ANNALS OF SURGERY
Vol. 218, No. 6, 791–799

Macrophage Elimination Increases Bacterial Translocation and Gut-Origin Septicemia but Attenuates Symptoms and Mortality Rate in a Model of Systemic Inflammation

Grard A. P. Nieuwenhuijzen, M.D.,*† Yuval Haskel, M.D.,‡ Qi Lu, M.D.,‡ Rodney D. Berg, Ph.D.,§ Nico van Rooijen, Ph.D.,‖ R. Jan A. Goris, M.D.,* and Edwin A. Deitch, M.D.‡

From the Departments of Surgery† and Microbiology and Immunology,§ Louisiana State University Medical Center, Shreveport, Louisiana, and the Department of Surgery, University Hospital St. Radboud, Nijmegen, and the Department of Cell Biology, Division of Histology, Vrije Universiteit, Amsterdam,‖ The Netherlands*

Objective

The central question tested in this study was whether dichloromethylene-diphosphonate (CL_2MDP) liposome-mediated elimination of hepatic and splenic macrophages would influence zymosan-induced bacterial translocation and the zymosan-induced generalized inflammatory response.

Summary Background Data

Both an uncontrolled activation of macrophages and the loss of intestinal barrier function have been implicated in the development of adult respiratory distress syndrome and multiple organ failure.

Methods

Macrophage elimination was accomplished by intravenous injection of 200 μL of CL_2MDP–liposome suspension. Control mice received an intravenous injection of 200 μL of phosphate-buffered saline. Two days later, the animals were challenged intraperitoneally with zymosan suspended in paraffin to determine a dose–response curve (0.1, 0.5, or 1.0 mg/g body weight). Twenty-four hours after zymosan challenge, signs of systemic stress were determined, and bacterial translocation to the mesenteric lymph node, liver, spleen, and blood was measured. A separate mortality study was performed with a dose of 1.0 mg/g of zymosan suspension.

Results

The incidence of the systemic spread of bacteria was significantly increased in the macrophage-depleted mice. Although systemic bacterial translocation was promoted by macrophage elimination, the systemic toxic response was significantly decreased in all macrophage-depleted groups ($p \leq 0.01$). The 12-day mortality rate was 0% in the macrophage-depleted groups and 27% in the control group ($p = 0.05$).

Conclusions

The lethal and toxic effects of zymosan appear to be related more to the excessive activation of macrophages than to the systemic spread of bacteria.

Both an uncontrolled inflammatory response[1] and the loss of intestinal barrier function leading to gut-origin septic states[2] have been clinically implicated in the development of adult respiratory distress syndrome and multiple organ failure (MOF). The experimental and clinical evidence supporting the macrophage and gut hypotheses of MOF have been recently reviewed.[3] Briefly, in the macrophage- or inflammatory-mediated model of MOF, the trigger that initiates the cascade of events leading to organ failure is the excessive and uncontrolled production and release of cytokines and other immunoinflammatory products. By contrast, in the gut hypothesis of MOF, the loss of intestinal barrier function results in the translocation of bacteria and/or endotoxin from the gut to systemic organs and tissue where they initiate, exacerbate, or perpetuate a systemic inflammatory response. The ability of gut-derived portal and systemic bacteria and endotoxin to activate Kuppfer cells and other tissue macrophage populations has been well documented experimentally.[3-5] Thus, there appears to be considerable physiologic overlap between the two hypotheses because the mediators that ultimately lead to organ injury in both hypotheses are similar.

Conceptually, macrophage elimination could disrupt the sequence of events leading to organ injury by dampening the inflammatory signal. However, because macrophages are involved in wound healing, antibacterial host defenses, and the metabolic response, impaired macrophage function can also have deleterious consequences. Consequently, it would be interesting to examine what influence macrophage depletion might have in a model of generalized inflammation associated with bacterial translocation. To accomplish this goal, we used the "liposome-mediated macrophage suicide technique" to deplete mice of splenic and hepatic macrophages[6] before challenge with the inflammatory agent, zymosan.[7] This was chosen as the inflammatory agent because it induces a generalized inflammatory response that is associated with bacterial translocation,[8] signs of sepsis,[9] and the development of a syndrome similar to MOF.[7]

† Recipient of a traveling fellowship of the Surgical Infection Society Europe.

Address reprint requests to Edwin A. Deitch, M.D., Department of Surgery, LSUMC School of Medicine, 1501 Kings Highway, Shreveport, LA 71130-3932.

Accepted for publication March 16, 1993.

MATERIALS AND METHODS

Animals

Ninety specific pathogen-free male mice (ICR; Harlan Breeding Laboratories, Prattsville, AL) weighing 21 to 25 g were used in these experiments. The mice were housed in autoclaved polystyrene cages under barrier-sustained conditions with controlled temperature (22 C), humidity, and lighting (12-hour light–dark cycles). They were fed standard laboratory chow (Diet 5001, Ralston Purina, St. Louis, MO) and acidified water (0.001 N HCl) *ad libitum*. The mice were maintained according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the experiments were approved by the Louisiana State University-Medical Center, Shreveport, Animal Care Committee.

