PERIPHERAL THINKING IN AD

By Selina Koch, Staff Writer

Researchers at the Weizmann Institute of Science have shown for the first time that systemic immune suppression may contribute to neuroinflammation, brain pathology and cognitive dysfunction in Alzheimer’s disease (AD), and that by taking the brakes off of peripheral immunity, companies may be able to develop therapies that recruit macrophages and T cells to treat the disease.

While the results only sketch the outline of the mechanism, they point to a central role for T regulatory cells (Tregs) in the process, making the cells a target for intervention.

Michal Schwartz, professor of neuroimmunology at Weizmann and principal investigator on the study, told BioCentury her group’s findings upend the long-standing dogma that “reducing inflammation within the CNS always requires immunosuppression.” Instead, she said the results “suggest that reducing inflammation in the brain requires boosting an immune response outside the CNS, to facilitate recruitment of immunosuppresive cells to the inflamed brain.”

Chronic neuroinflammation is thought to be a secondary feature of AD, in which ongoing deposition of Aβ persistently activates microglia and astrocytes — cells thought to mediate neuroinflammation through release of proinflammatory cytokines and other mechanisms.

Unlike neuroinflammation caused by acute injury or infection, which is typically transient, the inflammation in AD isn’t resolved because the activated glial cells can’t clear the Aβ as fast as it is deposited. The result is that the accumulated Aβ damages synapses and neurons, inducing more inflammation in an escalating cycle.

To break the cycle, researchers have largely focused on clearing Aβ, modulating microglial responses or inhibiting inflammation with steroids and NSAIDs.

But because inflammatory responses in tissues outside the brain can only be turned off via an active process involving recruiting immune cells with anti-inflammatory activity, Kutí Baruch, a postdoctoral researcher in Schwartz’s lab and first author on the study, hypothesized that the same might be true inside the brain.

In testing that idea, Schwartz said, “we found that transient depletion of regulatory T cells not only stops the progression of the disease, but could reverse some of the disease symptoms in a very dramatic way.”

While noting many details still need to be ironed out, she proposed a mechanism that centers on the role of Tregs in controlling the status of the choroid plexus — the epithelial barrier between the blood and CSF. In healthy brains, the choroid plexus acts as a site of immune surveillance that allows immune cell entry to the brain in response to acute injury or inflammation. But in AD, according to her team’s working model, Tregs suppress that transition and prevent immune cells from the periphery from being recruited to help resolve the inflammation inside the brain.

"Reducing inflammation within the CNS actually requires boosting an inflammatory response outside the CNS."

Michal Schwartz, Weizmann Institute

"You likely need immune cells to get into the CNS to perform an inflammation-resolving process," Baruch told BioCentury.

Schwartz added that if that turns out to be true, then her team’s findings could explain why anti-inflammatory compounds have largely proved disappointing in clinical trials for AD.

GATEWAY THEORY

Based on previous work from her group suggesting that the choroid plexus can act as a gateway for immune cell entry into the CNS during injury and disease, Schwartz and colleagues thought that immune cell trafficking across the choroid plexus might be impaired in AD, and that restoring choroid plexus function could help treat the disease.

The team measured expression levels of the leukocyte homing molecule ICAM-1 in the choroid plexuses of postmortem patient brains and in transgenic mice expressing multiple
AD-linked mutations. **ICAM-1** levels were lower in brains of patients and the AD mouse model than in healthy human or mouse control brains, respectively, and levels of three additional leukocyte homing molecules and IFNγ, a cytokine that controls the expression of all four trafficking molecules, were also lower in the choroid plexus of the mouse model than in controls.

Because Tregs suppress immune cell responses, the group hypothesized that Tregs could be involved in the loss of normal immune surveillance in the choroid plexus and tested whether depleting Tregs could activate immune effector cells and enhance IFNγ expression.

The researchers used diphtheria toxin to selectively and temporarily ablate Tregs in the AD mouse model, and found that in the absence of Tregs, the choroid plexus expressed higher levels of leukocyte trafficking proteins, and peripheral macrophages and T cells were allowed to enter the brain and surround plaques. The treated mice showed less neuroinflammation, including lower levels of proinflammatory cytokines and fewer activated microglia, in the hippocampus than controls. In addition, the treated mice had less than half as many amyloid plaques in the cortex and hippocampus than controls, and performed at levels comparable to wild-type mice in cognitive tests.

Pharmacological inhibition of Tregs with a small molecule tool compound that inhibited p300 produced similar results. Previous studies had shown the compound inhibited Treg function and enhanced immune responses in cancer models.

