FcγRIIb on Liver Sinusoidal Endothelium Clears Small Immune Complexes

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It has long been known that the ITIM-bearing IgG Fc receptor (FcγRIIb) is expressed on liver sinusoidal endothelial cells (LSEC) and that the liver is the major site of small immune complex (SIC) clearance. Thus, we proposed that RIIb of LSEC eliminates blood-borne SIC, thereby controlling immune complex-mediated autoimmune disease. Testing this hypothesis, we found most RIIb of the mouse, fully three-quarters, to be expressed in liver. Moreover, most (90%) liver RIIb was expressed in LSEC, the remainder in Kupffer cells. An absent FcγR in LSEC implied that the sole FcγR expressed. Testing the capacity of liver RIIb to clear blood-borne SIC, we infused mice intravenously with radio-iodinated SIC made of OVA and rabbit IgG anti-OVA. Tracking decay of SIC from the blood, we found the RIIb knockout strain to be severely deficient in eliminating SIC compared with the wild-type strain, terminal half-lives being 6 and 1.5 h, respectively. RIIb on LSEC, a major scavenger, keeps SIC blood concentrations low and minimizes pathologic deposition of inflammatory immune complex. The Journal of Immunology, 2012, 189: 000–000.

A crucial clue to the mechanism of SIC elimination emerged 30 y ago when it was observed that mab 2.4G2, specific for the binding sites of all mouse FcγR, blocked liver uptake of SIC infused intravenously into mice (3). This finding implicated liver sinusoidal FcγR in the elimination of SIC. Many concluded that the Kupffer cells (KC) were responsible for such liver elimination. Rather, as we have recently found, liver sinusoidal endothelial cells (LSEC) expressing FcγRIIb (RIIb) are responsible for virtually all mab 2.4G2 binding in mouse liver (12). Several converging lines of recent evidence (4, 12–18) now indicate that the predominant FcγR on LSEC is RIIb, the low-affinity ITIM-bearing inhibitory receptor studied most carefully on B cells, macrophages, and dendritic cells (reviewed in Refs. 19, 20).

We are led by the evidence presented above to propose that RIIb expressed on LSEC are responsible for the ongoing removal of SIC from the blood. This hypothesis has two predictions; first, that liver expression of RIIb is remarkably abundant; second, that a mouse strain lacking RIIb will fail to remove SIC from blood. We tested these two predictions, the first by immunofluorescence microscopy and immunoblotting, measuring the expression of RIIb in liver compared with the entire animal; and the second by comparing the rates of elimination of radio-iodinated SIC from the blood in wild-type (WT) and RIIb knockout (KO) strains of mice.

Neither test rejected the hypothesis. Rather, a very high fraction, fully three-quarters, of total body RIIb was found in the liver, most...
on LSEC. Further, the removal rate of SIC from blood of RIIb KO mice compared with WT mice was severely curtailed. These results confirm our hypothesis and suggest new ways of conceptualizing and treating immune complex-mediated diseases.

Materials and Methods

Ethics statement and animals

Male mice of age 12−15 wk were obtained from Taconic Laboratory. They were of strains BALB/c WT and RIIb KO on a BALB/c background [C.129S4 (B6)-FcγR2mum107cR-Itac2N12C.129S4 and model number 580-M]. The RIIb KO mice were generated and described previously (21). All protocols were approved by The Ohio State University Institutional Animal Care and Use Committee. Bleedings were performed under isoflurane anesthesia, and all efforts were made to minimize suffering.

Cells

RAW 264.7, A20, and COS-7 cells were obtained from American Type Culture Collection. COS-7 cells were maintained in DMEM supplemented with 10% FBS. RAW 264.7 and A20 cells were maintained in RPMI 1640 medium supplemented with 5% FBS. Bone marrow macrophages (BMM) were differentiated and with M-CSF as previously described (22).

