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Research Article

STREPTOZOTOCIN (STZ)-INDUCED DIABETES MELLITUS (DM) PROMOTES ALTERATION OF PERITONEAL MACROPHAGE MORPHOLOGYAND BEHAVIOUR

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ABSTRACT

The macrophages have multiple roles in glucose regulation. The abnormalities of macrophage functions may contribute to the development of systemic inflammation and insulin resistance associated with obesity and diabetes. The aim and objective of this study was to analyze the cytomorphology of rat peritoneal macrophages and their functionality during streptozotocin (STZ) induced condition. In present study two MØ subsets; large peritoneal MØ (LPM) and small peritoneal MØ (SPM) were identified. Increased cell aggregation and increased tendency of macrophage fusion were noticed in rats treated by STZ.The present study clearly indicates that STZ treated diabetes adversely affects peritoneal macrophages. Significant numbers of peritoneal macrophages were found to be pyknotic. Normal macrophages showed different stages of phagocytosis. But the necrotic macrophages in diabetic group were not able to phagocytose the charcoal particles. But it was interesting to notice that few diabetic peritoneal macrophages were completely laden by charcoal particles.Mean phagocytic index was significantly reduced in diabetic group. In present study increased cell aggregation and increased tendency of macrophage fusion may indicate inflammatory reaction which is the central sign of diabetes mellitus. These observations, thus, reveal peritoneal macrophages heterogeneity associated with diabetes mellitus (DM).

Keywords: Peritoneal Macrophages, Diabetes Mellitus, Streptozotocin,Inflammation, Aggregation, Phagocytosis

1. INTRODUCTION

Diabetes mellitus (DM) is a type of metabolic disease characterized by hyperglycemia. The DM exhibits an inflammatory phenotype, which is associated with dysfunction and failure of various organs [1, 2]. The macrophages have multiple roles in glucose regulation and also function in lipid metabolism as well as in the inflammation of adipose tissue, especially in the peritoneal cavity [3, 4].

To date, the most common macrophage sources are bone marrow, spleen, and peritoneal cavity. Compared to bone marrow-derived macrophages (BMDMs) and splenic macrophages (SMs), peritoneal macrophages (PMs) appear to be more mature with higher expression of inducible cytokines and are more stable in their functionality and phenotype [5].The expression of inflammatory mediators such as interleukin (IL)-6 is increased in adipose tissue macrophages in obesity [4]. Monocytes from diabetic subjects exhibit enhanced adherence to the endothelium [6, 7] suggesting an increased ability of tissue infiltration under diabetic conditions. These abnormalities therefore may contribute to the development of systemic inflammation and insulin resistance associated with obesity and diabetes.

Streptozotocin (STZ) has been extensively used to induce diabetes for various diabetes studies. The common mechanism of action of alloxan and streptozotocin includes degradation of pancreatic islet beta-cells by means of apoptosis [8, 9].

The aim and objective of this study was to analyze the cytomorphology of rat peritoneal macrophage and their functionality during STZ induced condition.

2. MATERIAL AND METHODS

2.1. Animals and housing

The laboratory experiments were performed using albino rat as a mammalian model. Normal young adult rats aged 8-10 weeks and weight 80-100 g were housed in polypropylene cages and were acclimatized in laboratory condition for a week with natural light and dark schedules prior to experimentation. The animals were fed standard rodent diet and water was provided *ad libitum*.

2.2. Treatment of animals

Rats were divided into experimental and their respective control groups. Each group consisted of 8 rats. Group I animals were treated with streptozotocin (STZ) to make them diabetic. In the experiment STZ was administered by i.p. injection in doses of 50 mg/kg body weight dissolved in 0.1 M ice-cold citrate buffer (pH 4.5) to overnight-fasted rats [10,11].

