3rd Venusberg Meeting on Neuroinflammation
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By Tom Fagan

Microglia Activation—Venusberg Meeting Questions M1, M2 Designations
In England, the M1 and the M2 motorways take you in almost opposite directions. Does the same happen in the brain? In-vitro, microglia can be driven to adopt M1 and M2 phenotypes, with M1 believed primed to drive inflammation and M2 to drive phagocytosis of debris. Researchers have tried to tie M1 and M2 status to pathology in various neurodegenerative diseases, but ironically, just as the concept has caught on in the field more broadly, leaders in neuroinflammation are beginning to question their usefulness. That was one of the main trends coming out of the 3rd Venusberg Meeting on Neuroinflammation, held 28 February to 2 March at the Biomedical Center, University of Bonn, Germany meeting. "It seems we need to place less emphasis on M1 and M2 now," said **David Morgan**, University of South Florida, Tampa. Many researchers at the meeting echoed that sentiment. "I think a major conclusion to come from this meeting is that we need to look more broadly at microglial gene expression," summed up **Michael Heneka**, University of Bonn, on the last evening. Others were less inclined to dismiss the classification so easily. "I think the jury is still out with regard to M1/M2. More functional studies need to be done to get a definitive answer," said **Joe El Khoury**, Massachusetts General Hospital, Charlestown. Heneka organized this conference, which drew researchers from Europe, North America, and Japan who study various neurodegenerative diseases, including Alzheimer's, multiple sclerosis, and motor neuron disease.

That microglia can both help and hinder the brain in Alzheimer's and other diseases (see ARF related news story) has created some confusion in the field. To try to sort it out, researchers have looked at these cells in terms of their M1 and M2 phenotypes. M1 genes include interleukin (IL) 1β, tumor necrosis factor α, interferon γ, and inducible nitric oxide synthase, while YM1, and IL-4 and -10 fall into the M2 bin. In Venusberg, however, researchers questioned the value of this distinction. **Richard Ransohoff**, Cleveland Clinic, Ohio, showed that, at least in some cases, M1 and M2 expression cannot distinguish good cells from bad.

Ransohoff studies multiple sclerosis, a neurodegenerative disease in which myelin membranes sustain selective injury. His group uses block face scanning electron microscopy to obtain detailed three-dimensional pictures of glia that initiate myelin damage, and contrasts them with glia that clear myelin from injured regions in the central nervous system of mice. Clearance of debris precedes repair and is therefore beneficial. To figure out which cells are responsible for the clearance, Ransohoff and colleagues

crossed mice that express red fluorescent protein in their circulating monocytes with others making green fluorescent protein in brain-resident microglia. The promoters of the monocyte-specific CC-chemokine type 2 receptor gene and the microglial fractalkine receptor gene drive production of the fluorescent proteins. In the double-transgenic mice, monocytes always turn up red, microglia green. The researchers induced experimental autoimmune encephalomyelitis (EAE) in the mice to mimic MS pathology, and then determined which cells went about causing injury and which were mopping up the damage.

Ransohoff reported that the monocytes were invariably the cells that seemed to yank myelin off axons, and often seemed to begin the process at the node of Ranvier, where myelin thins out. Microglia, on the other hand, seem to clear up myelin that has already detached from neurons. Without their red and green labels, could these cells be distinguished in any other way, for example, by M1 and M2 gene expression? Apparently not. Researchers in Ransohoff's lab separated monocytes and microglia by fluorescence-activated cell sorting and then carried out microarray expression analysis in collaboration with Oleg Butovsky at Brigham and Women's Hospital, Boston. The patterns overlapped. "The cells do express individual M1 and M2 genes, which are also nonspecific indicators of myeloid cell activation, but they can express both together or not at all, and when they do express them, it's not necessarily the full panel of genes," said Ransohoff. In short, M1 or M2 gene expression did not differentiate the cells. "I think the categorization has little value for studying microglia," said Ransohoff.

