REVIEW

Trafficking and retention of protein antigens across systems and immune cell types

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Abstract

In response to infection or vaccination, the immune system initially responds non-specifically to the foreign insult (innate) and then develops a specific response to the foreign antigen (adaptive). The programming of the immune response is shaped by the dispersal and delivery of antigens. The antigen size, innate immune activation and location of the insult all determine how antigens are handled. In this review we outline which specific cell types are required for antigen trafficking, which processes require active compared to passive transport, the ability of specific cell types to retain antigens and the viruses (human immunodeficiency virus, influenza and Sendai virus, vesicular stomatitis virus, vaccinia virus) and pattern recognition receptor activation that can initiate antigen retention. Both where the protein antigen is localized and how long it remains are critically important in shaping protective immune responses. Therefore, understanding antigen trafficking and retention is necessary to understand the type and magnitude of the immune response and essential for the development of novel vaccine and therapeutic targets.

Keywords Lymph node · Spleen · Mucosa · Immunology · Antigen processing · Vaccination · Infection · Antigen archiving · Antigen retention · Antigen persistence · Antigen trafficking · Lymphatic endothelial cell · Dendritic cell

Introduction

Protein antigen trafficking involves the transport of self or foreign proteins (antigens) through the tissue via lymphatics to the lymph node (LN) or through the blood to the spleen, where immune surveillance occurs. Antigen trafficking usually occurs within 1–3 days (Fig. 1a). While there are other types of antigens (e.g., lipids, polysaccharides, nucleic acids) this review will focus primarily on protein antigens. Current understanding of protein antigen trafficking has relied largely on the fluorescent conjugation of soluble protein antigens [1–5], labeled nanoparticles [6] or infrared dye (IR) conjugation of virus-like particles (VLP) [7] in mice.

Antigen location can be determined by immunofluorescence using highly sensitive detection methods or genetically engineering expression of fluorophores within the viral or bacterial genome, e.g., vaccinia virus labeled with mCherry or GFP [4, 8–11]. These tools have facilitated a greater understanding of how antigens are handled in different locations throughout the body and the cell types involved.

Protein transport through organ systems

In this section, we will discuss what is known about where protein antigens traffic upon entering the body, how protein antigens get to the LN and spleen, the mucosa, and new insights into how proteins traffic through the brain. We will also discuss tissue specific cell types involved in acquiring or trafficking protein antigens to shape the immune response.

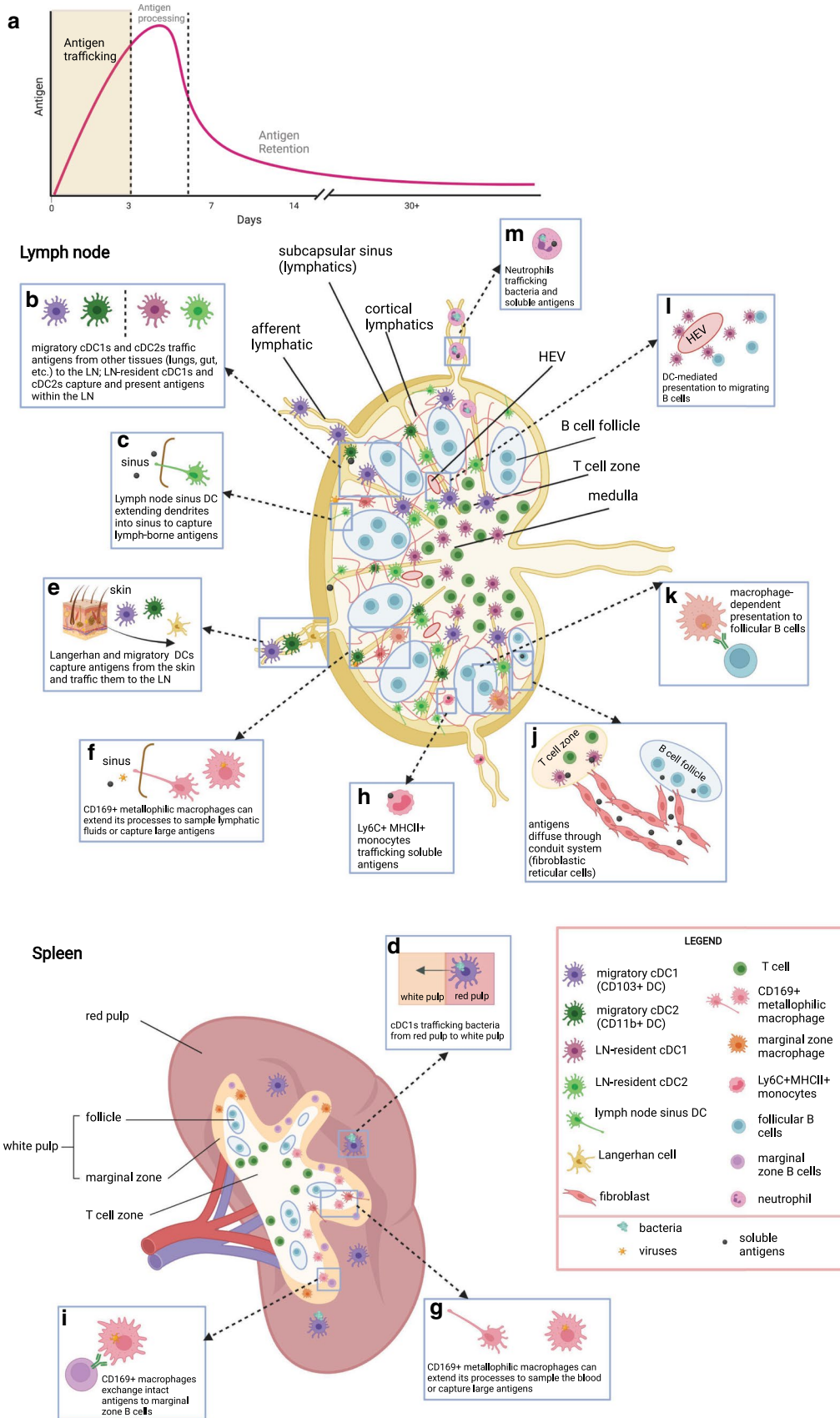### Antigen trafficking within the LN and spleen

The function of the LN is to survey antigens, pathogens and debris from lymphatic fluid that drains from the other tissues of the body (e.g., skin, liver, gut, lung, brain, etc.). The

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a

Antigen (y-axis)

Antigen trafficking

Antigen processing

Antigen Retention

Days

0   3   7   14   30+

Lymph node

subcapsular sinus (lymphatics)

cortical lymphatics

afferent lymphatic

m

Neutrophils trafficking bacteria and soluble antigens

b

migratory cDC1s and cDC2s traffic antigens from other tissues (lungs, gut, etc.) to the LN; LN-resident cDC1s and cDC2s capture and present antigens within the LN

l

HEV

DC-mediated presentation to migrating B cells

HEV

B cell follicle

T cell zone

medulla

c

sinus

Lymph node sinus DC extending dendrites into sinus to capture lymph-borne antigens

e

skin

Langerhan and migratory DCs capture antigens from the skin and traffic them to the LN

k

macrophage-dependent presentation to follicular B cells

f

sinus

CD169+ metallophilic macrophages can extend its processes to sample lymphatic fluids or capture large antigens

h

Ly6C+ MHCII+ monocytes trafficking soluble antigens

j

T cell zone

B cell follicle

antigens diffuse through conduit system (fibroblastic reticular cells)

Spleen

d

white pulp   red pulp

cDC1s trafficking bacteria from red pulp to white pulp

red pulp

follicle

white pulp

marginal zone

T cell zone

LEGEND

migratory cDC1 (CD103+ DC)

migratory cDC2 (CD11b+ DC)

LN-resident cDC1

LN-resident cDC2

lymph node sinus DC

Langerhan cell

fibroblast

T cell

CD169+ metallophilic macrophage

marginal zone macrophage

Ly6C+MHCII+ monocytes

follicular B cells

marginal zone B cells

neutrophil

bacteria

viruses

soluble antigens

i

CD169+ macrophages exchange intact antigens to marginal zone B cells

g

CD169+ metallophilic macrophages can extend its processes to sample the blood or capture large antigens

