Improved tumor imaging and therapy via i.v. IgG–mediated time-sequential modulation of neonatal Fc receptor

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The long plasma half-life of IgG, while allowing for enhanced tumor uptake of tumor-targeted IgG conjugates, also results in increased background activity and normal-tissue toxicity. Therefore, successful therapeutic uses of conjugated antibodies have been limited to the highly sensitive and readily accessible hematopoietic tumors. We report a therapeutic strategy to beneficially alter the pharmacokinetics of IgG antibodies via pharmacological inhibition of the neonatal Fc receptor (FcRn) using high-dose IgG therapy. IgG-treated mice displayed enhanced blood and whole-body clearance of radioactivity, resulting in better tumor-to-blood image contrast and protection of normal tissue from radiation. Tumor uptake and the resultant therapeutic response was unaltered. Furthermore, we demonstrated the use of this approach for imaging of tumors in humans and discuss its potential applications in cancer imaging and therapy. The ability to reduce the serum persistence of conjugated IgG antibodies after their infusion can enhance their therapeutic index, resulting in improved therapeutic and diagnostic efficacy.

Introduction
Radiolabeled and toxin-conjugated monoclonal antibodies are becoming increasingly important for cancer imaging and therapy (1, 2). To date, 2 radiolabeled antibodies for the treatment of non-Hodgkin lymphoma, 2 radiometal-labeled antibodies for imaging of colon and prostate cancer, and 1 drug-conjugated monoclonal antibody have been approved by the FDA. The blood half-life of intact IgG in humans is exceptionally long (23 days; refs. 3, 4). While this long serum persistence may allow for greater tumor targeting, it results in increased background activity and normal-tissue toxicity. As a consequence of these unfavorable pharmacokinetics, successful therapeutic uses of the approved conjugated antibodies have been exclusively limited to the highly sensitive and readily accessible hematopoietic tumors.

The neonatal Fc receptor (FcRn), a MHC class I–like protein highly expressed in neonatal rodent intestinal epithelium, human placental syncytiotrophoblasts, vascular endothelial cells, and hepatocytes, plays a central role in perinatal IgG transfer and protection of IgG from catabolism in adults (5–12). Genetic mutation or deletion of β2 microglobulin, a component of the FcRn heterodimer, has been shown to result in reduced serum IgG concentration both in mice and in humans (13–16). The hypothesized mechanism of IgG protection by FcRn involves nonspecific pinocytosis of circulating IgG followed by low pH-dependent (~6.0) binding of IgG to FcRn in the endocytic vesicles. The FcRn-IgG complex is transported away from the degradative lysosomal pathway to the cell surface, where IgG is released at the higher physiologic pH (pH ~7.4). IgG not bound by FcRn is degraded in the lysosomes (17).

One way of altering the blood clearance of intact antibodies is by engineering mutations that affect their binding to FcRn (18–22). The conserved amino acid residues (Ile353, His318, and His315) in the FcRn binding region of IgG are crucial for its binding to FcRn, and their genetic modification can prolong or shorten the blood half-life of IgG. However, this approach is not feasible as a general method to improve antibody pharmacokinetics because it reduces the delivery of the immunoconjugate to the tumor and also requires reengineering of each therapeutic or diagnostic antibody. In addition, the strategy is expensive and time-consuming, requiring regulatory approval for each modification. A more general and feasible approach would be to pharmacologically block the binding of the conjugated antibody to FcRn. We hypothesized that this could be accomplished via administration of high doses of IgG to competitively inhibit the binding of the conjugated antibody to the FcRn. Indeed, one proposed mechanism of the observed therapeutic benefit of high-dose i.v. IgG (IVIG) therapy in humoral autoimmune disease is the enhanced catabolism of pathogenic autoantibodies via FcRn blockade (23–26).

Here, we determined the mechanism and assessed the effectiveness of high-dose polyclonal IgG therapy in altering the pharmacokinetics of several different radiolabeled IgG antibodies in mice. We found enhanced blood and whole-body clearance of radioactivity, resulting in better tumor-to-blood image contrast and reduced normal-tissue radiation dose without compromising the targeting to the tumor or the resultant therapeutic response. Lastly, we demonstrated in humans the use of high-dose IgG therapy for tumor imaging, and we discuss its potential applications in imaging and therapy of cancer with conjugated antibodies.

