Differential Binding of Immunoglobulin A and Immunoglobulin G1 Immune Complexes to Primate Erythrocytes In Vivo

Immunoglobulin A Immune Complexes Bind Less Well to Erythrocytes and Are Preferentially Deposited in Glomeruli

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Abstract

Primate erythrocytes appear to play a role in the clearance of potentially pathogenic immune complexes (IC) from the circulation. This study was undertaken to compare the clearance from the circulation and tissue uptake of two monoclonal IC probes: one of which, IgG1-IC, was bound well by erythrocytes, the other of which, IgA-IC, was bound relatively poorly by erythrocytes. The IC probes were labeled with different iodine isotopes and infused either concommitantly or sequentially into the arterial circulation. The results indicate that, compared with IgG1-IC, IgA-IC bind less well to primate erythrocytes, are cleared from the circulation more quickly despite their smaller size, and show increased uptake in kidney and lung but decreased uptake in liver and spleen. Evidence is presented which suggests that this pattern of clearance from the circulation and systemic uptake of IgA-IC is the result of decreased binding of IgA-IC to circulating erythrocytes. These findings support the hypothesis that the primate erythrocyte-IC clearing mechanism may be critically important for the safe removal of IC from the circulation.

Introduction

Recent observations suggest that primate erythrocytes serve as a defense mechanism for removing potentially pathogenic immune complexes (IC)[1] from the circulation (1, 2). Erythrocytes rapidly intercept circulating IC by the binding of C3b sites on the IC to erythrocyte complement receptors, type one (CR1). The erythrocytes can carry the IC to reticuloendothelial organs and there deposit the IC (1, 2). The erythrocytes then return to the circulation apparently able, once again, to participate in this "primate erythrocyte-IC clearing mechanism" (1, 2).

The full biological consequences of the in vivo interactions between IC and erythrocytes, or the failure of IC to interact with erythrocytes, remain to be determined. In an effort to better understand how erythrocyte-IC interactions are affected by the biophysical properties of IC, we prepared a panel of mouse monoclonal anti-dinitrophenol (DNP) antibodies and then formed IC with DNP-bovine serum albumin (DNP-BSA). Examination of the binding properties of the individual IC preparations in vitro revealed that IgA-containing IC (IgA-IC) were bound relatively poorly by primate erythrocytes compared with IgG1-containing IC (IgG1-IC). This observation presented the opportunity to compare the fate in vivo of an IC probe that bound well to erythrocytes compared with that of an IC probe that bound relatively poorly to erythrocytes. Thus, the present study was undertaken to determine if there was a relationship between the magnitude of IC to erythrocyte binding, the rate of IC clearance from the circulation, and tissue uptake of IC. The results indicated that, compared with IgG1-IC, IgA-IC were bound less well by primate erythrocytes in vivo and were cleared from the circulation more quickly, despite their smaller size. There was also an enhanced uptake of IgA-IC in glomeruli. These data provide the first direct experimental evidence supporting the hypothesis that, in primates, the failure of IC to bind to erythrocytes results in the preferential uptake of these potentially pathogenic IC in vulnerable organs.

Methods

Preparation of DNP-BSA

DNP-BSA was prepared using 2,4-dinitrobenzene sulfonic acid (DNBS) as follows: equal amounts of ^{125}I-BSA and potassium carbonate were added to phosphate-buffered saline (PBS) (pH 7.2) to achieve a final BSA concentration of 1 mg/ml. DNBS (Eastman Kodak Co., Rochester, NY) was then added. By varying the concentration of DNBS, as well as the incubation times at 37°C, a panel of DNP-BSA compounds with varying hapten/carrier ratios was constructed. The reaction mixture was passed through a Dowex column to remove unreacted DNBS. The protein concentration was determined by the Lowry method and absorbance was measured at 360 nm for determination of DNP concentration. Specific activity was measured in a gamma scintillation spectrometer (model 5230, Packard Instrument Co, Inc., Downers Grove, IL). A preparation containing 25 DNP groups per BSA molecule was used to sensitize mice for hybridoma production, while preparations containing 40–60 DNP groups per BSA molecule were used to form IC.

Preparation of mouse monoclonal antibodies

IgA. IgA monoclonal antibodies reactive with DNP were produced from mouse myeloma MOPC 315 obtained from the American Type Culture Collection, Rockville, MD. Pristane-primed Balb/c mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were injected intraperitoneally with 10^6 MOPC 315 cells to raise IgA-containing ascites fluid.

1. *Abbreviations used in this paper:* CR1, CR2, and CR3, complement receptors types 1, 2, and 3, respectively; DNBS, 2,4-dinitrobenzene sulfonic acid; DNP, dinitrophenol; ELISA, enzyme-linked immunoassay; IC immune complex; IgA-IC, IgA-containing IC; IgG1-IC, IgG1-containing IC.

IgG1. Balb/c mice were sensitized with DNP-BSA and hybridomas were produced by the procedure described by Kohler and Milstein (3) as modified by Chesebro et al. (4). Anti-DNP antibody producing hybridomas were identified using a DNP-specific enzyme-linked immunoassay (ELISA). Wells containing hybridomas at limiting dilution were again screened for DNP reactivity, and cell populations in positive wells were expanded. These cells were used to produce IgG1-containing ascites fluid as described above for IgA.