Zymosan

The zymosan suspension was prepared as previously described.[10] The zymosan (Sigma Chemical, St. Louis, MO) was irradiated with 5 kGy and suspended by high-frequency vibration in 100 mL of liquid paraffin (European Pharmacopeia PA.S.68.81 CP846021). This suspension was sterilized by incubation in a water bath at 100 C for 80 minutes. The concentration of the zymosan in the paraffin suspensions varied based on the dose administered to ensure that each animal received the same volume in each animal group (approximately 1 mL/animal): 2.5 mg/mL for the 0.1-mg/g dose, 12.5 mg/mL for the 0.5-mg/g dose, and 25 mg/mL for the 1.0-mg/g dose. Before use, the zymosan suspension was warmed to 40 C and vibrated at high frequency for 15 minutes. Sterility was verified by incubation on blood–agar culture plates. The zymosan suspension was administered intraperitoneally, depending on the treatment group, in doses of 0.1, 0.5, or 1.0 mg of zymosan per gram body weight.

Dichloromethylene-Diphosphonate (CL_2MDP)-Containing Liposomes

Multilamellar liposomes were composed of phosphatidylcholine and cholesterol (molar ratio, 6:1) and contained CL_2MDP dissolved in phosphate-buffered saline (PBS, 0.125 g/mL). They were prepared as described previously.[6] In brief, 86 mg of phosphatidylcholine and 8 mg of cholesterol (Sigma) were dissolved in 10 mL of chloroform, and a lipid film was produced by low-vac-

uum rotary evaporation, which was dissolved in 10 mL of CL_2MDP. The suspension was kept at room temperature for 2 hours; after this, it was sonicated for 3 minutes in a water bath sonicator at 20 C and again kept for 2 hours at 37 C. The CL_2MDP–liposome suspension was then diluted in 100 mL of PBS and centrifuged at 100,000 × *g* for 30 minutes to remove free CL_2MDP. Then the CL_2MDP-containing liposomes were resuspended in 4 mL of PBS. CL_2MDP was a kind gift of Boehringer Mannheim (Mannheim, Germany).

Testing for Bacterial Translocation

The mice were killed by cervical dislocation. After cleaning the skin with 75% alcohol, the thorax was opened using sterile instruments, and approximately 0.5 mL of blood was withdrawn by heart puncture and cultured aerobically for 24 hours at 37 C in 50 mL of brain–heart infusion solution. A specimen of the lungs was removed, weighed, and placed in grinding tubes (Tri-R Instruments, Rockville Center, NY) containing sterile PBS. Subsequently, an incision was made through the skin and peritoneum of the abdomen. Using sterile technique the mesenteric lymph node complex (MLN), spleen, liver, and cecum were removed, weighed, and placed in grinding tubes. The organs were then homogenized, and aliquots (0.1 mL) of the homogenate were plated directly and after serial dilutions onto blood–agar plates to detect the total aerobic and facultative aerobic bacteria and onto MacConkey's agar to detect gram-negative bacilli. The plates were incubated aerobically for 24 hours at 37 C. The number of viable bacteria was determined as the number of colony-forming units per gram of tissue.

Experimental Protocol

To examine whether or not macrophage elimination could influence zymosan-induced bacterial translocation and toxicity, three doses of zymosan were tested. Before the zymosan challenge, the mice were randomly assigned to a macrophage-nondepleted "control" group and a macrophage-depleted group (n = 8 in each group). The macrophage-depleted groups received 200 μL of the CL_2MDP–liposome suspension and the macrophage-nondepleted groups, 200 μL of PBS by tail vein injection. Eighty-four hours later, the animals received an intraperitoneal injection of the zymosan suspension at a dose of either 0.1, 0.5, or 1.0 mg/g. Twenty-four hours after the zymosan injection, signs of systemic toxicity were determined, and the organs were tested for bacterial translocation. Systemic toxicity was graded in a blinded fashion and assessed by recording the extent of conjunctivitis, ruffled fur, lethargy, and diarrhea using a five-point scale (0, none; 1, minimal; 2, moderate; 3, severe; and 4, extensive).

An additional two control groups of six animals were tested to verify that neither the paraffin vehicle, the PBS, nor the CL_2MDP–liposome suspension alone induced bacterial translocation or systemic toxicity. One group received 200 μL of PBS, and the second group received the CL_2MDP–liposome suspension intravenously followed 2 days later by an intraperitoneal injection of the paraffin vehicle (0.04 mL/g) that did not contain zymosan.

Lastly, the mortality rate was assessed in two separate groups of control and macrophage-depleted mice (n = 15 per group) challenged with a dose of 1.0 mg/g of zymosan. These animals were followed for 12 days.

