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Review

Lymphatic cannulation models in sheep: Recent advances for immunological and biomedical research

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A B S T R A C T

Lymphatic cannulation models are useful tools for studying the immunobiology of the lymphatic system and the immunopathology of specific tissues in diseases. Sheep cannulations have been used extensively, as models for human physiology, fetal and neonatal development, human diseases, and for studies of ruminant pathobiology. The development of new and improved cannulation techniques in recent years has meant that difficult to access sites, such as mucosal associated tissues, are now more readily available to researchers. This review highlights the new approaches to cannulation and how these, in combination with advanced omics technologies, will direct future research using the sheep model.

1. Introduction

The lymphatic system drains nearly all the tissues in the body, and its analysis can provide direct monitoring of the local immune responses to infection and other disease states. Much has been written about the rationale for analyzing responses in local lymphatics rather than blood (Westermann and Pabst, 1990; Hein and Griebel, 2003), but technically it is much easier to sample blood in small laboratory animals, so blood remains the main window into the study of infection and immunity in many models. In larger animals, such as sheep, it is easier to cannulate lymphatic ducts and directly sample both the cellular and non-cellular components of lymph. Sheep have been used extensively as models for humans in the areas of physiology, responses to infection and inflammatory diseases, and in developmental immunology (Hein and Griebel, 2003). As a model for developmental immunology, the sheep reflects the fetal and neonatal timeline seen in humans. The sheep thymus becomes colonized by haemopoietic stem cell precursors during the first trimester of gestation (Mackay et al., 1986) as does the human thymus (Haynes et al., 1988). Lymphocyte recirculation is also found in the first trimester of sheep (Pearson et al., 1976) and humans (Horst et al., 1990), suggesting that this may be a general feature of fetal immune systems in mammals which have well-developed lymphoid systems at birth. Large animals are regaining popularity as pre-clinical models before human clinical trials, due to limitations that have appeared in translating the outcomes of mouse pre-clinical research into the clinic (Vilahur et al., 2011; Harding et al., 2013; Pinnapureddy et al., 2015), so it is timely to look at recent advances in cannulation techniques.

Large animal joints resemble those of humans in size and load-bearing stresses, making them particularly useful for studies of joint and bone diseases (Ghosh et al., 2012). Studies of osteoarthritis (Cake et al., 2008), inflammatory arthritis (Thorp et al., 1992; Abdalmula et al., 2014), asthma (Bischof et al., 2003) and peanut allergies (Van Gramberg et al., 2015) in sheep are crucial for setting up these models. The sheep model has been used for testing the efficacy of mesenchymal stem cell therapies in cartilage injury and restoration of the extracellular matrix of degenerate intervertebral discs (McCarty et al., 2009; Ghosh et al., 2012; Oehme et al., 2016), rheumatoid arthritis (Abdalmula et al., 2017), coronary endothelial cell dysfunction (Dooley et al., 2015) and myocardial ischemia (Dixon et al., 2009; Hamamoto et al., 2009).

In this review, we want to highlight changes that simplify the surgical approach to lymphatic cannulation in the sheep model, especially those techniques involving cannulation of the mucosal lymphatics, as these have historically been the most challenging. However, it is also important to recognize why monitoring changes in the contents of lymph are so informative. The areas of research that have benefited from lymphatic cannulation models range from the development of the immune system and the recirculating lymphocyte pool before and after birth, to the myriad of responses to infection, both cellular and non-cellular. More recently the focus has shifted to more complex immunofluorescent and molecular analysis. We will also discuss some of

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the advances made in molecular analysis of lymph and highlight research topics that would benefit from further analysis.

2. The lymphatic cannulation models

Lymphatic cannulation models in large animals have been well established and have been useful tools for immunological studies. They have been developed in large animal models such as sheep, cattle and goats (Linzell, 1960; Lascelles and Morris, 1961; Hartmann and Lascelles, 1966), in smaller animals such as dogs (Uhley et al., 1963) and juvenile pigs (Binns et al., 1985; Yen et al., 2015b; Yen and Davies, 2016), and in human volunteers (Bierman et al., 1953; Yawalkar et al., 2000; Olszewski, 2005). The rodent cannulations are usually confined to large ducts, such as the thoracic duct (TD) in rats (Gowans, 1957). Cannulation models in sheep have wide-ranging applications in biomedical studies. Both afferent (or pseudo-afferent) cannulations that access lymph draining from the tissue towards the lymph node (Hein et al., 2004; Schwartz-Cornil et al., 2006; de Veer et al., 2010) and efferent lymph cannulations that access lymph draining away from the lymph node (Lascelles and Morris, 1961; Yen et al., 2006, 2009, 2016) allow long-term monitoring of lymphatic contents, such as cells, antibodies, cytokines and connective tissue components such as glycosaminoglycans and collagens, in unanesthetized animals. While true afferent cannulations are possible, the ducts are very small and in practice pseudo-afferent cannulations are more common. With the latter technique, the lymph nodes are surgically removed at an earlier time-point and the afferent ducts are allowed to naturally re-anastomose with the efferent duct to form a combined pseudo-afferent duct. In addition to the relatively simple cannulations of peripheral lymph nodes there are also procedures for the long-term monitoring of mucosal associated lymph (Fig. 1). These methodologies include the catheterization of the hepatic lymphatic (Lascelles and Morris, 1961), the mammary efferent lymphatic draining the mammary glands (Linzell, 1960; Lascelles and Morris, 1961; Yen et al., 2016), the prefemoral (or sub-iliac) lymphatic (Hall, 1967), the popliteal lymphatic draining the lower hind limb (Hall and Morris, 1962), the efferent duct of the prescapular (or superficial cervical) lymph nodes (Glover and Hall, 1976), intestinal or jejunal lymphatic draining the small intestine (Lascelles and Morris, 1961; Hein et al., 2004), the tracheal trunks (or the cervical ducts) from the lateral retropharyngeal lymph nodes basically draining the head region (Schwartz-Cornil et al., 2006; Yen et al., 2006), the thoracic duct with thoracotomy (Lascelles and Morris, 1961; Staub et al., 1975) and the efferent lymphatic of the caudal mediastinal lymph node with thoracotomy (Staub et al., 1975) or the thoracic duct and the efferent lymphatic of the superficial cervical (or prescapular) lymph node on the left without thoracotomy for harvesting pulmonary lymph (Yen et al., 2009).

