Video Article

Intravital Microscopy of the Inguinal Lymph Node

Stephanie L. Sellers[1], Geoffrey W. Payne[2]

[1]Interdisciplinary Science, University of Northern British Columbia

[2]Northern Medical Program, University of Northern British Columbia

Correspondence to: Geoffrey W. Payne at payneg@unbc.ca

URL: http://www.jove.com/video/2551
DOI: doi:10.3791/2551

Keywords: Immunology, Issue 50, Intravital vital microscopy, lymph node, arteriole, vasculature, cellular trafficking, immune response

Date Published: 4/4/2011

Citation: Sellers, S.L., Payne, G.W. Intravital Microscopy of the Inguinal Lymph Node. *J. Vis. Exp.* (50), e2551, doi:10.3791/2551 (2011).

Abstract

Lymph nodes (LN's), located throughout the body, are an integral component of the immune system. They serve as a site for induction of adaptive immune response and therefore, the development of effector cells. As such, LNs are key to fighting invading pathogens and maintaining health. The choice of LN to study is dictated by accessibility and the desired model; the inguinal lymph node is well situated and easily supports studies of biologically relevant models of skin and genital mucosal infection.

The inguinal LN, like all LNs, has an extensive microvascular network supplying it with blood. In general, this microvascular network includes the main feed arteriole of the LN that subsequently branches and feeds high endothelial venules (HEVs). HEVs are specialized for facilitating the trafficking of immune cells into the LN during both homeostasis and infection. How HEVs regulate trafficking into the LN under both of these circumstances is an area of intense exploration. The LN feed arteriole, has direct upstream influence on the HEVs and is the main supply of nutrients and cell rich blood into the LN. Furthermore, changes in the feed arteriole are implicated in facilitating induction of adaptive immune response. The LN microvasculature has obvious importance in maintaining an optimal blood supply to the LN and regulating immune cell influx into the LN, which are crucial elements in proper LN function and subsequently immune response.

The ability to study the LN microvasculature *in vivo* is key to elucidating how the immune system and the microvasculature interact and influence one another within the LN. Here, we present a method for *in vivo* imaging of the inguinal lymph node. We focus on imaging of the microvasculature of the LN, paying particular attention to methods that ensure the study of healthy vessels, the ability to maintain imaging of viable vessels over a number of hours, and quantification of vessel magnitude. Methods for perfusion of the microvasculature with vasoactive drugs as well as the potential to trace and quantify cellular traffic are also presented.

Intravital microscopy of the inguinal LN allows direct evaluation of microvascular functionality and real-time interface of the direct interface between immune cells, the LN, and the microcirculation. This technique potential to be combined with many immunological techniques and fluorescent cell labelling as well as manipulated to study vasculature of other LNs.

Video Link

The video component of this article can be found at http://www.jove.com/video/2551/

Protocol

1. Reagent Preparation

1. Reagents to be used throughout the experiment should be prepared before starting the surgical protocol. Note that all solutions were made using sterile technique.
2. Physiological saline solution (PSS) is best prepared in two components: basic salt solution and sodium bicarbonate solution. Prepare both solutions at 20X concentration and store at 4°C. Sodium bicarbonate at 20X is 360.0mM $NaHCO_3$ (84.01g/mol) and basic salt solution at 20X concentration is 2638.0mM NaCl (58.44g/mol), 94.0mM KCl (74.56g/mol), 40.0mM $CaCl_2$•$2H_2O$ (147.02g/mol), and 23.4mM $MgSO_4$•$7H_2O$ (246.48g/mol). Solutions should be prepared in dH_2O and chemicals should be added one at a time and stirred until solution is clear prior to adding the next chemical
3. Prepare 2L of PSS by diluting equal volumes of sodium bicarbonate and basic salt solution and diluting to 1X concentration (for two 2L of PSS dilute 100mL of each sodium bicarbonate and basic salt solution in dH_2O
4. Place 2L volumetric flask containing PSS and PSS in 37°C water bath. Equilibrate the solution with 5% CO_2 / balance nitrogen gas for 1hr prior to start of surgical procedure.
5. Vasoactive chemicals (ex. Phenylephrine, acetylcholine, sodium nitroprusside) are best prepared at 1M concentration in dH2O and stored in 100µl aliquots at -20°C.