That seemed to be echoed by **Monica Carson's** research at the University of California, Riverside. Carson studies microglia and macrophages together in cell culture with neurons. She saw that the microglia remain quiescent when neurons in the culture are one week old and inactive, but change their morphology a week later, when neurons start to transmit across synapses. The microglia go from being flat and amorphous to assuming a more defined structure, showing that they can react to their environment and to the differentiation state of the co-cultured neurons.

Carson used these mixed cell cultures to study inflammation. She injected lipopolysaccharide into the brains of mice to induce an immune response and then co-cultured cells from those brains. She reported that macrophages that had infiltrated the brain began to kill the neurons, whereas microglia did not. However, she was surprised to find that the toxic macrophages were predominantly of the M2 phenotype, showing high expression of classic M2 markers, including YM-1, TGFb, and IL-1 receptor antagonist. This runs contrary to the view that M1 states are more toxic. Carson emphasized that other factors besides the cells' activation state determine toxicity. "We should not ignore the M1/M2 paradigm completely, as it may still be useful, but perhaps more important is the cells' provenance and context," she said. "Infiltrating macrophages were much more toxic, yet they correlated with M2 activation states."

Terrence Town, who recently moved across town from the Cedars Sinai Medical Center in Los Angeles to the University of Southern California, echoed the idea that context determines neuroinflammation in neurodegenerative diseases such as Alzheimer's. The

M1/M2 concept is oversimplified, Town said. He favors a microglial "continuum" of activation, where M1 and M2 represent extreme ends of the spectrum. He also argued that the field desperately needs functional markers. "Markers for phagocytosis, or pro- and anti-inflammatory states, would really be valuable," he said. Others agreed. "Research in the T cell field took off when people were able to associate markers with function," noted **Greg Cole**, University of California, Los Angeles. Morgan argued for microarray-based data to get a better picture of expression phenotypes.

El Khoury has taken steps in that regard using direct RNA sequencing (DRS). Developed originally for yeast, El Khoury's group applied this relatively new technique to mouse microglia. The technique requires very little mRNA, and avoids bias introduced by amplification steps or making cDNA. DRS captures mRNA using an oligo*dT* chip that binds mRNA polyA tails, then sequences the captured RNA directly, while using a highly sensitive camera to record the addition of nucleotides one at a time. Thousands of transcripts are sequenced together. The only disadvantage, said El Khoury, is that DRS cannot measure alternative splice variants. "You can only sequence what's at the 3' end of the transcript," he said.

El Khoury used DRS to sample what he calls the microglial "sensome." Microglia are constantly sensing their environment by sending out extensive processes (see ARF related news story), which he believes may have cell surface receptors unique to these surveying cells. He developed magnetic beads to capture the microglial surface marker CD11b, and with those beads, isolated microglia from mouse brain tissue by flow cytometry. He then compared the DRS transcriptome of the brain microglia to that of peritoneal macrophages.

From around 22,000 transcripts, El Khoury identified 2,300 that were specific to the glia. He narrowed that down with bioinformatics to a subset of transcripts that he believes represent the "sensome." This panel of markers, El Khoury claims, distinguishes microglia from astrocytes, macrophages, and other cells in the brain. It covers a broad array of different genes, including chemokines and purinergic receptors. El Khoury used proteomic analysis of 2-D polyacrylamide gels and dual in-situ hybridization to check the DRS data. The latter, for example, showed that sensome genes colocalized with CD11b, indicating they were specifically expressed in microglia.

Eventually, this new information could be used to sample changes during disease or immune challenge, said El Khoury. For now, he applies it to study aging in mice. Comparing the "sensome" of young and old mice, he found genes that appear to be upregulated as the animals age, and seem to be involved in sensing microbes. Neither monocytes nor macrophages show such expression pattern changes with age, he said. Some of those genes fall into the M2 classification. El Khoury speculated that microglia assume a more neuroprotective phenotype with age.