◀**Fig. 1** Trafficking of antigens to the lymph node and spleen. **a** Timing of antigen trafficking is approximately 1–3 days. **b** Migratory DCs, both cDC1 and cDC2, traffic different antigens from the periphery to the draining LN via the lymphatics. LN-resident cDCs can capture antigens that are passively transported through the lymphatics to the LN and present the antigens to naïve T cells [3, 62–64]. **c** Lymphatic sinus DC (cDC2) extend dendrites into the subcapsular sinus to acquire lymph-borne antigens [73]. **d** In the spleen, cDC1s can traffic bacteria, such as *Listeria monocytogenes*, from the red pulp to the white pulp [76]. **e** Langerhan cells (located in the epidermis) and dermal dendritic cells (DDC) (located in the dermis) traffic different antigens from the skin to draining LN via the lymphatics [82]. XCR1 + DDC (cDC1s) can acquire and traffic viral antigens, such as Herpes simplex virus 1 and vaccinia virus [19, 96–99]. **f** CD169 + metallophilic macrophages (also known as subcapsular sinus macrophages) capture incoming small antigens by extending their processes to sample the lymphatic fluids [11, 114, 115]. They can also capture larger, viral antigens, such as vesicular stomatitis virus [116]. **g** In the spleen, CD169 + metallophilic macrophage can sample the blood and capture large antigen in a similar manner as in the LN [11, 114–116]. **h** Ly6C + MHCII + monocytes can acquire soluble antigens, such as ovalbumin, and migrate to the LN [124]. **i** In the spleen, CD169+ metallophilic macrophages capture blood-borne pathogens and hold the antigens within non-degradative endosomal compartments. These macrophages exchange intact antigens to marginal zone (MZ) B cells [129, 130]. **j** Small, soluble antigens diffuse from the conduit pores and traffic to the B cells follicles or other parts of the LN cortex [17, 132]. **k** CD169 + metallophilic macrophages sequester antigens near the sub-capsular sinus to exchange unprocessed antigen to follicular B cells [11, 134]. **l** DCs concentrated near the high endothelial venule (HEV) mediates presentation of unprocessed antigens to migratory B cells [135]. **m** Neutrophils can transport bacteria (*Staphylococcus aureus)* and fluorescent ova from the site of infection to the lymph nodes via the afferent lymphatics [140–142]

organization and structure of the lymphatic vasculature in the tissues and the LN selectively allow entry to different locations within the LN based on antigen size [6]. The LN is surrounded by the lymphatic sinus which allows for entry of lymph through the afferent lymphatics and is home to large numbers of lymphocytes, macrophages, and antigen-presenting cells like dendritic cells (DCs) (Fig. 1). Most protein antigens that are encountered in the tissue (e.g., skin, lung, gut, brain) traffic via the lymph to the draining LN.

The LN is arranged with B cell follicles on the outer edge near the lymphatic sinus [12]. The cortical lymphatics reside in the space between the B cell follicles and branch into the cortex near the T cell zone [12]. The structural support of the LN is provided by several different types of fibroblasts that lymphocytes crawl along [13, 14]. The conduit system within the LN, comprised of fibroblastic reticular cells, begins at small holes in the subcapsular sinus and acts as a sieve where antigens and particulates smaller than 70kD or 4 nm can passively enter the system and the cortex of the LN (Fig. 1) [15–17]. Soluble protein antigens administered via immunization (30–50 kD) and viruses (20–100 nm) are small enough to passively flow through the lymphatics directly to the LN conduit system, while most bacteria (400–2000 nm) are too large to pass through the lymphatic capillaries in the LN and require active transport via migratory cells [6, 18, 19]. The ensuing immune response is largely affected by which type of antigen-presenting cells have access to the antigen. In the case of small antigens, both LN resident (passive transport) and migratory DCs (active transport) can acquire and present antigens (see DC subtypes in DC section). Follicular B cells, located in the B cell follicles, can also acquire small, soluble antigens that passively diffuse through the subcapsular sinus of the LN. Follicular B cells also encounter larger antigens which are actively transported by DCs and subcapsular sinus macrophages (discussed further in the B cells section).

High endothelial venules (HEV) are vascular structures within the lymph node important for the transport of lymphocytes from the blood. This function and maturation of the HEVs is highly regulated by CD11c + DCs [20]. During fever, the upregulation of ICAM-1 and CCL21 causes increased lymphocyte transport across HEVs [21]. Neutrophils [22] and plasmacytoid DCs (pDCs) [23] actively transit through HEVs during infection or inflammation. As neutrophils (97, 98) and pDCs (56) are capable of trafficking antigen to the LN it is possible for these cells to traffic protein antigens from the blood through the HEVs (see Neutrophil and Dendritic cells sections).

In contrast to the LN, the spleen acquires protein antigens and pathogens that arise in the blood. The spleen consists of the red pulp and the white pulp. The red pulp filters and surveys the blood for antigens, pathogens and dead or dying cells (particularly red blood cells). The white pulp largely acts like a LN within the spleen and is organized in a similar manner with specific B cell zones, T cell zones and a sinus (Fig. 1) [24]. Many different myeloid cell types reside in the red pulp, and can traffic to the white pulp in a similar manner as DCs, monocytes, neutrophils and T cells traffic from the tissue through the lymph and to the LN. As such, many of the cell types involved in trafficking of antigens are similar between the spleen and LN. Therefore, the immune system has two major immune cell "hubs" where antigens traffic to initiate an immune response. One, the LN, that surveys the lymph from the tissue and two, the spleen, that surveys the blood.

Antigen transport through the respiratory and gastrointestinal mucosa

While the major sites of immune cell priming are the LN and spleen, several other tissue sites have specialized systems for antigen detection and trafficking based on their location. Detection of antigens, particularly vaccine antigens, given orally or nasally, has identified unique mechanisms of antigen acquisition and transfer within intestinal or respiratory mucosa. Oral or nasal mucosal protein antigen vaccines

must be formulated to activate innate signaling pathways for antigen to bypass an environment that is permissive or tolerant to normal flora. Within the mucosa, protein antigens passively enter through the epithelium [25] or are captured by microfold (M) cells and then transported across the epithelium for uptake/transfer to epithelial DCs [26]. M cells express clusterin, Siglec-F, Annexin V, and UEA1 in mice [27–30], and so far human M cells are known to express clusterin [29]. M cells are a specialized epithelial cell type found within the epithelial barrier that allow antigen entry into the mucosal lymphoid follicles (Table 1). Antigens entering the airway mucosa are captured by nasopharynx-associated lymphoid tissue (NALT) M cells or respiratory M cells [31]. When proteins or pathogens enter through the epithelium they can encounter the lymphatic structures [32]. Within the small intestinal villi is the lacteal which is a specialized lymphatic capillary with highly permeable "button like" junctions whose main function is to take up chylomicrons for fat absorption as demonstrated in mice [33]. Antigens (vaccine or pathogen derived) can be actively captured by DCs reaching through the epithelium or passively transported through the epithelium [34, 35]. In either case, once through the epithelium, the antigens are carried by intestinal DCs [34] or respiratory DCs through the lymphatic vasculature. Both the lacteals and submucosal lymphatics at the Peyer's patches drain into the mesenteric lymphatics [36]. Passive antigen transport through the mucosal lymphatics has not been directly measured, though active cellular transport is well described [34, 35, 37–39]. In either case the antigens, passively or actively, would be transported into the mesenteric LNs [36]. Within the respiratory tract the antigens, either actively or passively, enter the lymphatics that drain to the mediastinal LNs in mice.

Antigens that pass through the M cells at intestinal or lung mucosal sites are carried by DCs to the lymphoid tissue where T and B cells can be activated for robust production of mucosal IgA. Mucosal vaccination is attractive because the vaccine initiated immune response is at the site where mucosal infections occur, and obviates the need for intramuscular injection. Mucosal vaccines licensed for clinical use in humans and animals are live attenuated or inactivated [40]. These vaccines include nasal (FluMist-Influenza A/B) and oral (Rotarix-rotavirus) delivery routes. Preclinical studies have attempted to target vaccine antigens to M cells to improve the efficiency of the immune response within the mucosal tissues in mice [41]. For example, vaccine antigens conjugated to an agonist (TLR2, 4, 9) [42] or a specific receptor expressed by M cells, such as a (1,2) fucose-containing carbohydrate [43], a Co1 ligand or a C5aR ligand, could target antigens to M cells. Targeting of antigens to M cells would optimize DCs recruitment to the follicle-associated epithelium where the DCs would become activated and promote an adaptive immune response [44, 45]. Other options for enhancing the immunogenicity of mucosal vaccines are virus-like particles and bacterial strains that overexpress antigens on their surface (reviewed in detail elsewhere [46]).

## Protein transport through the brain to the LN

The brain is a highly specialized organ where inflammatory responses must be minimized. New studies have determined that removal of proteins and protein aggregates requires a drainage system within the brain that is somewhat unique to other systems. The glymphatic system, primarily studied in rodents, acts to remove interstitial fluid and protein, protein aggregates and other solutes that mix with cerebral spinal fluid (CSF) in the paravascular space. This transport of solutes is acellular, but does require Aquaporin 4 water channels in the astrocytes that line the brain vasculature to passively direct fluid flow toward the perivenous space [47]. The interstitial fluid collects in the perivenous space and drains out of the brain toward the cervical lymphatic system in rodents and non-human primates [48]. While there is still some debate about how the glymphatic and traditional lymphatic system cooperate it is thought that dural sinuses and meningeal arteries are lined with lymphatics which drain from the glymphatic system to the cervical lymph nodes [49, 50]. Several recent and interesting studies evaluated lymphatic drainage in the brain using fluorescently tagged protein antigen or diffusible solutes (Evan's Blue) in mice. Following injection into the brain parenchyma of mice, these proteins were found to passively drain through the meningeal lymphatics to the deep cervical LNs and later the superficial cervical LNs [49–51]. While vaccination with protein antigens in the brain is not an attractive route of immunization, this mechanism of antigen trafficking is critical for removal of proteins and pathogens from the brain. Other studies have demonstrated the importance of sleep in the removal of protein via the brain glymphatics and lymphatics in mice [52, 53]. Further studies have linked the removal of proteins and protein aggregates to improved outcomes including decreased neural degeneration and dementia in mice and humans [54]. While this field is still relatively new and exciting, understanding how proteins traffic from the brain and through the deep cervical LN for clearance will improve our understanding of brain function, protein trafficking and immune activity. These studies clearly demonstrate how understanding protein and antigen trafficking can reveal novel discoveries that may improve overall health and well-being.

