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(54) Title: MODULATION OF IgG BINDING TO FcRn

(57) Abstract

Disclosed are mutant IgG molecules having altered amino acid sequences in the FcRn–binding region. These changes confere increased or decreased affinity for FcRn and thus, respectively, a decreased or increased rate of clearance from the systemic circulation. Such molecules can be attached to detectable labels or cytotoxic moieties for imaging tissues or for delivering cytotoxins. Also disclosed is a method for identifying IgG molecules with altered half–lives in circulation by contacting the molecules with FcRn.

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MODULATION OF IgG BINDING TO FcRn

Background of the Invention

The field of the invention is immunoglobulins.

Immunoglobulin G (IgG) is used intravenously to treat a number of diseases that involve immune deficiencies, including acquired immune deficiency syndrome (AIDS), idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, Guillaine-Barre Syndrome, and dermatomyositis. Recently, there has also been increasing use in immunosuppression in transplanted patients, and in specifically directed antibody therapy, such as monoclonal antibodies used as a form of cancer chemotherapy.

Previously, immunoglobulins were extracted from pooled whole blood, but the increasing risk of infection from AIDS and the related decrease in the available blood supply precipitated increased reliance on murine monoclonal antibodies for *in vivo* human therapy. Initially, antibodies from such sources had problems with short half-life and inciting an immune response against the murine proteins (the HAMA response), but both of these problems have been alleviated somewhat by the practice of humanizing the antibodies by combining the human constant region with the mouse variable region.

Although monoclonal antibodies now have a half-life similar to that of native human IgG, there are still situations where it would be desirable to have even more control over the length of time before immunoglobulins are catabolized.

This is especially true in treating immune deficiency conditions. When the concentration of IgG is increased above normal levels in the circulation, its half-life decreases. Therefore it is difficult to maintain higher-than-normal levels of the immunoglobulins during treatment, and patients require frequent

injections of the antibodies. On the other hand, in situations where the antibodies are being used as a means to target a chemotherapeutic agent, such as a radionuclide or a protein toxin, to a particular tissue or cell type, the dosage is often limited by the risk of damage to the bone marrow and other normal tissues due to non-specific binding. In such cases, a shorter-than-normal half-life would be desirable.

Summary of the Invention

The invention is based on the discovery that FcRn, in binding to IgG, sequesters it and protects it from degradation. FcRn is a receptor found on the intestinal surface of the neonate, and is responsible for the shuttling of maternal milk IgG from the intestinal lumen through the intestinal epithelial cell into the systemic circulation. It is now known also to be responsible for preventing IgG from being cleared from the animal's circulation. Using the guidance provided herein, one can create, using recombinant methods, an IgG molecule that has one or more amino acid additions, deletions, or substitutions (conservative or nonconservative) in the region that binds to FcRn, thereby either increasing or decreasing the molecule's affinity for FcRn. An increase in affinity would translate into the altered IgG's having a longer half-life *in vivo* than native IgG, while a decrease in affinity for FcRn would have the opposite effect.

Although monoclonal antibodies now have a half-life similar to that of native human IgG, there are still situations where it would be desirable to have even more control over the length of time before immunoglobulins are catabolized.

This is especially true in treating immune deficiency conditions. When the concentration of IgG is

increased above normal levels in the circulation, its half-life decreases. Therefore it is difficult to maintain higher-than-normal levels of the immunoglobulins during treatment, and patients require frequent injections of the antibodies. On the other hand, in situations where the antibodies are being used as a means to target a chemotherapeutic agent, such as a radionuclide or a protein toxin, to a particular tissue or cell type, the dosage is often limited by the risk of damage to the bone marrow and other normal tissues due to non-specific binding. In such cases, a shorter-than-normal half-life would be desirable.

An IgG with an increased half-life *in vivo* would be useful in treating conditions such as acquired immune deficiency syndrome (AIDS) or idiopathic thrombocytopenic purpura (ITP), where maintaining a higher-than-normal concentration of circulating IgG is desirable. A mutant IgG molecule that binds less strongly to FcRn and is therefore cleared more rapidly would be of benefit where the IgG is being used for chemotherapy or as a tumor imaging agent.

The mutated IgG of the invention would have amino acid substitutions in the FcRn-binding region only, resulting in altered half-life only, with no substantial change in overall immune function.

