Abstract

Understanding the pathophysiology of inflammatory disease processes at the cellular and biochemical level is a major challenge in basic research and drug development. The difficulty in recapitulating complex biological processes in vitro, in particular, raises the importance of robust and quantitative in vivo techniques to assess progressive inflammation and therapeutic intervention.

For example, the recruitment of monocytes and macrophages from the blood to sites of tissue infection or trauma is crucial for the induction of acute or chronic inflammation. To assess the biochemical changes that occur in acute inflammation, we used a simple model of acute neurotoxic-driven inflammation, carrageenan-injected paw edema (CPE), generated in mice. A carrageenan solution (1% in PBS) was injected in the right footpaw of each mouse to cause overt swelling and inflammation, and PBS was injected in each left footpad as a non-inflamed control site. We used non-invasive fluorescent imaging (Fluorescence Molecular Tomography™ [FMT]) to assess these changes. Values of the model by measuring edema with a near infrared (NIR) vascular leak and inflammatory cell capillaries and neutrophil elastase activity with NIR protease activable fluorophores. These provided robust and statistically significant signal increases in inflamed versus control paws and correlated well with changes in paw thickness.

In addition, a novel lipophilic cell-labeling agent was developed (VM3211) as a means to label primary macrophages, providing a tool to monitor the recruitment of these cells to the site of inflammation. This NIR agent effectively internalizes into cell membranes to provide a long-lasting signal for in vivo and in vivo studies with imperceptible effects on cell viability or function. To perform these macrophage-tracking studies, thioglycollate-elicited macrophages from donor mice were labeled with VM3211 and then transferred intravenously to normal syngeneic mice. The labeled cells were allowed to accumulate in the liver and lungs for 24h, and then the recipient mice were injected with carrageenan (right footpad) and PBS (left footpad). The active site of inflammation was used as a means to recruit other inflammatory cells, including the VM3211-labeled macrophages injected 24h earlier. Paw swelling and NIR FMT imaging was performed prior to carrageenan injection at 2, 24, 48, 72, and 168 h thereafter. Macrophages selectively trafficked into inflamed paws and accumulated there, showing statistically significant differences in macrophage influx by approximately 40%. As expected, the peak macrophage influx accounted for ~1% of the total injected cells (50,000 cells/mouse), and control experiments characterized the linearity of cell quantification for VM3211-labeled macrophages.

1 VM3211 Characterization

The cell-labeling agent VM3211 is a modified fluorophore designed to intercalate into cellular membranes. This agent has absorption and emission peaks at 676 nm and 696 nm peak in 1x PBS, respectively (λex=676 nm, λem=696 nm).

2 Cell Labling Methods & Validation

A RAW 264.7 cells

B Primary mouse macrophages

3 Paw inflammation Transfer Model

A Carrageenan-induced inflammation & macrophage transfer model

B Paw edema model validation

4 Timecourse of Macrophage Trafficking

A Reconstitution of BALB/c mice with VM3211-labeled macrophages

B Tracking VM3211-labeled macrophages

5 Linearity of VM3211-Labeled Cell Detection

A FMT Images: Direct paw implantation of VM3211-labeled macrophages

B FMT quantification of paw fluorescence

6 Dexamethasone Inhibition of Macrophage Recruitment

A Imaging results

B Paw swelling and fluorescence quantification

C Correlating swelling and macrophage influx

7 References