Cell types involved in protein antigen trafficking

Antigen trafficking within the LN, spleen, tissue, mucosa, and brain can occur passively, but ultimately leads to encounters with different immune cell types. Below we

Table 1 Overview of cell types that traffic or retain antigens

Cell Type	Location	Time	Function upon antigen encounter	Mouse Markers	Human Markers
LN-resident cDC1 / migratory cDC1	resident: LN-resident cDC1 located deep in the cortex; migratory: dermal DC (skin), CD103+ DC (lungs and gut)	traffic antigens for approximately 1-3 days	antigen trafficking, antigen processing, antigen presentation; acquire antigens via phagocytosis, macropinocytosis, receptor-mediated endocytosis (e.g. mannose receptor, DEC-205) [213]	CD45+, CD11c^{hi}, XCR1,+ CD11b- CD103+, CD8α+, CLEC9a+ (DNGR), MHC II^{mid} [56]; dermal DC: CD45+, CD11c^{mid} MHC CII^{hi}, XCR1+, CD11b-, Clec9a+ Langerin+/-, CD103+/- [83, 84]	CD141+ (BDAC3), CLEC9a+ (DNGR1) [56]; dermal DC: CD1c+, (BDAC1), CD11c+ [91]
LN-resident cDC2 / migratory cDC2	resident: lymph node sinus DC located near subcapsulary sinus; migratory: dermal DC (skin), CD11b+ (lungs and gut)	traffic antigens for approximately 1-3 days	antigen trafficking, antigen processing, antigen presentation; acquire antigens via phagocytosis, macropinocytosis, receptor-mediated endocytosis (e.g. dectin, DCIR2, DC-SIGN) [213]	CD45+, CD11c^{hi}, Esam+, CD301b+ (Mgl2), MHC II^{mid} [57], CD11b+, XCR1-; dermal DC: CD45+, CD11c^{mid}, MHCII^{hi}, either CD11b+ and SIRPα+ or low levels of CD11b and CX3CR1 [83, 84]	CD1c+ (BDAC1), SIRPα+ [59]; dermal DC: CD1c+ (BDAC1), CD11c+ [91]
Langerhan cell	skin to LN	traffic antigens for approximately 1-3 days	antigen trafficking, antigen presentation; receptor-mediated endocytosis (e.g. Langerin, DEC-205) [213]	CD45+, CD11c+, CD11b+, Langerin+ (CD207), Epcam+, Sirpα+ [83, 84]	CD1a+, Langerin+ (CD207), Birbeck granules+ [85]
CD169+ metallophilic macrophage	subcapsular sinus of LN or near marginal zone of spleen	hold antigens up to 3 days	antigen trafficking, antigen retention; endocytose antigens via FcγRII [129]	CD45+, CD11b+, F4/80+, MHC I+, MHC II+, CD169+ [113, 117, 120]	CD169+ [117]
Ly6C+MHCII+ monocytes	site of infection to LN	traffic antigen for 1-4 days	antigen trafficking	CD45+, CD11b+, MHC II+, Ly6C+ [122-124]	CD14+, CD16+ [122]
marginal zone B cell	marginal zone of spleen	traffic antigens for approximately 1-3 days	antigen trafficking; endocytose antigens via BCR [133]	CD45+, B220+, CD1d+, CD21+ [126, 127]; high IgM, low IgD [127, 128]	CD1c+ (BDCA1), CD19+ [126, 127]; high IgM, low IgD [127, 128]
follicular B cell	B cell follicles of LN and spleen	displayed peptide on MHC for approximately 24 hr	antigen trafficking; internalize B cell receptor-bound antigens [133]	CD45+, B220+, CD19+, MHC II+, CD23+ [126, 127]; low IgM, high IgD [127]	CD19+, HLA-DR+ [126, 127]; low igM, high IgD [127]
neutrophil	site of infection to LN	traffic antigens to LN (unknown time)	antigen trafficking	CD45+, CD11b+, Ly6G+ [137-139]	CD45+, CD11b+, MPO+, elastase+ [137-139]
microfold (M) cell	Peyer's patches	–	antigen exchange; transport proteins (secretary IgA) via dectin-1 and Siglec-5 [214]	CD45-, Siglec-F+, clusterin+, Annexin V+, UEA1+ [27-30]	clusterin+ [29]
follicular dendritic cell (FDC)	B cell follicles of LN	retained antigens for several weeks-years	antigen persistence; antigens held in antigen/antibody complexes [153, 157-161]	CD45-. CR1+ (CD35), CR2+ (CD21) [153, 157-161]	CR1+ (CD35), CR2+ (CD21), CR3+ [162, 163]
lymphatic endothelial cell (LEC)	sinuses of LN (subcapsular, cortical, medullary LECs)	archived antigens for 5+ weeks	antigen archiving/persistence, antigen exchange; antigen uptake via clathrin/caveolin-mediated endocytosis? [5]	CD45-, VEGFR3+, LYVE1+, Prox1+, podoplanin+ [5, 166-173]	CD45-, VEGFR3+, LYVE1+, Prox1+, podoplanin+ [5, 166-173]
Nr4a1+ and CD34+ fibroblasts	conduit of LN	up to 2 weeks	antigen persistence	CD45-, Nr4a1 or CD34 [5]	unknown

Yellow colored boxes indicate cells that traffic antigens. Purple colored boxes indicate cells that retain antigens. The location of each cell type and the timing of antigen trafficking or retention are shown. For the cell types that have known endocytosis receptors, we have listed them in the function column. For example, cDC1s use mannose receptor and DEC-205, cDC2s use dectin, DCIR2 and DC-SIGN [213], and M cells can transport proteins via dectin-1 and Siglec-5 [214]. Respective mouse and human markers for each cell type are also listed

will outline different immune cell types that are involved in actively acquiring and transporting protein antigens to the LN or spleen where they initiate the adaptive immune response. We will outline differences between tissue types.

Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells (APC) that are essential for the initiation and control of adaptive immune responses. DCs specialize in acquiring and processing antigens to present to naïve or memory T cells via major histocompatibility complex (MHC) molecules. DCs also express necessary co-stimulatory molecules that help in the activation of T cells. As sentinel APCs, DCs survey peripheral tissues, such as the skin, to acquire foreign antigens, migrate to the draining LN and present the antigens to T cells to initiate adaptive immune responses. There are several different classifications of DCs, both lymphoid and tissue specific, whose main functions are to acquire, traffic, process, and present antigens. These different classes are outlined below with a focus on their role in antigen acquisition, recognition and transport.

Within each of the tissues are cDC1 and cDC2, often classified based on the tissue type, while pDCs are typically only found in the secondary lymphoid organs and blood. The development of each DC subset is controlled by specific transcription factors. In both mice and humans, the cDC1 development and survival requires interferon regulatory factor 8 (IRF8) and basic leucine zipper ATF-like transcription factor 3 (BatF3) [55]. In mice, cDC1 are further defined by their expression of XCR1, CD103, and CD8α, while in humans, cDC1 are defined by their expression of CD141 (BDAC3) (Table 1) [56]. Expression of CLEC9a (DNGR1) on cDC1 is common between the two species. In contrast, cDC2s exhibit more heterogeneity than cDC1s. They have variable expression of Esam and CD301b (Mgl2) [57]. The cDC2s require IRF4 for their development in mice [58], while it is unclear if human cDC2 also required IRF4. Human cDC2s expressed CD1c (BDAC1) and SIRPα (Table 1). Brown et al. [59] demonstrated in mice that cDC2s can be further classified based on the presence or absence of T-bet. They described T-bet$^+$ cDC2A to have anti-inflammatory properties and express Runx3 and SREBF2 while T-bet$^-$ CDC2B have pro-inflammatory properties and rather express the transcription factor RORγt and the cell surface molecules, CLEC12A and CLEC10A (Table 1). In humans, only the cDC2B subset was found in the blood [59], but the group suggested that the cDCA population may be found in human lymphoid tissues [59]. In mice and humans, pDCs require IRF7, IRF8 and TCF4 for their differentiation and maintenance [55, 60, 61].