Nonstandard abbreviations used: BUN, blood urea nitrogen; CI, confidence interval; FcRn, neonatal Fc receptor; IVIG, i.v. IgG; RIC, radioimmunoconjugate.
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**Results**

**FcRn blockade enhances the clearance of radiolabeled IgG antibody.** BALB/c mice were injected i.v. with 2 μCi of indium-111-labeled (111In-labeled) humanized anti-CD33 IgG1 HuM195 antibody and simultaneously received either saline or 1 g/kg polyclonal human IgG i.p. (n = 4 per group). Enhanced whole-body clearance of radioactivity was observed in IgG-treated mice relative to the control group (Figure 1A). Mice were sacrificed 148 hours after injection with the radioimmunoconjugate (RIC) to measure the organ distribution of 111In activity (Figure 1B). Postmortem biodistribution revealed substantially lower 111In activity in blood (0.66 ± 0.11 versus 12.12 ± 0.48; P < 0.0001), as well as modest reductions in kidneys (5.91 ± 0.31 versus 8.63 ± 0.33; P = 0.001) and spleen (8.83 ± 0.05 versus 10.07 ± 0.49; P = 0.04) of IgG-treated mice relative to untreated controls. The liver 111In activity, however, was significantly greater in the IgG-treated mice (15.14 ± 0.61 versus 7.44 ± 0.25; P < 0.0001). To dissect the mechanism of the altered IgG pharmacokinetics, we performed a similar experiment using wild-type or FcRn-deficient (lacking the FcRn α chain; FcRn−/−) C57BL/6J mice. The whole-body elimination of radioactivity was faster in FcRn-deficient and IgG-treated wild-type mice than in untreated wild-type mice (Figure 1C). IgG treatment (1 g/kg i.p.; administered simultaneously with 111In-HuM195) did not alter whole-body clearance or organ distribution of 111In in FcRn knockout mice. Data are mean ± SEM. Scale of y axes varies in D and E.

**Figure 1**

FcRn blockade enhances the clearance of radiolabeled IgG antibody. (A) Whole-body 111In activity at various time points after injection with 111In-HuM195 antibody (n = 4 per group). IgG-treated mice had faster whole-body clearance of radioactivity. (B) Organ distribution of 111In activity in the same mice after the last whole-body measurement (144 hours) revealed accelerated blood clearance after IgG treatment. %ID/g, percentage of injected dose per gram. (C–E) Whole-body and organ distribution of 111In activity in wild-type and FcRn knockout mice with or without IgG treatment. Genetic absence of a functional FcRn, or its pharmacological inhibition by high-dose IgG administration, resulted in accelerated whole-body elimination (C) and blood clearance of 111In-HuM195 antibody at 44 hours (D) and 160 hours (E) after injection. IgG treatment (1 g/kg i.p.; administered simultaneously with 111In-HuM195) did not alter whole-body clearance or organ distribution of 111In in FcRn knockout mice. Data are mean ± SEM. Scale of y axes varies in D and E.
administration enhanced the blood clearance of the radiolabeled antibody in wild-type mice at 44 hours (mean difference, 7.95; 95% confidence interval [CI], 6.74 to 9.15; \( P < 0.001 \)) and at 160 hours (mean difference, 7.77; 95% CI, 5.33 to 10.22; \( P < 0.001 \)), but not in FcRn-deficient mice at 44 hours (mean difference, 0.30; 95% CI, −2.84 to 3.44; \( P > 0.05 \)) or at 160 hours (mean difference, 0.001; 95% CI, −2.44 to 2.45; \( P > 0.05 \)), suggesting that the altered antibody pharmacokinetics seen with IgG therapy was mediated by this receptor.