The antigen specificity and isotype of monoclonal antibodies from donors selected by the ELISA assay were verified by gel diffusion. Ascites fluid from mice injected with DNP-specific clones formed precipitin lines with DNP-BSA, DNP-histone, and DNP-poly-L-lysine, but not with native BSA. The isotype was determined by first incubating anti-DNP monoclonal antibody and DNP_{25}-BSA at equivalence, followed by solubilization of the washed precipitate in DNP_{25}-BSA excess. The solubilized IC were then placed in a central well surrounded by antisera specific for murine γ_1, γ_{2a}, γ_{2b}, γ_3, α and μ heavy chains (Zymed Laboratories, San Francisco, CA). The monoclonal antibody from the clone used in this study formed a sharp precipitin line with anti-γ_1 serum only, while all isotype-specific antisera formed more diffuse precipitin lines with IC prepared with polyclonal mouse anti-DNP antiserum. The IgA antibody contained λ light chains. The IgG1 antibody contained κ light chains.

Formation of IC

BSA was labeled with ^{125}I or ^{131}I via the chloramine-T method as previously described (1). The free unreacted ^{125}I or ^{131}I was removed using Dowex-1-Cl (Sigma Chemical Co., St. Louis, MO). Ascites fluid containing monoclonal antibody was combined with the ^{125}I-DNP-BSA or ^{131}I-DNP-BSA at equivalence. This reaction mixture was incubated at 37°C for 30 min, then at 4°C for 15 h. The resulting precipitate was centrifuged at 1,200 g for 10 min, washed once with PBS, and then centrifuged again at 1,200 g for 10 min. The precipitate was then resuspended in the original volume of PBS and centrifuged for 20 min at 10 g. The supernatant fluid was used as the IC preparation. To form a given IC preparation, typically 0.7 mg of ^{125}I- or ^{131}I-labeled DNP-BSA was reacted with an amount of ascites fluid needed to achieve conditions of equivalence. The amount of antigen in the IC solution infused in each of the in vivo clearance studies was ~0.06 mg.

Binding of IC to erythrocytes in vitro

The assay measuring the amount of IC binding to erythrocytes in vitro was performed as described previosuly (6). Briefly, washed, packed erythrocytes (buffy coat cells removed), IC, and serum (complement source) were incubated at 37°C for 10 min. The mixture was then centrifuged on 65% iced percoll (density = 1.10 g/ml) to separate erythrocyte-bound from unbound IC, and the percent binding calculated (6). In each in vitro experiment, portions of the IC-erythrocyte reaction mixtures were also incubated with heat-inactivated (56°C for 30 min) baboon serum to assess complement-independent binding. Negligible (<4%) binding occurred under these conditions. The ^{125}I-DNP-BSA preparations (without antibody) did not bind to erythrocytes in normal or heat inactivated serum.

Primate experiments

In vitro experiments were performed on blood samples from 18 female baboons (*Papio cyanocephalus*). In vivo studies were performed on five female baboons. Previous experiments have not identified any difference in vitro (6) or in vivo (3) between males and females with respect to erythrocyte-IC interactions. The primates used for the in vivo studies weighed 10–28 kg. All studies were performed under general anesthesia as described previously (1, 2). In vivo exps. 1–3 were terminal experiments in which the animals were killed immediately after completion of the experimental protocol. These primates were prepared with a double-lumen catheter placed in the ascending aorta just above the aortic valve for the simultaneous infusion of the two different IC probes. A second arterial catheter was placed at about the level of the renal arteries for arterial blood sampling. In vivo exps. 4 and 5 were performed under

sterile conditions and the animals were not killed. These primates were prepared with a single-lumen angiographic catheter placed in the ascending aorta just above the aortic valve and an arterial catheter placed in the abdominal aorta at about the level of the renal arteries. In addition, catheters were placed under fluoroscopic control in the renal vein (right or left) and the right hepatic vein. These procedures have been described previously (1, 2).

Experimental protocol. The IgA-IC and IgG1-IC solutions were adjusted before infusion so that each IC preparation contained 4×10^6 cpm in 15 ml. The dose of IC used in these experiments is regarded as a trace dose, since it is far below that which saturates erythrocyte CR1 or the hepatic phagocytic system (1, 2, 6). In addition, when larger doses of IC were given to the same primate in three sequential doses, there was no change in IC to erythrocyte binding, IC clearance from the circulation, or hepatic uptake of IC (2). Before infusing the IC, the primates were first infused with autologous ^{51}Cr-labeled erythrocytes, as described previously (1, 2). The erythrocytes were allowed to circulate for a minimum of 20 min. The infusion of IC then commenced. In exps. 1–3, the IgA-IC (^{131}I-labeled) were infused into one port and the IgG1-IC (^{125}I-labeled) were infused into the other port of the aortic arch double-lumen catheter. The IC were delivered by a 2-syringe constant infusion pump that simultaneously delivered 15 ml of each IC solution over 120 s. Arterial blood samples were taken at 30-s intervals for 180 s and then at 4, 5, and 15 min, as described previously (1, 2). At 15 min, the primates received a lethal dose of pentobarbital.

In exps. 4 and 5, the same protocol was used except as follows: (*a*) The IC probes were both labeled with ^{125}I and were infused sequentially rather than simultaneously. In exp. 4 the IgA-IC was infused first. After 15 min had elapsed from the start of the first IC infusion, the IgG1-IC was infused. In exp. 5, the IgG1-IC was infused first. After 15 min had elapsed from the start of the first IC infusion, the IgA-IC preparation was infused. (*b*) In addition to arterial blood samples, blood samples were obtained from renal and hepatic veins.

As each blood sample was obtained during the experiments, it was immediately placed on ice and remained on ice until processed as described below. Icing the blood samples immediately stops further binding of IC to erythrocytes and prevents release of IC already bound to erythrocytes (1, 2). Thus, icing the blood samples stabilizes the binding of IC to erythrocytes at the same level that was present in vivo (1). All processing of blood samples was done within 2 h of completing the experiment. Arterial blood pressure was monitored throughout the experiments and was normal and stable in all protocols. At the end of exps. 4 and 5, the position of the hepatic and renal vein catheters was confirmed angiographically.

