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Harnessing the Lymph Node Microenvironment

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Abstract

Purpose of review: To evaluate role of the lymph node (LN) in immune regulation and tolerance in transplantation and recent advances in the delivery of antigen and immune modulatory signals to the LN.

Recent findings: LNs are a primary site of immune cell priming, activation, and modulation, and changes within the LN microenvironment have the potential to induce specific regulation, suppression, and potentially tolerance. Antigen enters the LN either from tissues via lymphatics, from blood via high endothelial venules, or directly via injection. Here we review different techniques and materials to deliver antigen to the LN including microparticles or nanoparticles, *ex vivo* antigen presenting cell manipulation, and use of receptor conjugation for specific intra-LN targeting locations.

Summary: The promising results point to powerful techniques to harness the LN microenvironment and direct systemic immune regulation. The materials, techniques, and approaches suggest that translational and clinical trials in non-human primate and patients may soon be possible.

Keywords

Lymph node; suppression; tolerance; microenvironment; nanoparticles; microparticles

Introduction

Transplantation has made incredible advances in past sixty years since the development of the first immunosuppressants. Immunosuppression used clinically today focuses on controlling the “positive” immune response of activated T cells and donor-specific antibodies. These defensive strategies are not effective to induce tolerance, rely upon patients taking multiple medications daily, and have many side effects. Here, we shift our attention to the regulatory immune response and, specifically, the role of the LN.

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None.

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LNs are the site of antigen presentation and activation of T and B lymphocytes. The development of cell-mediated, antibody-mediated, or regulatory immunity is modulated by different kinetics and combinations of immune signals. These signals alter the local microenvironment to promote cell interactions that translate into a systemic response [1], [2]. Thus, modulation of the LN microenvironment and its interaction with antigen has the potential to induce specific regulation and suppression and, eventually, tolerance.

Lymph Node Structure and Function

Secondary lymphoid organs (SLO) form at predetermined locations and are highly dynamic structures. A review of the path an antigen may take through the LN highlights potential targets that may be exploited for the future development of immune regulatory regimens (Table 1).

Two Modes of Entry into the LN

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Antigen, which is either soluble or phagocytosed within an antigen presenting cell (APC), enters the LN via one of two routes: from tissues to the afferent lymphatics or from blood through the high endothelial venules (HEV). The manner in which a lymphocyte enters the LN dictates with which cell types it interacts, and ultimately its fate and response to antigen [13]. It is worth noting that naïve T cells primarily enter LNs while memory or activated T cells may bypass LN and traffick directly to sites of inflammation and antigen deposition. Nonetheless, if appropriate chemotactic and adhesions signals are present, both central and effector memory T cells can migrate to LNs. Thus, the approaches presented below may be applicable to primary and anamnestic responses as long as the different types of T cells are accounted for.

Lymphatic Vessels—Lymph drains from the interstitial space, enters lymphatic vessels, and is transported to LNs [3], [48]. CCL21 constitutively expressed on lymphatic vessels and CCR7 on T cells drives lymphocytes to the draining LN [5*], [20], [22]. Afferent lymph under homeostatic conditions is relatively low in cell density and comprised of mainly CD4⁺ T cells with effector memory phenotype and DC, with a smaller proportion of monocytes, B cells, and granulocytes [5*]. Regulatory T cell (Treg) migration from allografts to draining LNs is mediated by CCR2, CCR5, and CXCR3 [39], [43]. Treg migration to the draining LN is a prerequisite for efficient suppression [21*], [38], [39], [42], [43]. After reaching the afferent lymphatics of a draining LN, lymph travels around the periphery of the LN via the subcapsular sinus, through trabecular sinuses, and toward medullary sinuses, moving across lymphatic endothelial cells (LEC) into the LN parenchyma [5*], [17], [49].

High Endothelial Venules (HEV)—The second mode of entry into LNs is from the blood across specialized cuboidal endothelial venous cells, the high endothelial venules (HEV). Migration into the parenchyma involves a multi-step adhesion cascade [8], mediated by the chemokine ligands CCL19 and CCL21 on HEV and their receptor, CCR7, on many leukocytes.

Reticular Meshwork: a Specialized Stroma

The HEV and lymphatic sinuses are connected to the LN parenchyma by stroma. The specialized stroma, summarized in Table 1, is made of porous, reticulated fibers and extracellular matrix components that facilitate immune cell migration [19]. Two stromal cell types, LEC and FRC, are highlighted below.

Lymphatic Endothelial Cells (LEC)—LECs line lymphatic vessels and secrete of sphingosine 1-phosphate (S1P) which binds to S1PR1 on T cells. S1P-S1PR1 signaling regulates T cell LN migration and maintains T cell mitochondrial content, providing energy for constant migration and regulating the number of LN T cells [14], [23*]. Under inflammatory conditions, LECs upregulate chemokine expression to fine-tune leukocyte recruitment to the lymphatic vessels [15], [16], [23*] (Table 1). LECs use transcellular, in addition to paracellular pathways, to regulate solute transport into the LN in times of high flow, such as inflammation or injury [50].

Fibroblastic Reticular Cells (FRC)—FRCs line the lymphatic sinus and are specialized myofibroblasts. The FRC network acts as a scaffold that allows DC and T cells to traffic throughout the LN [27], [30]. FRC generate a CCL21 gradient that promotes T cell retention via CCR7 [5*], [36], [51]. Podoplanin (PDPN or gp38) expression on FRC regulates contractility [28], [26], that alters the spacing of FRC and T cells within the LN.

Zones of the LN

The LN can be divided into four major zones: medulla, paracortex, cortical ridge, and cortex.

Medulla—Antigen-presenting macrophages reside with the subcapsular sinus [52] and regulate lymphocyte entrance into LN parenchyma via MSR1 [6*] and antigen specific cytotoxic T lymphocyte (CTL) responses via CD169 [34]. The innermost medulla is the point of entry from afferent for immune cells.

Resident DC migrate to the paracortex (T cell zone) while migratory DC reside within the lymphatic sinus [53], [54], [55]. DC positioning regulate interaction with antigens and the extent and quality of T cell responses [56].

Paracortex—The paracortex is known as the T cell zone. Naïve CD4⁺ T migration from the medulla toward the paracortex is CCR7 dependent [36].

Cortical Ridge—The cortical ridge is between the regions of the HEV, the paracortex, and the germinal centers of the cortex. DC present antigen to activate helper and cytotoxic T cells [31].

Cortex—The cortex contains T and B cell zones, and in particular the germinal centers, the sites of B cell stimulation and maturation to antibody secreting cells [1], [44].

