How a Newly Discovered Body Part Changes Our Understanding of the Brain (and the Immune System)

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At this time of year, researchers, doctors, and recreational nerds alike turn to the American Association for the Advancement of Science (AAAS) for the list of the past year's most important scientific breakthroughs [1]. 2015 saw many significant advances that gained flashy and well-deserved press. The world witnessed the creation of an Ebola vaccine, the first fly-by of Pluto, and the discovery of a new human subspecies, *Homo naledi*. However, one critical breakthrough received far less press: the discovery of lymphatic vessels in the brain. Oh, you didn't hear about that? Well, that's probably just what these stealthy vessels would have wanted!

Your newest body part: it lives in the brain, but it's part of your immune system

The discovery of brain lymphatics (Figure 1) will open many new doors to understanding the brain, but it is also remarkable simply because there is so little about the human anatomy that has not yet been mapped. Indeed, because of these vessels' hidden location deep within the brain and their close proximity to prominent blood vessels, brain lymphatics had legitimately escaped the notice of the biomedical community. That is, until now.

To back up, what are lymphatic vessels? Lymphatics are like the highways of the immune system and are found throughout the body. They orchestrate immune surveillance of various bodily tissues so that specific immune responses can unfold in the body parts that need them. Lymphatics are similar to blood vessels, but they carry immune cells in a fluid called lymph. Lymph is the collection, or "drainage," of non-blood fluids that are found in tissues or organs. Like tiny streams converging, this fluid collects and is channeled into the network of lymphatic vessels, passes through lymph nodes, and makes its way back to the bloodstream.

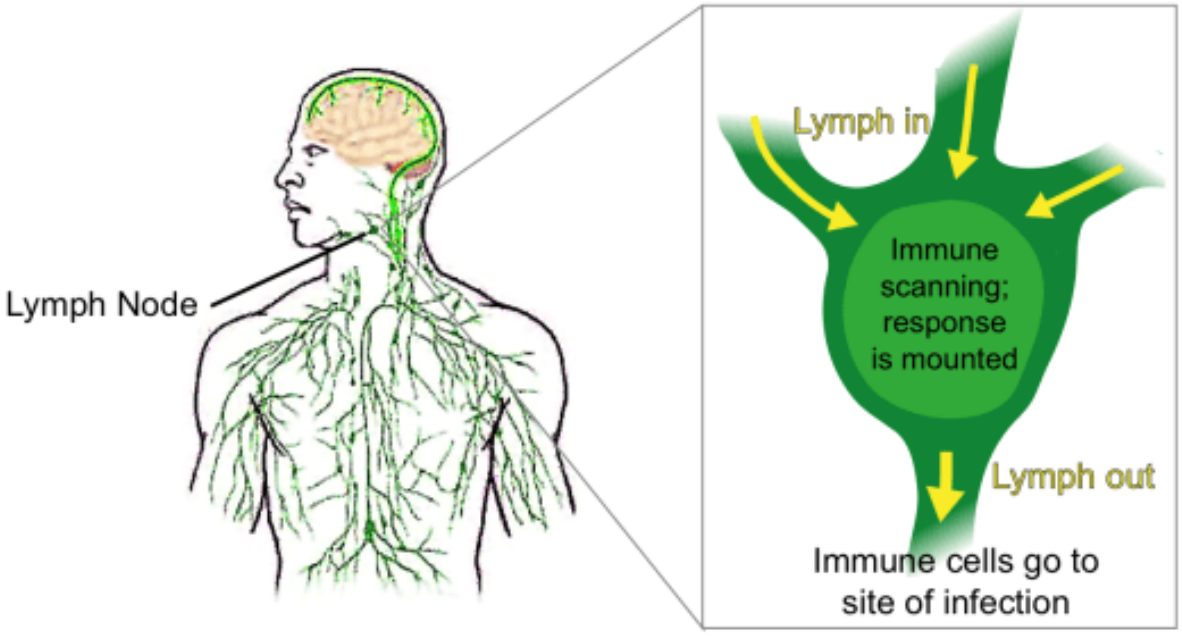

Figure 1: The body's lymphatic system. Lymphatic vessels drain lymph and immune cells from around the body through lymph nodes, then back to the blood stream. Lymph nodes sift through lymph and mount immune responses when they catch evidence of an infection. Depicted is a lymph node in the neck. (Figure adapted from University of Virginia Health System)
When a tissue is infected by a pathogen, like a virus, bacteria, or parasite, bits and pieces of the offending pathogen end up in the lymph. These pieces, along with immune cells from the infected tissue, reach the lymph node, and the cells in the lymph node then react to coordinate a specific immune response to the pathogen. Thus, the system not only allows for recirculation of bodily fluid, but it also provides a means for the immune system to sift through material from around the body in order to scan for infection. Without lymphatics, fluid would build up in body tissues, and there would be no way to alert the adaptive immune system to invading pathogens.

