Uppsala: Conference Puts Uppsala on Immunotherapy Map

28 June 2011. Founded in 1477, Uppsala University in Sweden has a long tradition of advancing the cutting edge of medicine, from its pharmacopeiac gardens tended by Carl Linnaeus of taxonomy fame, to a seventeenth-century anatomy theater hosting public operations and dissections. In this day and age, the institution aims to lead in part by hosting a conference on new technologies that are pushing toward earlier diagnosis and treatment of Alzheimer's disease and other neurodegenerative disorders, which are looming as twenty-first-century health crises of aging Western societies. Organized by **Lars Lannfelt**, **Martin Ingelsson**, and **Lars Nilsson**, the 2nd International Conference on Neurodegenerative Disorders: Immunotherapy and Biomarkers took place at Uppsala University 26-27 May 2011. At about 150 attendees, the small size of the meeting belied its impact. Most participants questioned by a reporter said this meeting targets the right niche at the right time, calling it "a keeper." With potential immunotherapeutics mushrooming, **Roger Nitsch**, University of Zurich, quipped that before long, Lannfelt's catalogue of antibodies might rival Linnaeus's one of plants.

Immunotherapy for AD started about a decade ago with Elan/Wyeth's active immunization program targeting soluble amyloid-β (see ARF related news story), and that is still the leading target of many academic and industry labs. At the same time, this meeting showed that programs targeting other forms of Aβ (see below), tau (see Part 2), and proteins involved in other neurodegenerative diseases (see Part 3) are rapidly catching up.

One reason immunotherapy has become so attractive to drug developers is that it bypasses the need for medicinal chemistry, noted **Peter Seubert**, from Elan Pharmaceuticals, San Francisco. Medicinal chemists tweak small-molecule drug candidates to improve safety, pharmacodynamics, and pharmacokinetics. Their work can be time consuming and expensive. Antigens and antibodies do not need such delicate manipulations. Another draw of immunotherapy is that, contrary to conventional wisdom, a small proportion of antibodies made by the immune system or injected into the bloodstream do appear to cross the blood-brain barrier into the central nervous system—something scientists at the meeting puzzled over but seemed to accept. A case in point is bapineuzumab, a humanized monoclonal antibody that is being closely watched by observers in research and beyond. It lowered brain amyloid when given intravenously to patients enrolled in a Phase 2 trial (see ARF related news story on Rinne et al., 2010). A current Phase 3 trial powered to test for cognitive outcomes is expected to end in 2012. Though no data are officially available yet, Seubert noted that the trial has not been scuppered by vasogenic edema, as some had expected following the appearance of this side effect in some patients in Phase 2 (see ARF related news story). In April 2009, Elan dropped the highest doses of bapineuzumab from the trial in an attempt to mitigate this risk (see ARF related news story). Later that year, JANSSEN Alzheimer Immunotherapy Research & Development, a subsidiary of Johnson & Johnson, acquired all rights to Elan's Alzheimer's immunotherapy program.

Brendon Binneman, from Pfizer Inc., Groton, Connecticut, reviewed ponezumab, this company's humanized mouse monoclonal antibody to the C-terminal end of Aβ (see ARF related news story and clinical trials). The company is awaiting results of a

Phase 2 trial begun in 2008, and will not present ponezumab data at ICAD next month in Paris, France. Binneman said Pfizer believes this antibody acts via the peripheral sink hypothesis, which posits that antibodies that mop up Aβ in the bloodstream help to coax the peptide out of the brain by tipping the blood/brain equilibrium. Binneman said the company likes to think of it as "brain dialysis." This would make it similar to Lilly's solanezumab, which is in Phase 3 (see ARF related news story). In Phase 1 safety trials, ponezumab had a long half-life of about 20 days in blood. Only about 0.5 percent of the infused protein made its way into the cerebrospinal fluid (CSF). After a single dose in humans, total plasma Aβ levels rise, as does CSF Aβ, which Binneman considered evidence that the peripheral sink idea might be working. The antibody appears to be safe so far, with no evidence of encephalitis or vasogenic edema. Ponezumab is modified to reduce activation of the innate and cellular immune systems, and it does not bind to Aβ well in tissue. These may be beneficial properties, said Binneman, because the antibody may be less likely to cause the type of blood vessel damage that leads to microhemorrhage or vasogenic edema.

