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Histamine H₂ Receptor Signaling in the Pathogenesis of Sepsis: Studies in a Murine Diabetes Model

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Type 1 diabetes enhances susceptibility to infection and favors the sepsis development. In addition, diabetic mice produced higher levels of histamine in several tissues and in the blood after LPS stimulation than nondiabetic mice. In this study, we aimed to explore the role of mast cells (MCs) and histamine in neutrophil migration and, consequently, infection control in diabetic mice with mild sepsis (MS) induced by cecum ligation and puncture. We used female BALB/c, MC-sufficient (WB/B6), MC-deficient (W/Wv), and NOD mice. Diabetic mice given MS displayed 100% mortality within 24 h, whereas all nondiabetic mice survived for at least 5 d. The mortality rate of diabetic mice was reduced to 57% after the depletion of MC granules with compound 48/80. Moreover, this pretreatment increased neutrophil migration to the focus of infection, which reduced systemic inflammatory response and bacteremia. The downregulation of CXCR2 and upregulation of G protein–coupled receptor kinase 2 in neutrophils was prevented by pretreatment increased neutrophil migration to the focus of infection, which reduced systemic inflammatory response and bacteremia. The downregulation of CXCR2 and upregulation of G protein–coupled receptor kinase 2 in neutrophils was prevented by pretreatment of diabetic mice given MS with compound 48/80. In addition, blocking the histamine H₂ receptor restored neutrophil migration, enhanced CXCR2 expression, decreased bacteremia, and improved sepsis survival in alloxan-induced diabetic and spontaneous NOD mice. Finally, diabetic W/Wv mice had neutrophil migration to the peritoneal cavity, increased CXCR2 expression, decreased bacteremia, and improved sepsis survival in alloxan-induced diabetic and spontaneous NOD mice. These results demonstrate that histamine released by MCs reduces diabetic host resistance to septic peritonitis in mice. The Journal of Immunology, 2013, 191: 000–000.
It was demonstrated that histamine levels are increased in plasma and tissues in diabetic rats, as well as in patients with diabetes mellitus (32, 33). Moreover, levels of histamine are higher in diabetic rats than in nondiabetic animals after LPS administration (34). In agreement, in this study, we found that histamine is increased in diabetic mice undergoing septic peritonitis. Moreover, we observed that histamine released by MCs decreases neutrophil migration to the infection site through interaction with H<sub>2</sub>, resulting in impaired host defense and a poor sepsis outcome in diabetic mice.

**Materials and Methods**

**Mice**

Female BALB/c, MC-sufficient (WB/B6), W/W<sup>v</sup>, and NOD mice were housed in facilities of Department of Pharmacology, School of Medicine of Ribeirão Preto at 23–25˚C with free access to water and food. Animal care and treatment was based on the Guide for the Care and Use of Laboratory Animals (35), and all experiments were approved by the Animal Research Ethics Committee of the School of Medicine of Ribeirão Preto (no. 001/2008).

**Diabetes induction**

Diabetes was induced with a single i.v. injection of alloxan (Sigma Chemical Company, St. Louis, MO) at a dosage of 50 mg/kg in saline (100 μl). The nondiabetic control group received an i.v. injection of saline alone. Blood samples were collected from the tail vein to measure blood glucose levels using a glucometer (Precision Xtra Abbott, Alameda, CA).

**Histamine measurement**

Mouse plasma histamine concentration was measured 6 h after surgery using an immunoenzymatic method with a manufactured kit (Cayman, Ann Arbor, MI).

**Sepsis model**

Sepsis was induced by CLP as previously described (36). In brief, mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by i.p. injection. A 1-cm midline incision was made on the anterior abdomen, and the cecum was exposed and ligated below the ileocecal junction without causing bowel obstruction. A single puncture was made in the cecum using an 18-gauge needle to induce SS, or three punctures were made using a 30-gauge needle to induce mild sepsis (MS). The cecum was squeezed to express its contents through the puncture holes and then placed back into the abdominal cavity. The peritoneal wall and skin incisions were closed. Sham-operated (Sham) animals were submitted to laparotomy but without punctures in cecum.

