

Functional Evaluation of Neutrophils Spheroidized by HRG

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[Background]

Histidine-rich glycoprotein (HRG) is 75 kDa plasma glycoprotein produced from the liver. In previous study, we reported that HRG treatment prevents lethality of sepsis model mice and HRG regulated spherical shape change, passage of microcapillary and production of extracellular ROS on the human neutrophils. Next, we analyzed functional evaluation of neutrophils spheroidized by HRG.

[Method]

Phagocytosis analysis: We quantified the area of fluorescence-labeled bacteria by pHrodo in the neutrophils. Viability analysis: The number of intact neutrophils were counted by the staining with calcein-AM. Determination of extracellular ROS production: After adding isoluminol, HRP and each test reagent to neutrophils, the intensity of luminescence at 30 minutes were measured.

[Result]

Neutrophils treated with HRG showed increased activity of phagocytosis in a dose-dependent manner. HRG also induced high survival rate. When Zymosan A was added to neutrophils, the increased ROS production was observed in the presence of HRG.

[Discussion]

The neutrophils treated with HBSS or HSA are firmly attached to the bottom of the plate and being stimulated with regard to ROS production. In contrast, HRG maintained the spherical shape of neutrophils, phagocytic activity and responsiveness to Zymosan A. These results suggested that HRG may act on neutrophils to suppress excessive adhesion to vascular endothelium under normal condition and induce the functional activation when neutrophils meet bacteria.