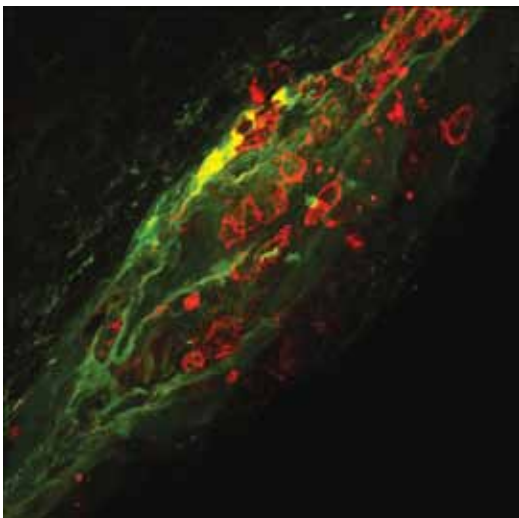


Recruitment of Plaque-devouring Macrophages Against Alzheimer's Disease

Terrence Town, PhD

Even as immune privilege protects neural cells from the threat of overly aggressive inflammatory responses in the brain, this complex mechanism enables neurodegenerative disorders to progress with little if any restriction by immune cells.

The cerebral amyloid plaque deposits characteristic of Alzheimer's disease damage neurons and set off a chronic, low-grade inflammatory process that is ineffective at best, and possibly deleterious. Our laboratory and others are investigating a variety of ways to convert this response into a therapeutic approach, and recent findings in genetically engineered mice suggest that peripheral macrophages that specialize in phagocytosis may be lured into the brain to attack the abnormal amyloid- β peptide and plaque buildup.



Laser scanning confocal image shows macrophages infiltrating the brain of an Alzheimer's mouse (the macrophages appear red and the blood vessel appears green). Reproduced from Town et al., *Nat. Med.* 2008, 14:681-687.

One subject of interest is the immunosuppressive molecule transforming growth factor- β (TGF- β), which regulates immune cell activation, inflammation and repair after injury. TGF- β 1 is increased in abundance in the brains of patients with Alzheimer's disease, possibly the result of the brain's attempt to quiet the inflammatory response around the amyloid deposits.

We hypothesized that blocking TGF- β signaling in peripheral macrophages would unleash overstimulation and activation of these cells, thereby promoting "runaway brain inflammation" and worsening Alzheimer's-like pathology. In a series of experiments, however, we observed the opposite effect. Counter to our expectations, when we bred Alzheimer's disease model mice to mice with genetic interruption of TGF- β and downstream Smad2/3 signaling (termed CD11c-DNR mice), progeny performed considerably better on several behavioral tests.

Laboratory studies showed that amyloid plaques were reduced by as much as 90 percent in these crossed mice by some analyses. Blockade of TGF- β appeared to lower the threshold for both recruitment of peripheral macrophages to brain regions with Alzheimer-like lesions and phagocytic activation. Recruited macrophages were especially voracious, engulfing more amyloid plaque on a per-cell basis than wild-type macrophages. Importantly, increased macrophage plaque clearance did not come at the cost of exacerbated brain inflammation, which could damage sensitive neurons.

Further analysis suggested that when the TGF- β -Smad2/3 signaling mechanism was blocked, signaling shifted to an alternative pathway, TGF- β -Smad1/5/8. The relevance of this shift for brain recruitment of peripheral macrophages and for increasing macrophage amyloid- β plaque

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Spontaneous Spinal CSF Leaks Should Be Considered With Onset of Unexplained Headaches

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Spontaneous intracranial hypotension was first described in 1938 but continues to be frequently misdiagnosed, sometimes for years.

The condition is caused by a spontaneous leak of spinal cerebrospinal fluid (CSF), and while symptoms vary, a patient typically experiences onset of a new headache that occurs when assuming an upright position and is relieved by lying down. These headaches are a direct result of the downward displacement of the brain due to loss of CSF buoyancy, but are often initially misdiagnosed as migraines, tension headaches, viral meningitis or malingering.

