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Glomerular Deposition of Immune Complexes Made with IgG2a Monoclonal Antibodies

Melva L. Gonzalez and Frank J. Waxman

The factors that determine whether immune complexes (IC) are cleared safely from the circulation or are deposited in vulnerable tissues such as glomeruli are not well defined. To better understand how IC are handled, the present study examined the fate in vivo of three model IC preparations with different immunochemical characteristics. Radiolabeled IC were constructed with murine IgG1, IgG2a, or IgG3 anti-DNP mAbs bound to DNP-BSA, designated IgG1 IC, IgG2a IC, and IgG3 IC, respectively. The IC were infused i.v. into BALB/c mice, and clearance and tissue localization of the three IC probes were compared. The results indicate that the major portion of each IC preparation was cleared from the circulation by the liver. However, compared with the other two probes, IgG2a IC were preferentially deposited in the kidney. Histologic examination revealed the presence of IgG2a IC in glomeruli. The enhanced renal uptake of IgG2a IC could not be attributed solely to such characteristics as IC size, Ag/Ab ratio, Ab charge, or affinity. However, the preferential renal deposition of IgG2a IC was abrogated by complement depletion. Thus, enhanced renal uptake in normal mice was complement dependent. These data suggest that interactions between IC and the complement system can influence the propensity of IC to deposit in tissues susceptible to IC-mediated injury. The Journal of Immunology, 2000, 164: 1071–1077.

Immune complexes (IC) are continuously formed as Abs encounter target Ags in the circulation. Normally, the IC are safely cleared from the circulation by the reticuloendothelial system (RES). In some circumstances, the IC can lodge in vulnerable tissues such as glomeruli and incite an inflammatory response that may ultimately lead to end-stage renal disease.

The development of IC-mediated renal disease is a complex process that involves both cellular and humoral elements of the immune response. There is increasing evidence that engagement of FcR may be responsible for many features of IC-mediated inflammation in experimental mouse models (1–4). The complement system also plays a central role in both the inflammatory events associated with IC-mediated disease and the normal clearance of IC from the circulation. In primates, C3b and C4b deposited on IC as a consequence of complement activation provide ligand sites for the complement receptor, type 1 (CR1) expressed on erythrocytes. The binding of C3b/C4b-coated bacteria to human erythrocytes in vitro by this mechanism was originally termed immune adherence (5). Studies in baboons revealed that erythrocyte CR1-adenent IC were carried to the RES, where the IC were deposited while the erythrocyte returned to the circulation (6–8). In nonprimates, IC are cleared from the circulation by a similar process in which the analogue to primate erythrocyte CR1, expressed in this case on platelets rather than erythrocytes, shuttles the IC to the RES (9, 10). Thus, in both primate and nonprimate models, the activation of the complement cascade by IC resulting in the generation of covalently bound C3b/C4b and subsequent adherence to complement receptors are critical events in the clearance of IC.

The immunochemical and physical characteristics of both the Ag and the Ab components of an IC can affect how well the IC activates the complement cascade. To better define how the Ab component of the IC affects complement activation, a panel of IC was constructed using murine anti-DNP mAbs and DNP-BSA. The interactions between these model IC probes, human erythrocytes, and human complement have been characterized extensively in vitro (11–13). These collective data indicate that the interactions between IC, complement, and erythrocyte CR1 are dependent on both the quantitative expression of CR1 and the immunochemical and physical characteristics of the Ab used to form the IC. Depending on the model system employed, both isotypic (11) and clonotypic (14) variables can affect complement activation. In some cases, the molecular orientation of the N-glycan linked to the C1q2 domain of IgG can also influence complement activation via the classical pathway (15).

While these studies have provided insight into how the Ab component of an IC modulates its interaction with the complement system in vitro, the ultimate test of the physiologic relevance of these data has awaited an examination of how the immunochemical and physical properties of model IC affect their fate in vivo. The present study was designed to compare the clearance and organ localization of three model IC probes made with representatives of the murine mAb panel. The results indicate that the complement system can play a role in the deposition of IC in the kidney.