Also claimed is a method of removing IgG from the blood of an animal by administration of soluble FcRn, which would complex with the circulating antibody and prevent it from being sequestered by cellular FcRn. This method would be especially useful in chemotherapies, to control the ratios of tumor-bound to circulating antibodies.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art

to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 is a graph illustrating clearance of intravenously injected ^{125}I-immunoglobulins in mice with and without β2-microglobulin. Approximately 1 x 10^7 counts per minute of labeled IgG was injected into the external jugular vein. Blood was collected at the time points indicated. Percent initial radioactivity = counts per minute per milligram blood at indicated time point x 100 per counts per minute per milligram blood at t_0 = 1 minute after injection. Mouse IgG1 is represented by the solid line, chicken IgY by a dashed line, β2m+/+ by circles, β2m+/- by triangles, and β2m-/- by diamonds; n = 5 for each group.

Figure 2 is a diagram illustrating partial amino acid sequences of IgG molecules used in this study (EU numbering). Alternative amino acid residues within an isotype are shown below the most common sequence. Sequences are from Kabat et al., 1991, Sequences of proteins of immunological interest, 5th edn., pg. 683,

NIH, Bethesda, MD. Predicted contacts with FcRn (from Burmeister et al., 1994, Nature 372:379) are underlined. Residues for binding to FcRn (Kim et al., 1994, Eur. J. Immunol. 24:2429; Raghavan et al., 1995, Biochemistry 34:14649) are marked with an asterisk.

Figure 3 is an illustration depicting the proposed role for FcRn in protecting IgG from degradation. IgG taken up in the fluid phase binds FcRn at acidic pH in early endosomes. IgG bound to FcRn is recycled to the plasma membrane and released, whereas unbound proteins are sorted to lysosomes and degraded.

Detailed Description

The altered IgG's of the invention are most readily prepared by standard recombinant DNA methods, e.g. site-directed mutagenesis or PCR. The regions of IgG to be mutated corresponds to amino acids 248 through 257, 308 through 314, and 429 through 436 of IgG. Within these regions, five particular amino acid residues have been identified as being important in FcRn binding, but others can be explored as well by using DNA primer-based site-directed mutagenesis, available commercially as a kit (Amersham, Arlington Heights, Illinois), and well known in the art. The Amersham kit can be used according to the manufacturer's instructions in order to mutate specific residues within the FcRn-binding region.

Preliminary *in vitro* comparison of the binding of non-native IgG molecules relative to native IgG is done by radiolabelling the IgG molecules, incubating them with cells which express FcRn, washing the cells, and then measuring the amount of radioactivity that remains in association with the cells. Suitable cells include endothelial cells, cells (such as human embryonic kidney cell line 293) transfected with a vector encoding FcRn,

or intestinal epithelial cells from suckling rats or mice. Alternatively, one could use a brush border fraction derived from such intestinal epithelial cells, prepared in accordance with standard methods (e.g., Wallace and Rees, 1980, Biochem. J. 188:9).

The mutant IgG antibodies can be tested for *in vivo* half life by radiolabelling with $Na^{125}I$ (DuPont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine is removed by gel filtration on Sephadex G-25 and aggregated immunoglobulins are removed by gel filtration on Sephadex G-200. The ^{125}I-immunoglobulin can then be diluted in 10% normal mouse serum to an injection concentration of 1 x 10^7 cpm / 150 μl. Anesthetized mice can be injected in the jugular vein with 150 μl of the radiolabelled immunoglobulin, and subsequently bled with capillary tubes from the retro-orbital sinus at serial time points following injection. Plasma would be collected by centrifugation and total radioactivity measured in a gamma counter and expressed as cpm/mg blood. The percentage radioactivity remaining in the blood after the last bleed would be calculated relative to the value 1 minute after injection. Protein-bound radioactivity would be measured by precipitation of the plasma in 10% TCA. The clearance curves for the various radiolabelled antibodies can then be plotted, revealing which produce antibodies with the desired characteristics. To test whether immune function is preserved, immunoprecipitation or ELISA assays can be used to ensure that the antibody does in fact complex with the antigen of interest.

For administration to human patients, the mutated immunoglobulins of the invention can be humanized by methods known in the art, e.g., monoclonal antibodies can be commercially humanized (Scotgen, Scotland; Oxford Molecular, Palo Alto, CA). The antibodies can then be

purified using known methods, such as absorption onto immobilized Protein A or immunoaffinity chromatography. Following purification, the antibodies of the invention can be administered to patients in a pharmaceutically acceptable excipient such as physiological saline. The antibodies of the present invention can be administered by any standard route including intravenously, intraperitoneally, intramuscularly, or subcutaneously. It is expected that the preferred route of administration will be intravenous.

As is well known in the medical arts, dosages for any one patient depends on many factors, including the patients general health, sex, size, body surface area, age, as well as the particular compound to be administered, time and route of administration, and other drugs being administered concurrently. Determination of correct dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology.