High-dose IgG administration enhances tumor contrast in immuno-PET imaging. With the foregoing evidence that blood clearance of radiolabeled antibodies was markedly accelerated with high-dose IgG administration, we hypothesized that this strategy would translate to contrast enhancement in imaging of tumors. SK-RC-52 or SW1222 xenograft-bearing athymic mice were injected with \( ^{111}\text{In} \)-labeled cG250 and A33 antibodies, respectively. High-dose IgG or saline was administered 24 hours after injection of the radiolabeled antibody to allow for tumor targeting of the radiolabeled antibody. Postmortem biodistribution 48 hours after high-dose IgG administration (Figure 2, A and B) showed 5- to 10-fold enhancement of the blood clearance of \( ^{111}\text{In} \)-labeled antibodies (cG250, 16.72 ± 1.24 versus 3.0 ± 0.26; \( P = 0.0004 \); A33, 5.83 ± 0.18 versus 0.59 ± 0.04; \( P < 0.0001 \)) without affecting their tumor uptake. Data are mean ± SEM. (C and E) PET images of representative tumor xenograft-bearing mice at the indicated time points following injection with \( ^{124}\text{I} \)-labeled cG250 (C) or A33 (E) antibody. (D) Excised tumors from mice in C. Enhanced tumor-to-blood contrast was seen in mice that received 1 g/kg human IgG, either 24 hours (C) or 6 hours (E) following \( ^{124}\text{I} \)-antibody injection. H, heart; T, tumor; B, urinary bladder.

**Figure 2**
High-dose IgG administration enhances blood clearance and tumor-to-blood contrast in immuno-PET imaging without affecting the uptake of radiolabeled antibody in the tumor. (A and B) Organ distribution of \( ^{111}\text{In} \) activity at 72 hours after injection with \( ^{111}\text{In} \)-cG250 (A) or \( ^{111}\text{In} \)-A33 (B). IgG administration (1 g/kg i.p.; administered 24 hours after \( ^{111}\text{In} \)-antibody) decreased the blood and renal \( ^{111}\text{In} \) activity without affecting the tumor uptake. Data are mean ± SEM. (C and E) PET images of representative tumor xenograft-bearing mice at the indicated time points following injection with \( ^{124}\text{I} \)-labeled cG250 (C) or A33 (E) antibody. (D) Excised tumors from mice in C. Enhanced tumor-to-blood contrast was seen in mice that received 1 g/kg human IgG, either 24 hours (C) or 6 hours (E) following \( ^{124}\text{I} \)-antibody injection. H, heart; T, tumor; B, urinary bladder.
The Journal of Clinical Investigation

The control mouse, was still readily discernible. The enhancement of tumor-to-blood contrast with high-dose IgG administration was even more pronounced in 124I-A33 imaging studies, in which tumors were visualized as early as 4 hours after injection (Figure 2E). At 18 hours after injection, IgG-treated mice displayed lower cardiac and higher urinary bladder activity, indicating enhanced blood clearance and metabolism and urinary elimination of radioactivity. By 48 hours after injection, although both groups showed similar tumor uptake, the IgG-treated mice displayed almost complete elimination of blood radioactivity and therefore enhanced tumor-to-blood contrast (Figure 2E and Supplemental Videos 1 and 2; supplemental material and available online with this article; doi:10.1172/JCI32226DS1).

IgG administration alters the pharmacokinetics and absorbed radiation dose from therapeutic actinium-225 antibody constructs. We tested whether high-dose IgG could enhance the blood clearance of actinium-225–labeled (225Ac-labeled) antibody and as a result alter the biodistribution of the decay daughters of 225Ac. The α particle–emitting daughters, mainly francium-221 (221Fr) and bismuth-213 (213Bi), generated in vivo from circulating 225Ac-antibody constructs have the propensity to accumulate and irradiate the renal parenchyma (27) (Supplemental Figure 1). We hypothesized that high-dose IgG administration would enhance blood clearance and decrease the transit time of 225Ac-HuM195 in the blood. This would decrease the 225Ac decays and therefore the generation of radioactive decay elements in the blood, resulting in decreased transport and accumulation of 221Fr and 213Bi in the kidneys. Mice were injected i.v. with 0.5 μCi 225Ac-labeled HuM195 antibody with or without simultaneous high-dose IgG administration (n = 4). The whole-body clearance of radioactivity, determined via measurement of characteristic energy of the γ-photons of 221Fr, was enhanced by IgG administration (Figure 3A). Mice were then sacrificed at 170 hours after injection, and the tissue distribution of 225Ac, 221Fr, and 213Bi activity in the same mice after the last whole-body measurement (168 hours). (C–F) Time course of the alteration of 225Ac-labeled HuM195 pharmacokinetics and that of 225Ac decay elements 221Fr and 213Bi after high-dose IgG administration. IgG treatment (1 g/kg i.p.; administered at the same time as 225Ac-HuM195) resulted in enhanced blood clearance of 225Ac-HuM195 antibody at 24 (C), 48 (D), 120 (E), and 192 hours (F) after injection. Data are mean ± SEM. Scale of y axes varies in B–F: Bl, blood; Ki, kidney; Li, liver.