Because spinal CSF leaks do not produce visible signs or symptoms, they usually are detected only if they are suspected and specialized diagnostic imaging is performed. Cranial MRI, for example, is often used to identify and monitor changes related to loss of CSF volume. The study of choice for accurately defining the location and extent of a leak is myelography with iodinated contrast followed by thin-cut CT or with gadolinium followed by MRI.

Connective tissue disorders involved

Spontaneous CSF leaks result from a weakness in the dura, with a generalized connective tissue disorder playing a crucial role in the majority of cases. About one-third of patients also report that a relatively trivial traumatic event preceded the onset of symptoms. The amount of fluid that leaks into the epidural space varies greatly and depends on the extent and type of dural defect.

For a small leak, bed rest is usually the first approach to treatment, giving the dura an opportunity to seal spontaneously. Increased oral hydration, supplemental caffeine and the use of an abdominal binder may also be recommended.

The epidural “blood patch” – an injection of autologous blood into the epidural space – is the mainstay of treatment. The placement of 10 to 20 mL of blood forms a dural tamponade, sealing the leak and bringing rapid symptom relief to about one-third of patients. We usually place a blood patch at two sites – first at the thoracolumbar junction and then in the lower lumbar area.

Most patients need more than one blood patch before permanent repair is achieved, and the procedure can be repeated at intervals of no less than five days. We also sometimes consider a large-volume patch consisting of 20 to 100 mL of blood.

Treating persistent leaks

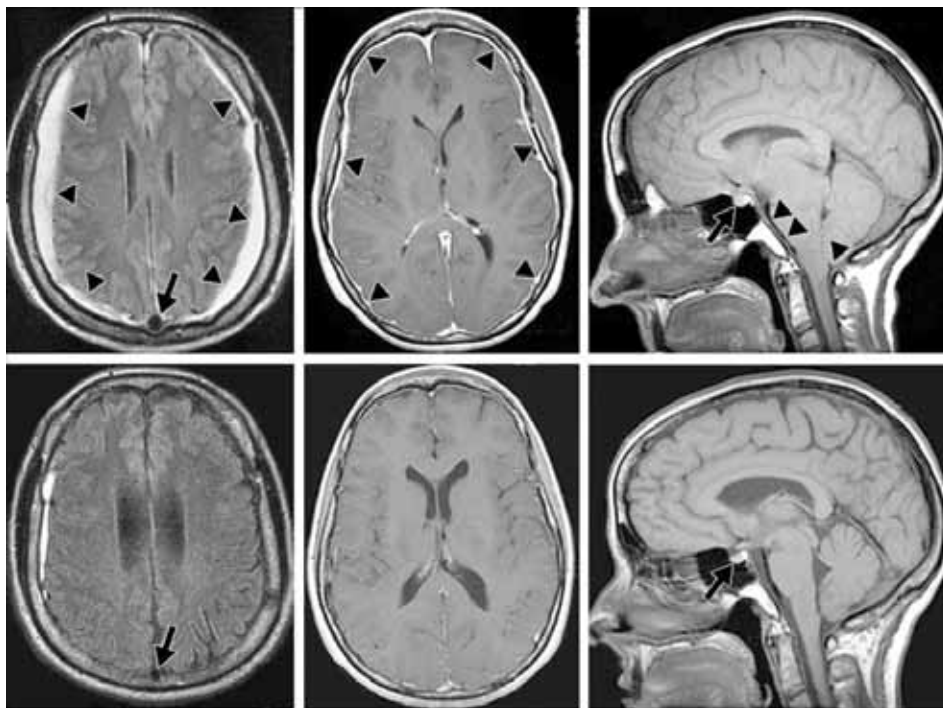
If blood patches fail to provide long-term relief, we perform a directed blood patch or apply a fibrin sealant. With either procedure, the exact site of the leak is identified for localized treatment. About one-third of our patients who do not experience symptom relief with blood patches will benefit from the percutaneous placement of this biosynthetic sealant.