Other scientists were impressed with these findings. "This is groundbreaking work," said Heneka, who emphasized that it will be important to relate these expression patterns to specific microglial functions. Others noted that this type of broad approach will be more

informative than relying on M1 and M2 expression patterns. "This work illustrates what is now possible in the microglial field," said Ransohoff.

Inflammatory Crosstalk Between Periphery and Brain

Talk about immune cells in the periphery and the central nervous system was traded back and forth during the 3rd Venusberg Meeting on Neuroinflammation, held 28 February-2 March 2013 at the Biomedical Center, University of Bonn, Germany. For decades, scientists have debated whether peripheral cells cross the blood-brain barrier and what signals egg them on. "I think it seems clear from this meeting that peripheral cells can enter the brain under certain circumstances," noted **David Morgan**, University of South Florida, Tampa. Other researchers agreed, though how often those circumstances occur remains unclear.

Evidence for infiltration of peripheral cells into the brain has been equivocal. Doubts lingered over methodology when studies used irradiation to ablate peripheral immune cells and then replaced them with fluorescently labeled cells to test if they could reach the brain (see ARF related news story). Irradiation can damage the blood-brain barrier and allow cells across. Even in the absence of irradiation, results were at odds. Mouse models of Alzheimer's disease lacking the chemokine receptors that drive migration of peripheral monocytes clear plaque more slowly than usual (see ARF related news story), suggesting that peripheral cells enter the brain. Yet other data suggests that peripheral immune cells are distinct from those in the CNS in AD models (see Mildner et al., 2011).

Notwithstanding the ambiguities, evidence has grown that peripheral cells can sometimes infiltrate the CNS, though they typically avoid a healthy brain. In experimental models of multiple sclerosis, for example, monocytes enter the brain and contribute to degeneration of myelin (see Ajami et al., 2011). At the Venusberg meeting, Richard Ransohoff from the Cleveland Clinic, Ohio, showed how CCR2-positive cells strip myelin from neurons in the CNS (see ARF related news story). Only peripheral monocytes express CCR2, a chemokine receptor that helps guide these cells to sites of damage.

What prompts peripheral cells to enter the brain? **Nicholas Varvel**, who works at the Hertie Institute, University of Tubingen, Germany, posited that neurodegeneration can be that trigger. Varvel chose a model of epilepsy to study infiltration of circulating monocytes. In his poster, he described how intraperitoneal injection of kainic acid into FVB mice, a strain particularly sensitive to this neurotoxin, induces robust epileptic activity. Kainate also causes rampant neurodegeneration in the CA3 region of the hippocampus within three days. By crossing FVB mice with a CCR2 reporter strain developed in Ransohoff's lab, Varvel tested if this neurodegeneration recruited circulating monocytes.

The CCR2 mice make red fluorescent protein (RFP) driven by the chemokine receptor promoter. In the crosses, Varvel saw recruitment of RFP-positive cells to the CA3 within two to three days of inducing epilepsy. "The finding suggests that not only do the cells migrate into the brain, but that they are also functional when they get there, because they are clustering around sites of neuronal damage," said Varvel. In fact, he was able to show

this in a second way. By backcrossing to the FVB strain, the neurodegenerative profile changed to envelop mostly cortical neurons. The CCR2 cells now traveled to the cortex, again targeting sites of damage. "This supports the idea that these monocytes are functional in the brain," said Varvel.

Could peripheral cells similarly enter the brain in Alzheimer's disease? People who have AD are susceptible to epilepsy-like seizures, and many transgenic AD mouse models have seizure activity as well. "That might be difficult to measure," suggested Varvel. He noted that his is a model of acute toxicity, whereas AD is chronic.

