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Intravital two-photon imaging of the gastrointestinal tract

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ABSTRACT

Live imaging of the gastrointestinal tract with two-photon microscopy (TPM) has proven to be a useful tool for mucosal immunologists. It provides deep penetration of live tissues with reduced phototoxicity and photobleaching and thus excels in deciphering dynamic immunological processes that require cell motility and last minutes through hours. The few studies that employed this technique in the gut have uncovered new aspects of mucosal immunity. They focused mainly on adaptive immunity in the small intestine and exposed the details of important interactions among several epithelial and hematopoietic cell types. TPM can be employed either on explanted tissue or intravitally, as has been practiced in our lab. Intravital TPM preserves physiological conditions more faithfully, but it is a demanding technique that requires dedicated personnel. To achieve success, the peristaltic motility of the intestine must be curbed, surgical and photonic damage must be minimized, and tissue degradation must be delayed and controlled for. Here we briefly review published studies that employed intravital TPM in the gut, describe our own technique for imaging the intestinal Peyer's patches (PPs) and villi, and present some observations we made using this technique.

1. Introduction

Biologists and physicians have recently come to acknowledge how critically the intestinal microbiome contributes to diseases such as obesity, IBD, and cancer (Blaser, 2014; Dalal and Chang, 2014). This acknowledgement renewed interest in the intestinal mucosal immune system—the main arm that mediates these microbial influences.

Researchers have long been using traditional tools of immunology, such as immunohistology and flow cytometry, to study histological and cellular aspects of mucosal immunity. These methods, however, provide limited insights into the dynamic physical interactions among the cells involved. To address this need, a few laboratories have started using two-photon microscopy (TPM) to dynamically image cellular behavior in live intestinal tissues of mice. Compared to other techniques for optical imaging, TPM provides deeper penetration (100–400 nm, depending mainly on tissue opacity). It also causes less phototoxicity and photobleaching—making it the tool of choice for long-term in vivo optical observation of intact tissues (Denk et al., 1990; Germain et al., 2006). Thus, TPM allows researchers to follow the interactions among the many hematopoietic, epithelial, and stromal cell types in the gut mucosa.

Previously, Xu et al. (2012) have described the technique for TPM of the live small intestine. They covered relevant issues, including tissue integrity and vascular permeability, and highlighted the behavior of dendritic cells and T cells in the intestine. Here we would like to complement their work by reviewing more recent advances in the field, describing our technique for imaging Payer's patches (PPs) and detailing novel observations we made.

In the intestines, TPM can be performed either on tissue explants or intravitally. In the former technique, intestinal sections are cultured in aerated media while being imaged (Chieppa et al., 2006; Coombes et al., 2013). In the latter technique, which we discuss here, an intestinal section is externalized for examination in anesthetized mice (Chennupati

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et al., 2010; Chieppa et al., 2006; Edelblum et al., 2012; Farache et al., 2013; McDole et al., 2012; Xu et al., 2012).

Tissue explants offer the advantage of greater stability and easier pharmacological manipulation, and are the only option for TPM of human tissues. However, since the vascular, lymphatic, and nervous connections of the tissue are severed, explants cannot fully mimic physiological conditions. Worse still, in our hands, such preparations are prone to artifacts introduced by the accelerated deterioration of the delicate mucosal tissue (Brokaw et al., 1998; Farache et al., 2013).

Intravital TPM preserved the physiological milieu more faithfully by keeping the mucosal environment as intact as possible. It should be remembered, though, that the surgical preparation of the tissue and its exposure to air may introduce artifacts that must be considered, and if possible, controlled for. This technique is also more demanding, as it requires surgical skills and control over several factors such as peristalsis, bleeding, blood flow, and core temperature.

In recent years, intravital TPM has been used by several groups to investigate diverse cell populations along the intestinal tract. In 2006, Chieppa et al. (2006) showed, using both explants and intravital imaging of the lamina propria (LP), how $CX_3CR1^{gfp/+}$ macrophages send unusual trans-epithelial extensions, which they termed balloon bodies, to sample *Salmonella typhimurium* from the gut lumen.

A related study, performed in our laboratory by Farache et al., used intravital TPM to examine the interaction of $CD103^+$ DCs with epithelial cells in the ileum. Following bacterial challenge with *S. typhimurium*, $CD103^+$ DCs were recruited from the LP to patrol the intestinal epithelium and sent dendrites to capture bacteria and deliver their antigens to the draining lymph nodes (Farache et al., 2013).