**Pharmacological treatment**

Nondiabetic mice were treated s.c. with histamine (3 μg or 10 μg in 100 μl saline) (Sigma Chemical Company, St. Louis, MO), 1 h before CLP and every 24 h afterward for a total of 5 d for survival rate analysis, or treated 1 h before CLP with 10 μg histamine per mouse for neutrophil migration, bacterial load, and CXCR2 expression evaluation. Ten days after alloxan treatment, diabetic mice were injected i.p. with pyrimoline (20 mg/kg, a selective H<sub>1</sub> antagonist), cimetidine (20 mg/kg, a selective H<sub>2</sub> antagonist), thioperamide (5 mg/kg, an H<sub>3/4</sub> antagonist), or saline 1 h before CLP. Ten days after saline or alloxan administration, diabetic and nondiabetic mice underwent MS induction, SS induction, or a sham operation as the control according to the CLP model (Supplemental Fig. 1A).

**Neutrophil migration to the peritoneal cavity**

Peritoneal cavity lavage (PCL) was performed after 4 or 6 h of sepsis induction using 3 ml PBS/EDTA 1 mM. Total cell counts were obtained using a cell counter (Coulter Analyzer; Coulter), and differential cell counting was conducted using cytocentrifuge slides (Shandon Southern Products) stained with May-Grunwald-Giemsa.

**Bacterial count**

Four or 6 h after CLP, the blood was collected and the peritoneal cavity was washed with 1.5 ml sterile PBS/EDTA. Aliquots of serial dilutions of these samples were plated on Muller-Hinton agar dishes (Difco Laboratories) and incubated at 37˚C. CFUs were analyzed after 18 h, and the results were expressed as log of CFU/10<sup>10</sup> ml.

**GRK2 immunofluorescence**

Immunofluorescence was used to analyze GRK2 expression on blood neutrophils at 6 h postsurgery using anti-mouse GRK2 (Y137; Abcam) as the primary Ab, as previously described (14). Cell nuclei were stained with DAPI. Fluorescent images were analyzed using an Olympus BX-50 epifluorescence microscope.

**Flow cytometry**

One hundred microliters of blood was stained using PerCP-Cy5.5-conjugated anti-mouse myeloid lineage differentiation Ag Gr-1 (Gr-1) (BD Bioscience, Franklin Lakes, NJ), PE-conjugated anti-CXCR2 mAb (R&D Systems, Minneapolis, MN), or the appropriate isotype controls. The cells were washed, fixed in 2% formaldehyde, and analyzed by flow cytometry in a FACSort flow cytometer using CellQuest software. Neutrophils were identified by their light scatter properties and high GR-1 expression. MC number was determined using double staining with PE-Cy5-conjugated anti-CD117 and FITC-conjugated anti-Flt3L (eBioscience, San Diego, CA). The GRK2 staining was evaluated by flow cytometry in neutrophils as previously described (37).

**Detection of serum TNF-α and CXCL-2 levels**

TNF-α and CXCL-2 levels were assessed in serum and PCL 6 h after surgery by ELISA. The results were displayed in picograms per milliliter as the mean ± SEM.

**Vascular leakage**

Evans blue dye (2.5% in 100 μl of PBS) was i.v. administered 30 min before CLP. Two hours after CLP, the mice were killed, and the peritoneal cavity was washed with 1.5 ml PBS/EDTA (1 mM). The cells were spun down, and the OD of the supernatant at 600 nm was measured. The concentration of Evans blue in peritoneal exudates was determined under a standard curve.

**Determination of the myeloperoxidase activity**

The extent of leukocyte accumulation in the lung was measured by myeloperoxidase assay as previously described (38). The absorbance of the tissue supernatant was compared with a standard curve of mouse peritoneal neutrophils, and the results were reported as the mean ± SEM of the total number of neutrophils per milligram of lung.