Conventional DCs

Conventional dendritic cells (cDC)s can present both peptide and lipid antigens to T cells. cDC1s are important in cross-presentation, which is the process by which exogenous antigens are displayed on MHC class I to be presented to naïve CD8$^+$ T cells (Fig. 1b). In the mouse, lymphoid-resident cDC1s express toll-like receptor (TLR) 3, 4, 9, 11, 12, 13, and STING, while migratory cDC1s express TLR1, 3, 6, 8, 11, 12, and STING [59]. In humans, lymphoid-resident cDC1s express TLR3, 8, 10, and STING. So far, it is only known that human migratory cDC1s express TLR3 [59]. In contrast, cDC2 generally uses the MHC class II pathway to present peptide antigens to CD4$^+$ T cells but can also cross-present soluble vaccine antigens via MHC class I to CD8$^+$ T cells under certain conditions in mice (Fig. 1b) [3, 62–64]. Based on analysis of differential gene expression, Brown et al. demonstrated that cDC2A subset express TLR1, 5, and 7 and cDC2B subset express TLR1, 2, 5, 6, 7, and 8 [59]. DCs can also present microbial lipid antigens via CD1 isoforms (CD1a, CD1b, CD1c). Interestingly, the presentation of lipid antigens via CD1b is not limited to the state of DC maturation. CD1b can present lipid and glycolipid antigens on immature DC and mature DC, while MHC class II is limited to presentation of peptide antigens only on mature DC [65, 66]. CD1b expressing DCs can present to αβ T cells [67, 68] and a recent study demonstrated that CD1b can be recognized by human γδ T cells [69].

Within each cDC subset there are both migratory and spleen/LN-resident populations. cDC migration from the tissue to dLNs requires transport via the lymphatic vasculature where the CCR7 expressing DCs are exposed to gradients of chemokines, specifically CCL19/21 [70–72]. For those antigens that are small enough to passively traffic through the lymphatics from the tissue and enter the subcapsular sinus, specific LN sinus (LS)-DCs (a subset of cDC2s) acquire the lymph-borne antigen (Fig. 1c) [73]. The LS-DCs are proximal to the LN lymphatic vasculature providing the LS-DCs with a unique opportunity to shape the immune response [73]. In contrast, LN-resident DCs (cDC1) located deep within the cortex of the LN present antigens to naïve CD8$^+$ T cells. Thus, cDC1s located near CD8$^+$ T cells often acquire cell-associated antigens and those antigens that require cross-presentation [1]. To aid in the active transport of antigens to the LN from the tissue are migratory DCs, which can localize to specific zones within the LN to prime T cells and appropriately shape the response. Splenic migratory DCs are also important for trafficking antigens acquired in the red pulp to the white pulp and require fibroblastic reticular cells [74, 75]. As an example, migratory cDC1s are thought to traffic *Listeria monocytogenes* from the red pulp to the white pulp in mice [76]. This process is thought to be co-opted by the bacteria to access the T cells within

the white pulp [76] (Fig. 1d). Thus, cDCs express different pattern recognition receptors, present different types of antigens, and localize to specific regions within the tissue and lymphoid organs. Therefore, which cDCs encounter and traffic protein antigens for presentation can dramatically affect the adaptive immune response.

Plasmacytoid DCs

Plasmacytoid DCs (pDCs) are highly inflammatory and have been described to produce nearly one thousand times more type 1 interferon than cDCs [77]. Like cDCs, pDCs express MHC class II molecules and costimulatory molecules. These pDCs can present endogenous antigens via MHC class I and II molecules [78, 79]. Although pDCs can phagocytose soluble antigens, such as ovalbumin or hen egg lysozyme, it is still debatable whether pDCs present exogenous antigens as efficiently as cDCs do, particularly in the presence of cDCs [80, 81]. For example, ovalbumin taken up by pDCs, when inhaled, can be transported from the lungs into draining LNs in mice [80]. Thus, while pDCs are not a major contributor to DC antigen trafficking, they can traffic antigens either in the absence of cDC or under specific circumstances that require them to do so.

Skin DCs

In the skin, cDCs are found primarily in the dermal layer. Skin-derived DCs are important in trafficking antigens from the skin via afferent lymphatics to draining LNs (Fig. 1e) [82]. The DC subsets that reside within skin are Langerhans cells (LC) and dermal dendritic cells (DDCs) which have both unique and redundant functions. LCs share a common ontogeny with macrophages and are found within the epidermis, while DDCs are of the cDC ontogeny and are found within the dermis. Murine LCs are defined by CD11b, Epcam, Sirpα, and Langerin (CD207) [83, 84], while human LCs are characterized CD1a, Langerin (CD207), and Birbeck granules (Table 1) [85]. LCs can take up antigens and migrate to skin-draining LNs for presentation to naïve T cells with the peak of migration occurring after 72 h [86, 87]. LCs are structurally linked to E-cadherin-expressing keratinocytes [88]. To egress the epidermis, E-cadherin expression is decreased and further migration is aided by CXCR4 and EpCAM [89, 90]. Similar to migratory DDCs, activated LCs upregulate CCR7 and follow the CCL19/CCL21 chemokine gradient to the draining LNs. In addition to Langerhans cells, DDCs in the mouse express numerous markers based on their location and function and can be classified as cDC1 or cDC2. The cDC1 DDCs express XCR1, Clec9A, and Langerin (CD207) and can be further delineated based on CD103 expression (+ or −), while the cDC2 DDCs express either CD11b and Sirpα or low levels of CD11b and CX3CR1 (Table 1) [83, 84]. Human DDCs are divided into human CD1a⁺ DDCs and human CD14⁺ DDCs [91]. These two subsets also express CD1c (BDAC1) and CD11c (Table 1). Following infection or intradermal vaccination, DDCs can phagocytose pathogenic material and antigens as well as upregulate CCR7 and MHC II to promote migration through the lymphatics and antigen presentation to naïve T cells within the draining LN [70, 92, 93]. The release of lipid mediators, cysteinyl leukotrienes, prostaglandin E_2 (PGE2) and expression of PD-L1 during inflammation aid in CCR7 recognition of CCL19/21, matrix metalloproteinase 9 (MMP9) expression and downstream signaling events [18, 94, 95]. When trafficking from the skin, dermal DCs are of critical importance. In bacterial skin infections, DDC migration to the LN is required for antigen presentation in the LN as most bacteria (400–2000 nm) are too large to pass through the lymphatic capillaries [6]. Further, XCR1⁺ dermal DCs, of the cDC1 lineage, were recently shown to be exceptionally important for cross-presentation of viral antigens from Herpes Simplex Virus-1 and Vaccinia Virus (VV) that infect skin cells, such as keratinocytes or dermal DCs [19, 96–99]. Thus, skin DCs are critically important for trafficking of antigens from the skin to the LN and mounting the appropriate immune response to each specific pathogen.

Pulmonary DCs

In the lungs, pulmonary cDCs traffic inhaled antigens to lung-draining LNs. Pathogen-derived or vaccine antigens are acquired by cDCs which then transport and present antigens in the lung-draining LNs. Within the lung tissue, migratory cDC1 have high expression of CD103 and low expression of CD11b (termed CD103⁺ DC). Conversely, migratory cDC2 express low levels of CD103 and high levels of CD11b (termed CD11b⁺ DC) [100]. Both cDC populations are represented at relatively similar frequency in the lungs. While CD103⁺ DCs are better at trafficking apoptotic cells, both are capable of trafficking viral antigens to draining LNs [101]. Trafficking of antigens by cDCs occurs as a result of chemokine receptor expression where decreased CCR6 expression and increased CCR7 promotes migration. The interaction of CCR7 with CCL19 and CCL21 ligands are similar in the lung and the skin [102]. Once the DCs arrive in the LNs, CD103⁺ DC typically cross-present the antigenic peptide to CD8⁺ T cells while the CD11b⁺ DC presents the antigenic peptide to CD4⁺ T cells. Surprisingly, in the case of soluble antigens, both cDC1 and cDC2 subsets can present antigens via MHC class I or MHC class II [101]. Therefore, pulmonary DCs have specific functions within the lung that aid in tailoring T cell responses to protect the host from respiratory infection.

Intestinal DCs

DCs that occupy the gut mucosa are found within the mesentery, LNs [103], Peyer's patches [104], the colon [103], and intestinal lamina propria [34]. Similar to other organs, DCs found within the gut tissue are comprised of cDC1 (CD103$^+$CD11b$^-$) and cDC2 (CD103$^-$CD11b$^+$). As foreign antigens pass through the intestinal epithelium they are captured within Peyer's patches by microfold (M) cells. These M cells are uniquely suited to facilitate antigen transfer to mucosal DCs [25]. In addition to the transfer of antigens from M cells to cDCs, subepithelial dome resident DCs that express high levels of lysozyme extend dendrites across the M cell-specific transcellular pores to sample luminal antigens [105] in a similar manner as cDC2s at the lymphatic sinus (LS-DC) of the LN [73]. Small intestine goblet cells also can capture small antigens and IgA complexes for transport to DCs [106, 107]. In addition to the commonly described DC subsets, gut cDC2s can differentiate into CD103$^+$CD11b$^+$ DCs in the presence of TGFβ to promote Th17 and T regulatory CD4 T cell subset generation within the gut [108]. Upon encounter with antigens, the CD103$^+$CD11b$^+$ DC subset can induce Th17 development by contributing to IL-23 and IL-6 production [109, 110]. Additionally, migratory intestinal cDC1s found within the mesenteric lymph can acquire intestinal antigens and lead to cross-presentation to CD8$^+$ T cells [111]. These migratory DCs can traffic either through the lacteal or directly through the Peyer's patches. The intestinal DCs therefore have unique features that are important for programming the immune response within the specific environment of the intestine.

