Application Note

Stepwise transmigration of T- and B cells through a perivascular channel in high endothelial venules

Introduction

Lymph nodes (LN) are major checkpoints for circulating T lymphocytes to recognize foreign antigens collected from peripheral tissue. Lymph nodes constantly recruit and return lymphocytes to and from the blood to facilitate rapid encounters between antigens and rare antigen-specific lymphocytes. Circulating lymphocytes in the blood enter the lymph nodes via high endothelial venules (HEVs), the wall of which is composed mainly of two cellular components, endothelial cells (ECs) and fibroblastic reticular cells (FRCs). Although efforts to elucidate post–trans-EC migration in HEVs have been made, a clear visualization and molecular mechanism of post–trans-EC migration, including the intra-PVC and trans-FRC migration of T- and B cells in HEVs, is still lacking. Herein, we clearly visualized the multiple steps of post-luminal T- and B-cell migration in popliteal lymph node, including trans-EC migration, intra-PVC crawling, and trans-FRC migration, using intravital confocal microscopy and fluorescent labelling of ECs and FRCs with different colors.

(Published info. : Life Science Alliance Vol. 4, Issue 8, e202101086 (2021))

Instrument

IVM-C (Intravital Confocal Microscopy system)

Methods

For simultaneous imaging of T- and B cells, T- and B cells isolate from spleens of actin-DsRed and actin-GFP mice, respectively, were intravenously injected to a wild-type mouse. To fluorescently visualize ECs and FRCs surrounding HEVs of a popliteal lymph node, we utilized actin-DsRed mouse and an anti–ER-TR7 antibody conjugated with fluorescent dye was injected into a footpad 12 h before imaging. For 3D-time-lapse imaging, 22 sequential z-stacks (170 × 170 μm, 512 × 512 pixels) with a 2-μm axial spacing were acquired at intervals of a minute for 2–3 h after injection of lymphocytes.