2.3. Peritoneal macrophages isolation and staining

Sterile phosphate buffered saline (PBS), pH 7.2 was injected into rat peritoneum and the abdomen was gently massaged and the aspirate was taken for macrophage study [12]. Peritoneal fluid was placed and smeared directly on sterilized glass slides and incubated at 37 °Cin a humid chamber for 3 hours. After incubation the nonadherent cells were removed by washing three times with PBS. The adherent macrophages were fixed by methanol and stained by Giemsa and observed under light microscope.Cell counting was performed by hemocytometer. Activated charcoal particles suspended in normal saline (0·9% NaCl) was injected into rat peritoneum and the aspirate was taken for phagocytosis study.

The phagocytic index was calculated as per the following formula.

$$\text{Phagocytic index} = \frac{\text{Number of cells with phagocytosed charcoal particle}}{\text{Total number of cells counted}} \times 100$$

3. RESULTS & DISCUSSION

3.1. Cytomorphology of macrophages

In the present study, significant changes were observed in the cytomorphology of peritoneal macrophages in the experimental group. Large peritoneal MØ (LPM), and small peritoneal MØ (SPM) were identified. Normal morphology was predominately found in the control group cells, showing a well-defined plasma membrane and intact nucleus. Significant number of treated macrophages showed membrane blebbing, rupture of plasma membrane, loss of viability and become pyknotic (Fig. 1).

3.2. Phagocytosis study

Different stages of phagocytosis of charcoal particles by peritoneal macrophages on glass slides were determined in both control and treated rats (Fig. 2). Control rat macrophages showed different stages of phagocytosis like formation of pseudopodia, attachment of charcoal particle on macrophage surface and tip of pseudopodia (Fig. 2A). But significant numbers of macrophages in diabetic group were not able to phagocytose the charcoal particles. But few macrophages were completely laden by charcoal particles in diabetic group(Fig. 2B).A significant number of peritoneal macrophages containing charcoal particles reduced in number in STZ treated group (Fig. 2C).

3.3. Cell aggregation

Different stages of aggregation in peritoneal macrophages of control rats were noticed (Fig. 3A). Increased cell aggregation (Fig. 3B1) and increased tendency of macrophage fusion was noticed in rats treated by STZ (Fig. 3B2).

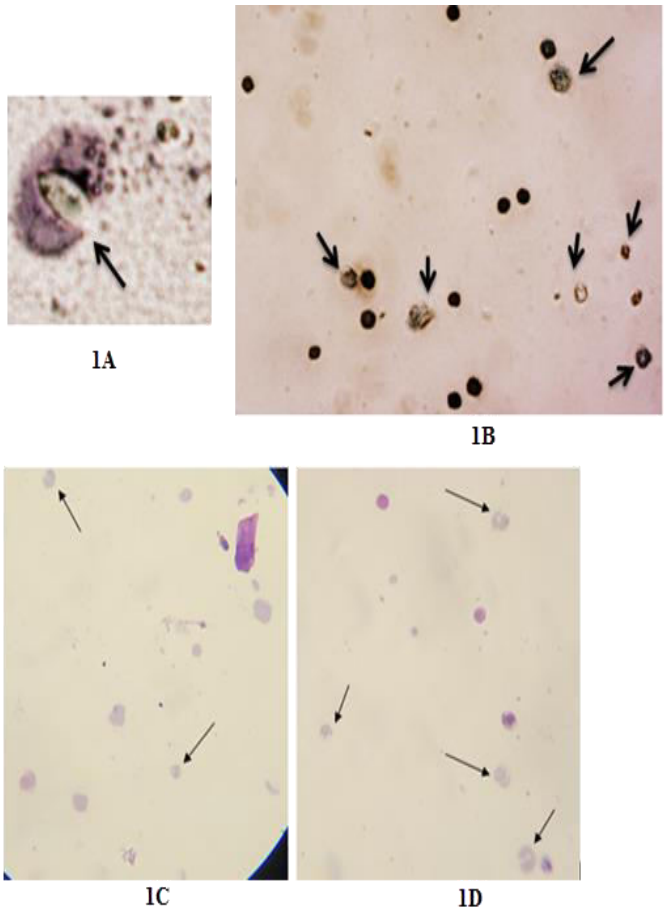

Fig. 1

Fig.1:(A) Giemsa stained STZ treated rat peritoneal macrophages showing rupture of plasma membrane (x400), (B,C,D) Giemsa stained STZ treated diabetic rat peritoneal pyknotic macrophages indicated by arrow (x100)

