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Shiga Toxin-2 Induces Neutrophilia and Neutrophil Activation in a Murine Model of Hemolytic Uremic Syndrome

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It has been demonstrated that infections due to Shiga toxins (Stx) producing *Escherichia coli* are the main cause of the hemolytic uremic syndrome (HUS). Although it is recognized that Stx damage the glomerular endothelium, clinical and experimental evidence suggests that the inflammatory response is able to potentiate Stx toxicity. Lipopolysaccharides (LPS) and neutrophils (PMN) represent two central components of inflammation during a gram-negative infection. In this regard, patients with high peripheral PMN counts at presentation have a poor prognosis. Since the murine model has been used to study LPS–Stx interactions, we analyzed the effects of Stx alone or in combination with LPS on the kinetics of neutrophil production and activation and their participation in renal damage. We observed a sustained neutrophilia after Stx2 injection. Moreover, these neutrophils showed increased expression of CD11b, enhanced cytotoxic capacity, and greater adhesive properties. Regarding the cooperative effects of LPS on Stx2 action, we demonstrated potentiation of neutrophilia and CD11b induction at early times by pretreatment with LPS. Finally, a positive correlation between neutrophil percentage and renal damage (assayed as plasmatic urea) firmly suggests a role for PMN in the pathogenesis of HUS. © 2000 Academic Press

Key Words: **Shiga toxin; neutrophils; hemolytic uremic syndrome.**

INTRODUCTION

The hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and nephropathy. Typically, HUS develops in young children as a vascular disease several days after the occurrence of diarrhea and bloody gastroenteritis (1), caused by gram-negative bacteria such as *Shighella dysenteriae* 1 (2, 3) or by particular serotypes of enterohemorrhagic *Escherichia coli* producing significant quantities of Shiga toxins (Stx) (4), also referred to as verotoxins or Shiga-like toxins (5). Since the beginning of the 1980s, it has become clear that Stx-producing *E. coli* infections are the main cause of HUS (6, 7). Stx exert their cytotoxic effects only when they can bind to a specific cell receptor (8), a neutral glycolipid known as globotriaosylceramide (Gb3) (9, 10). Gb3 receptors have been identified on renal epithelial, endothelial, and recently on glomerular mesangial cells (11). However, compelling evidence has indicated that inhibition of protein synthesis after Stx–receptor interaction is not sufficient to induce tissue injury and that other pathogenic factors would be necessary to develop HUS. Moreover, substances thought to be involved in endothelial damage and neutrophil products are increased in patients with HUS (12). In addition, lipopolysaccharides (LPS), a central inflammatory component in gram-negative infections, are powerful inducers of cytokines and their importance in disease development has been documented in several reports (13–15). In view of the association of infectious agents with many cases of HUS, reports of neutrophil leukocytosis in HUS are not surprising (16). In this regard, in the typical form of HUS, a high peripheral blood neutrophil (PMN) count at presentation indicates a poor prognosis, suggesting that neutrophils may play a role in pathogenesis (17, 18). There is evidence that activated PMN cause endothelial injury and it has been demonstrated that purified products from these cells can damage the glomerular basement membrane in a model of glomerulonephritis (19). In children with HUS, PMN have been found to adhere more avidly to endothelium and to damage the endothelial cell (20). More recently, increased levels of IL-8 have been detected in the serum during the acute phase of HUS (21). This cytokine promotes leukocyte adhesion *in vivo* and leads to recruitment of PMN to sites of tissue inflammation (22, 23). However, it is not known whether these are gram-negative-induced nonspecific effects due to LPS, or to Stx-derived specific effects, or both.

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Different animal models have been used to study histological alterations and LPS contributory effects to Stx pathogenicity (13, 14, 24, 25). The aim of this study was to analyze *in vivo* the effect of Stx type 2 (Stx2) on leukocyte populations, with special emphasis on neutrophils. Since during human natural infection Stx are accompanied by LPS, the mouse model makes it possible to investigate the effects of Stx, alone or in combination with LPS, on the kinetics of neutrophil production, activation, and their participation in renal damage.

We observed a sustained neutrophilia after a Stx2 injection, without alterations in other leukocyte populations. Circulating neutrophils showed increased expression of CD11b, greater adhesion to lung vessels, and increased cytotoxic capacity. Pretreatment with LPS potentiated neutrophilia and CD11b induction at early times after Stx2 injection. Finally, a positive correlation between neutrophil percentage and renal damage assayed as plasmatic urea suggests a role of PMN in the pathogenesis of HUS.