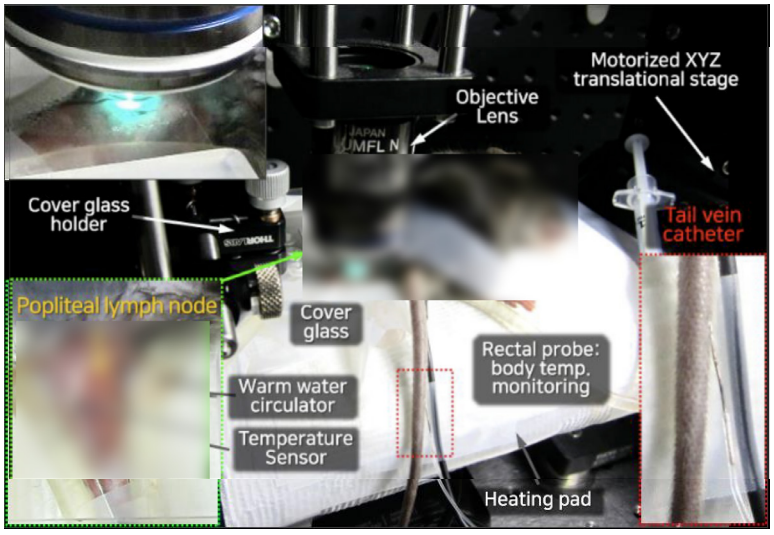

Fig 1. In vivo imaging of popliteal lymph node

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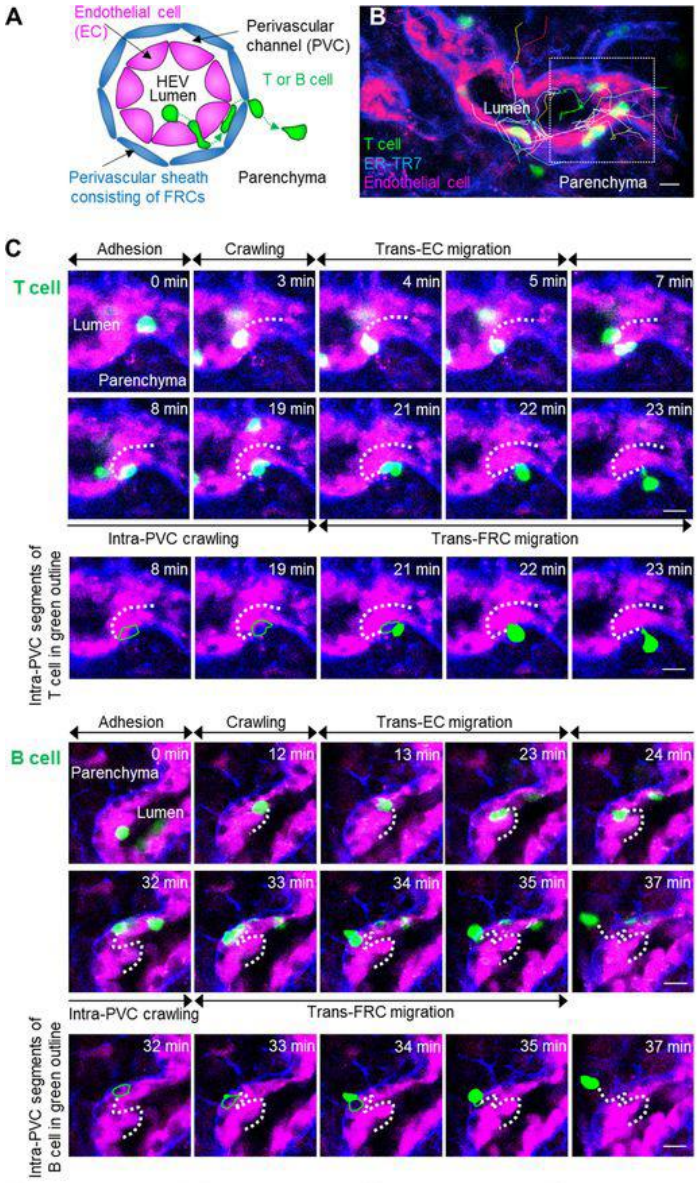

Fig 2. Intravital imaging of T- and B-cell transmigration across high endothelial venules via the perivascular channel consisting of endothelial cells (ECs) and fibroblastic reticular cells (FRCs).

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For in vivo imaging of popliteal lymph node, the popliteal fossa was shaved by a hair clipper and depilatory cream and the left popliteal lymph node was exposed with a scalp incision. Then, place a cover-glass over the imaging area on the mouse popliteal lymph node and warmed saline was topically applied to the exposed tissue to maintain the optimal temperature and humidity of the imaging tissue. During imaging, the body core and local imaging tissue temperature of mouse model were maintained by imaging glass heater and homeothermic control mount stage of IVM system.

Results & Discussion

Herein, we performed intravital imaging to investigate post-luminal T- and B-cell migration in popliteal lymph node, consisting of trans-EC migration, crawling in the perivascular channel (a narrow space between ECs and FRCs) and trans-FRC migration. The post-luminal migration of T cells occurred in a PNAd-dependent manner. Remarkably, we found hot spots for the trans-EC and trans-FRC migration of T- and B cells. Interestingly, T- and B cells preferentially shared trans-FRC migration hot spots but not trans-EC migration hot spots. Furthermore, the trans-FRC T-cell migration was confined to fewer sites than trans-EC T-cell migration, and trans-FRC migration of T- and B cells preferentially occurred at FRCs covered by CD11c+ dendritic cells in HEVs. These results suggest that HEV ECs and FRCs with perivascular DCs delicately regulate T- and B-cell entry into peripheral lymph nodes.

We believe that the popliteal lymph node imaging strategy can be a useful way to directly monitor the post-trans-EC migration dynamics of T and B cells in HEVs, and further investigate underlying molecular and cellular mechanisms involved in cellular dynamics or immune responses in lymph node. IntraVital Microscopy (IVM) system of IVIM technology enables intravital visualization of various cellular and molecular dynamics in your interest organ/tissue of live animal model with sub-cellular level resolution (~0.7㎛) and ultra-fast real-time frame rate (~100fps). IVIM's All-in-one imaging system for longitudinal repetitive in vivo monitoring is fully optimized for various application to any imaging organ, tissue and animal case study of your research.