If circulating monocytes are entering the brain in response to neuronal death, then to what signals are they reacting? Varvel found that glia in the damaged zones made CCL2, the chemokine that binds the CCR2 receptor. But ultimately, neurons must be sending out signals, probably fractalkine, suggested Varvel. Fractalkine and its cognate CX3CR1 receptor on glia are a primary neuron-glial signaling pathway in the brain.
In peripheral immune cells, other pathways are at work. **Mari Shinohara**, from Duke University, Durham, North Carolina, reported that inflammasome signaling drives infiltration of circulating immune cells into the brain in experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. Inflammasomes are large complexes that promote caspase 1 cleavage and maturation of interleukin 1β (IL-1β) and IL-18, and together the two cytokines boost expression of chemokines and chemokine receptors that guide monocyte migration. That may be how the inflammasome helps direct peripheral cells into the CNS, said Shinohara.

Previously, researchers in her lab had found that interferon- β (IFN-β), a first-line treatment for MS, suppresses a specific inflammasome that goes by the mouthful "nod-like receptor (NLR) family, pyrin domain-containing 3 type inflammasome," or NLRP3 for short (see Inoue et al., 2012). When researchers in Shinohara's group ablated NLRP3 in mice, T cells produced fewer chemokines and chemokine receptors than usual, and injections of myelin oligodendrocyte glycoprotein (MOG) failed to induce EAE. Compared to wild-type mice, NLRP3 knockouts had fewer IL-17-producing Th17 cells, which are thought to be particularly inflammatory and promote a variety of autoimmune diseases, including MS. Her group found that NLRP3 helped antigen-presenting cells activate Th17 cells.

Do fewer Th17 cells explain why these mice resist EAE? Not exactly. In Bonn, Shinohara showed that when she injected additional Th17 cells from MOG-immunized NLRP3 knockouts into the blood to replenish cell counts to normal, she still failed to induce EAE. Taking the same number of Th17 cells from MOG-immunized wild-type mice and injecting them into the periphery, however, did restore the immune response that leads to central encephalomyelitis. Those experiments indicated that quality, rather than the quantity, of the cells was crucial for EAE. The key was cell migration. "The Th17 cells activated by NLRP3-negative antigen-presenting cells are pathogenic but unable to migrate into the CNS," said Shinohara. When she injected Th17 cells from immunized NLRP3 knockout mice directly into the CNS, then the animals did develop EAE.

The work not only suggests that the NLRP3 inflammasome may have ramifications for MS treatment strategies. Only half of MS patients respond to IFN-β, suggesting that there may be alternate pathways driving cell migration into the CNS. In fact, Shinohara found that more aggressive immunization with the myelin oligodendrocyte glycoprotein antigen induced EAE even in the NLRP3 knockout. "It seems that there is also an inflammasome-independent pathway that induces EAE and possibly MS," said Shinohara. "If we could understand that other pathway, we may be able to target it for treatment."
Researchers at the meeting felt this was a good example of how inflammation and inflammatory signals in the periphery can lead to consequences in the central nervous system. "We need to develop a better understanding of how systemic inflammation affects the brain," said meeting organizer **Michael Heneka**, University of Bonn. He recently reported that among patients at an intensive care unit, cognition declined over the next six to 24 months in those who had developed sepsis. Magnetic resonance imaging also showed atrophy of the hippocampus (see Semmler et al., 2013).

Blessing or Curse? Peripheral Cytokines in the Brain
More friendly border than iron curtain, the blood-brain barrier lets all sorts of legitimate travelers pass. Could it also be letting some unsavory characters slip by? The subject of crosstalk between the peripheral circulation and the central nervous system permeated conversation at the 3rd Venusberg Meeting on Neuroinflammation, held 28 February-2 March 2013 at the Biomedical Center, University of Bonn, Germany. Researchers agreed that, in certain settings, circulating immune cells can enter the brain and create havoc (see ARF related news story). But they also emphasized that crosstalk occurs in the absence of infiltrating cells. Scientists argued that elevated plasma cytokines and other signaling molecules may profoundly affect the central nervous system, though not always negatively. While some proinflammatory cytokines might block neurogenesis in the brain, limit synaptic plasticity, or even cause cell death, others seem to encourage resident brain cells to take on protective roles, such as clearing amyloid-β plaques. Scientists have studied links between plasma cytokines and the brain for decades.