The invention also includes screening methods for identifying IgG molecules with altered circulating half-lives and binding affinities relative to native IgG.

Example 1: Increased clearing in mice that lack β2-microglobulin.

Animals. Mice heterozygous for the β2m gene disruption (Zijlstra et al., 1990, Nature 344:742) were mated and allowed to deliver. Tail tissue was taken from the pups for genomic DNA preparation to determine their β2m genotypes (Israel. et al., 1995, J. Immunol. 154:6246). The clearance experiments were done at 8 weeks of age. The mice were given 0.01% NaI in their drinking water one day prior to injection and throughout the period of monitoring clearance of the ^{125}I-immunoglobulins.

Preparation of ^{125}I-immunoglobulin for injection: Mouse IgG1, IgG2a, IgG2b and IgG3 (Cappel, Durham, N.C.) and chicken IgY (Cappel) were labeled with Na^{125}I (Dupont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine was removed by gel filtration on Sephadex G-25 and aggregated immunoglobulins were removed by gel filtration on Sephadex G-200. Precipitation in 10% trichloroacetic acid (TCA) showed that at least 90% of the ^{125}I in preparations of IgG1, IgG3, and IgY was bound to protein, and at least 80% of the radiolabel in IgG2a and IgG2b was bound to protein. The ^{125}I-immunoglobulin was diluted in 10% normal mouse serum to achieve approximately 1 x 10^7 cpm/150 μl for injection.

Clearance experiments: Under pentobarbitol anesthesia (65 μg/g body weight), the external jugular veins of β2m-/-, β2m+/-, and β2m+/+ mice were exposed and injected with approximately 150 μl of the ^{125}I-immunoglobulin diluted in 10% normal mouse serum. The mice were bled with capillary tubes from the retro-orbital sinus at serial time points following injection, under light isotharine anesthesia. Plasma was collected by centrifugation and total radioactivity was measured in a gamma counter and expressed as cpm/mg blood. After the last bleed, the animals were killed with CO_2. The percentage radioactivity remaining in blood was calculated relative to the value 1 min. after injection. Protein-bound radioactivity was measured by precipitation of the plasma in 10% TCA. This protocol has been approved by the Institutional Animal Care and Use Committee at Brandeis University.

Pharmacokinetic data analysis: The data from each animal were fitted to a double exponential model,

$$C = e^{b-k2t} + e^{a-(k1+k2)t},$$

by a non-linear least squares method with a multiplicative error structure. The parameters k1 and k2 were constrained to be positive. The area under the curve at infinity (AUC∞), mean residence time (MRT), terminal elimination half life ($t_{1/2}$), and the phase I half life were then calculated from a, b, k1, and k2. Means and s.e.m.s for these parameters were calculated for each genotype group. Differences between the groups were tested with an analysis of variance using Gabriel's procedure to make pairwise comparisons (Gabriel, 1978, J. Amer. Statistical Assoc. 73:724).

Results: The clearance curves for radiolabeled mouse IgG1 and chicken IgY are shown in Figure 1, which is a graph showing the clearance of intravenously injected ^{125}I-labelled immunoglobulins in mice with and without β2-microglobulin. The curves are biphasic, with phase 1 representing equilibration between the intravascular and extravascular compartments and phase 2 representing the elimination of the protein from the intravascular space. The pharmacokinetic parameters are shown in Table 1. The phase 1 half lives did not differ significantly for IgG1 and IgY, or between the three β2m genotypes. However, there were significant differences in the phase 2 (terminal) half lives ($t_{1/2\beta}$). Mouse IgG1 was degraded more rapidly in β2m-/- mice than in β2m+/- or β2m+/+ littermates. Specifically, the half life was 25 hours in the β2m-/- animals compared to 5 days for the β2m+/+ mice (Fig. 1). There was no significant difference between the clearance in heterozygous ($t_{1/2}$ = 123 h) and wild type mice, suggesting that the effect on IgG catabolism was not related to the dose of the β2m gene. The radioactivity left in the serum was 95-98% precipitable by TCA at all time points (data not shown), suggesting the radioactivity measured was indeed bound to IgG. There was little release of TCA-soluble

radioactivity into the systemic circulation during the time period studied.

Radiolabeled chicken IgY was cleared equally rapidly in wild type mice and in mice homozygous for the disruption in the β2m gene (Figure 1). The terminal half life of IgY (21-22 hours) was not significantly different than that of mouse IgG1 I in β2m-/- mice (Table 1).

Table 1.Pharmacokinetic parameters of ^{125}I-IgG clearance.