The brain: an immunological no-fly zone

The brain is considered an immune-privileged site, meaning it doesn't play by typical immune system rules. Compared to most other tissues, it has tighter barrier membranes separating, which restricts movement of cells and material into and out of the brain's parenchyma (the meat of the brain where neurons reside). Thus, immune cells from inside the brain can't travel to the lymph nodes to initiate an immune response the way they would in other

tissues like the lungs, skin, or gut. The result is that normal immune reactions within the brain are rare.

Immune privilege likely exists because an overzealous immune response can act as a double-edged sword. The immune system is important for maintaining healthy tissues, fighting off bugs, and cleaning up after injury, but too much activation can be severely damaging. T cells are a common subset of immune cells that are particularly important when it comes to offing viruses or bacteria. They are constantly on the lookout for invaders, and they can be cytotoxic (cell-killing), so they find and eliminate infected cells.

Autoimmune diseases like type I diabetes (T1D) or rheumatoid arthritis (RA) are examples of conditions caused by problematic immune responses, wherein the immune system mistakes a bit of self for a bit of invader and wreaks havoc on the tissue where that bit is found—the pancreas in T1D, and joints in RA. The immune system can also cause damage when it's doing its job right. Attacking an invading bug often comes at the cost of collateral damage to surrounding tissue, which must later be repaired. Thus, restricting immune system access to our most important tissue sites is a mode of self-preservation, especially for those sites that cannot easily repair after hosting a battle.

Neurons do not regenerate in adults the way most other cells do, making them difficult to replace or repair if they're damaged by a traumatic event like an immune response. This is likely the evolutionary reason for restricting immune access to the brain, and it's the reason why immune responses in the brain can be so devastating when they do occur. For a long time, scientists thought that the brain had a totally autonomous system of immune defense[1] based on brain-compatible immune cells called microglia, which live inside the parenchyma and never come and go the way a normal immune cell would.

Some other types of immune cells—ones that are great at capturing and exposing elements of pathogens—survey the brain for infections not by going into the brain, but by monitoring it from the outside. These cells sample material from the brain that gets into the cerebrospinal fluid (or CSF, the fluid that surrounds the brain and spinal cord). They show what they find to T cells, which circulate around the outside of the brain looking for signs of trouble, but typically stay within the CSF. If there is no sign of infection, the T

cells continue on their way. If they see evidence of a problem, they can be triggered to enter the parenchyma[2] (Figure 2).

Because of the brain's immune privilege, the lack of evidence for brain lymphatics was rarely called into question. It was believed that CSF could play the part of the lymphatic system in the brain [2]. Even so, conceptions of how CSF allowed information to be passed from the brain to the lymph nodes had been fuzzy at best. The proposed model entailed CSF first draining through nasal mucosa—that's right, snot!—and joining up again with lymphatics in the head and neck to ultimately channel through lymph nodes [3]. However, the discovery of brain lymphatics points to a far simpler route.