Macrophages

Macrophages are derived from the same myeloid progenitors as DCs [112]. Macrophages express MHC class I and II, co-stimulatory molecules and can promote pro- or anti-inflammatory immune responses [113] (Table 1). Unlike DCs, macrophages are essential for removing apoptotic and necrotic cell debris at the site of inflammation. Of particular interest in antigen trafficking is the subcapsular sinus macrophage or CD169$^+$ metallophilic macrophage (Table 1). These macrophages are strategically located near the subcapsular sinus of the LN or near the blood vasculature to capture incoming protein antigens and viruses (Fig. 1f, g). These CD169$^+$ macrophages extend cellular processes to sample the afferent lymphatic fluids and blood for protein and viral antigens (Fig. 1f, g) [11, 114, 115]. The CD169$^+$ macrophages can also capture large antigens, including vesicular stomatitis virus (VSV) antigens (Fig. 1f, g) [116]. These subcapsular macrophages have limited degradative properties, which allows them to hold antigens for up to

three days [117]. The duration of antigen allows for migrating B cells to arrive at the subcapsular sinus, where macrophages present the antigen to cognate B cells and initiate the humoral response [11]. The duration of antigen requires the expression of CD169, a sulfated glycoprotein [117] (Table 1). Furthermore, CD169$^+$ macrophages in the LN can capture and present lipid antigens to iNKT cells, which is critical in the activation and rapid secretion of IL-4 and IFNγ by iNKT cells [118]. The expression of CD1d on the CD169$^+$ macrophages allows iNKT to come into close proximity with these macrophages for antigen presentation.

Additional studies have illustrated how macrophages can both present antigens and exchange antigens with other cell types, such as DCs [119], to shape the immune response in the spleen. For example, like in the LN, splenic CD169+ macrophages can capture blood-borne pathogens and interact with CD8α$^+$ DC to cross-prime CD8$^+$ T cells (79). CD169 on the macrophages was critical for antigen transfer to the CD8α$^+$ DC (79). In addition, red pulp macrophages, expressing CD11c^{int} and F4/80^{hi}, acquired antigen via the mannose receptor to cross-present AdLGO adenovirus-expressing ovalbumin which led to control of early viral infection [120] (Table 1). In this study, *SpiC*$^{-/-}$ mice (SpiC is required for the development of red pulp macrophages) had a higher viral burden at an earlier timepoint compared to *Batf3*$^{-/-}$ mice (which lack cross-presenting cDC1s) and viral clearance was achieved at 10 days [120]. These findings demonstrate that red pulp macrophages are important during early viral infection by presenting viral antigens to CD8$^+$ effector T cells, while cDC1s help in later viral clearance. These studies demonstrate the importance of macrophages in both antigen exchange and antigen presentation which can shape the immune response.

Monocytes

Monocytes can participate in antigen surveillance and acquire antigens prior to migrating to the LNs during steady state [121]. Monocytes in mice are Ly6C$^+$MHCII$^+$ and suggested to be the CD14$^+$/CD16$^-$ subset in humans (Table 1) [122–124]. Immature monocytes, in addition to differentiated monocytes (monocyte-derived DCs or macrophages), can transport fluorescent ovalbumin at steady state or in an inflammatory state [124] (Fig. 1h). While Ly6C$^+$MHCII$^+$ monocyte populations are abundant at steady state, and increase dramatically during inflammation, other monocyte populations do differentiate into macrophages. Presentation of antigens by monocytes typically occurs via differentiation into monocyte-derived DCs [121]. The monocyte-derived DCs are thought to originate from monocytes, be similar to cDC2s in function, and expand during inflammation [121]. Thus, while monocytes participate in antigen trafficking

many of their presentation capabilities require maturation into a type of inflammatory DC.

B cells

B cells are of particular importance as they encounter antigens within specific regions of the LN and spleen. There are three types of B cells, transitional B cells, marginal zone (MZ) B cells, and follicular B cells found in the lymphoid organs. Transitional B cells are those B cells that have come from the bone marrow and have yet to fully differentiate. As such they will not be described in detail within this section. An additional B cell subset, B1 B cells, can also be found within the lymphoid tissue but largely comprise the B cell populations of the pleural and peritoneal cavities [125]. These B1 cells can be delineated into B1a and B1b and can produce antibodies, primarily IgM, to different types of antigens (reviewed in [125]) and have a minor role in antigen trafficking. Each of these B cell types has unique features that contribute to antigen handling, recognition and antibody responses via class-switch recombination. Below we will further discuss the major B cell types involved in protein antigen handling and responses in the lymphoid organs.

Marginal zone (MZ) B cells

MZ B cells, along with DCs, macrophages, and granulocytes, are positioned within the marginal zone of the spleen where they readily encounter blood-borne pathogens. The marginal zone is located where large amounts of blood enter the MZ from the circulation (Fig. 1). Interestingly, MZ B cells in humans are freely circulating and can be found in other tissues besides the spleen. In humans, MZ B cells have high expression of CD1c (BDCA1) and CD19 while in mouse, MZ B cells express B220, CD21 and CD1d (Table 1) [126, 127]. The MZ B cells in humans and mice express higher levels of IgM and lower levels of IgD (Table 1) [127, 128]. Blood-borne pathogens or small soluble antigens that enter the marginal zone are captured by CD169$^+$ metallophilic macrophages or MZ macrophages and endocytosed via FcγRIIB, allowing the antigens to be held within non-degradative endosomal compartments [129]. Upon encounter with MZ B cells the CD169$^+$ macrophages can recycle the intact antigens to the surface for recognition by the B cell receptor (Fig. 1i) [129, 130]. MZ B cells can quickly produce IgM antibodies in response to particulate bacterial antigens participating in very early defense against these pathogens [130]. Like other B cells, the MZ B cells can perform class-switch recombination to produce IgA or IgG, MZ B cells, however, require assistance from neutrophils and splenic sinusoidal endothelial cells [131]. MZ B cells are

therefore critical in the initial recognition of antigens and at later times can be presented with antigens by CD169$^+$ metallophilic and MZ macrophages to manipulate the immune response.

Follicular B cells

In the LN, small soluble antigens in the lymph diffuse in the subcapsular sinus into B cell follicles, where follicular B cells can encounter them (Fig. 1). These B cells express B220, CD19, MHC class II, CD23 in the mouse, and express CD19 and HLA-DR in human (Table 1) [126, 127]. Both also express high levels of IgD and low levels of IgM (Table 1) [127]. Analysis of antigen diffusion by electron microscopy or immunofluorescence suggests that antigens diffuse through pores or conduits in the subcapsular sinus to reach the follicular B cells and other areas of the LN cortex in mice (Fig. 1j) [17, 132]. Upon encounter with these antigens, follicular B cells internalize the B cell receptor bound soluble antigens and eventually (~ 24 h) display the antigenic peptide in the context of MHC class II for presentation to T cells which they encounter following CCR7 dependent T cell migration to the paracortex [133].

While small, soluble antigens can easily pass through the lymphatics and into the LN, larger antigens (e.g., VSV antigens) are captured by subcapsular sinus macrophages and DCs for recognition by follicular B cells. The subcapsular sinus macrophages act to sequester antigen near the sinus and B cell follicle where they can transfer unprocessed particulate antigens to follicular B cells (Fig. 1k) [11, 134]. The antigens are then internalized by follicular B cells which migrate to the edge of the follicle and interact with T cells and present the antigen [134].

During DC-mediated presentation to B cells, DCs endocytose antigens via FcγRIIB. Endocytosis via the FcγRIIB prevents degradation of the protein antigen via non-degradative endocytic compartments and promotes recycling of the antigen to the surface for recognition by the B cell receptor [129]. Indeed, a DC population in the paracortex presents intact and unprocessed antigens to B cells [135, 136]. DC populations concentrated in the paracortex around the HEV interact with migrating B cells as a mechanism for antigen recognition by the B cell and to promote T cell dependent antibody production (Fig. 1l) [135]. Upon encounter, the B cells slow down and interact with the DC for activation of the B cells, which can give rise to extrafollicular plasma cells [135]. Like MZ B cells, follicular B cells are important for shaping the immune response and thus trafficking antigen to the follicular B cells is critical for optimal antigen presentation, antibody production, and coordination of immunity.

