INFECTION AND IMMUNITY, Sept. 1987, p. 2074-2080 Vol. 55, No. 9
0019-9567/87/092074-07$02.00/0
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Effect of Kupffer Cell Phagocytosis of Erythrocytes and Erythrocyte Ghosts on Susceptibility to Endotoxemia and Bacteremia

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Received 12 January 1987/Accepted 29 May 1987

The phagocytosis of erythrocytes by macrophages has previously been shown to depress macrophage function. In this study we compared the effect of the phagocytosis of erythrocytes and erythrocyte ghosts by Kupffer cells on the duration of the depression of complement receptor clearance function and host defense against endotoxemia and bacteremia. Phagocytosis of erythrocytes and erythrocyte ghosts was induced in rats by the injection of rat erythrocytes or erythrocyte ghosts coated with anti-rat erythrocyte immunoglobulin G (EIgG and GIgG, respectively). The hepatic uptake of EIgG and GIgG (17.4 × 10^8/100 g) occurred during the first 30 min after injection. The digestion of phagocytized EIgG and GIgG, as assessed by electron microscopy, was complete at 24 and 3 h after injection, respectively. The depression of Kupffer cell complement receptor clearance function caused by EIgG and GIgG returned to normal by 6 h after injection of EIgG and by 3 h after injection of GIgG. Phagocytosis of EIgG depressed the survival rate after endotoxemia and bacteremia when endotoxin or bacteria were injected at 30 min after EIgG. The survival rate returned to normal when the endotoxin and bacteria were injected at 12 and 6 h after the EIgG, respectively. Phagocytosis of GIgG did not depress the survival rate after endotoxemia and bacteremia. Thus, compared with erythrocytes, erythrocyte ghosts are more rapidly digested after phagocytosis, depress complement receptor function for a shorter period of time, and cause less depression of host defense. These findings indicate that the contents of erythrocytes play an important role in the impairment of host defense caused by the phagocytosis of erythrocytes by Kupffer cells.

The phagocytosis of erythrocytes by macrophages is associated with a depression of macrophage function. Results of in vitro studies have demonstrated that phagocytosis of erythrocytes depresses the ability of macrophages to phagocytize and kill bacteria (8, 10). The in vivo phagocytosis of erythrocytes by Kupffer cells depresses the hepatic clearance of formalinized sheep erythrocytes and Kupffer cell complement receptor clearance function (14, 15). Erythrocyte phagocytosis also increases the mortality rate caused by endotoxin and bacterial infection (12, 14). We have also shown that the phagocytosis of a number of erythrocytes that depressed Kupffer cell complement receptor function also impaired host defense (14).

The contents of erythrocytes could be responsible for the depression of the macrophage host defense function. Hand (9) has demonstrated that interactions between hemoglobin and reactive products of oxygen metabolism inhibit oxidative bactericidal mechanisms. Erythrocytes contain large amounts of superoxide dismutase, catalase, and glutathione peroxidase which could neutralize the bactericidal function that is based on reactive products of oxygen metabolism (5, 16, 19).

In a previous study from this laboratory (15) it was demonstrated that the hepatic uptake of erythrocyte ghosts depressed host defense much less than intact erythrocytes. In this study we extend this work in several ways. (i) Erythrocytes and erythrocyte ghosts coated with anti-rat erythrocyte immunoglobulin G (EIgG and GIgG, respectively) were used so that the phagocytosis of erythrocytes and ghosts was more likely to be mediated by the same mechanism; (ii) the phagocytosis of erythrocytes and ghosts by Kupffer cells was verified and quantified; (iii) the length of time required for Kupffer cells to digest phagocytized erythrocytes and ghosts was determined; (iv) the duration of the depression of Kupffer cell complement receptor clearance function was determined; and (v) the time course of digestion and recovery of normal complement receptor function was compared with the duration of increased susceptibility to endotoxemia and bacteremia after the phagocytosis of erythrocytes and ghosts.

MATERIALS AND METHODS

Determination of hepatic uptake of EIgG and GIgG. Inbred, male Sprague-Dawley rats (weight, 200 to 250 g) were used for all experiments. Guidelines established by the National Institutes of Health (Bethesda, Md.) for the use of experimental animals were adhered to throughout this investigation. Rat erythrocyte ghosts were prepared by suspending packed erythrocytes in 25 volumes of 5 mM phosphate buffer (pH 8.0). After 30 min at room temperature, a 0.1 volume of 9% NaCl was added, and the suspension was incubated for 30 min at 37°C. The erythrocyte ghosts were washed 3 times and contained about 10% of the hemoglobin present in erythrocytes. Erythrocytes or erythrocyte ghosts coated with IgG were prepared as described previously (14). Briefly, washed erythrocytes or ghosts were incubated with anti-rat erythrocyte IgG (U.S. Biochemical Corp.) at 37°C for 30 min. The concentration of antibody used for the preparation of EIgG and GIgG was adjusted to obtain 65 to 80% hepatic localization of the EIgG at 10 min after injection of 2.9 × 10^8 EIgG per 100 g of body weight. For this study, EIgG, erythrocytes, GIgG or ghosts labeled with ^{51}Cr were injected intravenously (i.v.) at a dose of 17.4 × 10^8/100 g; and localization in the liver, spleen, and lungs was determined at several time points after injection. Separate groups of animals were evaluated at each time point. Organ localization of radioactivity was not corrected for the blood that was present in the organs.