β2m genotype (n)	Ig isotype	AUC∞[a] x 10^7	MRT[b] (hours)	Phase I half life (hours)	$t_{1/2}$[c] (hours)
+/+ (2)	mIgG1[d]	35 (8)[f]	167 (6)	3.1 (0.1)	120 (3)
+/- (4)	mIgG1	33 (3)	168 (14)	6.7 (1.7)	123 (10)
-/- (4)	mIgG1	8.3 (0.9)	29.7(3.6)	5.5 (0.4)	25 (3.6)
+/+ (3)	cIgY[e]	3.7 (0.2)	15.7(0.8)	5.4 (0.6)	22 (1.2)
-/- (3)	cIgY	3.8 (0.1)	18.7(3.0)	4.6 (1.2)	21 (0.5)

a = Area under the curve
b = Mean residence time
c = Terminal half life
d = Mouse IgG1
e = Chicken IgY
f = Mean (s.e.m.)

Pharmacokinetic parameters for the clearance of mouse IgG1, IgG2a, IgG2b and IgG3 compared in a separate experiment in β2m-/-, +/- and +/+ mice are shown in Table 2. The half lives of IgG1, IgG2a and IgG3 were significantly lower in β2m-/- mice than in β2m+/- or +/+ siblings. The half lives of IgG2b in mice of the three β2m genotypes were not significantly different, although degradation appeared degradation appeared more rapid in β2m-/- mice.

Table 2. Pharmacokinetic parameters of mouse ^{125}I-IgG subclass clearance.

Ig Isotype	β2m genotype (n)	AUC∞[a] x 10^7	MRT[b] (hours)	Phase I half life (hours)	$t_{1/2}$[c] (hours)
IgG1	+/+, +/1 (3)	10.7(2.0)[d]	114 (38)	2.7 (0.4)	84 (27)
IgG1	-/- (3)	3.3 (0.4)	31 (4)	3.8 (2.2)	25 (1.4)
IgG2a	+/+, +/- (3)	2.1 (0.4)	29 (2)	2.2 (0.3)	33 (1.3)
IgG2a	-/- (3)	1.7 (0.1)	16 (1)	2.0 (0.2)	21 (0.6)
IgG2b	+/+, +/- (3)	1.7 (0.04)	35 (1)	1.6 (0.1)	42 (1.9)
IgG2b	-/- (3)	1.1 (0.2)	12 (1)	2.3 (0.8)	33 (10)
IgG3	+/+, +/- (3)	8.4 (0.5)	97 (9)	2.7 (0.2)	76 (7.0)
IgG3	-/- (2)	2.5 (0.3)	16 (2)	4.4 (1.8)	24 (7.0)

a = Area under the curve
b = Mean residence time
c = Terminal half life
d = Mean (s.e.m.)

Example 2: Generation and Screening of IgG mutants.

Five amino acid residues (Ile 253, His 310, Gln 311, His 433, and Asn 434) in the conserved portion of IgG have been identified as being important in FcRn binding. These are shown in Figure 2, which is a diagram showing alignment of partial amino acid sequences of immunoglobulin molecules used in Example 1 (EU numbering). Alternative amino acid residues within an isotype are shown below the most common sequence (Kabat et al., 1991, Sequences of proteins of immunological interest, 5th edn., pp. 683, 692, NIH, Bethesda, MD). Predicted contacts (Burmeister et al., 1994, Nature:372:379) with FcRn are underlined. Residues required for the protection of IgG from rapid degradation and for binding to FcRn (Kim et al., 1994, Eur. J. Immunol. 24:2429; Raghavan et al., 1995, Biochemistry 34:14649) are marked with an asterisk. Other amino acids can be identified using known methods.

To create a mutant IgG molecule, a cDNA encoding the expressed IgG Fc-binding fragment is cloned from IgG hybridoma cells. Site-directed mutagenesis is used to

substitute specific amino acid residues in positions 248 through 257, 308 through 314, and 429 through 436, using a commercially-available DNA primer-based site-directed mutagenesis kit (Amersham, Arlington Heights, Illinois), according to the manufacturer's instructions.

The binding affinity of the mutant IgG antibodies to FcRn can be tested by immobilizing FcRn on a solid substrate e.g., a Sepharose™ bead, by standard methods. An anti-IgG monoclonal antibody (of other than IgG isotype) is labelled with ^{125}I (Dupont, Wilmington, DE) using the Iodogen™ method (Pierce Biochemical, Rockford, IL). Free iodine is removed by gel filtration on Sephadex G-200. The immobilized FcRn is contacted with 0.5 μg/ml of the test IgG plus the ^{125}I-labeled antibody (e.g. for 16-18 hours at 37°C), then washed. The amount of radioactivity remaining associated with the immobilized FcRn is measured, and the binding affinity calculated using well known methods. Alternatively, binding affinity may be evaluated using an ELISA assay known in the art.