**Statistical analysis**

The log-rank test was used to evaluate the survival rate, and the proportional risk model of Cox was used to identify the differences between groups. The Mann–Whitney <i>U</i> test was used to assess the following variables: blood and PCL bacterial count between vehicle-administered MS group and others variables. A <i>p</i> value < 0.05 was considered statistically significant.

**Results**

**Diabetic mice showed increased MC numbers after MS**

Alloxan-induced diabetes is a common experimental model used to study the pathophysiologic mechanisms involved in type 1 diabetes (39). Blood glucose levels in diabetic mice were increased 10 d after alloxan administration (Supplemental Fig. 1B), and these mice showed reduced body weight compared with nondiabetic mice (Supplemental Fig. 1C). We also evaluated the numbers of MCs (FceRI<sup>+</sup>/CD117<sup>+</sup> cells) in the peritoneal cavity during diabetes by flow cytometry. Interestingly, we observed higher
numbers of MCs in the peritoneal cavity of sham-operated (sham) diabetic mice compared with sham nondiabetic mice. In addition, diabetic mice had a significant increase in MCs in their peritoneal cavity compared with nondiabetic mice after MS induction. However, the number of MCs in MS diabetic mice did not differ from sham diabetic mice (Fig. 1A). We also observed a significant increase in the MC numbers in the peritoneal cavity of naive diabetic mice that did not undergo sham surgery compared with related nondiabetic mice (Supplemental Fig. 1D).

**MC degranulation contributes to sepsis onset in diabetic mice**

To evaluate the role of MCs in diabetic mice given sepsis, we pretreated diabetic mice with C48/80, an agent that induces MC-granule depletion (40), for 4 d before MS induction. Diabetic mice exhibited 100% mortality rate after MS, whereas all nondiabetic mice survived for 5 d after surgery (Fig. 1B). C48/80 pretreatment of diabetic mice enhanced survival rate to 42% after MS induction. As a control for sepsis induction, we induced SS in nondiabetic mice. The pretreatment of nondiabetic mice with C48/80 before induction of MS. SS was carried out in nondiabetic mice. The survival rates were evaluated daily for 5 d after CLP in nondiabetic mice and observed that they died within 3 d after surgery (Fig. 1B). The nondiabetic mice pretreated with C48/80 had a 33.3% survival rate after SS, whereas all SS mice that were not pretreated with C48/80 died. The survival rates of the pretreated mouse and nonpretreated mouse after MS were 85.7 and 100%, respectively, demonstrating that the pretreatment with C48/80 did not alter the MS survival rate (Supplemental Fig. 2D). It is important to mention that the pretreatment of diabetic mice with C48/80 had no effect on blood glucose levels or weight loss (Supplemental Fig. 1B, 1C).

We next examined the end points commonly associated with survival in the CLP model, including neutrophil migration to the focus of infection and the bacterial load 6 h after surgery. MS diabetic mice, which were more susceptible to sepsis, lost their ability to control infection, as demonstrated by a higher bacterial load in the peritoneal cavity and blood than MS nondiabetic mice (Fig. 1C, 1D). Mice undergoing SS also had a higher CFU content in these compartments compared with MS nondiabetic mice. Similar to our observations of survival rate, pretreatment of diabetic mice with C48/80 significantly decreased the CFU content in the peritoneal cavity and the blood of the MS diabetic mice (Fig. 1C, 1D). MS nondiabetic mice showed a significant increase in neutrophil migration to the peritoneal cavity compared with sham nondiabetic mice (Fig. 1E). In contrast, MS diabetic mice had decreased neutrophil migration in the peritoneal cavity, which were similar to the numbers observed in SS nondiabetic mice. Notably, pretreatment of diabetic mice with C48/80 significantly increased the neutrophil migration toward the focus of infection (Fig. 1E). Similarly, the directional migration (chemotaxis) toward CXCL-2 was significantly increased in these treated mice compared with MS nontreated diabetic mice (Fig. 1F). We also observed that the pretreatment with C48/80 of diabetic mice restored the random migration (chemokinesis) of blood neutrophils compared with nontreated diabetic mice after MS induction (Fig. 1F).