2. Animal Preparation

1. Determine the weight of the mouse and administer the initial dose of sodium pentobarbital at 90mg/kg by intraperitoneal injection. Throughout the procedure the mouse is continually monitored for anesthesia plane via toe pinch and careful examination of respiratory rate. Additional injections of sodium pentobarbital should be administered as needed at 20mg/kg to maintain anesthesia plane. The surgical plane of anesthesia is maintained in accordance with federal and institutional regulations as outlined in the animal care protocol approved by the Animal Care and Use Committee (ACUC) of the University of Northern BC.
2. Remove the hair from the abdominal and flank/hip area by shaver. Remove remaining loose hair by alcohol swab and patting the area with tape. This is sufficient as this experimental procedure is terminal.
3. Place the mouse on a clear surgical board in a supine position and attach the mouse to the board by taping each footpad to the board. Body temperature is maintained via an external heat lamp and monitored via rectal thermometer to ensure that hypothermia does not occur as a result of anesthetic.

3. Surgical Procedure

1. During the entire surgical procedure ensure exposed tissue is always kept superfused with equilibrated warmed PSS solution. It is also important to never make contact with the vasculature of interest with surgical instruments or injure the vessels by placing to much force on them by manipulating adipose tissue and connective tissue around them.
2. Place the mouse under the dissecting scope. Make a midline incision along the ventral surface of the abdominal cavity and retract the skin towards the mouse's spinal column.
3. Once the skin is retracted so that the LN is easily visible, pin the skin onto a pedestal of transparent Sylgard 184.
4. Remove the thin layer of connective tissue overlaying the area around the LN
5. Clear the adipose tissue overlaying and surround the lymph node until the microvascular bed is exposed. The main arterial segment lays adjacent to the LN and is commonly overlayed by the venule. The entire surgery takes approximately 30 minutes.

4. Imaging and Vessel Analysis

1. Once the vessel segment of interest is exposed under the dissecting scope, secure the preparation on the intravital microscope. Note that animal remains on the clear surgical board while on the intravital microscope throughout the duration of the experiment. The body temperature is maintained via heat lamp and measured via rectal thermometer as described during the surgical section. The anesthesia plane is also monitored throughout the experiment as described in section 2.1.
2. Set up the superfusion and suction lines. Superfusion of PSS occurs by gravity feed from a reservoir, into the water jacket heated reservoir (the water jacket is circulated and heated by the circulating waterbath) being perfused by 5% CO_2 balanced nitrogen, and down a drip line at a rate of 10ml/min. A suction line should be used to continue pull superfused PSS away from the preparation. Note that prior to the experiment the suction lines and water jacket are thoroughly cleaned and stored after each experiment. This ensures sterility prior to the next experiment.
3. Check the temperature of the superfusing PSS. Adjust the temperature of the circulating water bath and/or the rate of PSS superfusion to achieve a temperature of ˜37°C across the preparation.
4. Allow the vasculature to equilibrate with PSS for at least 60 minutes and quantify the vessel diameter using the video caliper. Typically, vessel diameter should be determined at multiple points (for example, at 40, 50, and 60 minutes) to ensure the vessel has stabilized.
5. Assess the health of the vessel using a smooth muscle specific agonist (ex. Phenylephrine, PE) and an endothelium specific agonist (ex. Acetylcholine, ACh). Concentration of agonist should be adjusted depending on the agonist, but for PE and Ach the vessel should be evaluated at each concentration by cumulative addition (10-5M to 10-9M) to the superfusate. Each agonist application should be separated by a wash phase (typically 30 minutes) using PSS until the vessel returns to resting diameter.
6. Following evaluation of vessel health, subsequent vasoactive drugs, florescent labeling, and cell tracing experiments can be conducted.
7. Following the end of the experiment the animal is given an overdose of sodium pentobarbital. The animal is then monitored to ensure that there is no response to toe pinch and lack of respiratory movement or heart beat. At such time this is followed by cervical dislocation.

5. Representative Results:

Following the equilibration period the preparation should yield a clear image that allows for identification of both the arteriole and venule that supplies the inguinal lymph node. There should be minimal adipose cells surrounding the vessels to allow for walls of the arteriole and venule to be clearly observed for the video calipers to be superimposed to calculate vessel diameter. The integral point of a successful preparation is the arteriole has a rich supply of blood moving through the lumen and that there is a significant degree of vascular tone (i.e. the arteriole has the ability to vasoconstrict and vasodilate to smooth muscle and endothelial specific agonists respectively).