Materials and Methods

Preparation of Ag

BSA was radiolabeled with 125I (Amersham Life Science, Arlington Heights, IL) using chloramine T and substituted with DNP, as previously described (11). The sp. act. of the IC was ~5 × 107 cpm per mg of protein. The hapten substitution ratio for preparations used in this study was 63–76 DNP groups per BSA molecule.

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3 Abbreviations used in this paper: IC, immune complex; CR1, type 1 complement receptor; CVF, cobra venom factor; RES, reticuloendothelial system; SLE, systemic lupus erythematosus.
Preparation of IC

The procedures for raising murine anti-DNP mAbs and constructing IC are described elsewhere (11). IC made with IgG1, IgG2a, and IgG3 mAbs used in this study are designated IgG1 IC, IgG2a IC, and IgG3 IC, respectively. The Ag/Ab ratio of each IC preparation was calculated as previously described (11).

IC clearance

Female BALB/c mice (Charles River Laboratories, Wilmington, MA) approximately 8–10 wk old were used for these experiments. Each mouse was injected i.m. with 100 μg of xylazine (Miles, Shawnee Mission, KS) and 10 min later with 1 mg of ketamine (Fort Dodge Laboratories, Fort Dodge, IA). Six minutes later, the animal was injected with 110 μg of soluble 125I-labeled IC via the lateral tail vein. The quantity of IC infused was based on preliminary experiments (not shown) that indicated this tracer dosage was the threshold amount required to give quantifiable and reproducible kidney deposition. Blood samples were obtained from the retroorbital plexus using a 44.7 μl heparinized micropipet at 0.5, 1, 1.5, 2, 4, 6, and 8 min postinjection. The radioactivity in each sample was measured in a scintillation counter, and clearance data expressed as the percentage of the total IC inoculum present in each blood sample.

Tissue deposition

Mice were killed by cervical dislocation at 8 min, and the spleen, kidneys, liver, and lungs were collected. The radioactivity in each organ was measured and expressed as the percentage of the total IC dosage infused. The kidney deposition data were corrected for blood flow using the following formula: corrected kidney cpm = measured kidney cpm – [(8.4 μl × (cpm per μl of blood))]. For this calculation, the volume of blood in the kidneys was calculated as 8.4 μl based on the average weight of the kidneys of 0.12 g and the assumption that the blood volume was 7% of kidney weight (16). The value of the cpm per μl of blood was calculated based on the radioactive content of the sample obtained at the final 8-min time point. When tissues were used for histological studies, the animals were injected with 110 μg of nonradiolabeled IC and killed at 8 min, and the organs were placed in liquid nitrogen (frozen sections), which were later stained with FITC-conjugated goat anti-mouse IgG (Sigma, St. Louis, MO) (17) or in B5 fixative (paraffin sections) that were later stained with alkaline phosphatase-conjugated sheep F(ab)2 anti-mouse IgG (Sigma) and counterstained with hematoxylin, as described elsewhere (17).

IC size

Isokinetic sucrose gradients were constructed to measure the IC size, as described elsewhere (6). Briefly, linear gradients were formed with 10–25% sucrose in 4.8-ml tubes blocked with 1% BSA at 4°C overnight. The gradients were centrifuged in a L7 Ultracentrifuge (Beckman, Palo Alto, CA) using a SW 50.1 rotor at 1000 rpm for 30 min at 20°C. Fractions were collected using a peristaltic pump, starting from bottom. Approximately 24 fractions were collected, and their 125I content was measured. The data are expressed as the percentage of the total IC sample applied to the gradient contained in each fraction. In the experiments reported in this work, approximately 91–97% of the total IC applied to the gradient was recovered in the aggregate collected fractions.