High systemic levels of proinflammatory cytokines and leukocyte sequestration in the lungs correlate with sepsis severity (41). Accordingly, we found higher levels of leukocytes in the lungs of diabetic MS mice and nondiabetic SS mice than in nondiabetic MS mice. Pretreatment of diabetic mice with C48/80 attenuated this leukocyte infiltration into the lungs after MS induction (Supplemental Fig. 2A). In addition, diabetic MS mice exhibited higher serum concentrations of TNF-α and CXCL-2 than nondiabetic mice.

**FIGURE 1.** MC granule depletion induces septic peritonitis control in diabetic mice. MC numbers were quantified in the peritoneal cavity using M-specific markers (CD117 and FcεR) in nondiabetic or diabetic mice 10 d after alloxan administration (A). Nondiabetic mice and diabetic mice were pretreated with saline or C48/80 before induction of MS. SS was carried out in nondiabetic mice. The survival rates were evaluated daily for 5 d after CLP surgery (B). Bacterial burden in the PCL (C), blood (D), and neutrophil numbers (E) into the peritoneal cavity was quantified 6 h after surgery. Blood neutrophils were obtained from sham-operated, nondiabetic (ND), and diabetic mice pretreated with saline or C48/80 after MS surgery. The chemotactic activity of blood neutrophils toward CXCL-2 (30 ng/ml) or RPMI 1640 (negative control) was determined in Boyden chambers (F). The results were expressed as the mean ± SEM (n = 5–7). These are representative graphs of at least two independent experiments. *p ≤ 0.05 compared with the nondiabetic sham-operated group, †p ≤ 0.05 compared with the nondiabetic MS group, ‡p ≤ 0.05 compared with the diabetic MS group.
abetic MS mice, which were reduced significantly by C48/80 pre-treatment (Supplemental Fig. 2B, 2C).

**MC degranulation promotes GRK2 upregulation and CXCR2 downregulation in neutrophils**

Reduced neutrophil migration to focus of infection can result from GRK2-mediated downregulation of CXCR2 in circulating neutrophils (16). To address this possibility, we analyzed CXCR2 expression by flow cytometry and GRK2 by immunofluorescence in circulating neutrophils after MS in alloxan-induced diabetic and nondiabetic mice. As shown in Fig. 2A, circulating neutrophils from MS diabetic mice exhibited a significant reduction in CXCR2 expression compared with those from both sham (nondiabetic and diabetic) and MS nondiabetic mice. Pretreatment of diabetic mice with C48/80 significantly prevented the CXCR2 downregulation in MS diabetic mice. Moreover, we observed that GRK2 protein expression was increased in neutrophils from MS diabetic mice and SS nondiabetic mice compared with the MS nondiabetic or sham-operated groups. The pretreatment of diabetic mice with C48/80 prevented this increased GRK2 expression compared with sham-operated nondiabetic mice. In addition, pretreatment with C48/80 prevented GRK2 induction in the neutrophils from these mice (Supplemental Fig. 2E).

