【Grant-in-Aid for Specially Promoted Research】
Biological Sciences

Title of Project : Molecular mechanism of the engulfment and degradation of dead cells by macrophages

Shigekazu Nagata
(Kyoto University, Graduate School of Medicine, Professor)

Research Area : Medicine, dentistry, and pharmacy

Keyword : Medical Chemistry

【Purpose and Background of the Research】

Apoptosis plays an important role in maintaining homeostasis of animals. However, due to few experimental systems, its molecular mechanism and physiological roles had been elusive. We showed Fas, a protein of the TNF receptor family, transduces an apoptotic signal. We then in collaboration with Dr. Golstein identified Fas ligand (FasL) that kills the cells, establishing a concept of "death factor". We found that two mouse mutations, *lpr* and *gld* are mutations of Fas and FasL, respectively. FasL works as an effector of CTL and NK cells. Administration of FasL rapidly killed the mice by causing acute hepatitis, suggesting that Fas-mediated apoptosis is responsible for the CTL-induced tissue destruction.

We found that a protease (caspase) cascade is activated in FasL-induced apoptosis, and identified an enzyme (CAD) for the apoptotic DNA fragmentation. We then showed that DNA of apoptotic cells and erythroid precursors is digested by DNase II in macrophages after they are engulfed. *DNase II*$^{-/-}$ mice suffer from lethal anemia in embryos, and polyarthritis in adults due to the IFNβ and TNFα produced from the macrophages. IFNβ gene induction in *DNase II*$^{-/-}$ macrophages is TLR-independent. We found that Eya, a Janus phosphatase, regulates the IFNβ gene expression.

Using the knowledge of the DNA degradation of dead cells, we established an assay for phagocytosis of apoptotic cells, and identified a soluble factor (MFG-E8) and a membrane protein (Tim-4) that stimulate the engulfment. MFG-E8 and Tim-4 bind phosphatidylserine (PS) on dead cells. MFG-E8 is expressed in the tingible-body macrophages in the spleens. Many apoptotic cells are left unengulfed in *MFG-E8*$^{-/-}$ tingible-body macrophages, and the mice develop SLE-type autoimmune diseases, confirming that apoptotic cells must be swiftly cleared to prevent the release of cellular components from dying cells. We also showed that nuclei from erythroid cells are phagocytosed in a PS-dependent manner.

【Research Methods】

In this project, we want to elucidate the molecular mechanism how apoptotic cells are engulfed by macrophages and how dead cells are degraded. In particular, we will study (1) how PS is exposed on the surface of apoptotic cells. Our goal is the identification of the enzyme(s) that mediates the PS exposure. (2) How Tim-4, a PS-receptor, and MFG-E8, a soluble protein that binds to PS, works for the engulfment of apoptotic cells. Or, does Tim-4 associate with other molecules for engulfment of apoptotic cells? What kinds of molecules are involved in engulfment of apoptotic cells? (3) How Eya is involved in the intracellular pathogens- induced IFN gene activation? We will determine the active site of its threonine-phosphatase domain in Eya and its tertiary structure. We are planning to determine the targets of the Eya phosphatase.

【Expected Research Achievements and Scientific Significance】

Dead cell generated during apoptotic cell death are engulfed and degraded by macrophages. We will elucidate the molecular mechanism of engulfment and degradation of dead cells. The outcome of this project will help our understanding of human diseases, in particular autoimmune diseases.

【Publications Relevant to the Project】

1. Okabe, Y., Sano, T. and Nagata, S.: Regulation of the innate immune response by threonine phosphatase of Eyes absent. *Nature* **460**: 520-524. 2009
2. Nagata, S., Hanayama, R. and Kawane, K.: Autoimmunity and the Clearance of Dead Cells. *Cell* **140**, 619-630, 2010

【Term of Project】 FY2010-2014

【Budget Allocation】 318,700 Thousand Yen

【 Homepage Address and Other Contact Information】

http://www2.mfour.med.kyoto-u.ac.jp/~nagata/
snagata@mfour.med.kyoto-u.ac.jp