Histologic Findings

To determine whether or not macrophage elimination altered the zymosan-induced intestinal mucosal injury, the ileums from at least three mice per group were analyzed by light microscopy. The specimens were recovered after death and fixed by luminal perfusion and immersion in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.4 overnight at 4 C. The tissue was then dehydrated to 95% ethanol and embedded in methyl methacrylate (JB-4, Polysciences, Warrington, PA). Semithin sections of 2 to 3 μm were cut on glass knives and stained with 1% toluidine blue.

To verify that the macrophages had been successfully eliminated by the time of zymosan administration, specimens of the spleens were removed, and cryostat sections of 8-μm thickness were cut. The sections were stained for acid phosphatase activity. Acid phosphatase activity was demonstrated by incubation with naphthol biphosphate and para-rosaniline for 30 to 45 minutes at 37 C.

Statistical Analysis

Continuous data were analyzed by analysis of variance (ANOVA). Between-group significance levels were assessed by using the Student–Newman–Keuls *post hoc* test. Data on bacterial levels were log transformed before analysis. Discontinuous data were evaluated by using the Fisher exact test when the smallest number in a comparison was smaller then or equal to 5. In all other cases, the chi square test with a continuity correction was used. When $p \leq 0.05$, the difference was considered significant.

RESULTS

Macrophage elimination was successfully achieved by the injection of liposomes containing CL_2MDP, as reflected by the absence of acid phosphatase activity in the

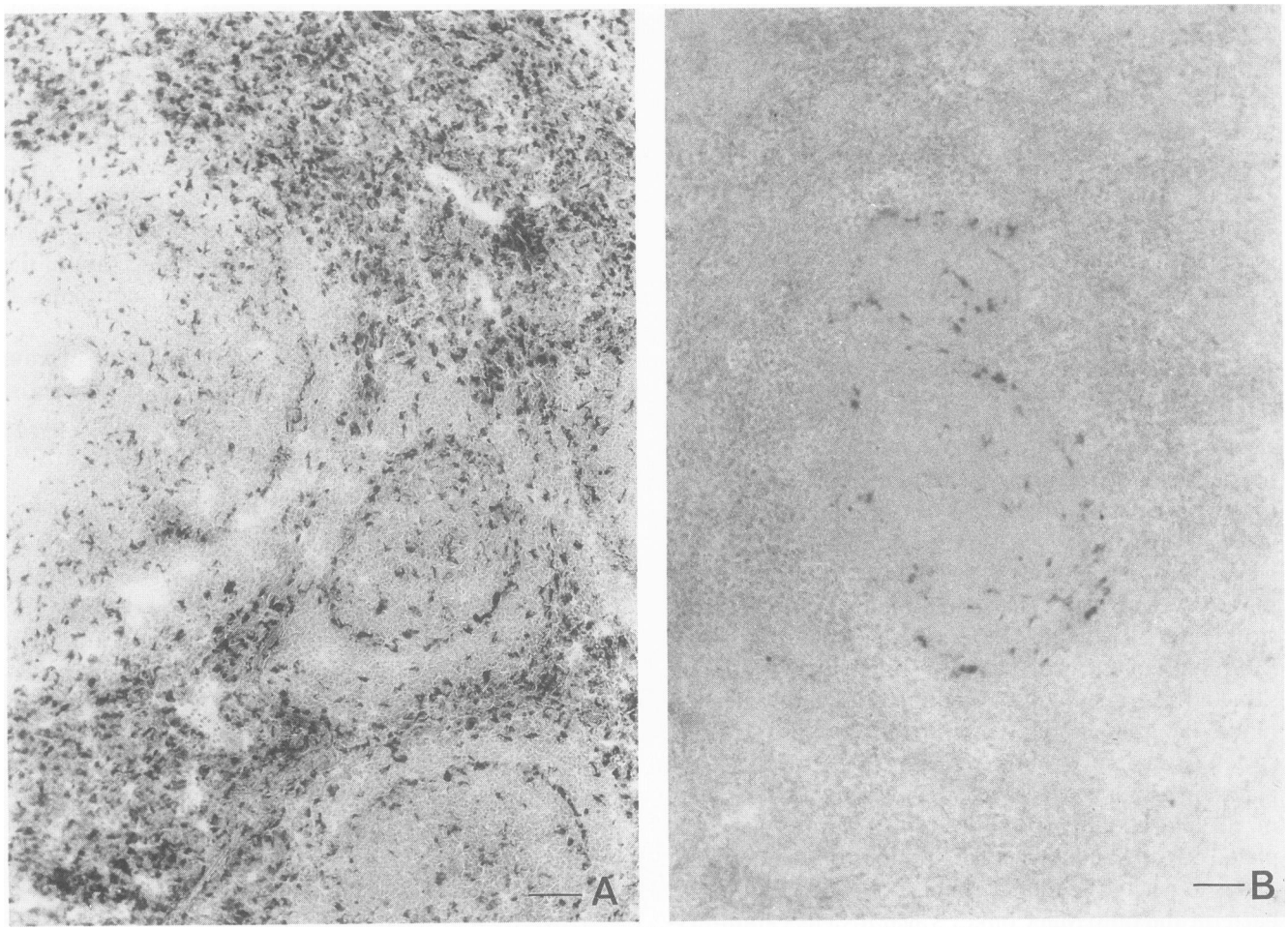

Figure 1. Splenic tissue sections stained for acid phosphatase 48 hours after pretreatment (× 100). (A) PBS pretreated mouse spleen in which macrophages are present throughout the tissue. (B) CL_2MDP-containing liposome-pretreated mouse spleen in which most macrophages have been eliminated. Some macrophages in the white pulp remained. Scale bar = 100 μm.