**H₂ antagonist treatment alleviates sepsis in diabetic mice**

As shown in Fig. 2B, MS diabetic mice produced higher levels of histamine compared with both sham (nondiabetic and diabetic) and MS nondiabetic mice. As previously shown (Fig. 1B), depleting MC granules with C48/80 decreased the severity of sepsis in diabetic mice after MS induction. This pharmacologic intervention also significantly decreased serum histamine levels in MS diabetic mice (Fig. 2B). This finding suggested that the high serum levels of histamine in diabetic mice may contribute to poor sepsis outcome. To test this hypothesis, we used histamine receptor antagonists in diabetic mice given MS. Treatment with an H₁ antagonist (pyrilamine) delayed the death of the mice, but all mice still died within 4 d of MS induction (Fig. 3A). In contrast, treating diabetic mice with an H₂ antagonist (cimetidine) increased the survival rate by ∼45%. The treatment of MS diabetic mice with an H₃/₄ antagonist (thioperamide) did not influence their survival rate (Fig. 3A). As shown in Fig. 3B, the combination of H₁ and H₂ antagonists had no effect on survival rates of diabetic mice after MS induction.

We next evaluated the effects of the H₂ antagonist on neutrophil recruitment to the focus of infection, blood bacterial load, and CXCR2 expression in circulating neutrophils in MS diabetic mice. H₂ blockade significantly restored the neutrophil migration to the peritoneal cavity of MS diabetic mice (Fig. 3C), resulting in a significantly lower bacterial load in the blood compared with nontreated MS diabetic mice (Fig. 3D). In addition, treatment with the H₂ antagonist increased CXCR2 expression in the neutrophils of the MS diabetic mice (Fig. 3E). Blood neutrophils from the MS diabetic mice have a reduced chemotactic response to CXCL-2, but not to fMLF, compared with the MS nondiabetic mice, excluding the possibility of cross desensitization for this receptor. In

**FIGURE 2.** MC granule depletion prevents CXCR2 internalization and histamine release during polymicrobial peritonitis in diabetic mice. Quantitative analysis of the mean intensity of fluorescence (MFI) for CXCR2 in blood cells with high expression of GR-1 (neutrophils) was evaluated by flow cytometry (A). Serum histamine levels were determined 6 h after surgery from several experimental groups as described earlier (B). Blue staining by DAPI represents neutrophil nuclei; red staining represents GRK2 expression in neutrophil cytoplasm (C) (original magnification ×400). An overlay of nuclei and GRK2 expression shows the qualitative analysis from several experimental groups. Results are expressed as the mean ± SEM (n = 5–7). These are representative graphs of at least two independent experiments. *p ≤ 0.05 compared with the nondiabetic sham-operated group, †p ≤ 0.05 compared with the nondiabetic MS group, ‡p ≤ 0.05 compared with the diabetic sham-operated group, §p ≤ 0.05 compared with the diabetic MS group.
addition, H₂ pretreatment of the MS diabetic mice significantly restored the chemotactic response to CXCL-2, compared with the chemotactic response of the blood neutrophils of the MS diabetic nontreated mice (Supplemental Fig. 3A).

To determine whether these observations in MS diabetic mice were attributable to differences in vascular permeability between the septic groups, we used Evans blue extravasation in the peritoneal cavity as an index for vascular leakage. The Evans blue content during the first 2 h after MS was prominently increased in both the nondiabetic and diabetic mice compared with nondiabetic sham-operated mice. H₂ antagonist treatment did not alter the Evans blue content in the peritoneal cavity of the MS diabetic mice (Fig. 3F).

Histamine is involved in septic peritonitis susceptibility

Our earlier results suggested that histamine negatively regulated neutrophil migration. To test this hypothesis, we first incubated neutrophils with histamine (1 and 10 μM) and observed a reduction in their chemotactic response to CXCL-2 (Fig. 4A). Accordingly, neutrophil incubation with histamine (10 μM) also promoted CXCR2 internalization (Fig. 4B). Pretreatment of neutrophils with an H₂ antagonist significantly prevented the histamine-induced CXCR2 downregulation (Fig. 4B). In addition, neutrophils incubated with histamine showed increased intracellular GRK2 expression compared with neutrophils incubated with RPMI (control). However, preincubation of neutrophils with an H₂ antagonist significantly diminished the histamine-induced upregulation of GRK2 expression (Supplemental Fig. 3B, 3C).