Processing of blood samples. The procedure for assessing IC binding to erythrocytes was described previously (1, 2, 6). Briefly, 0.5 ml of whole blood was placed on 2 ml of cold 65% percoll (density = 1.10 g/ml, Sigma Chemical Co.) and centrifuged at 350 g for 15 min. Under these conditions, an erythrocyte fraction (erythrocytes and IC bound to erythrocytes) migrates through the percoll to the bottom of the tube, while the leukocytes and IC not bound to erythrocytes remain on the surface of the percoll. The ^{51}Cr marker on the erythrocytes served as an indicator of the efficiency of the separation procedure. In all experiments, >99% of the ^{51}Cr was detected in the erythrocyte fraction, and <1% was present in the percoll fraction. The ^{125}I-cpm and the ^{131}I-cpm of the erythrocyte fraction and of the supernatant fluid were then determined in a gamma scintillation counter, and the percent of IC bound to erythrocytes was calculated as described previously (1, 2, 6). Most IC that are not bound to erythrocytes are free in the plasma since IC binding to blood leukocytes is quantitatively a minor event (2).

Tissue analysis. In exps. 1–3, tissue samples were taken from the following organs (the number after each organ indicates the number of specimens taken): brain (2), lung (2 peripheral, 2 or 3 central), heart (2, left ventricle), aorta (2, abdominal), stomach (2, central), small bowel (3, central), large bowel (3, central), skin (3, upper chest; 3, thigh), right kidney (1, central cortex), left kidney (1 or 2, central cortex), glomeruli (remaining cortex of both kidneys), medulla (1, both kidneys), liver (2 central, 2 peripheral), spleen (2 central, 2 peripheral), muscle (3, thigh).

Glomeruli were isolated by sieving techniques, as described previously (5). Each glomerular preparation was inspected under the microscope and was found to be composed of at least 85% glomeruli.

Analytical techniques

Antibody affinity determination. Association constants were determined for the two antibodies by equilibrium dialysis (7). Briefly, Plexiglass chambers were assembled from two complemetary halves, each containing an open end compartment with an 8,000-mol wt dialysis membrane (Spectrum Medical Industries, Los Angeles, CA) separating the two compartments. 1 ml of a given concentration of ascites fluid was added to one compartment of a series of chambers. To the opposite compartment was added 1 ml of ^{3}H-DNP–lysine (New England Nuclear, Boston, MA), with each chamber containing a different concentration. The chambers were incubated at room temperature on a table top shaker. At 24 h, duplicate 50-μl samples were removed from each compartment and assayed for radioactivity. The concentration of bound and free hapten was determined, and the inverse of these concentrations were plotted to determine association constants (8).

Isoelectric point determination. Isoelectric points of the monoclonal antibodies were determined by chromatofocusing (9). The ion exchanger (PBE 74) and eluting buffer (PBE 74, pH 3.5) were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). The PBE 74 was equilibrated with 0.025 M Imidazole-HCl, pH 7.3, before loading the antibodies.

Before chromatofocusing, the IgG1 was separated from ascites fluid by addition of Caprylic acid and 50% ammonium sulfate (10) and the IgA was separated from ascites by precipitation in 50% ammonium sulfate. Both monoclonal antibody preparations were then dialyzed against the equilibration buffer before loading on the column.

IC size determination. Isokinetic sucrose gradients were used according to the method of Johns and Stanworth (11), as described previously (1, 2).

IC complememt activation. The capacity of the IgA-IC and the IgG1-IC to activate complement was assessed by a standard method as follows: appropriate dilutions of serum were incubated with equal amounts of IgG1-IC, IgA-IC (based on equal cpm of ^{125}I-DNP–BSA) or with buffer alone at 37°C for 30 min. Specimens were then centrifuged to remove IC (>95% of the IC-cpm were recovered in the pellet). Each serum sample was then diluted serially and assayed for hemolytic activity by incubation of aliquots with sheep erythrocytes sensitized with rabbit IgG anti-erythrocyte (Cordis Laboratories Inc., Miami, FL) antibodies. After incubation at 37°C for 30 min, the samples were centrifuged at 1,500 g for 5 min to remove erythrocytes. The supernatant was then analyzed spectrophotometrically at 530 nm for free hemoglobin. To calculate CH_{50}, for each specimen the percent hemolysis was determined and the log of that value plotted against the log of the serum dilution.

Calculations. The ^{125}I and ^{131}I cpm were corrected for background, spillover and decay. All mean values are shown±1 SE of the mean. Statistical tests used are described in relation to the data.