Figure 3
High-dose IgG administration alters the pharmacokinetics of the therapeutic 225Ac-labeled HuM195 antibody. (A) Whole-body 221Fr activity (a decay product of 225Ac) at various time points after injection with 225Ac-HuM195 antibody (n = 5 per group). IgG-treated mice had faster whole-body clearance of radioactivity. (B) Organ distribution of 225Ac, 221Fr, and 213Bi activity in the same mice after the last whole-body measurement (168 hours). (C) Activity at 168% (ID/g) versus time after injection. (D) Activity at 168% (ID/g) versus time after injection. (E) Activity at 168% (ID/g) versus time after injection. (F) Activity at 168% (ID/g) versus time after injection. Data are mean ± SEM. Scale of y axes varies in B–F: Bl, blood; Ki, kidney; Li, liver.
Having shown in separate studies limiting radiation nephropathy while preserving the antitumor therapy study whether we could protect against the dose-enhancement of its blood clearance and that the tumor uptake of the antibody was not significantly altered, we evaluated in a renal radiation dose and toxicity from high-dose IgG administration significantly decreased the protection against the bone marrow toxicity of circulating 225Ac-HuM195 by enhancing its blood clearance. (B and C) Renal function of mice 35 weeks after injection with 500 nCi 225Ac-HuM195, with or without simultaneous IgG administration. (D) Kidney and liver histology 35 weeks after injection with 500 nCi 225Ac-HuM195. IgG treatment (1 g/kg i.p.; administered at the same time as 225Ac-HuM195) protected mice against the late radiation-induced morphological and functional damage to the kidneys. No hepatic histopathology was seen in either group. Data are mean ± SEM.

Figure 4

High-dose IgG protects animals against the toxicity of 225Ac antibody construct. (A) Total leukocyte count 12 days after injection in mice injected with escalating doses of 225Ac-HuM195. IgG treatment was protective against the bone marrow toxicity of circulating 225Ac-HuM195 by enhancing its blood clearance. (B and C) Renal function of mice 35 weeks after injection with 500 nCi 225Ac-HuM195, with or without simultaneous IgG administration. (D) Kidney and liver histology 35 weeks after injection with 500 nCi 225Ac-HuM195. IgG treatment (1 g/kg i.p.; administered at the same time as 225Ac-HuM195) protected mice against the late radiation-induced morphological and functional damage to the kidneys. No hepatic histopathology was seen in either group. Data are mean ± SEM.

received 0.3 µCi (P < 0.01) but not 0.6 µCi (P > 0.05) of 225Ac-labeled antibody. To test whether IgG therapy could mitigate the delayed radiation nephropathy, mice were injected with 0.5 µCi 225Ac-labeled HuM195 and sacrificed 35 weeks after injection to assess the functional and histopathological damage to the kidneys. We have shown previously that 0.35 µCi 225Ac-labeled HuM195 causes significant renal morphologic and functional damage at this time point (29). The blood urea nitrogen (BUN) and serum creatinine was significantly lower (Figure 4, B and C) in mice that received high-dose IgG (BUN, mean difference, 40.0 ± 5.1 mg/dl; 95% CI, 27.48 to 52.52 mg/dl; P = 0.0002; serum creatinine, mean difference, 0.50 ± 0.082 mg/dl; 95% CI, 0.30 to 0.70 mg/dl; P = 0.0009). Kidney sections from 225Ac-HuM195–injected control mice displayed diffuse subcapsular cortical atrophy, extensive tubulolysis with thickened and wrinkled basement membranes, glomerular crowding, and dilatation of the Bowman spaces (Figure 4D). The renal histopathologic damage seen in IgG-treated mice was significantly diminished in extent and severity, with only focal areas of tubulolysis and basement membrane thickening. Despite a greater radiation dose to the liver in IgG-treated mice, no significant morphologic damage was observed (neither group displayed histopathologic damage to the liver).

