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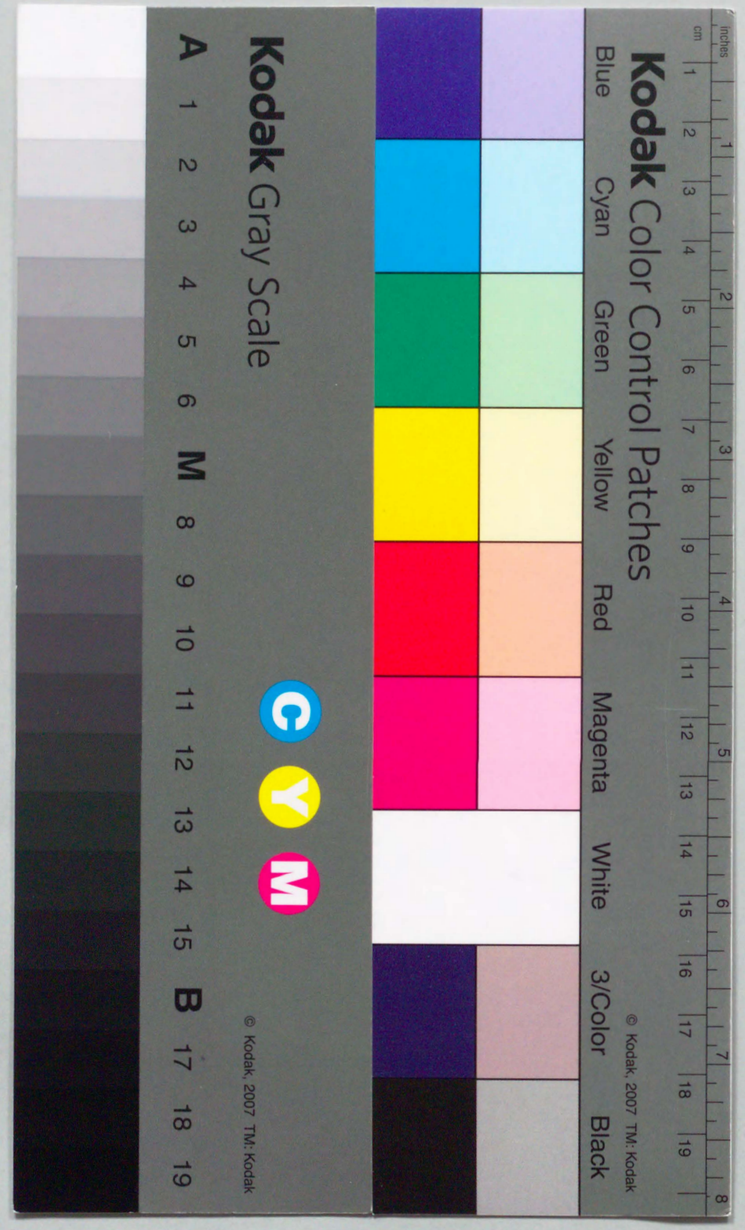

CHARACTERIZATION OF NEUTROPHIL ACTIVATION BY REPEATED INJECTION OF ENDOTOXIN IN RABBITS. ROLE OF NEUTROPHILS IN THE GENERALIZED SHWARTZMAN REACTION

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Characterization of neutrophil activation by repeated injection of endotoxin in rabbits. Role of neutrophils in the generalized Shwartzman reaction

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Abstract: The relationship between activated neutrophils and end-organ injury in endotoxemia was studied. The function of peripheral blood neutrophils (PMNs) in rabbits with the generalized Shwartzman reaction (GSR) was compared to that of PMNs rabbits receiving a single injection of endotoxin. The following results were obtained: (1) PMNs from rabbits with the GSR demonstrated enhanced adherence to endothelial cells and increased mitochondrial ATP production; (2) the GSR did not enhance chemotaxis and oxygen radical production of PMNs; (3) a single injection of endotoxin did not cause necrosis of visceral organs; (4) in vitro detachment of endothelial cells by PMNs was increased in rabbits with the GSR; (5) in vivo administration of monoclonal antibody (mAb) against CD11b/CD18 (Mac-1) suppressed the increase in PMN adherence; and (6) hemorrhagic necrosis did not occur when mAb to Mac-1 was injected. Thus, enhanced adherence of PMNs to endothelial cells appears to play a key role in endotoxin-induced end-organ injuries in this animal model. *J. Leukoc. Biol.* 53: 256–263; 1993.