Results

In vitro erythrocyte binding properties of IgA-IC and IgG1-IC. Fig. 1 demonstrates that, compared to IgG1-IC, IgA-IC bind poorly to primate erythrocytes in vitro. This is most evident under conditions in which the erythrocytes or serum (complement source) are limited (Fig. 1). Varying the incubation period from 10 to 60 min did not affect the relative binding of IgG1-IC vs. IgA-IC (data not shown). Fig. 2 demonstrates that, at limiting serum concentration, the amount of binding of IgA-IC to erythrocytes was always less than that of IgG1-IC. There were nevertheless considerable differences in the absolute amount of IgA-IC bound by erythrocytes in the 18 individual baboons. The IC binding capacity of the erythrocytes from each of the 18 baboons varied little when a second blood sample was obtained 7–14 d later (mean change for IgG1-IC: 1.6±1.5%; mean change
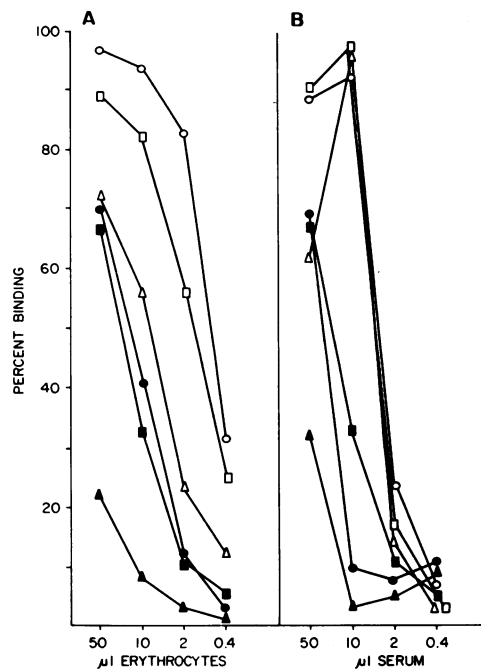

Figure 1. In vitro erythrocyte binding properties of IgA-IC and IgG1-IC incubated with varying volumes of baboon erythrocytes and baboon serum. In the representative experiment shown in *A*, 50 μl of IgG1-IC (open symbols) or IgA-IC (closed symbols), and 50 μl of baboon serum were incubated with varying volumes of packed erythrocytes from three baboons (each signified by a different symbol shape) for 10 min at 37°C. At 10 min, each reaction mixture was centrifuged on percoll, and the percent binding was calculated. The experiment shown in *B* was carried out under the same conditions with the following excpetions: the volume of packed erythrocytes was held constant at 50 μl and the volume of baboon serum was varied.
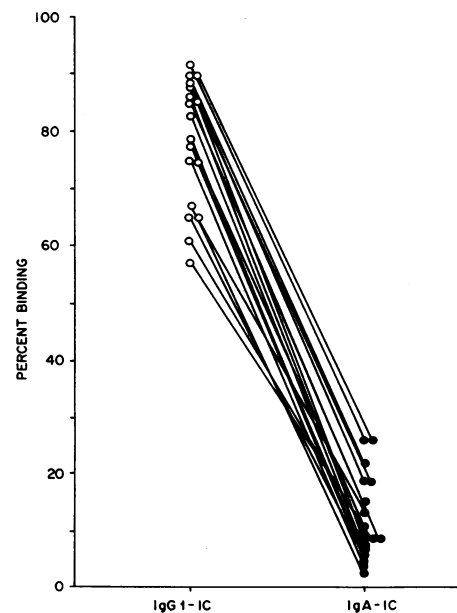

Figure 2. In vitro erythrocyte binding of IgA-IC and IgG1-IC incubated with limited volumes of baboon serum. Erythrocytes from 18 individual baboons were incubated with IgA-IC (●) or IgG1-IC (○) in the following proportions: 15 μl of packed erythrocytes, 15 μl of IC (containing ~3,500 ^{125}I-cpm), and 10 μl of baboon serum. The reaction mixtures were incubated for 10 min at 37°C, centrifuged on percoll, and the percent binding calculated.

for IgA-IC: $0.3\pm1.5\%$; both differences are not significantly different from zero by paired t test). Such differences in erythrocyte to IC binding may reflect differences in the density of erythrocyte CR1 receptors among baboons. Differences in density of erythrocyte CR1 receptors among humans have been noted previously and appear to be a genetically regulated characteristic (12).

In vivo erythrocyte binding properties of IgA-IC and IgG1-IC. Fig. 3 shows the sequential erythrocyte to IC binding data from the five in vivo experiments. As can be seen, the IgG1-IC were efficiently bound by erythrocytes in all experiments. In contrast, IgA-IC were bound less well by erythrocytes. In some primates the percent of IgA-IC bound by erythrocytes in vivo was better than that observed in the in vitro experiments. This may reflect the fact that in vivo there was a great excess of erythrocyte CR1 relative to IC, compared with the in vitro conditions used to assess IC to erythrocyte binding (Figs. 1 and 2).

