ELSEVIER

Journal of Immunological Methods 305 (2005) 152–161

JIM
Journal of
Immunological Methods

www.elsevier.com/locate/jim

Research paper

Probing leukocyte traffic in lymph from oro-nasal mucosae by cervical catheterization in a sheep model ☆

Isabelle Schwartz-Cornil [a,*], Mathieu Epardaud [a], Jean-Pierre Albert [b], Christian Bourgeois [b], Franck Gérard [b], Isabelle Raoult [b], Michel Bonneau [b,*]

[a] *Virologie et Immunologie Moléculaires UR892 INRA, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France*
[b] *Centre de Recherche en Imagerie Interventionnelle, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France*

Received 22 February 2005; received in revised form 25 May 2005; accepted 20 June 2005
Available online 15 August 2005

Abstract

Lymph nodes are instructed via the lymph about ongoing events in tissues both during the steady state and under provoked inflammation. In order to probe for tissue-to-node transduction mechanisms, we have developed a novel in vivo technique of pseudo-afferent lymph collection from the oro-nasal mucosae which represent the main portals of entry of micro-organisms and efficient routes for vaccination. After lateral lymph node resection of the head, a network of lymph ducts was reconstructed as checked by lymphography. Subsequent catheterization of the cervical lymph duct allowed the collection of cells that were shown to originate from the oro-nasal mucosae. These cells included dendritic cells, monocytes, granulocytes, memory $CD45RA^{neg}$ $CD2^{pos}$ integrin $\beta7^{lo}$ CD4 T cells, $CD25^{pos}$ CD4, CD8, γ/δ T cells, and B lymphocytes. This approach, which permits lymph collection over several weeks, opens a valuable and unique way to study leukocyte and particulate (micro-organisms, vaccines) trafficking from head tissue to nodes under homeostatic and immuno-stimulatory conditions in a highly physiological setting.

Keywords: Cell traffic; Lymph; Sheep; Oro-nasal mucosae

1. Introduction

Tissue micro-environmental factors play key roles in shaping immunity. For instance, delivery of immunogenic antigens via mucosal surfaces typically induces much higher amounts of IgA and Th2-type cytokines as compared to subcutaneous or intramuscular delivery. Furthermore, mucosal administration of high or repetitive doses of soluble antigens is an

Abbreviations: DC, Dendritic cells; PA, Pseudo-afferent; mAb, Monoclonal antibody; S.D., Standard deviation.

☆ This work was supported by institutional grants from the Institut National de la Recherche Agronomique.

* Corresponding author. Isabelle Schwartz-Cornil is to be contacted at VIM UR892 INRA, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France. Tel.: +33 134652641; fax: +33 134652873. Michel Bonneau, Cr2i-UEPSD INRA, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France. Tel.: +33 134652506.

E-mail addresses: isabelle.schwartz@jouy.inra.fr (I. Schwartz-Cornil), michel.bonneau@jouy.inra.fr (M. Bonneau).

0022-1759/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2005.06.019

efficient way to trigger systemic tolerance (Mowat and Viney, 1997). Distinct types of mucosal surfaces also determine subtle differences in the type of elicited response (Brandtzaeg et al., 1999). Nasal-targeted immunization induces antigen-specific immunity in the respiratory and reproductive tract, whereas gut-targeted immunization promotes the generation of protective immunity in the gastrointestinal-tract tissues (Brandtzaeg et al., 1999; Kiyono and Fukuyama, 2004). Nasal immunization also triggers higher level of IgG2a and IgG2b as compared to gut immunization (Zuercher and Cebra, 2002; Zuercher et al., 2002). Local cues, in addition to cell types per se, probably contribute to tissue influences on immunity, such as alimentary antigens, and both the xenobiotic and microflora that probably modulate the local cytokinic production. During immune responses, specific T and B cell responses are initiated in regional lymph nodes that are anatomically placed in proximity to their respective tissues. The physical connection between tissue and lymph node is made by the afferent lymph duct. Cells and substances from interstitial fluids that migrate from tissue to nodes via these ducts are probably crucial in linking tissue influence and immunity. However, the way in which local tissue conditions are transmitted from the periphery to regional draining lymph node is still an open question. Experimental set-ups based on pseudo-afferent lymph cannulation in cattle, sheep or rats permit the collection of lymph draining from skin (Bujdoso et al., 1989; McKeever et al., 1991), the intestinal region (Liu et al., 1998; Hein et al., 2004) and the liver (Matsuno et al., 1996). In these models, because afferent lymph ducts are too small, lymph nodes are surgically removed weeks before inserting a catheter in the previously efferent duct; this allows collection of so-called pseudo-afferent (PA) lymph cells that would be normally trapped in the nodes. These animal models have suggested that sampling PA lymph can provide information on tissue fragments migrating within lymphoid cells (Huang et al., 2000) and on the modulation of lymph cell traffic by regional influences (Mackay et al., 1992, 1996; Hein et al., 2004; Turnbull et al., 2005).

The mucosae of the nasal and oral area are prototypes of stratified or pseudo-stratified mucosal epithelia. These mucosae placed at the entry of the body are exposed to high concentrations of ingested and inhaled environmental factors, such as innocuous microorganisms, food antigens, allergens, and irritating substances. They are major sites for the entry of numerous viral, parasitic and bacterial pathogens. Oro-nasal mucosae have been efficiently used to generate therapeutic tolerance induction (Zheng et al., 2004) or to vaccinate (Etchart et al., 2001; Kiyono and Fukuyama, 2004), due to their convenient location and low content of degradative enzymes compared to the gut. Nasal vaccination appears to be the best route when attempting to elicit mucosal and systemic immunity in mice (Kiyono and Fukuyama, 2004). However how immunity is induced via the oro-nasal mucosae is poorly understood. Most studies addressing this question have relied on immunophenotyping of local tissue sections at different time points (Eyles et al., 2001; Zuercher et al., 2002). Thus intermediate dynamic events, such as antigen transportation mechanisms to inductive areas and cell recruitment from tissue to lymph have not been evaluated.

In order to provide a physiological model system to study the lymphatic traffic of cells and substances originating from the oro-nasal territories, we developed an original model of lymphatic cannulation in sheep designed to collect lymph from mucosae in the head. Because afferent lymph ducts are too small to be directly catheterized, we proceeded to the complete lateral resection of head lymph nodes 2 months before insertion of a catheter in the collecting cervical duct that does have a sufficient size. The resection step abolished the trapping of afferent lymph cells that do not leave lymph nodes and thus allowed the collection of pseudo-afferent lymph. Sheep are particularly appropriate for establishing such a model system, because of their size and relatively convenient housing conditions for experimental purposes. We were able to show that collected cells originate from the oro-nasal mucosae and include phagocytic cells such as dendritic cells (DC), monocytes and granulocytes, memory $CD4^{pos}$ T cells, $CD25^{pos}$ $CD4^{pos}$ T cells, $CD8^{pos}$ T cells, γ/δ T cells, B lymphocytes. Such cells could play an important role in linking mucosal facial surfaces to inductive lymph nodes. This model is physiologically relevant and a valuable tool to study, in real time, transduction to the node of information generated locally by commensal bacteria, pathogen and vaccines.