Key Words: neutrophils • adherence • Mac-1 antigen (CD11b/CD18) • endothelial detachment (cytotoxicity, immunologic) • generalized Shwartzman reaction (GSR)

INTRODUCTION

The generalized Shwartzman reaction (GSR) is a well-known model of organ injury induced by repeated injections of small amounts of endotoxin. In many reports, neutrophils and the coagulation cascade have been linked to the pathogenesis of the GSR [1–3]. We previously reported that the GSR is a useful model of clinical organ injury caused by endotoxins [4]. It has been reported that in this model neutrophils accumulate within 2 to 3 h after LPS injection and the hemorrhage and necr sis are complete after 9 to 12 h [5]. Neutrophils are the main infiltrating inflammatory cells from the initial step to the final stages of the GSR [5]. It has also been reported that these phenomena do not occur in granulocytopenia induced by injection of nitrogen mustard [6]. Overall, these experimental results suggest that neutrophils (PMNs) may play a crucial role in the pathogenesis of end-organ injury in the GSR. Further characterization of this model indicated that varying the interval between endotoxin injections can alter the course of end-organ injury [6].

In this study, the functional characteristics of PMNs were studied and compared with those of PMNs from a group of animals injected only once with endotoxin. A single injection did not cause histologic necrosis. Through comparison of the PMN activation state in the two groups, we sought to clarify the functional attributes crucial to end-organ injury in the GSR. The results help to differentiate between useful activation of host defenses by endotoxin and overactivation leading to organ injury.

MATERIALS AND METHODS

Reagents

Lipopolysaccharide from *Escherichia coli* (LPS, 026:B6) was purchased from Difco (Detroit, MI). Affinity-purified anti–Mac-1 monoclonal antibody (mAb) M1/70, which identifies CD11b of the Mac-1 adhesion molecule (CD11b/CD18) on granulocytes, macrophages, and natural killer cells [7], was kindly provided by Dr. A. Imaizumi (Tokyo Institute of Immunopharmacology Inc.). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 10 μl per 100 μl of medium], was purchased from Dojindo (Kumamoto, Japan). Bacterial factor (BF), a chemoattractant derived from bacteria, was collected from *E. coli* cultured fluid, passed through a 0.45-μm Millipore filter, and stored at –20°C prior to use. Opsonized zymosan (OZ) was prepared by incubating normal rabbit serum with a suspension containing zymosan A (Sigma) for 1 h at 37°C. Fetal calf serum (FCS; Gibco, Grand Island, NY) was heat inactivated at 57°C prior to use. HH (an endothelial cell line from the bovine carotid artery) was kindly provided by the Japanese Cancer Resources Bank [8].

Preparation of the rabbit general Shwartzman reaction

The rabbit GSR was induced by methods previously reported [4]. Thirty-three male JW rabbits, weighing 2.5

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Abbreviations: BF, bacterial factor; FCS, fetal calf serum; GSR, generalized Shwartzman reaction; HPF, high-power field; HUVEC, human umbilical vein endothelial cell; LPS, lipopolysaccharide; mAb, monoclonal antibody; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OZ, opsonized zymosan; PMN, neutrophil; TNF, tumor necrosis factor.

to 3.0 kg, were injected intravenously with either one or two doses of 0.04 mg/kg endotoxin (LPS 026:B6). Five rabbits were injected twice with the same dose of LPS at an interval of 36 h and made up the GSR model (E2 group). Five additional rabbits were injected with saline (1.0 ml) 36 h before LPS injection (E1 group). The control group was injected twice with saline at a 36-h interval and consisted of five rabbits.

Twelve rabbits were prepared for histologic examination. Necropsy was performed either 4 h (n = 3) or 24 h (n = 3) after the final LPS injection in the E1 and E2 groups. The liver and lung were fixed with a Bouin solution, embedded in paraffin, and stained with hematoxylin and eosin for examination by light microscopy.

Six rabbits were used for anti-Mac-1 mAb treatment and received 2.0 mg/kg mAb 10 min prior to the second endotoxin injection.

Isolation of neutrophils

PMNs were isolated from five rabbits in each group (total 15 rabbits) by the method of Kaija et al. [9] 24 h after the last LPS injection. Briefly, all rabbit PMNs were isolated from acid-citrate-glucose–anticoagulated blood obtained from the heart. Twenty milliliters of blood and 5 ml of anticoagulant (38 mg/ml acid-citrate-glucose) were mixed with 10 ml of 6% (w/v) dextran T-500 (Pharmacia, Piscataway, NJ) in normal saline. After sedimenting at 1g for approximately 30 min, the supernatant was layered over 56% Percoll (Sigma Chemical Co., St. Louis, MO) and centrifuged at 450g for 20 min. The erythrocyte-PMN pellet from this centrifugation was then suspended in 40 ml of 8.3% (w/v) ammonium chloride (pH 7.2) to produce erythrocyte lysis. After 7 min, PMNs were centrifuged for 10 min at 150g and washed twice in phosphate-buffered saline. This method for rabbit PMN isolation produces greater than 97% viability as judged by trypan blue exclusion and greater than 97% homogeneity as judged by light microscopic appearance.