LN Exit

Lymphocytes collect in the medulla of the LN and exit via the efferent lymphatics, which express endomucin, a ligand that prevents leukocyte adhesion [5*] [6*], [57]. T cell egress from the LN is mediated by the low concentration of S1P within the LN and toward the higher levels within lymph, a process regulated by T cell expression of S1PR1 and CD69 [45], [47]. T cell retention in the LN is promoted by leukocyte function associated antigen (LFA-1), common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1), and macrophage mannose receptor (MR) with its ligand L-selectin [5*]. T cell retention in the LN is also promoted by the drug FTY720 (fingolimod) [59]

After resolution of an immune response and elimination of the antigen, LNs resume a period of quiescence [58], in which the structure and content are altered relative to the pre-immune activation state [1].

Targeting Approach to a LN and Current Technologies

The approaches to target LNs mirror the entrance into a LN: interstitial tissue via afferent lymphatics, peripheral blood via the HEV, or direct entry via bioengineering techniques (Figure 1). It is worth noting that for all these approaches, addition of FTY720 may permit enhanced trafficking and retention of T cells within the LN [59], and thus further augment exposure to immune regulatory bioengineering methods and materials.

Peripheral Tissue to Draining LN

Nanoparticles (NP)—Small molecules enter lymphatic vessels either passively or after phagocytosis by APC, travel via interstitial flow into lymphatic capillaries, and drain into regional LNs [60]. One technique for targeting LNs uses nanoparticles (NP). NP are generally a collection of molecules between 1 nm – 100 nm, and can be solid, liquid, or gaseous [61]. NP flow and lymphatic uptake is influenced by particle size, hydrophobicity, charge, and weight [62]. NP measuring 25 nm efficiently and preferentially are taken up by LN resident macrophages and DC, compared to larger (100 nm) nanoparticles [60]. Most NP for medical applications are dispersed in a liquid and form micelles or emulsions. Micelles are a collection of amphiphilic surfactant molecules that aggregate in water and form a hydrophobic center, whereas nanoemulsions are two-phase mixtures consisting of a hydrophobic liquid core stabilized by a surfactant [61].

NP can be delivered by many routes (intramuscular (IM), subcutaneous (SC), hypodermic, microneedles [63], intraperitoneal (IP), or oral (PO). Microneedle patches may be used as a transcutaneous delivery system for NP in contrast to hypodermic needle [64*]. Oral administration is facilitated by self-emulsifying drug delivery systems (SEDDS), isotropic mixtures of drug, lipid, and surfactants that self-emulsify in the gastrointestinal tract and are passively absorbed into intestinal lymphatics [62].

To promote lymphatic drug delivery, nanosized polymer-based carriers have been developed to encapsulate drugs. Polyethylene glycol (PEG) modification nanoemulsions lower toxicity, increase half-life, and decrease immunogenicity by allowing the glycol units to form associations with water molecules and create a hydrophilic layer that prevents protein

adsorption and clearance by macrophages. Micelles, which encapsulate hydrophobic drugs, can be injected intradermally, drained via lymphatics, and enter draining LN efficiently to exert immunosuppressive effects [65].

NP have been extensively studied in vaccine development. Immunization subcutaneously of mice with an antigen and NP containing toll-like receptor (TLR) ligands (TLR4 and TLR7 ligands) enhanced antibody and T cell responses compared to immunization with a soluble antigen [66]. Blocking this pathway in a transplant model may alter the microenvironment, dampen T cell responses, and promote tolerance.

Typical limitations to peripheral tissue injection of NP, such as sequestration by the mononuclear phagocyte system (MPS) and nonspecific distribution in SLO [67], may play a less significant role when considering the use of NP to target LN. However, because lymphatic uptake and flow is a passive process and because NP may still undergo nonspecific cellular internalization [68], NP pharmacokinetics remains a major limitation to this approach.

Another major limitation of NP is the lack of site-specific bioavailability. Thus, NP conjugated with ligands to target specific cells have been developed. Potential targets for LN and lymphatics include LYVE-1, CLEVER-1, and MSR1 (Table 1). Opsonization, as proteins bind to the NP surface, form a protein corona, defunctionalize surface ligands, and reduce the NP bioavailability [69], [70]. Liu et al. developed a lipophilic oligonucleotide-albumin conjugate to exploit albumin “hitch hiking”, and produced a potent vaccine [71]. An approach to antigen-specific tolerance in transplantation may be through use of these amphiphilic conjugates that efficiently accumulate in the LN.

Exosomes & Macrophages—M1 macrophages secrete proinflammatory cytokines to support the T_H1 immune response, while M2 macrophages support Th2 immunity, suppressing immune response and promoting wound healing [72**]. This is due, in part, to the secretion by these cells of exosomes. Exosomes are similar in physical property to nanoparticles and liposomes, which consist of an amphiphilic bilayer with an aqueous core, and could be considered a “natural” NP.

Intra-LN macrophages and DC take up these exosomes, although the overall percent of cells within a LN that take up exosomes is small (~1%) [72**]. For vaccine development, M1 exosomes enhance the efficacy of the immune response to antigen [72**], and M2 exosomes may correspondingly alter the LN microenvironment to promote immune suppression when paired with a specific donor antigen. Marino et al. and Liu et al. showed that exosomes carry both donor and recipient MHC molecules, a phenomenon termed “cross-dressing”, to the LN and initiate allograft rejection [73*], [74] These findings are in contrast to the passenger leukocyte theory, which states that allograft rejection is initiated by recipient T cells recognizing donor leukocytes that traveled via lymphatics or blood to the regional LN [73*]. In a murine transplant model, they demonstrated a large number of “cross-dressed” recipient APCs (i.e. $CD11c^+$ DC and $CD20^+$ B cells) within the draining LN and spleen following transplantation, compared to a small number of donor leukocytes [73*].

NP coated with self-peptides bound to MHC class II molecules generate and expand an antigen specific CD4⁺ Treg type 1-like cells that abrogates autoimmunity [75] This approach could be applied to transplant recipients with donor-specific antigens loaded on to MHC-class II-NP complexes.

Intraperitoneal (IP) Route to LNs

IP injection represents a special case of peripheral tissue injection because antigen, taken up by APC or soluble, may drain toward a LN or enter the vascular system. Furthermore, mesenteric LN and Peyer's Patches have unique features compared to peripheral LN, including innate lymphoid cells, from mucosa-associated tissue [76], [77].

Shirali et al. demonstrated that after IP injection, mycophenolic acid-coated NP were internalized by DC and macrophages that then trafficked to the spleen and LNs. These NPs delayed allograft rejection and at a log-fold lower dose than systemic mycophenolic acid [78]. Within LN and spleen, mice had significantly increased PD-L1 expression by DC, suggesting this delivery technique leads to tolerogenic changes within the LN [78]. Similar results have been demonstrated with tacrolimus and cyclosporin A (CsA) NP, which suppressed effector T cells and decreased T cell proliferation [62], [79], [80].

