Modification of Glomerular Immune Complex Deposition in Mice by Activation of the Reticuloendothelial System

UNO BARCELLI, RICARDO RADEMACHER, YUET M. OOI, and BOON S. OOI,
Division of Nephrology, Department of Internal Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio 45267

ABSTRACT To determine the effect of activation of the reticuloendothelial system on the localization of immune complexes in the kidney, a model of passive serum sickness nephritis in the mouse was used, with activation of the reticuloendothelial system with *Corynebacterium parvum*. Groups of mice, control and *C. parvum*-treated animals, were injected with BSA-^{125}I-anti-BSA complexes containing 3 mg ^{125}I-anti-BSA. Blood was obtained at 5 min, at 3 h, and at 12 h, when the animals were killed. Blood concentrations of BSA-^{125}I-anti-BSA complexes were reduced in *C. parvum*-treated animals compared with controls. This appeared to be mediated by two effects, increased uptake of complexes in the liver and spleen, and enhanced degradation of immune complexes as measured by TCA-soluble radioactivity. In vitro studies using cultures of peritoneal macrophages also showed enhanced uptake of immune complexes. The amount of immune complexes deposited in the glomeruli of *C. parvum*-treated animals was reduced as determined by quantitation of radiolabeled material bound to isolated gomeruli and by immunofluorescence techniques. The results of the study emphasize the role of the reticuloendothelial system in the modulation of immune complex localization in the kidney and suggest a potential use of stimulants of the reticuloendothelial system in the therapy of immune complex nephritis.

INTRODUCTION

Previous studies have defined the role of the reticuloendothelial system in the clearance of soluble immune complexes infused into the circulation (1–5). It was shown that the bulk (up to 50%) of immune complexes was taken up by the liver, (2), indicating that the reticuloendothelial system serves as an important buffer against these potentially phlogistic substances. The reticuloendothelial system was also shown to be subject to saturation when it was presented with increasing amounts of immune complexes (3). Furthermore, when uptake of immune complexes by the liver and spleen was reduced, the amount deposited in the glomerulus was correspondingly increased (4, 5). Thus, alterations in reticuloendothelial system function may be important in modulating the localization of immune complexes in the kidney. Many forms of human and experimental nephritis arise from the entrapment of circulating immune complexes in the kidney. Such complexes may be presumed to have escaped processing by the reticuloendothelial system, either because the latter is saturated or defective. It is logical to propose that activation of the reticuloendothelial system under these circumstances may lead to enhanced clearance of soluble immune complexes by the liver, spleen, and other phagocytic cells, thereby decreasing the impact of such complexes on the kidney. The present study was designed to determine if such an effect would be achieved, using the passive model of serum sickness nephritis, and activating the reticuloendothelial system by *Corynebacterium parvum*.

METHODS

Production and isolation of antibody. Bovine albumin (Miles Laboratories, Inc., Ames Div. Elkhart, Ind.) was dissolved in 0.15 M NaCl at a concentration of 10 mg/ml and emulsified with an equal volume of complete Freund's adjuvant. Rabbits were immunized by intramuscular injection of 10 mg of the antigen; booster doses were given 2 wk after the initial injection, and thereafter at 4-wk intervals. Animals were bled 4 wk after the initial dose followed by weekly bleedings. Serum samples harvested were stored at −70°C; antisera from several rabbits were pooled for the experiments.

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Address reprint requests to Dr. B. S. Ooi.

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Purified anti-bovine serum albumin (anti-BSA)[1] was isolated by means of an immunoadsorbent column, using a modification of previously described techniques (6, 7). In brief, 0.3 ml of glutaraldehyde (final concentration 50% vol/vol) was added to 30 ml of BSA (40 mg/ml) with stirring. The mixture was allowed to settle at room temperature until a gel had formed. A volume of 30 ml of phosphate-buffered saline (PBS) was added to the gel, which was broken up by homogenizing for 10–15 s with a Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) at the lowest speed setting. A slurry of Sephadex was prepared by adding 75 ml of PBS, pH 7.4, to 15 g of coarse Sephadex G-25 (Pharmacia Fine Chemicals, Div. Pharmacia, Inc., Piscataway, N. J.). This slurry was mixed thoroughly with the homogenized gel. A 60 × 170-mm glass column was packed with the mixture, washed with 100 ml glycine buffer, pH 2.8, followed by PBS, pH 7.4 until the effluents gave a pH >7. An aliquot of 40 ml rabbit serum was decomplemented by heating at 56°C × 30 min, diluted with 80 ml of PBS, pH 7.4, and run through the column at 0.5 ml/min. The column was washed with 600–800 ml of PBS pH 7.4 until optical density at 280 nm was <0.03. Specific anti-BSA antibody was eluted from the column with 0.1 M glycine buffer pH 2.8. Fractions containing protein were pooled, pH adjusted to 7.4 using 0.1 M NaOH, and dialyzed against 0.15 M NaCl. After dialysis, the protein was concentrated through an Amicon XM-100 filter (Amicon Corp., Scientific Sys. Div., Lexington, Mass.). All procedures were done at 4°C. Monomeric anti-BSA antibody was obtained by ultracentrifugation at 100,000 *g* × 90 min with collection of the protein in the upper two-thirds of the specimen. Antibody was stored at −70°C until used. Purity of the antibody was established by immunoelectrophoresis. Sucrose density gradient ultracentrifugation (see below) of labeled antibody showed it to have a sedimentation coefficient of 7S. Trace labeling with ^{125}I (New England Nuclear, Boston, Mass.) of the antibody (sp act 1 μCi/mg) was done by the chloramine-T method (8).