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TABLE 1. Time course of hepatic, splenic, and pulmonary localization of erythrocytes, EIgG, and GIgG[a]

Time (h) after injection and cell type	No. of erythrocytes or ghosts (10^8) in[b]:		
	Liver	Spleen	Lungs
0.5			
Erythrocytes	1.64 ± 0.12	0.97 ± 0.13	0.43 ± 0.02
EIgG	19.73 ± 1.29	9.56 ± 0.25	0.26 ± 0.03
GIgG	31.84 ± 0.43	3.91 ± 0.33	0.57 ± 0.05
3			
Erythrocytes	1.35 ± 0.08	0.83 ± 0.09	0.48 ± 0.04
EIgG	22.00 ± 1.32	16.92 ± 0.85	0.13 ± 0.01
GIgG	30.70 ± 0.91	4.23 ± 0.26	0.36 ± 0.05
6			
Erythrocytes	1.20 ± 0.06	0.55 ± 0.03	0.39 ± 0.01
EIgG	21.53 ± 1.04	17.67 ± 0.46	0.09 ± 0.01
GIgG	29.15 ± 0.51	3.96 ± 0.37	0.25 ± 0.05
12			
Erythrocytes	1.63 ± 0.07	0.80 ± 0.07	0.44 ± 0.03
EIgG	22.96 ± 0.96	16.49 ± 1.04	0.09 ± 0.01
24			
Erythrocytes	1.35 ± 0.04	0.90 ± 0.07	0.41 ± 0.01
EIgG	19.44 ± 0.85	15.00 ± 0.61	0.12 ± 0.03

[a] Erythrocytes, EIgG, and GIgG were injected at a dose of 17.4×10^8/100 g.
[b] Values are the number of erythrocytes or erythrocyte ghosts (10^8) per organ and are the mean ± standard error with six animals per group.

Quantification of the number of phagocytized EIgG and GIgG. Quantitative electron microscopy was used to determine the time course of the digestion of phagocytized EIgG and GIgG. At the designated time after the injection of EIgG, the animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and heparinized (1 U/g), and the portal vein was cannulated. The liver was perfused with 20 ml of phosphate-buffered saline (0.9% NaCl and 50 mM phosphate buffer [pH 7.4]) at a perfusion pressure of 15 cm of water. This was followed immediately by perfusion with 20 ml of 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer containing 2 mM $CaCl_2$ (pH 7.4, 360 mosmol). Two equally spaced biopsy samples were taken from the left lobe of the liver, cut into 1-mm cubes, and immersed in the same fixative for 2 h. Tissue sections were washed twice for 15 min in 0.15 M sodium cacodylate buffer (pH 7.4), postfixed with 1% osmium tetroxide buffered in 0.15 M sodium cacodylate (pH 7.4) for 1.5 h, dehydrated in a graded series of ethanol and propylene oxide, and embedded in Poly Bed 812 (Polysciences). Thin sections were cut on a ultramicrotome (LKB Instruments, Inc., Rockville, Md.), mounted on 300-mesh copper grids, stained with uranyl acetate and lead citrate, and visualized in an electron microscope (JOEL 100CX).

To quantify the number of EIgG and GIgG ingested by Kupffer cells, 20 to 30 Kupffer cells per animal were randomly examined with the electron microscope. The percentage of Kupffer cells containing ghosts, intact erythrocytes, and/or partially digested erythrocytes was determined. Phagocytized erythrocytes were considered to be partially digested if they did not have an intact cell membrane and the cell contents no longer had a uniform dense appearance. Animals were studied at 0.5, 3, 6, 12, and 24 h after the injection of EIgG and 0.25, 0.5, and 3 h after the injection of GIgG.

Determination of Kupffer cell complement receptor clearance function. Complement receptor function was determined as described previously (3). Briefly, rat erythrocytes were coated with rabbit anti-rat erythrocyte IgM. Antibody was obtained from rabbits immunized with rat erythrocytes, and the IgM fraction of the antiserum was separated from the IgG fraction by gel filtration. Washed rat erythrocytes labeled with ^{51}Cr were incubated with the anti-erythrocyte IgM for 30 min at 37°C. The concentration of antibody used was adjusted to obtain 75 to 85% hepatic localization of EIgM at 10 min after injection of 2.9×10^8 EIgM per 100 g. Kupffer cell complement receptor clearance function was assessed from the hepatic localization of EIgM (2.9×10^8/100 g) at 10 min after i.v. injection.