Enhanced infiltration of neutrophils into the inflammatory site was observed in air-pouch inflammation (42) and bacterial peritonitis (43) in histidine decarboxylase–deficient mice, which cannot produce histamine, indicates that histamine inhibits neutrophil recruitment. Thus, we examined the role of histamine in neutrophil recruitment in the septic peritonitis model. In vivo, administration of 3 or 10 μg histamine per mouse decreased the survival rates by ~30 and 50%, respectively, 5 d after MS surgery (Fig. 4C). Histamine administration also significantly inhibited neutrophil migration to the focus of infection, resulting in impaired infection control as shown by reduced bacterial clearance in the peritoneal cavity and an enhanced bacterial load in the blood (Fig. 4D, 4E). Moreover, systemic histamine administration significantly reduced the CXCR2 expression in the blood neutrophils in MS mice (Fig. 4F). Moreover, the H₂ antagonist treatment significantly prevented the downregulation of CXCR2 expression in neutrophils from mice given MS (Fig. 4F).

MC deficiency protects diabetic mice from SS

Next, we evaluated sepsis progression in mice lacking MCs (W/W°) and their littermate controls (WB/B6). Our results showed that nondiabetic W/W° mice were more susceptible to MS than control WB/B6 nondiabetic mice (Fig. 5A). We observed a significant decrease in neutrophil numbers in the peritoneal cavity accompanied by increased bacterial load at the focus of infection and in the blood of MS W/W° nondiabetic mice compared with MS WB/B6 nondiabetic mice (Fig. 5B–D). In contrast, diabetic W/W° mice with MS showed ~25% improvement in the survival rate compared with WB/B6 diabetic mice with MS (Fig. 5A). In parallel, we observed that diabetic WB/B6 mice given MS had a significant reduction in neutrophil migration and increased peri-
toneal cavity and blood bacterial loads compared with nondiabetic WB/B6 MS mice. In contrast, diabetic W/Wv mice displayed higher neutrophil numbers and reduced bacterial loads in the peritoneal cavity and blood compared with diabetic WB/B6 mice after the MS induction (Fig. 5B–D). Analysis of blood neutrophils by flow cytometry showed that MC deficiency did not affect CXCR2 expression on neutrophils from nondiabetic mice with MS, but prevented CXCR2 downregulation on neutrophils after MS induction in diabetic mice (Fig. 5E).

**H2 antagonist treatment improves infection control in NOD mice**

Finally, we used NOD mice, which spontaneously develop type 1 diabetes, to confirm the role of H2 in sepsis pathogenesis in diabetic mice. We first observed a significant increase in MC numbers (FceRI+/CD117+ cells) in the peritoneal cavity of NOD mice after MS induction (Fig. 5B–D). Analysis of blood neutrophils by flow cytometry showed that MC deficiency did not affect CXCR2 expression on neutrophils from nondiabetic mice with MS, but prevented CXCR2 downregulation on neutrophils after MS induction in diabetic mice (Fig. 5E).

**Discussion**

The factors that determine whether a particular infection in a diabetic mouse or patient will be successfully controlled or progress to sepsis are not well understood. Neutrophils from diabetic mice without any apparent infections already have functional abnormalities, including decreased phagocytic capacity (44) and chemotactic responses (45). In addition, increased numbers of tissue MCs have been found in clinical diabetes (46). Studies using experimental models also demonstrated that MCs are more abundant in different tissues of diabetic rats compared with related nondiabetic rats (47). Consistent with these findings, we observed that the increase in peritoneal MC numbers in diabetic mice coincided with the severity of sepsis and impaired neutrophil migration during septic peritonitis.