splenic tissues of the CL_2MDP-treated mice (Fig. 1). Neither the paraffin vehicle nor the CL_2MDP–liposome suspension promoted bacterial translocation in the absence of a zymosan challenge (Table 1) or induced signs of systemic toxicity (data not shown). By contrast, bacterial translocation occurred in the mice receiving zymosan in a dose-dependent fashion (Table 1), with more systemic spread being observed in the animals receiving 0.5 or 1.0 mg/g than in the animals receiving 0.1 mg/g of zymosan. The incidence of bacterial translocation to the MLN was similar between the macrophage-depleted and -nondepleted groups. However, macrophage depletion was associated with a significantly higher incidence of the systemic spread of bacteria after challenge with the higher two doses of zymosan (Table 1). This was especially true for the lung (Table 2). The indigenous bacterial flora, as reflected in the cecal population levels, was clearly increased by zymosan administration (Table 1). The cecal bacterial population levels were not higher in the macrophage-depleted groups than in the comparable nondepleted groups after zymosan challenge. Thus, the increase in the systemic spread of translocating bacteria observed after macrophage depletion does not appear to be related to changes in the cecal bacterial population levels (Table 1).

By contrast with the incidence of bacterial translocation, the magnitude of bacterial translocation to the MLN and the systemic organs, expressed as the number of colony-forming units of total aerobic bacteria (includes gram-negative enteric and gram-positive facultative aerobes), was similar between the macrophage-depleted and -nondepleted groups (Table 3). This was also true when the magnitude of only gram-negative enteric bacterial translocation was quantified (data not shown).

Zymosan-induced mucosal injury appeared to occur in a dose-dependent fashion and ranged from slight vil-

Table 1. INFLUENCE OF MACROPHAGE DEPLETION ON INCIDENCE OF BACTERIAL TRANSLOCATION AND CECAL POPULATION LEVELS

Group	N	Bacterial Translocation to MLN (%)	Systemic Bacterial Translocation (liver/spleen/lung/blood) (%)	Cecal Population Levels (log CFU/g)	
				Aerobes	Gram-negatives
Paraffin					
Controls	6	0/6 (0)	0/24 (0)	5.14 ± 0.05	4.20 ± 0.25
Macrophage depleted	6	0/6 (0)	0/24 (0)	5.07 ± 0.15	4.13 ± 0.16
Zymosan 0.1 mg/g					
Controls	12	6/12 (50)	1/48 (2)	6.95 ± 0.44‡	6.77 ± 0.48‡
Macrophage depleted	11	0/11 (0)*	4/44 (9)	6.26 ± 0.21‡	5.65 ± 0.25‡
Zymosan 0.5 mg/g					
Controls	8	7/8 (88)‖	10/32 (31)‖	10.25 ± 0.10§	10.18 ± 0.11§
Macrophage depleted	8	6/8 (75)‖	19/32 (59)*‖	9.18 ± 0.40§	9.00 ± 0.47§
Zymosan 1.0 mg/g					
Controls	8	7/8 (88)‖	11/32 (34)‖	9.27 ± 0.43§	9.17 ± 0.43§
Macrophage depleted	8	8/8 (100)‖	23/32 (72)†‖	9.69 ± 0.35§	9.60 ± 0.33§

* $p \leq 0.05$ and † $p \leq 0.01$, control *vs.* macrophage-depleted group of same zymosan dose group. ‡ $p \leq 0.05$ and § $p \leq 0.01$, zymosan *vs.* paraffin. ‖ $p \leq 0.01$, zymosan 1.0 mg/g or 0.5 mg/g *vs.* 0.1 mg/g (Fischer exact test or ANOVA with Student Newman Keuls post hoc).

lous tip edema at the lowest zymosan dose to almost complete disruption of the mucosal architecture at the highest dose (Fig. 2). Because the magnitude of histologic damage to the terminal ileum was similar between the groups, the increased incidence of systemic bacterial translocation in the macrophage-depleted mice did not appear to be caused by an increase in the extent of injury to the mucosal barrier.

