Hyaluronan in the lymphatics: the key role of the hyaluronan receptor LYVE-1 in leucocyte trafficking

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Abstract
LYVE-1, a close relative of the leucocyte receptor, CD44, is the main receptor for hyaluronan (HA) in lymphatic vessel endothelium and a widely used marker for distinguishing between blood and lymphatic vessels. Enigmatic for many years because of its anomalous HA-binding characteristics, the function of LYVE-1 has just recently been identified as that of a lymphatic docking receptor for dendritic cells, selectively engaging with their surface HA glycocalyx to regulate entry to peripheral lymphatics and migration to downstream lymph nodes for immune activation. Furthermore, LYVE-1 is also exploited by HA-encapsulated Group A streptococci for lymphatic invasion and host dissemination. Consistent with a role in lymphatic trafficking, the interaction of LYVE-1 with HA and its degradation products can also activate intracellular signaling pathways for endothelial junctional retraction and lymphatic endothelial proliferation. Here we outline the latest findings on the receptor in the context of its peculiar biochemical properties and speculate on how the interaction of LYVE-1 with different HA sizes and conformations might variably influence cell function as a consequence of avidity and receptor crosslinking. Finally, we evaluate evidence that LYVE-1 can also bind growth factors and associate with kinase-linked growth factor receptors and conclude on how the LYVE-1•HA axis may be exploited as a target to either block inflammation or tissue allograft rejection, or potentiate vaccine and drug delivery.

Keywords: Lymphatic, hyaluronan, LYVE-1, CD44, trafficking

Abbreviations: DC; Dendritic cell, bmDC; bone marrow derived DC, HA; Hyaluronan, HMW; High Molecular weight, GAG; Glycosaminoglycan

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Introduction

The lymphatic network is a primary compartment of the adaptive immune system, within which antigen presenting cells and other leukocytes migrate from tissues to draining lymph nodes for immune surveillance and for the generation and regulation of cellular immune responses [1-4] [5]. As an integral part of this immune function, the lymphatics filter fluids and macromolecules from the surrounding interstitium, including matrix components that undergo regulated turnover during normal homeostasis and tissue injury. In particular, the large and ubiquitous glycosaminoglycan hyaluronan (HA; $GlcNAc_{\beta1-4}GlcUA_{\beta1-3})_n$) undergoes rapid turnover during inflammation, and a proportion of its breakdown products are conveyed *via* lymphatic vessels for terminal degradation in draining lymph nodes [6]. LYVE-1 (Lymphatic Vessel Endothelial Receptor-1), a member of the Link superfamily closely related to the leucocyte HA receptor CD44 and the subject of this minireview, is the major HA receptor in the endothelia of afferent lymphatic vessels and lymph node sinuses, and a widely used marker for discriminating between blood and lymphatic networks [6-11]. Expressed within the distinctive loose junctions of initial lymphatics, at the tips of the interdigitating endothelial flaps that open during leucocyte entry [12-14], LYVE-1 has a location indicative of an important physiological function. Originally predicted either to play a role in HA catabolism, or to act as the lymphatic counterpart of CD44 in regulating leucocyte trafficking, efforts to define the true physiological function of LYVE-1 were long confounded by difficulties in demonstrating significant binding of HA to the endogenous receptor in lymphatic endothelium either *in vitro* or *in vivo* [6, 15]. More recently however, a growing appreciation that the 3-D organisation of HA can be important for receptor binding led to the discovery that LYVE-1 favours interaction with large polyvalent HA-protein complexes rather than free HA chains [11, 16]. Most notably, LYVE-1 has now been confirmed to function as a lymphatic trafficking receptor, and it is currently understood that its preference for engagement with pericellular HA rather than free ambient HA mediates docking of HA-coated leucocytes to lymphatic vessels *in vivo* [16, 17], much as CD44 can mediate HA-dependent adhesion of T cells and DCs at the immune synapse [18].

Sharing some 44% overall similarity in primary sequence with CD44, LYVE-1 is a 340 residue integral membrane receptor containing an extended form of the conserved HA-binding Link module at the end of a 211-212 residue extracellular domain, a 21 residue transmembrane anchor and a 63 residue cytoplasmic tail **(Figure 1** [7, 8]**)**. Like CD44, the consensus Link module in LYVE-1 is predicted to comprise a sandwich of two beta sheets made up of six beta strands (β1-β6) stabilised by a pair of conserved disulphide bonds [19]. This in turn is bracketed by two additional beta strands (β0 and β7) linked by a third conserved disulphide bond [20, 21]. However, two further strands (β8 and β9) that may be critical for conformational switching between "on" and "off" states of the HA-binding domain in CD44 (see below) are not well conserved in LYVE-1, implying a likely difference in its' functional regulation [21, 22]. Connecting the HA-binding domain to the plasma membrane in both receptors is a heavily O-glycosylated stalk region which, intriguingly in LYVE-1 **(Figure 1)**, contains an unpaired cysteine residue through which the receptor forms disulphide-linked homodimers [23] - a unique feature whose functional significance is discussed in further detail below. Unlike CD44 which is widely expressed in epithelial, mesenchymal, haemopoietic and blood (but not lymphatic) endothelial cell lineages, LYVE-1 is largely confined to lymphatic endothelia where it displays a punctate pattern of distribution on the luminal and basolateral plasma membrane surfaces as well as variable retention in a perinuclear, ER-derived intracellular compartment [8, 24, 25]. Notably however, the receptor is also expressed in liver and spleen sinus vascular endothelia, in pulmonary endothelia [8, 26, 27], and in a rare macrophage sub-population resembling so-called immune-regulatory M2–like cells with endothelial progenitor potential that are occasionally present in inflamed and tumour tissue [27-30]. The function of LYVE-1 in blood endothelium is not yet known, however it may as suggested mediate HA uptake as part of the reticulo-endothelial system for waste clearance [27]. Equally uncertain, the receptor in macrophages has been suggested to regulate HA-mediated lymphangiogenesis (see eg. [31]). For this current review however, we focus specifically on the role of LYVE-1 in lymphatic trafficking, its molecular mechanism and its consequences for inflammation and immunity. We detail how LYVE-1 interactions in lymphatic endothelium are critically dependent on avidity, and how this avidity is harnessed by HA arrays on the surface of dendritic cells (DCs), macrophages and likely other leucocytes for their docking, entry and onward migration within lymphatic capillaries as well as immune priming in downstream lymph nodes. In addition, we consider how the unusual properties of LYVE-1•HA interactions differ from those of the leucocyte HA receptor CD44 and how they tune LYVE-1 for its particular function within the low-shear environment of lymphatics as distinct from blood vessels. Finally, we consider the properties of

LYVE-1 as a signalling receptor for endothelial junctional relaxation and lymphangiogenesis, as well as how these functions might be influenced by HA size and whether they involve binding of LYVE-1 to additional growth factor ligands.

LYVE-1, a lymphatic docking receptor for HA-coated leucocytes

In the initial models of LYVE-1 function, it was hypothesised that the receptor mediated leucocyte entry to lymphatic vessels indirectly, by assembling an HA pericellular matrix on the surface of lymphatic endothelium, to which migrating leucocytes expressing CD44 could adhere [32]. On the contrary however, it has emerged that LYVE-1 mediates transmigration by engaging with HA arrayed on the DC surface in the form of an endogenously synthesized leucocyte glycocalyx [16, 17] (**see Figures 2 and 3).** Although earlier reports had shown that DCs and other leucocytes synthesise an HA matrix that can mediate adhesion to T cells for immune activation or antigen cross-presentation [18, 33, 34], a role in mediating lymphatic entry had not been widely envisaged. In our own recent studies using either biotinylated HA-binding protein (bHABP) or biotinylated versican G1 domain (bVG1) as probes, we confirmed mouse bone marrow-derived DCs and human monocyte-derived DCs generate this endogenous HA surface coat *via* the hyaluronan synthase HAS2, an enzyme capable of generating very high molecular weight polymer chains (up to 2 x 10^6 Da) equivalent to contour lengths of several micrometres [16, 17, 35]. Notably, the levels of both cellular and surface glycocalyx HA increase in bmDCs subjected to *in vitro* LPS-induced maturation [17], a differentiation state similar to DCs that migrate *via* lymph in inflamed tissues. Indeed, the interaction between the surface HA glycocalyx and LYVE-1 is pivotal for adherence of mature bmDCs to lymphatic endothelium and subsequent transmigration, as evidenced by the finding that both processes are impaired by the mouse LYVE-1 HA blocking mAbs C1/8 and 2125, or digestion of the DC cell surface using purified hyaluronidase in established *in vitro* assay systems [17]. Intriguingly, HA-mediated adhesion triggers the formation of LYVE-1 lined ring-like structures in the endothelium at points of intimate DC contact, as observed by high resolution *in vitro* confocal imaging (**Figure 2**). These LYVE-1 rich entities gradually surround the DCs and eventually envelop them as they penetrate further into the endothelial monolayer, and transit to the undersurface. In view of the similarities between these lymphatic endothelial rings and the transmigratory cups that form between leucocytes and blood vascular endothelium [36, 37], they have been termed lymphatic transmigratory cups [17]. Moreover, like their blood vascular counterparts, there is ambiguity as to whether the LYVE-1-enriched cups assemble at interendothelial junctions or within the main endothelial cell body and hence whether they support paracellular or transcellular migration, or indeed both [38]. How the HA glycocalyx is anchored to the DC surface is a subject for speculation. Although DCs, like most migrating leucocytes, express CD44, the receptor does not appear to be required for glycocalyx assembly or retention (Johnson LA and Jackson DG unpublished), and hence the HA chains must either be held indirectly through complex formation with other binding proteins as discussed later in this minireview, or remain anchored to the membrane-bound HA synthase as a consequence of the high processivity of the enzyme [35].