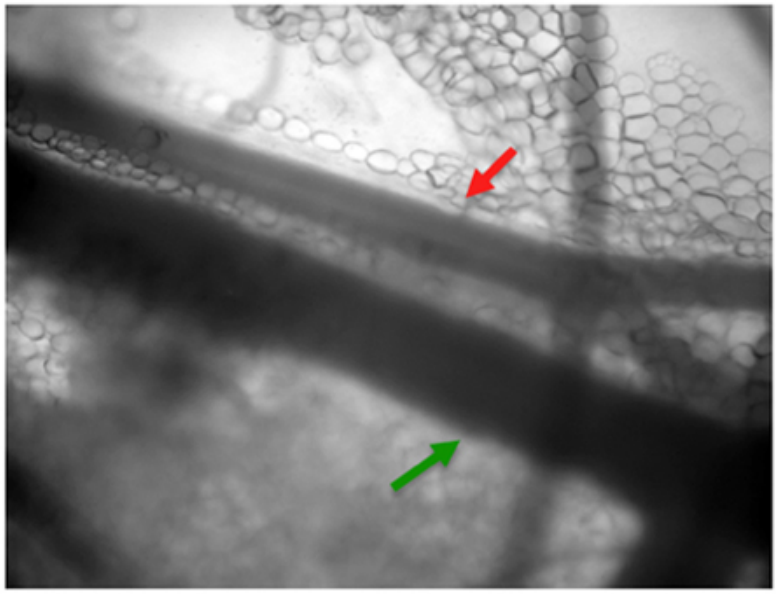

Figure 1. Intravital microscopy image of inguinal lymph node feed arteriole (red arrow) feeding the lymph node equilibrated with physiological saline solution and running along side the main venule (green arrow).

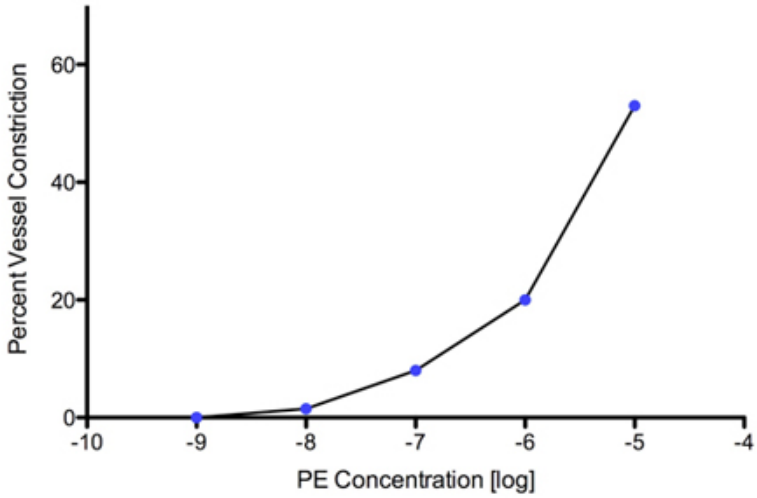

Figure 2. Normal vasoactive response to superfused phenylephrine (PE) by cumulative addition to physiological saline from the concentrations 10^{-9}M to 10^{-5}M. The presence of a robust vasoconstriction is suggestive of normal smooth muscle function present in the arteriole.

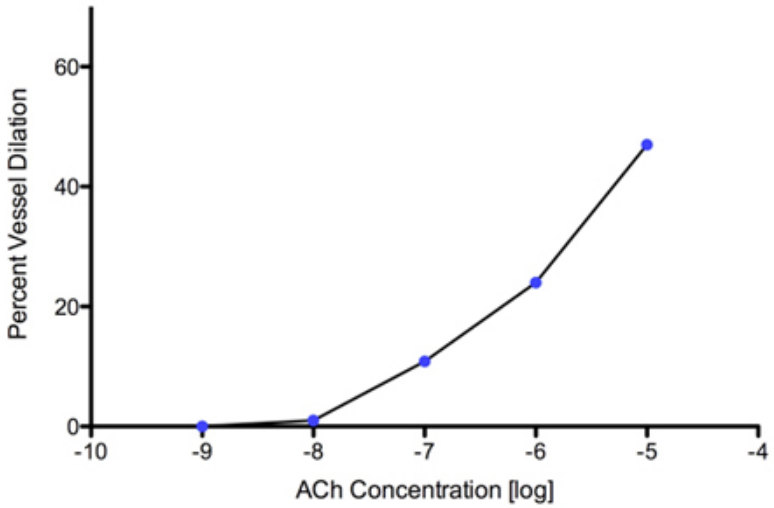

Figure 3. Normal vasoactive response to superfused acetylcholine (ACh) by cumulative addition to physiological saline from concentrations 10^{-9}M to 10^{-5}M. The presence of a robust vasodilation is suggestive of normal endothelial function present in the arteriole.