Young, Hein and Hay (Young et al., 1997) have described the techniques for sheep lymphatic cannulations of efferent or pseudo-afferent lymph for the prefemoral, popliteal and the superficial cervical (prescapular) lymph nodes, and these can be applied to other subcutaneous lymph nodes (Fig. 1). Methods for cannulating the intestinal trunk, the hepatic trunk, and the thoracic duct in the thorax (Lascelles and Morris, 1961) are also outlined in the article. However, the lymphatic vessels of some mucosal lymph nodes and the thoracic duct can be difficult to access so that the surgery can take longer, leading to more physiological stress to the animal and longer recovery times.

In recent years, several novel methodologies for collecting lymph from these difficult sites, or refinements of the existing methodologies in sheep have been developed. In particular, there are new methodologies for harvesting lymph draining a variety of mucosal sites (Hein et al., 2004; Schwartz-Cornil et al., 2006; Yen et al., 2006, 2009, 2016). These novel approaches have expanded the application of lymphatic cannulation methodologies in research. They also offer the potential for investigating the pathobiology at specific mucosal sites, response to novel vaccine or adjuvant formulations, pharmacokinetics of drugs and response to drugs and for investigating connections between different mucosal locations. These recent lymphatic cannulation methodologies are outlined in Fig. 1 and will be reviewed in detail.

2.1. Harvesting lymph draining oronasal mucosae

Efferent or pseudo-afferent lymph draining the oronasal mucosae on the left and right sides of the cephalic and cranial cervical region can be harvested by cannulating the tracheal trunk of the corresponding side (Schwartz-Cornil et al., 2005, 2006; Yen et al., 2006). Lymph from the four major lymph nodes in the head and cranial neck regions - the parotid, mandibular, medial retropharyngeal and lateral retropharyngeal lymph nodes that is streamed into the tracheal trunk can then be collected (Fig. 1). Of these four lymph nodes, the lateral retropharyngeal lymph node is the key center to which lymph from the other lymph nodes drains and from which the tracheal trunk initiates, though anatomical variations are observed (Tanudimadja, 1973). Apart from the four major lymph nodes described above, small and inconstant lymph nodes such as pterygoid and hyoid lymph nodes may also be found in the head and the cranial cervical region (Tanudimadja, 1973).

Schwartz-Cornil et al. (2005, 2006) reported methodologies for harvesting pseudo-afferent lymph draining the oronasal mucosae. Being able to collect dendritic cells from the oronasal mucosae in live animals expands the potential for studying mucosal immunity. However, difficulties in removing all lymph nodes in the head and neck regions may occur when inconstant lymph nodes are present. Surgical approaches for catheterizing the tracheal trunks to collect efferent lymph are similar to those for harvesting pseudo-afferent lymph. Surgical procedures, including intra-operative images illustrating the insertion of a cannula into the tracheal trunk, have been described previously (Yen et al., 2006), but the general techniques for lymphatic cannulation will also be summarized later in this review.

The tracheal trunk cannulation model has been applied to examining both cellular and antibody responses following nasal vaccines such as an ISCOMATRIX® influenza vaccine delivery (Scheerlinck et al., 2006; Yen et al., 2006), and represents an important tool for studying the effects of vaccine delivery by this route.

2.2. Harvesting pulmonary lymph without thoracotomy

The collection of pulmonary lymph has been difficult in the past, because it has involved opening the chest cavity to access the ducts (Staub et al., 1975; Yen et al., 2009). Our group has developed a cannulation model that uses a less invasive approach for collecting pulmonary lymph from the thoracic duct without thoracotomy - as depicted in Fig. 1 (Yen et al., 2009).

Another major advantage of using a surgical approach without a direct incision in the chest wall is that it reduces the biases in immune assessment that could result from the damage-associated molecular patterns associated with tissue injuries in the pulmonary region, as it is known that both damage-associated molecular patterns and pathogen-associated molecular patterns can trigger innate immunity (Reinhart et al., 2012). In addition, this less invasive technique can include the collection of an “in-built” control lymph (efferent lymph from the superficial cervical lymph nodes) in a single surgical operation. The control lymph enhances the comparisons of lymph samples from the target organ (the lungs) to that of the non-target organ at the same time point.

As depicted in Fig. 1, lymph from the major intra-thoracic lymph nodes, including the caudal mediastinal, tracheobronchial and cranial mediastinal lymph nodes, and draining the lungs and the heart re-enter the circulation via the thoracic duct. The thoracic duct terminates in the external jugular vein near the confluences of the external jugular and subclavian veins on the left. Whether pulmonary lymph enters the cardiovascular system via the thoracic duct or the right lymphatic duct appears to vary both between species and between individuals. This