Preparation of immune complexes. This was done by a previously reported technique (9). Quantitative precipitin curves were constructed using BSA, and monomeric ^{125}I-anti-BSA antibody to determine the point of equivalence. Immune precipitates were prepared at equivalence; incubation was done at 37°C × 1 h followed by 4°C × 24 h. The precipitates were washed three times with cold normal saline, and suspended in the same solution. Five times excess of antigen was added to the suspension and pH lowered to 2.4 by the stepwise addition of 0.1 N HCl. Precipitates were completely solubilized at this pH. The pH was then re-adjusted to 7.4 with 0.1 N NaOH. Soluble immune complexes were stored at 4°C for 48 h and centrifuged at 10,000 rpm × 30 min before use.

Sucrose density gradient ultracentrifugation analysis. The size of complexes was measured by sucrose density gradient ultracentrifugation analysis using previously reported techniques (10). Serum samples obtained from animals at the end of 3 h were diluted 1:10 with PBS before analysis. In brief, 200 μl of a sample was applied to 10–40% sucrose gradients and ultracentrifuged at 35,000 rpm for 16 h at 4°C using an SW50.1 rotor in a Spinco ultracentrifuge (Model L2) (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Sedimentation coefficients were estimated by the method of Martin and Ames (11), using the markers BSA human IgG, Clq, and IgM. Fractionated material showed two peaks, a 7S peak (presumed to be unreacted anti-BSA because ultracentrifugation analysis of complexes formed by using labeled albumin did not show any label in the 7S region, thereby excluding the possibility that this represented small sized immune complexes) and another peak containing 13.6S and larger sized immune complexes. For convenience, the latter peak will be designed as the 13.6S fraction. The counts under each peak were estimated and the percentage of counts representing the 7S and the 13.6S fractions were calculated. After totalling TCA precipitable counts injected and in the serum samples, the distribution of these counts in each fraction was determined.

Animal experiments. C57 BL/6J female mice 18–20 g (Jackson Laboratories, Bar Harbor, Maine) were divided into two groups. One group was pretreated 7 d before the experiment with an intraperitoneal injection of 150 μl (1 mg dry wt) *C. parvum* (Burroughs Wellcome Co., Triangle Park, N. C.); the other group served as controls. Animals received potassium iodide in their drinking water 48 h before the experiment. The experimental protocol was determined by preliminary experiments in which different doses of complexes were administered to untreated animals who were killed at varying time intervals, and kidneys examined by immunofluorescence. It was established that the optimum dose of immune complexes was one containing 3 mg antibody and time of killing 3 h after the injection. The experimental protocol consisted of giving immune complexes containing 3 mg antibody dissolved in 0.3 ml normal saline via the tail vein. 20-μl blood samples were obtained from the retro-orbital venous plexus at 5 min and at 3 h after the injection. To determine if the effect of *C. parvum* treatment on the in vivo clearances of immune complexes could be sustained, another set of experiments, using control and *C. parvum*-treated mice were subjected to the same protocol as for the 3 h experiments except that the experiment was terminated at 12 h. Serum was also obtained during killing for determinations of (*a*) size of complex by sucrose density gradient ultracentrifugation, (*b*) fractions of TCA soluble and insoluble radioactivity. TCA soluble radioactivity was used as a measure of degradation of immune complexes. The equivalent amount of ^{125}I-anti-BSA degraded was calculated from the specific activity of antibody protein and the measured number of ^{125}I-TCA soluble counts.