MATERIALS AND METHODS

Mice. BALB/c mice were bred in the animal facility at the Department of Experimental Medicine, Academia Nacional de Medicina, Buenos Aires. Male mice aged 8–16 weeks and weighing 20–24 g were used throughout the experiments. They were maintained under a 12-h light–dark cycle at a temperature of 22 ± 2°C and fed with standard diet and water *ad libitum*. The experiments performed herein were conducted according to principles set forth in the Guide for the Care and Use of Laboratory Animals (National Institute of Health, 1985).

Stx2 preparation. Stx2 was kindly provided by Dr. Sugiyama Junichi (from Denka Seiken Co. Ltd., Nigata, Japan). Purity was analyzed by the supplier, showing only one peak in HPLC. Stx2 preparation was checked for endotoxin contamination by the *Limulus amoebocyte* lysate assay given that 1 IU/ml is equal to 0.1 ng/ml of United States Pharmacopea standard *E. coli* endotoxin (26). Stx2 preparation contained less than 40 pg LPS/μg of Shiga toxin protein.

Stx2 was tested for cytotoxic activity on Vero cells as previously described (7) at the Instituto Nacional de Enfermedades Infecciosas, ANLIS, "Dr. C. G. Malbran" (Buenos Aires, Argentina). Briefly, Vero cells were grown in Eagle's minimum essential medium with Earle salts and nonessential amino acids (Gibco Diagnostics, Madison, WI) supplemented with 7% fetal calf serum (Sigma Chemical Co., St. Louis, MO), 0.03 M glutamine, 50 μg/ml gentamicin, and 2.5 μg/ml fungizone in microtiter plates (Nunc, Intermed, Roskilde, Denmark). Aliquots (50 μl) of serial twofold dilutions of the samples containing Stx2 were added to each well (25,000 Vero cells) and incubated for 3 days at 37°C in 5% CO_2. Vero cells were examined daily for cytotoxicity. The 50% cytotoxic dose (CD_{50}) corresponded to the dilution required to kill 50% of the Vero cells: the CD_{50} was ~0.063 pg.

Stx2 treatment. The same batch of Stx2 preparation was used throughout the experiments. In a previous paper (15) we evaluated the *in vivo* lethality by serial dilutions in pyrogen-free saline. We chose a dose of 12.5 ng/kg (approx. 250 pg/mouse), which induced a mortality of >50% between 3 and 4 days after injection.

Endotoxin preparation and treatment. To modulate Stx2 pathogenicity we used commercial LPS from *E. coli* 0111:B4 (Sigma). The LPS O111:B4 lot used throughout the experiments had a LD_{50} of 150 μg/mouse. It was diluted in sterile saline prepared with pyrogen-free distilled water immediately before use at the desired concentration. Mice were given 5 μg of LPS by ip injection at 1 h before iv injection with 250 pg of Stx2 or alone, simultaneously with Stx2.

Blood leukocyte counts. Blood samples were obtained by puncture of the retroorbital plexus at different times after Stx2 or LPS injections. Total leukocyte counts were determined in a Neubauer chamber by means of an optical microscope after dilution of blood samples in a 2% acetic acid solution. The percentage of circulating neutrophils was determined after differential cell counts on May–Grúnwald Giemsa-stained blood smears. These percentages were confirmed by cytometric studies.

Bone marrow cell analysis. The animals were killed with an overdose of ether immediately after the collection of the blood samples. The right femur bone marrow was eluted with 2 ml of complete RPMI 1640 medium. Differential leukocyte analysis was performed on cytocentrifuged smears stained by the May–Grunwald Giemsa method. Determination of myeloperoxidase (MPO) was determined as described below and results were expressed per 10^6 cells.