Peripheral Blood to LN

Active LN Targeting via HEV—Naïve and memory T cells circulate between blood and LNs, and lymphocyte trafficking to the LN is regulated by lymphocyte L-selectin [9], [10], [81] and HEV peripheral node addressins (PNAds). PNAd expression is increased in draining LNs following transplantation [82*]. Azzi et al. developed an immunoregulatory microparticle (MP) to target the LN using an antibody that binds PNAd to deliver tacrolimus. This MP prolonged murine cardiac allograft survival [82*]. Increased survival was associated with a reduction of CD4⁺ effector T cells in the LNs [82*]. The trafficking of only 0.4 – 1.6% of the MP to the draining LN was low, probably related to its large size (2-5 µm) and the physical constraints of the FRC molecular sieve [29]. The results suggest that further development using PNAd or other HEV molecular markers (Table 1) will focus on engineering of MP physical characteristics.

Generation of antigen-specific Treg within the LN—Because the tolerogenic effects in transplantation are often to be due to Tregs, another approach to altering the LN microenvironment centers on the generation of antigen-specific Tregs. This has been demonstrated in a chronic diabetic autoimmune murine model in which IV delivery of NP coated with disease relevant peptide and MHC promoted Treg development and suppressed antigen-specific presentation by APC within the LN [83].

The aryl hydrocarbon receptor (AhR) pathway in DC, which can be induced with a mucosal ligand (ITE), controls the generation and differentiation of Tregs and Th17 cells [84], [85]. Yeste et al. treated DC with NP that co-administered antigen with ITE *ex vivo*, thereby activating the AhR pathway, and induced a tolerogenic phenotype via expansion of FoxP3 Tregs *in vivo* [86]. While the intra-LN Tregs were not evaluated in this study, it is

conceivable that this technique may influence the microenvironment of the LN after IV administration.

Manipulation of APC ex-vivo—In response to infection or inflammation, DC mature, upregulate MHC and costimulatory molecules, and produce a variety of cytokines that promote the activation of naïve T cells and their differentiation into effector T cells, a mechanism that has been exploited (*in vitro* or *in situ*) for cancer and infectious vaccine development [87], [88], [89]. For tolerance development in transplantation, manipulation of APCs *ex-vivo* has been performed using donor-specific transfusion (DST) or IV injection of antigen-coupled ethylene carbodiimide splenocytes (ECDI-SP) techniques.

DC maturation is inhibited by Tregs via CTLA-4 and LAG3 co-inhibitory ligands, TGF- β signaling, and IL-2 [90]. Leukemia inhibitory factor (LIF) is a member of the IL-6 family that promotes Treg development and immune tolerance [91], and the use of LIF NP-bound APC with DST significantly, though modestly, prolonged allograft survival in a murine cardiac transplant model, which correlated with an expansion of antigen-specific FoxP3⁺ cells within LN in murine and NHP *in vitro* models [92].

ECDI-SP can induce tolerance to autoantigen in transplant models by recipient CD11c⁺ DCs that internalize donor ECDI-SP and selectively upregulate negative costimulatory molecules (PD-L1 and PD-L2) in the spleen and increase Tregs in draining LN [93], [94], [95] [96]. This approach was shown to be safe and feasible in a phase 1 trial for multiple sclerosis patients [97]; however, the complexity required using *ex vivo* manipulation and high numbers of the cellular carriers for the ECDI-SP technique has since shifted focus toward biodegradable NP.

Direct LN Manipulation

Because the drainage and trafficking of antigen from peripheral tissue to a draining LN is variable and relatively inefficient [32], one mechanism that bypasses this process is the direct deposition of antigen via intra-LN (i.L.N.) injection [98], [99]. This method concentrates the antigen of interest at the site of T and B cell priming, which may drive more encounters of the injected antigen with the resident APC population and promote tolerance [100]. Tumor (“foreign”) cells directly injected into LN demonstrated swift rejection secondary to the generation of CTLs; however, tolerance was observed when tumors developed outside of the LN and tumor antigen was cross-presented to CD8⁺ T cells in the draining LN [101]. The tolerogenic milieu is associated with plasmacytoid DC [102] and CD4⁺ and CD8⁺ Treg cell subtypes [103]. Given these encouraging results, a phase I clinical trial for i.L.N. allergen immunotherapy is on-going ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01982474) Identifier NCT01982474).

Our group has explored the generation of double emulsion MP that encapsulates rapamycin, a peptide fragment specific for the pathogenesis of multiple sclerosis (MS), or the combination of both and the direct LN injection of these MP [98], [104]. Recently we demonstrated in a murine model of MS that a single i.L.N. dose of MP with peptide fragment and rapamycin, given at the peak of disease, significantly reversed the MS disease severity as measured by clinical limb paralysis scores [105**]. Furthermore, these results

were associated with the polarization of CD4⁺ T cells toward a Treg phenotype within the cortical ridge of the LN and in non-injected tissue (i.e. spleen, pooled LNs), decreased IL-17 and GM-CSF production, and antigen-specific tolerance [105**].

The limitations of i.L.N. injection include the rapid clearance of antigen by flushing of afferent lymph [106], technical challenges of LN mapping, and uncertainty of exact injection within the LN zones. Perhaps as microneedle development progresses, this technique could be utilized to yield a more consistent and evenly dispersed MP distribution within the node. This method also is dependent on the delivery of encapsulated, disease relevant self-antigen [105**], which in our current transplantation paradigm might only be applied to planned living donor transplantation.

Conclusions

LNs are the primary site of immune cell priming, activation, and modulation. Direct targeting of LNs may be accomplished by considering the mechanisms of entry into the LN: from peripheral tissue via lymphatic vessels, from blood via HEV, or via direct injection. The use of NP, MP, and similar biotechnologies has allowed exploration and discovery of how manipulations of the LN microenvironment may affect systemic antigen-specific immune responses. While the application of many of these techniques is still in its infancy for transplantation, the delivery of approved immunosuppressive medications via NP is feasible and may help to reduce the systemic burden of medications currently required by most transplant recipients. More novel techniques like harnessing M2 exosomes or exploring clinical application of i.L.N. injection may be further away from clinical trials; however, they hold significant potential for promoting tolerogenic microenvironments following transplantation.