To determine the effect of *C. parvum* injection on renal function, measurements of serum creatinine were done on sera obtained from a group (five animals each) of control and *C. parvum*-treated mice using an autoanalyzer method. The geometric mean serum creatining (mean±SD) of the controls and *C. parvum*-treated mice were 0.38 mg/100 ml (0.33–0.43 mg/100 ml) and 0.36 mg/100 (0.30–0.42 mg/100 ml), respectively ($t = 0.63$, $P < 0.3$).

Specific organ uptake of immune complexes. The specific localization of BSA-^{125}I-anti-BSA complexes in the liver and spleen was determined 3 h after the injection of complexes. Mice received an injection of 200 μg of human monomeric ^{131}I-IgG as an intravascular blood marker 5 min before killing. The ^{125}I and ^{131}I counts per min in the organs were determined and the ^{125}I-specific uptake was calculated as follows:

$$^{125}\text{I-specific uptake} = {}^{125}\text{I counts per minute/organ} - \frac{^{131}\text{I counts per minute/organ}}{^{131}\text{I counts per minute/milliliter blood}} \times {}^{125}\text{I counts per minute/milliliter blood.}$$

In separate experiments using three control and an equal number of *C. parvum*-treated mice, peritoneal washings were obtained at 3 h and counted for radioactivity.

The concentration of antibody in the organ was calculated from the specific activity of the antibody protein.

[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; PBS, phosphate-buffered saline.

Specific localization of immune complexes in isolated glomeruli. Mouse glomeruli were isolated by the following procedure. Kidneys were removed from mice during killing. Cortices were dissected from medullae, and cortical tissue pressed through a 200-mesh stainless steel sieve (74 μm pore Diam), and passaged material suspended in PBS. The suspension was forced through a 25-gauge needle and allowed to stand in ice for 30 min. The supernatant was suctioned off and the sediment resuspended in PBS and centrifuged at 1,000 rpm × 5 min. The supernate was discarded and resuspended in 0.5 ml PBS and layered on top of a 72% sucrose solution and centrifuged at 1,000 rpm for 15 min. The resultant pellet was suspended in 0.2 ml PBS, and passed once more through the 25-gauge needle. Glomeruli were stained with a 0.1% solution of toluidine blue and counted in a hemacytometer. The preparations contained ~80% glomeruli with some tubules. Glomeruli from four kidneys were pooled and counted for ^{125}I and ^{131}I radioactivity. ^{125}I-anti-BSA bound to glomeruli was determined after correction for blood counts (using ^{131}I-IgG counts as the blood marker) using the formula previously described. In the first series of experiments, one kidney from each animal was processed for isolation of glomeruli to quantitate the amount of antibody bound, and the other kidney processed for histologic analysis. A second series of experiments was done for the specific purpose of quantitating the amount of immune complexes localized in the glomeruli. Control and *C. parvum*-treated groups consisted of four animals in each group. A blood sample was obtained at 5 min to ensure that comparable amounts of complexes had been infused into the animals; these mice were killed and kidneys processed as described.

Histologic examination of kidneys. Cortical wedges of kidney tissue were obtained from killed animals and processed for fluorescence and light microscopy by previously described techniques (12). For fluorescence microscopy, a fluorescein-conjugated goat anti-rabbit IgG antiserum (N. L. Cappel Laboratories, Cochranville, Pa.) was used; monospecificity of antisera was monitored by immunoelectrophoresis. A semiquantitative scale of grading the extent of fluorescent deposits was used, with a scale from 0 to 4, each unit indicating one quadrant of a glomerulus having a significant amount of IgG deposited. Previous studies have validated this method of scoring (12). All comparisons were done independently by two observers, with no knowledge of the states of the animals from which the kidneys were obtained.

In vitro experiments using peritoneal macrophage cultures. The methods for harvesting and maintaining mouse peritoneal macrophages have previously been reported (13). The technique of assessing Fc receptor function is that of Bianco (14), previously reported in detail (14). In brief, immune complexes were prepared by incubating 1 ml of 5% vol/vol sheep erythrocytes with 1 ml of a subagglutinating dilution of rabbit IgG anti-sheep erythrocytes (Cordis Laboratories Inc., Miami, Fla.) in Hanks' balanced salt solution for 30 min at 37°C. The cells were then washed three times and resuspended in 10 ml of Dulbecco's modified Eagle minimum essential medium. Macrophage monolayers were prepared on glass coverslips kept in petri dishes. They were overlayed with erythrocyte complexes at a final concentration of 0.5%. After 1 h of incubation at 37°C, the coverslips were then fixed with glutaraldehyde 2% for 10 min, stained with Giemsa, and viewed microscopically. The ingestion index was the percentage of macrophages that ingested erythrocytes × average number of erythrocytes ingested per macrophage. At least 100 macrophages were examined, and triplicated cultures done for each group.