Charles Dinarello, University of Colorado, Denver, noted data from the Framingham Study that suggests people with the highest levels of circulating interleukin 1β (IL-1β) are almost three times more likely to develop Alzheimer's disease (see Tan et al., 2007). In contrast, people with more interleukin 1 receptor antagonist in their blood are less likely to have dementia, said Dinarello. He argued that peripheral cytokines, including IL-1β and other highly inflammatory ones such as IL-18, may predispose people to dementia and other degenerative conditions. IL-18, activated by IL-1β, drives apoptosis, and Dinarello showed that in a heart failure model, the IL-18 antagonist IL-18 binding protein (IL-18BP) rescues damage caused by IL-1β. Blocking IL-1β and IL-18 activation also limits damage in human myocardial tissue following ischemia (see Pomerantz et al., 2001). While these experiments implicate these cytokines in the heart, researchers have yet to dig into IL-18's actions in the brain, said Dinarello.

Epidemiological evidence suggests a link. Dinarello noted an Italian study that found higher circulating IL-18BP in centenarians than in the general population, hinting that

less free IL-18 promotes health (see Gangemi et al., 2003). People with AD have lower blood IL-18BP levels than age-matched controls, hence, higher free IL-18, as do people with certain systemic disorders. What these correlations mean was debated at the Venusberg meeting. Researchers noted it's difficult to separate cause and effect here, but Dinarello countered that emerging evidence points to peripheral cytokines profoundly influencing the central nervous system. "If they can get into the brain, they can cause damage," he said.

Many researchers at the meeting were intrigued by the link between systemic illnesses and dementia. **Clive Holmes**, University of Southampton, U.K., discussed an idea he has pursued for some time, namely that systemic inflammation can lead to central nervous system disease, including "sickness behavior," which resembles some of the neuropsychiatric symptoms of delirium, though it is a separate entity. Sickness behavior correlates with increased risk for AD (see ARF related news story). Holmes has reported that AD patients decline faster if they had a recent systemic infection, traumatic event (see Holmes et al., 2003), or elevated proinflammatory cytokines in their blood (see Holmes et al., 2009).

Animal models may help test correlations between circulating molecules and disease. **Tony Wyss-Coray**, Stanford University, Palo Alto, California, gave an update on his parabiosis model for studying blood factors that contribute to aging. In this model, two congenital mice share their blood systems, allowing Wyss-Coray to measure the effect of young blood on older animals, and old blood on younger. This led him to identify blood factors that appear to mediate aging, including the chemokine CCL11. Simply delivering CCL11 to young animals yields phenotypes associated with older mice, including less neurogenesis in the brain (see ARF related news story).

How else might blood factors influence tissue inside the CNS? In Bonn, Wyss-Coray said his group addressed this question by looking for differences in gene expression in hippocampal tissue from old/old and old/young parabiosis pairs. Researchers in his lab identified several gene networks that are altered in old animals exposed to young blood. The most highly upregulated turned out to be involved in synaptic plasticity. The group confirmed this at the protein level, looking at factors such as pCREB, Egr1, and cFOS. "The data indicate that factors in young blood reactivate the brain," said Wyss-Coray. In keeping with this, he reported greater synaptic spine density in older mice exposed to young blood.

How to achieve this effect without parabiosis? Wyss-Coray claimed that injections of young-mouse plasma improved cognition in older animals. "All this points to the possibility of some degree of rejuvenation, with neurogenesis, spine density, synaptic plasticity, memory, and inflammation all improving," he said.