Quantitative immunoblot

The expression of RIIb and Syk in various organs was quantified by immunoblot as follows: organs (kidney, spleen, ileum, liver, heart, and lung) from three sacrificed mice were removed and weighed. Small pieces of ∼100 μg from each organ were homogenized with a glass homogenizer in a lysis buffer composed of 25 mM HEPES, 20 mM NaH2O2·10H2O; 100 mM NaF; 4 mM EDTA; 2 mM Na3VO4; 1% Triton X-100; 0.34 mg/ml PMSF; 0.01 mg/ml aprotinin, and 0.01 mg/ml leupeptin. Lymph nodes from axillary, mesangial, inguinal, and submandibular regions were pooled. Bone marrow cells were flushed from femurs, centrifuged at 376 × g for 5 min, and lysed. Blood (∼80 μl) was obtained from three mice via the retroorbital plexus using heparinized capillaries, and the erythrocytes were lysed by dilution in 10 vol of 20% PBS at room temperature for 10 min. Cells remaining were spun down at 376 × g × 5 × 5 × 5 × 5 for 5 min and lysed. All lysates were incubated on ice for 30 min and centrifuged at 18,407 × g for 15 min. The protein concentrations of spun lysates were estimated using the bicinchoninic acid protein assay.

Organ lysates (35 μg) along with lysates of control standards (0, 3, 6, 9, and 12 μg of lysates from A20 cell line for RIIb blots and 0, 5, 10, 15, 20 μg of lysates from RAW 264.7 cells for Syk blots) were separated on 8−16% gradient SDS-polyacrylamide gels, and the proteins were transferred to nitrocellulose membranes (0.45 μm). The membranes were blocked with 5% milk for 30 min and then incubated overnight with primary rabbit anti-mouse RIIb (kind gift from John Cambier) and rabbit anti-Syk (Santa Cruz Biotechnology) Abs at concentrations of 1:5000 at 4˚C. The bands with 5% milk–PBS prior to incubation overnight at 4˚C with primary Abs. The primary Abs rabbit anti-mouse RIIb, goat anti-mouse RIIb (kind gift from Mark Coggeshall), rabbit anti-mannose re- ceptor (Santa Cruz Biotechnology), and rabbit anti–FcγR-chain (Millipore) were all used at dilutions of 1:25. The 2.4G2 was used at a concentration of 20 μg/ml. After three washes with PBS, the sections were incubated with secondary Abs for 1 hr at room temperature. Nuclei were stained with DAPI for 10 min, and the sections were mounted under coverslips in Prolong gold (Invitrogen). Control incubations included isotype controls along with their respective secondary Abs and also secondary Abs alone. The images were acquired in the Olympus Fluoview 1000 Laser Scanning Confocal Microscope equipped with a spectral detection system for a finer separation of fluorochromes (FV 1000 spectra) using a ×60 oil immersion lens at room temperature. Image analyses were done using Fluoview software (Olympus version 2.1.39). In Fig. 4, to overcome the inherent limits of resolution in the z-dimension in our confocal microscopes, we used ultrathin cryosections of liver tissue as the substratum for high-resolution immunofluorescence microscopy as we have described (27−29). Preparation of tissue and ultrathin cryosections were described in detail earlier (30).

Quantitative microscopy

The relative liver expression of RIIb between LSEC and KC, shown in Fig. 3 (bottom row), was quantified from immunofluorescence images dual-stained for RIIb (green) using goat anti-RIIb Ab and anti-F4/80 (red) in the following manner. The total intensity of RIIb (green) in the images was calculated using ImageJ software. From this total, we subtracted the intensity of RIIb measured in all cropped KC, identified with F4/80. Thus, the total intensity of RIIb minus KC intensity of RIIb equaled the RIIb intensity of LSEC. Optical sections with a total area of 6.3 mm2 were analyzed from each of three different mice.

The fluorescence intensities from mannose receptor (MR) (green) and RIIb (red) channels in the ultrathin sections represented in Fig. 4 were obtained using Leica TCS SP5 confocal microscope software. Randomly selected signal intensities (n = 100−200) from merged color images were cross-sectioned eight times at 45˚ angles, and the intensities of both color channels of eight sections over the LSEC cross-sectional distance from luminal to abluminal surface were recorded. Data were analyzed using a paired t test.