Statistical analysis. Except for comparisons of immunofluorescence scores of renal biopsies, which were done by the Wilcoxon rank test, all other comparisons were performed by the t test.

RESULTS

Blood levels of 7S and 13.6S fractions. Analysis by sucrose density gradient ultracentrifugation revealed that injected BSA-anti-BSA material resolved into two peaks (Fig. 1), a 7S peak and a peak containing 13.6S and larger complexes (designated the 13.6S peak). Ultracentrifugation analysis showed that serum samples obtained at 3 and 12 h from *C. parvum*-treated mice contained a reduced amount of 13.6S material compared with untreated animals, indicating greater removal of 13.6S fractions (Fig. 1). The calculated amount of the two fractions at 5 min, at 3 h, and at 12 h, are given in Table I and Table III. Comparable amounts of radiolabeled material were administered into the two groups of mice, as indicated by the concentrations of ^{125}I-anti-BSA (7S + 13.6S fractions-see Tables I and III). At 3 h, the levels of total ^{125}I-anti-BSA (7S + 13.6S fractions) and of 13.6S fractions were significantly lower in *C. parvum*-treated mice compared with control animals (Fig. 2 and Table I), the respective

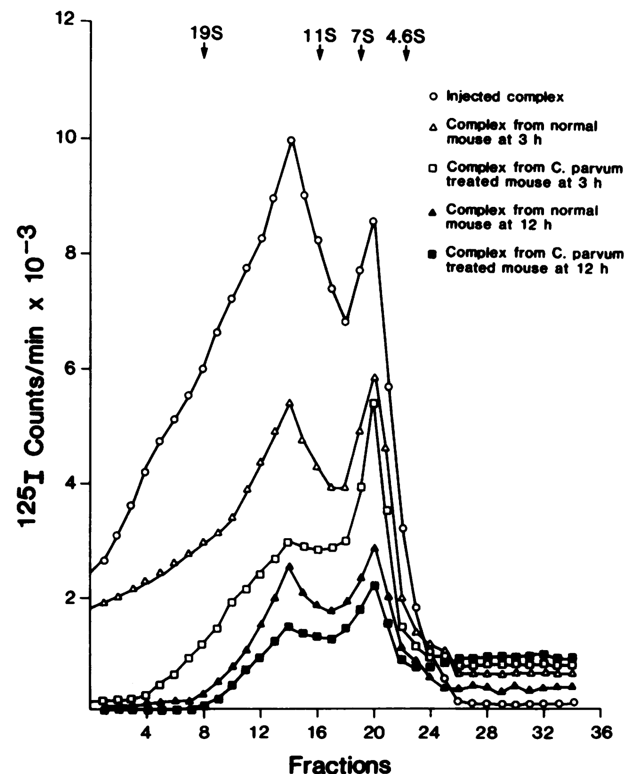

FIGURE 1 Representative analysis of injected material and serum from control and *C. parvum*-treated mice by sucrose density gradient ultracentrifugation. Note the resolution of injected material into two peaks, 7S, and 13.6S. By comparison with the profile of fractionated serum from a control mouse, the serum of the *C. parvum*-treated mouse showed a reduced 13.6S peak.

TABLE I
Blood Levels of ¹²⁵I-Anti-BSA of Controls and C. Parvum-treated Mice at 5 Min and at 3 h

Animals	5 min 7S C	5 min 7S CP	5 min 13.6S C	5 min 13.6S CP	3 h 7S C	3 h 7S CP	3 h 13.6S C	3 h 13.6S CP	¹²⁵I-anti-BSA equivalent degraded C	¹²⁵I-anti-BSA equivalent degraded CP
	$\mu g/ml$				$\mu g/ml$				$\mu g/ml$	
1	419	349	1,258	1,046	218	253	226	175	15	62
2	387	389	1,161	1,168	232	227	348	217	28	53
3	419	387	1,257	1,161	295	237	359	226	41	194
4	357	432	1,072	1,297	309	290	462	266	40	90
5	385	377	1,154	1,130	314	316	557	303	65	97
6	385	370	1,155	1,109	431	321	799	328	75	113
7	381	385	1,144	1,156	489	298	908	361	ND	ND
Geometric mean	390	383	1,170	1,150	315	275	473	261	39	93
Mean±SD	368–412	359–409	1,106–1,237	1,078–1,228	234–423	239–317	289–772	202–337	22–69	58–147
Statistical significance	$t = 0.51$, $P < 0.6$		$t = 0.52$, $P < 0.6$		$t = 1.08$, $P < 0.3$		$t = 2.84$, $P < 0.02$		$t = 2.86$, $P < 0.02$	