The importance of the HA glycocalyx for DC adhesion/transmigration and the likely reason why such a ubiquitous polysaccharide might initiate the process is suggested from the physical characteristics of HA polymers. Because of their glucuronic acid composition and potentially long chain lengths (> 1 μm, [39]), the constituent HA molecules have the capability to form a negatively charged shell around the DC that would extend well beyond the reach of other adhesion receptors, especially the much smaller (approx. 20nm) integrins [40], restricting their accessibility and making HA the primary component for initial DC contact with the endothelium. Given the fast kinetics and unusual mechanics of LYVE-1 • HA bond formation and rupture (Bano F. Banerji S, Jackson DG and Richter R, unpublished) as described later, the relatively weak LYVE-1• HA interaction may therefore generate a low-friction, sliding or "snail-slime" like interface that would allow DCs to migrate along the surface of the endothelium in search of entry points by amoeboid movement [41, 42] prior to transmigration. It is likely that these weak interactions would then be supplemented by more conventional firm interactions involving DC integrins, activated by endothelial-sequestered chemokines such as CCL21 [43-45], to provide the traction and directionality required during and after the transit process [46, 47]. Although the DC HA glycocalyx appears to remain largely intact during transendothelial migration, we speculate it may undergo local redistribution or turnover through the action of endogenous endothelial hyaluronidases to expose these underlying integrins during diapedesis **(illustrated in Figure 2).** It is also possible that newly-transited leucocytes might disengage from the endothelium through localised LYVE-1 shedding, as the molecule has recently been shown to undergo ADAM 17 and MT1-MMP catalysed cleavage at discrete sites (F226-E229 and A235-L236

respectively, **see Figure 1**) close to the LYVE-1 transmembrane anchor [48, 49]. Besides LYVE-1, lymphatic endothelial cells express a variety of other adhesion molecules including ICAM-1, VCAM-1, E-selectin, L1CAM, Macrophage mannose receptor, ALCAM, CD137 (4-1BB), CD99 and CLEVER-1, many of which have been shown to play roles in leucocyte adhesion / transmigration [50-56]. Notably, the interaction between ICAM-1 and β2 integrins is critical for DC transmigration both *in vitro* and *in vivo* in response to inflammation [57-60], and also intraluminal crawling after transit [46, 47]. Undoubtedly, the sequence of these and other adhesive interactions must be carefully choreographed during leucocyte transmigration **(illustrated in Figure 2)**, as is the case in the blood vasculature, and much further research will be needed to chart their relative contributions and the order in which they occur.

LYVE-1 and lymphatic trafficking *in vivo*

Confirmation that docking of LYVE-1 with the HA glycocalyx mediates DC trafficking *in vivo* has come from visualisation of these events in wild-type and LYVE-1-deficient mice, using high resolution confocal imaging [17]. Although the first phenotypic analyses of constitutive *Lyve1*$^{-/-}$ mice showed the architecture of lymphatic capillaries and their ability to drain tissue fluids are overtly normal, and that the cellular composition of lymphoid tissues remains largely unaltered, these studies did not fully assess the impact of *Lyve1* deletion on DC migration [61]. When this was rigorously re-examined using a model of skin contact-hypersensitivity induced by topical application of the chemical allergen oxazolone combined with FITC-painting **(Figure 3)**, and comparisons made between *Lyve1*$^{-/-}$ animals and their wild-type littermates, it was found that the absence of the receptor significantly impaired migration of endogenous CD11c^{+} dermal DCs to draining lymph nodes [17]. Indeed, when these events were imaged over time in the skin of *Lyve1*$^{-/-}$ mice injected with mature CMFDA labelled bmDCs, the migrating cells were found to accumulate at the basolateral surface of dermal lymphatic capillaries **(see Figure 3),** consistent with a role for LYVE-1 in mediating adhesion and intravasation. In contrast to the delay seen in gene-deleted mice, a more sustained impairment of DC intravasation and lymph node trafficking was observed when oxazolone-sensitised wild-type mice were administered with LYVE-1 HA-blocking mAbs [17]. These distinct behaviours indicate the likelihood of some redundancy in LYVE-1 function and suggest that compensatory mechanisms can make up for the loss of the receptor during embryonic development. The importance of LYVE-1 for lymphatic trafficking was also demonstrated by *ex vivo* skin crawl-out assays, which showed that transit of endogenous DCs through dermal lymphatics was greatly diminished in both mAb-treated and *Lyve1*$^{-/-}$ mice [17]. Likewise, the reciprocal importance of the HA glycocalyx for DC trafficking was underlined by the demonstration that combined treatment of mature bmDCs with purified hyaluronidase and the HA synthase inhibitor 4-methylumbelliferone (4-MU, Hymecromone®) reduced their migration to draining LNs by as much as 50% in adoptive transfer studies. Indeed, from an endogenous dermal DC population that was considerably heterogeneous in terms of HA glycocalyx expression, virtually all of the cells transiting through the lymphatics stained positively for the glycosaminoglycan by immunofluorescence microscopy. Efficient lymphatic entry and trafficking may therefore be a property reserved for cells that possess a sufficiently dense HA matrix to engage optimally with LYVE-1. Given the enhanced HA biosynthesis [62, 63], antigen presentation and tissue migratory properties of fully differentiated DCs, the populations trafficking in these studies most likely constitute mature dermal DCs, inflammatory monocyte-derived DCs and Langerhans cells that have acquired antigen for delivery and immune priming in draining lymph nodes. The physiological importance of the LYVE-1 • HA axis for such immune priming is underlined by studies showing that CD8 T-cell responses to viral vaccine and ovalbumin peptide are significantly impaired either by *Lyve1* gene deletion or by the administration of LYVE-1 HA-blocking antibodies, making LYVE-1 a potential target for therapeutic blockade in appropriate pathological contexts [17].

In addition to its importance for DC trafficking, there is mounting evidence that the LYVE-1 • HA axis also mediates the trafficking of macrophages in lymph. Like DCs, both human and murine monocyte-derived macrophages can synthesise HA and assemble a surface HA glycocalyx, and in a similar manner to DC maturation, synthesis has been shown to increase markedly during *in vitro* LPS/interferon-induced differentiation of monocytes to inflammatory M1-like macrophages [62, 63]. Indeed, such macrophages were shown to adhere and transmigrate inflamed LEC monolayers *in vitro*, in a LYVE-1 dependent manner [16]. The likely *in vivo* significance of these properties is that they allow macrophages to exit inflamed tissues *via* draining lymphatics, a physiological clearance route that is thought to be essential for the resolution of inflammation and the restoration of normal homeostasis [64, 65], although this has been disputed [66]. Accordingly, in a mouse model of sterile peritonitis elicited by Biogel® bead injection, the late-stage exit of M1-like macrophages from the inflamed peritoneum to draining mediastinal nodes

is significantly impaired in both *Lyve1*$^{-/-}$ mice and wild-type mice administered with LYVE-1 HA blocking mAbs (Bhattacharjee, S. and Jackson, DG, unpublished). Likewise, in a mouse model of myocardial infarction in which immune-regulatory M2-like monocytes involved in repair of the ischemic heart are normally cleared *via* lymph, exit is again attenuated in *Lyve1*$^{-/-}$ animals, leading to macrophage accumulation, aggravated inflammation and delayed cardiac repair [67]. While these findings clearly attest to the importance of LYVE-1 for leucocyte trafficking in inflamed tissues they also expose an apparent paradox. Although the synthesis of HA and assembly of an HA surface glycocalyx are increased in DCs and macrophages in response to inflammation, as is their capacity for migration *via* lymph, the levels of LYVE-1 in neighbouring lymphatics have been found to decrease [68, 69] - *via* uptake through an early endosome/lysosomal pathway that can be activated in LECs by cytokines including TNFα and IL-1 [68]. This contrasts markedly with receptors such as ICAM-1 and VCAM-1 that are upregulated in LECs under similar conditions [57, 68, 69]. One possible explanation for this unusual phenomenon is that LYVE-1 down-modulation constitutes a homeostatic mechanism for limiting lymphatic trafficking and immune activation during inflammation. Conversely, one might speculate that LYVE-1 uptake is part of a mechanism for receptor translocation that facilitates delivery of leucocytes (or appropriate HA complexes) across inflamed lymphatic endothelium. Resolving this apparent enigma should be achievable through video imaging of the process in lymphatic endothelium and the use of appropriate inhibitors of vesicular uptake and re-cycling.