Immunofluorescence from anti–FcγR-chain (green) was colocalized with signals from mab F4/80 and mab anti-F4/80 (red) in the following manner. The total intensity of RIIb (green) in the images was normalized to the background as described for Fig. 4, with the following modification. Individual LSEC (n = 60) were cropped from images obtained from two WT livers, and Mander’s overlap coefficient was expressed as percent colocalization. By cropping the LSEC, we avoided pixels from nuclei nonspecifically stained with the rabbit RIIb Ab. Signal from mab 2.4G2 colocalization with immune fluorescence from goat anti-RIIb in individual LSEC (n = 194) was quantified manually by scoring the number of LSEC that were positive for 2.4G2 (green) and goat anti-RIIb (red) (Fig. 2, bottom row).

RT-PCR

Total RNA extracted from TRizol lysates of organs and cell lines were transcribed to cDNA using Thermoscript RTase H-reverse transcriptase and amplified using primers specific for mouse RIIb. The RNA isolation protocol, primer sequences, and PCR conditions were described earlier (32). The RT-PCR products were resolved by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and imaged using Quantity One software.

ENDOTHELIAL FcRIIB CLEARS IMMUNE COMPLEXES
Quantitative precipitin reaction and small immune complex preparation

A single lot of affinity-purified rabbit IgG anti-OVA Ab (Immunology Consultants) was “calibrated” for its interaction with OVA (Sigma) by a quantitative precipitin reaction to derive a point of equivalence (POE) (2, 5, 33). Specifically, microfuge tubes containing serially diluted OVA at concentrations ranging from 6.5 to 156 μg/ml were each incubated with 200 μg/ml rabbit anti-OVA Ab in a total volume of 500 μl of PBS at pH 7.4 for 1 h at room temperature and 18 h at 4°C. The mixtures were centrifuged at 2000 × g for 10 min; the pellets were dissolved in glycin HCl buffer (0.05 M glycine and 0.15 M NaCl pH 2.3); and the protein concentrations of pellets and supernatants were determined by UV absorbance at 280 nm.

The calibration curve of the precipitin reaction yielded a distinct POE for Ag and Ab concentrations of 26 and 200 μg/ml, respectively. To prepare SIC for determination of particle size by dynamic light scattering (DLS) and for infusion into mice, we increased the protein concentrations 5-fold greater than the concentration used in the precipitin curve. SIC were prepared by mixing rabbit anti-OVA Ab at 1000 μg/ml (~5 × 200) with OVA (Ag) at 1500 μg/ml (26 × 5 × 15); a 15-fold excess over the POE. Ag concentration determined in the calibration curve, attempting to duplicate the conditions used by others (2, 3, 7). The mixture was incubated for 1 h at room temperature and 18 h at 4°C and was centrifuged at 2000 × g for 1 min. SIC size was measured by DLS. To prepare trace-labeled SIC for infusion into mice, radio-iodinated rabbit anti-OVA Ab (iodinated as described later) was added to unlabeled Ab at 1:500 mass ratio prior to mixing Ag with Ab exactly as described earlier.

Radio-iodination of Ab

Affinity-purified rabbit IgG anti-OVA Ab was dialyzed against PBS pH 7.4 containing 1.06 mM KH2PO4, 155 mM NaCl, and 2.97 mM Na2HPO4,7H2O and radio-iodinated by a modified chloroglycouril method using chloroglycouril-coated tubes (Pierce). Briefly, at room temperature 1 μCi of Na125I (Pierce) was activated in a chloroglycouril-coated tube for 6 min, and the activated Na125I was transferred to 100 μl of Ab at 1.5 mg/ml. The iodination reaction was terminated at 9 min by adding 50 μl of 10 mg tyrosine/ml in PBS at pH 7.4. The radio-iodinated Ab was separated from free radio-iodine using PD-10 desalting columns equilibrated with 0.1% fish gelatin in PBS. The precipitability of labeled Ab in 12.5% iced TCA was >95%. Labeling efficiency was ~50%. The cpm/μg of Na125I-labeled Ab was 1.4 × 106 and 3.2 × 106 in two experiments.