C, control; CP, *C. parvum*-treated mice; ND, not done.

mean concentrations being 536 and 788 $\mu g/ml$ for 7S + 13.6S fractions ($t = 2.28$, $p < 0.05$), and 261 and 473 $\mu g/ml$ for 13.6S fractions ($t = 2.84, P < 0.02$). The effect was sustained as shown by levels of 13.6S and 7S + 13.6S fractions at 12 h (Fig. 3 and Table III). Additionally, at 12 h, levels of 7S fractions of *C. parvum*-treated animals showed a small but significant decrease compared with levels in control animals (Table III).

Blood levels of degraded ¹²⁵I-anti-BSA antibody. The concentrations of degraded ¹²⁵I-anti-BSA antibody at 3 and at 12 h are given in Tables I and III and show that the amount of labeled antibody degraded by *C. parvum*-treated animals was significantly increased compared with that of controls; mean levels in control and *C. parvum*-treated animals being 39 and 93 $\mu g/ml$, respectively at 3 h; ($t = 2.86$, $P < 0.02$, Table I); by 12 h the differences were even more pronounced being 23 $\mu g/ml$ for controls and 97 $\mu g/ml$ for *C. parvum*-injected animals ($t = 7.92$, $P < 0.001$, Table III). The data suggest enhanced degradation of immune complexes by *C. parvum*-treated animals. Incubation of serum from *C. parvum*-treated animals with labeled immune complexes did not lead to increased deiodination compared with normals, thereby excluding the effect of serum.

Specific organ uptake of immune complexes. The values for the uptake of ¹²⁵I-anti-BSA in liver, spleen at 3 and at 12 h, and for kidney at 3 h for the two groups of mice are given in Tables II and III. Mean uptake in the liver at 3 h was significantly increased in *C. parvum*-treated mice, mean uptake being 461 and 668 μg per organ ($t = 6.57$, $P < 0.001$) in control and experimental groups, respectively. The effect was present at 12 h, mean uptake being 271 and 437 μg in control and experimental groups, respectively ($t = 4.6$, $P < 0.001$). Splenic uptake in the *C. parvum* group was

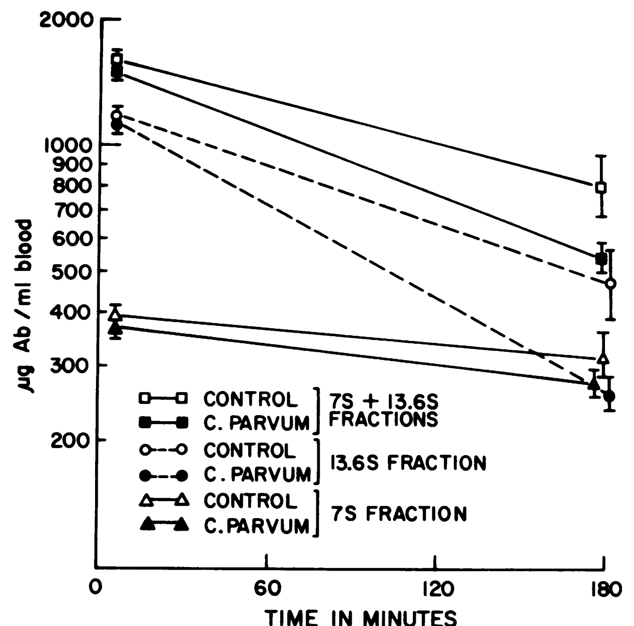

FIGURE 2 Blood levels (mean±SE) of ¹²⁵I-anti-BSA and its fractions at 5 min and at 3 h in control and *C parvum*-treated mice. An aliquot of BSA-¹²⁵I-anti-BSA containing 3 mg antibody was given intravenously into the mice. By sucrose density gradient ultracentrifugation analysis the distribution of 7S and 13.6S fractions was obtained and the concentration of each calculated. At 3 h, the levels of total (7S + 13.6S) ¹²⁵I-anti-BSA material and 13.6S fractions were significantly lower in *C. parvum*-treated mice compared with normal.