But are any of these age-associated differences related to neurodegenerative disease? To test this, Wyss-Coray and colleagues compared gene expression and blood phenotypes among healthy controls and people with a semantic variant of frontotemporal dementia. Using pathway analysis software from Ingenuity Systems, they found elevated expression

of networks involving interleukin 12 (IL-12) and IL-23 in the dementia patients. Independent research suggests that IL-12/23 signaling may drive autoimmunity, and exacerbate pathology and cognitive decline in mouse models of AD (see ARF related news story).

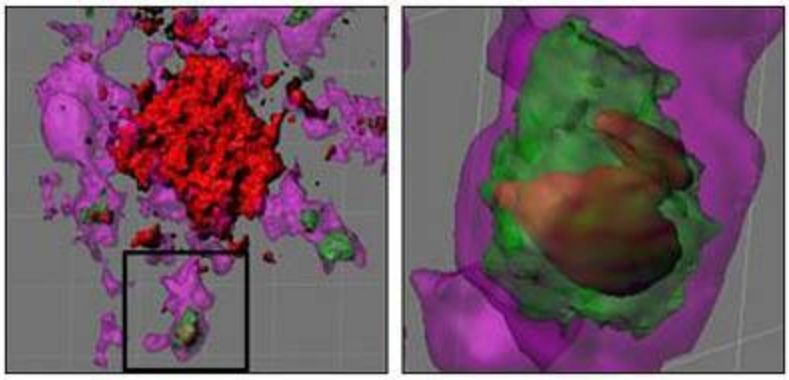
Wyss-Coray's group used a similar network-based approach to analyze blood phenotypes, measuring a panel of 50 proteins expressed by immune cells. The scientists found three subsets of markers more highly represented in plasma from FTD patients than controls. Some, including CD27, are known to contribute to hyperactivation of T cells. "Again, we seemed to have convergence around factors that contribute to autoimmunity," said Wyss-Coray.

What does this mean for FTD and other neurodegenerative diseases? For example, could ustekinumab, an IL-12/23 antibody currently used to treat the autoimmune skin disorder psoriasis, benefit dementia? Researchers at the Venusberg meeting were reluctant to speculate, but Wyss-Coray noted that autoimmune diseases were more common in FTD patients than would be expected by chance. Many of the affected patients have autoimmune skin diseases, but systemic lupus erythematosus and other disorders were represented as well.

For all the talk about cytokines and inflammation being bad for the brain, **Terrence Town**, University of Southern California, elaborated on a different idea, namely that of "good" neuroinflammation. Unlike mouse models in AD, Town's transgenic rat model of AD recapitulates most cardinal pathologies of the disease, including Aβ accumulation, Aβ oligomers, gliosis, tau accumulation, synaptic loss, neuronal loss, and cognitive decline (see ARF related news story). In this model, microglial activation preceded obvious plaque formation, which hints that these cells may be attempting to prevent Aβ accumulation, said Town.

Town previously reported that blocking anti-inflammatory signals in peripheral macrophages promoted clearance of Aβ plaques in transgenic mice rather than exacerbating pathology, as might be expected if tipping the balance toward inflammation was bad (see ARF related news story). That result came from blocking TGF-β signaling. In Bonn, Town reinforced this finding by reporting that blocking other anti-inflammatory pathways had a similar effect. Town crossed PSAPP AD model mice with IL-10 and IRAKM knockouts. Both IL-10 and IRAKM quell inflammation, the latter being the only member of the interleukin 1 receptor-associated kinase family that suppresses innate immunity.

Both crosses accumulated fewer Aβ plaques than the PSAPP strain. They seemed to activate microglia, showing elevation of microglial markers Iba1+ and CD45 in the brain. In the IRAKM-negative animals, plaque burden fell while free Aβ rose in the brain, prompting Town to suggest that some dynamic process was going on to reduce plaques. He showed 3-D confocal microscopy reconstructions of brain tissue that demonstrated colocalization of Aβ with the phagolysosomal vesicle marker, LAMP1, demonstrating plaque phagocytosis by activated innate immune cells in vivo (see image below).