As well as decreasing plaque burden and improving cognitive performance, the inhibitor increased the number of cells expressing IFNγ in the choroid plexus 10-fold, and increased the fraction of IFNγ-secreting cells in the spleen from 18% to 30% compared with vehicle, which suggested the compound enhanced peripheral immune activity.

Finally, to show that turning up peripheral immune suppression could exacerbate AD pathology, the team used all-**trans** retinoic acid, which enhances the number, stability and function of Tregs. The result was an increase in numbers of Tregs in the spleen and levels of amyloid plaques and soluble Aβ in the brain, and worsened performance on a spatial memory task compared with vehicle.

Together, the data supported the idea that Tregs contribute to neuroinflammation in AD. Schwartz noted that, unlike anti-Aβ antibodies and other interventions that target early stages of AD, Treg depletion was also effective in mice with advanced disease.

In the AD model her team used, the mice normally develop amyloid plaques when they are about two months old, but her team performed the experiments when the mice were four to 10 months old, when plaque pathology and cognitive impairments had progressed to more advanced stages.

"We’re not only dealing with early stages of Alzheimer’s; we’re dealing with the stage when the disease is full-blown and you want to reverse it,” she told BioCentury.

**REBALANCING ACT**

Although the team showed that after depleting Tregs, IFNγ and a variety of leukocyte trafficking proteins were up-regulated in the choroid plexus, Schwartz acknowledged the link between those data and reduced AD pathology in the mice established a correlation — not a cause — and that more work needs to be done to flesh out the mechanism.

However, Eugene Butcher, a professor of pathology at Stanford University, told BioCentury the study is important, even without a clear mechanism, because it supports a small but growing body of evidence implicating peripheral immune cells in AD pathology, and offers "the hope that we will eventually be able to treat or prevent Alzheimer’s disease in patients through targeted manipulation of the peripheral immune system.”

"People used to think that the problem with Alzheimer’s was too much inflammation, but we're coming to realize that the opposite is actually true."

Terrence Town, USC
Butcher is also director and co-founder of Leuvas Therapeutics Inc., a start-up working on blocking specific leukocyte-vascular interactions to treat AD and epilepsy.

Terrence Town, professor of physiology and biophysics at the University of Southern California, told BioCentury the Schwartz group’s results were “compelling,” and said the idea that Tregs contribute to AD by suppressing immunity in the periphery complements work from his own lab showing that loss of the immune suppressor IL-10 had a beneficial effect in a mouse model of AD. However, he noted that while the effects in his group’s study seemed to be “occurring at the level of the brain itself,” the Schwartz group’s study “demonstrates that peripheral immune suppression also plays a role” in the disease.

He said findings from the past 10 years have transformed the field’s thinking about the role of the inflammation in AD in at least two ways. First, it has become increasingly clear that the brain is not the sterile compartment the field once thought it was. Instead, it’s now well-established that under certain conditions, immune cells can enter the brain through the blood-brain barrier (BBB) and likely the choroid plexus as well, he said. He noted that can work either to benefit or harm the brain. While regulated immune cell entry can be beneficial, unregulated inflow of immune cells in the context of BBB breakdown can exacerbate neuroinflammation, especially in the case of multiple sclerosis (MS) and other autoimmune conditions where the infiltrating immune cells will attack brain cells.

Second, he said, “People used to think that the problem with Alzheimer’s was too much inflammation, but we’re coming to realize that the opposite is actually true.”

Town emphasized that the problem in AD is not lack of an innate immune response, but induction of the wrong type of response. “You get these low levels of pro inflammatory cytokines that are produced, but the microglia are just kind of spinning their wheels and not effectively clearing the Aβ.”

He said the Schwartz team’s study suggests that interventions that “rebalance” the peripheral immune system might have therapeutic potential in AD.

Schwartz told BioCentury she thinks AD patients may benefit from some of the therapies being developed to boost immune responses in cancer patients. In addition to investigating the molecular mechanisms of the Treg effect, she said her lab is evaluating the ability of “a variety of cancer immunotherapeutic approaches” to treat mouse models of AD.

Schwartz declined to disclose the patent and licensing status of her team’s work.

COMPANIES AND INSTITUTIONS MENTIONED
Leuvas Therapeutics Inc., Mountain View, Calif.
Stanford University, Stanford, Calif.
University of Southern California, Los Angeles, Calif.
Weizmann Institute of Science, Rehovot, Israel

TARGETS AND COMPOUNDS
Aβ - β-amyloid
ICAM-1 (CD54) - Intercellular adhesion molecule-1
IFNγ - Interferon γ
IL-10 - Interleukin-10
p300 (EP300) - E1A binding protein p300

REFERENCES