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Sepsis-induced impairment of neutrophil chemotaxis on a microfluidic chip

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ABSTRACT

This study aimed to design a microfluidic chip to measure neutrophil chemotaxis, which is a convenient assay to assess the severity and prognosis of sepsis, and to study the mechanisms involved in the variation of neutrophil chemotaxis. Neutrophil chemotaxis was investigated in this microfluidic device by measuring the migration speed of neutrophils following the LPS concentration gradient stimulus. Neutrophils of 32 sepsis patients were divided into three groups according to the seriousness of physician-diagnosed sepsis, and 12 healthy individuals served as controls. Statistical significance was set at an alpha value of $P < 0.05$. Neutrophil chemotaxis was significantly decreased following the seriousness of sepsis. By contrast, in septic neutrophils, the expression of TLR2 was significantly increased, whereas the expression of CXCR2 was significantly decreased. Neutrophil chemotaxis in sepsis was significantly reduced as compared to healthy individuals. We speculated that impaired neutrophil chemotaxis in sepsis was probably mediated by the TLR2-CXCR2 pathway.

1. Introduction

Sepsis, as a primary cause of death in the Intensive Care Unit (ICU) is caused by a series of systemic inflammatory responses and ultimately a syndrome that is induced by severe infection that has a great tendency to develop to severe sepsis, and septic shock with a case fatality rate exceeding 50%.

Neutrophils play an important role in the non-specific cell-mediated immune system, and it is at the forefront of resisting microbial pathogens [1–3]. Neutrophils have an innate ability to migrate to the site of infection through complex mechanisms where they play a significant role in reducing the systemic inflammatory response and effectively removing the infectious pathogen [4,5]. Numerous studies have found that neutrophil chemotaxis is closely related to the occurrence and development of sepsis [6–8]. Therefore it is ideal to observe neutrophils chemotaxis in sepsis *in vitro* for septic diagnosis and treatment.

Microfluidic chip technology has the advantages of high flux and integration that could be used to simulate the body's physiological environment to study cell function and monitor cell activity under conditions of real-time control [9–11]. Microfluidic chip technology has established itself as a cell research platform with unlimited potential. Moreover, microfluidic chips can advantageously save on the need for extensive dosing of precious cellular resources and expensive reagents. In general, by using this technology, it is possible to control the micro-environment and dynamically track live cells [12,13].

One of the benefits of microfluidic chips and neutrophil chemotaxis in the capacity to visualize and digitize images that are challenging or impossible using traditional techniques *in vitro*. We aimed to differentially document the differences in neutrophil chemotaxis between healthy individuals and sepsis patients using microfluidic chips. Moreover the neutrophil expression levels of the toll-like pattern recognition receptor TLR2, and the chemokine receptor CXCR2 in septic neutrophils were assayed by flow cytometry to explore the altered mechanisms that are involved in neutrophil chemotaxis.

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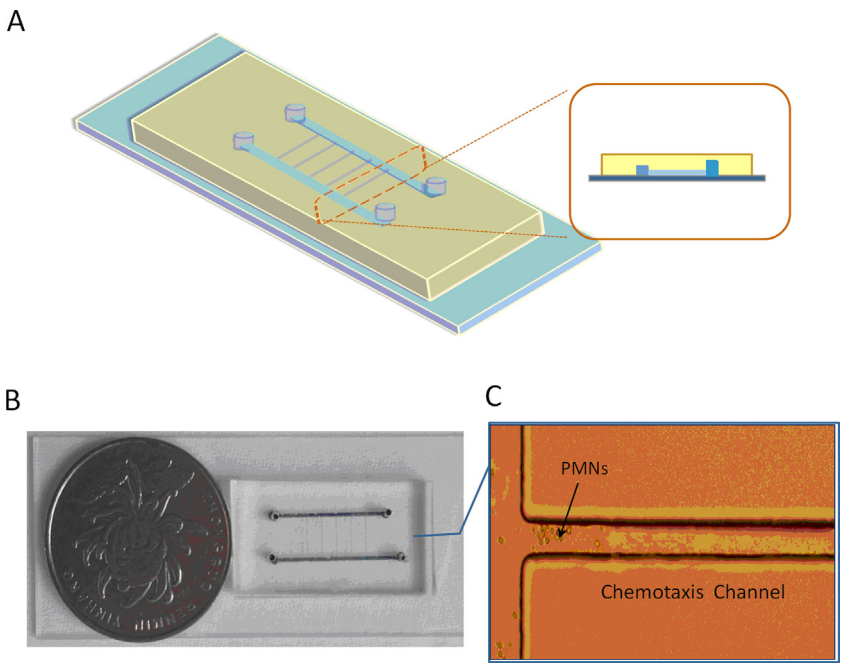