Lars Nilsson, also from Uppsala University, described preliminary work using mAb158 to develop a PET ligand for protofibrils in the brain. To test the concept, he labeled the antibody with radioactive iodine I-125, and found this left the antibody's immunoreactivity intact. He then tried to detect the radiolabeled antibody in mouse brain in vivo by SPECT, but found that it lacked the sensitivity of PET. Changing tack, Nilsson infused the mice with antibody, then removed the whole brain and imaged it ex vivo. After a single injection, the antibody peaked in the brain after three days but took almost a month to disperse. This is much longer than PIB, AV-45, and other amyloid ligands take to vacate the brain. Nilsson is trying to optimize the ligand by using F(ab) fragments and I-124, which has a shorter half-life. He did say that the antibody can detect protofibrils in the mouse brain at young ages before amyloid has accumulated, suggesting that if this ligand could be perfected, it might serve as an early marker of pathology and complement current plaque ligands.

Other immunotherapies aimed at different Aβ species are in various preclinical stages. **Cynthia Lemere**, Brigham and Women's Hospital, Boston, and **Thomas Bayer**, University of Gottingen, Germany, collaborate with the German biotech company Probiodrug AG, based in Halle, Germany, to target truncated Aβ with a cyclized glutamate (pyroglutamate) at the amino end. Pyroglutamate Aβ3-42 is reputed to be highly toxic and likely to form oligomers (see ARF related news story). In Uppsala, Lemere reported on her prevention and therapeutic tests in mice of a monoclonal antibody from Probiodrug. When given intraperitoneally over 32 weeks as a prophylactic to young APP/PS1 mice, the antibody reduced cortical plaque deposits and lowered Aβ and pyrogluAβ in the hippocampus and the cerebellum. It calmed inflammatory gliosis as judged by the number of microglia sprouting the inflammatory marker CD45. As a therapy given to 23-month-old mice for seven weeks, the antibody had a less robust effect, showing only trends for decrease in pyrogluAβ and reduction in cerebellar pathology. In addition, Lemere reported on an active vaccination trial in six-month-old J20 mice. Given monthly over eight months, pyrogluAβ3-9, coupled to keyhole limpet hemocyanin as an adjuvant, elicited an immune response that generated mostly IgG1, IgG2a, and IgG2b antibodies. Immunized animals had fewer fibrillary plaques in the brain than controls, less gliosis, and yielded less guanidine hydrochloride soluble Aβ. Interestingly, mice inoculated with a control, full-length Aβ accumulated less pyrogluAβ than mice immunized with

the truncated pyroglutamate antigen. Lemere said that this is most likely because the former vaccine prevents the deposition or promotes the removal of plaques that trap pyroglutamate Aβ. The point of the comparison, she said, was to test if pyrogluAβ acts as a seed for plaques. "In terms of plaque pathology, we saw reductions with both antigens. I do think pyroglutamate Aβ is a seed, but I don't think it is the only seed," she told ARF.