Determination of lung MPO activity. Lung tissue MPO activity was determined following a previously described method (27) with minor modifications. Before being removed, the lung vessels were flushed to discard circulating blood. Under embutal anesthesia, the hepatic artery was cut and 2 ml of saline was gently perfused through the retroorbital sinus. The lungs were removed from the thorax, blotted with gauze to remove blood, then minced, and pressed gently through a fine screen to obtain a single cell suspension. The cells were washed and suspended in 200 μl of phosphate-buffered saline (PBS) supplemented with $Ca^{2+}Cl$ (1 mM)/$Mg^{2+}Cl$ (1 mM) and Triton X-100 (2%). After

two freeze–thaw cycles, samples were centrifuged at 2000 rpm for 10 min. The supernatants (100 μl) were placed in a 96-well plate and reacted with H_2O_2 (12%) in the presence of 100 μl of *o*-phenylenediamine (0.35 mg/ml in citrate/phosphate buffer). After 15 min of incubation while shaking at 37°C, the reaction was stopped by addition of 100 μl of 2 M H_2SO_4. The MPO activity was determined as change in absorbance at 490 nm in a microtiter plate reader (Organon Tecnika, Argentina).

Immunofluorescence flow cytometry. Measurement of the expression of membrane markers on peripheral blood cells was done by direct immunofluorescence flow cytometry. Fluorescein isothiocyanate-conjugated rat monoclonal antibodies (MoAb) against murine CD3, CD4, and CD8 and goat anti-mouse immunoglobulins was provided by Pharmingen (San Diego, CA). Phycoerytrin-conjugated anti-CD11b antibody was purchased from Immunotech (France). After a blood sample was obtained, erythrocytes from saline- and Stx2-treated mice were lysed by hypotonic shock and cells were washed with cool PBS and incubated with MoAb for 30 min at 4°C. The antibody concentration of the final reaction mixture was 2 μg/ml to 1×10^6 cells. After two washes with PBS, the cells were suspended in 0.4 ml of ISOFLOW (International Link, S.A., Buenos Aires, Argentina) and the fluorescence was measured with a Beckton–Dickinson FACScan. The analysis was made on 10,000 events on each sample by using the Cell Quest program (Becton–Dickinson).

Antibody-dependent cellular cytotoxicity (ADCC). Peripheral blood neutrophils from individual mice were obtained by layering on a Ficoll–Hypaque density gradient as previously described (28). Purified neutrophils from saline- and Stx2-treated mice were suspended at 1×10^6 cells/ml in 1640 medium supplemented with 2% fetal calf serum (GIBCO) and 50 μg/ml gentamycin (Sheering Corp., ESSEX, Argentina). The viability of these suspensions containing 97% polymorphonuclear cells was always higher than 90% by the trypan blue exclusion test. ADCC was assayed by the chicken red blood cell (CRBC)–anti-CRBC system as previously described (29). The reaction was performed by reacting 1×10^5 PMN with 2×10^5 ^{51}Cr-labeled CRBC sensitized with subagglutinating amounts of specific rabbit IgG. After incubation for 18 h at 37°C, the culture plate was centrifuged and the radioactivity of supernatants and pellets was measured. The mean amount of ^{51}Cr released was expressed as the percentage of total radioactivity. Spontaneous release was always less than 3% and it was subtracted to obtain specific cytotoxicity, which was depicted.

Urea studies. Blood was obtained by puncture of the retroorbital plexus. Biochemical determinations of urea in serum were performed in a CCX Spectrum autoanalyzer (Abbott Diagnostics System, Buenos Aires, Argentina) following standardized instructions.

Statistical analysis. All data correspond to the mean ± SEM of individual mice. Statistical differences were determined using the one-way analysis of variance (ANOVA), and $P < 0.05$ was considered significant. Individual groups were compared using the unpaired Student's *t* test. The Pearson's correlation test was used for calculating correlation.

RESULTS

Effect of Stx2 on Circulating Leukocytes

Since LPS frequently accompany Stx in natural infections, the direct effect of Stx2 on the absolute number and percentage of circulating leukocytes could not be determined in humans. However, the murine model makes it possible to investigate the influence of Stx2 on leukocyte populations.

We have previously established that mice inoculated with 250 pg of Stx2 developed severe neurological symptoms, coma, and death after 3–4 days of Stx2 injection (26). Therefore, mice injected with this amount of Stx2 were bled 72 h later for differential leukocyte counts. Compared with saline-injected animals, Stx2-treated mice showed an increase in total blood leukocytes, due to the neutrophil population (Table 1).