Analysis of SIC using DLS

The particle size of SIC was estimated by DLS (BI-200SM Research Goniometer System; Brookhaven Instrument, Holtsville, NY) under the following conditions: detection angle 90°, laser wavelength 633 nm, temperature 20°C, measurement duration 2 min. The particles were diluted in 0.22-μm-filtered PBS. Fe3O4 particles of size range 200–250 (BD Biosciences), 10, and 5 nm (NN Laboratories) were analyzed to check the precision of the instrument and were found to have mean diameters of 209, 12, and 20 nm, respectively, suggesting imprecision at low particle size. The standard latex particles of size 40, 92, and 100 nm were found to have mean diameters of 42, 93, and 98 nm, respectively. The mean diameters by volume of Ag and Ab at a concentration of 1 mg/ml were 6 ± 1 and 5 ± 1 nm, mean ± variance of two observations, respectively. The mean diameter by volume of particles at the POE was 1228 ± 774 nm. The mean diameter by volume of SIC particles prepared at 3-fold excess Ag was measured to be 220 ± 14 nm. The characterization of SIC prepared at 15-fold excess Ag is presented in Fig. 7B and the text. All samples showed a monodispersed distribution with insignificant outliers and aggregates.

Clearance kinetics of SIC

To characterize in vivo SIC clearance kinetics, we infused by tail vein freshly prepared radio-iodinated SIC containing 1.4 × 106 and 1.9 × 106 cpm in 58 μl. Two identical experiments were performed 3 mo apart, in each infusing three WT and three RIIb KO mice matched for sex and age. The mice were bled of ~15 μl blood via the retro-orbital plexus at post-infusion times of 1, 5, 10, 20, 30, and 60 min. To adjust for different body weights of individual mice, the blood concentrations of radioactive SIC (cpm/10 μl blood) were normalized to an average value of dose/body weight among animals (i.e., a dose of 1.6 × 106 cpm/25.43 g body weight). Because a semilog plot of blood concentrations of iodinated SIC from both strains indicated biphasic decay, we used a biexponential decay model to fit the SIC concentration–time profile. Half-lives were calculated by 0.693/slope, where the slope was obtained from the biexponential decay analysis. Independent two-sample t test was performed to compare the means of two genotypes. Differences between WT and KO strains were considered significant at p < 0.05.

Quantification of SIC in various organs

Mice (n = 3) were infused intravenously with freshly prepared radio-iodinated SIC containing 1.1 × 106 cpm in 58 μl and were sacrificed at 25 min. The mice were bled of ~20 μl via the retro-orbital plexus; organs (liver, kidney, lung, spleen, and heart) were removed and weighed. SIC in the weighed portions of each organ were measured and quantified after factoring total organ weight.

Results

Most RIIb of the mouse is in the liver

We recently noticed that the expression of RIIb on LSEC was astonishingly high, far in excess of what we had perceived to be expressed on other RIIb reservoirs of the body such as spleen, lymph nodes, B cells, and macrophages (12). More precisely, assessing in our mind’s eye the brightness and extent of RIIb fluorescence in a microscopic field of view of liver sections, and multiplying this by the three dimensions of the liver, the body’s largest internal organ, the total quantity of RIIb would be far greater than that of any of the other RIIb sites of the body (data not shown). It has long been known that a like assessment of the human liver with a specific anti-RIIb Ab gives the identical impression by immunolabeling (14). Quantifying our visual impression, we measured by immunoblotting the expression of RIIb in lysates of the liver and spleen and most of the other major organs and tissues of the body (Fig. 1). Consistent with our visual impression, we found that of the total body pool of RIIb, fully 72 ± 5% (n = 3) was expressed in the liver, and the remaining 28% was spread among the other organs and tissues of the body, each being less than 10%. By mobility of the anti-RIIb identified bands, the liver appeared to express mostly the b2 protein isoform but also some b1, whereas, as expected, most of the RIIb in spleen was b1 (Fig. 1A). Our immunoblots of organ lysates affirm that this RIIb KO strain shows no evidence for the RIIb bands (data not shown). The band with b1 mobility in the kidney lane, as well as unidentified bands at 75 and 45 kDa in many lanes, were artifacts based on their presence in tissues from RIIb KO mice (data not shown). Further studies of kidney tissue sections examined by immunofluorescence (IF) microscopy with three anti-RIIb Abs showed no evidence for the expression of RIIb in the kidneys (data not shown).