Clearance of IgA-IC and IgG1-IC from the circulation. Fig. 4 shows the clearance from the circulation of IgA-IC and IgG1-IC. As can be seen, the IgA-IC were cleared from the circulation faster than IgG1-IC in all experiments. This is shown by the fact that in each experiment IgA-IC and IgG1-IC were given at the same rate and in the same number of cpm, but in each experiment the peak IC level achieved was less for IgA-IC than for IgG1-IC (mean ratio IgA-IC/IgG1-IC at 120 s was 0.80 ± 0.02, $P < 0.001$). The more rapid clearance of IgA-IC was seen whether the IgA-IC was infused simultaneously with IgG1-IC (exps. 1–3) or sequentially (exps. 4 and 5).
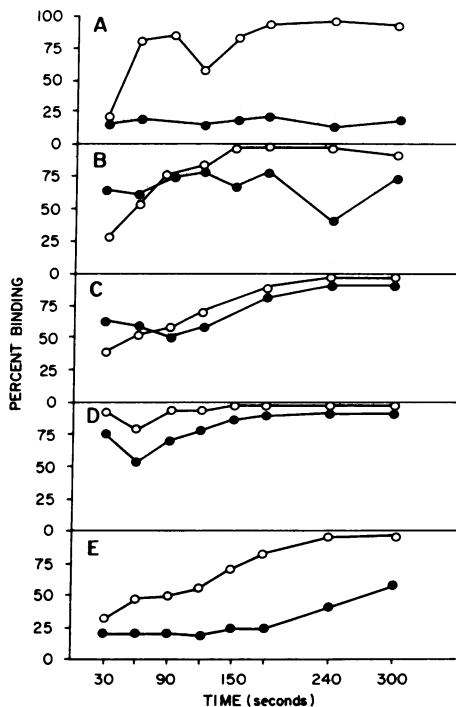
Figure 3. Binding of IC to baboon erythrocytes in vivo. In the experiments shown in *A, B,* and *C* (exps. 1–3), IgG1-IC (o) and IgA-IC (●) were infused concomitantly through a double lumen catheter. In the experiments depicted in *D* and *E* (exps. 4 and 5), the IgG1-IC (o) and IgA-IC (●) were infused sequentially at 15-min intervals. In each experiment the IC were infused at a constant rate beginning at time 0 and ending at 120 s. To assess IC to erythrocyte binding, blood samples were obtained periodically, centrifuged on percoll, and the percent IC to erythrocyte binding was calculated.
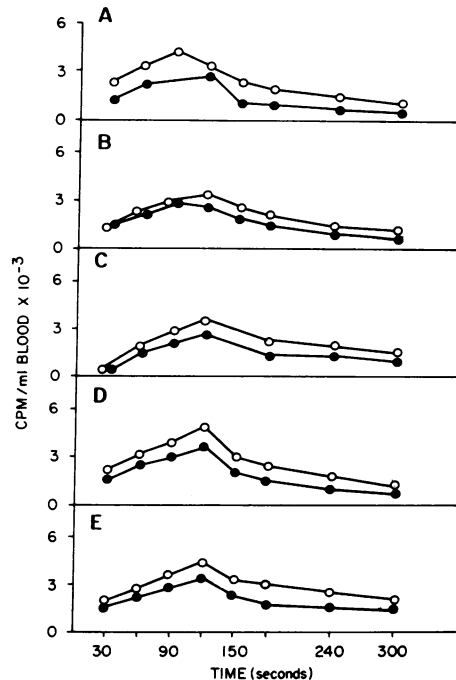
Figure 4. Clearance of IC from the circulation of baboons. IgG1-IC (o) and IgA-IC (●) were infused as described in the legend for Fig. 3. Blood samples were obtained periodically, centrifuged on percoll, and the total ^{125}I- or ^{131}I-cpm/ml of blood was calculated by adding the erythrocyte-bound cpm to the unbound cpm.

Clearance of IC from the circulation in relation to erythrocyte binding. To assess whether the speed with which the IC were cleared from the circulation was related to the degree to which IC were bound to erythrocytes, the IC clearance and the IC to erythrocyte binding events were analyzed during the period of rapid clearance, the 60-s period commencing immediately after cessation of the IC infusion. As discussed previously (1, 2), this time period is chosen for analysis because the most rapid clearance of IC from the blood occurs during this time period. In addition, during this time period the blood IC level is affected only by the rate of IC clearance, whereas before that time period, the blood IC level is affected by both the rate of IC infusion and the rate of IC clearance. At the start of the period of rapid clearance, $90\pm8\%$ of the IgG1-IC and $70.4\pm7\%$ of the IgA-IC injected were still present in the circulation.

To compare in the same primate, the rate of IC clearance to the level of IC to erythrocyte binding for each IC probe, the ratio percent IC bound to erythrocytes (IgA-IC/IgG1-IC), and the ratio, IC clearance rate (IgA-IC/IgG1-IC), were calculated from the data of the period of rapid clearance as follows: *percent IC bound to erythrocytes* for a given IC probe was taken as the mean of the percent IC bound at the 120-, 150-, and 180-s intervals; IC clearance rate for a given probe was taken as (cpm/ml of blood at 120 s—cpm/ml of blood at 180 s)/cpm/ml of blood at 120 s. The data for all experiments is shown in Fig. 5. As can be seen, the greater the disparity in IC to erythrocyte binding between IgA-IC and IgG1-IC, the faster was the clearance of IgA-IC relative to IgG1-IC ($r = 0.958$, $P < 0.02$).

Tissue uptake of IgA-IC and IgG1-IC. The primates used in exps. 1–3 were killed at 15 min after beginning the IC infusion. Thus, tissue IC levels reflect tissue uptake since relatively little catabolism of IC can occur in that period of time (1, 2). The
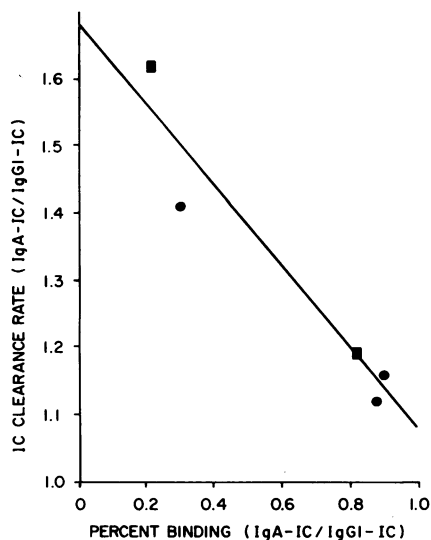
Figure 5. Clearance of IC from the circulation of relation to IC to erythrocyte binding. The ratio: (percent IgA-IC bound to erythrocytes/ percent IgG1-IC bound to erythrocytes) is shown in relation to the ratio: (IgA-IC clearance rate/IgG1-IC clearance rate) during the period of rapid clearance. See text for definitions. The circles represent exps. 1–3. The squares represent exps. 4 and 5.