In order to investigate whether this neutrophilia was accompanied by alterations in other blood cells we also analyzed the percentage of circulating leukocyte populations such as B and T lymphocytes and their $CD4^+$ and $CD8^+$ subsets by cytometric studies. Although we found a lower number of B lymphocytes after Stx2 treatment, there was no significant difference among B or T populations and subpopulations between saline- and Stx2-treated mice (Fig. 1). On the contrary, the percentage of CD11b-positive cells, a marker of myeloid population, was significantly increased in mice treated with Stx2.

Since neutrophilia induced by inflammatory stimuli such as LPS could be accompanied by a reduction in the number of neutrophils in the bone marrow (30), we analyzed whether a similar effect could be observed in Stx2-treated mice. The number of neutrophils present in the bone marrow was quantified by MPO content and immunohistochemistry 72 h after Stx2 injection. The total number of cells obtained from bone marrow and MPO quantification was similar in saline- and Stx2-treated mice (ng MPO/10^6 bone marrow cells: Saline, 2.3 ± 0.12; Stx2, 3.1 ± 0.95). Similar results were obtained at 24 and 48 h after Stx2 injection (not shown). These findings were confirmed by histochemistry observations (not shown).

TABLE 1

Absolute and Relative Numbers of Blood Leukocytes[a]

	Leukocytes (cells/mm^3) $\bar{X} \pm$ SEM	PMN (cells/mm^3) $\bar{X} \pm$ SEM	Mononuclears cells (cells/mm^3) $\bar{X} \pm$ SEM
Saline ($N = 16$)	3741 ± 283 (100%)	541 ± 80 (14%)	3200 ± 194 (86%)
Stx2 ($N = 16$)	4100 ± 309 (100%)	1326 ± 70 (32%)*	2774 ± 254 (67%)

[a] The numbers of PMN and mononuclears cells were counted in a Neubauer chamber 72 h after Stx2 injection. The percentage of each cellular population is expressed in parentheses.

* $P < 0.05$ compared to the saline group.

Time Course of Stx2-Induced Neutrophilia

The kinetics of Stx2-induced neutrophilia were studied in comparison with those of LPS-induced neutrophilia (Fig. 2). As previously described, the ip injection of LPS (5 μg/mouse) induced a great drop in neutrophil counts as early as 60 min, followed by neutrophilia which reached its maximal level at 24 h after LPS injection (31). In contrast, Stx2 induced a slow but sustained increase in neutrophil counts up to 72 h after Stx2 inoculation, the time at which the mice were sacrificed. In addition, the potential reciprocal influence between Stx2 and LPS on neutrophilia was studied. We had previously established that LPS treatment (5 μg/mouse) 1 h before Stx2 injection significantly enhanced Stx2 toxicity (15). Therefore, the consequences of the combined effects of Stx2 and LPS on PMN were analyzed. The results show that after an injection of LPS plus Stx2, the early drop (1 h) and the rebound effect (24 h) in neutrophil counts induced by LPS were observed, as well as the neutrophilia induced by Stx2 at 72 h. This indicates that the effects of LPS and Stx2 on neutrophilia may be two independent phenomena.

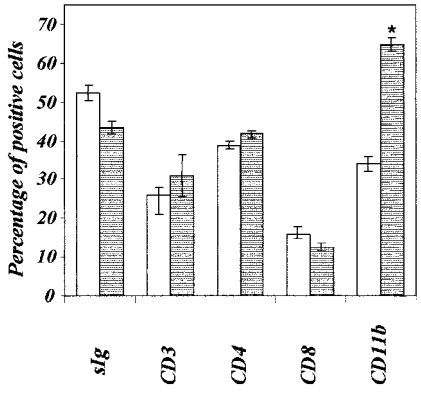

FIG. 1. Percentage of peripheral blood leukocytes. At 72 h post saline (white bars) or Stx2 injection (gray bars), mice were bled by retroorbital plexus puncture. After erythrocyte lysis by hypotonic shock, 100 μl of blood was stained with specific MoAb against markers of different leukocyte populations, as described under Materials and Methods. Each bar represents the arithmetic mean ±SEM of the percentage of positive cells ($n = 4$–7 mice). *$P < 0.05$ compared to the saline-treated group.