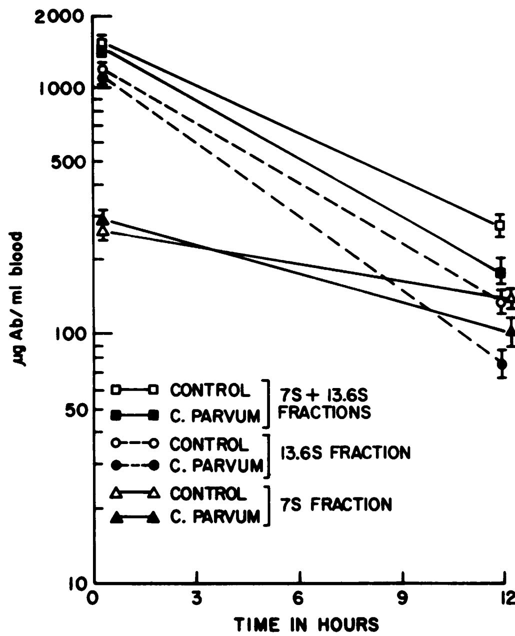

FIGURE 3 Blood levels (mean±SE) of ^{125}I-anti-BSA and its fractions at 5 min and at 12 h in control and *C. parvum*-treated mice. (See legend of Fig. 2 for methods).

even more pronounced, mean uptake by this organ being increased by about five times that of control mice at 3 h and being increased by almost nine times at 12 h (Tables II and III).

The weights of the liver and spleen were obtained in the two groups of mice at 12 h. The mean weights (±SD) of control and *C. parvum*-treated livers were 950±78 mg and 1,556±248 mg, respectively. The mean weights (±SD) of spleens were 76±17 mg and 327±97 mg for control and experimental groups, respectively. The mean uptake of immune complexes per mg liver tissue was identical in both control and *C. parvum*-treated animals, being 0.29 µg/mg liver tissue. However, uptake of immune complexes per milligram splenic tissue was higher in *C. parvum* mice than in control mice, being 0.15 µg/mg splenic tissue compared with 0.08 µg/mg splenic tissue (t = 4.9, $P < 0.001$).

Using glomerular isolation techniques, the amount of ^{125}I-anti-BSA bound to glomeruli was quantitated and was found to be reduced in *C. parvum*-treated animals being 91 ng/8,000 glomeruli compared with 180 ng/8,000 glomeruli in normal mice (t = 6.54, $P < 0.01$) Table II. At 12 h, immune complexes could be not detected in kidneys of either control or *C. parvum*-treated animals. This finding was probably related to the markedly reduced blood levels of 13.6S fractions.

To exclude the possibility that differences in blood levels could be attributable to differences in peritoneal content of immune complexes, peritoneal washings were counted. The mean concentrations of ^{125}I-anti-BSA (±SD) of control and *C. parvum*-treated mice were 4±1 µg and 18±1.7 µg, respectively (t = 12.1, $P < 0.01$). Although the differences were significant, it should be noted that the absolute amount constituted only a minute fraction of the quantity infused.

Histologic examination. By using light microscopy, there were only minor mesangial changes that could not be readily quantitated. The differences were more

TABLE II
Specific Organ Localization of Immune Complexes of Controls and C. Parvum-treated Mice at 3 h

Animals	Liver		Spleen		(ng/8,000 glomeruli)		Immunofluorescence score (0–4+)	
	C	*C. parvum*	C	*C. parvum*	C	*C. parvum*	C	*C. parvum*
	µg/organ		µg/organ					
1	374	568	24	39	Data pooled		3.2	1.0
2	513	682	20	98	Data pooled		3.5	0.5
3	425	647	16	98	Data pooled		3.3	1.0
4	442	738	18	82	Data pooled		3.5	0.8
5	484	618	20	55	Data pooled		3.0	0.9
6	544	702	12	120	Data pooled		3.0	0.8
7	445	721	15	111	Data pooled		1.2	0.3
Mean±SD	461±57	668±61	18±3.9	86±30	180±20.0	91±12.0	3.2	0.7
Statistical significance	t = 6.57 $P < 0.001$		t = 6.05 $P < 0.001$		t = 6.54 $P < 0.01$		$P < 0.002$ by Wilcoxon rank test	

C, control.
* Data for glomerular localization were pooled from these animals and from additional experiments (see Methods); individual values were (nanograms per 8,000 glomeruli) 161, 178, 201 for control mice and 80, 91, 104 for *C. parvum*-treated mice.