Based on these results, we evaluated the role of MCs in the increased sepsis susceptibility of diabetic mice using several approaches. Pretreatment of diabetic mice with C48/80 induced marked neutrophil migration to the focus of infection, which was associated with decreased bacteremia and an improvement in the survival rate after MS. In agreement, we observed that the high...
TNF-α and CXCL-2 levels in the serum of diabetic mice undergoing septic peritonitis were decreased by C48/80 treatment. The improved control of septic peritonitis in C48/80-pretreated diabetic mice could not be attributed to glucose metabolism effects, as the drug did not alter hyperglycemia. C48/80 pretreatment was previously demonstrated to prevent neutrophil migration impairment and systemic mediator release after SS (23). A different study showed that, after MC stabilization using cromolyn sodium, mice were protected from a lethal dose of i.v. Gram-negative bacteria (48). Overall, our results implicate MCs in both neutrophil migration impairment and systemic inflammatory mediator release in response to septic stimulus in diabetic mice.

Chemokines and their receptors play a pivotal role in regulating the activation and migration of leukocytes through the extracellular matrix (49). The importance of CXCLFR and chemokines and their receptors for host defense against infection has been demonstrated in various models (50, 51). In humans (52) and experimental sepsis (13), reduced neutrophil chemotaxis and migration were associated with decreased CXCR2 expression on circulating neutrophils. Similarly, we found that CXCR2 expression in neutrophils from diabetic mice undergoing MS was significantly reduced during MS in diabetic mice, but neutrophils. Similarly, we found that CXCR2 expression in neutrophils from diabetic mice undergoing MS was significantly reduced during MS in diabetic mice, but

**FIGURE 5.** MC deficiency promotes resistance of diabetic mice to polymicrobial peritonitis. Survival of W/Wv or WB/B6 nondiabetic (ND) and diabetic mice upon MS was evaluated daily for 5 d after CLP surgery (A). The number of neutrophils into the peritoneal cavity (B) and bacterial burden in the PCL (C) or the blood (D) were quantified 4 h after surgery. Quantitative analysis of the mean intensity of fluorescence (MFI) for CXCR2 in blood cells with high expression of GR-1 (neutrophils) was evaluated by flow cytometry (E). Results were expressed as the mean ± SEM (n = 5–7). These are representative graphs of at least two independent experiments. *p ≤ 0.05 compared with the nondiabetic sham-operated WB/B6 group, #p ≤ 0.05 compared with the nondiabetic WB/B6 MS group, = p ≤ 0.05 compared with the diabetic WB/B6 MS group.
endothelial cells. These studies support our finding that the antag-
expression in endothelial cells is promoted by histamine via H1
chemotaxis (60). On the other hand, increased adhesion molecule
agonist reversed the inhibitory effect of histamine on neutrophil

FIGURE 6. H2 signaling favors sepsis development in NOD mice. MC
numbers in the peritoneal cavity (A) and serum histamine levels (B) were
measured 6 h after surgery. Survival rates of prediabetic or NOD mice
pretreated and posttreated with an H2 antagonist were determined daily for
5 d after CLP surgery (C). The number of neutrophils into the peritoneal
cavity (D) and bacterial burden in the PCL or the blood (E) were quantified
6 h after surgery. Results are expressed as the mean ± SEM (n = 4–6).
These are representative graphs of at least two independent experiments.
*p ≤ 0.05 compared with the sham-operated prediabetic NOD group, *p ≤ 0.05 compared with the prediabetic NOD MS group, *p ≤ 0.05 compared with the NOD MS group.

released by MCs account for neutrophil migration failure and sepsis
onset in diabetic mice.

Chemotactic deactivation may be an important mechanism in
inhibition of neutrophil chemotaxis in some syndromes charac-
terized by recurrent bacterial infections. Cold urticaria is one such
syndrome (57), where neutrophil chemotactic defects are related to
high levels of IgE and histamine in serum (58, 59). In addition,
functional studies reported that the chemotaxis of neutrophils in
normal and atopic subjects is inhibited by histamine, and this
inhibition is more evident in atopic individuals. Furthermore, the
incubation of neutrophils from these individuals with an H2 an-
tagostant reversed the inhibitory effect of histamine on neutrophil
chemotaxis (60). On the other hand, increased adhesion molecule
expression in endothelial cells is promoted by histamine via H1
(61, 62). Histamine regulates the expression of its own receptors
on endothelial cells (63). Thus, histamine can either directly sup-
press the neutrophil chemotaxis activity via H2 or indirectly induce
neutrophil rolling, adhesion, and transmigration via H1 acting on
endothelial cells. These studies support our finding that the antag-
onism of H1 and H2 did not alter the survival rates in MS diabetic
mice; however, blocking only H2 increased the survival rates.