Further testing in a cell-based system may also be carried out. Confluent layers of FcRn-expressing cells (e.g., cells transfected with a vector encoding FcRn) are incubated with 0.4 μ/ml ^{125}I-labeled native or mutant IgG to allow IgG-FcRn binding (e.g., overnight 16-18 hours at 37°C), and washed with medium (complete RPMI, 10% FCS; Gibco, Grand Island, NY). Cells are then detached by incubation with 5 mM Na_2EDTA in 50 mM phosphate buffer (pH 7.5) for 5 minutes. The cells are pelleted and resuspended in 2 ml 2.5 mg/ml CHAPS, 0.1 M Tris-HCl (pH 8.0), 0.3 mg/ml PMSF, 25 μg/ml pepstatin and 0.1 mg/ml aprotinin and incubated for 30 minutes at room temperature. The suspension is centrifuged at 12,000 x g for 30 minutes and the amount of radioactivity in pellets and supernatants determined as an indication of the level

of binding of the test IgG to FcRn. Alternatively, the test IgG can be unlabelled, and its binding detected with a labelled anti-IgG antibody as described above.

Final studies on *in vivo* clearance of the mutant IgG molecules relative to the native IgG are conducted as described in Example 1.

Example 3: Therapeutic use of IgG with increased or decreased rate of clearance.

For administration to human patients, mutant IgG molecules can be humanized by methods known in the art, e.g, monoclonal antibodies can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Mutant IgG molecules can be purified using known methods, such as absorption onto immobilized Protein A or immunoaffinity chromatography. Following purification, the mutant IgG molecules of the invention or immunologically active fragments thereof can be administered to patients in a pharmaceutically acceptable excipient such as physiological saline. The mutant IgG molecules or other compounds of the invention, e.g., mutant IgG molecules linked to therapeutic agents such as cytotoxic moieties (e.g., radionuclides or toxic polypeptides such as ricin, *Pseudomonas* exotoxin A, and diphtheria toxin), can be administered by any standard route including intraperitoneally, intramuscularly, subcutaneously, or intravenously. It is expected that the preferred route of administration will be intravenous. These compounds can be administered systemically to the bloodstream.

As is well known in the medical arts, dosages for any one patient depends on many factors, including the patients general health, sex, size, body surface area, age, as well as the particular compound to be administered, time and route of administration, and other

drugs being administered concurrently. Dosages for compounds of the invention will vary, but a preferred dosage for intravenous administration is approximately 1 μg to 500 μg/ml/blood volume. Determination of correct dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology. The optimal dosage may be adjusted according to the condition of the patient and response of the patient to therapy.

Where the IgG carries a cytotoxic moiety, as for cancer chemotherapy, rapid clearance would be desired and so mutant IgGs with reduced FcRn binding are chosen. For therapeutic use of nontoxic IgG molecules, a decreased clearance rate is desired, and so mutant IgG molecules with increased affinity for FcRn are selected.

Example 4: Diagnostic use of IgG with increased rate of clearance. Labelled IgG molecules of the invention can be used diagnostically. In these situations, increased clearance of IgG is desirable so that nonspecifically bound labelled antibody is quickly removed from the body, thereby reducing background. Hybridoma strains producing IgG molecules specific for target tissue, e.g., cancer cells, are used, and the IgG molecules are mutated as described in Example 2. Testing for clearance is also conducted as in Example 2. IgG molecules with decreased binding to FcRn, compared to wild type molecules, are chosen.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the

invention, which is defined by the scope of the appended claims.

Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

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(ii) TITLE OF THE INVENTION: MODULATION OF IgG BINDING TO FcRn

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(v) COMPUTER READABLE FORM:
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(D) SOFTWARE: FastSEQ Version 2.0

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Lys Asp Val Leu Thr Ile Thr Leu Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ile Met His Gln Asp Trp Leu
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Glu Gly Leu His Asn His His
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Lys Asp Val Leu Met Ile Ser Leu Ser Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ile Gln His Gln Asp Trp Met
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Glu Gly Leu His Asn His Leu
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Asp Val Leu Met Ile Ser Leu Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ile Gln His Gln Asp Trp Met
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

His Glu Gly Leu Lys Asn Tyr Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Asp Ala Leu Met Ile Ser Leu Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Gln His Gln Asp Trp Met
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Glu Ala Leu His Asn His His
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val Ser Thr Gln Asp Trp Leu
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Glu Ala Leu Pro Met Arg Phe
1 5

What is claimed is:

1. A non-naturally occurring IgG molecule having an altered amino acid sequence compared to native IgG, and which binds to FcRn with increased affinity relative to native IgG, the alteration comprising an addition or deletion of an amino acid within the FcRn-binding region of native IgG, or a substitution of an amino acid within said region.