Ab charge and affinity

The charge of each mAb was determined by isoelectric focusing as previously described (18). The relative affinities of each Ab were compared using an inhibition ELISA, as described elsewhere (19).

IC clearance in cobra venom factor (CVF)-treated mice

Mice were injected i.p. with doses of 10 U of CVF (Naja naja kaouthia, Quidel, San Diego, CA). The animals received a total of three doses at 8-h intervals. Three hours after the final dose, serum was obtained from normal and CVF-treated mice to measure the degree of complement depletion (20, 21). Nunc Maxisorp Immunoplates (Fisher Scientific, Pittsburgh, PA) were coated with 50 μl of goat anti-mouse C3 Ab (Cappel Organon Teknika, Durham, NC) at a 1/750 dilution in PBS and then blocked with 1% BSA. Two-fold serial serum dilutions were added starting at 1/10,000, and the wells were incubated for 3 h at room temperature. The plate was washed and incubated with HRP-conjugated goat anti-mouse C3 Ab (Cappel), and finally, color development was measured at OD490 at each serum concentration. Comparison of C3 present in sera from normal vs CVF-treated mice indicated that CVF treatment caused an 80–85% complement depletion in the experiments reported in this work (data not shown). This is most likely an underestimate of the actual degree of functional complement depletion achieved since the polyclonal anti-mouse C3 Ab employed as both the capture and reporter Abs may have bound residual inactive C3 cleavage fragments in the serum of CVF-treated mice.

Statistical analysis

Multiple groups were compared by ANOVA, and paired groups were compared by Student’s t test or the Spearman correlation test.

Results

Clearance of IC from circulation

To compare the clearance of the three model IC, mice were infused i.v. with radiolabeled IC and periodic blood samples were obtained. All three model IC preparations were quickly cleared from the circulation, reaching a stable level within 8 min (Fig. 1). Assuming a maximum total blood volume of 2 ml for a 25-g mouse (16), upon completion of the IC infusion and in the absence of any clearance, approximately 2.2% of the infused IC should theoretically be contained in each 44.7-μl blood sample. Thus, the data indicate that by the time IC infusion had been completed and the first blood sample had been obtained at 30 s, approximately 34–51% of the infused IC had already been cleared. Of the three model IC preparations, IgG1 IC were cleared the most quickly and efficiently. IgG3 IC were cleared more slowly, but eventually reached approximately the same level as observed for IgG1 IC. At 8 min, approximately 10% of the IgG1 IC and IgG3 IC inocula remained in the circulation. In contrast, approximately twice as much of the IgG2a IC inoculum remained in circulation at 8 min. Thus, IgG2a IC were less efficiently cleared, compared with IgG1 IC or IgG3 IC.

Deposition of IC in tissues

At 8 min, mice were killed and IC deposition in the liver, lungs, spleen, and kidneys was measured (Fig. 2). The majority of circulating IC were deposited in the liver (Fig. 2A) with relatively little splenic clearance (Fig. 2B). A considerable portion of the IC also deposited in the lungs (Fig. 2C). Compared with the other two IC preparations, IgG3 IC were more efficiently cleared by the liver and commensurately less well trapped in the lungs. The most noteworthy finding was that the IgG2a IC were preferentially deposited in the kidneys (Fig. 2D). To determine whether the observed trapping of IC in the kidney reflected actual tissue deposition, the aorta
was ligated a few millimeters above and below the renal arteries, exposing a section approximately 1 cm in length. Approximately 1–2 ml of PBS was then injected into the aorta. The kidneys became visually pale after this procedure, indicating that the perfusion had washed out the major portion of residual blood from the renal vasculature. Perfusion of kidneys from mice injected with radiolabeled IgG2a IC by this procedure dislodged, 20% of the IC trapped by the kidney (data not shown). Thus, renal trapping of IgG2a IC seemed to be relatively stable even though only 8 min had elapsed after IC infusion and the radioactivity detected represents actual tissue deposition rather than transitory adherence within the renal vasculature.