High-dose IgG administration prevents normal-tissue toxicity while preserving the therapeutic effect. Having shown in separate studies that high-dose IgG administration significantly decreased the renal radiation dose and toxicity from 225Ac-labeled antibody via enhancement of its blood clearance and that the tumor uptake of the antibody was not significantly altered, we evaluated in a tumor therapy study whether we could protect against the dose-limiting radiation nephropathy while preserving the antitumor efficacy. Mice that received 0.2 or 0.3 µCi 225Ac-A33 had substantially inhibited tumor growth and enhanced survival (Figure 5, A and B). All but 2 mice in the group that received 0.2 µCi 225Ac-A33 plus high-dose IgG and all but 1 mouse in the 0.2 µCi 225Ac-A33 group were cured of their tumors, defined as having no detectable tumor at 140 days after implantation. All mice that received 0.3 µCi 225Ac-A33 (with or without IgG administration) were cured of tumors; these mice were sacrificed 30 weeks after tumor implantation, and their liver and kidney sections were examined by light microscopy. No significant liver pathology was observed in either group (Figure 5C). However, kidneys of mice that received 0.3 µCi 225Ac-A33 without IgG therapy displayed moderately severe radiation damage with focal areas of basement membrane thickening with tubulolysis. IgG-treated mice showed complete protection against this delayed radiation nephropathy.

124I-A33 immuno-PET imaging in patients that received high-dose IgG administration. Serial images and blood radioactivity levels were obtained in patients that received high-dose IgG 2 days after injection with 124I-A33. Initial images showed predominantly blood pool activity (Figure 6, A and B); however, significant blood clearance was seen at the 160-hour time point, and clear visualization of A33 antigen–positive, metastatic aortocaval lymph node was observed (Figure 6, C–E). The A33 antigen positivity of the aortocaval node was confirmed by immunohistochemistry following surgical resection. A decrease in the blood radioactivity was seen following IVIG administration (Figure 6F).

Discussion

Monoclonal antibodies represent one of the fastest-growing classes of new therapeutic and diagnostic agents, with nearly 2 dozen FDA-
The biodistribution and whole-body clearance data in mice indicate that high-dose IgG therapy markedly increased the blood clearance of the radiometal-labeled antibody, which was followed by gradual elimination of the radioactivity from the liver and thus enhanced whole-body clearance. For whole-body clearance and postmortem biodistribution studies, radiometals (111In or 225Ac) were used to label antibodies instead of radioiodine because iodine can be rapidly released from tissues following endocytosis and catabolism of radiiodinated antibodies and may therefore lead to an overestimation of the normal-organ and whole-body clearance. For the same reason, although enhancement in liver uptake of radioactivity was seen following high-dose IgG administration in biodistribution experiments, no liver uptake was seen in PET images, which used 124I as a radiolabel. For PET studies, in which enhanced tumor-to-blood contrast is desirable, 124I provides a quantitative measure of the blood clearance and tumor targeting. We showed the versatility of this approach in altering the pharmacokinetics of IgG antibodies by testing 3 different radiolabeled antibodies, each of which has been used in patients with respective tumor types. 225Ac-HuM195, an antibody construct currently in human clinical trials for patients with advanced myeloid malignancies, does not have a suitable mouse tumor model. Therefore, biodistribution and imaging studies in tumor-bearing mice were performed with radiolabeled cG250 or A33 antibodies because of the availability of reliable mouse tumor xenograft models.

