Targeting Immune Cells Within Lymph Nodes

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Advances in our understanding of immunomodulation have resulted in the exploration and development of a range of new therapeutics to treat and prevent conditions such as cancer, immune and metabolic disease, infection and allergy. Immunotherapeutic strategies require engagement with immune cells, including B cells, T cells and antigen presenting cells, such as dendritic cells (DCs). Delivery to immune cells is challenging as they are unevenly distributed in the body and, concentrated in specific regions of the lymph nodes and lymphoid organs, which are difficult to access via traditional drug delivery routes through the blood. Writing in this issue of Nature Nanotechnology, Alex Schudel et al describe a two-stage approach to maximise drug delivery to the lymph nodes and to lymph node-resident immune cells, inspired by the trafficking route of particulate antigens[1].

Antigens and macromolecules are transported from the interstitial space to downstream lymph nodes via lymphatic vessels [2, 3, 4]. Access to the lymphatics is dependent on molecular size. Small molecules (<20 kDa) are typically rapidly cleared into the blood following injection, whereas high molecular weight molecules (>20 kDa) and particles (10-100 nm in diameter) drain into the lymphatics [2, 4, 5].Preferential lymphatic access occurs because tight junctions between endothelial cells in blood capillaries restrict macromolecular entry, whereas lymphatic capillaries have open button-like junctions and higher permeability [2, 4, 5]. For materials with diameters >100 nm, lymphatic access is limited as they get trapped in the interstitial matrix [2, 4, 5]. Therefore, to reach the lymph nodes, efforts have focussed on delivering macromolecules or nanoparticles into the interstitial space[5, 6, 7] or on oral delivery of lipid-like (pro)drugs[8, 9, 10] that associate with similarly sized colloidal lipoproteins in intestinal absorptive cells. These strategies improve lymphatic uptake and lymph node exposure [2, 4, 5, 6] and thus, immunomodulation [2, 4, 9, 10] , perhaps most notably for vaccines [2, 5, 6, 7].

Following entry into the lymphatics, access to distinct immune cell populations within the lymph node is further controlled by the anatomical structure [2, 3, 11]. Lymph that drains into the node (via afferent lymphatic vessels) enters into a channel (the subcapsular sinus) that surrounds the node, thereby providing a rapid flow path for fluid to leave the node via the exiting (efferent) lymphatic vessel (Fig. 1). The sinus is lined by barriers cells including macrophages and DCs, which phagocytose materials from the inflowing lymph [2, 3, 11]. B and T cells are compartmentalised into cortical and paracortical regions [2, 3, 11], which can be accessed via narrow conduits of 3-5 nm in diameter that only allow entry of smaller molecules with a molecular weight ~<70 kDa or very small particles (2-5 nm)[2, 11]. Therefore, lymph node drug delivery faces the difficulty that macromolecules or nanoparticles have greatest lymphatic uptake, but at the same time cannot access the smaller conduits within the lymph node and thus, the majority of lymph node cells.

Schudel et al[1] overcome this challenge by applying a two-stage approach (Fig. 1). They use nanoparticles with a diameter of ~27 nm to preferentially access the lymphatics (and thus, the draining lymph nodes) following intradermal injection. Deeper access into the draining lymph node is then

achieved by programmed release of the payload prior to cellular uptake. The authors conjugated thiol-reactive oxanorbornadiene (OND) linkers to thiolated poly(propylene sulphide) nanoparticles, which have previously been shown to accumulate in lymph nodes after intradermal injection [5, 7]. The OND linkers fragment via a retro-Diels-Alder reaction, which allows the programming of release half-lives in the range of hours to days. Released cargo can then penetrate deep into the draining lymph node to access immune cells in the cortex and paracortex. Similar effects were observed for other particles and for fluorophores conjugated directly to the nanoparticle core or to the surface. The authors demonstrate the therapeutic benefit of the two-stage approach by conjugating a thiol terminated CpG oligonucleotide (a Toll-like receptor 9 (TLR9) ligand) to the nanoparticles via an OND linker with an intermediate release half-life. Following intradermal injection of the nanoparticles, more TLR9 expressing T cells, B cells and DCs are found in the draining lymph node, which cannot be achieved by administration of CpG alone or CpG conjugated via a disulphide linker that only releases cargo after internalisation into cells. In an EL4 model of murine lymphoma, in which secondary tumours develop in the draining lymph node, intradermal administration of this platform resulted in a reduction in tumour burden in the draining lymph node and the primary tumour.

As efforts to prevent and treat disease via immunomodulation increase, mechanisms to tailor the delivery of therapeutic cargoes and vaccines to lymph nodes and specific cell populations within lymph nodes will become increasingly important. The work by Alex Schudel et al provides insight into the impact of release kinetics on the spatial and cellular disposition of lymph node-targeted cargos. Strategies to target and release cargo within specific cell populations have been widely explored in the context of drug targeting to tumours, but have been less robustly evaluated in lymph nodes thus far.