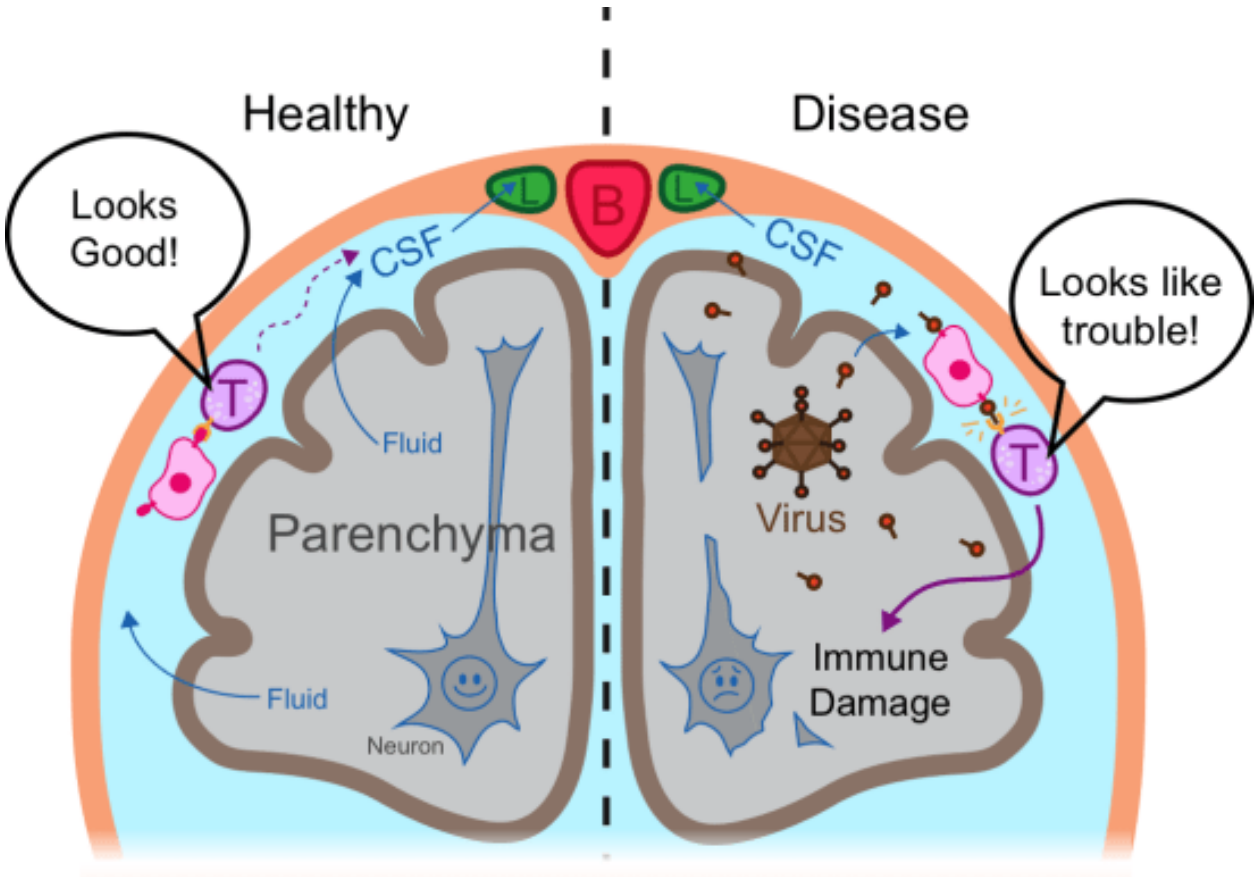

Figure 2: Immune surveillance in the CNS. Immune cells (pink) surveying the CSF present what they see to circulating T cells (purple). Lymphatics (green) provide a route for CSF and circulating immune cells to reach lymph nodes in the neck and return to the rest of the body.
In a healthy brain (left), T cells don't see a problem and circulate back to the rest of the body. In a diseased brain (right), the T are alerted to infection and respond by infiltrating the parenchyma, which can cause immune-mediated damage. (Cells not to scale).

Brain lymphatics: immune privilege redefined?

This past year, researchers at the University of Virginia (UVA) led by Jonathan Kipnis and Antoine Louveau were analyzing the brains of healthy mice, and happened upon what looked like well-organized immune cells—specifically T cells—throughout brain tissue [1]. They weren't roaming willy-nilly through the tissue; something was confining them. After looking more closely, they identified lymphatic vessels in the brain, draining CSF and its contents into the neck's lymphatics and to the lymph nodes below [3, 4]. Independently, a group of researchers in Switzerland, led by Kari Alitalo and Aleksanteri Aspelund, made the same discovery [5], making it highly unlikely that the finding is a fluke. Why hadn't this been seen before?

The sophisticated imaging technology the researchers used had never before been applied to this task. Their study involved looking at large, intact brain samples from mice, as well as monitoring live mouse brains. In both cases, the teams specifically labeled the brain's structures and cells with differently colored fluorescent tags. For example, T cells might be tagged with red fluorescence, blood vessels with green, and so on. This strategy allowed them to visualize these lymphatic systems by fluorescent microscopy [4].