Although surgery is typically reserved as a last option, surgical repair is safe and offers the ability to address large leaks and more complex structural abnormalities. Our experience suggests that about 10 percent of patients will have a recurrence, regardless of the treatment approach employed.

Commonly misdiagnosed

Incidence of spontaneous intracranial hypotension is estimated at five per 100,000, although comprehensive population-based statistics are not available. Women are more likely to be sufferers, with a female-to-male ratio of 2:1, and while symptoms may begin at any time in life, onset usually occurs in the fourth or fifth decade, with peak incidence around age 40.

Although the classic positional headache is highly suggestive of this condition, headache patterns vary among patients, and other symptoms – mostly related to sensations in the head and face – may occur. Not all orthostatic headaches are caused by CSF leaks, but because these conditions are often misdiagnosed initially, they should always be considered as a possible cause of unexplained headaches.



Top row: Preoperative MRIs in patients with spontaneous intracranial hypotension.
Bottom row: Postoperative MRIs.



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Tissue Engineering Strategies Seek to Repair Degenerative Disc Disease at the Source

Frank L. Acosta, Jr., MD

Low back pain is the most common health problem for people between the ages of 20 and 50, and degenerative disease of the intervertebral disc plays a significant role.

Medical and surgical therapies may help to alleviate symptoms, but they do not address the underlying pathophysiologic process of degeneration.

Cellular dysfunction in the nucleus pulposus is considered an underlying factor in disc degeneration. Cells decrease in number and density while undergoing a variety of biological and functional changes. These lead to molecular and biomechanical alterations that perpetuate the degenerative process. Tissue engineering strategies seek to stop the cycle and rebuild the disc by introducing an invigorated cell population capable of synthesizing matrix.

A number of studies are underway to identify the cells and mechanisms best suited for this daunting task. The intervertebral disc is the largest avascular tissue in the body. Lacking a direct blood supply and receiving nutrients by diffusion through thick tissue, the nucleus pulposus is a harsh environment even for healthy native cells. Implanted cells must be capable of surviving in these difficult conditions and producing matrix.

Chondrocytes, the cells found in cartilage, appear to be uniquely equipped for the low-oxygen, low-glucose environment of the nucleus pulposus, expressing several

proteins (HIF-1alpha, GLUT1 and GLUT3) that enable them to undergo glycolytic metabolism.

Several recent animal studies and a clinical trial have focused on the use of autologous disc chondrocytes for nucleus pulposus repair. The human study found that implantation of autologous chondrocytes reduces back pain and may improve disc height in patients after lumbar discectomy for symptomatic disc herniation.

The use of autologous cells, however, has several limitations, including:

1. The procurement of cells is an invasive procedure. Patients who may not need a discectomy would still have to undergo a procedure to collect cells in addition to the later procedure to implant the culture-expanded graft.
2. The harvesting of chondrocytes from a healthy disc can potentially accelerate degeneration at that level. On the other hand, harvesting cells from an already degenerated disc extends the timeline to treatment because the graft cells must be expanded and the annulus must heal to ensure containment of the new cells after injection.
3. Cells harvested from a degenerated disc may be poorly equipped to rebuild matrix, having undergone the biologic and functional changes inherent in degeneration.

Allogeneic juvenile chondrocytes derived from articular surfaces may overcome these and other challenges, including limitations of supply and immediate availability. Like those in the nucleus pulposus, cells from articular surfaces exist in relatively hypoxic conditions.

I have been involved in recent animal (pig) studies documenting that these pre-differentiated but committed chondrocytes can survive and function – synthesizing cartilage-like matrix – *in situ* for at least 12 months after transplantation. In contrast, undifferentiated allogeneic mesenchymal stem cells were unable to survive in the avascular environment of the disc.

These findings support further study of the use of allogeneic juvenile chondrocytes as one option in tissue engineering strategies for disc repair.



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clearance potential is currently under investigation. If this pathway shift *per se* is a critical determinant of these beneficial macrophage responses, this opens up the possibility for an entirely new set of pharmacological targets for the treatment of Alzheimer's disease.