CD11b Expression on Circulating Neutrophils

Quantitative and qualitative changes in the expression of CD11b molecules on PMN are an early consequence of neutrophil activation (32). Taking this into account, the effect of Stx2 and LPS on CD11b expression in PMN was evaluated. As shown in Fig. 3, while LPS induced a rapid and transient increase in CD11b expression, Stx2 induced a minor but sustained increase in the expression of this marker up to the death of the animals. The LPS plus Stx2 group showed an initial increase in the expression of CD11b, although this effect disappeared at 24 h and was not significant at 72 h, probably due to the late anti-inflammatory effects induced by LPS to its own action (33) which could counteract the Stx2 action.

Effect of Stx2 on Neutrophil Adherence to the Lung

Since endothelial injury is a central event in Stx2 toxicity and PMN adherence to vessels could play an important enhancing role in this damage, Stx2 effect on *in vivo* PMN adherence to lung vessels was analyzed. As previously described, there is a strong correlation between the number of intravascular neutrophils and the lung content in the enzyme marker MPO (34). Therefore, this parameter was measured to quantify the total neutrophil sequestration in the lungs 72 h after Stx2 injection. Stx2 induced a significant enhancement in MPO content (ng MPO/lung: Saline, 0.43 ± 0.08; Stx2, 0.72 ± 0.09, $P < 0.05$). These findings suggest that PMN are profoundly modified by Stx2 treatment, in such a way that neutrophils readily invade and adhere to vessels.

Effect of Stx2 on Neutrophil Cytotoxicity

Frequently, functions dependent on the presence of receptors for the Fc portion of immunoglobulin G

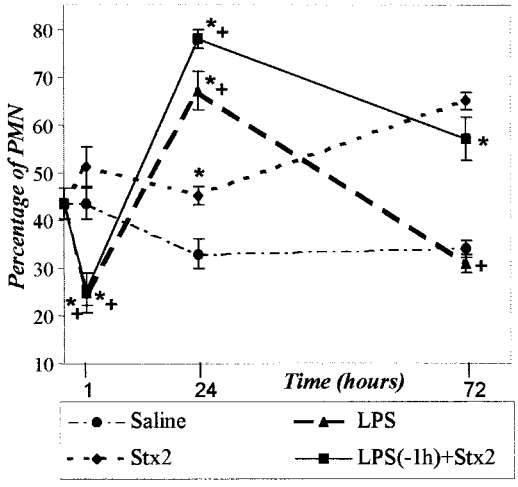

FIG. 2. Kinetics of Stx2-induced neutrophilia. Groups of mice were bled at different times before (time 0) and after treatments as indicated in the figure. The neutrophil population was identified and gated using forward scatter/side scatter (FSC/SSC) dot-plot profiles. Points depicted represent the percentage of gated cells. This percentage was coincident with the percentage of CD11b-positive cells for each mouse. Each point represents the arithmetic mean ±SEM of 7–9 mice for 1 h posttreatment and the mean ±SEM of 15–25 mice for all other times. $*P < 0.05$ compared to the saline-treated group; $+P < 0.05$ compared to the Stx2 group.

(FcγR) are enhanced in activated neutrophils (35). Therefore, we analyzed the capacity of peripheral blood PMN from individual Stx2-treated mice to mediate ADCC (a FcγR-dependent cytotoxicity). As shown in Fig. 4, Stx2 treatment induced a significant increase in ADCC at every time evaluated.

Correlation between Percentage of Neutrophils and Renal Damage Induced by Stx2

Since the death of the animals in the murine model of HUS is associated with renal damage, the course of the disease could be followed by determining uremia after Stx2 injection (36). In mice treated with saline or LPS, normal levels of urea were found at 24, 48, and 72 h after treatment (range 45–60 mg%). However, in the group of mice injected with Stx2, increasing levels of uremia were found 48–72 h posttreatment (range 150–357 mg%). In order to analyze the relationship between the number of PMN and the outcome of the disease, individual values of percentage of PMN and uremia were correlated. As shown in Fig. 5, there was a significant positive correlation between the percentage of PMN and urea concentration in mice treated with Stx2, suggesting a relationship between neutrophilia and the severity of the Stx2 damage.