Among other leucocyte populations known to migrate in lymph, T lymphocytes have also been reported to biosynthesise HA and hence may present the glycosaminoglycan on the cell surface [70, 71]. Consequently, one might speculate these cells also utilise LYVE-1-based adhesion mechanisms for lymphatic entry and trafficking. Furthermore, it is noteworthy that LYVE-1 is expressed abundantly in the lymph node cortical and medullary sinuses across which lymphocytes transit to the cortex and through which they exit to the efferent lymphatics for eventual re-entry to the venous circulation [7, 8]. Consequently, it would not be surprising if the receptor is found to mediate HA-dependent cell transit events in lymph nodes as well as peripheral tissues. In contrast, neutrophils which can migrate in dermal lymphatics in response to bacterial infection do not appear to elaborate an HA glycocalyx, and instead employ β2 integrins to adhere to lymphatic endothelium and intravasate by a separate LYVE-1 independent mechanism involving chemorepellant lipoxins and matrix metalloproteinases [72, 73]. Synthesis of an HA surface glycocalyx and trafficking *via* LYVE-1 may therefore be a feature reserved for less motile leucocyte populations.

Lastly and perhaps unsurprisingly, the LYVE-1•HA axis can be exploited by microbial pathogens, notably the HA-encapsulated Group-A haemolytic streptococcus S. pyogenes that causes human tonsillitis. These streptococci are known to invade the pharyngeal epithelium via CD44 [74], but can also disseminate further *via* lymph to cause post-infection sequelae such as rheumatic fever, toxic shock syndrome and necrotizing fasciitis. In a mouse model of infection, virulent *S. pyogenes* strains were shown to bind LYVE-1 in afferent lymphatics *via* their HA capsule, in a manner analogous to the DC HA glycocalyx, and their dissemination to lymph nodes was similarly impeded by LYVE-1 HA blocking mAbs or *Lyve1* gene deletion [75]. Interestingly, sequestration of *S. pyogenes* in the draining lymph node sinuses appears to facilitate their progressive spread *via* efferent lymph and may allow them to manipulate the host immune response for enhanced survival (Siggins M, Jackson DG and Sriskandan S, unpublished). Hi-jacking of the LYVE-1 • HA axis by Group-A streptococci may also underlie their role in the pathogenesis of erysipelas, an erythematous skin rash characterized by fever, swelling and lymph node hypertrophy in which the microbe invades the superficial lymphatics in the upper dermis [76]. This contrasts with the comparatively localized, non-disseminating pattern of infection in impetigo, a common skin disease caused by S. aureus, which lack an HA capsule and hence cannot engage with LYVE-1 [77].

The critical importance of HA binding avidity for LYVE-1 function

A key question at the heart of LYVE-1 function is how the receptor confers lymphatic endothelium with the ability to discriminate between leucocyte HA glycocalyx and the far more abundant levels of ambient HA encountered in the surrounding matrix and interstitial fluid, to fulfill its function in leucocyte docking and lymphatic trafficking. Based on insight gained from detailed structure/function analyses, and comparison with its counterpart receptor CD44 on leucocytes, much of the explanation lies in the distinct binding properties of LYVE-1 and its organisation within the lymphatic endothelium – the features of which are illustrated in **Figure 4**.

Firstly, LYVE-1 is so far unique amongst Link superfamily HA receptors in functioning predominantly as a covalent disulphide-linked homodimer, an association that is essential for *in vivo* function and enhances the binding affinity of the monomer for HA some 60x fold through avidity [23].

As deduced by small-angle X-ray scattering (SAXS) analyses, the individual homodimers adopt an open-scissors arrangement in which the Link modules are situated at the tips and the disulphide bridge within the LYVE-1 juxtamembrane domain forms the hinge [23]. Whether the molecules undergo re-arrangement upon HA-binding to form a closed-scissors, more upright conformation **(see Figure 4)** has yet to be determined. Indeed, it is likely that the intermolecular disulphide, which is particularly labile [23], undergoes reduction in response to changes in redox conditions, thus allowing functional regulation of the receptor through homodimer association/dissociation *in vivo*. Regardless, a key consequence of homodimerisation is that the register of the two HA-binding domains must occur in a head-to-head orientation that is incompatible with contiguous binding by the same HA chain without the introduction of an intervening (i.e. reversing) loop **(Figure 4)**. Consistent with this arrangement, the efficient displacement of bound HMW•HA from LYVE-1 homodimers was shown to require competition with longer HA oligosaccharides (> 40mers) than expected if the paired binding domains were more closely apposed [78]. Importantly, the spontaneous formation of loops within free HA chains would be entropically unfavourable, but might be stabilised within higher order HA complexes. Hence the physical structure of LYVE-1 may actually pre-dispose the receptor for binding HA when organised within dense surface arrays or glycocalyces as opposed to free HA chains.

Secondly, in common with CD44, native LYVE-1 binds HA chains with relatively low affinity (K_D 8- 125μM) as determined by Biacore analyses with recombinant soluble receptor constructs, and thus stable adhesion would require the co-ordinate tethering of individual sugar chains by many neighbouring LYVE-1 molecules [23, 79]. Importantly, for LYVE-1 this dependence on binding avidity is particularly stringent, and consequently native lymphatic endothelial cells display little default binding even to free high-molecular weight HA polymers [11, 16, 79]. Such behaviour likely derives from physical restriction of LYVE-1 lateral mobility through its association with the underlying cortical actin cytoskeleton (Stanly, T, Jackson DG and Eggeling, C. unpublished, **and see Figure 4**) and mutual charge repulsion *via* LYVE-1 sialylated O-linked glycan chains [15]. Nevertheless, as has been demonstrated for CD44 [80, 81], LYVE-1 can be induced to bind bHA polymers when they are organised in supramolecular protein complexes **(Figure 4)**, for example with the small HA-binding protein TSG-6, or as streptavidin cross-linked aggregates, as such assemblages have the capacity to recruit the receptor from its normally diffuse distribution on the endothelial cell surface into discrete microclusters that support polyvalent interactions [16]. Evidently this "supersize" requirement is also satisfied by HA assemblages in the leucocyte glycocalyx and the surface capsule of Group A streptococci in view of their ability to recruit LYVE-1 into transmigratory cups [17, 75]. Although such assemblages may exert their effects primarily through increased HA packing density, it is also possible that they do so by organizing the constituent HA chains within higher order complexes. The formation of such complexes, which can comprise TSG-6, the matrix proteoglycan versican, the IαI (inter-alpha trypsin inhibitor) heavy chain (variably termed HC•HA and SHAP; Serum Hyaluronan Associated Protein) and the crosslinking protein pentraxin has been shown to dramatically enhance HA-binding to CD44 in other tissue contexts [81-85]. Moreover, monocyte-derived macrophages and myeloid cell lines have been reported to express both mRNA and protein for versican, TSG-6 and IαI heavy chains [62, 63, 86]. The composition of the leucocyte HA glycocalyx and its relation to the strength and duration of LYVE-1 binding will no doubt become clearer as biochemical and physical studies progress further in this area.