The plasma clearance of 124I-A33 in humans, although resulting in favorable image contrast, was not as pronounced as that seen in mice that received the same dose (1 g/kg) of polyclonal human IgG. The blunted effect in humans may be explained not only by biologic differences, but also by the 8-fold higher affinity of human IgG for mouse FcRn than for human FcRn (37). Therefore, higher doses of IgG may be required in humans to achieve the effects seen in mice. Petkova, Roopenian, and colleagues have addressed this issue by generating mice that express human FcRn and radiometal accumulation in these organs. Despite an estimated doubling of the radiation dose to the liver, no significant hepatic pathology was observed in IgG-treated animals. A possible explanation for this observation is the relative radioresistance of the liver (35). Our previous data in mice and nonhuman primates, in which high doses of 225Ac-HuM195 resulted in severe renal toxicity but no hepatic toxicity, are consistent with this finding (29, 36).

The MHC-1, like FcRn, binds IgG and albumin at distinct sites and protects both proteins from catabolism (30). However, this protective capacity of FcRn is saturable. It has been shown that the plasma half-life of IgG is inversely proportional to its serum concentration and that the plasma half-life of radiolabeled γ globulin is shorter in patients with monoclonal gammopathy (31, 32). Because it is FDA approved and large amounts are readily available, we used human polyclonal IgG to pharmacologically inhibit the murine FcRn, which is promiscuous and can bind human IgG with high affinity (33).

Biodistribution studies in mice revealed enhanced blood clearance and a higher liver uptake of the radiolabeled antibody following high-dose IgG therapy. An enhancement in liver uptake was expected because it was previously reported that FcRn is expressed in hepatic endothelium and the hepatocytes (5, 12, 34) and that blockade of FcRn should result in degradation of the radiolabeled IgG and radiometal accumulation in these organs. Despite an estimated doubling of the radiation dose to the liver, no significant hepatic pathology was observed in IgG-treated animals. A possible explanation for this observation is the relative radioresistance of the liver (35). Our previous data in mice and nonhuman primates, in which high doses of 225Ac-HuM195 resulted in severe renal toxicity but no hepatic toxicity, are consistent with this finding (29, 36).

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Another strategy, developed by Vaccaro et al., uses administration of engineered IgG antibodies with high affinity and reduced pH-dependent binding to FcRn that are not readily released at the cell surface during exocytosis (40). As a consequence, low doses of engineered antibodies were required relative to wild-type polyclonal IgG to enhance blood clearance of endogenous antibodies. A relatively longer duration of action of this therapeutic via its inability to dissociate at the cell surface may be useful in autoimmune disorders, in which sustained degradation of endogenous pathogenic autoantibodies is desirable. However, for imaging or therapy with conjugated antibodies, in which a transient FcRn blockade to sufficiently accelerate the blood clearance of injected diagnostic or therapeutic antibody is desirable, an optimally timed high-dose IgG therapy is preferable.

In summary, we have shown that it is possible to effectively control the blood half-lives and therefore the therapeutic index of targeted IgG antibodies via pharmacological modulation of their interaction with the protective FcRn. The described approach resulted in enhanced tumor contrast and reduction of normal-tissue toxicity for tumor imaging and therapy.

Methods

Mice. Female BALB/c, C57BL/6J (wild-type as well as FcRn−/−), and athymic nude mice (4–8 weeks of age) were purchased from Taconic, The Jackson Laboratory, and the National Cancer Institute, respectively. All animal studies were conducted according to the NIH Guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use committee at Memorial Sloan-Kettering Cancer Center.

Cells lines and xenografts. SW1222 and SK-RCS2 cell lines (expressing A33 antigen and carbonic anhydrase IX, respectively) were cultured in RPMI medium supplemented with 10% fetal calf serum at 37°C in 5% CO2. Xenografts were established in athymic nude mice by subcutaneous inoculation of 5 x 106 cells in 200 μl.

Preparation, quality control, and administration of RICs. Humanized anti-CD33 IgG1 (HuM195) antibody was purchased from Protein Design Labs. Chimeric anti–carbonic anhydrase IX (cG250) and humanized anti-A33 antigen (huA33) were a gift from Ludwig Institute of Cancer Research. 125I (Oak Ridge National Laboratory) and 111In (PerkinElmer) were conjugated to antibodies using a 2-step labeling method, as described previously (41, 42). Iodo-Gen method was used to radioiodinate antibodies with 124I (18). Radiochemical purity and immunoreactivity of the RIC were determined as described previously (42). Mice were anesthetized with isoflurane and injected i.v. with the RIC in 100 μl.

