the sequence given in SEQ ID NO: 25 and wherein the variable domain of the light chain comprises a sequence having at least 90% identity to the sequence given in SEQ ID NO: 16 and wherein the antibody molecule has the sequence given in SEQ ID NO: 1 for CDR-H1, the sequence given in SEQ ID NO: 2 for CDR-H2, the sequence given in SEQ ID NO: 3 for CDR-H3, the sequence given in SEQ ID NO: 4 for CDR-L1, the sequence given in SEQ ID NO: 5 or SEQ ID NO: 7 for CDR-L2 and the sequence given in SEQ ID NO: 6 for CDR-L3.

7. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 6, wherein the antibody or binding fragment is a scFv, Fv, Fab or Fab' fragment.

8. An anti-FcRn antibody Fab' fragment according to claim 7 having a heavy chain comprising the sequence given in SEQ ID NO: 33 and a light chain comprising the sequence given in SEQ ID NO: 20.

9. An anti-FcRn antibody Fab' fragment according to claim 4 having a heavy chain comprising the sequence given in SEQ ID NO: 63 and a light chain comprising the sequence given in SEQ ID NO: 55.

10. An anti-FcRn antibody Fab fragment according to claim 7 having a heavy chain comprising the sequence given in SEQ ID NO: 29 and a light chain comprising the sequence given in SEQ ID NO: 20.

11. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 10, wherein the antibody or binding fragment is conjugated to a polymer for example selected from starch, albumin and polyethylene glycol.

12. An anti-FcRn antibody or binding fragment thereof according to claim 11, wherein the polymer is PEG, for example with a molecular weight in the range 5 to 50kDa.

13. An anti-FcRn antibody according to any one of claims 1 to 6, wherein the antibody is a full length antibody.

14. An anti-FcRn antibody according to claim 13, wherein the full length antibody is selected from the group consisting of an IgG1, IgG4 and IgG4P.

15. An anti-FcRn antibody according to claim 4, claim 13 or claim 14 having a heavy chain comprising the sequence given in SEQ ID NO: 37, SEQ ID NO:39 or SEQ ID NO:73 and a light chain comprising the sequence given in SEQ ID NO: 20.

16. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 6 wherein the antibody or binding fragment thereof is a Fab-dsFv having a heavy chain comprising the sequence given in SEQ ID NO: 42 and a light chain comprising the sequence given in SEQ ID NO: 40.

17. A humanised anti-FcRn antibody or binding fragment thereof which binds an epitope of human FcRn which comprises residues A81, G83, G84, K85, G86, P87, N113, E115, W131, P132, E133, L135, A136 and Q139 of human FcRn extracellular domain (SEQ ID NO: 48).

18. The humanised anti-FcRn antibody or binding fragment thereof according to claim 17, which binds an epitope which comprises residues A81, G83, G84, K85, G86, P87, N113, E115, W131, P132, E133, L135, A136, Q139, L82, Y88, L112 and D130 of human FcRn extracellular domain (SEQ ID NO: 48).

19. An anti-FcRn antibody or binding fragment thereof according to claim 17 or 18 which further binds one or more residues selected from the group consisting of L82, Y88, L112 and D130.

20. An anti-FcRn antibody or binding fragment thereof according to any one of claims 17 to 19, which has a binding affinity for human FcRn of 150pM or less at both pH 6 and at pH 7.4.

21. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 20, which blocks binding of human IgG to human FcRn.

22. An anti-FcRn antibody or binding fragment thereof according to any one of claims 1 to 21 which does not bind human β2 microglobulin (SEQ ID NO:72).

23. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody according to any one of claims 1 to 16.

24. A cloning or expression vector comprising one or more DNA sequences according to claim 23.

25. A vector according to claim 24 wherein the vector comprises (i) the sequence given in SEQ ID NO: 30, 32, 34 or 36 and the sequence given in SEQ ID NO: 21 or 24 or (ii) the sequence given in SEQ ID NO: 38 and the sequence given in SEQ ID NO: 22, or (iii) the sequence given in SEQ ID NO: 74 and the sequence given in SEQ ID NO: 22 or (iv) the sequence given in SEQ ID NO: 41 and the sequence given in SEQ ID NO: 43.

26. A host cell comprising one or more cloning or expression vectors according to claim 24 or claim 25.

27. A process for the production of an antibody having binding specificity for human FcRn, comprising culturing the host cell of claim 26 and isolating the antibody.

28. A pharmaceutical composition comprising an anti-FcRn antibody or binding fragment thereof as defined in any one of claims 1 to 22 in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

29. A pharmaceutical composition according to claim 28 comprising other active ingredients.

30. An antibody or binding fragment thereof as defined one in any one of claims 1 to 22 or a composition as defined in claim 28 or 29 for use in therapy.

31. An antibody or binding fragment thereof as defined in any one of claims 1 to 22 or a composition as defined in claim 28 or 29, for use in the treatment of an autoimmune disease, such as myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, idiopathic thrombocytopenic purpura and thrombotic thrombocytopenic purpura.

32. Use of an antibody or binding fragment thereof as defined in any one of claim 1 to 22 or a composition as defined in claim 28 or claim 29 in the manufacture of a medicament for the treatment of an autoimmune disease, such as myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, idiopathic thrombocytopenic purpura and thrombotic thrombocytopenic purpura.