Thirdly, the character of the HA-binding interaction in LYVE-1 is unusual and rather different to that in CD44 [6, 20, 21]. Although structure-based modelling of the Link domains suggests that in both receptors the bound sugar lies in a similar orientation within a shallow binding cleft, in LYVE-1 the binding surface has a different topography and is lined by predominantly charged or polar residues (eg. Y87, I97, R99, N103, K105, K108 in hLYVE-1), unlike the aliphatic and aromatic residues (Y42, Y79, I88, I96, C97, A98, A99 and Y105) that make up the equivalent surface in CD44 **(see Figure 1)**. Accordingly in LYVE-1, HA-binding is mediated predominantly by electrostatic interactions, in contrast to H-bonds and hydrophobic interactions in CD44 [21, 79]. This is also reflected in the fact that HA binding to both soluble LYVE-1 and LYVE-1 transfected cells is disrupted by low salt concentrations (≥150 mM), whereas CD44 • HA-binding remains intact even at salt concentrations above 2M [79]. In addition, the HA-binding domain in LYVE-1 may have a different degree of conformational flexibility to that of CD44, in which the c-terminal portion undergoes significant conformational change when the binding of HA induces the receptor to switch between its low and high-affinity states [20, 21]. Indeed in CD44 it has been reported that two constituent beta strands β8 and β9 become partially disordered in the high-affinity state, re-orienting the

Link domain relative to the membrane-proximal stalk [22]. Moreover, it is thought that rapid transition between the low and high affinity binding states *in vivo* is induced by the mechanical forces encountered during high-shear blood flow, thus enabling CD44 to mediate the distinctive rolling interactions of leucocytes on HA in capillary venules [22]. In LYVE-1, the Link domain appears to lack the β8 / β9 extension, perhaps reflecting the distinct functional requirements of the receptor in lymphatic vessels. Moreover, recent studies using atomic force spectroscopy to study the mechanics of HA- binding and unbinding at the single molecule level have revealed some unique features of LYVE-1. Unlike CD44, where the individual bonds along each HA chain form slowly and rupture sequentially (conventional sticking bonds), in LYVE-1 they form rapidly, are weaker and rupture collectively (sliding bonds, see ref. [87], Bano F, Banerji S, Jackson DG. and Richter R. unpublished). How these properties might contribute to the physiological function of LYVE-1 as a lymphatic docking receptor can again be appreciated when one considers the particular physiological environment in which the receptor operates, as summarized in **Figure 5**. For example, in the tissues surrounding lymphatic vessels, the level of shear stress ($\leq$ 5 $\mu N/cm^2$) is extremely low and the weak, sliding interaction between LYVE-1 and the HA glycocalyx would facilitate the amoeboid locomotion employed by DCs for intravasation, directed by cues from the chemokine CCL21 sequestered around the vessel surface. This contrasts with the high shear stress conditions (50 $\mu N /cm^2$) in post-capillary blood, where the sticking or Velcro® - like interaction between leucocyte CD44 and endothelial HA enables their capture from flow **(Figure 5** [88, 89]**)**.

LYVE-1 downstream signalling and endothelial permeability

As detailed above, the unusual biophysical properties of LYVE-1 and the avidity-dependent nature of the LYVE-1 • HA interaction allows the receptor to selectively dock with migrating leucocytes *via* their surface glycocalyx. However, docking is not the only function of the receptor, as LYVE-1 can also transduce intracellular signals that regulate endothelial junctional permeability. As described earlier in this review, the inter-endothelial junctions of lymphatic capillaries are stabilised by both tight and adherens junctional proteins, and in particular VE-cadherin, a homophilic adhesion molecule concentrated at the overlapping cell borders, that holds them together in a button-like manner [7-9]. During the process of leucocyte intravasation, it is thought these junctions transiently unbutton, enabling migrating cells to more easily transit to the vessel lumen. Observations made with *in vitro* cultured LEC monolayers indicate that contact with DCs triggers an increase in junctional permeability, as assessed by a reduction in electrical resistance (Johnson LA, and Jackson DG unpublished). More importantly, purified HA can itself promote rapid LEC junctional retraction through VE-cadherin disruption [25, 90], and interaction of HA with LYVE-1 has been shown to activate the MAP kinase/*Erk*, and *Src* non-receptor tyrosine kinase signalling pathways, the latter of which leads ultimately to phosphorylation of VE-cadherin on key tyrosine residues Y658 and Y731 that regulate junctional integrity (Wang YJ, and Jackson DG unpublished). Intriguingly, our recent studies have revealed these events are elicited by low MW HA (ie ~80mers), and at concentrations in the submicromolar (0.1μM) region, well below the measured K_D for LYVE-1 (8-125μM), implying that signalling can be triggered even at low levels of receptor occupancy. Furthermore, while both low and high molecular weight HA can induce junctional retraction, the kinetics differ with chain length and are faster and more sustained in the case of low MW HA, indicating that LYVE-1 signalling may be determined by the degree of receptor crosslinking imposed by the polymers (see below). Importantly, LYVE-1 has been confirmed as the cognate receptor for these HA-mediated signalling events by means of shRNA knockdown controls, thus excluding the possible complication of Toll-like receptor activation by contaminating endotoxins [90]. Exactly how LYVE-1 integrates with the appropriate intracellular signalling pathways to achieve junctional opening is not fully clear. The 63 residue cytoplasmic tail is highly conserved between species and lacks any apparent endogenous catalytic activity. Although it contains motifs for phosphorylation, there is no indication that LYVE-1 is itself phosphorylated either constitutively or as a result of ligand binding [7, 8]. Significantly, the pathways for HA-induced signalling via LYVE-1 for VE-cadherin phosphorylation can be blocked by selective inhibitors of EGFR, PDGFR and VEGFR receptor tyrosine kinases as well as *src* kinase, which suggests that LYVE-1 may well recruit the endogenous kinase activity of these growth factor receptors for downstream signalling in response to HA engagement (Wang YJ, and Jackson DG unpublished). There is also evidence to support this notion from co-immunoprecipitation studies, which indicate LYVE-1 can associate physically with VEGFR and PDGFR in LECs [90]. As these tyrosine kinase-linked growth factor receptors can activate multiple signalling cascades involving *src*,

Ras MAPK/*Erk*, PI3K and *Akt* kinases, it is possible that LYVE-1 signalling complexes regulate not only endothelial junctional permeability but also endothelial cell proliferation, as discussed in the next section.

Role of the LYVE-1•HA axis in lymphangiogenesis

Lymphatic proliferation or lymphangiogenesis, the generation of additional vessels by sprouting and branching from existing structures is a fundamental process during embryonic development [91, 92]. Moreover, it is critically important in the adult for tissue repair, following injury, infection and inflammation when the expansion in vessel numbers allows for lymph-borne transit of DCs for immune priming, as well as later for clearance of phagocytes and inflammatory debris during resolution [93]. The process of lymphangiogenesis is under the complex control of a large variety of growth factors belonging to the vascular endothelial growth factor (VEGF, A, C and D), fibroblast growth factor (FGF-2) and angiopoietin families (Ang1/2) and their endothelial signal transducing receptors VEGFR2/3 and Neuropilins, FGFRs and TIE-1/2. Interestingly however the process is also activated by degraded HA fragments that act as pro-inflammatory danger signals, (also known as DAMPs; damage associated molecular patterns) and recent studies indicate that LYVE-1 is the receptor mediating these effects.

In the first such study using the murine lymphatic derived SVEC4-10 cell line, Wu et al. [94] showed that HA oligosaccharides of 4-10 sugars in length induce a modest increase in lymphatic endothelial proliferation, migration and tube formation *in vitro* in a process that was disrupted by LYVE-1 inhibitory mAbs or LYVE-1 siRNA knockdown. This correlated with activation of the mitogenesis-associated MAPK/*Erk* and Sphingosine 1 phosphate (S1P1/*Edg*-3) signalling pathways. Curiously, the ability of HA to induce such responses was strictly dependent on short chain length as HMW HA polymers had no significant effect [95]. These early findings have been corroborated and extended by rigorous studies of human and murine lymphangiogenesis by Sleeman and colleagues [96] using a combination of primary lymphatic endothelial cell, explanted tissue and whole animal experiments, stemming from their earlier observation [97] that the levels of low MW HA fragments in colorectal tumour interstitial fluid correlated with lymphatic invasion and nodal metastasis. In particular, they showed that physiologically relevant concentrations (1-10 μg/ml, equivalent to low mM) of HA 8-26mer oligosaccharides promote a sizeable (2-7 x fold) increase in *in vitro* LEC proliferation, *in vitro* lymph node lymphangiogenesis and *ex vivo* lymphatic vessel outgrowth that requires *Lyve1* gene expression but is independent of TLR4 [96]. Again, in agreement with the earlier studies, no lympho-proliferative responses were seen with HMW • HA.