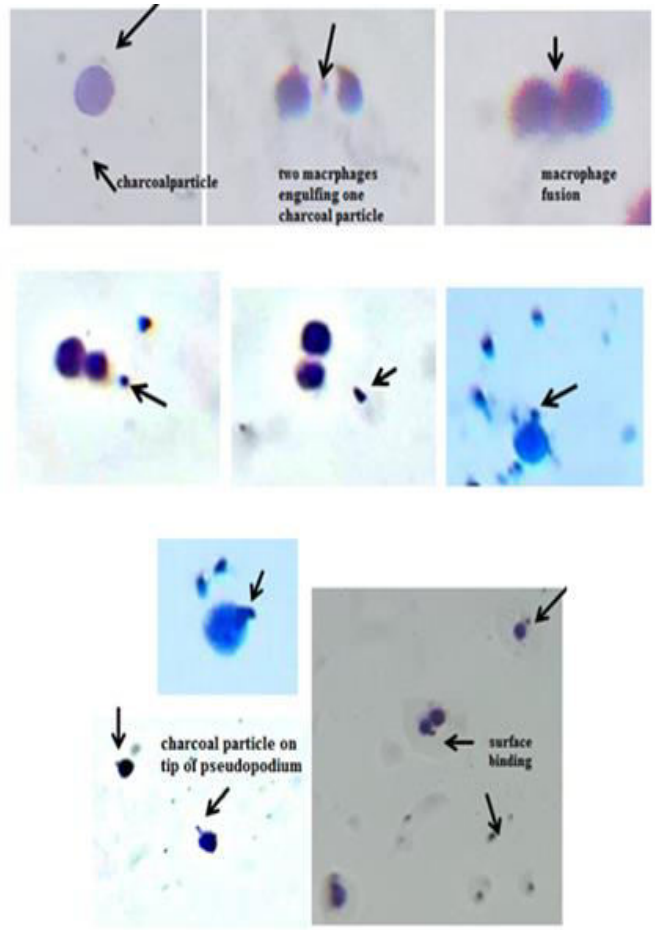

Fig. 2A

Fig. 2A: Different stages of phagocytosis in control rat peritoneal macrophages observed like formation of pseudopodia, attachment of charcoal particle on macrophage surface and tip of pseudopodia. Phagocytosis of charcoal by macrophages was indicated by arrow (x400)

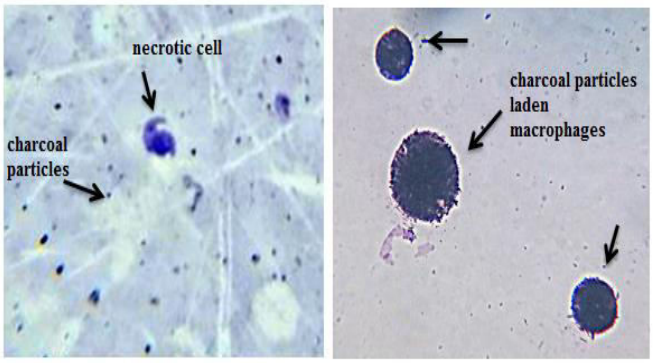

Fig. 2B

Fig. 2B: Necrotic macrophages and charcoal particles laden macrophages in diabetic condition indicated by arrow (x400)