Discovering a new body part in the 21st century is in itself remarkable, and the scientific significance of this finding is equally extraordinary. While it doesn't necessarily upend ideas of the brain's immune privilege, it demonstrates a specific connection between the brain and the immune system—one that was previously thought to be nonexistent—and indicates that these two systems may be far more intertwined than previously believed. The specific implications of this finding are still unresolved, but it is now thought that defects in lymphatics could factor into diseases where immune dysfunction harms the brain, like Multiple Sclerosis and Alzheimer's [4, 2].

In healthy individuals, it's likely that the immune system uses brain lymphatics for immune surveillance. While the brain's microglia still cannot exit the parenchyma, cells in the CSF and lymphatics could shuttle information back to nearby lymph nodes via the lymphatic vessels (Figure 2). They would thus be able to efficiently communicate immunological

messages about the brain in an “outgoing” direction without disrupting the barriers that keep the brain safe—although all of this remains to be verified.

Could immune access to the brain be important for reasons beyond host defense?

A related and equally intriguing idea is that the immune cells that survey the brain from the outside, like those in the lymphatics, are communicating messages to brain cells in an “incoming” direction. They could be passing along information about what is going on in the peripheral immune system. Additionally, recent studies suggest that the incoming messages could somehow influence cognitive functions [6].

A hint at a role for immune cells in neurological health comes from the observation that cognitive functions can be sharp or dull depending on the status of the immune system—whether it is fully competent or compromised. This relationship has been explored by researchers examining cognitive function in healthy versus immunocompromised mice. The healthy mice can easily fight off typical infections, and they behave like any normal mouse would in cognitive tests involving mazes and other stimuli. The immunocompromised mice, which don’t have T cells, get significantly lower scores on the same cognitive tests given to the healthy mice.

Researchers uncovered evidence that the immune systems of the mice may be directly responsible for these behavioral differences. They demonstrated this by using bone marrow transfers to swap the immune systems of the two types of mice. The findings were striking: the typical cognitive defects seen in immunocompromised mice were remedied by receiving healthy marrow. Conversely, the healthy mice receiving immunocompromised bone marrow started to behave more poorly in the tests [6].

How could the immune system be affecting cognition? Among many possible explanations, some scientists speculate that T cells and brain-resident microglia are responsible for these cognitive effects—that T cells just outside the brain could be communicating with brain-resident microglia and orchestrating their behavior, and that microglia could in turn affect neuronal signaling. This is plausible because microglia are not only in charge of fighting pathogens in the brain, but also play a role in brain homeostasis, meaning they keep everything in line day-to-day. Microglial cells clean up

debris from old cells that die or from occasional erroneous cellular processes, which aren't necessarily pathogenic. They also help refine connections between neurons in the brain, keeping signals sharp and specific. The theory is that T cells outside the brain signal to the microglia to perform these duties. Without the T cells, microglia might be dysfunctional, leading to a build-up of debris or connections that go haywire, ultimately disrupting proper cognitive function.

Another possibility is that systemic or local levels of chemicals produced by a normal immune system, but not by a weakened one, influence the chemical context of the brain. This could affect the cognitive functions of mice by influencing any of the diverse cells in the brain, even the neurons themselves.

Whether the lymphatic-bound T cells, like the ones observed by the researchers at UVA, are actively promoting the cognitive wellbeing of mice or humans remains to be proven. However, the immune system is clearly involved. With these findings, and with the discovery of the brain's lymphatics, the picture of neuroimmune interactions continues to develop. Brain lymphatics show that the nervous system is using the typical structures and tools of the classical immune system, meaning we've been wrong about some basic ways in which the brain and immune system are connected.

It is not yet clear whether lymphatics and cognition are part of the same neuroimmunology story, but these two examples demonstrate that communication between the brain and the immune system is a two-way street, and powerful in ways that the scientists of yesterday might have never anticipated. It seems that it's high time to welcome the brain back from its pedestal of separation from the immune system, and start a new conversation about how studying neuroimmune interactions could begin to address a multitude of problems in neurological function and disease.

Marie Siwicki is a first year graduate student in Harvard's PhD program in Immunology and is currently studying how neurons in the intestines interact with the immune system.

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Further reading:

On the body's system of Lymphatics:
http://emedicine.medscape.com/article/1899053-overview

National Institutes of Health – news brief on discovery of brain lymphatis:
http://www.nih.gov/news-events/nih-research-matters/lymphatic-vessels-discovered-central-nervous-system