The administration of zymosan was associated with the development of signs of systemic toxicity that occurred in a dose-dependent fashion (Fig. 3). However, the macrophage-depleted mice receiving either 0.5 or 1.0 mg/g of zymosan had lower symptom scores than did the macrophage-nondepleted animals receiving comparable doses of zymosan. In addition, the 12-day mortality rate after a 1.0-mg/g zymosan challenge was significantly lower in the macrophage-depleted than in the macrophage-nondepleted mice (Fig. 3).

Table 2. DISTRIBUTION OF THE INCIDENCE OF SYSTEMIC BACTERIAL TRANSLOCATION AMONG THE TESTED ORGANS

Group	Liver	Spleen	Lung	Blood
Zymosan 0.5 mg/g				
Controls	37.5	50	25	12.5
Macrophage depleted	75	50	100*	12.5
Zymosan 1.0 mg/g				
Controls	50	50	25	12.5
Macrophage depleted	87.5	62.5	87.5†	50

Values are percentages.

* $p \leq 0.01$ and † $p \leq 0.05$, control *vs.* macrophage-depleted group of same zymosan dose group using the Fischer exact test on the actual numbers.

DISCUSSION

The central question asked in this study was what effect elimination of splenic and hepatic macrophages would have in a systemic inflammatory model associated with bacterial translocation. To answer this question, we used the CL_2MDP–liposome-mediated macrophage suicide technique[6] to eliminate the hepatic and splenic macrophage populations. In this technique, liposomes are used as carriers for the CL_2MDP because liposome-encapsulated CL_2MDP is easily and preferentially ingested by macrophages. Once phagocytized, the liposomal bilayers are disrupted by lysosomal enzymes, resulting in the intracellular release of CL_2MDP and the subsequent death of the macrophages. Which macrophage populations will be eliminated depends on the route by which the CL_2MDP-containing liposomes are administered.[11] Because liposomes cannot cross the endothelium or epithelium, only hepatic and splenic macrophages will be eliminated after an intravenous injection. Macrophages in organs without sinusoids like the lung, mesenteric lymph nodes, or lamina propria of the intestine will not be eliminated using the intravenous administration route. After these populations of macrophages have been killed, the animal will remain macrophage depleted until these macrophage compartments are repopulated by

Table 3. INFLUENCE OF MACROPHAGE DEPLETION ON THE MAGNITUDE OF BACTERIAL TRANSLOCATION

Group	MLN	Liver	Spleen	Lung
Zymosan 0.5 mg/g				
Controls	2.49 ± 0.20	3.32 ± 0.52	3.04 ± 0.18	2.84 ± 0.11
Macrophage depleted	3.04 ± 0.25	2.87 ± 0.28	3.00 ± 0.21	3.10 ± 0.30
Zymosan 1.0 mg/g				
Controls	3.34 ± 0.34	3.87 ± 0.47	4.25 ± 0.41	4.72 ± 0.56
Macrophage depleted	2.77 ± 0.17	3.00 ± 0.41	2.69 ± 0.13	3.28 ± 0.35

Data are expressed as $\log^{10}$ CFU/g of aerobic bacteria in positive organs; there are no significant differences.

bone marrow-derived macrophages. Repopulation requires at least 7 days in the liver and, depending on the macrophage subpopulation, 7 to 30 days in the spleen; therefore, this technique appears to be a suitable approach to study the effects of macrophage depletion *in vivo*.[11] Zymosan, which is a cell wall preparation from the yeast *Saccharomyces cerevisiae,* was chosen as the inflammatory agent because it induces a generalized inflammatory response that has been associated with dose-dependent bacterial translocation,[8,10,12,13] signs of sepsis,[9,14] and the development of a syndrome similar to MOF.[7]

The elimination of macrophages did not result in an attenuation of bacterial translocation; therefore, zymosan-induced bacterial translocation does not appear to be mediated directly by macrophages or their products. However, zymosan-induced toxicity, as measured by the symptom scores and mortality, did appear to be macrophage mediated. These results are consistent with earlier studies documenting that zymosan-induced bacterial translocation occurs in macrophage function-deficient C3H/HeJ-mice,[10] although mortality and morbidity rates are significantly less in these macrophage-deficient animals compared with normal mice.[15] Thus, these results indicate that zymosan-induced toxicity and zymosan-induced bacterial translocation are independent phenomena and, therefore, probably mediated by different mechanisms.

The exact reason why the incidence of systemic bacterial translocation was higher in the macrophage-depleted animals cannot be determined from the results of this study. However, the most likely explanation is that the translocating bacteria that reach the liver and spleen are more likely to survive when macrophages are absent than when they are present. Furthermore, in the absence of hepatic and splenic macrophages, circulating endotoxin will not be efficiently cleared, and endotoxemia has been documented to promote bacterial translocation even in macrophage-deficient mice.[16]