Bayer and colleagues raised antibodies against pyrogluAβ3-38 (see Wirths et al., 2010 and Wirths et al., 2010). They chose this antigen because it aggregates more slowly than pyrogluAβ3-42, and fast aggregation might complicate the immune response. The scientists identified the antibodies by their binding to human brain tissue in immunohistochemical assays. In biochemical assays, Bayer was surprised to find that one, 9D5, had an unusual binding pattern. It does not react with full-length Aβ1-42, nor with Aβ3-42 monomers, dimers, or oligomers greater than 20-mers. Under native conditions, it does seem to bind pyroglutamate Aβ oligomers in the 4- to 20-mer range, and it can block aggregation and reduce toxicity of pyrogluAβ. In three-month-old 5xFAD and APP/PS1KI mice, 9D5 detected no antigen. At six months it did detect some Aβ species, and immunoreactivity in neurons and microglia grew from there over the course of the next six months, Bayer said. Unlike generic Aβ antibodies, 9D5 did not react with plaques in brain tissue of non-demented people. When asked if this means plaques are not a reservoir of pyrogluAβ peptides, Bayer replied that plaques react strongly to other antibodies that specifically recognize pyroglutamate Aβ. In contrast, 9D5 reacts with plaques to a minor extent, showing preference for neurons, microglia, and blood vessel walls. This would fit the theory that small amounts of pyrogluAβ oligomers in the 4- to 20-mer range can form seeds for aggregation, said Bayer. He also presented a small mouse study indicating that 9D5 reduces plaque load and stabilizes cognition as judged by an elevated plus maze test. Injected once per week (10 mg/Kg) into 4.5-month-old 5xFAD mice, the antibody reduced both pyroglutamate Aβ and full-length Aβ plaques. Bayer believes this finding suggests that these small, 4- to 20-mer, pyroglutamate Aβ oligomers act as seeds for aggregation.

For his part, Lannfelt expressed concern about procedural artifacts in the study of truncated pyrogluAβ, specifically the suggestion that brain tissue that has been stored for some time contains much more pyrogluAβ than fresh brain tissue. Lemere replied that this has not been an issue in her hands. On the contrary, she said, leaving tissue too long in fixative can dramatically reduce pyrogluAβ signals, something researchers should keep in mind. Lemere told ARF that she found a distinct difference between tissue that is briefly fixed (two weeks) and routinely fixed (months to even years). In the latter, the fixative seems to mask staining for pyroglutamate Aβ, most likely because of molecular crosslinks that form when tissue sits in formaldehyde.

Lannfelt, who co-founded the Swedish biotech company BioArctic Neuroscience, is developing antibodies to protofibrillar forms of Aβ. The pharma company Eisai Inc. is now testing a humanized monoclonal antibody (BAN2401) in Phase 1. Both Lannfelt and Eisai's **Andrew Satlin** summarized this program's status at the AD/PD meeting in Barcelona last April (see ARF related news story).

In addition, Lannfelt and colleagues are using antibodies to Arctic peptides to develop assays for protofibrillar Aβ. **Frida Ekholm-Pettersson**, from Uppsala University,

described a sandwich ELISA test using the monoclonal antibody mAb158, the mouse forerunner of BAN2401. The ELISA detects protofibrils in extract from human AD patients, but not from people who had frontotemporal dementia. Ekholm-Pettersson said she has not tested the assay on samples from people with other neurodegenerative diseases yet. To confirm that the assay does indeed detect protofibrillar species, she separated synthetic Aβ by ultracentrifugation and analyzed fractions by atomic force microscopy. The antibody detected Aβ species of median size, that is, in the second of three size fractions. The researchers are still characterizing the structures. The ELISA also detects protofibrils in human plasma, but the researchers ran into interference problems when trying to assay cerebrospinal fluid. They are still working on resolving those issues. Ekholm-Pettersson cautioned that one of the problems with this, and similar assays, is that human fluids can contain heterophilic antibodies that recognize immunoglobulins from other species. In this case, it appears that human antibodies recognize the mouse monoclonals. She depleted human fluids of IgG immunoglobulins to overcome this interference.