2. A non-naturally occurring IgG molecule having an altered amino acid sequence compared to native IgG, and which binds to FcRn with decreased affinity relative to native IgG, the alteration comprising an addition or deletion of an amino acid within the FcRn-binding region of native IgG, or a substitution of an amino acid within said region.

3. The IgG molecule of claim 1, in which at least one of the following amino acids conserved in native IgG is substituted with another amino acid: Ile 253, His 310, Gln 311, His 433, Asn 434.

4. The IgG molecule of claim 2, in which at least one of the following amino acids conserved in native IgG is substituted with another amino acid: Ile 253, His 310, Gln 311, His 433, Asn 434.

5. A purified preparation of the IgG of claim 1.

6. A purified preparation of the IgG of claim 2.

7. A method of therapy comprising identifying an animal having a condition treatable with IgG and administering a therapeutically effective amount of the molecule of claim 1 to the animal.

8. A method of therapy comprising identifying an animal having a condition treatable with IgG and administering a therapeutically effective amount of the molecule of claim 2 to the animal.

9. The method of claim 7, wherein said condition is selected from the group consisting of idiopathic thrombocytopenic purpura (ITP), Kawasaki disease, acquired immunodeficiency syndrome (AIDS), Guillain-Barre Syndrome, and dermatomyositis.

10. A composition comprising the molecule of claim 2 complexed to a cytotoxic moiety.

11. The composition of claim 10, wherein the cytotoxic moiety is a toxic polypeptide or a radionuclide.

12. A composition comprising the molecule of claim 2, wherein the molecule comprises a cytotoxic radionuclide.

13. A therapeutic method comprising indentifying an animal in need of treatment with an immunotoxin, and administering a therapeutically effective amount of the molecule of claim 10 to the animal.

14. A method of imaging a particular tissue in an animal, comprising

(a) providing the IgG molecule of claim 2, wherein the IgG molecule binds preferentially to said tissue and is detectably labelled; and

(b) administering the IgG molecule to the animal in an amount effective to permit the tissue to be imaged.

15. A method for identifying an IgG molecule with an altered half-life in circulation relative to native IgG, comprising

(a) contacting a molecule of FcRn with a candidate non-native IgG *in vitro*, and

(b) determining whether the candidate non-native IgG binds to FcRn with an affinity higher or lower than that of native IgG, wherein an affinity higher than that of native IgG indicates that the candidate non-native IgG has a half life in circulation greater than that of native IgG, and an affinity lower than that of native IgG indicates that the candidate non-native IgG has a half life in circulation shorter than that of native IgG.

16. The method of claim 15, wherein the FcRn is soluble FcRn.

17. The method of claim 15, wherein the FcRn is bound to a cell.

18. A method of increasing the rate of clearance of circulating IgG, comprising

(a) identifying an animal in need of an increased rate of clearance of circulating IgG, and

(b) administering soluble FcRn to the animal in an amount sufficient to increase the rate of clearance of circulating IgG.