Glia Gobble Aβ
3-D confocal reconstructions of glial cells (purple) phagocytosing and digesting Aβ (red) via lysosomes (green).
Images courtesy of Terrence Town and David Gate

“If it’s targeted, inflammation can be beneficial," said Town. "So we shouldn’t find it surprising that promoting specific types of microglial activation can lead to plaque clearance."

Glial Imaging—Amid Slow Progress, EU Project Takes Up Challenge
Calls for functional markers emanated loud and clear from the 3rd Venusberg Meeting on Neuroinflammation, held 28 February-2 March 2013 at the Biomedical Center, University of Bonn, Germany. Markers for brain-resident microglia and infiltrating macrophages, that is. Despite renewed interest in the role of immune cells in Alzheimer's and related diseases, researchers remain hamstrung by difficulties detecting these cells and figuring out what they do in disease. Developing markers for in-vivo imaging has proven particularly vexing. In Venusberg, members of Imaging of Neuroinflammation in Neurodegenerative Diseases (INMiND), a European consortium seeking immune targets for diagnostic and therapeutic applications, reported on potential new tracers. Other researchers lamented that these all bind the same target, a mitochondrial transporter. INMiND principal investigator, **Andreas Jacobs**, Westfälische Wilhelms-Universität, Münster, Germany, told Alzforum that tracers for other microglial targets, including enzymes and cell surface receptors, are being developed, but no leading contender has yet to emerge.

For decades, researchers have studied glia in vivo by imaging TSPO, a transporter in the outer mitochondrial membrane. PK11195, a ligand developed in the early 1980s, binds this protein with high affinity and has been used to image microglia in a variety of human conditions, including Alzheimer's (see ARF related news story), stroke, viral encephalitis, and more recently to identify inflammation in soldiers who have suffered traumatic brain injury (see Ramlackhansingh et al., 2011). However, the ligand has its drawbacks. "For one, it tells nothing about the nature of microglia, or why they are active," noted INMiND investigator **David Brooks**, Imperial College London, U.K. PK11195 lacks specificity, poorly penetrates the brain, and has a weak signal-to-noise ratio.

Against this background, researchers in Europe conceived of INMiND to develop probes for microglial imaging and study the basic mechanisms that underscore neuroinflammation. The project grew from a Diagnostic Molecular Imaging (DiMI) project the EU funded from 2005 to 2010.

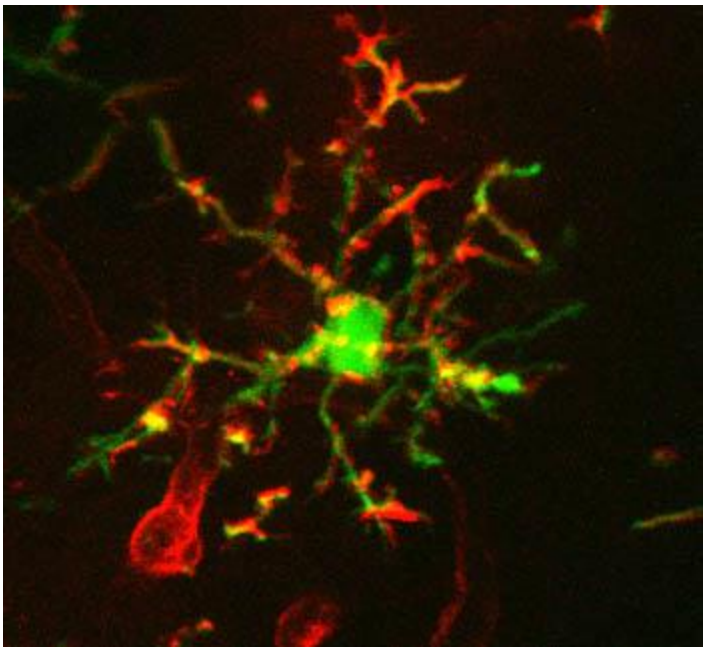
When that ended, Jacobs, together with about 50 of the neuroscience partners from DiMI, applied for fresh EU funds. "The idea was to focus on the microglial aspect of neuroinflammation," Jacobs told Alzforum.