Moving to uptake of soluble antigens, McDole et al. used TPM to examine the interaction of DCs with goblet cells in the epithelium of the small intestine. Following administration of fluorescent dextran or ovalbumin to the gut lumen, these antigens accumulated in goblet cells and were then delivered to $CD11c\text{-}YFP^{hi}$ cells for presentation to T cells (McDole et al., 2012).

Intravital TPM also allowed Chennupati et al. (2010) to compare the motility of γδ T cells found in peripheral lymph nodes (LNs) with TCR $\gamma\delta^+$ intraepithelial lymphocytes (IELs) found in the small intestine. They concluded that unlike the LN population, the IEL population is isolated and self-renewing. They also noted that these cells were relatively sessile. The motility of γδ T cells in the jejunum was examined more closely by Edelblum et al. This group reached a different conclusion, showing that γδ IELs migrate vigorously within the epithelium using a mechanism dependent on occludin (Edelblum et al., 2012). Our own observations confirm that IELs are highly motile.

Unlike the villi of the small intestine, PPs have seldom been studied. So far, these structures have only been imaged using TPM of explanted tissue (Lelouard et al., 2012). This study showed how mononuclear phagocytes, termed LysoDCs, extend their dendrites though a transcellular pore in the M cell to capture particulate antigens.

Here we explain our methodology for imaging the intestinal villi and PPs in the externalized ileum of anesthetized mice. We discuss the advantages, limitations, and pitfalls involved, and demonstrate some findings obtained with this technique.

2. Methods

2.1. Mice

We used mice of the following strains: C57BL/6, CD11c-YFP (Lindquist et al., 2004), $CX_3CR1^{gfp/+}$ (Jung et al., 2000), and actin-CFP mice (Hadjantonakis et al., 2002). Mice were maintained in a specific pathogen-free facility under conditions approved by the institutional animal care and use committee of the Weizmann Institute of Science and were used for experiments at the age of 8–10 weeks.

2.2. Surgery and anesthesia

Before imaging, mice were anesthetized by i.p. injection of 100 mg/kg ketamine + 15 mg/kg xylazine + 2.5 mg/kg acepromazine. Anesthesia was supplemented hourly with half this dose for up to 4 h. Mice were placed on a heated stage, their core temperature was measures and kept at 37 °C, and they were supplied with oxygen through a mask (Fig. 1, left).

A 1 cm incision was made in the abdominal skin and the muscular wall along the midline under semi-sterile conditions. A section of the ileum (the distal part of the intestine) was carefully externalized with tweezers. For PP imaging, the section was chosen to include a centrally located PP. To reveal the mucosal surface, a 15 mm incision was made along the intestine using a cautery. Two additional perpendicular incisions were made to fully expose the luminal surface. To maintain blood supply, the incision site was chosen to avoid the vascular plexus and be as far as possible from the site of imaging (Fig. 1, right). To prevent dehydration, the intestine was placed on a saline-soaked tissue paper (Kimwipe, Kimberley-Clark) and was repeatedly moisturized during imaging. To maintain physiological temperature, thermal putty was used to conduct heat from the stage to the preparation.

To immobilize the tissue, we used a custom-built imaging chamber combined with tissue adhesive. The imaging chamber consisted of a nylon washer fitted with a cover glass bottom and 5 insect pins at the perimeter (Fig. 1, right). The tissue adhesive used was *n*-butyl cyanoacrylate veterinary adhesive (Vetbond, M3) (Chieppa et al., 2006; McDole et al., 2012), which is commonly used in surgeries and causes minimal tissue damage. The outlines of the exposed tissue was marked on the cover glass, a syringe needle was used to apply minute amounts of the adhesive to the bottom of the glass surface along these marks, and the cover glass was replaced on the tissue preparation.

2.3. Intravital two-photon imaging

In vivo imaging was performed using the Ultima® two-photon microscope (Perkin Elmer) fitted with a Ti-sapphire Deep See Mai Tai® pulsed laser (Spectraphysics) with water-immersed 20× 0.95 NA or 40× 0.8 NA objectives (Olympus). The excitation wavelength was set to 890 nm. To create time-lapse sequences, we typically scanned 50 to 150 μm of tissue depth, at 3 μm Z-steps every 20 to 40 s. Images were reconstructed and analyzed using Volocity® software (Perkin Elmer).