Preparation of human umbilical vein endothelial cells and adherence assay

Neutrophil adherence activity to human umbilical vein endothelial cells (HUVECs) was examined. HUVECs were harvested and grown as previously described [10]. They were used in the second passage for the adhesion assay, which was performed using our original method as previously described [11]. Briefly, HUVECs (1.5×10^4 cells/well) were spread sufficiently over a culture-treated flat-bottom 96-well microplate (Nunc, Denmark). After 1 to 2 days, HUVECs had grown to subconfluence. One hundred microliters of PMNs [1×10^6 cells/ml in RPMI 1640 medium (Nissui, Japan), 2% FCS] from each group were added and coincubated for 30 min at 37°C. After cultivation, the plate was washed vigorously twice with RPMI to remove the nonadherent neutrophils. The plate was dried, fixed with methanol, and stained with Giemsa solution (Wako, Japan) for 10 min. The total number of neutrophils adhering to the HUVECs in five high-power fields (HPF) of each well was then counted by inverted microscopy (×200).

Colorimetric MTT (tetrazolium) assay

An MTT assay was performed according to the method of Mosmann [12] to identify mitochondrial activation [13]. Briefly, PMNs from each rabbit were placed in a Nunc 96-well microplate at a concentration of 1×10^5 cells/well in 0.1 ml of medium 199 (Sigma) containing 10% FCS and 50 μg MTT. The plate was incubated for 4 h at 37°C in a 5% CO_2 atmosphere. Acid-isopropanol (100 μl of 0.04 N HCl in isopropanol) was then added to each well and mixed thoroughly to dissolve the dark blue crystals. After waiting a few minutes at room temperature to ensure that all crystals were dissolved, we measured the optical absorbance of each well on an automatic plate reader (Easy Reader, SLT-labinstruments, Austria) using a test wavelength of 570 nm and a reference wavelength of 620 nm.

Chemotaxis

PMN chemotaxis was assessed by a modification of Boyden's chamber method [14,15]. All assays were done in duplicate. Millipore filters (type SMWP, Millipore Corp., New Bedford, MA) with a pore diameter of 3 μm were used. The upper compartment of each chamber contained 200 μl of cell suspension and the lower contained 200 μl of 5% BF medium. After incubation for 90 min at 37°C in a 5% CO_2–95% air atmosphere, the chambers were disassembled and the filters fixed with ethanol and stained with Mayer's hematoxylin (Muto Pure Chem., Japan). Five fields were selected at random for inspection under high-power light microscopy (×200). Chemotactic activity was expressed as the total number of migrated cells found in the five fields examined.

Chemiluminescence

PMN oxygen radical production was measured using the method of Easmon et al. [16] with chemiluminescence stimulated by luminol-dependent OZ and recorded using a Luminometer (LKB Wallac model 1250, Turku, Finland). Briefly, 5×10^5 PMNs, 8×10^{-6} mol luminol, and 2 mg of OZ were added to a 3-ml polystyrene container; the final volume was 1 ml. The solution was introduced into an exclusive chamber at 37°C and chemiluminescence determined in duplicate. The value was then expressed in mV.

Endothelial cell lysis and detachment assay

To estimate the cytotoxic activity of isolated PMNs, ^{51}Cr release and detachment asssays were performed with a modification as previously described [17, 18]. HH endothelial cells were grown to confluence over 24 h on 24-well flat-bottom microplates. During the last 16 h of this culture, sodium chromate (^{51}Cr) was added to the wells (111 kBq/well). At the beginning of the assay the monolayers were carefully washed three times with RPMI 1640, 2% FCS. PMNs in RPMI 1640 supplemented with 10% FCS were added to the monolayers as indicated, at a final volume of 1.0 ml/well, and incubations were performed at 37°C for 90 min (unless otherwise noted). Endothelial cell lysis was determined by measuring the ^{51}Cr release in the 500-μl cell-free supernatant. Detached endothelial cells were suspended by repeated careful pipet-