Complement receptor function was determined at 0.5, 3, 6, 12, and 24 h after the injection of EIgG and at 0.5, 3, and 6 h after the injection of GIgG. There were small variations in the hepatic uptake of each preparation of EIgM; therefore, each individual experiment was completed with a single preparation of EIgM. Controls received an injection of erythrocytes that were not coated with IgG.

Endotoxemia. Endotoxin (*Salmonella enteritidis*, lipopolysaccharide B; Difco Laboratories, Detroit, Mich.) was injected i.v. at a dose of 0.2 mg/100 g. The endotoxin was injected at 0.5, 3, 6, 12, and 24 h after the injection of EIgG and at 0.5 h after the injection of GIgG. Controls received an equal volume of phosphate-buffered saline. None of the animals died before 6 h after the injection of endotoxin, and the survival rate was recorded at 24 h.

Bacteremia. *Pseudomonas aeruginosa* PA1348A were grown on nutrient agar plates for 18 h, washed twice in phosphate-buffered saline, and enumerated by determining the optical density against pour plate standards. The bacteria were injected i.v. at a dose of 8.75×10^8 per animal at 0.5, 3, 6, and 12 h after the injection of EIgG and at 0.5 h after the injection of GIgG. Controls received an equal volume of PBS. The survival rate was recorded 48 h after injection of the bacteria. This strain of *P. aeruginosa* is resistant to killing by serum when grown on nutrient agar (4).

Statistics. Data were expressed as the mean ± standard error of the mean. Two group comparisons were analyzed by the Student's *t* test, and multiple group comparisons were analyzed by the one-factor analysis of variance. Survival data were analyzed by the Fisher exact test. The level of confidence was placed at 95% for all experiments.

RESULTS

The hepatic uptake of EIgG occurred during the first 0.5 h after injection (Table 1). There were only small changes in the hepatic localization of EIgG from 0.5 to 24 h after injection. Splenic localization of EIgG increased during the first 3 h after injection. Localization of EIgG in the lungs was less than 1% of the injected dose. The total localization of EIgG in the liver and spleen at 3 h after injection was 84% of the injected dose. At this time point label recovery was 92% (including blood radioactivity), so most of the erythrocytes were removed from the blood by the liver and the spleen. Rat erythrocytes not coated with antibody showed low organ localizations, most of which was probably due to the blood content of the organs.

As with the EIgG, hepatic uptake of GIgG occurred during the first 0.5 h after injection (Table 1). Splenic uptake increased only slightly at 3 h, probably because the hepatic uptake of GIgG was 85% of the injected dose and so few GIgG remained in the blood to be taken up by the spleen. Hepatic uptake of ghosts not coated with antibody was 22.56

$\times 10^8 \pm 1.96 \times 10^8$ ghosts at 30 min after injection. This high hepatic uptake of ghosts may account for the greater hepatic uptake of GIgG than EIgG.

Electron microscopic examination of the livers of noninjected animals or animals injected with erythrocytes not coated with antibody showed that less than 2% of the Kupffer cells contained phagocytized erythrocytes. In animals injected with 17.4×10^8 EIgG per 100 g, more than 35% of the examined Kupffer cells contained intact erythrocytes at 0.5 h after injection (Fig. 1). This value was unchanged at 3 h after injection, decreased to 15.8% by 6 h, and was less than 4% at 12 and 24 h. Thus, half of the ingested erythrocytes were disrupted by 6 h, and essentially none of the Kupffer cells contained intact erythrocytes by 12 h. The percentage of Kupffer cells containing partially digested erythrocytes was less than 5% at 0.5 h, reached a peak of 29% at 6 h, was 25% at 12 h, and decreased to 0% at 24 h. Electron micrographs showing examples of a phagocytized intact erythrocyte and partially digested erythrocytes at 0.5, 6, and 12 h are presented in Fig. 2.

An electron micrograph of a Kupffer cell that phagocytized an erythrocyte ghost is shown in Fig. 2. The time required for the digestion of phagocytized erythrocyte ghosts was much less than that for erythrocytes (Fig. 3). At 15 min after injection a greater percentage of Kupffer cells had phagocytized erythrocyte ghosts than had phagocytized erythrocytes at 30 min, which is consistent with the greater hepatic uptake of labeled ghosts by the liver. The percentage of Kupffer cells containing ghosts had already decreased by 30 min and was less than 10% by 3 h after injection. Less than 10% of the Kupffer cells had ghosts attached to their surfaces at 15 min after injection.

There was a clear discrepancy between the presence of ^{51}Cr in the liver and the documentation of digestion of erythrocytes and ghosts by electron microscopy (Table 1 and Fig. 1 and 3). There was little decrease in the radioactivity in the liver after the phagocytosis of ^{51}Cr-labeled erythrocytes or ghosts over the 24- and 6-h observation periods, respectively. The histological data, however, showed total digestion of the phagocytized erythrocytes and ghosts over these respective time periods.

