Measuring Respiratory Burst in Alveolar Macrophages

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Abstract

Alveolar macrophages are white blood cells in the lungs which become activated and undergo a change in cell shape and increase of metabolic activity as they encounter foreign particles. The increase of the oxidative metabolism is a process called "respiratory burst." The main goal of this work is to automate the quantitative measurement of respiratory burst in cultures of alveolar macrophages in order to evaluate the hypothesis that the uptake of particles causes a small respiratory burst that results in the enhancement of the respiratory burst stimulated subsequently by a receptor mediated agaonist. Superoxide radical production is used as a marker for detecting the respiratory burst. When stained with nitroblue tetrazolium, superoxide forms a precipitate which is observable using light microscopy. We use image processing techniques to compute histograms of precipitate levels in images of cell cultures. We also consider different experimental and image acquisition conditions in order to explore the tradeoff between the challenges posed to the image processing and the statistical significance of the measurements.

1. Introduction

Alveolar macrophages are white blood cells in the lungs which function as a first line of defense against infection by foreign organisms. As macrophages encounter foreign particles, they become activated and undergo a change in cell shape and increase of metabolic activity. In particular, the increase of the oxidative metabolism is a process called "respiratory burst" in which the main oxygen species produced are superoxide anion and hydrogen peroxide. The specific hypothesis this work considers is that uptake of particles causes a small respiratory burst that results in enhancement of the respiratory burst stimulated subsequently by a receptor mediated agaonist. This is based on previous observations in which low doses of exogenous hydrogen peroxide enhance subsequent stimulation of the respiratory burst.

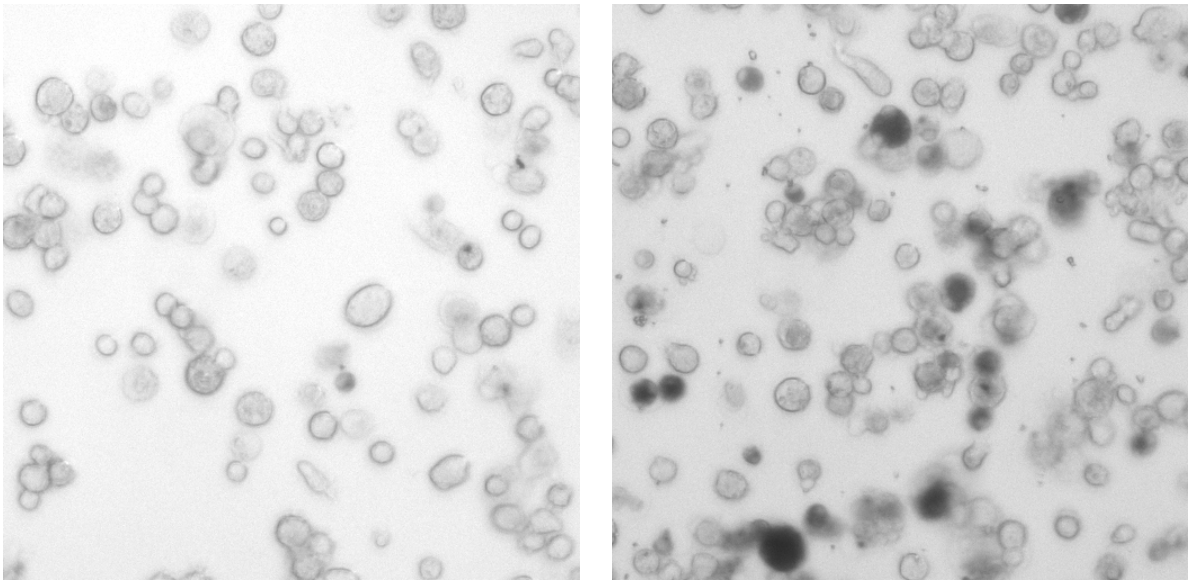
The main goal of this work is to automate the quantitative measurement of respiratory burst in cultures of alveolar macrophages. Superoxide radical production is used as a marker for detecting the respiratory burst. A common method for detecting superoxide is through the reaction of nitroblue tetrazolium (NBT). Staining with NBT results in the formation of a precipitate in active cells (those undergoing respiratory burst) which is observable using light microscopy.

Previous approaches for measuring superoxide production involve manually labelling cells imaged using light microscopy [1]. Manual approaches are problematic in that 1) labelling the cells is time- and labor-intensive; 2) the labelling can be subjective and dependent on the observer; and 3) the limited number of cases that an observer can differentiate results in a coarsely quantized scale of categories that does not accurately reflect the continuous nature of the phenomenon.

Our investigation into automated methods for measuring superoxide production consists of two thrusts. First, we investigate image processing techniques, such as the generalized Hough transform and image segmentation, for extracting the individual cells in order to measure the precipitate. The second thrust explores issues of scale and cell density. In particular, images are acquired at different optical resolutions and for cultures that have been prepared with different cell populations. Images with fewer cells are easier to process but might not result in measurements that are sufficiently statistically significant to evaluate the proposed hypothesis. We examine the tradeoffs with regard to this issue.

2. Our Approach

Images of a control culture and a culture that has been exposed to an agent (silica) that induces respiratory burst are shown in figures 1(a) and 1(b) respectively. The precipitate resulting from superoxide production is indicated by the darker cells in 1(b). Respiratory burst is generally not an on or off process so we need to record varying levels of precipitate. A histogram of cell counts over a range of precipitate levels is thus a useful measurement of respiratory burst. The histogram can be normalized to account for different total cell counts and for different overall lighting conditions.



(a) (b)

Figure 1. Images showing alveolar macrophages. (a) Control culture and (b) culture that has been exposed to an agent (silica) that induces respiratory burst

Extracting the individual cells is a critical step in computing the histograms of precipitate levels. This is a challenge since 1) the cells are frequently grouped and touching; and 2) the shapes and sizes of the cells vary. We consider a number of image processing techniques for extracting the cells. The generalized Hough transform is an extension of the standard Hough transform to general parameterized shapes. Circular regions are detected by transforming edge-enhanced images to a location-radius parameter space. In addition to the Hough transform, we also consider image segmentation techniques. This includes standard approaches, such as the watershed algorithm, as well as more novel approaches, such as graph partitioning active contours [3] and variational segmentation based on anisotropic diffusion [2].

We also consider different experimental and image acquisition conditions. Specifically, cultures are prepared with different cell populations and images are acquired at different optical magnifications. This produces images that vary along two dimensions: 1) the number of cells in an image; and 2) the pixel resolution. It is easier to measure precipitate levels in images with fewer, well separated cells imaged at higher resolutions. However, the resulting histograms are computed for smaller distributions and are not as statistically significant. We explore this tradeoff to find the optimal image processing technique combined with experimental and image acquisition conditions. Manually constructed ground truth measurements are used for evaluation.

References

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