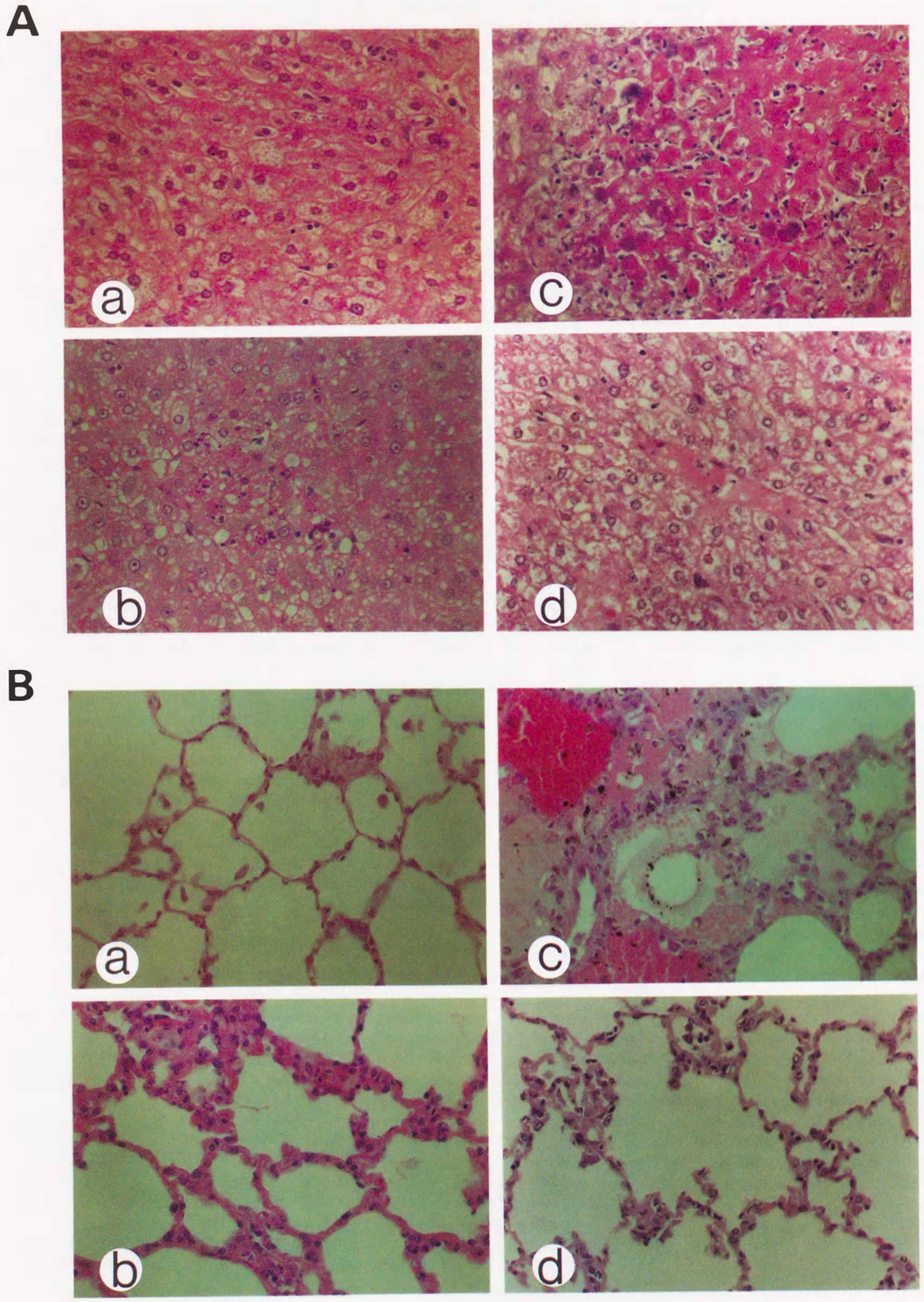

Fig. 1. (A) Typical light microscopic findings for the liver (×217). (a) E1 rabbit (single injection of LPS) 4 h after LPS injection: neutrophil accumulation. (b) E2 rabbit (GSR group) 4 h after the last LPS injection: increased neutrophil accumulation. (c) E2 rabbit 24 h after LPS injection: tissue necrosis. (d) Anti–Mac-1 mAb (2.0 mg/kg)–pretreated E2 rabbit 24 h after LPS injection: no necrosis. (B) Histologic sections of the lung (×217). (a) E1 rabbit (single injection) 4 h after LPS injection. (b) E2 rabbit (GSR group) 4 h after LPS injection. (c) E2 rabbit 24 h after LPS injection. (d) Anti–Mac-1 mAb–treated E2 rabbit 24 h after LPS injection.