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References:

1. Katakai T, Hara T, Sugai M, Gonda H, Shimizu A. Lymph node fibroblastic reticular cells construct the stromal reticulum via contact with lymphocytes. *J Exp Med* 2004 9 20;200(6):783–795. [PubMed: 15381731]
2. Warren KJ, Iwami D, Harris DG, Bromberg JS, Burrell BE. Laminins affect T cell trafficking and allograft fate. *J Clin Invest* 2014 5;124(5):2204–2218. [PubMed: 24691446]
3. Swartz MA, Lund AW. Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat Rev Cancer* 2012 2 24;12(3):210–219. [PubMed: 22362216]
4. Forster R, Braun A, Worbs T. Lymph node homing of T cells and dendritic cells via afferent lymphatics. *Trends Immunol* 2012 6;33(6):271–280. [PubMed: 22459312]

- 5*. Hunter MC, Teixeira A, Halin C. T Cell Trafficking through Lymphatic Vessels. *Front Immunol* 2016 12 21;7:613. [PubMed: 28066423] Review that thoroughly explores different mechanisms and molecular pathways involved in T cells trafficking specifically through lymphatic vessels and into the lymph node.
- 6*. Iftakhar-E-Khuda I, Fair-Makela R, Kukkonen-Macchi A, Elimä K, Karikoski M, Rantakari P, et al. Gene-expression profiling of different arms of lymphatic vasculature identifies candidates for manipulation of cell traffic. *Proc Natl Acad Sci U S A* 2016 9 20;113(38):10643–10648. [PubMed: 27601677] Identifies unique molecular markers of afferent (CD169 and MSR1) and efferent (endomucin) lymphatic endothelium
7. Johnson LA, Banerji S, Lawrance W, Gileadi U, Prota G, Holder KA, et al. Dendritic cells enter lymph vessels by hyaluronan-mediated docking to the endothelial receptor LYVE-1. *Nat Immunol* 2017 7;18(7):762–770. [PubMed: 28504698]
8. Girard JP, Moussion C, Forster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat Rev Immunol* 2012 11;12(11):762–773. [PubMed: 23018291]
9. Carlow DA, Gossens K, Naus S, Veerman KM, Seo W, Ziltener HJ. PSGL-1 function in immunity and steady state homeostasis. *Immunol Rev* 2009 7;230(1):75–96. [PubMed: 19594630]
10. Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. *Immunol Rev* 2009 7;230(1):97–113. [PubMed: 19594631]
11. Ruddle NH. High Endothelial Venules and Lymphatic Vessels in Tertiary Lymphoid Organs: Characteristics, Functions, and Regulation. *Front Immunol* 2016 11 9;7:491. [PubMed: 27881983]
12. Ager A High Endothelial Venules and Other Blood Vessels: Critical Regulators of Lymphoid Organ Development and Function. *Front Immunol* 2017 2 3;8:45. [PubMed: 28217126]
13. Brinkman CC, Burrell BE, Iwami D, Nakayama Y, Warren KJ, Xiong Y, et al. Anatomy of tolerance. *Curr Opin Organ Transplant* 2013 8;18(4):393–401. [PubMed: 23838643]
14. Fang V, Chaluvadi VS, Ramos-Perez WD, Mendoza A, Baeyens A, Rivera R, et al. Gradients of the signaling lipid SIP in lymph nodes position natural killer cells and regulate their interferon-gamma response. *Nat Immunol* 2017 1;18(1):15–25. [PubMed: 27841869]
15. Johnson LA, Clasper S, Holt AP, Lalor PF, Baban D, Jackson DG. An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium. *J Exp Med* 2006 11 27;203(12):2763–2777. [PubMed: 17116732]
16. Vigl B, Aebischer D, Nitschke M, Iolyeva M, Rothlin T, Antsiferova O, et al. Tissue inflammation modulates gene expression of lymphatic endothelial cells and dendritic cell migration in a stimulus-dependent manner. *Blood* 2011 7 7;118(1):205–215. [PubMed: 21596851]
17. Card CM, Yu SS, Swartz MA. Emerging roles of lymphatic endothelium in regulating adaptive immunity. *J Clin Invest* 2014 3;124(3):943–952. [PubMed: 24590280]
18. Chang JE, Turley SJ. Stromal infrastructure of the lymph node and coordination of immunity. *Trends Immunol* 2015 1;36(1):30–39. [PubMed: 25499856]
19. Kedl RM, Tamburini BA. Antigen archiving by lymph node stroma: A novel function for the lymphatic endothelium. *Eur J Immunol* 2015 10;45(10):2721–2729. [PubMed: 26278423]
20. Riedel A, Shorthouse D, Haas L, Hall BA, Shields J. Tumor-induced stromal reprogramming drives lymph node transformation. *Nat Immunol* 2016 9;17(9):1118–1127. [PubMed: 27400148]
- 21*. Brinkman CC, Iwami D, Hritzo MK, Xiong Y, Ahmad S, Simon T, et al. Treg engage lymphotoxin beta receptor for afferent lymphatic transendothelial migration. *Nat Commun* 2016 6 21;7:12021. [PubMed: 27323847] With an islet allograft transplant model, Tregs specifically use lymphotoxin to migrate from the allograft to draining LN.
22. Vaahhtomeri K, Brown M, Hauschild R, De Vries I, Leithner AF, Mehling M, et al. Locally Triggered Release of the Chemokine CCL21 Promotes Dendritic Cell Transmigration across Lymphatic Endothelia. *Cell Rep* 2017 5 2;19(5):902–909.
- 23*. Mendoza A, Fang V, Chen C, Serasinghe M, Verma A, Muller J, et al. Lymphatic endothelial SIP promotes mitochondrial function and survival in naïve T cells. *Nature* 2017 6 1;546(7656): 158–161. [PubMed: 28538737] Demonstrates that SIP is essential for T cell migration and trafficking to LNs because SIP signaling pathway provides energy to naïve T cell mitochondria.