Because our method of quantifying tissue expression of proteins from band densities in immunoblots entailed a large multiplicative factor for liver due to its relative size, we also measured the expression of the tyrosine kinase Syk, reasoning that a molecule expressed largely in the spleen would appear highly expressed in liver if our method were artifactually amplifying the extent of liver expression (34). Confirming the validity of our method, 63 ± 6% of total body Syk was expressed in the spleen but less than 10% in liver and all other organs except the ileum (13%) where B cells and macrophages are prevalent (Fig. 1B).

LSEC expression of RIIb confirmed with three anti-RIIb Abs

Within the liver, RIIb is predominantly expressed in LSEC (12, 14, 17, 35). We confirmed this conclusion by IF microscopy using three anti-RIIb Abs; that is, mab 2.4G2 and two polyclonal Abs from goat and rabbit, the last directed toward the cytoplasmic tail sequence of RIIb. We found that all three Abs gave similar and mostly overlapping signals in LSEC (Fig. 2); these signals were specific for RIIb in that liver sections from RIIb KO mice showed
no LSEC binding (data not shown). None of these Abs gave positive signals with endothelium of portal and hepatic veins. All three Abs showed a weak staining pattern with KC as we reported earlier for mab 2.4G2 (12).

Within the liver, most RIIb is in LSEC

We quantified by IF microscopy of liver sections the relative expression of RIIb between LSEC and KC by colocalizing the binding of two anti-RIIb Abs with the KC marker F4/80 (Fig. 3). We found that of the total liver RIIb pixel intensity, 90% was expressed in LSEC and 10% in KC.

RIIb expression in LSEC predominates toward the apical membrane

Having earlier found that RIIb in the endothelium of the human placenta is expressed in the interior of the cell and not at the plasma membrane (28), we used the same strategy to localize RIIb in mouse LSEC, examining ultrathin (<100 nm) sections of liver by three-color IF microscopy, comparing anti-MR binding, which is relatively specific for LSEC (12), with anti-RIIb Ab binding (Fig. 4A). Quantifying the pixels of the two colors in merged images (Fig. 4B), we concluded that, unlike its localization in human placental endothelium, RIIb does not predominate in the interior of the cell; rather it appeared to be more highly expressed toward the apical portion of the LSEC, whereas MR was more diffusely spread throughout the cell.

The RIIb mRNA isoform expressed in liver is b2

Having found that LSEC-expressed RIIb constitutes nearly all RIIb of liver (Fig. 3), we realized that we could easily determine the mRNA isoform of RIIb in LSEC by RT-PCR analysis of whole-liver mRNA, comparing band sizes with known sizes from RIIb-expressing cells characterized earlier by others. Such an experiment indicated that the predominant isotype in liver was b2 with only a trace band of b1 being seen in the agarose gels (Fig. 5). We have analyzed the published cDNA sequence of rat RIIb from LSEC that was not isotyped in the original publication and find that the isotype was b2 and not b1 (17). Thus, human, rat, and mouse RIIb, which are highly expressed in LSEC, are of the b2 isotype (14, 16, 17).

Fcγ-chain is expressed only in KC and not in LSEC

We were unable to determine with confidence whether other members of the FcγR family were expressed in LSEC because of a dearth of reliable Abs to RI, RIII, and RIV that would be useful in IF microscopy. However, as all three of these agonist receptors require for function the association of the Fcγ-chain (36–38), we realized that by assessing Fcγ expression in LSEC, we could...
indirectly assess the expression of the agonist members of the FcγR family in mouse. By three-color IF microscopy, we found FcγR to be expressed only in the KC of the liver and not in the LSEC (Fig. 6A). Quantifying the colored signals in these images, we affirmed that FcγRγ was a distinctive feature of KC and not LSEC (Fig. 6B). Thus, it seems likely that RI, RIII, and RIV are not expressed in LSEC.