ting, and 500 μl of this suspension was removed. Detachment values were calculated by subtracting half of the ^{51}Cr counts measured in the lysis samples drawn from the same wells from the ^{51}Cr counts measured in the detachment samples. Control monolayers remained visually confluent after performing this procedure. The maximal ^{51}Cr content was determined in wells receiving 500 μl of 1 N NaOH and reached about 10^5 dpm. Furthermore, measurements of endothelial cell lysis and detachment were corrected for nonspecific ^{51}Cr content by subtracting the ^{51}Cr counts measured in lysis/detachment samples from wells without cells (in these wells spontaneous ^{51}Cr release and endothelial cell detachment ranged from 1 to 3% of the total ^{51}Cr content).

Effects of anti–Mac-1 monoclonal antibody

Rabbit leukocytes have adhesion molecules similar to human CD11/CD18 [19]. Six additional GSR rabbits were injected intravenously with anti–Mac-1 mAb (M1/70, 2.0 mg/kg) 10 min before the second LPS injection, and necropsy was performed either 4 h ($n = 3$) or 24 h ($n = 3$) after the last LPS injection with specimens prepared as above.

Three rabbit necropsies were performed 24 h after injection, and isolated PMNs were examined in functional assays.

Statistics

Differences between groups were evaluated for significance using Student's *t*-test.

RESULTS

Histologic findings on the rabbit generalized Shwartzman reaction

Neutrophils accumulated in the liver and lung tissue within 4 h after a single injection of LPS (**Fig. 1Aa** and 1Ba), but by 24 h these neutrophils had disappeared in the E1 group (data not shown) [19]. In the E2 group (rabbits with the GSR), significant neutrophil accumulation was found in lung and liver at 4 h as shown in Figure 1Ab and 1Bb and was followed by marked hemorrhagic necrosis 24 h after the second injection of LPS (Fig. 1Ac and 1Bc). At this time point, there were many neutrophils in the necrotic lesions and in fibrin thrombosis within vessels. **Table 1** demonstrates the neutrophil count per five high-power fields in each group 4 h after injection of endotoxin. Many neutrophils accumulated in the lung and liver in the GSR group, approximately seven times the level seen in the E1 group. These results suggest that accumulated neutrophils may play an important role in the end-organ injury of the GSR.

TABLE 1. Neutrophil Sequestration (Neutrophil Counts/5 HPF) and Effect of Treatment with M1/70 mAb[a]

	Lung	Liver
E1 group	20.8 ± 8.3	52.0 ± 10.8
E2 group	141.0 ± 22.9	187.6 ± 37.3
Anti–Mac-1 mAb	85.8 ± 14.9*	57.8 ± 10.7*

[a]Values are means ± SD of five sections.
*$P < .01$.

Adherent Activity of PMNs

The first step in the function of PMNs is adhesion to the endothelium. We examined the adherent activity of PMNs to HUVECs in each group of rabbits. As shown in **Figure 2,** the number of PMNs adhering to the HUVECs increased in both groups receiving LPS. Double injection of LPS significantly enhanced adherence compared to a single injection (control 100%, E1 182 ± 53%, E2 295 ± 73%; $P < .05$).

MTT assay of PMNs

As illustrated in **Figure 3a,** MTT assay revealed that the PMNs isolated from both groups receiving endotoxin were activated. The degree of PMN mitochondrial activation in the E1 group was 126.7% of that in the control group. Activation in the E2 group was 167.0% of that in the control group. These results indicate that PMNs in the E2 group were activated to a much greater extent than in the E1 group based on the mitochondrial level ($P < .05$) (Fig. 3a).

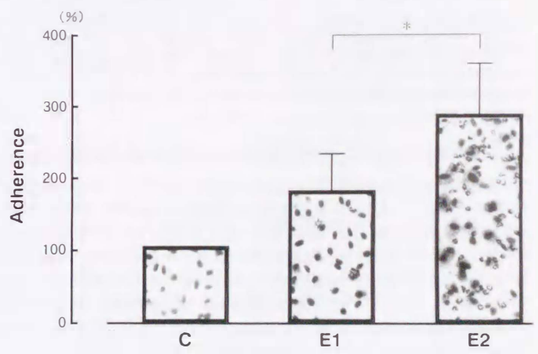

Fig. 2. In vitro adherent activity of PMNs in each group. Total number of PMNs adhering to the endothelial cells in five high-power fields. Values are means ± SD of five samples. The microscopic appearance of neutrophil adherence to human umbilical vein endothelial cells is shown in the bars (×330). (C, control (100%); E1, single injection of LPS (182 ± 53%); E2, GSR group (295 ± 73%). *$P < .05$