However, challenges remain; for example, release from the OND linkers was initiated at the point of conjugation, and thus, nanoparticles have to be administered shortly after formation. For clinical translation, triggers would need to be identified that initiate cargo release prior to dosing or ideally within the lymph node in order to improve stability and control the site and time of release. Individual disease states might also require tailored release profiles to enable access to appropriate target cells. It is also known that different carriers (for example, nanoparticles, dendrimers, liposomes, proteins or lipoproteins) show distinct distribution within lymph nodes[5, 7] and thus, release mechanisms will have to be adapted to specific nanoparticle designs. Furthermore, local intradermal administration provides access only to the downstream draining lymph nodes, but in diseases associated with multiple lymph nodes or lymph nodes that are not accessible by injection, delivery will be more challenging. However, clinical translation is in sight, as lymph drainage processes and lymph node structures appear to be similar across species.

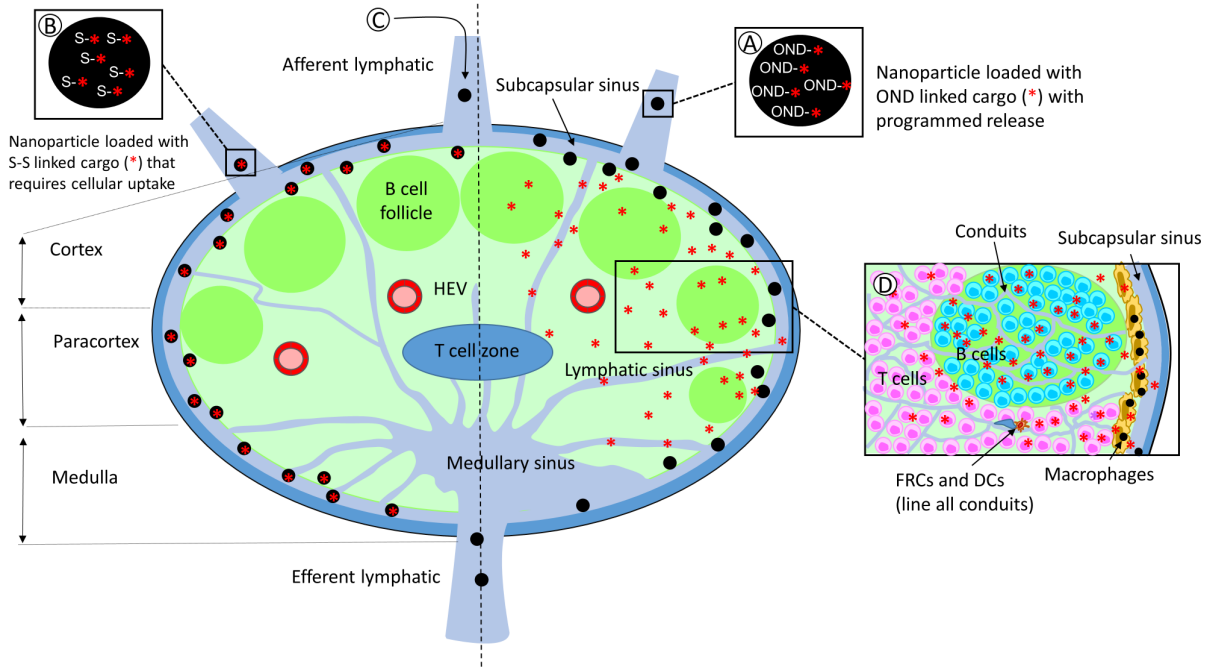

Figure 1. Two-stage delivery to the lymph node-resident immune cells. (A) Poly(propylene sulphide) (PS) thiolated (S) nanoparticles (NPs) (27 nm diameter) were linked to cargo via a thiol-reactive oxanorbornadiene (OND) linker for programmed release or **(B)** via a disulphide linker that requires cell uptake prior to release. **(C)** After intradermal injection, the nanoparticles are taken up via the draining lymphatic vessels and transported to the lymph node through the afferent lymphatic vessels. The afferent lymphatic vessels join the subcapsular sinus, which surrounds the lymph node and joins the efferent lymphatic vessel that exits the node. The nanoparticles are transported along this pathway or they are taken up by barrier cells (e.g. macrophages) that line the subcapsular sinus if they are too large to penetrate the lymph node. **(D)** The OND linked cargo is released at programmed rates. The small molecular size of the cargo enables penetration into the conduits that enter the cortex and paracortex of the lymph node, which are rich in B cells and T cells. The conduits are formed by fibroblastic reticular cells and dendritic cells. The release of the cargo within the lymph node allows access to a range of immune cells (T cells, B cells, dendritic cells and macrophages).

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