Kupffer cell complement receptor clearance function was depressed at 0.5 and 3 h after the injection of EIgG (Fig. 4). There was a small but statistically significant depression at 6 h after injection. Complement receptor function was not depressed at 12 and 24 h after the injection of EIgG. After injection of GIgG receptor function was depressed at 0.5 h but not at 3 h (Fig. 5). Receptor function was also depressed at 1 h after the injection of GIgG (data not shown). Thus, complement receptor function was depressed for a longer period of time following the phagocytosis of EIgG than that of GIgG, and recovery of normal receptor function was associated with the digestion of the phagocytized erythrocytes or ghosts.

The survival rate following endotoxemia was decreased when endotoxin was injected at 0.5, 3, or 6 h after the injection of EIgG (Table 2). The survival rate following bacteremia was depressed when *P. aeruginosa* were injected at 0.5 or 3 h after the injection of EIgG (Table 3). The survival rate following endotoxemia and bacteremia was not depressed by the injection of GIgG. Thus, the phagocytosis of erythrocytes had a much greater effect on host defense than phagocytosis of erythrocyte ghosts, and recovery of normal host defense was associated with the disruption of erythrocytes that were phagocytized by Kupffer cells.

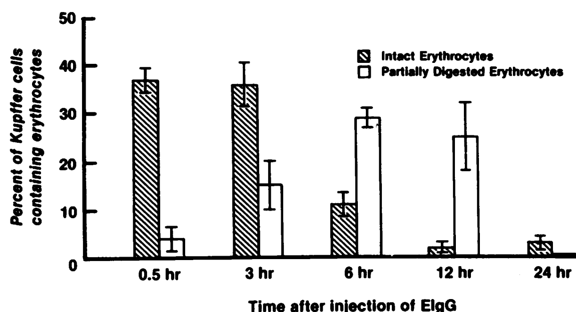

FIG. 1. Time course of the digestion of erythrocytes that were phagocytized by Kupffer cells. EIgG were injected at a dose of 17.4 $\times 10^8$/100 g, and separate groups of animals were evaluated at the inidcated times after injectin. By using quantitative electron microscopic methods, 20 to 30 Kupffer cells were evaluated per animal for the presence of intact erythrocytes, partially digested erythrocytes, or both. Values for the mean ± standard error with six animals per group.

DISCUSSION

Results of previous work from this laboratory (14) have shown that the hepatic uptake of EIgG, injected at a dose of 2.9×10^8/100 g, reached a maximum value by 10 min after injection. In this study we have shown that the hepatic uptake of a larger dose of EIgG (17.4×10^8/100 g) occurred entirely during the initial 30 min after injection. After injection, the EIgG activate complement and become coated with C3b/C3bi, which, in turn, mediates the binding to Kupffer cells (7). Within 30 min, the C3b/C3bi on the surface of the EIgG is converted to C3d, which does not bind to Kupffer cells. Prior depletion of complement with cobra venom factor results in very low hepatic uptake of EIgG, indicating that Fc receptors on Kupffer cells play little role in the in vivo hepatic clearance of EIgG (7, 14). Therefore, the time course of the hepatic uptake of EIgG is explained by the deposition and subsequent degradation of opsonic complement components on the EIgG. While Fc receptors are not responsible for the clearance of EIgG, these receptors are necessary for the phagocytosis of EIgG. This is demonstrated by the observation that EIgM, which do not interact with Fc receptors, bind to Kupffer cells but are not phagocytized and then are released back into the blood when the opsonic complement factors are degraded (7, 14). In this study electron microscopic observations confirmed that the hepatic uptake of EIgG and GIgG was the result of binding to and phagocytosis by Kupffer cells. EIgG and GIgG did not bind to endothelial cells, which is consistent with the results of a study by Muto and Fujita (17).

In contrast to Kupffer cells, Fc receptors on splenic macrophages can mediate the clearance of EIgG (7). This is indicated by the increase in splenic uptake of EIgG between 30 min and 3 h after injection. Splenic uptake of EIgG or GIgG did not increase after 3 h or 30 min, respectively, because at these times nearly all of the injected dose was localized in the liver and spleen. Localization of EIgG and GIgG in the lungs never exceeded 1% of the injected dose.

