Analysis of oxidative stress in immune cells

Naomi Kamimura, Kiyomi Nishimaki, Yoshiko Iwai

Dept. Biochem. Cell Biol., Grad. Sch. Med., Nippon Med. Sch.

Reactive oxygen species (ROS) is produced in immune cells during immune responses and is necessary for host defense and inflammation. Furthermore, ROS acts as signals for gene expression and is required for T cell proliferation and activation. While low levels of ROS play important roles in cell activation, high levels of ROS induce significant damage to cells. To monitor redox state in living cells we generated transgenic mice expressing a green fluorescent protein (roGFP) whose fluorescence varies with redox state (J Invest Dermatol. 34, 1701-1709, 2014). Since the redox state may change during *in vitro* analysis it should be necessary to fix the cells. Here we evaluate the fixation methods to analyze redox state *in vitro*. We compared aldehyde and organic solvent-based fixation methods. To fix redox state of roGFP protein N-ethylmaleimide which react thiol and modify cysteine residues in protein was used. Splenocytes were isolated from roGFP mice and treated with hydrogen peroxide (oxidized state) or DTT (reduced state) to induce the maximum oxidation and reduction status. Oxidized or reduced cells fixed with various fixation methods were accessed by flow cytometry. Organic solvents lead to a severe loss of fluorescence of roGFP protein. On the other hand, fixation with aldehyde and N-ethylmaleimide was useful to maintain fluorescence and redox status of roGFP. This system should be a powerful and convenient tool for analyzing redox state in various types of immune cells *in vitro*.