Neutrophils

Neutrophils (Lin$^-$, CD45$^+$, CD11b^{hi} in mouse and human, Ly6C$^+$ in mouse, MPO$^+$, Elastase$^+$ in human) [137–139] are the first responders to sites of inflammation and aid in the recruitment of other leukocytes through the release of mediators (Table 1). While many of the recruited neutrophils contract via cell death at the site of inflammation, emerging evidence has demonstrated that neutrophils have the capacity to traffic antigens to LNs (Fig. 1m) [140, 141]. In beautiful imaging studies, higher numbers of photoconvertible neutrophils were detected within the draining LNs only when *Staphylococcus aureus* was administered to the mouse ear compared to scarification without the bacteria [141]. These findings implicate neutrophil migration as an additional mechanism of antigen transport, at least during bacterial infection. Further evidence indicated neutrophils migrate to the LN via the lymphatic vasculature and can traffic fluorescent ovalbumin administered in Complete Freund's Adjuvant [142]. Neutrophil migration was dependent on TNFα produced from tissues, as well as CD11b and CXCR4, but not CCR7 (though CCR7 was important at homeostasis) [140]. Thus, neutrophils are another mechanism of antigen transport from the site of infection or injury that is important for modulating the immune response.

Conclusions for protein transport

Antigen can traffic both passively and actively. Active antigen trafficking requires several different cell types and the movement of these cells from the tissue, through the blood, lymph and into the spleen and LNs. Passive antigen trafficking to the LN or spleen does not require cellular transport and instead results in antigen acquisition by LN or spleen resident cells (DCs, macrophages, B cells, etc.). The timing of these trafficking events occurs within the first week after activation (Fig. 1a). This is a coordinated process that is required for detection of the antigen and/or pathogen and for forming the immune response. As discussed above, the cell types that encounter the antigen shape the type of immune response and work together with other cell types to amplify the response and clear the infection.

Protein antigen retention

In addition to antigen trafficking several studies have also highlighted the importance of antigen retention after vaccination [3–5] or after infection [143–148]. This type of antigen retention (also called antigen persistence, residual antigen, or antigen archiving) has been described as antigen remaining within the lymphoid tissue in mice in the absence of infection and beyond the peak of the immune response
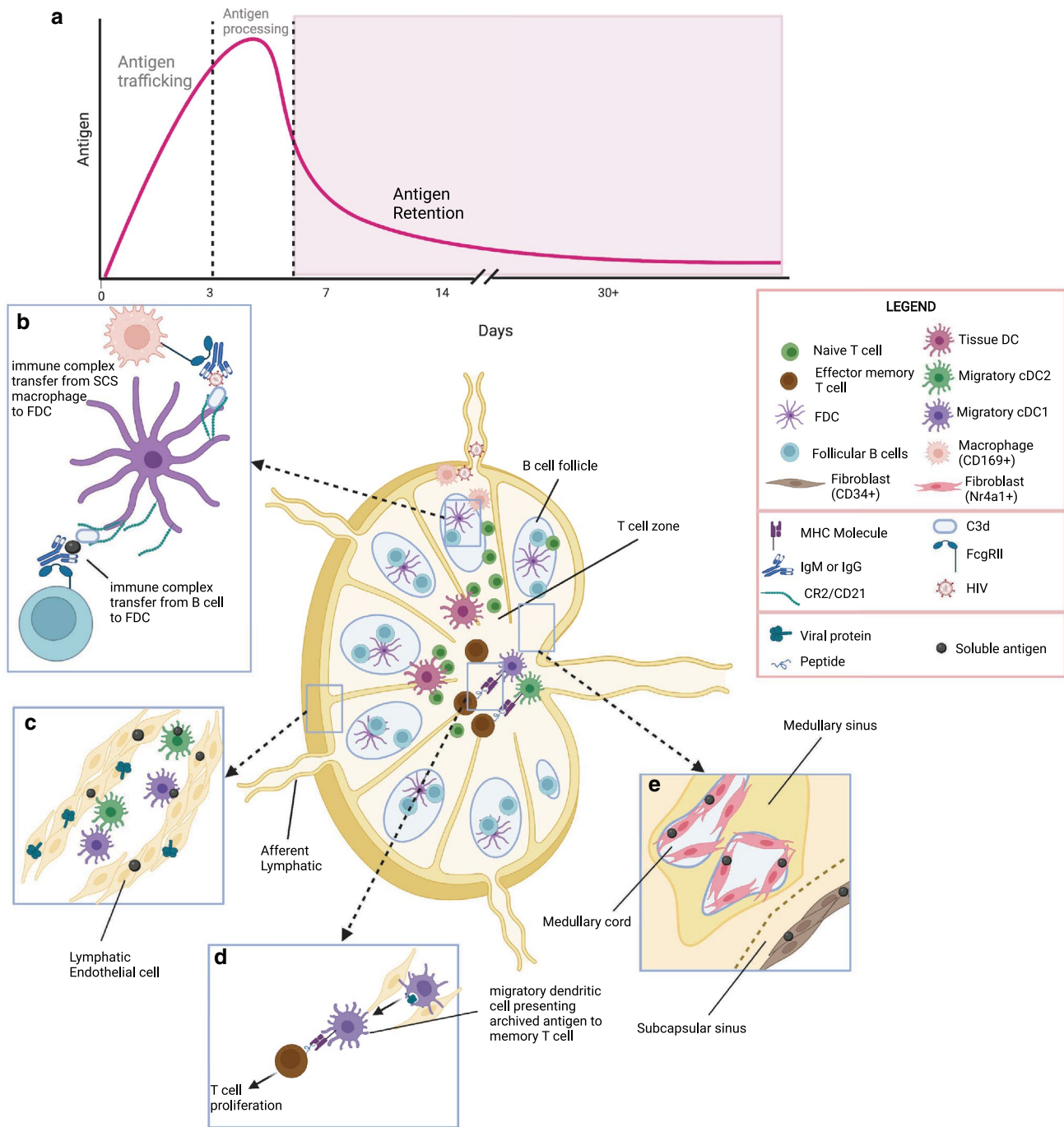
(Fig. 2a). This antigen persistence is different from chronic antigen or antigen depots. Chronic antigen is defined as antigen that remains due to chronic infection and antigen depots. In chronic depots, antigen is maintained for long periods of time at an injection site [149] ultimately trapping cells in the site where they become tolerized or die. In the case of chronic infection, antigen is present as a result of unchecked viral replication which causes clonal exhaustion [150–152]. As stated above, antigen archiving, persistence, or residual antigen is described as antigen that remains beyond the primary immune response and that is beneficial to immunity.

Cell types involved in antigen retention

As discussed above there are various documented cases in which vaccine antigens or viral antigens remain in the host for beyond detection of virus in humans, or beyond the primary immune response in mice. In most cases there is no longer detectable virus at the late time points in which either antigen-specific responses are detected or viral or vaccine proteins are detected (Fig. 2a). These factors led to remaining questions about which cell types are involved in the retention of non-replicating virus or protein antigens. Interestingly, all detection of antigens post-infection or vaccination is within lymph node stromal cells (LNSC) which require minimal turnover. Below are the LNSCs that have been described to be capable of antigen retention.

Follicular dendritic cells

Follicular dendritic cells (FDCs) are a radio-resistant stromal fibroblast subset that can capture and harbor antigen over extended periods of time (Fig. 2b) [144, 153–156]. The mechanism by which FDCs hold antigen is largely through antigen/antibody complexes and their interaction with CR1 (CD35) and CR2 (CD21) (Table 1) [153, 157–161]. CR1 and CR2 are also expressed by human FDCs, along with CR3 [162, 163] (Table 1). FDCs can acquire very small antigens directly via the LN conduit system or larger antigens from B cells (Fig. 2b) [16, 164, 165]. More specifically, immune complexes (antigen, IgM or IgG, and C3d/C3b opsonins) are captured by subcapsular sinus macrophages before being transferred to follicular B cells. The FDCs are then thought to acquire the antigen from B cells as a result of high expression of CR2 which could potentially strip the follicular B cell of its antigen–antibody complex (Fig. 2b) [165]. It was shown in mice that the duration of antigen persistence of antigen–antibody complexes within FDCs is quite long and involves the recycling of the unprocessed antigen stored in endosomes to the FDC surface for presentation to B cells [153]. It is predicted that FDCs acquisition and storage of

Fig. 2 Retention of foreign antigen within the lymph node. **a** Antigen retention occurs approximately 5–30 + days after the initial onset of vaccination [3–5] or infection [143–148] with a foreign antigen. **b** Follicular dendritic cells retain HIV and protein antigens via immune complexes bound to the CR2 receptor (CD21) [148–151]. **c** Soluble vaccine and viral antigen are retained by lymphatic endothelial cells for up to 5 weeks post vaccination [3, 5]. The LECs exchange the antigen to migratory DCs for the antigen to be presented in the lymph node to memory T cells to promote effector memory function [4]. Although the location within the lymph node is unknown, both influ-

enza (nuclear protein) and VSV have suggestive evidence of long-term antigen retention within the lymph node [4, 202]. **d** Respiratory viral antigen within the lung draining lymph node has been shown to result in antigen-specific effector memory T cells for over one month after infection [147, 148, 198]. These effector memory T cells patrol the lumen of the respiratory tract and can protect against secondary infection from influenza [144, 197]. **e** CD34$^+$ fibroblasts within the subcapsular sinus and Nr4a1$^+$ fibroblasts within the medullary cord retain soluble vaccine antigen for up to 2 weeks [5]

antigen is a mechanism by which memory B cells can react to FDC held antigens to maintain memory B cell function. The purpose behind FDC antigen–antibody complex retention is in germinal center development, B cell activation, affinity maturation, and B cell memory.