Kupffer cells are well known to be capable of digesting phagocytized erythrocytes (18). There are few reports in the literature, however, on the time required to digest a given number of ingested erythrocytes. In this study more than 3 h was required before there was a decrease in the proportion of Kupffer cells containing intact erythrocytes. By 24 h

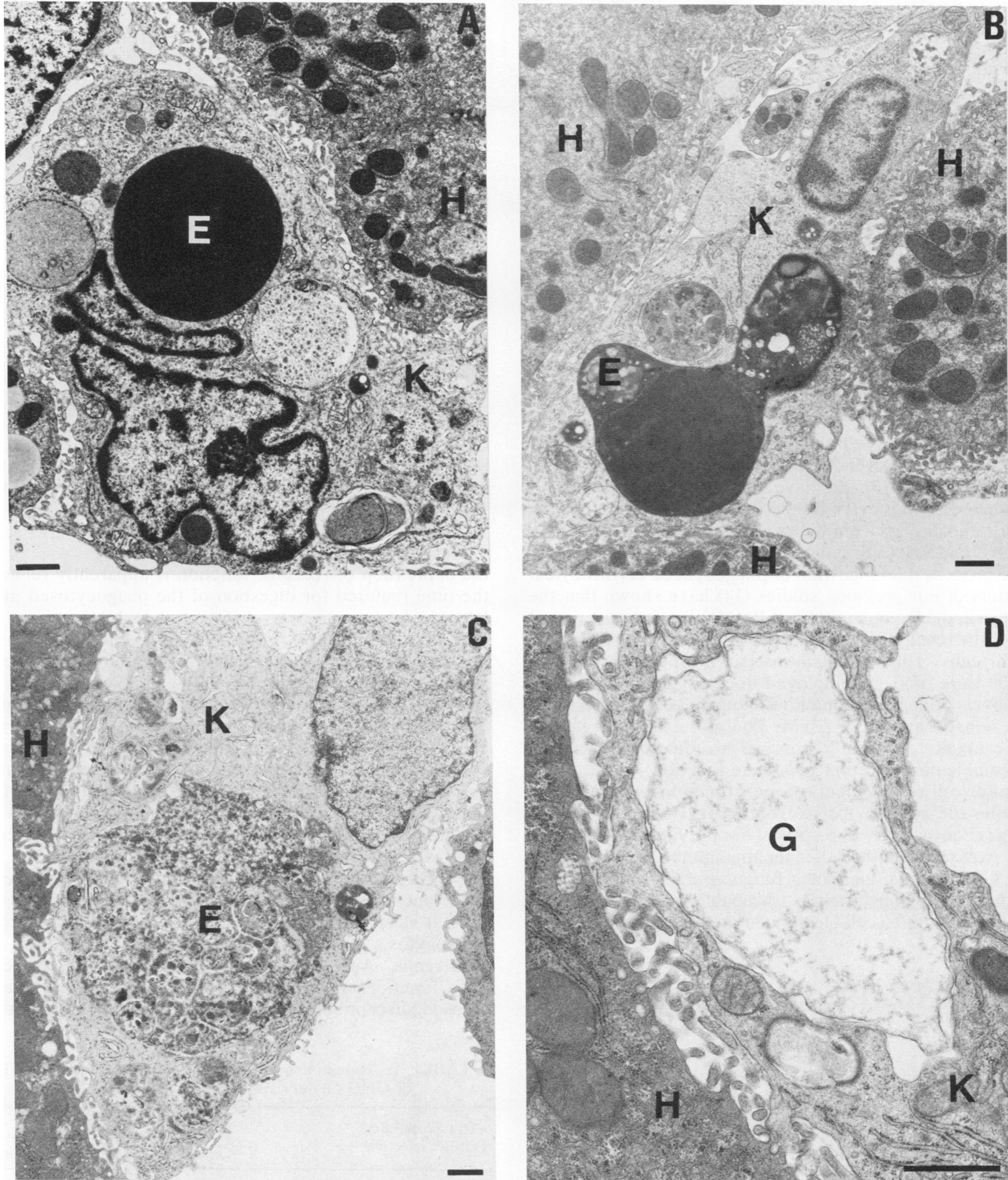

FIG. 2. Transmission electron micrographs showing the phagocytosis and digestion of erythrocytes and erythrocyte ghosts. (A) A Kupffer cell (K) containing an intact erythrocyte (E) at 0.5 h after injection of EIgG. H, hepatocyte. (B) A Kupffer cell containing a partially digested erythrocyte (E) at 6 h after the injection of EIgG. (C) A Kupffer cell containing a partially digested erythrocyte (E) at 12 h after the injection of EIgG. (D) A Kupffer cell containing an erythrocyte ghost (G) at 0.5 h after the injection of GIgG. Bars, 1 μm. Magnifications: panel A, ×6,750; panels B and C, ×5,780; panel D, ×14,530.

phagocytized erythrocytes were completely digested by the Kupffer cells. The digestion of phagocytized ghosts by Kupffer cells proceeded much more rapidly than the digestion of erythrocytes. This faster digestion of ghosts occurred in spite of the fact that a larger number of ghosts than erythrocytes were phagocytized and that the percentage of Kupffer cells containing phagocytized ghosts was initially greater than the percentage containing erythrocytes.