The apparent potency of HA oligosaccharides (in the micromolar concentration range) for LYVE-1 mediated lymphangiogenesis is redolent of that observed for endothelial junctional relaxation as described above, and again seems to indicate that binding to only a proportion or a functionally discrete subpopulation of LYVE-1 molecules in lymphatic endothelium is required to trigger the proliferative response. Moreover, the selective ability of short HA oligosaccharides to induce proliferation, suggests that such triggering may actually be repressed by polyvalent receptor crosslinking. This notion that the quality of LYVE-1 binding is determined by HA size and valency is in line with recent theories put forward to explain a similar phenomenon for CD44 (see e.g. [98]), for which a clear dichotomy exists between the pro-inflammatory, pro-angiogenic effects of low MW HA and the often anti-inflammatory, immunosuppressive effects of high MW HA [99-101] that include signalling for contact inhibition of growth [102]. The concept of superselective binding, whereby multivalent HA polymers and higher order complexes target high receptor surface densities for binding through avidity fits well with the known properties of both LYVE-1 and CD44 in this regard [100, 101]. Exactly how differential LYVE-1 crosslinking might alter the pattern of downstream signalling is still unclear. However, the problem is a tractable one, and the application of techniques such as differential phosphoproteomic analysis should shed new light on this area in the future.

LYVE-1, more than just a HA receptor ?

While the primary function of LYVE-1 is that of a HA receptor, a number of reports suggest this may not be its' sole function, and that the molecule can bind further ligands through interactions independent of those involving the main consensus HA binding site. Most notably, Huang and colleagues have proposed that LYVE-1 serves as a high-affinity receptor for the c-terminal basic regions of growth factors including PDGF and VEGF, based on their studies of the protein they originally termed CRSBP-1 (C-terminal Retention Sequence Binding Protein) in liver homogenates and

transfected NIH 3T3 fibroblasts [103, 104]. Subsequently, this was found to be identical to LYVE-1 by gene cloning [104, 105]. Curiously, these workers proposed that CRSBP-1/LYVE-1 serves the dual function of retaining newly-synthesised growth factors on the cell surface and handling their rapid internalisation for autocrine stimulation. Surprisingly for a receptor involved in peptide retention, CRSBP-1 bound its growth factor ligands with an affinity (K_D app <1 nM) some three orders of magnitude higher than that of their known heparan-sulphate proteoglycan co-receptors (K_D app > 1μM), and binding was displaced by a variety of anionic and cationic compounds including free heparin, polylysine, protamine and the polysulphonated naphthylurea suramin, indicative of a rather unspecific interaction [104]. Importantly, the binding site for these c-terminal peptides on LYVE-1/CRSBP-1 was reported to be distinct from the HA binding surface, on the basis that the peptide ligands and HA failed to show mutual displacement. Specifically, the site has been proposed to lie downstream of the Link module, within a short region containing a spacing of 5 acidic residues termed the AAAR (Acidic Amino Acid Rich domain [106]), although the basis for this conclusion is not clear and experimental evidence to support the notion is currently lacking.

The ability of LYVE-1 to sequester growth factors is also supported by the findings from another independent study using a completely different methodology. Employing purified recombinant LYVE-1 in a proprietary (AlphaScreen®) luminescence proximity assay for ligand screening, Platanova et al. detected binding to FGF-2, and to a lesser extent PDGF and VEGF that was apparently mediated by the receptor N-linked sugar chains, based on the observation that binding was lost after digestion with the glycoproteinase PNGase-F [107]. Like their c-terminal peptides described earlier by Boensch et al [103, 104], these full-length growth factors could also be displaced by heparin, perhaps indicating an interaction with terminally sialylated glycans, as LYVE-1 lacks GAG modifications [7, 15]. Moreover, such LYVE-1•FGF2 interactions were shown to have functional significance, since they promoted endothelial proliferation and tubulogenesis both *in vitro* and *in vivo* through signalling pathways associated with the signal-transducing FGFR1 receptor [107]. While the notion of growth factor-binding has important implications for LYVE-1 function, it is currently difficult to reconcile the conflicting findings from these two independent studies. Nevertheless, the concept of LYVE-1 as a co-receptor for growth factors is certainly attractive in explaining its apparent association with tyrosine kinase-linked receptors and their co-operation in LYVE-1 downstream signalling. Further developments in this intriguing area are eagerly awaited.

Summary, remaining issues and future therapeutic potential

As outlined in this minireview, the discovery that LYVE-1•HA axis mediates leucocyte docking and entry to lymphatic vessels has advanced our understanding not just of LYVE-1 itself, but also lymphatic trafficking in general. Coming after many years of research, the identification of such an important physiological function for this enigmatic receptor will finally raise its profile from that of a passive lineage marker to an active participant in immunity and tissue inflammation. Nevertheless, research into LYVE-1 is still in its relative infancy and there remain large gaps in our knowledge of precisely how the receptor functions within the lymphatics and even more so within the specialized blood vascular and macrophage compartments where the receptor can also be expressed. To date almost all research on LYVE-1 has been confined to the skin and dermis and we can only anticipate its role in other prominent lymphatic beds including the intestines, the brain and the CNS. Indeed even in the dermal lymphatics only the specific function of LYVE-1 in mediating vessel entry has been established. Importantly, LYVE-1 is expressed on both membrane surfaces of lymphatic endothelium [8] and hence may also mediate leucocyte crawling within initial lymphatic capillaries, where directional movement is thought to rely more on chemotaxis than active lymph flow. Likewise, as the receptor is expressed at high levels in downstream lymph nodes, particularly cortical and medullary sinuses it may also facilitate the access of migrating T-cells (which reportedly also synthesise HA [33, 70]) to the paracortex and/or exit in efferent lymph [1, 108]. No doubt these important issues will be resolved by intralymphatic injection of *Lyve1*$^{-/-}$ and wild-type reporter mice and tracking of cell migration in real-time by intravital videomicroscopy. With respect to the leucocyte HA pericellular matrix itself, as emphasized earlier in this review, it will be important to determine how the configuration and surface organization of the glycosaminoglycan by lymph-migrating DCs and macrophages enables their appropriate engagement with LYVE-1, and to identify the additional binding proteins involved. Besides lymphatics, there are also fundamental questions to be addressed about the role of LYVE-1 in its less well-studied non-lymphoid tissue locations. It is not clear for example what function is served by LYVE-1 in liver and spleen vascular sinuses. Does it mediate HA-dependent leucocyte capture from the blood circulation in these organs, in analogous fashion to CD44, or does it instead play a role in HA sequestration or scavenging [109, 110] ? What is the function of LYVE-1 in M2-like tissue

macrophages in inflamed tissues ? Since macrophages, in contrast to LECs, can also express CD44, do the two HA receptors function co-operatively or separately ? Does LYVE-1 mediate the trafficking of memory or regulatory T-cell subsets in lymph [47]? Which growth factors or other ligands are bound by LYVE-1 in these different cell types and what function(s) does such binding fulfill ?

Finally, the LYVE-1•HA axis has potential as a new target for therapeutic intervention. Because the lymphatics are so intimately involved in the development of inflammation, and lymph-borne trafficking is so fundamentally important for immune priming, such targeting could in principle be used to block unwanted immune or associated inflammatory responses in any appropriate disease context. In the first instance however, one might speculate that LYVE-1 blocking mAbs or small molecule inhibitors could have anti-inflammatory effects in immune-mediated skin conditions (eg psoriasis and atopic dermatitis) that are driven primarily by inflammatory DCs and T cells recruited to the affected dermis [111, 112] – particularly if their trafficking to lymph nodes should prove to be involved. If LYVE-1 is also found to regulate leucocyte trafficking in gut-associated lymphatics, its target range could potentially be extended to therapies for inflammatory bowel disease. Alternatively, in the field of tissue transplantation, LYVE-1•HA mediated DC trafficking might be targeted to prevent corneal allograft rejection – a frequent outcome of corneal transplant surgery, where inflammation-induced vascularization and lymphangiogenesis in this normally immune-privileged site enables alloantigen-loaded DCs to migrate to regional lymph nodes for immune activation [113-116]. Indeed, given the evidence that HA can promote inflammatory lymphangiogenesis through LYVE-1 signalling [94-96] and that new corneal lymphatics can arise from LYVE-1^+ $CD11b^+$ macrophages [117], such blockade could potentially be synergistic. While these are exciting possibilities, they are still speculative, and further research will be required to confirm efficacy in appropriate experimental disease models. As a corollary to blockade, augmenting LYVE-1•HA interactions might be exploited to boost either HA-mediated vaccine adjuvancy [118] or drug delivery [119, 120]. Realising this aim will require further knowledge of how the composition and structure of HA complexes (including the leucocyte HA glycocalyx) specifies LYVE-1 binding affinity, so that drugs and vaccines can be packaged for maximal lymphatic uptake and tissue delivery. Most importantly, advancing the therapeutic potential of LYVE-1 will require detailed investigation into the 3-D structure of the receptor and its distinctive HA-binding domain, in order to appreciate how its mode of ligand interaction defines its respective functions in leucocyte trafficking, endothelial signaling and lymphangiogenesis. Understanding how LYVE-1 homodimers associate on the cell surface to engage the leucocyte glycocalyx and how current HA blocking mAbs disrupt this engagement should inform the design of new and even more effective function blocking or function enhancing agents for future clinical exploitation.