Discussion

Intravital microscopy of the inguinal lymph node presented here provides the ability to image microvasculature of the lymph node *in-vivo*. Thus, it facilitates a means of direct, real-time observation. Imaging of the LN microvasculature is a unique site that allows one to study the interface between the immune response and the vasculature. Using this preparation, focus can be directed specifically at the immune response, alterations in the vasculature, or at interaction between the two.

As with all experimental approaches, standard intravital microscopy has both advantages and limitations. Standard IVM, such as the preparation described here, can easily be modified, and has been previously demonstrated by the authors to allow epifluorescent microscopy via the introduction of fluorescent tracer dyes or labeled cell populations. Although standard IVM does not give the possibility of three dimensional imaging and tracing such as would be given by two-photon microscopy or angiography, cell tracking is still achieved in two dimensions allowing cell-to-cell and cell-to-vasculature interaction to be observed and quantified and in conjunction with *in-vivo* administration and subsequent staining with fluorescent antibodies can be used to give data on protein/marker expression in real-time.

Notably, alternative imaging techniques require a static environment for images; clamping of the surgical area or the addition of a coverslip on a flat preparation is frequently needed. This limits, if not eliminates, the ability to actively perfuse vascular or other mediators over the preparation to evaluate vascular integrity and physiology etc. or the use of other techniques such as conducted vasodilation experiments within the IVM preparation. Furthermore, standard IVM does not require a completely flat preparation and is not affected by motion, such as that generated by the breathing of the animal. This allows standard IVM to be used in more surgical areas with greater reproducibility. This is exemplified by the inguinal lymph node preparation described here. Given the size and shape of the lymph node, the preparation can not be made flat without injuring the tissue and the location of the node gives rise to significant motion due to animal respiration. Such issues would be difficult to overcome by other methods, but are easily dealt with using the standard IVM preparation described.

In summary, the preparation detailed above can be combined with any number of other biochemical, vascular, and/or immunological techniques such as transfer of subsets of activated or labeled cells, induction of hypoxia, and over-expression of depletion of vascular mediators. However, additional applications are dependent on the health of the initial preparation. Therefore, it is vital to always test the health of the vasculature being imaged and evaluated. Key points to achieving a health preparation are ensuring the preparation is constantly perfused with equilibrated PSS at body temperature and minimizing contact and stress placed on the surgical area during surgery.

Disclosures

No conflicts of interest declared.

Acknowledgements

This work is funded by the Canadian Institutes of Health Research, Canadian Foundation for Innovation, University of British Columbia Centre for Blood Research and University of Northern BC.

References

1. Bearden S.E., Payne G.W., Chisty A., Segal S.S. Arteriolar network architecture and vasomotor function with ageing in mouse gluteus maximus muscle. J Physiol. 561(Pt 2):535-45 (2004).

2. Looft-Wilson R.C., Payne G.W., Segal S.S. Connexin expression and conducted vasodilation along arteriolar endothelium in mouse skeletal muscle. J Appl Physiol. 97(3):1152-8 (2004).
3. Payne G.W., Madri J.A., Sessa W.C., Segal S.S. Histamine inhibits conducted vasodilation through endothelium-derived NO production in arterioles of mouse skeletal muscle. Faseb J. (18): 280-286 (2004).
4. Smeda J.S., Payne G.W. Alterations in autoregulatory and myogenic function in the cerebrovasculature of Dahl salt-sensitive rats. Stroke. 34(6):1484-90 (2003).
5. de With M.C., de Vries A.M., Kroese A.B., van der Heijden E.P., Bleys R.L., Segal S.S., Kon M. Vascular anatomy of the hamster retractor muscle with regard to its microvascular transfer. Eur Surg Res. 42(2):97-105 (2009).
6. Domeier T.L., Segal S.S. Electromechanical and pharmacomechanical signalling pathways for conducted vasodilatation along endothelium of hamster feed arteries. J Physiol. 579(Pt 1):175-86 (2007).