(38), which should serve as a good surrogate for preclinical evaluation of diagnostic and therapeutic human IgG antibodies. The pharmacokinetic behavior of human IgG is also expected to differ in mouse and human systems. Moreover, the routes of administration of high-dose IgG were also different in mouse and human studies (i.p. and i.v., respectively). Plasma IgG concentrations of approximately 10 and 35 mg/ml in mice and humans, respectively, should suffice to functionally inhibit the ability of FcRn to protect IgG from catabolism (26). Based on basal plasma IgG concentration and its volume of distribution, 2 daily 1 g/kg doses (the recommended therapeutic dose for autoimmune disorders) should result in a peak plasma IgG concentration of 40 mg/ml (39) and may be sufficient to saturate the FcRn in humans.

Previous studies have tried to mutate key amino acid residues in the IgG molecule that are critical for FcRn binding in order to generate antibodies with altered binding to FcRn (18, 38, 40). Although enhanced blood clearance of fragments with low affinity for FcRn was seen, their tumor uptake was also compromised (18). An important advantage of the high-dose IgG therapy described here, besides obviating the need for engineering each antibody, is that it allows for timing of the IgG administration to clear excess circulating antibody after tumor targeting has occurred. This is crucial, because the time required for optimal tumor targeting varies with each antigen-antibody-tumor system. For example, optimal tumor targeting in mice with A33 and cG250 was seen at 4 and 24 hours after injection, respectively, and therefore the IgG was administered to mice after 6 or 24 hours, respectively.

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### Results and Discussion

The pharmacokinetics of human IgG in mouse and human systems. Moreover, the routes of administration of diagnostic and therapeutic human IgG antibodies. The pharmacokinetic behavior of human IgG is also expected to differ in mouse and human systems. Moreover, the routes of administration of high-dose IgG were also different in mouse and human studies (i.p. and i.v., respectively). Plasma IgG concentrations of approximately 10 and 35 mg/ml in mice and humans, respectively, should suffice to functionally inhibit the ability of FcRn to protect IgG from catabolism (26). Based on basal plasma IgG concentration and its volume of distribution, 2 daily 1 g/kg doses (the recommended therapeutic dose for autoimmune disorders) should result in a peak plasma IgG concentration of 40 mg/ml (39) and may be sufficient to saturate the FcRn in humans.

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#### Preparation, quality control, and administration of RICs

Humanized anti-CD33 IgG1 (HuM195) antibody was purchased from Protein Design Labs. Chimeric anti–carbonic anhydrase IX (cG250) and humanized anti-A33 antigen (huA33) were a gift from Ludwig Institute of Cancer Research. 125I (Oak Ridge National Laboratory) and 111In (PerkinElmer) were conjugated to antibodies using a 2-step labeling method, as described previously (41, 42). Iodo-Gen method was used to radioiodinate antibodies with 124I (18). Radiochemical purity and immunoreactivity of the RIC were determined as described previously (42). Mice were anesthetized with isoflurane and injected i.v. with the RIC in 100 μl.

Another strategy, developed by Vaccaro et al., uses administration of engineered IgG antibodies with high affinity and reduced pH-dependent binding to FcRn that are not readily released at the cell surface during exocytosis (40). As a consequence, low doses of engineered antibodies were required relative to wild-type polyclonal IgG to enhance blood clearance of endogenous antibodies. A relatively longer duration of action of this therapeutic via its inability to dissociate at the cell surface may be useful in autoimmune disorders, in which sustained degradation of endogenous pathogenic autoantibodies is desirable. However, for imaging or therapy with conjugated antibodies, in which a transient FcRn blockade to sufficiently accelerate the blood clearance of injected diagnostic or therapeutic antibody is desirable, an optimally timed high-dose IgG therapy is preferable.