Tempering the general enthusiasm about immunotherapy at this conference, **Dave Morgan**, University of South Florida, Tampa, offered some cautionary notes. For one, he is troubled that some antibodies can have acute effects in mouse models. Since Aβ takes a long time to accumulate, Morgan said he finds it surprising that a single antibody dose can repair the brain. 3D6, the mouse precursor to bapineuzumab, for example, reverses cognitive defects in Tg2576 animals after a single dose. Lannfelt echoed this concern, saying that if accumulation takes decades, then perhaps it is best not to try to remove it too quickly. Aβ trapped in blood vessel walls can cause microhemorrhages, for instance, which may be exacerbated if therapy is too aggressive (see ARF related news story). Morgan showed that age may play a role in this. Looking at young mice with aggressive Aβ accumulation and older mice with more gradual pathology, his group found that microhemorrhages are much more prevalent in the older mice. Monocyte recruitment into the brain was also more rampant in the older mice. To some extent, this effect might be due to age-related differences in the immune system. The overriding point, Morgan said, is that age—which mice model poorly—is an important factor to keep in mind when developing immunotherapies for AD.

Uppsala: Is Tau Immunotherapy Taking Off?

29 June 2011. Targeting extracellular Aβ with immunotherapy is one thing, but could antibodies even drive the clearance of tau, a predominantly intracellular protein? In the last few years, scientists have turned their attention to vaccinating against various forms of this potentially toxic protein, and the strategy seems to pay some dividends, at least in mice. Tau immunotherapy was well represented with new data at the 2nd International Conference on Neurodegenerative Disorders: Immunotherapy and Biomarkers, which took place 26-27 May 2011 at Uppsala University, Sweden. Even big pharmaceutical companies are now getting in on the act.

Martin Citron, Eli Lilly, Indianapolis, Indiana, is best known for his studies on the β-secretase that cleaves amyloid-β precursor protein (APP), but in Uppsala he confessed to being a bit of a closet tauist. He noted that the AD field has come to

appreciate in the last five to 10 years that amyloid pathology is an early event in the disease, and that by the time patients become demented, interventions targeting Aβ may be too late. Two alternative strategies are to diagnose and target Aβ earlier, or to go for targets that lie downstream. Citron thought tau might fit the latter category because tau pathology continues to worsen in the symptomatic phase of the disease. The problem, he said, has been figuring out how to target it.

Citron reported on a passive immunotherapy strategy using antibodies (PHF1 and MC1) that recognize neurofibrillary tangles. He injected these into two different models: the JNPL3 mouse (Lewis et al., 2000), which expresses a human tau transgene containing the P301L mutation that causes frontotemporal dementia, and a mouse expressing human tau with the P301S mutation that has more aggressive pathology and also more consistent transgene expression (Allen et al., 2002). He treated two-month-old JNPL3 mice for four months with PHF1 and MC1 (15 mg/kg three times per week for two months, then 10 mg/kg twice per week). While neither antibody treatment altered total tau levels in the mice, they did lower the amount of insoluble tau stained by the AT8 antibody. Citron said the JNPL3 model varied greatly from animal to animal. For quantitative analysis, he developed an AT8-based enzyme-linked immunosorbent assay (ELISA) for insoluble tau. Citron believes that a 64 kDa, hyperphosphorylated tau fragment recognized by AT8 is the biochemical correlate of neurofibrillary tangles. He said the 64 kDa tau correlates with the extent of tangle pathology in both Alzheimer's disease and transgenic mouse models. The AT8 ELISA detected no signal from brain tissue samples taken from human controls, but did detect tau in tissue from AD patients. He said neither the PHF1 nor MC1 interfere with the assay, indicating that it is suitable for monitoring the effects of these antibodies in vivo. With this ELISA, Citron detected significant reduction of AT8 tau reactivity in brain extracts from the treated JNPL3 mice compared to controls. Whether the ELISA could be used as a diagnostic test is unclear. That would require a test of biological fluid, such as CSF, and Citron said it is unlikely that much of the insoluble tau would make its way there.