The lack of concurrence of hepatic localization of ^{51}Cr and histological data on erythrocyte and ghost digestion by

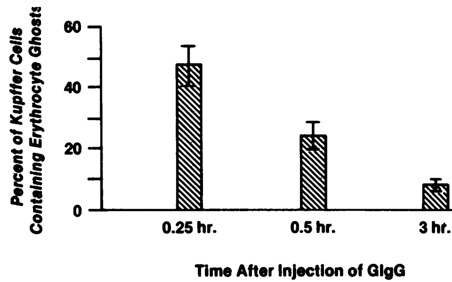

FIG. 3. Time course of the digestion of erythrocyte ghosts that were phagocytized by Kupffer cells. GIgG were injected at a dose of 17.4×10^8/100 g, and separate groups of animals were evaluated at the indicated times after injection. By using quantitative electron microscopic methods, 20 to 30 Kupffer cells were evaluated per animal for the presence of intact erythrocyte ghosts. Values are the mean ± standard error with six animals per group.

Kupffer cells indicates that hepatic localization of ^{51}Cr cannot be used as an index of erythrocyte digestion. Other investigators (11, 13, 20) have shown that the uptake of ^{51}Cr-labeled erythrocytes by the liver is followed by a slow loss of radioactivity from the liver. The results of this study indicate that the loss of radioactivity from the liver is a much slower process than digestion of phagocytized erythrocytes.

Results of our previous studies (14) have shown that the decrease in the hepatic uptake of EIgM following injection of EIgG is the result of an impairment of the binding of EIgM to Kupffer cells. This conclusion is based on the observation that the dose of EIgG employed does not depress complement levels or hepatic blood flow enough to cause a decrease in the hepatic uptake of EIgM. Therefore, the decrease in hepatic uptake of EIgM represents a reduction in Kupffer cells complement receptor clearance function.

Kupffer cell complement receptor function was depressed following the phagocytosis of both EIgG and GIgG. This finding is consistent with results of our previous work (15), in which we showed that the hepatic uptake of ghosts depresses the hepatic clearance function and which suggests that the act of phagocytosis is capable of depressing the subsequent Kupffer cell clearance function. The duration of

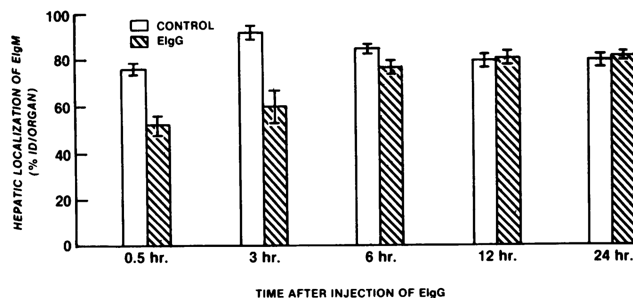

FIG. 4. Time course of the depression of the Kupffer cell complement receptor clearance function after the injection of EIgG. Unlabeled EIgG were injected at a dose of 17.4×10^8/100 g, and the complement receptor function was evaluated at the indicated times after injection. Labeled EIgM were injected at a dose of 2.9×10^8/100 g, and hepatic localization was determined 10 min after injection. Values are expressed as the percentage of the injected dose (%ID) of EIgM localized in the liver and are the mean ± standard error with six animals per group. There was a significant difference ($P < 0.05$) between the control and the experimental groups at the 0.5-, 3-, and 6-h time points.

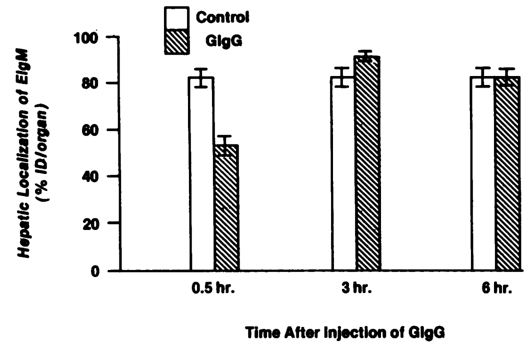

FIG. 5. Time course of the depression of the Kupffer cell complement receptor clearance function after the injection of GIgG. Unlabeled GIgG were injected at a dose of 17.4×10^8/100 g, and the complement receptor function was evaluated at the indicated times after injection. Labeled EIgM were injected at a dose of 2.9×10^8/100 g, and hepatic localization was determined 10 min after injection. Values are expressed as the percentage of the injected dose (%ID) of EIgM localized in the liver and are the mean ± standard error with six animals per group. There was a significant difference ($P < 0.05$) between the control and experimental groups at the 0.5-h time point.

the depression of receptor function is apparently related to the time required for digestion of the phagocytosed material.

In previous studies (14, 15) it has been shown that the phagocytosis of erythrocytes is associated with increased mortality following endotoxin shock and bacterial infection. We have also shown (14, 15) that the injection of erythrocytes that are not coated with antibody are not phagocytized and do not increase the mortality rate after endotoxemia or bacteremia. Results of this study extend these observations by demonstrating that the recovery of normal host defense function requires about the same amount of time as that required to at least partially digest the phagocytized erythrocytes.