In summary, we have shown that it is possible to effectively control the blood half-lives and therefore the therapeutic index of targeted IgG antibodies via pharmacological modulation of their interaction with the protective FcRn. The described approach resulted in enhanced tumor contrast and reduction of normal-tissue toxicity for tumor imaging and therapy.
IgG treatment in mice. Mice were injected i.p. at indicated time points with 1 g/kg purified polyclonal human IgG (Gammunex; Talecris) or an equal volume (250 μl) of saline. In studies that evaluated the effect of high-dose IgG on pharmacokinetics and toxicity of radiolabeled antibodies in non–tumor-bearing mice (Figures 1, 3, and 4), high-dose IgG was administered i.p. simultaneously with i.v. injection of the radiolabeled antibody. However, studies that tested the potential practical application of this strategy in terms of its effect on tumor targeting, imaging and therapy with radio-labeled antibodies, high-dose IgG administration was delayed by either 6 hours (imaging studies with 124I-A33) or 24 hours (imaging studies with 124I-labeled cG250, biodistribution studies with 111In-labeled cG250 and A33, and therapy studies with 225Ac-A33) after injection of the radiolabeled antibody to allow for effective tumor targeting.

Estimation of whole-body radioactivity and organ distribution. Whole-body activity in mice was measured at various time points using a high-purity germanium detector (HPGe; Ortec) and expressed as percent of the injected dose (corrected for physical decay to the time of injection). The photopeaks at 218 keV, 440 keV, and 171 keV were used to assay 221Fr, 213Bi, and 111In activity, respectively. For biodistribution studies, mice were sacrificed at the indicated time points after RIC injection (7–10 μCi antibody/mouse); their blood, kidneys, livers, and tumors (for some experiments) were harvested, weighed, and counted; and the percentage injected dose of activity per gram tissue was calculated, as described previously (27, 42).

225Ac-HuM195 toxicity studies in non–tumor-bearing mice. To evaluate the effectiveness of high-dose IgG in mitigating early bone marrow toxicity of 225Ac-labeled antibody, mice were injected with 0.3 or 0.6 μCi of 225Ac-HuM195 and injected simultaneously with either saline or 1 g/kg IgG i.p. Mice were euthanized 12 days after injection of the radiolabeled antibody, and their peripheral blood leukocyte count was measured. To study the effect of high-dose IgG on delayed radiation nephropathy, mice were injected with 0.5 μCi 225Ac-HuM195 with simultaneous administration of either saline or 1 g/kg IgG i.p., sacrificed 35 weeks after 225Ac-HuM195 injection, and evaluated for functional changes and histopathology in kidneys and liver.

Tumor therapy studies. Mice bearing SW1222 colon carcinoma xenografts were randomly assigned to various experimental groups that received either 1% human serum albumin (untreated; n = 5), 0.3 μCi 225Ac-labeled nonspecific IgG, isotopic antibody (225Ac-HuM195; n = 5), 0.2 or 0.3 μCi 225Ac-labeled huA33 antibody (225Ac-A33; n = 7), or 0.2 or 0.3 μCi 225Ac-labeled huA33 antibody along with 1 g/kg IgG (n = 7) administered i.p. 24 hours after injection of the RIC. Treatments were administered 16 days after implantation of xenografts. Tumor size was measured with calipers, and tumor volume was calculated as 0.52 × d1 × d2 × d3, where d1 is the smaller diameter and d2 is the larger diameter. Animals were followed long-term for survival advantage of the respective treatments. The mice that received 0.3 μCi 225Ac-A33 with or without 1 g/kg IgG were sacrificed 30 weeks after tumor implantation to evaluate the gross and histopathologic damage to the kidneys and liver.

Clinical and anatomic pathology. Blood was collected after mice were euthanized, and BUN and serum creatinine (LX 20 analyzer; Beckman Coulter) were determined. Liver and kidneys were harvested, fixed, processed, and paraffin-embedded. Sections (2–3 μm) were stained with H&E and Masson trichrome and examined with an Olympus BX45 light microscope.

Micro-PET imaging of mice. Tumor-bearing mice were injected i.v. with 250–280 μCi 124I-labeled cG250 or huA33 antibody. The antibody dose was 60–75 μg/mouse. Normal thyroid uptake was blocked by administration of potassium iodide in drinking water. Mice were anesthetized with isoflurane and imaged with a Focus 120 microPET camera (Concorde Microsystems) at specified time points after injection. An energy window of 420–580 keV and a coincidence timing window of 6 ns was used. The resulting list-mode data were sorted into 2-dimensional histo-