19. The method of claim 18, comprising administering IgG to the animal concurrently with the soluble FcRn.

20. The method of claim 18, wherein the circulating IgG comprises IgG complexed with a cytotoxic moiety.

21. The method of claim 18, wherein the circulating IgG is a tumor imaging agent.

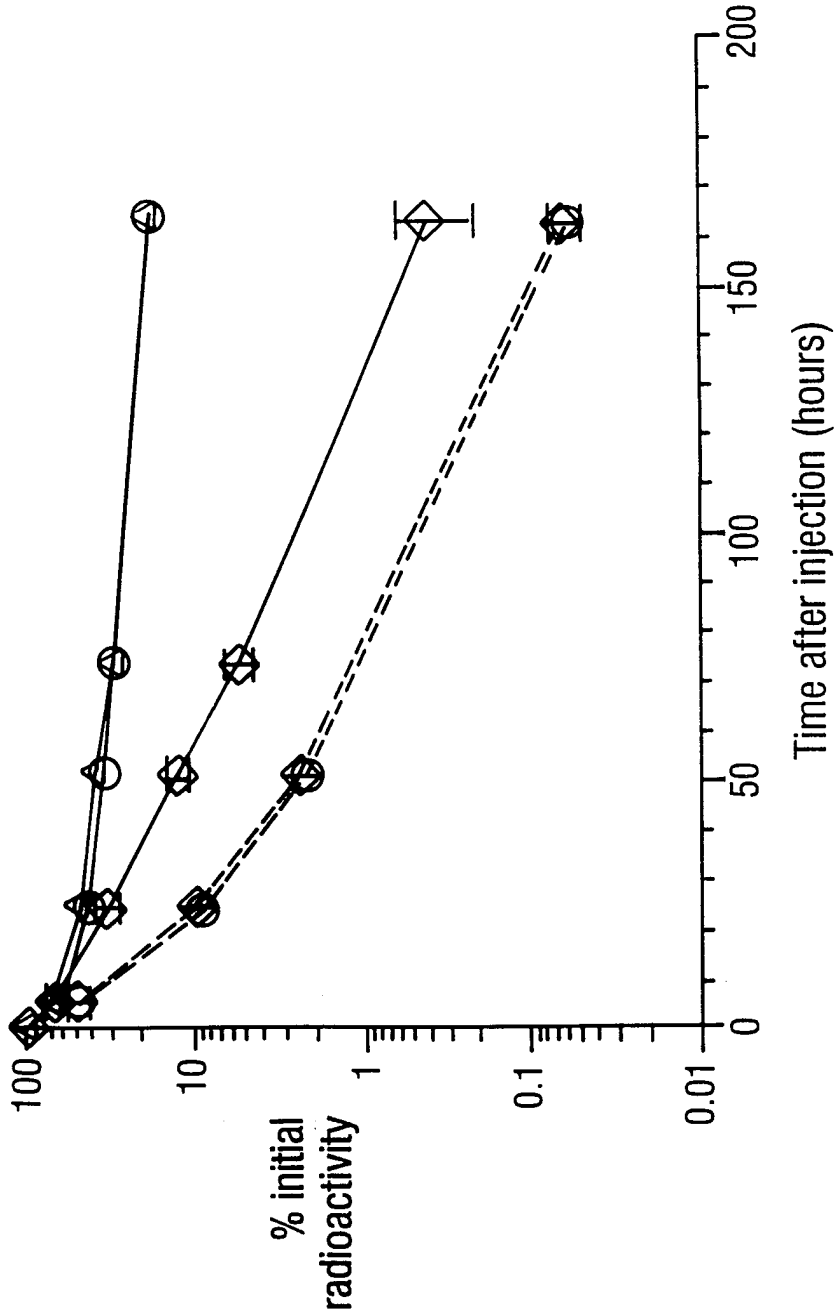
100
10
1
0.1
0.01
% initial radioactivity
0
50
100
150
200
Time after injection (hours)

FIG. 1

```
          248                    *                    257
mIgG1     Lys Asp Val Leu Thr Ile Thr Leu Thr Pro  (SEQ ID NO: 1)
                  Thr      Leu      Val

          308       *   *          314
mIgG1     Ile Met His Gln Asp Trp Leu              (SEQ ID NO: 2)

          429               *    *       436
mIgG1     His Glu Gly Leu His Asn His His          (SEQ ID NO: 3)

          248                                      257
mIgG2a    Lys Asp Val Leu Met Ile Ser Leu Ser Pro  (SEQ ID NO: 4)
              Asn

          308                    314
mIgG2a    Ile Gln His Gln Asp Trp Met              (SEQ ID NO: 5)

          429                         436
mIgG2a    His Glu Gly Leu His Asn His Leu           (SEQ ID NO: 6)
                  Val                 His

          248                                      257
mIgG2b    Lys Asp Val Leu Met Ile Ser Leu Thr Pro  (SEQ ID NO: 7)
                                             Ser

          308                    314
mIgG2b    Ile Gln His Gln Asp Trp Met              (SEQ ID NO: 8)

          429                         436
mIgG2b    His Glu Gly Leu Lys Asn Tyr Tyr           (SEQ ID NO: 9)

          248                                      257
mIgG3     Lys Asp Ala Leu Met Ile Ser Leu Thr Pro  (SEQ ID NO: 10)

          308                    314
mIgG3     Ile Gln His Gln Asp Trp Met              (SEQ ID NO: 11)

          429                         436
mIgG3     His Glu Ala Leu His Asn His His          (SEQ ID NO: 12)
```

FIG. 2A

	248 257	
cIgY	Pro Gly Glu Leu Tyr Ile Ser Leu Asp Ala	(SEQ ID NO: 13)
	308 314	
cIgY	Val Ser Thr Gln Asp Trp Leu	(SEQ ID NO: 14)
	429 436	
cIgY	His Glu Ala Leu Pro Met Arg Phe	(SEQ ID NO: 15)