Lymphatic endothelial cells

Lymphatic endothelial cells (LECs) line the lymphatic vasculature and are important for draining interstitial fluid, antigens, and immune cells from the periphery to the draining LN. They express VEGFR3, LYVE1, Prox1, and Podoplanin in mice and human [5, 166–173] (Table 1). Within the LN, LECs are comprised of subcapsular, cortical and medullary LECs [171] that are defined by specific markers that distinguish function as well as location [172, 173]. We described an additional role for LECs in the retention or archiving of viral and vaccine antigens for more than 5 weeks which was dependent on innate immune activation in mice (e.g., polyI:C and other TLR agonists) (Fig. 2c) [4]. Notably, antigens associated with vaccinia infection (VV-ova) but not *Listeria monocytogenes* infection (LM-ova) were archived [4]. Whether it is the type of innate immune activation that is required for LEC antigen archiving (e.g., viral versus bacterial) or the size of the pathogen (e.g., 20–100 nm versus 400–1000 nm) is still unclear.

LECs also have an important role in maintaining peripheral tolerance [2, 174–177]. However, in the case of antigen archiving, LECs exchange antigens with DCs to promote productive immune responses (Fig. 2c) [3]. Importantly, LEC antigen archiving was important for enhancing the CD8$^+$ T cell response during a secondary challenge in the mouse [4]. Future studies are required to understand what defines whether an LEC archives or presents a foreign antigen [2, 178]. One potential clue is that antigen archiving is dependent upon addition of an innate stimulus [4]. While the process by which metallophilic macrophages, DCs, or FDCs endocytose and maintain unprocessed antigens involves FcγRIIB, it is unclear how LECs maintain unprocessed antigens. However, based on transcriptional data of antigen high LEC populations, we identified caveolin-mediated endocytosis as a potential candidate by which LECs endocytose antigens [5]. Future studies of LEC antigen archiving are required to fully understand if antigens are held within caveosomes and if they are recycled back to the surface.

Antigen that resides in the host for long periods of time within the LN, is important for immune memory, and resides within LECs of the LN, is termed antigen archiving. Antigen that is held within other cell types or undescribed cell types with less information about the impact of the antigen duration has been termed antigen persistence. The difference between archiving and persistence is largely semantic, however we will use the terminology interchangeably referring to the process as persistence or archiving as described by the authors in the publications reviewed.

Other LN fibroblasts

The uniquely described ability of FDCs to retain antigen–antibody complexes suggests that other fibroblasts [179] may be able to perform this type of function. However, there are limited data describing other fibroblastic reticular cells as a cell type that acquires or retains foreign antigens, in fact, the majority of the data suggest otherwise [3, 4]. However, in our recent report we discovered fibroblast subsets, based on their transcriptional signature, within the draining LN of mice [179], retained low but detectable levels of barcoded antigen within the Nr4a1$^+$ and CD34$^+$ fibroblasts for 2 weeks (Table 1 and Fig. 2e) [5]. Interestingly, these subsets are in close proximity to the lymphatics that drain the vaccine antigens and could be an alternative source by which LECs acquire antigens over time for transfer to DCs [3]. Thus, previously unrecognized participation of antigen retention by LN fibroblasts may also have an important part in manipulating the immune response, though how this may occur can only be speculated.

Vaccines and infections that cause antigen retention

As mentioned above, all antigen retention occurs within the lymph node stromal cell (LNSC) populations. As the studies on LNSCs and their capacity to acquire and hold antigens is limited, additional studies are required to fully understand this process during different types of infections/vaccinations. In the section below, we will highlight what is known about the types of vaccinations and infections that have been demonstrated or predicted to result in prolonged vaccine or viral antigen within the host LNSCs.

Vaccination

Vaccine antigens can remain in the host due to vaccine antigen depots or vaccine antigen persistence. Antigen depots arise from substances within vaccinations, such as alum, and release vaccine antigens over time as the depot is broken down. Antigen depots are minimally beneficial to the cellular immune response [149]. Vaccine antigen persistence arises due to small soluble antigens being acquired by stromal cells within the LN. Studies of vaccine antigen persistence in mouse models have detected small soluble protein antigens, including viral proteins, within the draining LNs for up to 5 weeks post vaccination (Fig. 2c) [3–5]. Similarly, intradermal injection of virus-like particles (VLP)

s, was detected for as long as 6 days within the four draining LNs as measured in vivo using an IR dye to image the mouse daily [7]. In this study it was not determined if the duration of the VLP was longer than 6 days or if 6 days was the limit of detection with the IR dye or by which cell type the antigen was held.

In another report, the length of antigen persistence was directly associated with the amount of antigen administered as well as the signals received [4]. As such, the induction of an active immune response acted to facilitate an increased duration of antigen within the LN [4]. This report demonstrated that the persistent antigen was held by LECs [4]. The process was termed antigen archiving. Antigen persistence/archiving was independent of the type of protein or the type of TLR agonist, though antigen size was a determinant and some TLR agonists were better than others [4]. As an example, conjugation of the antigen to a TLR agonist resulted in a robust CD8 antigen-specific response and increased antigen persistence/archiving by LECs in mice [5]. It is noteworthy that antigen-TLR conjugation increased the duration of DC antigen presentation [180] similar to other antigen-TLR conjugate vaccine platforms [181, 182] and potentially increased the duration of antigen within the DC and possibly LEC or fibroblasts [5]. Whether the DC retained the antigen or received the antigen from another cell type (e.g., LEC, FDC or other fibroblast) was not demonstrated. However, in another report we documented the process of LEC–DC antigen exchange which occurred with archived antigen [3]. These findings suggest that detection of antigen within DCs using this methodology may be due to antigen exchange rather than DC antigen retention. Thus, vaccination with a spectrum of vaccine adjuvants and antigens can initiate antigen archiving and benefit long-term protective immunity.

In addition to LEC antigen archiving following vaccination, other reports have demonstrated the long-term retention of antigen–antibody complexes by FDCs following immunization [153, 183]. In these studies, immune complexes were detected following immunization with phycoerythrin (PE) and were taken up by B cells which were handed off to FDCs [153]. The unprocessed injected antigen–antibody complexes were detected for up to 16 days after immunization and predicted to be much longer based on in silico modeling [153, 183]. These findings also demonstrated that antigen amount and antigen half-life (depending on the protein antigen administered) would affect the duration within the FDC [183]. Indeed, the recycling of antigen by the FDCs was important in both mouse and human for promoting memory B cell responses. However, new transcriptional data also suggest FDC-retained antigens may have a regulatory function via PD-L1-PD-1 interactions between FDCs and T cells [184].

Together these data demonstrate a significant role for LNSCs in regulating antigen persistence/archiving

following vaccination. Whether current vaccines, such as mRNA-based vaccines, induce antigen retention by either LECs or FDCs is yet to be determined. Based on the premise of these types of vaccines, translation of mRNA into protein, it is possible that mRNA vaccine encoded proteins are retained by LNSCs. That said, the location of the vaccine encoded protein and whether LNSCs have access to the protein, would likely influence whether or not this is possible. Additionally, while the exact mechanisms of how antigens are retained are still not well understood there are several key pathways that could initially be targeted (eg. Caveolin mediated endocytosis, CR2 or FcγRII) to promote antigen retention. As we begin to better understand how antigens become archived or persist, testing if those pathways will be valuable to target in new vaccines will be necessary.

HIV

HIV is a chronic infection that results in the depletion of CD4$^+$ T cells, leading to acquired immunodeficiency syndrome (AIDS). The virus's ability to integrate into the genome of host cells that survive infection can lead to re-infections. Latent infection in CD4$^+$ memory T cells is the cause of most of the virus' persistence phenotype [185]. Another form of long-term viral reservoir is in myeloid cell types [185]. Even following antiretroviral therapy, HIV antigens can be detected within viral reservoirs. These viral reservoirs are known to contribute to the continual relapse of HIV infection [186]. It has been suggested that infectious HIV is kept in FDC reservoirs via complement receptor 2 (CD21/CD35) binding to complement C3d [187] (Fig. 2b and Table 1). Heesters et al. demonstrated that FDCs isolated from HIV-infected individuals could transmit the virus when co-cultured with uninfected human CD4$^+$ T cells [187]. Furthermore, when HIV$^+$ FDCs were pre-treated with soluble CD21-Ig and then co-cultured with uninfected human CD4$^+$ T cells there was a significant reduction in HIV RNA copy number compared to the isotype control [187]. These findings suggest that C3d binding to CD21 promotes the release of infectious HIV. Intriguingly, HIV antigens have been detected in FDCs for years after an infection [188, 189]. Most notably, the HIV antigen p24, a capsid protein, was detected in FDCs in mice for nine months after initial infection [190]. Additionally, macrophages and microglia can harbor p24 antigen in vitro making the cells that survive viral infection an additional viral reservoir [191]. However, it remains unclear whether these cells are harboring viral antigen from a primary infection, or if the infected cells are continuing to make new viral particles. Thus, while HIV is considered a chronic infection with viral reservoirs, the question still remains whether FDCs can retain viral antigens

in antigen–antibody complexes or if the virus hijacks the ability of the FDC to retain antigen–antibody complexes within non-degradative FcγRII positive endosomal compartments to protect the HIV from degradation [153].