**Quantitative precipitin curve and SIC characterization**

We prepared SIC using a quantitative precipitin curve described 76 y ago by Heidelberger and Kendall (5). Calibrating a single lot of affinity-purified rabbit IgG anti-OVA Ab, we determined the concentrations of Ab and OVA at the POE where the immune complexes precipitated maximally (Fig. 7A). We then prepared SIC at a concentration of Ag 15-fold greater than the POE concentration, conditions found by others to yield SIC (2, 3, 5). Measured by DLS, these SIC had a mean diameter by volume of ∼39 nm with a range from 20 to 150 nm (Fig. 7B).

**SIC are not cleared efficiently in the RIIb KO mouse strain**

Testing a prediction of our central hypothesis, that the RIIb of LSEC serves the purpose of removing SIC from circulation, we measured the elimination rate of SIC from the bloodstream of both WT and RIIb KO strains. Mice were infused by tail vein with trace radio-iodinated SIC prepared at 15 times Ag excess, as described earlier; the decay of gamma-radiation from peripheral净值归
blood was followed over the course of 1 h; and the data in cpm
were plotted in semilogarithmic fashion (Fig. 8). Indeed, our
prediction was not falsified. Rather, we found that the rate of SIC
clearance from the RIIb KO strain was dramatically retarded
compared with the WT strain. Specifically, both curves described
biexponential decay with early and late phases. Decay was most
obvious during the late phase, from 10 to 60 min, where half-
lives were 87 ± 6 min and 367 ± 222 min for WT and KO
mice, respectively (mean ± SD, n = 5 to 6, p = 0.0001), indicating a 3-fold lengthier survival of SIC in the absence of RIIb.
In contrast, decay rates during the early 1- to 5-min period were
not statistically different between the two strains, half-lives for
WT and RIIb KO strains being 12 ± 6 min and 19 ± 10 min,
respectively (n = 5 to 6, p = 0.2) (Fig. 8). Note also that the cpm
at 1 min were >20% lower in WT compared with the KO strain
(see Discussion).

Quantification of SIC in various organs
Documenting that infused SIC homes mostly to liver, we autopsied
three mice 25 min after infusion of radio-iodinated SIC. Of the
total administered dose, we recovered 72 ± 6% in blood, 27 ±
2% in liver, 0 ± 1% in lung, 1 ± 0% in spleen, and 0 ± 1% in
kidney (mean ± SD). Thus, nearly all of the cleared SIC (96%)
was found in the liver.

Discussion
Our results allow two major conclusions. First, RIIb is highly
expressed on the endothelial cells of the liver sinusoids. This im-
pression is easily reached by simple inspection of a section of liver
stained with anti-RIIb Ab. But, wanting to quantify our visual im-
pression, we have measured by immunoblotting the amount of RIIb
expressed in the major organs of the body and have concluded that

FIGURE 6. FcγRIIb clears immune complexes. (A) The confocal IF image in the left column illustrates the expression pattern of
γ-chain (green). The middle column shows the location of KC shown using anti-F4/80 (red) in the top panel and the location of LSEC using mab anti-
2.4G2 (red) in the bottom panel. The merged color image shows the colocalization of signal from γ-chain with signals from F-4/80 and 2.4G2 along with
DIC and DAPI staining of nuclei. Scale bars, 10 μm. (B) Quantitative colocalization analysis was applied to all images represented in (A). The graph
represents the percentage of signal from γ-chain Ab (green) colocalizing with signal from F4/80 or 2.4G2 (red) Ab and vice versa in the top panel of (A)
and also for the bottom panel (mean ± SD). Using a random-effects linear regression model, the data from bars 1 and 3 and also between bar 2 and 4 were
analyzed. *p < 0.001.