Glomerular IC deposition

To further localize the site of IgG2a IC deposition in the kidneys, tissue samples from mice infused with unlabeled IC were stained to detect the presence of Ig. For comparison, kidney samples from mice injected with IgG1 IC were also examined. The data indicated that the IgG2a IC were localized in glomeruli (Fig. 3B). In contrast, significant glomerular localization of IgG1 IC was not detected (Fig. 3A). In glomeruli, vascular and luminal Ig deposits were frequently observed in kidneys from mice injected with IgG2a IC, but only rarely in mice injected with IgG1 IC. Similar results were obtained in experiments in which IC deposited were visualized by immunofluorescence (data not shown). These data indicate that IgG2a IC are preferentially deposited in glomeruli.

FIGURE 2. Tissue deposition of IC. Animals were killed 8 min after IC infusion, and their spleen, kidneys, liver, and lungs were collected. The radioactive content of each organ is expressed as a percentage of the total infused IC dosage. There was no significant difference in the deposition of the three IC preparations in either the liver or the spleen. Significantly less IgG3 IC were deposited in the lungs, compared with the IgG1 IC and the IgG2a IC (p = 0.0001 by ANOVA). Significantly more IgG2a IC were deposited in the kidneys compared with IgG1 IC and IgG3 IC (p = 0.0001 by ANOVA). The data represent the mean values ± 1 SEM of individual determinations of the groups of mice enumerated in the legend of Fig. 2.

FIGURE 3. Histologic analysis of renal IC deposition. A representative kidney section from a mouse injected with IgG1 IC is shown in A, while a corresponding section from a mouse injected with IgG2a IC is shown in B. Glomeruli are denoted by arrows and IC deposits are stained red. Every panel is representative of tissue sections from groups of three mice.
IC size
Properties such as IC size (22), Ab affinity (23), charge (24–26), and Ag/Ab ratio (27, 28) may affect the clearance and tissue localization of IC. To determine how these variables contributed to IC clearance and particularly to the renal deposition of IgG2a IC, the three Abs used in the experiments shown in this study, or IC constructed with these Abs, were characterized by these criteria.

Sucrose gradient analysis revealed that the model IC used in this study consisted of large heterogeneous molecular populations (Fig. 4). These data are consonant with previous characterizations of similarly constructed model IC made with rabbit polyclonal (6, 7, 29) or murine anti-DNP mAbs (8). The relative size of the model IC preparations was IgG3 IC > IgG1 IC > IgG2a IC. Since larger IC are generally more efficiently cleared from the circulation than smaller IC, these data are consistent with the observation that IgG2a IC are cleared less efficiently than the other IC probes used in this study (Fig. 1). However, in view of evidence that larger IC tend to be more pathogenic (30), these data do not explain the enhanced glomerular deposition of IgG2a IC.

IC charge
There is evidence that cationicity favors the deposition of molecules in glomeruli by charge interaction (24–26). To determine whether the enhanced renal deposition of IgG2a IC could be attributed to charge, the three Abs were compared by isoelectric focusing (Fig. 5). The spectrotype of each of the Abs consisted of multiple bands. Evidence presented elsewhere (15) indicates that these spectrotypic variants represent distinct glycoforms. The data shown in Fig. 5 revealed that the isoelectric mobility of the IgG2a Abs was intermediate between that of IgG1 and IgG3 Abs. Thus, the preferential renal deposition of IgG2a IC could not be attributed solely to charge.

Ab affinity
The relative affinity of the Abs used to form the model IC probes was compared (Fig. 6). The data indicate that all three Abs had comparable affinities, thus excluding this variable as a primary cause of enhanced renal uptake of IgG2a IC.