A major finding of this study was that elimination of hepatic and splenic macrophages significantly decreased the toxicity and mortality rates associated with the zymosan challenge, even though macrophage elimination potentiated the degree of bacterial translocation. This observation indicates that the early deaths observed after zymosan challenge are related more to the activation of macrophages and the subsequent release of their products than to the translocating bacteria. Thus, in essence, elimination of splenic and hepatic macrophages disassociated zymosan-induced toxicity from zymosan-induced bacterial translocation. This apparent disassociation between toxicity and the extent of bacterial translocation or infection was consistent with recent advances in our understanding of the biology of the septic response. Until relatively recently, although it was believed that bacterial pathogens or their products, such as endotoxin, were directly responsible for the pathophysiologic manifestations of the septic response, it is now clear that this is not the case.[1,17,18] Instead, it is the host's immunoinflammatory response to the invading bacteria and their products (especially endotoxin) that are the direct mediators of the septic response. In fact, one of the major conceptual advances of the last decade has been the realization that sepsis and infection are not synonymous and that the septic state can even occur in the absence of infection or bacteria.[7,19] This realization explains why in uninfected patients with major inflammatory diseases, such as pancreatitis, and traumatized victims who have sustained severe tissue injuries that induce a major inflammatory response a septic syndrome develops that may be clinically indistinguishable from uncontrolled infection.[1,2,19,20]

Understanding the relative contribution of infection or other inflammatory stimulants, such as zymosan, as distinct from their induced inflammatory response in the evolution of organ injury and death is of major potential importance. For example, using the paradigm that the host is an innocent bystander who is being directly injured by invading bacteria, one potential goal of therapy would be to augment the host's inflammatory and immune responses and, thereby, increase the ability to fight infection. By contrast, if the paradigm is modified to take