Depression of the Kupffer cell complement receptor clearance function has been shown (2, 3) to be associated with several states of depressed host defense, including injury, phagocytosis of particulate material, endotoxemia, and bacteremia. Additionally, the complement receptor clearance function was depressed by doses of EIgG that increased susceptibility to endotoxemia and bacteremia (14).

TABLE 2. Time course of the effect of phagocytosis of EIgG or GIgG on survival rate after endotoxin shock

Cell type and time (h) after injection[a]	% Survival[b]	
	Control	Experimental
EIgG		
0.5	75	35[c]
3	80	25[c]
6	75	15[c]
12	85	85
24	85	100
GIgG, 0.5	65	45

[a] Endotoxin (*S. enteritidis*) was injected at a dose of 0.2 mg/100 g at the indicated times after injection of 17.4×10^8 EIgG or GIgG per 100 g.

[b] Survival was recorded at 24 h after the injection of endotoxin. There were 20 animals per group.

[c] $P < 0.05$.

TABLE 3. Time course of the effect of phagocytosis of EIgG or GIgG on survival rate after bacterial infection

Cell type and time (h) after injection[a]	% Survival[b]	
	Control	Experimental
EIgG		
0.5	90	20[c]
3	80	20[c]
6	95	85
12	90	100
GIgG, 0.5	80	95

[a] *P. aeruginosa* was injected at a dose of 8.75×10^8 at the indicated times after injection of 17.4×10^8 EIgG or GIgG per 100 g.
[b] Survival was recorded at 48 h after the injection of the bacteria. There were 20 animals per group.
[c] $P < 0.05$.

In this study we have demonstrated that the time course of the EIgG-induced depression of the complement receptor clearance function is similar to that of the depressed host defense. The phagocytosis of ghosts, however, did not depress host defense but did depress complement receptor function. This may have been due to the brief depression of the Kupffer cell receptor function after the phagocytosis of ghosts.

The rationale for implicating complement receptor function in host defense is strengthened by the observation that individuals with a hereditary deficiency of CR3 (the complement receptor that binds C3bi) have severe recurrent bacterial infections (6, 21, 22). In some cases the deficiency may not be restricted to CR3 and includes other important surface proteins on lymphocytes, granulocytes, and macrophages (6, 22).

The mechanism of the increased susceptibility to bacterial infection and endotoxin shock following the phagocytosis of erythrocytes by Kupffer cells remains to be determined. The difference in the effect of erythrocyte or ghost phagocytosis on host defense may simply be due to the greater mass of material that is internalized when erythrocytes are phagocytized. The nature of the erythrocyte contents, however, may also be important in this regard. Erythrocytes contain large amounts of superoxide dismutase, catalase, and glutathione peroxidase that could neutralize reactive oxygen metabolites and thereby decrease bactericidal function (5, 16, 19). Erythrocytes have been shown to prevent hydrogen peroxide-mediated damage to lung endothelial cells and leukemia cells (1, 23). Also, it has been shown that erythrocyte phagocytosis depresses macrophage antibacterial function and that hemoglobin can scavange reactive oxidative products (8–10). Therefore, the contents of erythrocytes appear to be capable of interfering with the host defense function of macrophages. The recovery of normal macrophage function, when partially digested erythrocytes were still present within Kupffer cells, may be due to inactivation of enzymes and denaturation of hemoglobin in the harsh environment of the phagolysosome. Additionally, the recovery of normal host defense function may be mediated by more than just the time required to regenerate or recycle receptors to the Kupffer cell surface, because the phagocytosis of erythrocyte ghosts did not depress host defense function. Other factors such as the impairment of bacterial clearance or depression of several aspects of macrophage function that may be associated with the depressed complement receptor function could contribute to the impairment of host defense against infection.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants GM-26102 from the Institute of General Medical Sciences and RR-01923 from the Division of Research Resources. Fred L. Minnear is a recipient of Public Health Service Research Career Development Award K04-HL-01830.

We thank A. L. Baltch (Veterans Administration Medical Center, Albany, N.Y.) for generously supplying the *P. aeruginosa* PA1348A culture. The use of Kevin Barron's electron microscopy facilities at the Veterans Administration Medical Center, Albany, N.Y. is gratefully acknowledged. The excellent technical assistance of Carol Fryc and the secretarial assistance of Marueen Davis are also acknowledged.