Figure legends

Figure 1. Main structural features of LYVE-1 and comparison with CD44
(A) Structure-based models of human LYVE-1 [79] and CD44 [20, 21] as integral membrane receptors, each containing an extended HA-binding domain (coloured coral/salmon with annotated secondary structure elements) at the terminus of a highly glycosylated membrane-stalk region, depicted as an extended rod (sugar chains in red). Although shown as a monomer, LYVE-1 forms disulphide-linked homodimers *via* an unpaired cysteine (yellow symbol) in the stalk region as discussed in the text [23] and depicted diagrammatically in Figure 4. Note the dimensions of the stalk region, the transmembrane anchor and the cytoplasmic tail are not drawn to scale. (B) The full amino acid sequences of the LYVE-1 and CD44 extracellular domains are shown in a pairwise alignment [79]. Highlighting indicates the boundary of the HA-binding domain in each case, which comprises the consensus Link module (coral) and its additional N- and C-terminal extensions (salmon), as well as the positions of the six conserved cysteine residues that form 3 critical disulphide bridges (C1-C6, C2-C5, C3-C4) stabilizing the overall domain fold. The additional cysteine residue (C201) that forms a critical inter-chain disulphide in the LYVE-1 homodimer is circled yellow. The five HA-binding residues in LYVE-1 identified to date by site-directed mutagenesis are coloured green and those in CD44 (3 of which are precisely conserved) are coloured red [21, 79]. Assignments for the ten beta strands (β0-β9) and two α chains that make up the extended CD44 HA-binding domain (as determined from the crystal structure [20, 21]) are shown above the sequence, and their predicted equivalents in LYVE-1 based on homology and JPRED / JUFO secondary structure prediction algorithms are shown below [79]. Note in LYVE-1, the assignments of β8 and β9 strands are uncertain and hence these are not depicted. The juxtamembrane sites identified for cleavage of LYVE-1 from the cell surface by ADAM17 and MT-MMP1 [48, 49] are annotated and boxed in red.

Figure 2. LYVE-1•HA interactions involved in docking, entry and migration of DCs in lymphatic vessels The cartoons (panels A-D) depict the various stages of DC entry and migration in initial lymphatic vessels mediated by interactions between LYVE-1 on the endothelial surface (lined red) and HA on the DC surface (lined dark green). Although the images depict entry by a paracellular route at endothelial junctions, it is also possible that entry occurs by the transcellular route (see text). The inset (panel B) shows a confocal microscopic image of a fluorescent dye-labelled mouse bmDC (CMFDA, green) being partly enveloped by a LYVE-1 transmigratory cup (thick red lining) following its adhesion to an *in vitro* cultured primary mLEC monolayer prior to transmigration [17]. Exposure of underlying adhesion molecules on the DCs such as β2 integrins (yellow symbols) initially concealed by HA, is proposed to occur after glycocalyx re-distribution or partial turnover (panels C and D), allowing their participation in adhesion during the latter stages of diapedesis and intraluminal crawling (see text).

Figure 3. DC trafficking in sensitized mouse skin and its disruption in *Lyve1*$^{-/-}$ mice
The upper panel (A) shows the experimental strategies used to track the lymphatic migration of endogenous and adoptively transferred bmDCs in mice sensitised with topical oxazolone using FITC skin-painting and fluorescent CMFDA dye-loading respectively [17, 57]. The confocal images in the lower panel (B) show the presence of CMFDA labelled bmDCs (green) in the lumen of dermal lymphatics (red) in wild-type *Lyve1*$^{+/+}$ mice and their absence due to impeded entry in the case of *Lyve1*$^{-/-}$ mice [17].

Figure 4. Key characteristics of LYVE-1 that allow avidity-dependent HA-binding
Lollipop representations of LYVE-1 depict the key features of receptor homodimerisation and surface clustering (panels A-B) that contribute to the affinity of the receptor and its capacity to bind selectively to higher-order HA structures, in particular the leucocyte HA glycocalyx and streptococcal HA surface capsule through avidity. Panel A depicts the splayed open-scissors conformation of the homodimer compared to the upright monomer as evidenced from a low-resolution structure (envelope in grey with individual monomer units in red and blue) determined by small angle X-ray scattering analyses shown in the inset. The values shown for the HA-binding constants were determined by equilibrium and single-cycle Biacore analyses with recombinant hLYVE-1 constructs [23]. The cartoon depiction of the LYVE-1•HA complex on the right in panel A indicates a possible re-arrangement of the homodimer upon binding to HA and highlights the opposing register of the two constituent HA-binding domains (red, with white arrows) that would necessitate formation of an intervening loop within a single co-ordinately bound HA chain (green). The alternative possibility that homodimers bind two separate HA chains is not

depicted. Panel B shows the restriction in LYVE-1 lateral mobility, imposed by interaction of the cytoplasmic tail with the underlying cortical actin cytoskeleton (see text) and clustering of the receptor induced by higher order HA complexes, critical for avidity-dependent binding [16]. The orange and purple components represent putative HA-binding and cross-linking proteins.

Figure 5. Comparison of HA-mediated leucocyte trafficking in the lymphatic and blood vasculatures
The Figure highlights key functional differences between LYVE-1 and the closely related CD44 receptor that contribute to their distinct physiological roles in leucocyte transmigration within the lymphatics and blood vasculature respectively.

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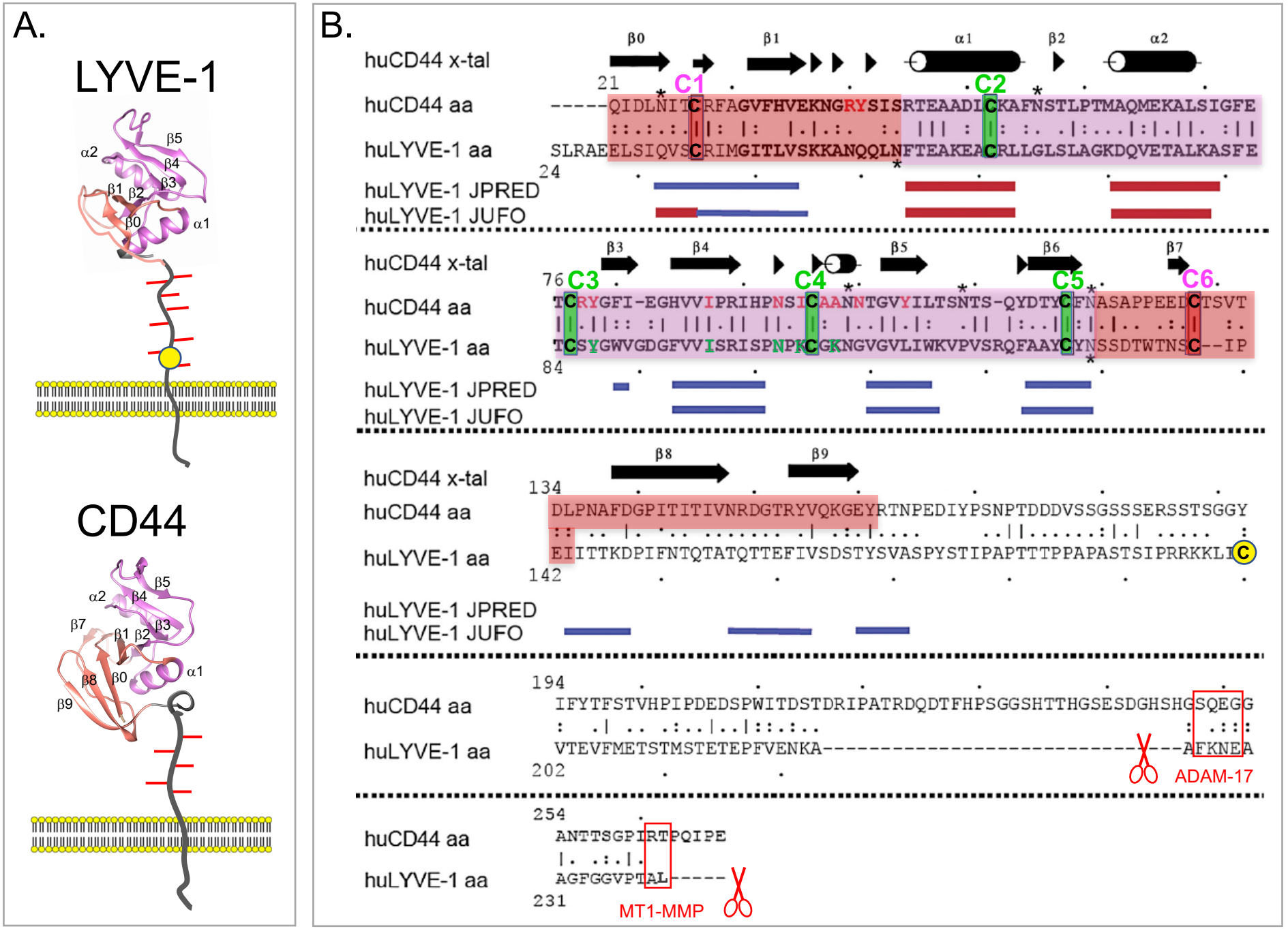