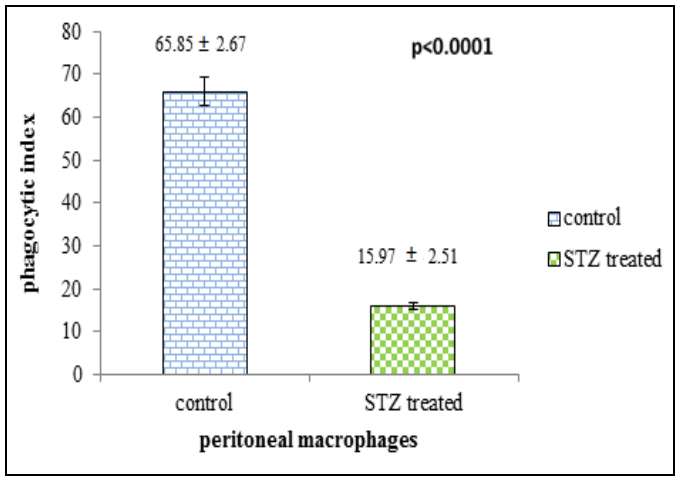

Fig. 2C

Fig. 2C: Mean phagocytic index in normal and diabetic group. Values are expressed as Mean ± SEM.

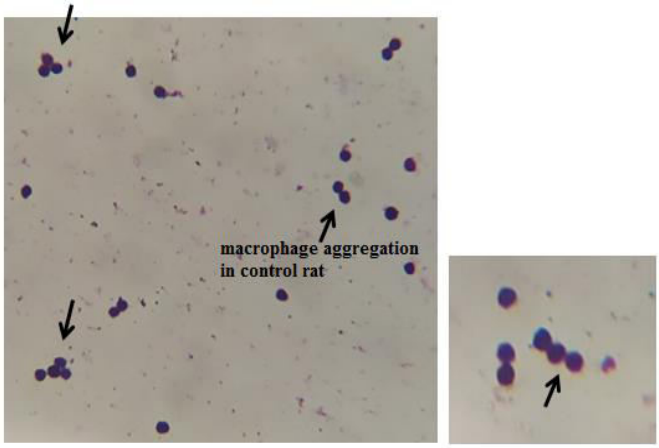

Fig. 3A

Fig. 3A: Different stages of aggregation in control rat peritoneal macrophagesindicated by arrow (x100).