data on tissue uptake for exps. 1–3 are shown in Table I. As can be seen, IgG1-IC were preferentially deposited in liver and spleen. In contrast, IgA-IC were preferentially deposited in lung and kidney. The consistency of the patterns of tissue uptake of IgA-IC vs IgG1-IC is shown in Fig. 7. Table I also shows the 2.7–4.7-fold increase in the glomerular deposition of IgA-IC relative to IgG1-IC. Note that at the time of killing the blood level of IgA-IC was lower than that of IgG1-IC in each experiment (Table I). Thus, the greater IgA-IC content of isolated glomeruli cannot be attributed to passively retained IgA-IC in the capillaries of
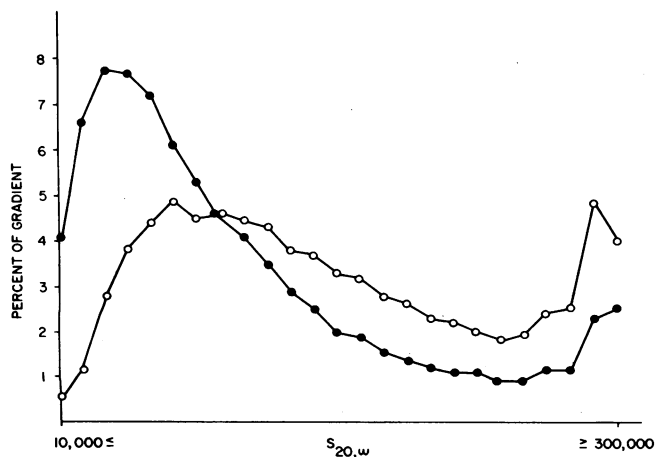

Figure 6. Relative size of IgA-IC vs. IgG1-IC. Aliquots of each IgA-IC (●) and each IgG1-IC (o) preparation from all experiments were separately subjected to isokinetic sucrose gradient centrifugation (n = 5). Each point represents the average value for the same fraction for the given IC preparation. The average SEM of the individual fractions is 0.4% for the IgG1-IC and 0.6% for the IgA-IC. The range of the SEM is 0.1–0.9% for the IgG1-IC and 0.2–1.7% for the IgA-IC. cpm bound to the tube represented an average of <5% for the IgG1-IC and <6% of the IgA-IC applied to the gradient.

the isolated glomeruli. Indeed, the opposite is true, because, at the end of the experiment, the blood level of IgG1-IC exceeded that of IgA-IC, IgA-IC deposited in glomeruli is actually greater than that shown.

By comparing all of the estimated cpm in glomeruli to the total cpm in renal cortex, it can be calculated that in exps. 1–3, 1.8, 4.3, and 7.4%, respectively, of IC in kidney were deposited in glomeruli. Since glomeruli comprise ~3–5% of the glomerular capillary network (13), and since that approximate percentage of IC in kidney were deposited in glomeruli, it is evident that glomeruli trap IC roughly in proportion to their volume (or surface area). This finding is consistent with our previous studies, using IC composed of polyclonal antibodies, in both primates (unpublished observations) and nonprimates (5, 13).

In exps. 4 and 5, fractional IC uptake by liver was estimated by measuring arterial-venous IC concentration differences. Consistent with our previous observations, there was nearly complete extraction of IC from the hepatic circulation for both IgA-IC and IgG1-IC. For exp. 4 there was >90% extraction of both IgA-IC and IgG1-IC. For exp. 5 there was >97% extraction of both IgA-IC and IgG1-IC. Thus, the greater hepatic uptake of IgG1-IC cannot be ascribed to decreased capacity of the liver to trap IgA-IC. Rather, the decreased IgA-IC uptake by liver demonstrated in exps. 1–3 must be ascribed to decreased delivery rate of IgA-IC to liver. This is due to the lower blood level of IgA-IC which, in turn, is the result of a greater rate of IgA-IC deposition elsewhere in the vascular network.

In exps. 1–3, tissue levels of IC were also measured in all of the other major organs, as described in Methods. However, in all of these organs, the ^{125}I and ^{131}I cpm/gram of tissue were <100 cpm/g of tissue, and most were <50 cpm/g of tissue. Because of the low cpm/g of tissue in these organs, the ^{125}I and ^{131}I cpm that is passively retained in the vascular space of these tissue contributed greatly to the total cpm/g of these tissues. This introduces a considerable error in estimating tissue uptake of IC, as discussed previously (5, 13). Thus, under these experimental conditions, it was not possible to reliably estimate tissue IC uptake in organs other than kidney, lung, liver, and spleen. The range of the percent of the IgA-IC and of the IgG1-IC deposited in the major organs is as follows: liver IgA-IC, 31–58%; IgG1-IC, 40–67%; spleen IgA-IC, 6–18%; IgG1-IC, 7–21%; lungs IgA-IC, 15–15.8%; IgG1-IC, 7–11%; kidneys IgA-IC, 0.44–1.6%, IgG1-IC, 0.20–0.56%. These findings are similar to those we previously obtained using an IC probe composed of rabbit antibody/BSA (1).

Biophysical properties of the IC

Size of IgA-IC vs. IgG1-IC. Large IC are cleared from the circulation more rapidly than small IC (2, 14). To assess the possibility that differences in IC size could explain differences in IC clearance rates, portions of the IgA-IC and IgG1-IC preparations used in each experiment were centrifuged on isokinetic sucrose gradients. The size profile of the IC preparations, compiled from all experiments, is shown in Fig. 6. These data indicate that the IgA-IC were actually smaller than the IgG1-IC. Thus, IgA-IC were cleared more rapidly from the circulation, despite their smaller size.

Antibody affinity. Antibody affinity, assessed by equilibrium dialysis, was similar for the IgA and IgG1 antibodies (5.53 × 10⁶ vs. 3.82 × 10⁶ liters/mole, respectively).