A.
LYVE-1
α2
β5
β4
β3
β1 β2
β0
α1
CD44
α2
β5
β4
β7
β3
β1 β2
β8
β0
α1
β9
B.
huCD44 x-tal
huCD44 aa
huLYVE-1 aa
huLYVE-1 JPRED
huLYVE-1 JUFO
C1
C2
C3
C4
C5
C6
ADAM-17
MT1-MMP

Fig 1

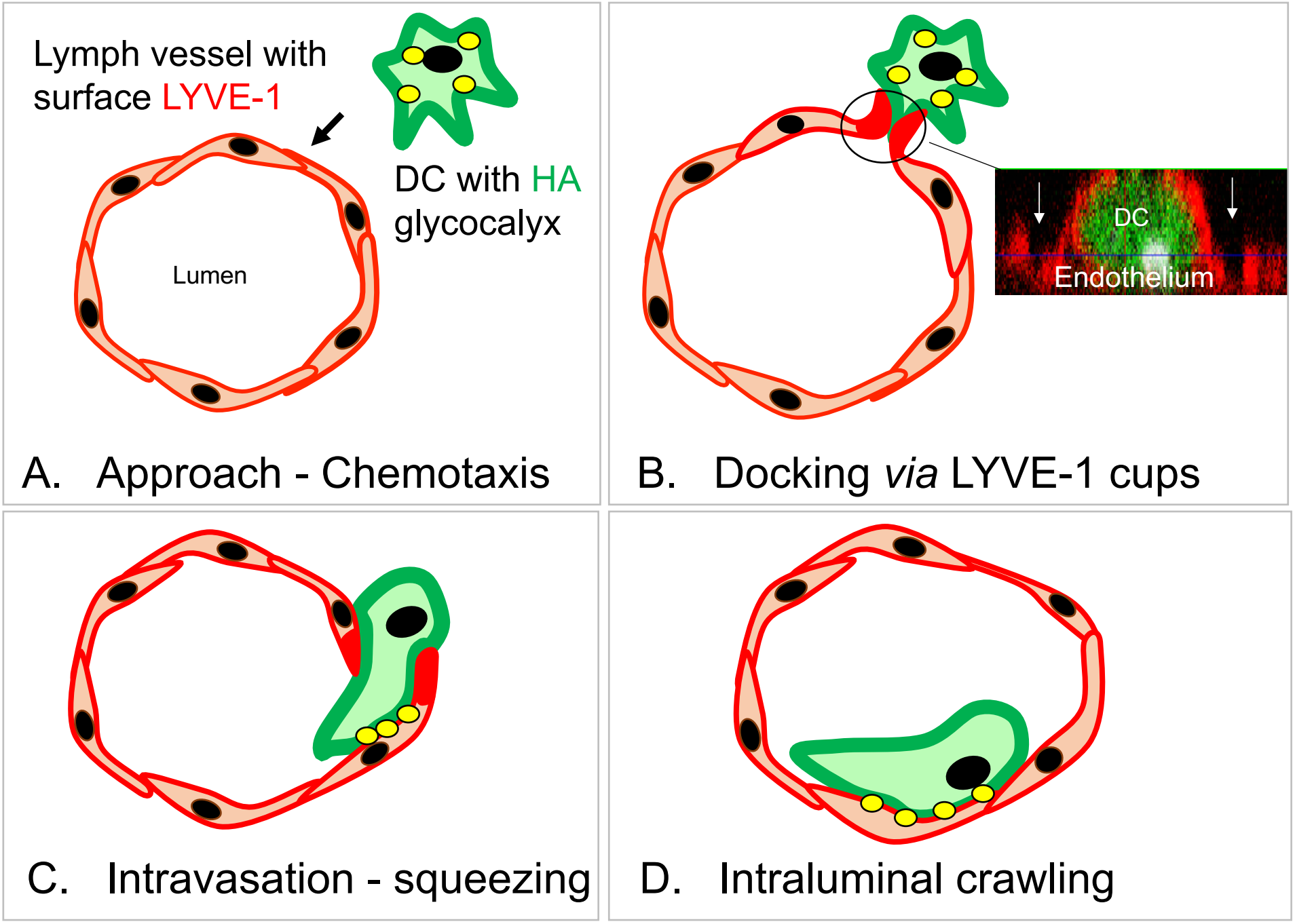
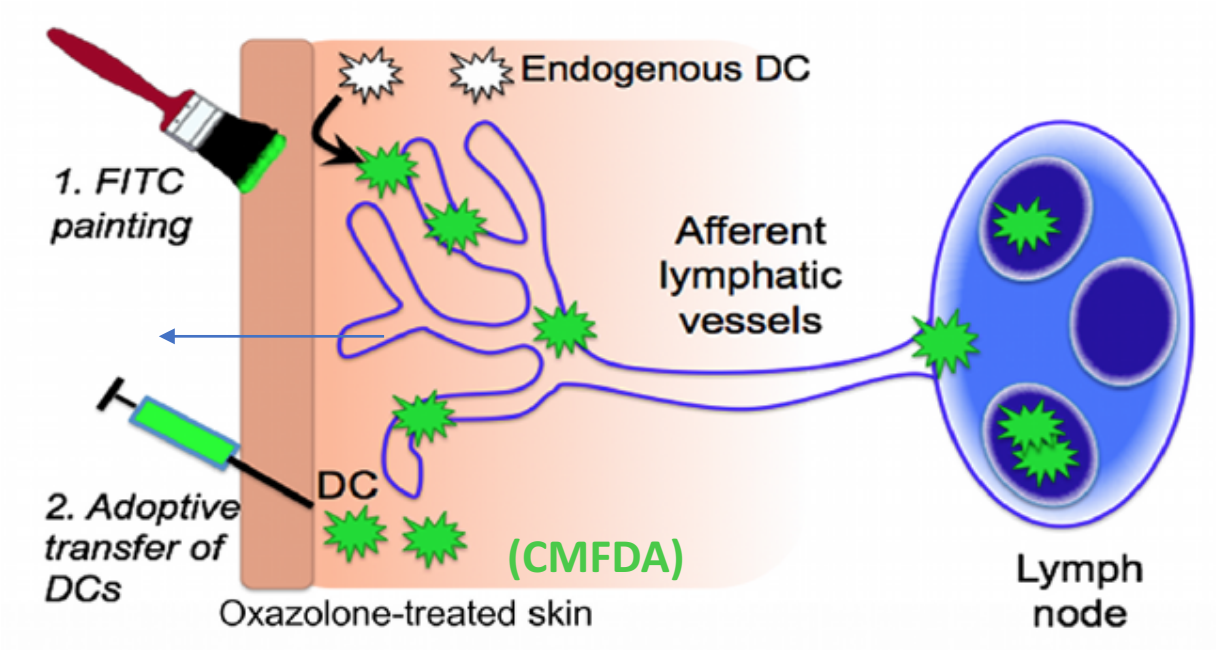

Lymph vessel with surface LYVE-1
DC with HA glycocalyx
Lumen
DC
Endothelium
A. Approach - Chemotaxis
B. Docking *via* LYVE-1 cups
C. Intravasation - squeezing
D. Intraluminal crawling

Fig 2

A


Endogenous DC
1. FITC painting
Afferent lymphatic vessels
DC
2. Adoptive transfer of DCs
(CMFDA)
Oxazolone-treated skin
Lymph node