FIGURE 7. (A) Quantitative precipitin curve. The graph shows a quantitative precipitin curve plotting the protein concentrations of immune precipitates
(pellets) from a series of tubes incubating increasing concentrations of Ag and a fixed concentration of Ab. The peak marks the POE. (B) Particle size
measurement. Particle size distribution of SIC prepared at a 15-fold Ag excess was measured using DLS; the data obtained with Dynapro software are
presented. The graph plots particle mean (± variance) diameter in nm on the x-axis, and the y-axis shows the percent distribution of total particles cal-
culated using the relative amount of particles [G(d)] at each size.
The third remarkable element of our second major conclusion is that a specialized endothelium, the LSEC, is the predominant cell responsible for SIC elimination in that 90% of liver RIIb is expressed on LSEC. The LSEC, according to the literature, is a vigorous scavenger of bloodstream detritus, expressing a variety of surface receptors for blood-borne material, displaying abundant coated vesicles and lysosomes appropriate for a disposal mechanism (45), and in our hands eliminating blood-borne virus with remarkable efficiency (12). Further, the uptake of immune complex by LSEC has been well documented by others (4, 13, 16, 46). We note parenthetically that FcyR-expressing endothelium has been found elsewhere only in the human placenta, and there, too, the isofrom expressed is RIIb (28).

The absence of the small fraction (10%) of total liver RIIb expressed on KC cannot be responsible for the slow rate of immune complex elimination in the RIIb KO mouse. Rather, the absence of RIIb on KC would be predicted to render the KC more vigorously endocytic due to overactive agonistic FcyR unopposed by the inhibitory RIIb. The result would be a more rapid decay of blood-borne SIC in the KO strain compared with the WT strain, the exact reverse of what we found.

We find the decay kinetics of SIC in the RIIb KO strain noteworthy on two accounts. First is the striking decrease in the rate of elimination during the latter of the two biexponential elimination phases, from 5 to 60 min, indicating that RIIb is largely responsible for the removal of blood-borne SIC. Second is the observation that the 1-min point on the WT curve is >20% lower than the 1-min point of the KO curve (p = 0.002, Fig. 8) despite the strains not only being matched for age and sex but also adjusted for radioisotope dose and body weight; and, further, that the period from 1 to 5 min shows similar rates of decay in both strains. We interpret these kinetics to suggest that during the first minute after infusion, the LSEC RIIb becomes saturated with SIC that pinocytose into (likely) coated vesicles, disappearing from the LSEC surface such that for the next 5 min, no more RIIb-specific elimination occurs. Then, after 5 min, RIIb begins to reappear on the LSEC surface, either by re-expression of endocytosed receptor, suggested by others (16), or by biosynthesis; and an ongoing elimination of SIC ensues at a vigorous rate (half-life ~90 min). This supposition suggests a fruitful path of insight into the mechanism of elimination and warrants detailed investigation.

We call attention to old observations that the repetitive infusion of SIC, such as those used here in our study, causes serum sickness with characteristic microscopic findings of glomerulonephritis (6, 7, 47). We speculate that LSEC RIIb, mediating the elimination of SIC, may critically attenuate the manifestations of serum sickness. One would further anticipate that a strain of mouse lacking only LSEC RIIb may be more susceptible to serum sickness and other soluble immune complex-mediated diseases, whereas targeted elimination of RIIb in B cells and myeloid cells would not produce like effects. Likewise, animals harboring mutants of the RIIb gene that result in diminished or defective receptor expression may as well be more susceptible to immune complex-mediated diseases. Such mutants have been described although their analyses appear to have focused on B cell and myeloid RIIb expression, whereas the evaluation of RIIb expression on LSEC would be expected to reveal more telling results (20, 48, 49).

Summarizing for clarity, risking oversimplification, we would propose that SIC are constitutively eliminated by LSEC RIIb, but when this capacity is overwhelmed, the escaping immune complexes become large and lodge in end organs to result in disease. Alterations in the expression and function of RIIb would modify this course of events.
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Disclosures
The authors have no financial conflicts of interest.

References

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