DISCUSSION

Research in the last few years has established a link between enteric infection with Stx producing *E. coli* and HUS (16, 18). It is well recognized that damage to the glomerular endothelium is the main site of injury by Stx in HUS. However, clinical and experimental evidence suggests that the inflammatory response is able to potentiate Stx toxicity. In this regard, LPS and neutrophils represent two central components of inflammation during a gram-negative infection. Thus, there is a consensus of opinion that in epidemic HUS

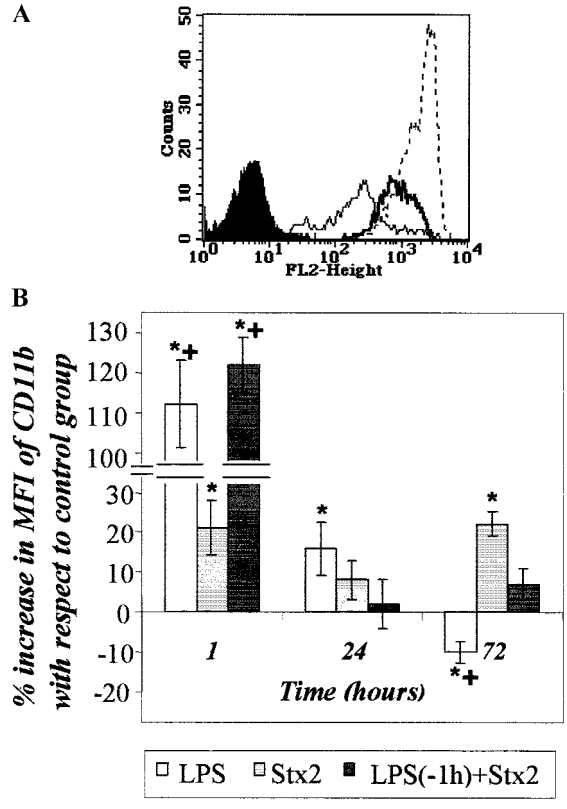

FIG. 3. CD11b membrane expression on circulating neutrophils. Groups of mice were bled at different times after the treatments indicated in the figure. After erythrocyte lysis by hypotonic shock, 100 μl of blood was stained with specific MoAb against murine CD11b as described under Materials and Methods. The neutrophil population was identified and gated using FSC/SSC dot-plot profiles. Cells into neutrophil gate were analyzed for the density of fluorescence. (A) A representative histogram of CD11b expression on neutrophils 72 h after saline (thick line), Stx2 (dashed line), or LPS (thin line) injection is shown. The filled in histogram represents the isotype-matched control. The ordinate and the abscissa represent the cell number and the fluorescence intensity, respectively. (B) The density of fluorescence for each group is expressed as the percentage of increase with respect to the mean fluorescence intensity (MFI) in control mice. Each bar represents the arithmetic mean ±SEM of 7–9 mice for 1 h posttreatment and the arithmetic mean ±SEM of 15–25 mice for all other times. $*P < 0.05$ compared to the saline-treated group; $+P < 0.05$ compared to the Stx2 group.

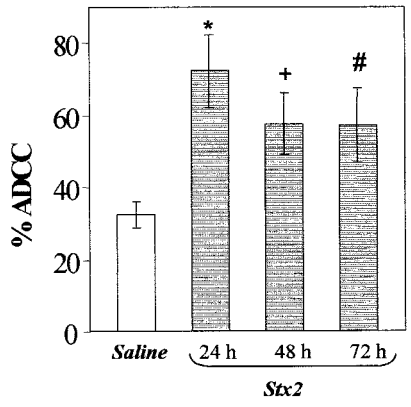

FIG. 4. Effect of Stx2 treatment on neutrophil ADCC. Saline- and Stx2-treated mice were bled at the times indicated in the figure and PMN isolated as described under Materials and Methods. PMN (1×10^5 cells) were incubated for 18 h at 37°C with sensitized ^{51}Cr-labeled CRBC (2×10^5). The percentage of cytotoxicity was measured as described under Materials and Methods. Each bar represents the mean ±SEM of 8 mice per group. *$P < 0.0001$, +$P < 0.01$, and #$P < 0.05$ compared to the saline-treated group.

and in murine models, LPS directly, or indirectly through the secretion of cytokines (i.e., TNF-α, IL-6, and IL-8), can amplify Stx cytotoxic potential (13, 14, 18). Previous data in children with HUS have suggested that endothelial damage might critically depend on neutrophil-specific proteases released by activated leukocytes (20). Since leukocytosis is a common feature of gram-negative infectious diseases, it is not surprising that children with the epidemic form of HUS present high counts of neutrophils. Moreover, LPS alters the surface characteristics of endothelial cells via a cascade of cytokines which renders them adhesive to neutrophils (37). However, direct involvement of Stx in modulating leukocyte populations has not been previously evaluated. The use of a murine model of HUS, based on intravenous injection of Stx, makes it possible to analyze the contributory role of the two central components of this disease: Stx and LPS.