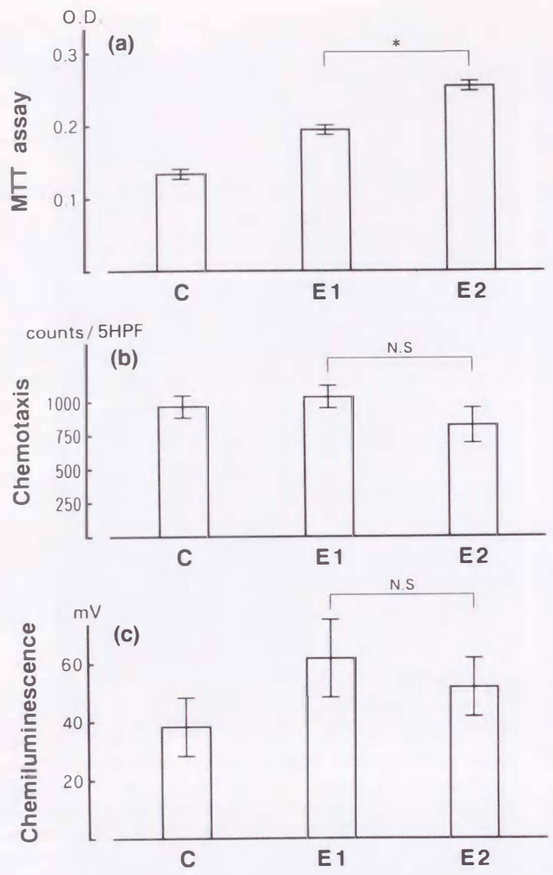

Fig. 3. Comparison of PMN functional assays in the indicated groups. Values are means ± SD of samples. (a) MTT assay as a marker for activation of PMNs from each group 24 h after LPS injection. C, control; E1, single injection of LPS (126.7% of control); E2, GSR group (167.0% of control). $*P < .05$. (b) PMN chemotaxis in response to 5% bacterial factor. C, control; E1, single injection of LPS; E2, GSR group. NS, no significant differences between groups. (c) PMN oxygen radical production estimated by chemiluminescence stimulated by opsonized zymosan. C, control; E1, single injection of LPS; E2, GSR group. NS, no significant differences between E1 and E2 groups.

Chemotaxis and chemiluminescence of PMNs

We examined PMN chemotactic activity in response to BF. There was no significant difference in PMN chemotaxis between the E1 and E2 groups (Fig. 3b). In addition, we examined PMN chemiluminescence, which measures neutrophil phagocytic activity and release of oxygen radicals [16]. A single injection of endotoxin enhanced PMN chemiluminescence, but a second injection resulted in no further increase (Fig. 3c).

Endothelial cell lysis and detachment by PMNs

To determine the capacity of PMNs to cause endothelial damage, we examined endothelial cell lysis and detachment. Endothelial cell damage was measured by release of intracellular ^{51}Cr or detachment of endothelial cells [17, 18]. **Table 2** shows endothelial cell lysis and detachment. Control PMN lysis was consistently below 2.0% (mean ± SD, 0.78 ± 0.64%). Endothelial cell lysis in the E1 and E2 groups was not different from that in the control (E1 2.50 ± 1.76%, E2 2.60 ± 1.21%). Detachment in control media and by control PMNs was below 3.0%. Endothelial cell detachment of the E1 group was 5.62 ± 2.20% (mean ± SD), which was a significant increase ($P < .05$). The endothelial cell detachment of the E2 group was further increased to 11.04 ± 3.66% ($P < .05$), almost twice that of the E1 group and more than four times the rate of control PMN detachment. **Figure 4** shows all experiments on endothelial cell detachment by PMNs in each group.

TABLE 2. Endothelial Cell Lysis and Detachment by PMNs[a]

	Endothelial cell lysis (%)	Detachment (%)
Control	0.78 ± 0.64	2.70 ± 1.66
E1 group	2.50 ± 1.76	5.62 ± 2.20*†
E2 group	2.60 ± 1.21	11.04 ± 3.66*

[a]Values are means ± SD of five samples.
*$P < .05$.
†$P < .01$.