24. Malhotra D, Fletcher AL, Astarita J, Lukacs-Kornek V, Tayalia P, Gonzalez SF, et al. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat Immunol* 2012 4 1;13(5):499–510. [PubMed: 22466668]
25. Dubrot J, Duraes FV, Potin L, Capotosti F, Brighthouse D, Suter T, et al. Lymph node stromal cells acquire peptide-MHCII complexes from dendritic cells and induce antigen-specific CD4(+) T cell tolerance. *J Exp Med* 2014 6 2;211(6):1153–1166. [PubMed: 24842370]
26. Astarita JL, Cremasco V, Fu J, Darnell MC, Peck JR, Nieves-Bonilla JM, et al. The CLEC-2-podoplanin axis controls the contractility of fibroblastic reticular cells and lymph node microarchitecture. *Nat Immunol* 2015 1;16(1):75–84. [PubMed: 25347465]
27. Brown FD, Turley SJ. Fibroblastic reticular cells: organization and regulation of the T lymphocyte life cycle. *J Immunol* 2015 2 15;194(4):1389–1394. [PubMed: 25663676]
28. Kumar V, Dasoveanu DC, Chyou S, Tzeng TC, Rozo C, Liang Y, et al. A dendritic-cell-stromal axis maintains immune responses in lymph nodes. *Immunity* 2015 4 21;42(4):719–730. [PubMed: 25902483]
29. Rantakari P, Auvinen K, Jappinen N, Kapraali M, Valtanen J, Karikoski M, et al. The endothelial protein PLVAP in lymphatics controls the entry of lymphocytes and antigens into lymph nodes. *Nat Immunol* 2015 4;16(4):386–396. [PubMed: 25665101]
30. Novkovic M, Onder L, Cupovic J, Abe J, Bomze D, Cremasco V, et al. Topological Small-World Organization of the Fibroblastic Reticular Cell Network Determines Lymph Node Functionality. *PLoS Biol* 2016 7 14;14(7):e1002515. [PubMed: 27415420]
31. Cremasco V, Woodruff MC, Onder L, Cupovic J, Nieves-Bonilla JM, Schildberg FA, et al. B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. *Nat Immunol* 2014 10;15(10):973–981. [PubMed: 25151489]
32. Andorko JI, Hess KL, Jewell CM. Harnessing biomaterials to engineer the lymph node microenvironment for immunity or tolerance. *AAPS J* 2015 3;17(2):323–338. [PubMed: 25533221]
33. Gray EE, Cyster JG. Lymph node macrophages. *J Innate Immun* 2012;4(5–6):424–436. [PubMed: 22488251]
34. Komohara Y, Ohnishi K, Takeya M. Possible functions of CD169-positive sinus macrophages in lymph nodes in anti-tumor immune responses. *Cancer Sci* 2017 3;108(3):290–295. [PubMed: 28002629]
35. Kumamoto Y, Hirai T, Wong PW, Kaplan DH, Iwasaki A. CD301b+ dendritic cells suppress T follicular helper cells and antibody responses to protein antigens. *Elife* 2016 9 22;5:10.7554/eLife.17979.
36. Braun A, Worbs T, Moschovakis GL, Halle S, Hoffmann K, Bolter J, et al. Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. *Nat Immunol* 2011 8 14;12(9):879–887. [PubMed: 21841786]
37. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immunol* 2009 6;10(6):595–602. [PubMed: 19412181]
38. Ochando JC, Yopp AC, Yang Y, Garin A, Li Y, Boros P, et al. Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3+ regulatory T cells. *J Immunol* 2005 6 1;174(11):6993–7005. [PubMed: 15905542]
39. Zhang N, Schroppel B, Lal G, Jakubzick C, Mao X, Chen D, et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. *Immunity* 2009 3 20;30(3):458–469. [PubMed: 19303390]
40. Levine AG, Medoza A, Hemmers S, Moltedo B, Niec RE, Schizas M, et al. Stability and function of regulatory T cells expressing the transcription factor T-bet. *Nature* 2017 6 15;546(7658):421–425. [PubMed: 28607488]
41. Pabbisetty SK, Rabacal W, Volanakis EJ, Parekh VV, Olivares-Villagomez D, Cendron D, et al. Peripheral tolerance can be modified by altering KLF2-regulated Treg migration. *Proc Natl Acad Sci U S A* 2016 8 9;113(32):E4662–70. [PubMed: 27462110]
42. Huang MT, Lin BR, Liu WL, Lu CW, Chiang BL. Lymph node trafficking of regulatory T cells is prerequisite for immune suppression. *J Leukoc Biol* 2016 4;99(4):561–568. [PubMed: 26543091]

43. Xiong Y, Ahmad S, Iwami D, Brinkman CC, Bromberg JS. T-bet Regulates Natural Regulatory T Cell Afferent Lymphatic Migration and Suppressive Function. *J Immunol* 2016 3 15;196(6):2526–2540. [PubMed: 26880765]
44. Weinstein JS, Herman EI, Lainez B, Licona-Limon P, Esplugues E, Flavell R, et al. TFH cells progressively differentiate to regulate the germinal center response. *Nat Immunol* 2016 10;17(10):1197–1205. [PubMed: 27573866]
45. Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y, Cyster JG. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. *Science* 2005 9 9;309(5741):1735–1739. [PubMed: 16151014]
46. Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol* 2012;30:69–94. [PubMed: 22149932]
47. Shiow LR, Rosen DB, Brdickova N, Xu Y, An J, Lanier LL, et al. CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* 2006 3 23;440(7083):540–544. [PubMed: 16525420]
48. Chary SR, Jain RK. Direct measurement of interstitial convection and diffusion of albumin in normal and neoplastic tissues by fluorescence photobleaching. *Proc Natl Acad Sci U S A* 1989 7;86(14):5385–5389. [PubMed: 2748592]
49. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. *Nat Rev Immunol* 2003 11;3(11):867–878. [PubMed: 14668803]
50. Triacca V, Guc E, Kilarski WW, Pisano M, Swartz MA. Transcellular Pathways in Lymphatic Endothelial Cells Regulate Changes in Solute Transport by Fluid Stress. *Circ Res* 2017 4 28;120(9):1440–1452. [PubMed: 28130294]
51. Worbs T, Mempel TR, Bolter J, von Andrian UH, Forster R. CCR7 ligands stimulate the intranodal motility of T lymphocytes in vivo. *J Exp Med* 2007 3 19;204(3):489–495. [PubMed: 17325198]
52. Phan TG, Green JA, Gray EE, Xu Y, Cyster JG. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nat Immunol* 2009 7;10(7):786–793. [PubMed: 19503106]
53. Gerner MY, Torabi-Parizi P, Germain RN. Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens. *Immunity* 2015 1 20;42(1):172–185. [PubMed: 25607462]
54. Hampton HR, Bailey J, Tomura M, Brink R, Chtanova T. Microbe-dependent lymphatic migration of neutrophils modulates lymphocyte proliferation in lymph nodes. *Nat Commun* 2015 5 14;6:7139. [PubMed: 25972253]
55. Russo E, Teijeira A, Vaahomeri K, Willrodt AH, Bloch JS, Nitschke M, et al. Intralymphatic CCL21 Promotes Tissue Egress of Dendritic Cells through Afferent Lymphatic Vessels. *Cell Rep* 2016 2 23;14(7):1723–1734. [PubMed: 26876174]
56. Gerner MY, Casey KA, Kastenmuller W, Germain RN. Dendritic cell and antigen dispersal landscapes regulate T cell immunity. *J Exp Med* 2017 10 2;214(10):3105–3122. [PubMed: 28847868]
57. Zahr A, Alcaide P, Yang J, Jones A, Gregory M, dela Paz NG, et al. Endomucin prevents leukocyte-endothelial cell adhesion and has a critical role under resting and inflammatory conditions. *Nat Commun* 2016 2 2;7:10363. [PubMed: 26831939]
58. Miller MJ, Safrina O, Parker I, Cahalan MD. Imaging the single cell dynamics of CD4+ T cell activation by dendritic cells in lymph nodes. *J Exp Med* 2004 10 4;200(7):847–856. [PubMed: 15466619]
59. Hobson JP, Rosenfeldt HM, Barak LS, Olivera A, Poulton S, Caron MG, et al. Role of the sphingosine-1-phosphate receptor EDG-1 in PDGF-induced cell motility. *Science* 2001 3 2;291(5509):1800–1803. [PubMed: 11230698]
60. Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil CP, et al. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nat Biotechnol* 2007 10;25(10):1159–1164. [PubMed: 17873867]
61. Hussein GA, Pitt WG. Micelles and nanoparticles for ultrasonic drug and gene delivery. *Adv Drug Deliv Rev* 2008 6 30;60(10):1137–1152. [PubMed: 18486269]