The results presented herein demonstrate that a single injection of Stx2 induced a slow but sustained rise in peripheral blood neutrophils, without modifying other leukocyte populations. This neutrophilia was not accompanied by a reduction in the number of neutrophils present in the bone marrow. Although the mechanism by which the absolute and relative increase of granulocytes remains undetermined, it can be speculated that the slow progression to neutrophilia, caused by Stx, can be accompanied by an increased production on PMN in the bone marrow without any apparent change in their number.

Although migration of circulating leukocytes from blood into surrounding tissues is a critical step of the inflammation necessary for host defense, excessive accumulation of leukocytes can be harmful and can lead to damage. CD11b/CD18 (Mac-1, complement receptor 3) is a β2 integrin that mediates several processes in neutrophils, such as adhesion to the endothelium, phagocytosis, superoxide production, and other activation events (38). Indeed, CD11b/CD18-deficient mice are severely compromised in leukotriene B4-induced leukocyte adhesion to vessel wall *in vivo* (39), and their PMN have reduced spreading and oxygen radical generation compared to those of normal PMN (40). Since the regulation of these functions is achieved by quantitative and qualitative changes in the expression of CD11b/CD18 on PMN (38), the increased expression of this molecule after Stx2 injection (Fig. 3) is consistent with a process of PMN activation in circulation. The enhancement of ADCC induced by Stx2 (Fig. 4) could be a concomitant event to CD11b up-regulation, since we and others have demonstrated that this β2 integrin cooperates as a cotransductor with the receptor for immunoglobulin G (FcγR) necessary to mediate ADCC (41, 42). Regarding the cooperative effects of LPS on Stx2 inflammatory action, we demonstrated a potentiation of neutrophilia and CD11b induction at early times. The loss of synergism at later times could be a consequence of a late anti-inflammatory effect induced by LPS to its own action (33).

As an additional proof of PMN activation, we have also demonstrated an enhanced adhesion of neutrophils to lung vessels after Stx2 *in vivo* treatment. This neutrophil activation is a complex phenomenon that could be the consequence of a direct effect of Stx2 on PMN or of an indirect effect mediated by active products released upon the damage of the endothelium. These results are in agreement with previous *in vitro* studies which demonstrate that Shiga toxin increases neutrophil adhesion to endothelial cells pretreated with TNF-α (43). Damaged or stimulated endothelial cells secrete cytokines, chemokines, nitric oxide, platelet-activating factor, IL-1, and IL-8 (44, 45), all of which are strong stimuli for neutrophils. Release of

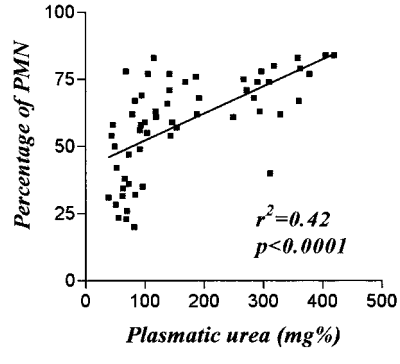

FIG. 5. Correlation between the percentage of PMN and plasmatic urea. The percentage of PMN in peripheral blood was determined at 24, 48, and 72 h after injection of Stx2. Plasma urea was simultaneously measured in the same samples.

enzymes and other constituents by degranulating PMN may be especially important in subsequent vascular injury (46). Finally, the positive correlation between the percentage of neutrophils and renal damage strongly suggests an active role of PMN during the evolution of the disease.

Previous reports in typical HUS patients have shown increased levels of IL-8 in the serum and high neutrophil counts. However, the question of whether the observed changes were induced by Stx, LPS, or both cannot be answered (47). In this regard, our data have demonstrated that Stx2 by itself is able to induce neutrophilia and the activation of PMN (increased ADCC, adhesion to lung, and expression of CD11b). Moreover, the significant correlation between neutrophilia, PMN activation, and renal damage suggests that PMN could represent an important cell in disease activity. Further understanding of the interaction of Stx, neutrophils, and endothelium is essential because modulation of these factors may be of therapeutic benefit in HUS.

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