B

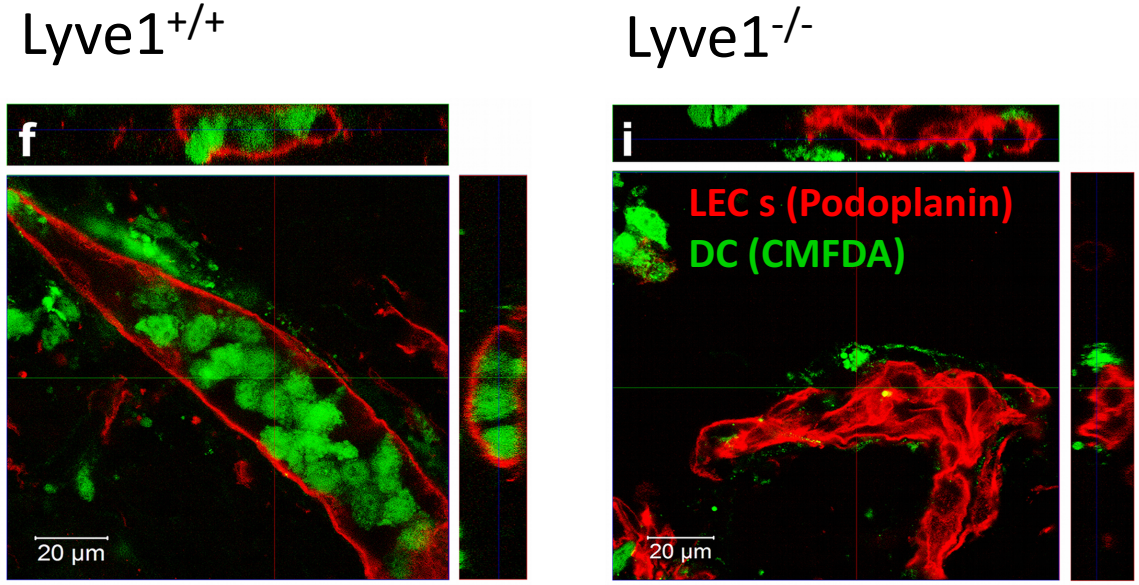

Lyve1+/+
Lyve1-/-
f
i
LEC s (Podoplanin)
DC (CMFDA)
20 µm
20 µm

Fig 3

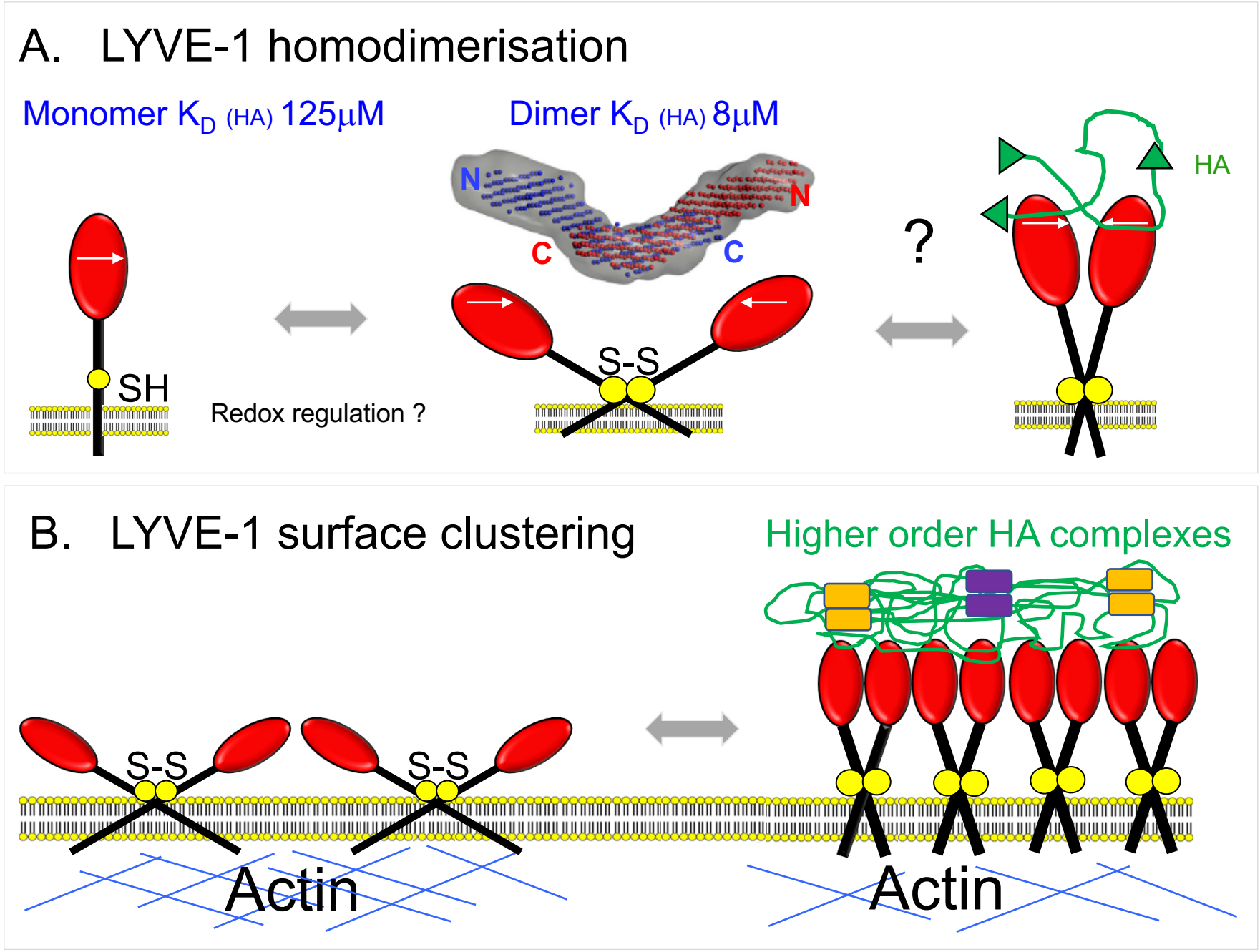
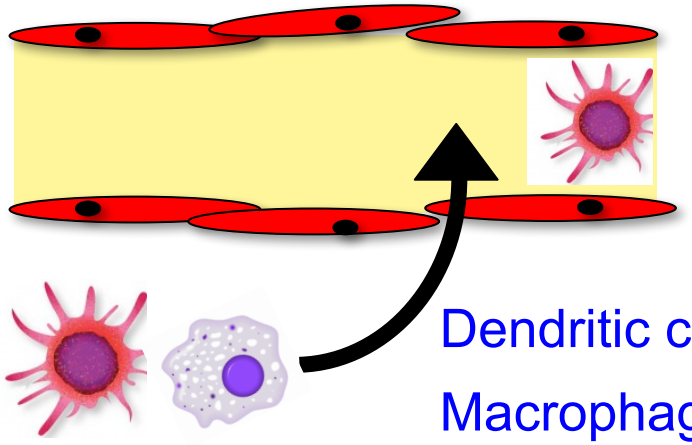

A. LYVE-1 homodimerisation
Monomer K_D (HA) 125µM
Dimer K_D (HA) 8µM
N
N
C
C
SH
S-S
Redox regulation ?
?
HA
B. LYVE-1 surface clustering
Higher order HA complexes
S-S
S-S
Actin
Actin

Fig 4

LYVE-1•HA

Leucocyte entry to lymph - Migration to lymph nodes

Low shear ~ 5μN (0.5 dynes) /cm^2



Dendritic cells

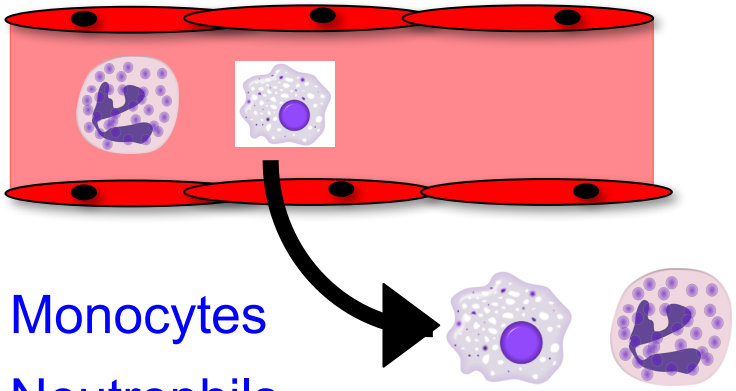
Macrophages

- HA is on leucocyte surface
- LYVE-1 is on endothelium
- Receptor functions as homodimer
- Fast binding kinetics
- Low-friction Sliding interaction with HA
- Amoeboid migration and intravasation

CD44•HA

Leucocyte exit from blood - Recruitment to inflamed tissue

High shear ~ 50μN (5 dynes) /cm^2



Monocytes

Neutrophils

- CD44 is on leucocyte surface
- HA is on endothelium
- Receptor functions as monomer
- Slower binding kinetics
- Velcro-like interaction with HA
- Rolling adhesion and extravasation

Fig 5