TABLE III
Blood Levels of ¹²⁵I-anti-BSA and Tissue Uptake of Immune Complexes at 12 h

Animal	Blood levels* of ¹²⁵I-anti-BSA								¹²⁵I-anti-BSA equivalent degraded		Tissue uptake			
	5 min				12 h									
	7S		13.6S		7S		13.6S				Liver		Spleen	
	C	CP	C	CP	C	CP	C	CP	C	CP	C	CP	C	CP
	μg/ml				*μg/ml*				*μg/ml*		*μg/organ*			
1	287	302	1,148	1,133	116	108	112	89	18	153	210	375	2.8	88
2	257	313	1,166	1,252	133	98	119	75	24	56	326	310	3.7	69
3	281	325	1,279	1,150	137	116	142	84	25	70	351	425	4.9	43
4	261	280	1,280	1,118	147	115	148	87	18	120	325	529	7.8	38
5	265	248	1,292	1,210	149	101	155	68	29	99	224	490	8.8	40
6	294	301	1,102	1,128	130	82	130	66	16	114	218	475	7.2	46
7	327	277	1,157	1,180	148	88	143	67	42	103	241	459	6.5	60
Mean	273*	291*	1,201*	1,166*	137*	100*	135*	76*	23*	97*	271‡	437‡	5.9‡	55‡
Mean±SD	240–311	265–318	1,126–1,281	1,119–1,216	125–150	88–114	119–152	66–86	17–33	69–137	211–331	363–511	3.7–8.1	36.6–73.4
Statistical significance	$t = 1.06$ $P < 0.4$		$t = 1.01$ $P < 0.4$		$t = 5.11$ $P < 0.001$		$t = 8.57$ $P < 0.001$		$t = 7.92$ $P < 0.001$		$t = 4.6$ $P < 0.001$		$t = 6.9$ $P < 0.001$	

C, control; CP, *C. parvum*-treated animals.
* Geometric mean.
‡ Arithmetic mean.

evident by fluorescence microscopy, and semiquantitative grading showed significantly increased amounts of IgG in the glomeruli of normal animals compared with *C. parvum*-treated animals at 3 h; the mean score for *C. parvum*-treated animals was 0.7 for normal mice 3.2 ($P < 0.002$ by Wilcoxon rank test). Immunofluorescent appearances of representative glomeruli from the two groups of animals are depicted in Fig. 4. At 12 h, glomeruli of both groups of animals did not show any staining for rabbit IgG.

In vitro macrophage culture studies for Fc receptor function. The mean ingestion index (±SE) of macro-

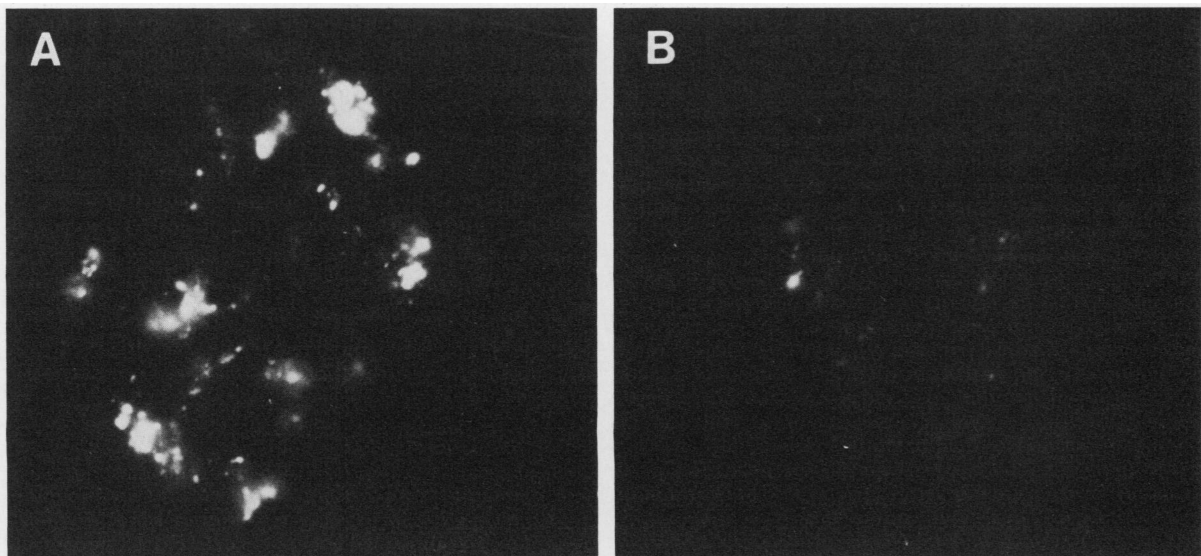

FIGURE 4 Immunofluorescent localization of rabbit IgG in glomeruli from kidneys of control (4A) and *C. parvum*-treated mice (4B) at 3 h. Sections of kidney tissue were layered with fluorescein conjugated goat anti-rabbit IgG (×540).

phages of control and *C. parvum*-treated mice in triplicate cultures were 537 (±68) and 1,054 (±78), respectively ($t = 5.0, P < 0.01$).