Citron used the P301S model to measure behavioral effects, since the JNPL3 animals show no behavioral phenotype until they are quite old. In the P301S mice, tau pathology damages the spinal cord and the animals have severe motor problems by five months of age. Citron treated two-month-old animals for three months with twice-weekly doses (15 mg/kg) of both PHF1 and MC1. Treated mice performed better on the rotarod and lost less weight than did controls who got an equal amount of a generic immunoglobulin G. Similar to the JNPL3 animals, total tau stayed unchanged, but the ELISA revealed a decline in AT8-reactive insoluble tau. Tangle pathology is rampant in the P301S animals, but the immunotherapy attenuated it. Citron noted that the antibody treatment also appeared to improve neurodegeneration.

How does this passive immunotherapy work? Citron does not know, but said the simplest hypothesis would be that it neutralizes extracellular tau, which somehow is driving pathology. Tau is an intracellular protein, but evidence is accumulating that tau can be secreted from cells and taken up by others (see ARF related news story and ARF news story on Frost et al., 2009). Alternatively, the antibodies might be taken up into the cells and neutralize tau there, he said.

Einar Sigurdsson, New York University, also broached the subject of how tau immunotherapies work. He agreed that multiple mechanisms—extracellular and intracellular—are likely involved in tau immunotherapy. Sigurdsson's group was the first to show that active immunization against tau can rescue phenotypes in mouse models and might be a viable strategy for tauopathies (see ARF related news story on Asuni et al., 2007 and ARF related news story on Boutajangout et al., 2010). His group also reported that passive tau immunotherapy clears tau pathology in mice and stems their functional decline (see Boutajangout et al., 2011). To address how these antibodies work, Sigurdsson has isolated immunoglobulin Gs (IgGs) from vaccinated mice, labeled them with a fluorescent tag (FITC), and then re-injected them into mice to see where they go. He showed that they not only enter the brain, but also seem to get inside neurons where they colocalize with tau. Interestingly, he said, the antibodies do not penetrate the brains of control mice—at least in quantities this method can detect. He believes a damaged blood-brain barrier explains why the antibodies gain access to the brains of the transgenic animals.

How do the antibodies get inside neurons? Sigurdsson said that work on ex-vivo slice cultures suggests cells take up the antibodies and that they colocalize with endosomal/lysosomal markers, such as LAMP2. Isolated lysosomal fractions from these cells contain the antibodies and tau. Sigurdsson's group presented some of these data at the International Conference on Alzheimer's Disease in Hawaii last July. He said that it is well known that several receptors on most cells, including neurons, bind antibodies. He speculated that antibodies enter via receptor-mediated endocytosis, traveling in endosomes that then fuse with autophagosomes containing tau aggregates. The antibodies would then drive disassembly of the aggregates, freeing tau up for lysosomal degradation. Alternatively, he suggested, antibodies might diffuse into cells through damaged membranes, bind to tau aggregates on the inner surface of the plasma membrane, and get processed by autophagosomes. Sigurdsson is also interested in a ubiquitin ligase system that binds antibodies attached to viruses and targets them for destruction. He thought this system might be involved in the tau antibody response as well.

Sigurdsson's lab has newer monoclonals in development. One of them, 4E6G7, is a phospho-tau-specific antibody. It reduced tau pathology and also rescued cognition in the radial arm maze, a closed-field symmetrical maze, and in an object recognition test as well. Even though treatment began late, he saw improvements, said Sigurdsson. He thought this bodes well for translation to human studies.

Scientists are also looking to other tau immunotherapy strategies, including specifically targeting tau oligomers. **Rakez Kayed**, University of Texas Medical Branch, Galveston, reviewed his lab's progress in this approach. He has made polyclonal (T22) and monoclonal (TOMA) antibodies to tau oligomers (see ARF related news story). In Uppsala, Kayed reported that his group isolated a specific tau oligomer directly from human AD brain by using the antibodies to immunoprecipitate tau from soluble fractions enriched with extracellular material. The oligomer is a dimer or possibly a trimer. Kayed said he is not sure which, because oligomer migration on electrophoresis gels does not always predict size. He said they also established a causative relationship between this putative tau dimer/trimer and memory deficits in mice. Specifically, injecting it into the brains of normal mice weakened their ability to remember novel objects.