INMiND comprises a collaboration among 20 academic partners in Europe, one in Australia, and seven small to medium private enterprises, said Jacobs. Through its Seventh Framework Programme (FP7), the EU funds the project to the tune of €12 million over five years. An additional €8 million comes from the partner academic institutions in 12 countries.
Jacobs told Alzforum that INMiND will study the earliest stages of disease, focusing on familial Alzheimer's, familial Parkinson's disease, and on patients with mild clinical impairment who test positive for brain amyloid and are at great risk of progressing to Alzheimer's dementia (see Okello et al., 2009). INMiND investigators are planning a clinical trial to treat MCI patients while following microglial activation with molecular imaging. "We want to see if we can modify plaque load, postpone AD, and tell how microglia are involved in that process," said Jacobs. The imaging agent has yet to be selected, he said.

Therein lies the challenge. Better imaging agents are emerging ever so slowly. Researchers in France, including INMiND investigator Bertrand Tavitian at the Atomic Energy Commission in Orsay, have developed a new range of TSPO ligands that have better profiles than PK11195, but they also have problems, most notably binding only weakly to people who have certain TSPO isoforms (see ARF related news story). That complicates analysis, said Brooks, because it divides populations into high and low binders. Distinguishing people with AD, for example, from controls then becomes problematic, he said, because high-binder controls take up more of the ligand than low-binder AD patients. Most new TSPO ligands, including DPA-714, and the DAA family of ligands (see Owen et al., 2011) exhibit this differential binding.

Brooks noted that GE-180, a TSPO ligand being developed by GE Healthcare, where Brooks worked previously, shows a better binding profile than PK11195, but so far has only been tested in animal models. Uptake and retention of GE-180 in brain areas that express TSPO compare well with that of other ligands, but it appears to be cleared more rapidly from low-binding regions, improving its signal-to-noise ratio. How this ligand binds to TSPO isoforms remains to be seen.
Researchers at the Venusberg meeting questioned why TSPO continues to be a focus for ligand development. In summing up the meeting, **Hugh Perry**, University of Southampton, challenged the community to come up with something better. Some researchers said cell surface markers, rather than a mitochondrial transporter, should be more thoroughly investigated. Others noted that new research presented at the meeting has turned up a suite of microglial-specific markers (see ARF related news story) that offer fertile hunting ground for new ligands.

In this vein, **Olga Garaschuk**, University of Tübingen, Germany, suggested plant lectins. She reported that isolectin B4 binds tightly to glia when injected into the brains of transgenic mice. IB4 binding overlapped with green fluorescent protein expression driven by a microglial promoter. It also bound to blood vessels, but Garaschuk did not see that as a major detriment, since vessels and microglia are easily distinguished. Tomato lectin was slightly better (see image below), lighting up tiny microglial processes that IB4 missed (see also Schwendele et al., 2012).



Lectins Label Microglia
DyLight 594-conjugated tomato lectin (red) labels microglia expressing enhanced green fluorescent protein (green). *Image courtesy of David Brooks, Imperial College London*

How do lectins work? They have high affinity for sugar coatings, which are rich on microglia. Tomato lectin binds N-acetylglucosamine oligomers, while α-galactose-rich proteins attract IB4. Garaschuk noted that, unlike other lectins that induced long-term potentiation when injected into the brain, tomato lectin seems to lack biological activity. Microglia also appear fully motile when they bind the ligand.

Brooks noted other non-TSPO targets that might prove useful, including cannabinoid and purinergic receptors. Jacobs said that receptors often make poor targets, suggesting instead that imaging arginase activity might prove useful, as activated microglia upregulate this enzyme. The idea here would be to make a ligand that can be metabolized by arginase into a compound that gets trapped in the cell.