Introduction

Neutrophils are a type of white blood cell. They play an extremely important role in protecting our bodies from infection. They are the rapid responders of the immune system and are the first type of immune cells to be recruited to sites of infection.

Neutrophils are constantly being made in the bone marrow at a rate of approximately 1.2 million per second\(^3\). They are produced from multipotent stem cells which mature into neutrophils before being released into the blood stream. Here they only survive for two to three days\(^3\). Upon detecting chemicals released from invading pathogens, neutrophils slide to the walls of blood vessels which allows them to exit circulation and migrate towards and then destroy invading microorganisms, recruit other immune cells and prevent infection.

To destroy invading microorganisms neutrophils release enzymes and large amounts of damaging molecules known as ROS which target bacteria. They also engulf bacteria in a process known as phagocytosis and produce large structures of DNA called NETs which entangle bacteria.

1. Why grow neutrophils?

Bacterial and fungal infections are commonplace when patient neutrophil numbers drop\(^2\). A culturing methodology to produce neutrophils from stem cells could be used to study diseases in which neutrophil production is disrupted, disorders which are collectively labelled neutropenia.

As this figure clearly shows, chemotherapy causes a drop in neutrophil numbers. This is due to chemotherapy being toxic to the bone marrow and leaves patients prone to life threatening infections. Stem-cell derived neutrophils, if produced on a large scale, could be transfused into patients to prevent infection post chemotherapy.

2. Producing neutrophils in the lab

We start with blood from healthy donors

Stem cells are isolated by magnetic separation

Stem cells are cultured in the lab

Cultured neutrophils 17 days later

As we have shown, we can genetically modify stem cells without affecting their ability to mature into neutrophils in the lab. This will allow us to produce modified neutrophils with novel functions such as destroying bacteria which normally evade the immune system.

3. Lab grown neutrophils function normally

If cultured neutrophils function in a similar way to normal neutrophils, they could be used as a model system to examine neutropenia and as a transfusion product following chemotherapy. We therefore tested the different mechanisms by which neutrophils destroy microorganisms, comparing cultured neutrophils (shown in red) with primary neutrophils isolated from healthy blood (shown in blue).

As shown above, cultured neutrophils release microbe-targeting enzymes when stimulated (left), as well as large amounts of ROS (right).

Cultured neutrophils phagocytose green fluorescently labelled bacteria (left) and produce a comparable number of NETs as primary neutrophils (right). Collectively, these experiments showed us that cultured neutrophils function normally and could be used a novel model system or therapeutically.

4. Working towards a therapeutic dose

Our current culture protocol routinely produces 500 million cultured neutrophils, around 10% of a therapeutic dose. We are currently working to optimise the culturing protocol to promote stem cell expansion to scale up neutrophil production. We will then progress to assess how cultured neutrophils behave in circulation in mice and ultimately healthy volunteers. Only then could cultured neutrophils be transfused into patients with neutropenia.

Meanwhile, we have shown that we can genetically modify stem cells without affecting their ability to mature into neutrophils in the lab. This will allow us to produce modified neutrophils with novel functions such as destroying bacteria which normally evade the immune system.

Summary and next steps

We have developed a methodology to produce cultured neutrophils from donor stem cells. We have shown that cultured neutrophils function normally; they possess anti-microbial activity which is comparable to primary neutrophils isolated from blood. Therefore, this methodology could be used as a source of patient-matched neutrophils which could be transfused into patients with neutropenia. We can also use this model system to examine diseases which cause neutropenia, as well as to potentially produce neutrophils with novel functions.