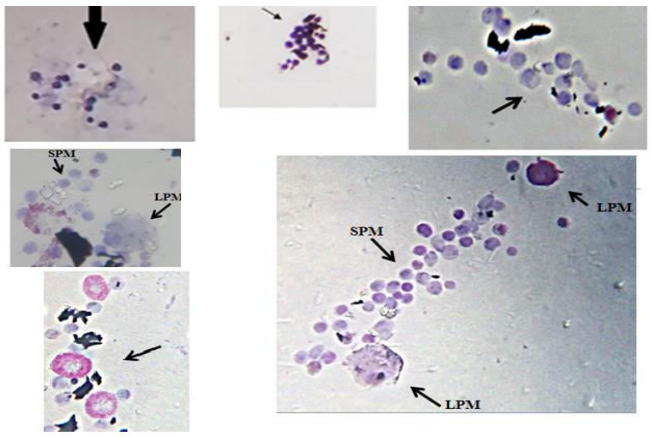

Fig. 3B1

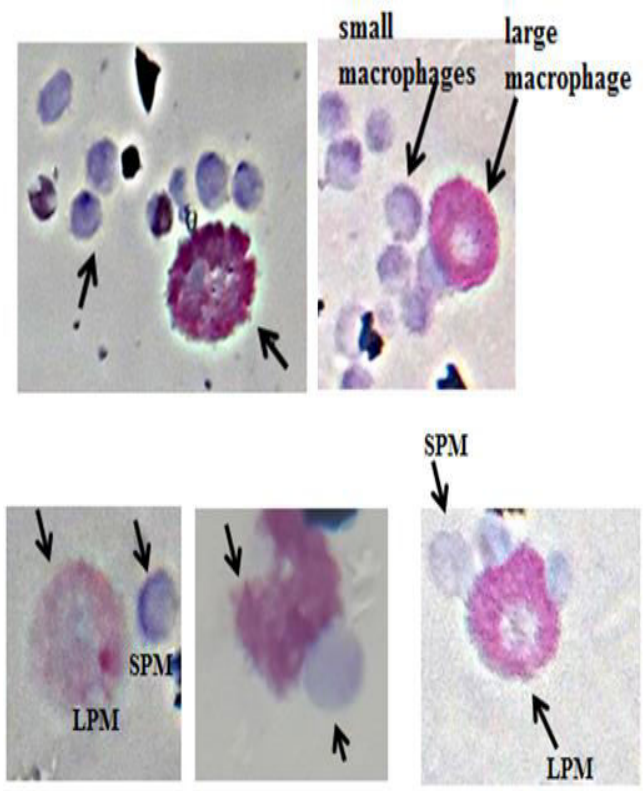

Fig. 3B2

Fig. 3: (B1) Increased cell aggregation and(B2) increased tendency of macrophage fusion was noticed in rats treated by STZ (x400), LPM= Large peritoneal macrophage (MØ), SPM= small peritoneal macrophage (MØ)

Streptozotocin (STZ) has been extensively used to induce diabetes in laboratory animals. STZ has been found to be a better chemical inducer for diabetes than alloxan [8].Guria *et al.*, 2018 exhibited that STZ treated rat developed atrophy of pancreatic islets and damages of liver and decrease of glucose tolerance [13].

The peritoneal cavity (PerC) is a unique compartment within which a variety of immune cells reside, and from which macrophages (MØ) are commonly drawn for functional studies. In present study two MØ subsets that coexist in PerChave been identified. One, provisionally called the large peritoneal MØ (LPM), the second subset, referred to as small peritoneal MØ (SPM), these findings corroborated current studies on MØ heterogeneity [14, 15]. Increased cell aggregation and increased tendency of macrophage fusion was noticed in rats treated by STZ (Fig. 3B1 and B2). It may relate inflammatory reaction. Some studies revealed that monocytes isolated from humans with type 1 diabetes display an inflammatory phenotype and secrete higher levels of proinflammatory cytokines, such as IL-6 and IL-1β, than do monocytes from subjects without diabetes [16-19].

The present study clearly indicates that STZ treated diabetes adversely affects peritoneal macrophages [20]. Significant numbers of peritoneal macrophages were found to be pyknotic. This study corroborated the previous study where peritoneal macrophage death was confirmed by alloxan treatment [21]. Normal macrophages of control group showed different stages of phagocytosis (Fig. 2A). But the necrotic macrophages in diabetic group were not able to phagocytose the charcoal particles. But it was interesting to notice that few diabetic peritoneal macrophages were completely laden by charcoal particles (Fig. 2B). The phagocytic efficiency of control and STZ treated macrophages was examined by calculating phagocytic index. Mean phagocytic index was significantly reduced in diabetic group (Fig. 2C).

Experimental evidence [22] indicated that when macrophages are unable to internalize foreign bodies via phagocytosis they acquire a fusogenic phenotype to form foreign body giant cells (FBGC). The mechanism behind this transformation is unclear and unanswered. But researchers showed that macrophage fusion was induced by interleukin-4 (IL-4) [23].Present study also showed fusogenic behaviour of macrophages (Fig. 3B2).

Espinoza *et al.*, 2012 reported both classically activated macrophages and alternatively activated macrophages populations are central players in diabetes, the first one triggering inflammatory responses which initiates pancreatic β cell death during type 1 diabetes, whereas the second population decreases hyperglycemia, and inflammation in the pancreas, thereby negatively regulate type 1 diabetes [1].Obesity is an important factor in the development of type 2 diabetes; classically activated macrophages are dominant cell population involved in the establishment of the inflammatory profile, insulin resistance, and activation of inflammatory signals during the development and progression of this disease [1].

Some current theory supports that weight gain during diabetes induces local inflammation and chemokine production to promote recruitment of circulating pro-inflammatory monocytes. Recruited monocytes differentiate into an M1 macrophage phenotype. Increased cytokine production from M1 macrophages impairs glucose tolerance [3, 4, 24].In present study increased cell aggregation and increased tendency of macrophage fusion may indicate inflammatory reaction which is the central sign of diabetes mellitus.

4. CONCLUSION

Present observations, thus, reveal peritoneal macrophages heterogeneity associated with diabetes mellitus (DM). But further experiments are in progress to solve this paradox.

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Conflict of interests

There is no conflict of interests regarding the publication of this paper.

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