Vaccinia virus

Vaccinia is an enveloped virus belonging to the poxvirus family. Vaccinia virus is largely tropic to livestock and was initially discovered by Edward Jenner in milk maids [192]. Since this discovery, vaccination with vaccinia largely eradicated the smallpox virus. The use of this virus in laboratory animals to understand viral pathogenesis and host–pathogen interactions has increased our understanding of how viruses in general affect the host. Since vaccinia virus is small it can directly traffic to the lymphatics, particularly when the virus is administered subcutaneously in mice and is not given ample time to infect skin cells [4, 19]. Recent studies with mice have demonstrated that virus administered via scarification, which results in infection of the keratinocytes and DDCs, caused the lymphatic vasculature to "close" to impede viral dissemination to the draining LN [19]. While virus is unable to enter the lymphatics, clearance of the virus does require DC migration. The DCs required for trafficking and promoting clearance of the skin infection are langerin+DDCs which promote robust CD8$^+$ T cell responses [96–98]. Vaccinia viral proteins, but not pathogenic virus, can also be retained within the skin-draining LN for at least five weeks post footpad injection (Fig. 2c) [3, 4, 193]. The cell type required for harboring the vaccinia viral antigens is the LECs [3, 4]. The archival of these viral antigens appears to be important for modulating T cell responses, similar to vaccination [4], rather than FDC retention of antigens which is important for B cell responses [3, 4, 153, 165]. Therefore, vaccinia viral infection is the only virus that has been demonstrated to result in LEC antigen archiving.

Respiratory viruses: influenza and sendai

Each year the influenza virus (flu) infects, on average, one in five people around the world and causes five-hundred thousand deaths [194]. Despite high vaccination rates the flu virus remains highly prevalent in the population due to high antigenic drift and results in a flu vaccine efficacy of lower than 50% in some years [195]. Even in populations with high vaccination rates, the amount of antigenic drift can increase due to evolutionary pressure [196]. Thus, developing new vaccination strategies that mimic the protective immune response to flu are increasingly important.

Several reports have now demonstrated that influenza antigen is retained in mice for weeks after the peak of the immune response and beyond the detection of replicating virus [143, 148]. Specifically, viral influenza antigen can be detected for as late as 30 days in draining mediastinal LNs in mice (Fig. 2d) [148]. The residual flu antigens found in the LNs are thought to periodically stimulate T cell proliferation and maintain an effector-like phenotype for the duration of antigen retention [144, 197] this is similar for other respiratory viruses such as Sendai virus [147, 198] (Fig. 2d). Thus, persistence of respiratory viral antigens promotes highly active viral antigen-specific T cells for a duration that is in line with seasonal respiratory infections. Perhaps, the increased number and effector phenotype of the flu specific CD8$^+$ T cells may be important for minimizing re-infection during the flu season. Based on the duration of flu antigens within the lung draining LNs and the substantial benefit to protective immunity, it is tempting to speculate that flu antigens are also "archived" by LECs in a similar to vaccinia virus.

Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is a member of the Rhabdovirus family. VSV is transferred horizontally from insects to mammals [199] and host survival is linked to a strong type-I-interferon response [200]. VSV infection in humans is rare, but outbreaks in cattle can have significant economic impact estimated at a loss of $100–200 per infected cow [201]. As with both influenza and vaccinia viral infection, VSV antigen was measurable in mice more than 6 weeks after infection in draining LNs, well beyond detection of the infectious virus (Fig. 2c) [202]. The location of antigen retention within the LNs was not explored, however, based on our understanding of antigen persistence/archiving and the turnover of hematopoietic cells within the LN, it is likely the antigen is within a LNSC.

COVID-19

Some individuals infected with SARS-CoV-2 have long-term symptoms for as long as 110 days, dubbed as "long-haulers" [203]. It is unclear the cause of these long-hauler symptoms, however, one study has shown SARS-CoV-2 antigen in biopsies of human enterocytes from a patient 92 days after the onset of symptoms [204]. It is unclear if the virus is infecting and replicating within these cells or if the viral antigen is being retained to protect from future infections. Growing evidence suggests that in patients who no longer have detectable respiratory swab viral loads, these patients can have fecal samples with detectable viral loads for nearly five weeks after negative respiratory swabs [205]. This suggests that SARS-CoV-2 infection may become chronic in these individuals. However, whether SARS-CoV-2 antigens, like other viral antigens [144–146, 148, 206], persists or is archived as a mechanism to stimulate either B or T cells

for longer periods of time is unknown. It seems most likely that symptoms in "long-haulers" is due to chronic infection rather than persistence or archiving. However, it is possible that a reservoir of SARS-CoV-2 persists within a LNSC using a mechanism of persistence similar to HIV.

Conclusions

Here we have outlined cell types, tissues, and types of protein antigens and infections required for antigen dispersal, persistence and archiving. While numerous factors are responsible for where protein antigens traffic, which cell types are important for protection against pathogens and the antigens they release, and how antigens are retained for future stimulation, we still have much to understand. Of particular interest is how the location of the protein antigen can determine the type of cell-mediated response required, i.e., the type of antibody response, the type of Th response, and cytotoxic response. Indeed, these responses are much more well described than antigen persistence or archiving, but there are still gaps in our understanding due to lack of visibility of antigens upon entry into the host. As single cell technology and high-resolution imaging improves our detection of antigens, it will be important to utilize the technology to enhance our understanding of antigen trafficking. There is still much to learn about the retention, persistence, and archiving of protein antigen. Key areas of investigation should include which viruses and/or other pathogens can initiate antigen archiving. We highlighted several types of infections here and only one has been demonstrated to initiate LEC antigen archiving (Vaccinia Virus). Interestingly, LECs have also been shown to take up Chikungunya viral RNA, however, the duration of the virus within LECs is unknown [207]. Others, like flu and VSV, that have similar effects on memory T cell responses are potential candidates for viruses that induce LEC antigen archiving. Additionally, based on the positive impact of LEC antigen archiving on memory responses and the possibility of achieving antigen archiving through vaccination, future vaccine platforms should attempt to leverage this process. Finally, as very little has been described regarding how viral or protein antigens are acquired by non-hematopoietic cells or how they are released or exchanged, future studies should focus on these mechanisms. Manipulation of the processes required for antigen archiving may ultimately lead to improved vaccines that provide robust memory to novel pathogens that we encounter. As mentioned above, determining if known mechanisms of antigen acquisition, such as caveolin-mediated endocytosis or FcγRII/CR2, can be targeted to enhance different cell types involved and the processes required for antigen retention will be important for improving vaccines. Furthermore, determining the best route of vaccination

(intradermal, subcutaneous, intramuscular, intranasal, oral, systemic, etc.) for inducing antigen retention will be required to optimize the long-term protective capabilities of different vaccines.

Thus far, antigen retention by lymph node stromal cells (LNSC) following vaccination or infection has only been shown to benefit protective immunity. Whether there are circumstances in which vaccine or viral antigens, in the presence of an adjuvant, are retained by LNSCs to result in immune tolerance has yet to be described. However, many studies have demonstrated that LNSCs are major contributors to maintaining peripheral tolerance through their expression of peripheral tissue antigens in mice and humans [174, 175, 208–212]. Further, LECs that acquire whole ovalbumin protein after injection participate in T cell tolerance in the absence of an adjuvant in mice [2] in a similar manner to how LECs present peripheral tissue antigens (via expression of PD-L1) [175]. Whether this antigen acquisition and retention by LNSCs could be a protective mechanism in which LNSCs retain antigens to mute allergic responses in the absence of inflammation is unknown. Similar to DCs, LNSCs seem to require an activation signal (infection, adjuvant, inflammation), to proceed down the path of antigen retention rather than antigen presentation and immune tolerance. Only when a vaccine adjuvant was added to ovalbumin did LECs archive the ovalbumin for longer than 1 week [4] which was later exchanged at 2 weeks post-vaccine with a professional APC rather than being presented by the LEC [12]. The possibility still remains that viruses, as with HIV, can co-opt the process in which LNSCs retain antigens to maintain latent infection [187]. Indeed, a recent study demonstrated that Chikungunya virus is acquired by MARCO + LECs [207]. Whether these LECs act as a reservoir for the virus or are participating in immunity against the virus is still unknown. Much work still needs to be done to understand the mechanisms of antigen retention by LNSCs and the implications for immunity, allergy, and viral evasion.

Declarations

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