Ag/Ab ratio
The relationship between Ag/Ab ratio and glomerular IC deposition was also examined. As shown in Fig. 7, there was consider-
mice, but continued to decline in complement-depleted mice. A similar clearance pattern was observed with IgG2a IC. Significantly lower levels of IgG2a IC were detected in complement-depleted compared with normal mice at the 2-, 4-, 6-, and 8-min time points.

Hepatic deposition was increased for IgG1 IC and IgG3 IC in complement-depleted compared with normal mice (Table I). In contrast, there was no significant difference in hepatic deposition of IgG2a IC in complement-depleted vs normal mice. Comparable deposition in the lungs and spleen was observed using all three IC probes. However, there was a striking difference in the deposition of IgG2a IC in kidneys under conditions of complement depletion (Table I). While complement depletion had no effect on kidney deposition of IgG1 IC or IgG3 IC, kidney deposition of IgG2a IC was decreased to less than 50% of the level observed in normal mice (0.26 ± 0.04% vs 0.57 ± 0.06%). Thus, enhanced kidney deposition of IgG2a IC in normal mice was complement dependent.

**Discussion**

The main discoveries reported in this study are the observation that IgG2a IC lodge in glomeruli and the determination that enhanced renal deposition by these IC is complement dependent. These findings underscore the role of the complement system in the clearance and handling of IC. The identification and characterization of IC with a predilection for renal deposition also provide a model system for exploring the mechanism of IC deposition in tissues vulnerable to IC-mediated injury.

The physical characteristics of the Ag and Ab components of IC can influence IC clearance. As has been previously reported for similar IC made with both polyclonal (6, 7) or mAbs (8), all three IC types used in this study consist of heterogeneous populations of large molecules. However, the average size of the IgG2a IC preparations was somewhat smaller than that of the other IC probes. The smaller size of the IgG2a IC may, in part, explain why these IC are cleared from the circulation less efficiently than the IgG1 IC or the IgG3 IC. In fact, there is evidence that larger size favors adherence to primate erythrocyte CR1 (29), which in turn may facilitate IC clearance by the cells expressing complement receptors.

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Relation between Ag/Ab ratio and the amount of IC deposited in the kidneys. Each datum point represents the Ag/Ab ratio of one mouse plotted against the percentage of IC deposited in the kidneys of that mouse. The composite data reflect groups of 16 mice injected with IgG1 IC (squares), 20 mice injected with IgG2a IC (circles), and 13 mice injected with IgG3 IC (triangles). Statistical analysis using the Spearman correlation test revealed no significant correlation between Ag/Ab ratio and kidney deposition.

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** Effect of complement depletion on IC clearance. The clearance of IC in CVF-treated mice (solid circles) was compared with the clearance of IC in untreated mice (open circles). Groups of CVF-treated and untreated mice were injected with the same preparation of IC to minimize the variables of Ag/Ab ratio and IC size. IgG1 IC were injected into 6 CVF-treated and 6 untreated mice. IgG2a IC were injected into 10 CVF-treated and 10 untreated mice. IgG3 IC were injected into 5 CVF-treated mice and 3 untreated mice. Levels of circulating IgG2a IC were lower in CVF-treated vs untreated mice at the 2-, 4-, 6-, and 8-min time points (p = 0.037, 0.004, 0.00065, 0.00034, respectively, by Student’s t test for unpaired data). Each datum point represents the mean value ± 1 SEM of all the mice injected with each preparation.
The negatively charged glomerular basement membrane presents a surface attractive for cationic molecules (24–26). However, in the present study, IgG3 IC were more cationic than IgG2a IC. Thus, charge does not appear to be the primary determinant of renal IC deposition in this model system. Likewise, variables such as Ab affinity and the Ag to Ab ratio of IC can affect biological properties of IC, including complement activation (31). However, neither of these variables appears to play a significant role in tissue localization in the present study.

