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PURETECH'S LYMPHATIC LEAP

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PureTech Health plc is launching a new branch of inquiry into the lymphatic system following discoveries that the network extends into the brain and could be used to deliver immunotherapies directly to tissues.

While research into the links between the immune system, brain and gut has been growing, the lymphatic system -- a network of vessels and lymph nodes through which immune cells travel and encounter antigens -- has remained relatively unexplored.

The topic was one focus of PureTech's inaugural Brain, Immune, Gut (BIG) Axis Summit in January, which discussed how the finding that the CNS contains lymph vessels could change the way immunologists and neurologists think about CNS disorders.

The provocative discovery overturned longstanding dogma that the brain, unlike the rest of the body's organs, had no such vessels.

It's early days for sizing up the finding's translational potential, but hypotheses are starting to flow. One idea is that the build up of toxic aggregates in neurodegenerative diseases may result from deficient drainage of interstitial fluid from the brain, which could serve as a target.

The summit also discussed opportunities beyond neurodegeneration, including those PureTech is exploring through its Glyph technology, which it licensed last year from Monash University.

With the technology, PureTech hopes to use the lymphatic system to transport and distribute therapeutics, including to specific lymph nodes. Another application it could enable is controlling trafficking of lymphocytes to treat autoimmune disease.

The Glyph technology was developed by Christopher Porter, professor and director of drug delivery, disposition & dynamics at Monash University.

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"In the last five years or so, the biology world has awakened to the idea that the lymphatic system, and the cells in it, are intimately involved in a number of disease states," Porter told BioCentury.

Yet most drug development and delivery still centers on the circulatory system, which Porter says misses a large part of the picture.

"There is so much interest in lymphocytes right now, but everyone is dosing into the blood and trying to target them in the circulatory system, where there are actually very few lymphocytes. The process of antigen presentation is also happening in lymphoid tissues, and that's where we should be targeting them," said Porter.

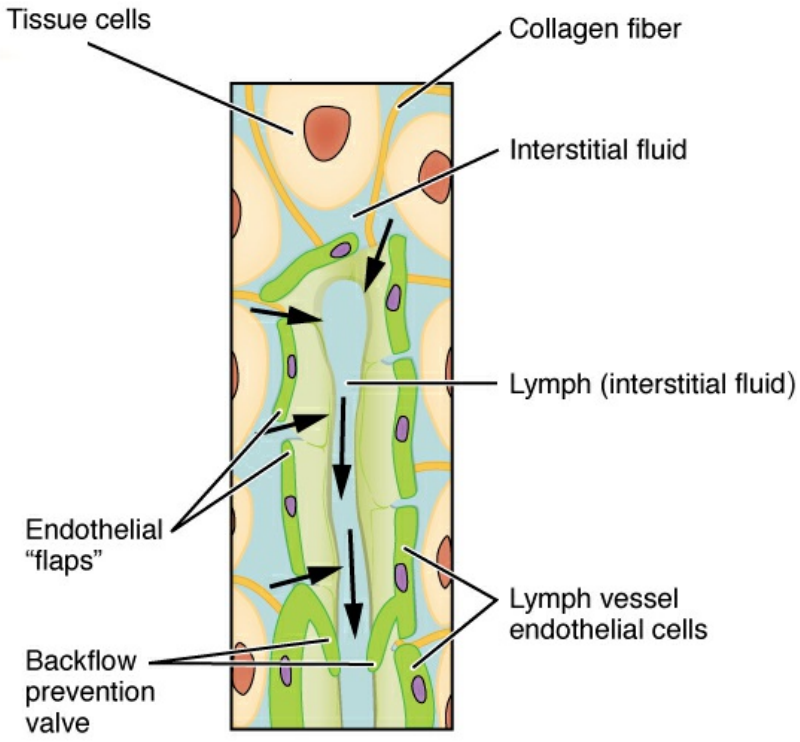
In addition, the gut-immune-brain axis could also underlie the mounting evidence linking the microbiome to gut immunity, and to CNS disorders such as Parkinson's disease and autism.

The hypothesis is that gut bacteria, or the factors they produce, enter interstitial fluid that drains into the regional lymphatic system. Immune cells within the lymph vessels and nodes could detect those signals, and disseminate immune responses locally or throughout the network (see "Lymphatic Flow"). A pair of *Science* papers published earlier this month support the hypothesis, showing disease states including hyperglycemia and autoimmunity cause intestinal microbiota to leak out of the gut into lymphatic structures, contributing to systemic inflammation and exacerbation of symptoms.