62. Cho HY, Choi JH, Oh JJ, Lee YB. Self-Emulsifying Drug Delivery System for Enhancing Bioavailability and Lymphatic Delivery of Tacrolimus. *J Nanosci Nanotechnol* 2015 2;15(2):1831–1841. [PubMed: 26353739]
63. Indermun S, Luttge R, Choonara YE, Kumar P, du Toit LC, Modi G, et al. Current advances in the fabrication of microneedles for transdermal delivery. *J Control Release* 2014 7 10;185:130–138. [PubMed: 24806483]
- 64*. Li J, Zeng M, Shan H, Tong C. Microneedle Patches as Drug and Vaccine Delivery Platform. *Curr Med Chem* 2017;24(22):2413–2422. [PubMed: 28552053] Review of current microneedle technology and potential future applications
65. Dane KY, Nembrini C, Tomei AA, Eby JK, O'Neil CP, Velluto D, et al. Nano-sized drug-loaded micelles deliver payload to lymph node immune cells and prolong allograft survival. *J Control Release* 2011 12 10;156(2):154–160. [PubMed: 21864593]
66. Kasturi SP, Skountzou I, Albrecht RA, Koutsouanos D, Hua T, Nakaya HI, et al. Programming the magnitude and persistence of antibody responses with innate immunity. *Nature* 2011 2 24;470(7335):543–547. [PubMed: 21350488]
67. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol* 2015 9;33(9):941–951. [PubMed: 26348965]
68. Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. *J Control Release* 2010 8 3;145(3):182–195. [PubMed: 20226220]
69. Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat Nanotechnol* 2013 Oct;8(10):772–781.
70. Salvati A, Pitek AS, Monopoli MP, Prapainop K, Bombelli FB, Hristov DR, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat Nanotechnol* 2013 2;8(2):137–143. [PubMed: 23334168]
71. Liu H, Moynihan KD, Zheng Y, Szeto GL, Li AV, Huang B, et al. Structure-based programming of lymph-node targeting in molecular vaccines. *Nature* 2014 3 27;507(7493):519–522. [PubMed: 24531764]
- 72**. Cheng L, Wang Y, Huang L. Exosomes from M1-Polarized Macrophages Potentiate the Cancer Vaccine by Creating a Pro-inflammatory Microenvironment in the Lymph Node. *Mol Ther* 2017 7 5;25(7):1665–1675. [PubMed: 28284981] Evaluated using a M1 exosomes derived from proinflammatory macrophages as an immunopotentiator for cancer vaccine development in a melanoma model.
- 73*. Marino J, Babiker-Mohamed MH, Crosby-Bertorini P, Paster JT, LeGuern C, Germana S, et al. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. *Sci Immunol* 2016 7 14;1(1):aaf8759. In murine heart and islet transplantation models, large numbers of recipient cells displayed donor MHC molecules via donor exosome fusion, and these cells trafficked to the regional LN.
74. Liu Q, Rojas-Canales DM, Divito SJ, Shufesky WJ, Stolz DB, Erdos G, et al. Donor dendritic cell-derived exosomes promote allograft-targeting immune response. *J Clin Invest* 2016 8 1;126(8):2805–2820. [PubMed: 27348586]
75. Clemente-Casares X, Blanco J, Ambalavanan P, Yamanouchi J, Singha S, Fandos C, et al. Expanding antigen-specific regulatory networks to treat autoimmunity. *Nature* 2016 2 25;530(7591):434–440. [PubMed: 26886799]
76. Mackley EC, Houston S, Marriott CL, Halford EE, Lucas B, Cerovic V, et al. CCR7-dependent trafficking of RORgamma(+) ILCs creates a unique microenvironment within mucosal draining lymph nodes. *Nat Commun* 2015 1 9;6:5862. [PubMed: 25575242]
77. Klose CS, Artis D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol* 2016 6 21;17(7):765–774. [PubMed: 27328006]
78. Shirali AC, Look M, Du W, Kassis E, Stout-Delgado HW, Fahmy TM, et al. Nanoparticle delivery of mycophenolic acid upregulates PD-L1 on dendritic cells to prolong murine allograft survival. *Am J Transplant* 2011 12;11(12):2582–2592. [PubMed: 21883921]

79. Azzi J, Tang L, Moore R, Tong R, El Haddad N, Akiyoshi T, et al. Polylactide-cyclosporin A nanoparticles for targeted immunosuppression. *FASEB J* 2010 10;24(10):3927–3938. [PubMed: 20547662]
80. Tang L, Azzi J, Kwon M, Mounayar M, Tong R, Yin Q, et al. Immunosuppressive Activity of Size-Controlled PEG-PLGA Nanoparticles Containing Encapsulated Cyclosporine A. *J Transplant* 2012;2012:896141. [PubMed: 22545201]
81. von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med* 2000 10 5;343(14):1020–1034. [PubMed: 11018170]
- 82*. Azzi J, Yin Q, Uehara M, Ohori S, Tang L, Cai K, et al. Targeted Delivery of Immunomodulators to Lymph Nodes. *Cell Rep* 2016 5 10;15(6):1202–1213. [PubMed: 27134176] Created MP with an antibody (MEC79) that binds PNA^d and targets the HEV of draining LN. While LN overall uptake of the MP was low, the group demonstrated improved allograft survival in association with decreased effector T cells draining toward the lymph node in a murine cardiac allograft model.
83. Tsai S, Shameli A, Yamanouchi J, Clemente-Casares X, Wang J, Serra P, et al. Reversal of autoimmunity by boosting memory-like autoregulatory T cells. *Immunity* 2010 4 23;32(4):568–580. [PubMed: 20381385]
84. Hauben E, Gregori S, Draghici E, Miglia vacca B, Olivieri S, Woisetschlager M, et al. Activation of the aryl hydrocarbon receptor promotes allograft-specific tolerance through direct and dendritic cell-mediated effects on regulatory T cells. *Blood* 2008 8 15;112(4):1214–1222. [PubMed: 18550851]
85. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* 2010 9 15;185(6):3190–3198. [PubMed: 20720200]
86. Yeste A, Nadeau M, Burns EJ, Weiner HL, Quintana FJ. Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 2012 7 10;109(28):11270–11275. [PubMed: 22745170]
87. Tacke PJ, de Vries IJ, Torensma R, Figdor CG. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. *Nat Rev Immunol* 2007 10;7(10):790–802. [PubMed: 17853902]
88. Tenbusch M, Ignatius R, Nchinda G, Trumpfheller C, Salazar AM, Topfer K, et al. Immunogenicity of DNA vaccines encoding simian immunodeficiency virus antigen targeted to dendritic cells in rhesus macaques. *PLoS One* 2012;7(6):e39038. [PubMed: 22720025]
89. Macri C, Dumont C, Johnston AP, Mintern JD. Targeting dendritic cells: a promising strategy to improve vaccine effectiveness. *Clin Transl Immunology* 2016 3 18;5(3):e66. [PubMed: 27217957]
90. Probst HC, Muth S, Schild H. Regulation of the tolerogenic function of steady-state DCs. *Eur J Immunol* 2014 4;44(4):927–933. [PubMed: 24652744]
91. Metcalfe SM, Watson TJ, Shurey S, Adams E, Green CJ. Leukemia inhibitory factor is linked to regulatory transplantation tolerance. *Transplantation* 2005 3 27;79(6):726–730. [PubMed: 15785381]
92. Park J, Gao W, Whiston R, Strom TB, Metcalfe S, Fahmy TM. Modulation of CD4⁺ T lymphocyte lineage outcomes with targeted, nanoparticle-mediated cytokine delivery. *Mol Pharm* 2011 2 7;8(1):143–152. [PubMed: 20977190]
93. Luo X, Pothoven KL, McCarthy D, DeGutes M, Martin A, Getts DR, et al. ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms. *Proc Natl Acad Sci U S A* 2008 9 23;105(38): 14527–14532. [PubMed: 18796615]
94. Getts DR, Turley DM, Smith CE, Harp CT, McCarthy D, Feeney EM, et al. Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1⁺ and IL-10-producing splenic macrophages and maintained by T regulatory cells. *J Immunol* 2011 9 1;187(5):2405–2417. [PubMed: 21821796]
95. Kheradmand T, Wang S, Bryant J, Tasch JJ, Lerret N, Pothoven KL, et al. Ethylenecarbodiimide-fixed donor splenocyte infusions differentially target direct and indirect pathways of

