

Single Molecule Studies of Exocytic Events*

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KEY WORDS: Single molecules, live cell imaging, fluorescence microscopy, exocytosis, immunoglobulins, FcRn

The transport of immunoglobulin G (IgG) within and across cells is a central aspect of humoral immunity. The MHC Class I-related receptor, FcRn, is known to play an important role as an IgG transporter. However, despite extensive biochemical studies of the FcRn-IgG interaction, little is known about the intracellular trafficking pathways taken by FcRn and its IgG ligand. We have taken the approach of using live cell fluorescence imaging at the single molecule level to analyze the exocytosis of IgG following recycling from endothelial cells. The use of dual color acquisition has allowed us to visualize exocytic events that involve both IgG and FcRn.

Our studies show that exocytosis can occur by means of multiple modes that range from complete fusion of the exocytic vesicle with the plasma membrane to a slower release mode ('prolonged release') that only involves partial mixing of membrane contents. The slower release mode is characterized by periodic, stepwise release of IgG molecules. Imaging at the single molecule level shows that even for prolonged release, diffusion of FcRn molecules into the plasma membrane can occur, indicating that FcRn is directly involved in IgG exocytosis. Analyses of single molecule tracks of IgG and FcRn during exocytic release demonstrate that both receptor and ligand can migrate back to the epicenter of the release site within a period of several seconds or less. This may provide a mechanism for FcRn retrieval. In addition, the diffusion rates of the tracked FcRn and IgG molecules are similar. Taken together, our data suggest that IgG can remain receptor bound for at least several seconds post-exocytosis. Our analyses show that single molecule imaging can be used to provide novel insight into how IgG levels are regulated at different body sites.

*This research was supported in part by the National Institutes of Health (grants R01 AI50747 and R01 AI39167).