LITERATURE CITED

1. **Agar, N. S., S. M. H. Sadradeh, P. E. Hallaway, and J. W. Eaton.** 1986. Erythrocyte catalase; a somatic oxidant defense? J. Clin Invest. **77**:319–321.
2. **Cuddy, B. G., D. J. Loegering, and F. A. Blumenstock.** 1984. Depression of *in vivo* clearance function of hepatic macrophage complement receptors following thermal injury. Proc. Soc. Exp. Biol. Med. **176**:443–451.
3. **Cuddy, B. G., D. J. Loegering, F. A. Blumenstock, and D. M. Shah.** 1986. Depression of hepatic macrophage complement receptor clearance function following injury. J. Surg. Res. **40**: 216–224.
4. **DeMatteo, C. S., M. C. Hammer, A. L. Baltch, R. P. Smith, N. T. Sutphen, and P. B. Michelsen.** 1981. Susceptibility of *Pseudomonas aeruginosa* to serum bactericidal activity. J. Lab. Clin. Med. **98**:511–518.
5. **Eaton, J. W., M. Boraas, and N. L. Etkin.** 1972. Catalase activity and red cell metabolism. Adv. Exp. Biol. Med. **28**:121–131.
6. **Fischer, A., R. Seger, A. Durandy, B. Grospierre, J. L. Virelizier, F. LeDeist, C. Griscelli, E. Fischer, M. Kazatchkine, M. Bohler, B. Deschamps-Latscha, P. Trung, T. Springer, D. Oliver, and C. Mawas.** 1985. Deficiency of the adhesive protein complex lymphocyte function antigen 1, complement receptor type 3, glycoprotein P150,95 in a girl with recurrent bacterial infections. Effects on phagocytic cells and lymphocyte functions. J. Clin. Invest. **76**:2385–2392.
7. **Frank, M. M., A. D. Schreiber, J. P. Atkinson, and C. J. Jaffe.** 1977. Pathophysiology of immune hemolytic anemia. Ann. Intern. Med. **87**:210–222.
8. **Gill, F. A., D. Kay, and W. E. Hook.** 1966. The influence of erythrophagocytosis on the interaction of macrophages and salmonella *in vitro*. J. Exp. Med. **124**:173–183.
9. **Hand, W. L.** 1984. Inhibition of cell-free oxidative bactericidal activity by erythrocytes and hemoglobin. Infect. Immun. **44**: 465–468.
10. **Hand, W. L., and N. L. King-Thompson.** 1983. Effect of erythrocyte ingestion on macrophage antibacterial function. Infect. Immun. **40**:917–923.
11. **Jandl, J. H., A. R. Jones, and W. B. Castle.** 1957. The destruction of red cells by antibodies in man. I. Observations of the sequestration and lysis of red cells altered by immune mechanisms. J. Clin. Invest. **36**:1428–1459.
12. **Kay, D., F. A. Gill, and E. W. Hook.** 1967. Factors influencing host resistance to salmonella infections: the effects of hemolysis and erythrophagocytosis. Am. J. Med. Sci. **254**:205–215.
13. **Loebl, E. C., C. R. Baxter, and P. W. Curreri.** 1973. The mechanism of erythrocyte destruction in the early post-burn period. Ann. Surg. **178**:681–686.
14. **Loegering, D. J., and F. A. Blumenstock.** 1985. Depressing hepatic macrophage complement receptor function causes increased susceptibility to endotoxemia and infection. Infect. Immun. **47**:659–664.
15. **Loegering, D. J., G. J. Grover, and M. J. Schneidkraut.** 1984. Effect of red blood cells and red blood cell ghosts on reticuloendothelial system function. Exp. Mol. Pathol. **41**:67–73.
16. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol.

Chem. **244**:6049–6053.

17. **Muto, M., and T. Fujita.** 1977. Phagocytic activities of the Kupffer cell: a scanning electron microscopic study, p. 109–119. *In* E. Wisse and D. L. Knook (ed.), Kupffer cells and other liver sinusoidal cells. Elsevier/North-Holland Publishing Co., Amsterdam.
18. **Nelson, J. C., N. G. Ibraham, and R. D. Levere.** 1984. Erythroclasia and bilirubin metabolism, p. 189–220. *In* S. M. Reichard and J. P. Filkins (ed.), The reticuloendothelial system. A comprehensive treatise, vol. 7A. Plenum Publishing Corp., New York.
19. **Paglia, D. E., and W. N. Valentine.** 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. **70**:158–169.
20. **Schneidkraut, M. J., and D. J. Loegering.** 1984. Effect of extravascular hemolysis on the RES depression following thermal injury. Exp. Mol. Pathol. **40**:271–279.
21. **Schrieber, R. D.** 1984. The chemistry and biology of complement receptors. Springer Semin. Immunopathol. **7**:221–249.
22. **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalsteig, and D. C. Anderson.** 1984. Inherited deficiency of the Mac-1,LFA-1, p150,95 glycoprotein family and its molecular basis. J. Exp. Med. **160**:1901–1918.
23. **Toth, K. M., D. P. Clifford, E. M. Berger, C. W. White, and J. E. Repine.** 1984. Intact human erythrocytes prevent hydrogen peroxide-mediated damage to isolated perfused rat lungs and cultured bovine pulmonary endothelial cells. J. Clin. Invest. **74**:292–295.