Short Communication

Mice with Streptozotocin-Induced Hyperglycemia are Susceptible to Invasive Enteric Bacterial Infection

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SUMMARY: Diabetes mellitus and diabetes are becoming increasingly burdensome worldwide, particularly in developing countries such as India. Diabetic patients are susceptible to infection with pathogenic bacteria, particularly those causing invasive enteric infections. In this study, we observed changes in the pathophysiological features of mice with streptozotocin-induced hyperglycemia. In our experiments, both hyperglycemic and control mice were infected with pathogenic enteric bacteria—non-typhoidal Salmonella, Shigella flexneri, or Vibrio parahaemolyticus. Morbidity, mortality, and bacterial load were all higher in the diabetic mice than in the control mice, and the phagocytic and bactericidal activities of peritoneal macrophages isolated from hyperglycemic mice were lower than they were in the controls. We hypothesize that hyperglycemia leads to a downregulation of the innate immune response, which in turn increases vulnerability to enteric bacterial infection.

Diarrheal disease accounts for an estimated 3.6% of the total daily global burden of disease and is responsible for 1.5 million deaths each year, of which an estimated 842,000, corresponding to 58% of the total, occur in developing countries (1). Furthermore, patients with insulin-dependent diabetes mellitus (IDDM) or non-insulin-dependent diabetes mellitus (NIDDM), both of which are associated with a variety of genetically determined complications, are at increased risk of infection (2), with gastrointestinal (GI) dysfunction occurring in as many as 75% of diabetic patients (3,4). Recent studies have shed light on the association between diabetes mellitus (DM) and bacterial infection, as well as how the synergy between these causes other complications. Invasive infections with Staphylococcus aureus or beta-hemolytic streptococci have been shown to be more common in diabetic patients, and East Asian patients with uncontrolled diabetes were more likely to suffer from constipation (5,6). Delayed emptying of the small intestine and the consequent stagnation of fluid may lead to bacterial overgrowth syndrome, resulting in diarrhea and abdominal pain. The duration of diabetes and the degree of glycemic control are the major determinants of the incidence and severity of GI problems in patients with DM (7,8).

Globally, the primary causative agents of gastroenteritis in both developed and developing countries are Shigella flexneri, Vibrio parahaemolyticus, and various strains of the Salmonella enterica serovar Typhimurium (9–11). While the resulting disease is usually mild and self-limiting, it can be fatal, particularly in immunocompromised individuals (11).

Following these discoveries, we investigated the impact of diabetes on susceptibility to enteric diarrheagenic bacteria, such as S. Typhimurium, S. flexneri, and V. parahaemolyticus, as well as the severity of the resulting disease. First, we developed a chemically induced diabetic mouse model by treating Swiss albino mice intraperitoneally with low doses (40 mg/kg) of streptozotocin (STZ) continuously for 5 days (12). A group of 15 diabetic mice and another group of 15 non-diabetic control mice were each then divided into 3 groups of 5 mice (A, B, and C). Two weeks after cessation of STZ treatment, significant physiological changes, including higher levels of glucose in the blood, plasma, and urine as well as reduced body weight, were observed in the treated mice compared to the control group (Fig. 1). Both the diabetic and non-diabetic group A mice were orally infected with S. Typhimurium IDH 0233 (1 × 10^9 cfu/ml), while the group B mice were intraperitoneally infected with S. flexneri 2a strain 2457T (1 × 10^5 cfu/ml) (13), and the group C mice were orally infected with V. parahaemolyticus IDH 3525 (1 × 10^9 cfu/ml, O3:K6) (14). All animal experiments were performed according to the National Institute of Cholera and Enteric Diseases (NICED) Animal Ethical Committee guidelines (Approval no. PRO/117/June 2015–June 2018). Pathophysiological changes to body weight, mortality, and intestinal

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Fig. 1. Development of Streptozotocin (STZ) treated hyperglycaemic mice. After low doses of STZ treatment, plasma, blood, and urine were checked in adult mice for 35 days ($n=5$). Body weights were taken for 4 weeks. Each point represents the mean ± SD value for 15 animal grouped in 3 groups ($n=3$, each group) ($^*p<0.05$, $^{**}p<0.005$).

**Salmonella Typhimurium infection**

A) B) C) D) E) F)

**Shigella flexneri 2a infection**

G) H) I)

**Vibrio parahaemolyticus infection**

colonization were monitored. Significant decreases in body weight following infection with all 3 pathogens, as well as increases in mortality and intestinal colonization, were observed in the diabetic mice in comparison to the non-diabetic mice (Fig. 2). Following these results, we hypothesized that hyperglycemia affects the intestinal innate immune system and consequently leads to disease. Hyperglycemia induces apoptosis in the cells...
of the nervous system, which diminishes the intestinal defense mechanisms (15) that are regulated by these cells, including the secretion of anti-microbial peptides, lysozyme, gastric acid, intestinal defensins, and mucus (16). Additionally, hyperglycemia directly inhibits the secretion of gastric acid, a vital first-line defense against intestinal pathogenic bacteria, which increases intestinal colonization (16).

Macrophages, essential immune system components, are involved in 3 fundamental homeostatic activities: host defense, wound healing, and immune regulation. It is generally accepted that phagocytic functions such as chemotaxis, phagocytosis, and respiratory burst are impaired in the polymorphonuclear (PMN) cells and monocytes/macrophages from diabetic individuals compared to those from healthy individuals (17–19). Accordingly, we isolated peritoneal macrophages from both hyperglycemic and control mice, infected these cells with the pathogenic bacteria described previously, and measured the bactericidal activity of the macrophages across 2 different time intervals. After 1 hour we noted that the bacterial load in the control macrophages was higher than in macrophages from diabetic mice; however, after 24 hours, the macrophages from diabetic mice had a higher bacterial load than those from normal mice (Fig. 3). Previously, PMN cells from diabetic subjects suffering from melioidosis reportedly from normal mice (Fig. 3). Previously, PMN cells from diabetic mice had a higher bacterial load than those in macrophages was higher than in macrophages from diabetic individuals. We conducted the present study to investigate the susceptibility of mice with STZ-induced diabetes to invasive enteric bacterial infection. Our results strongly indicate that impairments in the immune systems of diabetic mice make them more susceptible to diarrheagenic bacterial infection. This work adds to our understanding of the mechanisms by which DM leads to the development of pathogenic enteric bacterial infection.

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Conflict of interest None to declare.

REFERENCES