It is noteworthy that the IC probe with the greatest propensity to lodge in glomeruli is of the IgG2a isotype. The data reported in this work do not differentiate isotypic from clonotypic factors that may mediate this behavior. However, the results of a study using expanded panels of IC constructed with 14 independently derived IgG1, IgG2a, and IgG3 mAbs indicate that while not all IgG2a IC preferentially deposit in the kidney, no isotype except IgG2a formed IC with a predilection for renal deposition, and that enhanced renal deposition was independent of variables such as Ab charge or affinity, Ag/Ab ratio, or IC size (Gonzalez and Waxman, manuscript in preparation). There is evidence suggesting that IgG2a and IgG2b Abs play a role in the pathogenesis of the murine model of SLE. In both NZB and NZB/W mice, the onset of clinical disease occurs in close temporal relationship to the isotype switch from IgM to IgG2a and IgG2b anti-DNA Abs (32, 33). There is also an overrepresentation of IgG2a and IgG2b Abs in kidney eluates from MRL/lpr mice, and IgG2a is the predominant Ab in kidney eluates from NZB and NZB/W mice (34). The anti-Sm autoantibody response in MRL/lpr mice is largely restricted to the IgG2a isotype (35). Moreover, 80% of the anti-chromatin Abs in the sera of MRL/lpr mice are of the IgG2a or IgG2b isotypes (36). The majority of MRL/lpr serum rheumatoid factors are reactive with the IgG2a isotype (37).

There is also evidence that strains of mice that do not spontaneously develop SLE develop progressive glomerulonephritis when treated at birth with IFN-γ (38), a cytokine that promotes the isotype switch to IgG2a (39, 40). B cells from MRL/lpr mice with active disease responded to IFN-γ by producing higher levels of IgG2a Abs than B cells from mice that had not yet developed clinical disease (41). Moreover, recent data indicate that IFN-γ receptor deletion prevents autoantibody production and glomerulonephritis in NZB/W mice (42). Although IFN-γ may cause physiological changes that are independent of its role in driving the isotype switch to IgG2a, the collective data strongly suggest an association between IgG2a/IgG2b Abs and renal disease.

The reason that IgG2a and IgG2b Abs are evidently involved in the pathogenesis of murine SLE is unknown. However, it is of interest that IC made with IgG2a or IgG2b mAbs are uniquely susceptible to factor I-mediated release from erythrocytes (12). Thus, the development of renal disease in the murine model may be favored by the unusually rapid factor I-mediated cleavage of C3b bound to IgG2a- or IgG2b-containing IC, causing inefficient adherence to the platelet analogue of primate erythrocyte CR1 and ultimately resulting in renal deposition.

Consonant with this model, clearance data presented in this work indicate that IgG2a IC are less efficiently cleared from the circulation than the other IC probes. In primates, IgA IC that were weak complement activators bound relatively poorly to erythrocyte CR1 and were preferentially deposited in glomeruli (8). Presumably, circulating IC displaying multiple ligand sites for complement receptors exist in a dynamic balance between adherence to the complement receptor and their subsequent release as a consequence of factor I-mediated cleavage of C3b/C4b to fragments that no longer bind to the complement receptor. When these events occur on an IC surface, such as that expressed by IgG2a Abs, that renders the C3b/C4b molecules more susceptible to factor I-mediated cleavage or on an IC that serves as a poor complement activator, the dynamic balance would tend to favor the off reaction with respect to immune adherence. In this case, a greater percentage of IC would be free in the circulation rather than attached to a circulating cellular complement receptor at any given time point. Other factors such as diminished erythrocyte CR1 expression (43, 44) and hypocomplementemia (45) that are associated with exacerbation of IC-mediated diseases such as SLE would also tend to tilt the balance away from IC bound to complement receptors toward the alternate state of IC free in the circulation. When these free circulating IC transit glomeruli, they may be more readily trapped therein compared with IC bound to circulating cellular complement receptors during transit. Thus, the combination of factors, including an IC surface that is a poor complement activator or on which bound C3b/C4b are highly susceptible to factor I, reduced erythrocyte CR1 expression, and hypocomplementemia, may all act in concert to interfere with the normal complement-dependent clearance mechanism and to thereby favor IC deposition in the kidney.