Antibody charge. The pI of IgA vs. IgG1, assessed by chromatofocusing, showed that the IgA antibodies were more anionic

*Table I. Organ Uptake of IgGl-IC vs. IgA-IC**

	Baboon 1			Baboon 2			Baboon 3		
	IgG1-IC	IgA-IC	IgA-IC/IgG1-IC	IgG1-IC	IgA-IC	IgA-IC/IgG1-IC	IgG1-IC	IgA-IC	IgA-IC/IgG-IC
Blood	368	268	0.7	240	146	0.6	648	418	0.6
Brain	11	46	4.2	22	101	4.6	23	71	3.1
Lung	2,111	4,192	2.0	1,705	2,526	1.5	1,322	1,918	1.5
Liver	10,608	9,288	0.9	8,149	6,300	0.8	5,651	4,732	0.8
Spleen	11,108	9,183	0.8	28,512	22,204	0.8	27,690	24,405	0.9
Kidney									
Cortex	210	353	1.7	461	1,680	3.6	285	894	3.1
Medulla	141	121	0.9	74	77	1.0	169	213	1.3
Glomeruli	80	228	2.8	595	1,599	2.7	506	2,367	4.7

* Data are expressed as cpm/gram of tissue except for glomeruli. For glomeruli, data are expressed as cpm/glomerular isolate. Minimal (<100 cpm/g) deposition of IC was observed in heart, aorta, stomach, small and large intestine, skin, or muscle (data not shown).

than the IgG1 antibody (pI 3.95–3.50 for IgA vs. 5.76–5.34 for IgG1). Thus, despite the presumed greater electronegativity of the IgA-IC, the IgA-IC showed greater uptake by glomeruli.

Complement activation. IgA-IC and IgG-IC were tested in two different baboon sera. In the first serum, CH_{50} was reduced from 99 to 68 μm by IgA-IC and to 49 μm by IgG1-IC. In the second serum, CH_{50} was reduced from 164 to 139 μm by IgA-IC and to 70 μm by IgG1-IC. Thus, both IgA-IC and IgG1-IC activate complement with IgG1-IC being the better activator.

Discussion

This study shows that, compared with IgG1-IC, IgA-IC bind less well to circulating erythrocytes, are cleared from the circulation more quickly and at a rate that is inversely related to the degree of binding to erythrocytes in vivo. In addition, compared with
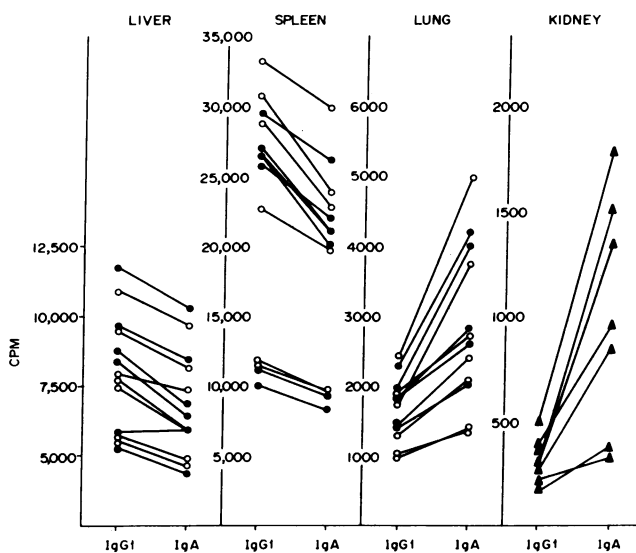

Figure 7. The IgA-IC and IgG1-IC content of each specimen from the major organs of the primates in exps. 1–3. Each point represents that cpm/g of a single specimen. The ^{131}I-cpm and ^{125}I-cpm of the same specimen are joined by a line. Values for central portions (●) and peripheral portions (o) are shown for liver, spleen, and lung. Values for whole cortex (▲) are shown for kidney.

IgG1-IC, IgA-IC are taken up in lesser amounts in liver and spleen but greater amounts in kidney and lung.

Differences in the biophysical properties of IC, which are generally recognized as being factors determining IC clearance from the circulation and tissue localization, do not in themselves appear to be an adequate explanation for the differential fate in vivo of IgA-IC vs. IgG1-IC. With respect to IC size, IgA-IC were smaller than IgG1-IC. Since smaller IC are cleared from the circulation more slowly than larger IC (14), it is clear that differences in IC size between IgA-IC and IgG1-IC is not an adequate explanation for the more rapid clearance of IgA-IC from the circulation. With respect to IC charge, IgA-IC were more anionic than the IgG1-IC. Since electronegativity of molecules tends to diminish their uptake by glomeruli (15–19), it is clear that differences in IC charge between IgA-IC and IgG1-IC cannot explain the greater uptake of IgA-IC in glomeruli. Finally, antibody affinity also affects IC clearance (20–22). However, IgA and IgG1 showed comparable affinity constants. Thus, differences in affinity, charge, or size do not appear to be an adequate explanation for the findings of the present study.

We suggest that the differences in clearance from the circulation and tissue uptake between IgA-IC vs. IgG1-IC is best explained by the fact that IgA-IC bind less well to circulating erythrocytes. The hypothesis is as follows: IC that do not bind well to circulating erythrocytes will tend to be free in the plasma, and IC that are free in the plasma are asumed to be more susceptible to depositing in tissues than are IC that are bound to erythrocytes (2). Thus, all other factors held constant, conditions that lead to decreased binding of IC to erythrocytes in vivo should lead to the following predictable consequences:

(a) Accelerated clearance of IC from the circulation: because IC free in the plasma are susceptible to uptake by liver and spleen plus all other sites in the circulation (2); by contrast, IC bound to erythrocytes are susceptible to uptake mainly by liver and spleen (1, 2). Since splenic and hepatic blood flow combined are only ~22% of cardiac output,[2] many circuits of the vascular system will be required before IC bound to erythrocytes can be

2. This estimate of splenic and total hepatic (hepatic artery plus portal vein) blood flow is based on unpublished microsphere blood flow studies, performed under experimental conditions comparable with the present study, in the dog (13) and the baboon (1).

removed from the circulation. Thus, conditions which promote binding of IC to erythrocytes should delay the clearance of IC from the circulation. On the other hand, conditions that inhibit binding of IC to erythrocytes should accelerate the clearance of IC from the circulation.