We also found that interrupting TGF- β signaling pharmacologically, not just genetically, increased amyloid phagocytosis by macrophages. Assuming that these findings are applicable to the human syndrome, they open the possibility that a

drug could be developed and administered peripherally yet have central nervous system effects – specifically, recruitment of plaque-clearing macrophages into the brain.

Accumulating evidence suggests that despite the unique "immune privileged" environment of the brain, the immune system has the potential to become a powerful therapeutic ally in the fight against neurodegenerative disease. The challenge remains to discover the molecular mechanisms that regulate the immune system's beneficial potential and to unlock them at the right place and time.



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Engineered Drugs Attack Specific Molecules in Cells to Stop Diseases Where They Start

Julia Ljubimova, MD, PhD

“Nanoconjugates” are the latest evolution in the development of molecular drugs designed to perform specific tasks by inhibiting several defined targets or pathways among the molecules and structures inside cells. Nanoconjugate drug delivery systems are overcoming hurdles that often thwart conventional medicine, with greater specificity than smaller molecules and better targeting than non-conjugated drugs.

In nanoconjugate systems, “modules” are attached to a delivery vehicle by strong (covalent) chemical bonds, which prevent the components from being damaged or separated in tissues/plasma transit. A nanoconjugated platform and its parts exist as a single chemical unit, with each entity serving a specific purpose in a predetermined sequence of events.

Whereas a variety of substances have been used to create delivery platforms, several polymers have been appealing because some are biodegradable, some are non-toxic and others are non-immunogenic. We found naturally derived polylactic acid (PLGA) to be ideal because it has all of these properties – biodegradability, non-toxicity and non-immunogenicity – and used it as a backbone to create a family of nanoconjugates that target tumors and release anti-tumor agents into tumor cells. It is different from other nanomedicine drugs that deliver and/or release the drugs at a tumor’s site, not tumor cells.

Polycefin, as we named this nanoconjugate system, is a macromolecule of 20 to 30 nanometers in size. It was developed from a highly purified form of polylactic acid derived from a single-cell organism, *Physarum polycephalum*. We have used it in laboratory

and animal studies to target cancer-specific molecules of high-grade brain tumors, invasive breast cancers and metastases.

Polycefin not only functions as a transport vehicle but also targets proteins involved in the development of tumor microvessels. Once it has served these purposes (active drug delivery and release into the tumor cells), it is completely “digested” by the body, leaving no harmful residue behind.

Hitting multiple targets in a single attack

The complexity and adaptability of cancer cells demand a multi-dimensional treatment approach. Multi-targeting – attacking several molecular targets, or markers, simultaneously – is not a new idea in cancer treatment, but nanoconjugates take multi-targeting to a new level.

Because of their size and molecular weight, nanoconjugates such as Polycefin have an enhanced “permeability and retention” effect, which allows them to readily pass through a tumor’s “leaky” blood vessels and accumulate inside a tumor. By attaching a tumor-specific antibody to our drug delivery shuttle, we improved tumor-specific targeting and penetration, and vastly increased drug accumulation within tumors.

Components engineered to work in concert

Once in the bloodstream, cancer-fighting drugs must penetrate two barriers – blood vessel walls and tumor cell walls – before reaching their targets. Brain cancer-fighting drugs have a third challenge – the blood-brain barrier. The ultimate attack on a tumor cell depends on a complex, well-

choreographed chain of biochemical events that defeats these and other obstacles. Each of the modules selected for inclusion on the shuttle performs a specific task advancing the process.

In laboratory and animal studies, we have attached a variety of modules, including monoclonal antibodies that target specific cell-surface receptors, antisense oligonucleotides that interrupt gene-encoded protein synthesis, and antibodies that allow Polycefin to permeate the blood-brain barrier and the blood-brain-tumor barrier.

Based on our findings, we have published several articles documenting that the nanoconjugate system effectively offers multiple targeting with a single carrier. We also have shown that tumor growth is slowed and survival time is significantly increased in animal models of human brain tumors. Human clinical trials are expected to begin in the near future.



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