Under conditions of complement depletion, clearance from the circulation of all three types of IC was accelerated. This is consistent with data obtained in primates indicating that complement depletion accelerates IC clearance (7). Interestingly, hepatic uptake of IgG1 IC and IgG3 IC was significantly increased in complement-depleted vs normal mice. This may reflect differences in the rate of complement-dependent solubilization (46) in the normocomplementemic vs complement-depleted state. Under conditions of complement depletion, complement-mediated solubilization would be reduced and the relative size of IC would consequently remain larger than in the normocomplementemic state in which complement-mediated solubilization reduces IC size. The larger average relative size of circulating IC in the complement-depleted state would render the IC more susceptible to RES clearance, resulting in the increased hepatic deposition of IgG1 IC and IgG3 IC observed in the present study.

### Table 1. Comparison of tissue deposition of IC in normal and complement-depleted mice

<table>
<thead>
<tr>
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<th>% IC Deposited in Normal Mice</th>
<th>% IC Deposited in CVF-Treated Mice</th>
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<tbody>
<tr>
<td>IgG1 Liver</td>
<td>38.83 ± 2.36</td>
<td>49.02 ± 0.710</td>
</tr>
<tr>
<td>Lung</td>
<td>45.05 ± 1.36</td>
<td>42.93 ± 6.507</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.67 ± 0.200</td>
<td>1.28 ± 0.070</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.348 ± 0.011</td>
<td>0.395 ± 0.039</td>
</tr>
<tr>
<td>IgG2a Liver</td>
<td>50.4 ± 1.69</td>
<td>51.36 ± 1.79</td>
</tr>
<tr>
<td>Lung</td>
<td>31.78 ± 3.19</td>
<td>35.94 ± 3.47</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.48 ± 0.320</td>
<td>1.69 ± 2.17</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.570 ± 0.060</td>
<td>0.256 ± 0.039</td>
</tr>
<tr>
<td>IgG3 Liver</td>
<td>65.17 ± 1.68</td>
<td>76.3 ± 1.95</td>
</tr>
<tr>
<td>Lung</td>
<td>15.67 ± 3.18</td>
<td>10.14 ± 0.524</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.4 ± 5.200</td>
<td>1.94 ± 0.144</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.220 ± 0.001</td>
<td>0.294 ± 0.040</td>
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*p = 0.002 by Student’s t test for unpaired data.

*p = 0.004 by Student’s t test for unpaired data.

*p = 0.009 by Student’s t test for unpaired data.
In contrast to IgG1 IC and IgG3 IC, complement depletion had no effect on the clearance of IgG2a IC by the liver. Evidence presented elsewhere indicates that the particular murine IgG2a mAb used to form IgG2a IC in the present study displays its sole carbohydrate moiety, a CIg2-linked N-glycan, in a manner that makes it relatively accessible to lectins and to glycosidases (15). It is possible that the relatively accessible orientation of the N-glycan may also render it more accessible to hepatic receptors for carbohydrates (47) and that these receptors play a more central role in the clearance of this IC compared with the other IC preparations used in this study. Alternatively, IgG2a IC may be a relatively poor target for complement-mediated solubilization. In this case, IgG2a IC size would be similar in the normocomplementemic vs the complement-depleted state, and hepatic clearance would be equivalent.

While preferential kidney deposition of IgG2a IC was observed in normal mice, complement depletion abrogated this enhanced IC deposition. Thus, the enhanced deposition of IgG2a IC in normocomplementemic mice is complement dependent. Evidently, the complement system can act to both clear IC safely and induce IC trapping in tissues vulnerable to IC-mediated injury. Further studies of how these model IC probes interact with the complement system may provide additional insight on the pathogenesis of IC-mediated diseases.

References