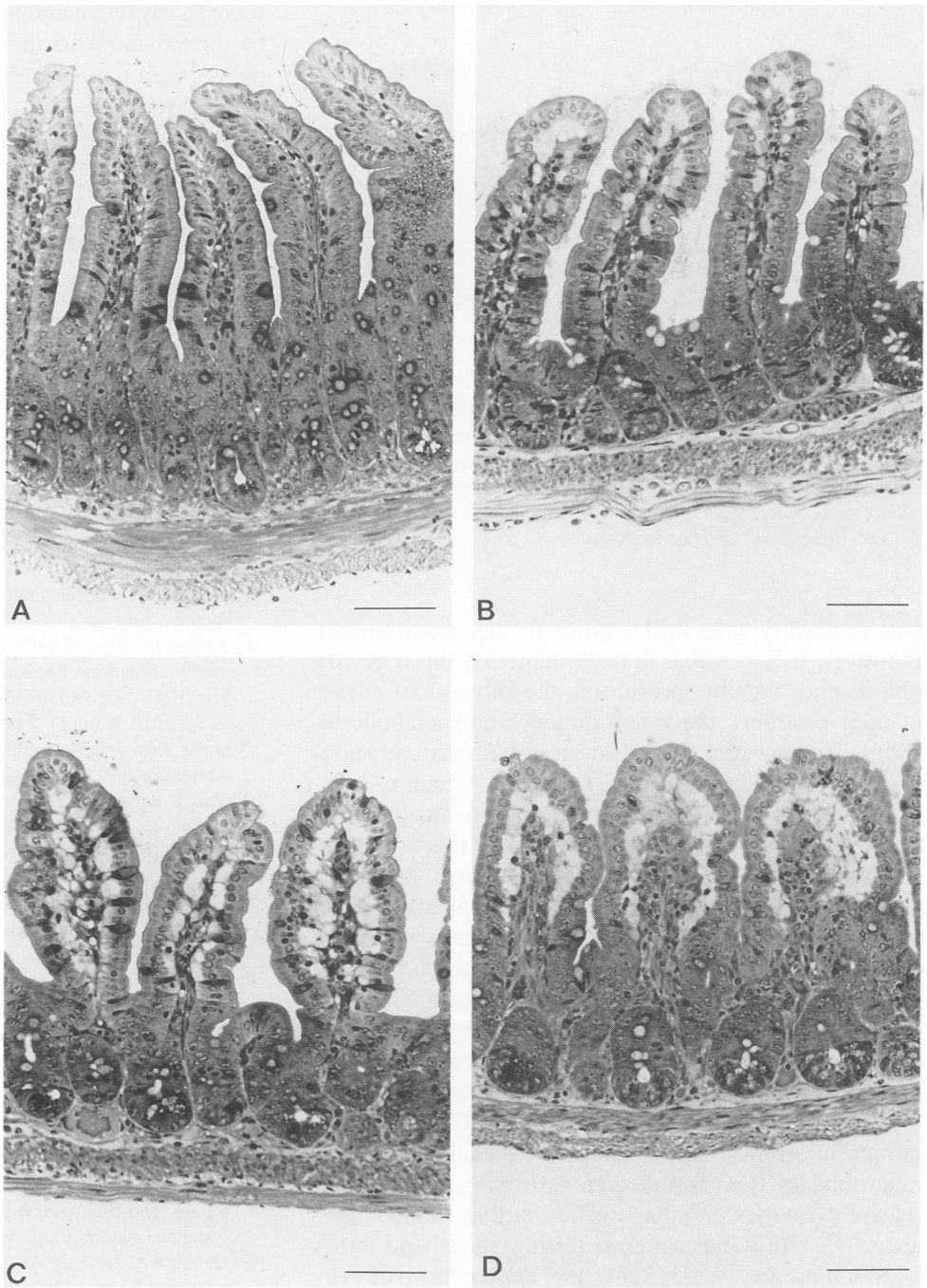

Figure 2. Plastic sections of distal ileum 24 hours after zymosan administration stained with toluidine blue (× 220). (A) Normal mucosa from a control mouse. (B) Administration of 0.1 mg/g of zymosan resulted in lifting of the villus tip epithelium. (C) A dose of 0.5 mg/g of zymosan caused more pronounced edema of the villus. (D) Administration of 1.0 mg/g of zymosan led to a complete disruption of the normal architecture of the mucosa. Scale bar = 100 μm.

into account that the host is being injured by an excessive or uncontrolled inflammatory response, the goal of treatment will include attempts to modulate the immunoinflammatory response and, thereby, limit toxicity. In this context, the results of the present study indicate that selective elimination of hepatic and splenic macrophages can be beneficial in a model of systemic inflammation.

This dissociation between bacterial translocation and toxicity does not mean that bacterial translocation is not an important phenomenon, although in this model the systemic spread of bacteria does not appear to be related directly to the mortality and morbidity rates. For example, germ-free animals survived zymosan challenge better than did animals with a normal gut flora,[7] as did cefoxitin-treated[13] mice. In these models, because elimination or control of the gut flora reduced the mortality rate, bacteria appear to be an important factor, but not the only factor, in the pathophysiology of zymosan-induced death.

Elucidation of the mechanisms by which depletion of hepatic and splenic macrophages reduces the mortality

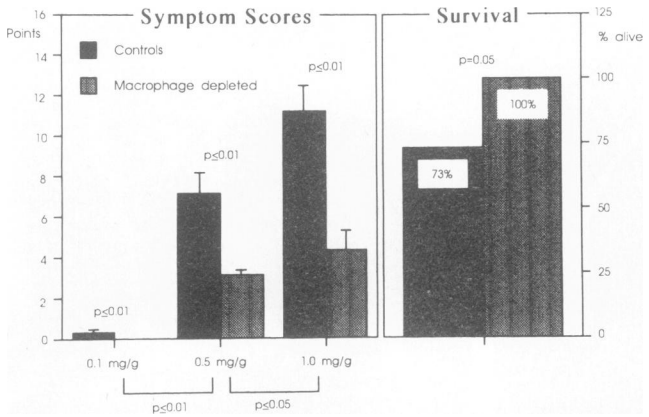

Figure 3. Symptom scores were dose dependent and significantly lower in the macrophage-depleted mice compared with those in the control animals. After 1.0 mg/g of zymosan suspension, the 12-day survival rate was significantly better in the macrophage-depleted mice than that in the the control mice. (Fisher exact test on the actual numbers and ANOVA with Student-Newman-Keuls *post hoc* test).

and morbidity rates will require further examination. However, it is possible that by depleting these macrophages and, thereby, preventing the subsequent release of their products, the inflammatory and metabolic responses to zymosan and gut-derived bacteria and endotoxin were attenuated. In addition, because posttraumatic immunosuppression has been attributed to dysfunctional macrophages,[21] restoring this immunosuppression might be another mechanism by which elimination of macrophages could lead to an attenuation of the zymosan-induced illness. Although this concept is consistent with studies demonstrating that elimination of alveolar or peritoneal macrophages is associated with an increase in local immune responsiveness,[22,23] it does not seem likely because bacterial spread was increased in the macrophage-depleted groups. However, it is not known what is the indirect effect of the CL_2MDP-containing liposomes, that is, the effect mediated by dying macrophages. It is possible that these dying macrophages release cytokines or other mediators that induce tolerance.[24,25] Until experiments testing these and other possibilities are carried out, the explanation of why macrophage depletion improved survival must remain speculative. Furthermore, these results should not unconditionally be extrapolated to other animal models or to humans. For example, although Kupffer cell blockade by gadolinium chloride in the cecal ligation and puncture (CLP) model results in an attenuation of the CLP-induced suppression of systemic cell-mediated immunity, the mortality rate is also increased.[26] Recently, it was shown that the intensity of bacterial translocation was significantly associated with the mortality rate in a combined model of burn and blood transfusion.[27]

In summary, this experiment showed that the observed early lethal and toxic effects of zymosan appear to be related more to the excessive activation of macrophages than to the systemic spread of bacteria. This correlates well with the clinical impression that the inflammatory response and not the systemic spread of bacteria determines the outcome.[28]

Acknowledgments

The authors thank M. Love for technical assistance and Boehringer Mannheim for kindly providing the Cl_2MDP.