FIG. 2B

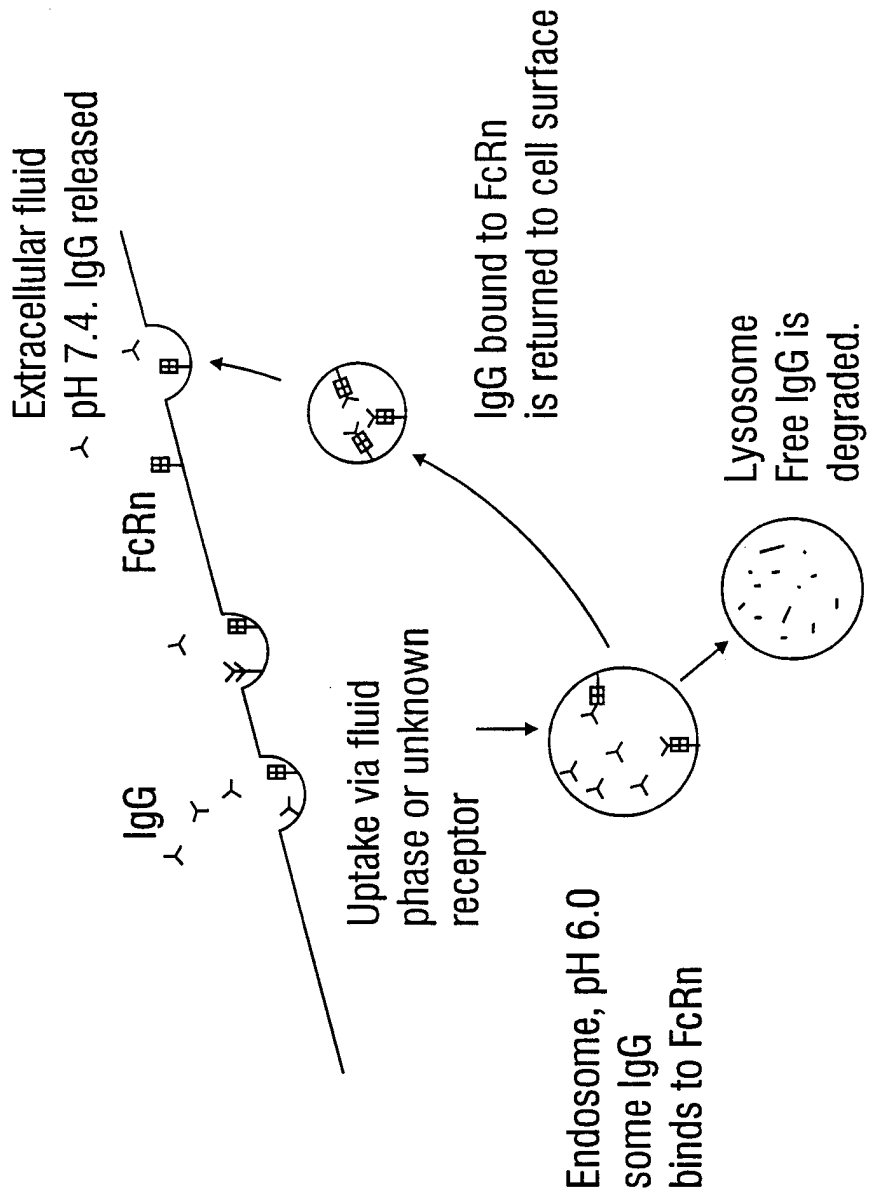
Extracellular fluid
pH 7.4. IgG released
FcRn
IgG
Uptake via fluid
phase or unknown
receptor
IgG bound to FcRn
is returned to cell surface
Endosome, pH 6.0
some IgG
binds to FcRn
Lysosome
Free IgG is
degraded.

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21437

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C07K 16/00

US CL :424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 143.1; 530/387.1, 387.3, 388.1, 388.22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Examiner's Antibody files.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG (file=medline), key words: antibod? FcRn, neonate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	US 5,623,053 A (GASTINEL et al.) 22 April 1997, see entire document.	1-21
X --- Y	RAGHAVAN, M. et al., Analysis of the pH dependence of the neonatal Fc receptor/Immunoglobulin G interaction using antibody and receptor variants, Biochemistry 1995, Vol. 34, pages 14649-14657, see entire document.	1-2 ------- 1-21
Y	SIMISTER, N. E. et al., An Fc receptro structurally-related to MHC Class I antigens, Nature, 12 January 1989, Vol. 337, pages 184-187, see entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 MARCH 1998	Date of mailing of the international search report 06 APR 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THOMAS CUNNINGHAM Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21437

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STORY, C. M. et al., A major histocompatibility complex Class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus, J. Exp. Med., December, 1994, Vol. 180, pages 2377-2381, see entire document.	1-21