FIGURE: LYMPHATIC FLOW

One of the main functions of the lymphatic system is to drain interstitial fluid from tissues. When unwanted components are released from tissue cells and blood vessels into the extracellular environment, they form the interstitial fluid. That fluid then passes into lymphatic vessels through openings, dubbed endothelial "flaps," between the lymph vessel endothelial cells that line the passageways. The flaps are big enough to allow entry of large molecules such as nanoparticles and certain lipids that do not readily diffuse through capillaries.

Once inside the lymph vessel, the interstitial fluid, now called lymph, flows in one direction and drains into the lymph nodes. In the lymph nodes, antigen-presenting cells educate lymphocytes with information about the contents of the interstitial fluid, programming the cells to go back into circulation to attack pathogens and other antigens present in the tissues. *Source: CFCF/Wikimedia Commons*



PureTech CSO Joseph Bolen thinks that line of research is also likely to present translational opportunities.

"All of a sudden, the ways we think about the origins of disease are turned upside-down. The field stopped to say: 'wait, here are a bunch of mechanisms we've never thought about that might be playing a major role in many different diseases,'" said Bolen.

BRAIN DRAIN

The first evidence of a role for lymphatics in the brain came from a 2015 publication from Jonathan Kipnis at the University of Virginia, which used two-photon live imaging to show the presence of lymphatic vessels in the mouse CNS. His lab is now investigating the function of those vessels in Alzheimer's disease and multiple sclerosis.

Last year, Daniel Reich at NIH extended the observation to humans, using specialized MRI techniques to provide live images of flowing lymph in the dura mater, and identifying the vessels and their structure.

Kipnis is chair of the department of neuroscience and director of the Center for Brain Immunology and Glia at UVA. Reich is chief of the Translational Neuroradiology Unit at NIH's National Institute of Neurological Disorders and Stroke (NINDS).

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Since systemic diseases of the lymphatic system often involve impaired drainage of interstitial fluid, the CNS discovery raised the question as to whether impaired drainage in the brain might play a role in neurodegenerative diseases.

"We think of Alzheimer's as a disease of the neurons that are affected by amyloid β, but what if it is really a disease of the lymphatics, caused by improper drainage that leads the deposits to accumulate?" said Bolen.

According to Bolen, CNS lymphatics are different from all other lymphatic networks except those of the gut, because they require constant upkeep and flow to function.

Reich thinks hydrocephalus and other disorders characterized by pressure in the brain could also be addressed by modulating lymphatic flow.

But the first step is to interrogate the function of the CNS lymph vessels, he argued.

"Someone needs to start looking at these vessels in diseases. Are they present? Enlarged? Proliferating? We don't have a method to dive into function in humans, but this could be easily done in the mouse," said Reich.

Bolen agreed. "The fact that lymphatic vessels exist in the brain is relatively new, and the information about what distinguishes those vessels from others is unknown," he told BioCentury. "We still don't know the actual specific surface molecules for lymphatic vessels in different tissues."

PureTech spokesperson Allison Mead Talbot told BioCentury the discovery of CNS lymphatics has given the company "opportunity and interest" in harnessing the system to address various neuroinflammatory conditions, although she would not disclose specific plans.

GLYPH OF HEALTH

For PureTech, utilizing lymphatics for drug delivery may be one of the most near-term projects.

The Glyph technology from Porter's lab at Monash preferentially transports drugs through the lymphatic system, rather than the circulatory system.

Beyond trafficking white blood cells and draining interstitial fluid, the lymphatic system's other major function is transporting lipids. Lipids from digestion are naturally taken up into lymphatic vessels rather than capillaries. Porter's team designed a prodrug system that conjugates therapeutics to a lipid structure, allowing them to be directly transported into the immune vessels.

In 2014, Porter published in the *Journal of Controlled Release* on a triglyceride mimetic prodrug that was designed to enter the natural triglyceride hydrolysis and resynthesis pathway. The prodrug increased lymphatic transport of mycophenolic acid (MPA) 80-fold and concentrations of MPA in lymphocytes 103-fold.

According to Porter, the benefits of lymphatic transport include more efficient access to lymphocytes, higher drug concentrations than in systemic circulation, and fewer systemic and hepatic toxicities.

