X-ray Microscopy Reveals A Fine Surface Structure of Macrophages

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INTRODUCTION

Since X-ray microscopy is well suited for high resolution and sensitivity for microscopic analysis of biological specimens without the use of conventional sample processing such as fixation, which causes dehydration, and staining required for electron microscopy, it enables analysis of fine fragile structures of hydrated cells. The high-resolution X-ray microscope XM-1 at the Advanced Light Source has been developed and utilized for the analysis of biological specimens (1,2). Macrophages play a key role in host defense against a variety of invading pathogens by phagocytosis, secretion of cytokines, presenting antigens to lymphocytes, and other functions (3). The initial step of the process required for such functions is the recognition of pathogens by the surface of macrophages (4). Therefore, the structural as well as physicochemical property of macrophage surfaces should be critical. To date, most studies on the surface structure of macrophages have been based on electron microscopic techniques, which require specimens to be fixed and stained, meaning dehydrated and non live cells are utilized. In the present study, the surface structure of hydrated macrophages was investigated by X-ray microscopy.

MATERIALS AND METHODS

Macrophages: Peritoneal macrophages were obtained from 8-week-old female BALB/c mice at 4 days after intraperitoneal injection of thioglycollate broth. The macrophage suspensions in 10% fetal calf serum (FCS)-RPMI 1640 medium supplemented with antibiotics (penicillin and streptomycin) were cultured on a window with silicon nitride film for 2 hrs at 37°C in 5 % CO_2. Nonadherent cells were removed by washing of the window with the medium. The resulting macrophage monolayers hydrated with the medium on the window were analyzed by the X-ray microscope. In some experiments, macrophage monolayers were fixed with 2 % glutalaldehyde.

X-ray microscope: The soft X-ray microscope XM-1 at the Advanced Light Source was utilized for this study. Soft X-rays of 2.4 nm wavelength were used. Images were recorded directly on an X-ray sensitive charge-coupled device using x2,400 magnification and stored digitally. Field of each view was about 10 microns. Exposure time was 1 sec.

RESULTS AND DISCUSSION

Macrophage surface structures have a pivotal role in the process of phagocytosis as well as in the communication of macrophages with other immune cells, such as lymphocytes. Therefore, precise structures should be studied for understanding macrophage functions, which are critical in the defense system of the host. The results obtained from the present study clearly showed the presence of fluffy surface structures of macrophages (Fig. 1). These structures observed by X-ray microscopy have not been observed by ordinary

electron microscopy. Since a biological spacemen for electron microscopy requires a series of processes, such as fixing, dehydration, and staining, fragile or hydrated structures of a specimen may be altered by the processing. Therefore, the observed surface structures of macrophages by X-ray microscopy indicate that there are some structures, which are sensitive to the process for electron microscopy of macrophages. The function of the fluffy structures is not known, but it seems likely that macrophages attach firmly on the surface of the supporting body, in this case the silicon nitride film, by the fluffy structure. Thus, the findings in this study indicate that X-ray microscopy may reveal some structures of biological specimens, which are not well recognized by ordinary microscopes, including electron microscopy.

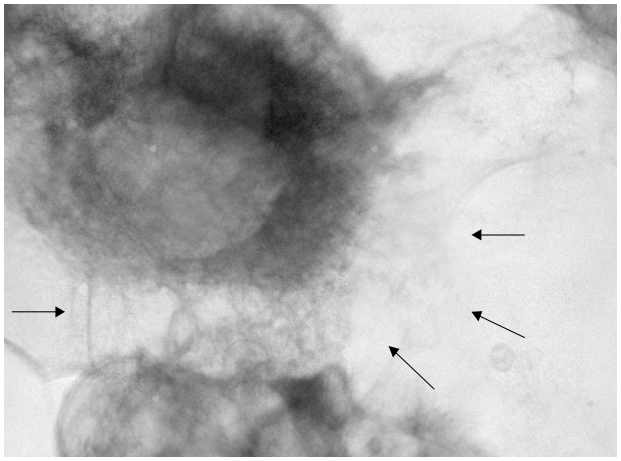

Figure. Tiled X-ray image of murine peritoneal macrophage in the medium. The macrophage was live, not fixed, and not stained. Arrows indicate the fluffy structure of macrophage.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (HL60329).

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