DISCUSSION

The present study provides data which show that activation of the reticuloendothelial system by an agent such as *C. parvum* leads to reduced deposition of immune complexes in the kidney in the model of passive serum sickness nephritis in the mouse. Decreased localization of complexes in the kidney of *C. parvum*-treated mice was demonstrated by two methods with concordant results: by quantitation of radiolabeled complexes bound to isolated glomeruli, and by immunofluorescent methods visualizing decreased amounts of mesangial deposits (by comparison to untreated mice). This effect was achieved by two mechanisms: enhanced uptake of immune complexes by the liver and the spleen, and augmented degradation as determined by increased concentrations of TCA-soluble radioactive material in the circulation. The net effect was to diminish the plasma level of immune complexes with beneficial consequences to the kidney. The mechanism of enhanced uptake of immune complexes by the liver appears to be different from that by the spleen. The amount of immune complexes taken up by the liver of C. parvum-treated mice increased *pari passu* with its weight, suggesting that increased delivery may be a mechanism. In the case of the spleen, the quantity of complexes taken up increased more than could be accounted for by the increase in weight, suggesting that there may be additional mechanisms such as enhanced Fc receptor-mediated functional activity. Insight into the mechanism of augmented uptake of immune complexes by the spleen during activation may in part be explained by our observations on macrophages in culture which show that there is an increase in the ingestion index of macrophages from *C. parvum*-treated animals. This accords with previous studies showing an expansion in the number of Fc receptors of the stimulated macrophage (15). Additionally, studies by Bianco et al. (16) have also demonstrated an alteration of receptor function upon macrophage activation, complement receptor-mediated ingestion being a property of the activated cells. The relative importance of the two mechanisms can be determined by decomplementation studies. The processing of immune complexes by macrophages has been previously studied by in vitro techniques (17, 18). The enhancing effect of complement has been demonstrated and shown to be due to increased uptake of complexes by these cells (18). The mechanisms for increased in vivo degradation of immune complexes shown in this study remain to be elucidated.

The results of the current investigation extend the observations of Ford (19) who showed that stimulation of the reticuloendothelial system of mice by estradiol followed by endotoxin led to decreased localization in the kidney as visualized by immunofluorescent techniques. However, the mechanism by which this effect was obtained was not clarified. Studies by Atkinson and Frank (20) have also shown increased clearance of particulate immune complexes (IgM-coated erythrocytes) by the reticuloendothelial system in Bacille Calmette-Guerin infected animals. More recently, Raij and Keane (21) have reported in abstract form the finding that zymosan activation of the reticuloendothelial system leads to reduced deposition of soluble aggregates in the rat kidney. However, zymosan also gives rise to complement depletion, and this may potentially affect the ability of macrophages to degrade immune complexes in view of the evidence for an enhancing effect of complement in the processing of soluble complexes, and the alteration of receptor function of such macrophages (16, 18).

The significance of our observations relates to the potential use of such immunostimulants as a modality in the treatment of various forms of human immune complex-mediated nephritis. Immune complexes have been detected in the sera of patients with diffuse proliferative glomerulonephritis, lupus nephritis, membranoproliferative glomerulonephritis, and other forms of nephritis (22–24). Studies of reticuloendothelial function in some of these forms of human renal disease have also shown impaired splenic clearance of immune complexes in these patients (25, 26). It is uncertain if this impairment is a primary defect, or if it is a reflection of saturation of the reticuloendothelial system by the complexes (of interest, studies of Finbloom and Plotz (27) and by Knutson et al. (28) have shown that the ability of NZB/W female mice to clear soluble immune complexes was unimpaired). Regardless of the mechanism for this event, it appears reasonable to suppose that activation of the reticuloendothelial system may exert a beneficial effect on the kidney by the mechanisms described in this model. An additional salutory effect may be inferred from recent observations that point to the monocyte as being important in removal of tissue immune complexes (29). Currently, macrophage and reticuloendothelial system activating agents such as *C. parvum* and Bacille Calmette-Guérin are being used in the therapy of some forms of human cancer, and underline the potential applicability of the results of this study to the management of patients with immune complex mediated renal disease. It is recognized that the use of such immunostimulants may give rise to an enhanced antibody response, which may limit their usefulness. However, it is expected that the functional capacity of the reticuloendothelial system will also be increased, and allow it to deal effectively with the increased loads of immune complexes; studies

of saturability of the reticuloendothelial system under these conditions are in progress to determine if this is the case.

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