In additional experiments, we evaluated the in vivo role of
histamine in septic peritonitis. Histamine administration to non-
diabetic mice impaired neutrophil migration in a CXCR2-dependent
manner. To gain further insight regarding the influence of hista-
mone on CXCR2 regulation, we confirmed that histamine reduced
neutrophil chemotaxis and promoted the CXCR2 desensitization
associated with GRK2 upregulation. A previous study demon-
strated that histamine stimulates the random motility (chemo-
kinesis) and inhibits the directional motility (chemotaxis) of
neutrophils in vitro. This study also showed that cAMP levels in
neutrophils are significantly increased after histamine incubation and
decreased after treatment with an H2 antagonist (64). Com-
plementing these studies, we demonstrated an additional effect of
histamine in neutrophil migration by induction of CXCR2 re-
ceptor desensitization perhaps via GRK2 upregulation. In support,
another study also found that histamine-deficient mice control
Escherichia coli infection better than wild-type mice, which was
related to high leukocyte migration (43). Also, importantly, his-
tamine is induced during acute M. tuberculosis infection and may
dampen protective immunity by augmenting the inflammatory
response associated with this disease (65).

MCs have been proposed to enhance survival by augmenting
intracellular neutrophil killing through either IL-6 production (66)
or through the action of MC proteases (67). In general, our find-
ings support that activation of local MCs is crucial for host de-
defense during bacterial infection because nondiabetic W/Wv mice
had more sepsis mortality than WB/B6 nondiabetic mice. In con-
trast, we showed that diabetic W/Wv mice had lower sepsis mor-
tality than WB/B6 diabetic mice. In agreement, other studies have
shown that systemic MC activation exacerbates mortality during SS
(24, 68). Although MCs may induce neutrophil recruitment and
activation, and could have a protective role during acute local in-
fection, these effects can be overcome and ultimately become det-
rimental during systemic and severe infections. In addition, along
with improved survival, diabetic W/Wv mice undergoing MS had
lower numbers of bacteria in the blood and increased neutrophil
migration into the peritoneal cavity that were associated with
decreased CXCR2 internalization, indicating that MC deficiency
confers resistance to septic peritonitis in diabetic mice.

NOD mice spontaneously develop a form of diabetes that closely
resembles the human disease (69). Accordingly, NOD mice were
more susceptible to MS than prediabetic mice, with 50 and 25%
mortality rates, respectively. Septic peritonitis was exacerbated in
NOD mice when compared with prediabetic mice because these
mice were unable to clear the polymicrobial infection, which then
progresses to bacteremia. These effects correlated with increased
MC numbers, increased serum histamine levels, impaired neu-
rophil migration, and downregulation of CXCR2 expression on
blood neutrophils. H2 pretreatment of NOD mice promoted neu-
rophil chemotaxis and migration associated with maintenance of
CXCR2 expression and, consequently, a better control of bacterial
infection.

In conclusion, this study shows that MC degranulation induces
neutrophil migration failure through CXCR2 receptor desensiti-
zation by a mechanism that is dependent on augmented GRK2
expression and that ultimately results in sepsis development. In
addition, to our knowledge, we provide the first evidence that MCs
appear to exert these harmful effects through the systemic release
of histamine. Taken together, our results suggest that inhibition of
histamine-mediated signaling through H2 antagonism might con-
fer protection against sepsis onset in a diabetic host.

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Disclosures
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