Effects of Anti-Mac-1 Monoclonal Antibody

Injection of anti–Mac-1 monoclonal antibody (M1/70) 10 min before the second LPS injection reduced neutrophil accumulation in the liver and lungs examined 4 h after a second LPS injection (Table 1). Furthermore, there was no hemorrhagic necrosis 24 h after the last LPS injection (Fig. 1Ad and 1Bd).

To determine whether these results were caused by suppression of PMN adherence to vascular endothelium, we assessed the adherence of PMNs isolated from M1/70-injected rabbits 24 h after the second LPS injection. Adherence and chemiluminescence of PMNs isolated from M1/70-treated rabbits are shown in **Table 3.** The adherence of PMNs from M1/70-treated rabbits was reduced to the levels of the E1 group despite two injections of LPS. Moreover, the PMNs from M1/70-injected rabbits revealed no alteration in chemiluminescence.

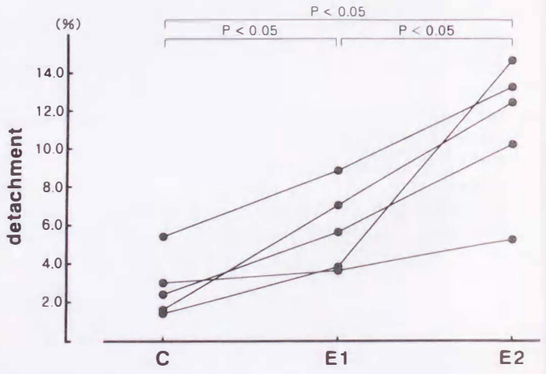

Fig. 4. Endothelial cell detachment assay in each group. C, control; E1, single injection of LPS, with PMNs obtained 24 h after LPS injection (5.62 ± 2.20, mean ± SD); E2, GSR group, with PMNs obtained 24 h after LPS injection (11.04 ± 3.66, mean ± SD). Connected points represent samples examined the same day under the same endothelial cell conditions. Values are means of five samples. Both the media alone and the control group demonstrated detachment of <3.0%.

TABLE 3. Endothelial Adherence and Chemiluminescence of PMNs Isolated from M1/70-Treated Rabbits[a]

	Chemiluminescence (mV)	Adherence (%)
Control	40.0 ± 9.7	100
E1 group	63.7 ± 15.0	182 ± 53.0
E2 group	53.0 ± 10.3 NS[b]	295 ± 73.0*
Anti–Mac-1 mAb	64.0 ± 15.6 NS[b]	178 ± 25.5*

[a]Values are means ± SD of five samples.
[b]NS, not significant.
*$P < .05$.

DISCUSSION

We studied the activation state of PMNs in the generalized Shwartzman reaction induced by a small amount of exdotoxin injected twice intravenously at an interval of 36 h. These results were compared with those obtained after a single injection of the same dose of endotoxin. In the group injected once (group E1), PMNs were activated but histologic injury did not occur, as previously reported [2, 5]. On the contrary, PMNs activated in the GSR (E2 group) resulted in hemorrhagic necrosis of the lungs and liver (Fig. 1Ac and 1Bc). Endotoxins mediate the formation and release of many cytokines in vivo [20, 21], and some of these inflammatory cytokines enhance neutrophil function in vitro [22–25]. Therefore it has been suspected that neutrophils activated by endotoxin in vivo can cause vascular endothelial damage, multiple organ failure, and the histologic findings in the septic animal model shown here [26, 27]. It is well known that neutrophils play mainly a bioprotective role, primarily against bacteria. There have been no reports in which the properties of activated neutrophils that contribute to organ injury are proved conclusively. We examined the various components of PMN activation during induction of the GSR to clarify which PMN functions play a crucial role in organ injury of the GSR.

Circulating PMNs must adhere to endothelium in order to function [11]. Figure 2 shows the adherence to HUVECs by PMNs from each group of rabbits. Remarkable enhancement in PMN adherence was noted with a significant increase in the GSR group. Figure 2 also shows a representative high-power field within the bar demonstrating PMN adherence to the HUVECs. The PMNs of the E1 group were also adherent to HUVECs, but to a lesser degree than in the GSR group. This suggests the possibility that destruction of normal tissues that should not be attacked at random can be caused by overadhesion to normal endothelium.

Adherent PMNs demonstrate chemotaxis toward inflammatory stimulants as the second step in neutrophil activation. Figure 3b shows chemotactic activity toward bacterial factor. Chemotaxis of PMNs in the GSR was not significantly different but appeared decreased from that of the E1 group PMNs. This could indicate that PMNs in the GSR, once adherent to the endothelium, stay fixed to this surface rather than passing through the endothelial junctions. Either enhanced adherence or diminished chemotaxis could cause PMN accumulation on the endothelium compatible with the findings presented in Figure 1Ab and 1Bb and Table 1.