References

1. Goris RJA, te Boekhorst TPA, Nuytinck JKS, Gimbrere JSF. Multiple organ failure: generalized autodestructive inflammation? Arch Surg 1985; 120:1109–1115.
2. Border J, Hassett J, LaDuca J, et al. The gut origin septic states in blunt multiple trauma (ISS-40) in the ICU. Ann Surg 1987; 206: 427–488.
3. Deitch EA. Multiple organ failure: pathophysiology and potential therapy. Ann Surg 1992; 216:117–134.
4. Keller GA, West MA, Cerra FB, Simmons RL. Macrophage mediated modulation of hepatic function in multiple-system failure. J Surg Res 1985; 39:555–563.
5. Callery MP, Kamei T, Mangino MJ, Flye W. Organ interactions in sepsis: host defense and the hepatic-pulmonary macrophage axis. Arch Surg 1991; 126:28–32.
6. Van Rooijen N. The liposome-mediated macrophage "suicide" technique. J Immunol Methods 1989; 124:1–6.
7. Goris RJA, Boekholtz WKF, van Bebber IPT, et al. Multiple-organ failure and sepsis without bacteria. Arch Surg 1986; 121:897–901.
8. Deitch EA, Ma W, Ma L, et al. Protein malnutrition predisposes to inflammatory-induced gut-origin septic states. Ann Surg 1989; 211:560–566.
9. Schirmer WJ, Schirmer JM, Naff GB, Fry DE. Systemic complement activation produces hemodynamic changes characteristic of sepsis. Arch Surg 1988; 123:316–321.
10. Deitch EA, Specian RD, Grisham MB, Berg RD. Zymosan-induced bacterial translocation: a study of mechanisms. Crit Care Med 1992; 20:782–788.
11. Van Rooijen N. Liposome-mediated elimination of macrophages. Res Immunol 1992; 143:215–219.
12. Mainous M, Tso P, Berg RD, Deitch EA. Studies of the route, magnitude, and time course of bacterial translocation in a model of systemic inflammation. Arch Surg 1991; 126:33–37.
13. Deitch EA, Kemper AC, Specian RD, Berg RD. A study of the relationships among survival, gut-origin sepsis, and bacterial translocation in a model of systemic inflammation. J Trauma 1992; 32: 141–147.
14. Gabriel Z, Romaschin A, Liauw S, et al. Regional hemodynamic and metabolic effects of chronic hyperdynamic sepsis in unrestrained rats. Surg Forum 1992; 43:38–39.
15. Van Bebber IPT, Speekenbrink RGH, Schillings PHM, Goris RJA. Endotoxin does not play a key role in the pathogenesis of multiple organ failure: an experimental study. *In* Schlag G, Redl H, eds. Second Vienna Shock Forum. Vienna: Alan R. Liss, 1989, pp 419–423.
16. Deitch EA, Berg R, Specian R. Endotoxin promotes the translocation of bacteria from the gut. Arch Surg 1987; 122:185–190.
17. Border JR. Sepsis, multiple systems organ failure, and the macrophage. Arch Surg 1988; 123:285–286.

18. Tracey KJ, Lowry SF. The role of cytokine mediators in septic shock. Adv Surg 1990; 23:21–56.
19. Goris RJA, van Bebber IPT, Mollen RMH, Koopman JP. Does selective decontamination of the gastrointestinal tract prevent multiple organ failure? Arch Surg 1991; 126:561–565.
20. Allardyce DB. Incidence of necrotizing pancreatitis and factors related to mortality. Am J Surg 1987; 154:295–299.
21. Faist E, Mewes A, Strasser T, et al. Alteration of monocyte function following major trauma. Arch Surg 1988; 123:287–292.
22. Thepen T, van Rooijen N, Kraal G. Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. J Exp Med 1989; 170:199–208.
23. Soesatyo M, Biewenga J, van Rooijen N, et al. The in situ immune response of the rat after intraperitoneal depletion of macrophages by liposome-encapsulated dichloromethylene diphosphonate. Res Immunol 1991; 142:533–540.
24. Deitch EA, Specian RD, Berg RD. Induction of early-phase tolerance to endotoxin-induced mucosal injury, xanthine oxidase activation and bacterial translocation by pretreatment with endotoxin. Circ Shock 1992: 36:208–216.
25. Freudenberg MA, Galanos C. Induction of tolerance to lipopolysaccharide-D-galatosamine lethality by pretreatment with LPS is mediated by macrophages. Infect Immun 1988; 56:1352–1357.
26. Callery MP, Kamei T, Flye W. Kupffer cell blockade increases mortality during intra-abdominal sepsis despite improving systemic immunity. Arch Surg 1990; 125:36–41.
27. Fukushima R, Gianotti L, Wesley Alexander J, Pyles T. The degree of bacterial translocation is a determinant factor for mortality after burn injury and is improved by prostaglandin analogs. Ann Surg 1992; 216:438–445.
28. Marshall J, Sweeney D. Microbial infection and the septic response in critical surgical illness. Arch Surg 1990; 125:17–23.