allorecognition for induction of transplant tolerance. *J Immunol* 2012 7 15;189(2):804–812. [PubMed: 22696445]

96. Chen G, Li J, Chen L, Lai X, Qiu J. ECDI-fixed allogeneic splenocytes combined with alpha1-antitrypsin prolong survival of rat renal allografts. *Int Immunopharmacol* 2015 5;26(1):43–49. [PubMed: 25749496]
97. Lutterotti A, Yousef S, Sputtek A, Stürner KH, Stellmann JP, Breiden P, et al. Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis. *Sci Transl Med* 2013 6 5;5(188):188ra75.
98. Jewell CM, Lopez SC, Irvine DJ. In situ engineering of the lymph node microenvironment via intranodal injection of adjuvant-releasing polymer particles. *Proc Natl Acad Sci U S A* 2011 9 20;108(38):15745–15750. [PubMed: 21896725]
99. Senti G, Kundig TM. Intralymphatic immunotherapy. *World Allergy Organ J* 2015 3 7;8(1):9–014-0047–7. eCollection 2015.
100. Maldonado RA, LaMothe RA, Ferrari JD, Zhang AH, Rossi RJ, Kolte PN, et al. Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance. *Proc Natl Acad Sci U S A* 2015 1 13;112(2):E156–65. [PubMed: 25548186]
101. Preynat-Seauve O, Schuler P, Contassot E, Beermann F, Huard B, French LE. Tumor-infiltrating dendritic cells are potent antigen-presenting cells able to activate T cells and mediate tumor rejection. *J Immunol* 2006 1 1;176(1):61–67. [PubMed: 16365396]
102. Gerlini G, Urso C, Mariotti G, Di Gennaro P, Palli D, Brandani P, et al. Plasmacytoid dendritic cells represent a major dendritic cell subset in sentinel lymph nodes of melanoma patients and accumulate in metastatic nodes. *Clin Immunol* 2007 11;125(2):184–193. [PubMed: 17827069]
103. Battaglia A, Buzzonetti A, Baranello C, Ferrandina G, Martinelli E, Fanfani F, et al. Metastatic tumour cells favour the generation of a tolerogenic milieu in tumour draining lymph node in patients with early cervical cancer. *Cancer Immunol Immunother* 2009 9;58(9):1363–1373. [PubMed: 19172271]
104. Andorko JI, Tostanoski LH, Solano E, Mukhamedova M, Jewell CM. Intra-lymph node injection of biodegradable polymer particles. *J Vis Exp* 2014 1 2;(83):e50984 doi(83):e50984. [PubMed: 24430972]
- 105**. Tostanoski LH, Chiu YC, Gammon JM, Simon T, Andorko JI, Bromberg JS, et al. Reprogramming the Local Lymph Node Microenvironment Promotes Tolerance that Is Systemic and Antigen Specific. *Cell Rep* 2016 9 13;16(11):2940–2952. [PubMed: 27626664] Direct i.L.N. injection of a MP with disease specific antigen (MOG) using a MS murine model demonstrated permanent disease reversal when MOG and rapamycin MP were delivering to peripheral LN.
106. Pape KA, Catron DM, Itano AA, Jenkins MK. The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. *Immunity* 2007 4;26(4):491–502. [PubMed: 17379546]

Key Points:

- LNs are a key nexus for integration of immune signals.
- LNs can be accessed by blood, tissue, and direct routes for delivery of immune signals.
- LNs can be specifically programmed to generate suppressive and tolerogenic immune responses.
- Recent advances in bioengineering techniques and materials demonstrate that LN programming is attainable.

Figure 1.

Targeting strategies of the LN include via afferent lymphatics (pink panel), bloodstream (green panel), or direct injection (blue panel). Lymphocytes (pink), DC (blue), other cells (green), or artificial nanoparticles (NP) may enter the lymph node using either afferent lymphatic or arterioles, and exit from the lymph node is via the efferent lymphatic. Lymph enters the LN via the afferent lymphatic, moves around the periphery via the subcapsular sinus, down into the depth of the node via the trabecular sinuses, and enters the medullary sinuses at the innermost aspect of the lymph node. Entry into the parenchyma of the LN from the intravascular route is primarily at the medulla and via the high endothelial venules (HEV). The main zones of the lymph node are illustrated here from the innermost medullary

cords, paracortex (T cell zone), cortical ridge, and cortex (B cell zone, in which germinal centers (GC) are generated.

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Table 1.