PMNs that have accumulated in inflammatory lesions by chemotaxis release cytotoxic substances such as oxygen radicals as the third step in immune function. Therefore we measured oxygen radical production by chemiluminescence for opsonized zymosan as shown in Figure 3c. Oxygen radical production may already be maximally enhanced by one injection of endotoxin. Many investigators have reported that cytotoxic activity of PMNs is due to the enhancement of oxygen radical production [26, 28–30]. Our results show no significant difference between the E1 and GSR groups. However, GSR PMNs increased oxygen radical production more than the control group. In addition, Figure 3a shows that mitochondrial activity of PMNs in the GSR group was higher than in the E1 group. The mechanism underlying the enhancement of mitochondrial activity remains unclear. The putative increase in mitochondrial activity may be due to an increase in mitochondrial number or to activation of individual mitochondria. Gerlier and Thomasset [31] reported that the MTT assay was useful for quantifying the activation level of cells, so we used it as an aspect of characterizing PMN activation.

The PMNs in this study were isolated from the peripheral blood 24 h after the injection of endotoxin. No PMNs were obtained from organ tissue itself. Therefore, we chose to use an in vitro endothelial cell injury assay to determine whether peripheral blood PMNs can cause end-organ injury. Detachment of endothelium caused by PMNs significantly increased in the GSR (Fig. 4, Table 2). This indicates that the enhanced adherence and increased mitochondrial activity of PMNs induced by in vivo LPS priming can activate these cells to cause endothelial cell injury. These results help differentiate between the usual activation of host defenses by endotoxin and overactivation leading to organ injury with repeated exposure.

Activated PMN adherence to endothelium is mediated primarily by the Mac-1 (CD11b/CD18) adhesion molecule [32, 33]. We previously reported that Mac-1 participates in the PMN accumulation within vital organs and that this accumulation could be inhibited by anti–Mac-1 mAb injection in a murine model [11]. Adhesion molecules similar to CD11/CD18 were detected in rabbit PMNs and cross-reacted with anti–Mac-1 mAb [19]. Therefore, we used anti–Mac-1 mAb (M1/70) to inhibit the GSR. If end-organ injury of the GSR was caused mainly by enhanced adherence, injury should be controlled by anti–Mac-1 mAb. M1/70 cross-reacted in the rabbit and inhibited the increased adherence of PMNs. There was no effect on chemiluminescence (Table 3). Histologically, neutrophil accumulation at 4 h after the second endotoxin injection was not detected after anti–Mac-1 mAb treatment (Table 1). In addition, there was no hemorrhagic necrosis as seen in the GSR (Fig. 1Ad and 1Bd). Of note, Mac-1 expression on the PMNs obtained from rabbits with the GSR was not increased as measured by

flow cytometry (data not shown). The ability of anti–Mac-1 mAb to block these phenomena means that the enhanced adherence of PMNs in the GSR acts through Mac-1 adhesion molecules. It has been reported that a change in binding affinity by an altered molecular structure can be shown in ICAM-1, a counterreceptor for Mac-1 [34, 35]. However, ICAM-1 could not explain the enhanced in vitro adherence of PMNs from E2 rabbits, since the target endothelial cells were not exposed to LPS. We suspect that enhancement of adherence by PMNs from rabbits with the GSR may be regulated by an affinity control mechanism of Mac-1 adhesion molecules.

The exact origin of the increased adherence of PMNs in the GSR is still unclear. Either tumor necrosis factor (TNF) or interleukin-1 can produce the Shwartzman reaction in addition to LPS [36]. Therefore, we measured TNF in each rabbit's serum by bioassay. One hour after LPS injection, TNF levels increased in the serum (data not shown). However, TNF activity did not increase further in the serum of rabbits in the E2 group compared to E1 rabbits and therefore could not explain the additional increase in adherence. We suspect that cytokines may act as adherence primers in the GSR. It has been reported that there are variable changes in expression of mediator receptors on human PMNs primed by TNF-α in vitro [37]. However, many of the mechanisms of priming of neutrophils by cytokines remain unclear.

In conclusion, the increased adherence of PMNs to the endothelium plays a significant role in tissue injury in the GSR. Dramatic necrosis can be seen in histologic specimens. The increased PMN adherence is regulated by Mac-1 adhesion molecules. Further pathophysiologic studies may assist in the prevention and treatment of end-organ injury in clinical endotoxemia.

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