Fig. 1. Overview of the neutrophil chemotaxis device. The unit consisted of a gel channel (100 μm high and 100 μm wide) for collagen loading and gelling, a cell channel (50 μm high and 100 μm wide) for cell introduction and culture, and five migration channels (50 μm high and 50 μm wide) for generating a linear LPS concentration gradient and detecting cellular chemotaxis.

2. Materials and methods

2.1. Microfluidic device design and fabrication

We designed and fabricated a microfluidic device for neutrophil chemotaxis *in vitro*. The specific layout of the microfluidic chip is shown in Fig. 1. Two parallel main channels intersected five parallel smaller channels vertically. One of the main channels was filled with a collagen gel that was spiked with lipopolysaccharide (LPS), while another was set for the introduction of polymorphonuclear neutrophils (PMNs). Due to free diffusion, a concentration gradient of LPS was formed in five smaller channels. PMNs will move in the smaller channel towards gel channels driven by the LPS concentration gradient. Using a collagen gel containing the LPS, the aim was to generate a concentration gradient and to reduce the shear stress acting on the PMNs. The microfluidic chip is fabricated following a previously described procedure [14]. The only difference is that two layers of photoresist (SU8, Microchem, Newton, MA) were patterned sequentially on one silicon wafer to form one photolithography mask containing two different channel heights.

2.2. Preparation of the collagen gel

The chemokine LPS (final concentration: 100 μg/ml; Sigma) was incorporated into 3D type I collagen lattices that were synthesized predominantly from non-pepsinized rat-tail collagen (final concentration: 1 mg/ml; BD Biosciences). The procedure was as follows: collagen was mixed with LPS solution, to which was added 0.1 M NaOH (final concentration: 1.2% v/v), followed by the addition of 10 × PBS. In addition, the final pH of collagen was adjusted to 7. All the above procedures were done over iced water. Once ready, the LPS-collagen solution was introduced into one main channel of the microfluidics device previously sterilized by ultraviolet irradiation. The device was placed at room temperature for 30 min, allowing collagen to polymerize.

2.3. Sample preparation

In the control group, 16 blood samples were derived from 12 healthy volunteers without a history of chronic diseases like hypertension, diabetes and inflammation for nearly a month. In the test group, 32 sepsis patients that were admitted to the emergency department of the Affiliated Zhongshan Hospital of Dalian University from July to December of 2014 were chosen as the experimental test group. According to related diagnostic criteria [15], 32 sepsis patients were further divided into a general sepsis group with 12 cases, a severe sepsis group with 10 cases and a septic shock group with 10 cases. The experiment was carried out within two hours after collection of each blood sample. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Affiliated Zhongshan Hospital of Dalian University.

2.4. Neutrophil isolation

Neutrophils were isolated from fresh whole blood by the modified Ficoll-Dextran method [16]. Briefly, 2 ml of whole blood was diluted in 2 ml of complete blood thinners, and centrifuged at 500*g* for 30 min in a swing-bucket rotor at room temperature. The sample was divided into five layers and the two lower bands containing most of the neutrophils were carefully aspirated and washed with PBS. After treating with erythrocyte lysis buffer, the cells were washed three times. The band of cells that deposited at the bottom of the tube was collected and resuspended in RPMI-1640, supplemented with 10% calf serum to a density of 10^6 cells/ml. Neutrophil viability was more than 95% as assessed by Trypan blue exclusion assay, and neutrophil purity exceeded 95%, as evaluated by Wright-Giemsa staining.

2.5. Neutrophils chemotaxis measurement

This was done by infusing 20 μl of neutrophil suspension into the main channel of a microfluidics chip, excess liquid outside of the