"We can get drug concentrations in the lymphatic system maybe 10-100 times higher than in the blood because lymph fluid has a lower volume, and it distributes in a different way. Drug in the blood can move in and out of circulation, which further dilutes it," said Porter. "We don't have to worry about systemic toxicities either because the lymphatics don't have the systemic exposure of blood-based delivery."

In addition, trafficking through lymphatics avoids first-pass metabolism and may improve exposure for many drugs.

Porter told BioCentury the prodrug system might be optimally used in immune disorders of the gut because it specifically targets intestinal lymphatics. An obvious first choice would be intestinal autoimmune processes such as inflammatory bowel disease (IBD), he said.

Porter's lab has also done early work on systemic lymphatic delivery. The same principle -- that large, lipophilic molecules pass into the lymph instead of capillaries -- might work with subcutaneous drug delivery anywhere in the body, he said.

In initial studies, his team has shown that subcutaneous injection of a macromolecule or nanoparticle into a capillary bed will cause it to "drop into the lymph and drain to the downstream lymph node." He has evaluated this technique to deliver various chemotherapies for several types of cancers in the lymph nodes.

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Joseph Bolen, PureTech

PureTech has a license to the prodrug technology targeting intestinal lymphatics. PureTech is not disclosing the drugs it might pair with the technology or the indications it might pursue.

There are few competitors in this field so far. Vedantra Pharmaceuticals Inc. is targeting lymph nodes systemically to increase immunotherapy efficacy. Its approach is to encapsulate therapeutics in lipid nanoparticles called Interbilayer-Crosslinked Multilamellar Vesicles (ICMVs) or conjugate them to amphiphilic constructs that bind albumin to target antigen-presenting cells in the lymph nodes directly.

Vedantra's Research Director Peter DeMuth told BioCentury both preclinical delivery vehicles are injected subcutaneously and are designed to deliver vaccines and immunotherapies to lymph nodes that wouldn't otherwise efficiently traffic there due to size.

The major difference between the Vedantra and Glyph technologies is the route of delivery. Vedantra is focusing on cancer and infectious diseases. PureTech says its Glyph technology enables development of novel approaches for tackling disease progression for disorders of the gut, as well as for cancer, autoimmunity and neuroinflammation.

DIRECTING TRAFFIC

PureTech has about eight other technologies that haven't been disclosed yet, but Bolen said he is particularly interested in regional trafficking in the lymphatic system and its impact on disease.

"Lymphatic vessels drain to about 600 lymph nodes in the body, where the immune system can survey the fluid and junk cells thrown out by tissues. The question is: why are there 600 instead of 1?" he said.

He believes the answer lies in the regional nature of the lymphatic system. "We are beginning to understand that regional lymph nodes educate immune cells about problems, and actually program them to go back to the same specific tissue for which that lymph node is responsible," he said.

He said in the skin, a metabolite of vitamin D is known to program T cells in the lymph nodes to return to the skin, and the cells aren't trafficked properly without it. A similar signaling system has been seen in the GI system. "One could speculate that if lymph node systems act regionally throughout the body, there are similar signaling programs that teach immune cells to go back to each tissue."

Bolen expects signaling programs and molecules, or "zip codes," exist that traffic lymphocytes to each tissue in the body. The next task is to uncover those codes.

He envisions targeting and modulating those pathways to prevent the abnormal lymphocyte trafficking behind autoimmune diseases such as multiple sclerosis.

"What we do as an industry is inhibit things, that's where people will undoubtedly take this first, but I think there might also be a later opportunity in cell therapies," said Bolen.

Given the rapid advances in T cell isolation, engineering and delivery in the industry, it may be possible to program the "zip codes" into therapeutic T cells to bring them straight to diseased tissues, limiting systemic exposure and toxicity.

PureTech hasn't disclosed whether it will pursue these particular approaches. But Mead Talbot said it is conducting additional undisclosed research in lymphatics to expand its internal immunology pipeline, and views the lymphatic system as a potential avenue for treating cancer, autoimmunity and neuroinflammation.

COMPANIES AND INSTITUTIONS MENTIONED

Monash University, Clayton, Australia
National Institutes of Health, Bethesda, Md.
PureTech Health plc (LSE:PRTC), Boston, Mass.
University of Virginia, Charlottesville, Va.
Vedantra Pharmaceuticals Inc., Cambridge, Mass.

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