Structure and function of the lymph node

Structure	Function	Molecular Markers	References
Lymph Node Entry			
Afferent Lymphatic and Sinuses	<ul style="list-style-type: none"> • Allow efficient cell and antigen entry into lymph node 	<i>T cell retention, trafficking to LN:</i> <ul style="list-style-type: none"> ◆ LYVE-1 ◆ Podoplanin ◆ CLEVER-1 ◆ MSRI ◆ PLVAP (MECA-32) 	[3], [5*], [4], [6*], [7]
Arteriole and High Endothelial Venules (HEV)	<ul style="list-style-type: none"> • Major site of extravasation of lymphocytes from the blood into lymph node via multistep adhesion process, which occurs at the cortical ridge 	<i>Adhesion:</i> <ul style="list-style-type: none"> ◆ ICAM1 ◆ ICAM2 <i>Lymphocyte extravasation:</i> <ul style="list-style-type: none"> ◆ CCL21 ◆ CCL19 ◆ CXCL12 ◆ CXCL13 <i>Lymphocyte trafficking:</i> <ul style="list-style-type: none"> ◆ PNAd 	[8], [9], [10], [11], [12]
Lymph Node Zones			
Medulla	<ul style="list-style-type: none"> • Innermost layer, which is comprised of lymphocytes, plasma cell, macrophages, and the medullary cords and sinuses • Point of entry from afferent lymph and exit via the efferent lymph 	SIP	[13], [14]
Paracortex (T Cell Zone)	<ul style="list-style-type: none"> • Surrounds the medulla and is comprised of T cells, DC, and FRC • Site of T cell activation 		[13]
Cortical Ridge	<ul style="list-style-type: none"> • Marks boundary between paracortex and cortex, comprised of FRC and HEV • Antigen presentation 		[13]
Cortex (B Cell Zone)	<ul style="list-style-type: none"> • Outermost layer of lymph node comprised of B cells, B cell follicles, GC, macrophages, and FDC 		[13]
Reticular Meshwork			
Lymphatic Endothelial Cells (LEC)	<ul style="list-style-type: none"> • Line the lymphatic capillaries including and subcapsular and medullary sinuses • Capture and retain antigen • Regulate lymph flow 	<i>Migration of APC:</i> <ul style="list-style-type: none"> ◆ CCL21 ◆ CCL19 ◆ CXCL12 ◆ CCL1 <i>Migration of T cells:</i> <ul style="list-style-type: none"> ◆ SIP ◆ VCAM-1 <i>Immune suppression:</i> <ul style="list-style-type: none"> ◆ PD-L1, ◆ iNOS 	[6*], [15], [16], [17], [18], [19], [20], [21*], [22], [23*]

Structure	Function	Molecular Markers	References
Fibroblastic Reticular Cells (FRC)	<ul style="list-style-type: none"> •Specialized myofibroblast cells •Secrete and remodel extracellular matrix •Serve as scaffold for DC and T cells to migrate •Act as a conduit for transporting lymph from sinuses into the parenchyma •Antigen presentation via MHC class II •Induce T cell tolerance 	<i>Dynamic scaffold:</i> <ul style="list-style-type: none"> ◆Podoplanin ◆ER-TR7 ◆Laminin <i>Suppress T cell expansion, promote tolerance:</i> <ul style="list-style-type: none"> ◆iNOS ◆IDO ◆PD-L1 ◆Cox-2 <i>Naïve T cell trafficking to paracortex:</i> <ul style="list-style-type: none"> ◆CCL19 ◆CCL21 ◆CXCL12 	[2], [13], [18], [20], [24], [25], [26], [27], [28], [29], [30]
Blood Endothelial Cells (BEC)	<ul style="list-style-type: none"> •Line blood vessels within the lymph node 	CD31	[18]
Marginal Reticular Cells (MRC)	<ul style="list-style-type: none"> •Stromal cells under subcapsular sinus at periphery of follicles 	<ul style="list-style-type: none"> ◆CXCL13 ◆ER-TR8 ◆Podoplanin ◆RANK ligand 	[8]
Follicular Dendritic Cells (FDC)	<ul style="list-style-type: none"> •Specialized reticular fibroblasts within B cell follicles that capture and present antigen to B cells, crucial to germinal center formation 	<i>Naïve B cell homing to follicles:</i> <ul style="list-style-type: none"> ◆CXCL13 <i>Support GC response:</i> <ul style="list-style-type: none"> ◆BAFF 	[8], [18], [19], [31]
Regulatory Immune Cells			
Macrophages	<ul style="list-style-type: none"> •Phagocytes that clear pathogen and dying cells from lymph, focused in the subcapsular and medullary sinuses •Induce antigen specific cytotoxic T cell response 	<i>Induce antigen specific CTL:</i> <ul style="list-style-type: none"> ◆CD169 <i>Regulates lymphocyte migration from sinus into parenchyma:</i> <ul style="list-style-type: none"> ◆MSR1 	[6*], [32], [33], [34]
Dendritic Cells (DC)	<ul style="list-style-type: none"> •Potent antigen presenting cells 	<i>Binds podoplanin:</i> <ul style="list-style-type: none"> ◆CD11c ◆CCR7 ◆CLEC-2 <i>Regulates expansion of B cells within GC:</i> <ul style="list-style-type: none"> ◆CD301b <i>Maintain reticular cells:</i> <ul style="list-style-type: none"> ◆LTβR ligands 	[4], [8], [26], [28], [35]
B Lymphocytes	<ul style="list-style-type: none"> •Development of humoral immunity to antigen 	<i>Germinal center migration:</i> <ul style="list-style-type: none"> ◆CXCR5 	[8]
T Lymphocytes	<ul style="list-style-type: none"> •Promote cytotoxic immune response to antigens presented 	<i>LN homing:</i> <ul style="list-style-type: none"> ◆CD62L ◆WNK1 <i>Retention within LN, migration to paracortex</i> <ul style="list-style-type: none"> ◆CCR7 ◆MSR1 <i>HEV extravasation:</i> <ul style="list-style-type: none"> ◆CXCR4 <i>Naïve T cell survival and LN retention/egress:</i>	[5*], [6*], [8], [22], [23*], [36], [37]

Structure	Function	Molecular Markers	References
Regulatory T Cells (Treg)	•Modulate immune system by downregulating T cell induction and proliferation and promote tolerance	◆SIPRI <i>Immunosuppressive function:</i> ◆Foxp3 ◆T-bet <i>Migration to LN:</i> ◆KLF2	[21*], [38], [39], [40], [41], [42], [43]
Follicular Helper T Cells (T _{FH})	•Assist B cell affinity selection within germinal center	<i>GC regulation:</i> ◆IL-21 ◆IL-4 ◆CD40L	[44]
Lymph Node Exit			
Efferent Lymphatics	•Return lymph and lymphocytes, mainly T cells, to circulation	<i>T cell egress:</i> ◆Endomucin ◆SIP1 ◆CD69 <i>T cell retention:</i> ◆LFA-1 ◆CLEVER-1 ◆MSR1	[5*], [6*], [8], [45], [46], [47]
HEV and Venules	•Entry of lymphocytes from blood circulation into LN	<i>T cell retention:</i> ◆CCL21 ◆CCR7	[5*], [12]