(b) Diminished IC uptake by liver and spleen: because decreased binding of IC to erythrocytes leads to increased uptake of the IC dose at nonhepatic, nonsplenic sites in the circulation (2). Thus, less IC is available for deposition in liver and spleen.

(c) Enhanced IC uptake by lung and kidney: because of the major organ systems, lung and kidney have the highest blood flow per gram of tissue.[3] Thus, these organs will have the highest rates of contact between circulating IC and the vascular endothelium, in which the IC may become entrapped. Thus, lung and kidney should be the major nonhepatic, nonsplenic sites of IC uptake.

The results of the present study provide direct experimental support for each of the above predictions. The findings and interpretation of the present study are also consistent with our previous study on the effect of complement depletion on the operation of the erythrocyte-IC clearing mechanism (2). In that study, complement depletion abrogated the binding of IC to erythrocytes in vivo. Coincident with diminished erythrocyte-to-IC binding, we found accelerated clearance of IC from the circulation and enhanced nonhepatic, nonsplenic uptake of IC, just as in the present study. It is noteworthy that these concomitants of decreased binding of IC to erythrocytes were observed under conditions quite different from the present study. This further strengthens the interpretation that the differential fate of IgA-IC vs. IgG1-IC observed in the present study is due, specifically, to effects of impaired binding of IgA-IC to erythrocytes.

The reason why IgA-IC bind poorly to primate erythrocytes compared with IgG-IC is not clear. The IgA-IC were smaller than IgG1-IC, and previous observations indicated that the degree of IC to erythrocyte binding increases with increasing IC size (6, 23). However, the IgA-IC were sufficiently large to achieve binding, if size alone were the determinant (see Fig. 6). Thus, the greater binding of IgG-IC to erythrocytes, relative to IgA-IC, cannot be explained simply on the basis of greater size of IgG1-IC. It is possible that IgG1-IC may generate more C3b sites than IgA-IC, or that the configuration of C3b sites on IgG1-IC facilitates IC-to-erythrocyte binding to a greater extent than IgA-IC. In in vitro studies we found that IgG1-IC activated complement better than IgA-IC, but the differences were not striking. Other investigators have also shown that IC made from the MOPC-315 antibody used in the present study can acitvate the complement system (24). The presumed greater electronegativity of IgA-IC might have been another factor diminishing binding of IgA-IC to erythrocytes, which normally have a negatively charged surface (25). Finally, IC erythrocyte binding is a reversible process (26). Thus, the lower level of binding of IgA-IC might have been due to a greater susceptibility of these IC to be released from the erythrocyte membrane by plasma factors.

The present study also seems relevant to the problem of IC-mediated vasculitis in the following way. Hypocomplementemia is seen in virtually all patients with systemic vasculitis associated with deposition of IC (27). Apparently, the hypocomplemen-

temia is the result of high rates of complement consumption caused by a large IC load which, in turn, is apparently responsible for the widespread vascular injury. The striking exception to this generalization is found in patients with vasculitis mediated largely by IgA-containing immune complexes (Henoch-Schonlein purpura) (27). These patients are not hypocomplementemic, despite the fact that the vasculitis is often widespread and the deposits contain large amounts of IgA-IC that have activated the alternative complement pathway (27). The data of the present experiments suggest that in Henoch-Schonlein purpura, one possible reason for extensive IC deposition but no hypocomplementemia is that IgA-IC can induce relatively widespread tissue injury at relatively low rates of IC production, because IgA-IC are not efficiently bound to erythrocytes and, therefore, are not efficiently cleared from the circulation. Thus, with IgA-IC-mediated disease, it is possible that extensive vasculitis can be caused by relatively small IC loads, and for this reason, hypocomplementemia does not develop.

The present study provides evidence that the erythrocyte CR1 system is a key component of a rapid and efficient mechanism for safely removing IC from the circulation. However, the erythrocyte CR1 system is also capable of functioning in other ways to defend against IC-mediated disease. Specifically, in vitro studies have shown that the erythrocyte CR1 can serve as a cofactor, which greatly increases Factor I-mediated degradation of C3b and C4b (28). These actions inhibit the further activation of the complement cascade by causing degradation of the C3 convertase and the C5 convertase (28). Other in vitro studies have shown that IC-bound C3b attached to erythrocyte CR1 are "processed" on the surface of the erythrocyte by Factor I, which results in release of the IC from the erythrocyte with the C3b moeity on the IC cleaved to C3d. This processing of C3b to C3d, which can then interact with CR2 receptors, may represent an immunologic signal (29).

The way in which the clearing function and the processing function of the erythrocyte CR1 system relate in vivo is not clear. However, the greater speed of the erythrocyte clearing mechanism, relative to the erythrocyte CR1 processing mechanism, suggests that, in vivo, the first option is for the erythrocyte-bound IC to be delivered to liver and spleen for disposal. If that mechanism is impaired because, for example, the mononuclear phagocyte system of liver and spleen are unable to efficiently take up IC, then the processing function of the erythrocyte CR1 system may become dominant.

Acknowledgments

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