TOMA injected into the brain reduced tau pathology in eight-month-old P301L and 14-month-old Tg2576 mice. Injected intravenously into the latter, the antibody rescued memory deficits as judged by a novel object recognition test. When given as a single intravenous injection (30 μg), TOMA reduced tau oligomers detected biochemically and immunohistochemically. Surprisingly, Kayed said, it did not reduce AT8-reactive tau species phosphorylated at serine 202/threonine 205), which is in contrast to other immunotherapies directed at tau, including those of Citron and Sigurdsson above. Kayed did not want to speculate why AT8-reactive tau was not reduced. He did claim that TOMA recognizes oligomers in human cerebrospinal fluid (CSF), suggesting that these oligomers, present at ng/ml levels, could become the basis for a new diagnostic test. In three separate experiments, two of them blinded, his group measured these oligomers in CSF taken from 25 AD patients and 25 normal controls, Kayed told the audience.

Uppsala: Immunotherapy—Not Just for AD Anymore

9 July 2011. As immune-based therapies targeting Aβ and tau are beginning to show promise for the treatment of Alzheimer's, what about other disorders of the nervous system? At the 2nd International Conference on Neurodegenerative Disorders: Immunotherapy and Biomarkers, which took place 26-27 May 2011 at Uppsala University, Sweden, researchers outlined potential immunotherapies for Parkinson's and other synucleinopathies, amyotrophic lateral sclerosis, and even prion diseases. The last have been a tough nut to crack, because the immune system strongly recognizes prion protein as "self" and fails to mount a response to prion antigens. The variety of different immune strategies discussed at the meeting spoke to how seriously scientists are pursuing this approach.

Eliezer Masliah, University of California, San Diego, reported on immunotherapies against α-synuclein, the principal component of Lewy body aggregates that characterize Parkinson's and other synucleinopathies. Masliah told the audience that he is not interested in targeting Lewy bodies per se, but toxic α-synuclein oligomers. Though α-synuclein is predominantly an intracellular protein, it also extrudes from cells in different forms (see Lee et al., 2005), and synuclein toxicity propagates from cell to cell (see ARF related news story). Oligomers extruded from cells could be mopped up by antibodies, suggested Masliah. Together with **Dale Schenk** and **Dora Games** at Elan Pharmaceuticals, San Francisco, California, Masliah previously developed an active α-synuclein vaccine that helps rid mice of the protein (see ARF related news story on Masliah et al., 2005). At Uppsala, he reported on a passive immunotherapy.

To test it, Masliah and colleagues used an α-synuclein transgenic mouse model of Lewy body disease in which the protein deposits in the temporal cortex and hippocampus. These mice lack full motor control and have poor cognitive skills (Masliah et al., 2000). Masliah reported on a monoclonal antibody, 9E4, that recognizes the C-terminal end of the protein and labels intraneuronal synuclein aggregates in brain tissue. When six-month-old α-synuclein mice received twice-weekly intraperitoneal injections of 9E4 for six months, they outperformed control transgenic mice on both the rotarod test of motor function and the Morris water maze

test of spatial learning and memory. The treated transgenic mice performed about as well as wild-type controls.

How does 9E4 rescue this phenotype? By fluorescent labeling, Masliah and colleagues tracked the antibody as it entered the central nervous system, where it labeled granular structures inside neurons of the temporal cortex and the CA1 of the hippocampus. The antibody colocalized with α-synuclein, and also with lysosomal markers including LC3. The results hinted that 9E4 protects mice by penetrating deep into the brain, reacting with α-synuclein, and ferrying it to lysosomes for degradation. In support of this, the treatment reduced the amount of α-synuclein in the cortex and hippocampus, while a control IgG had no effect. Treated mice exhibited more post-synaptic density 95 and synapsin 1 protein in the brain, suggesting synapses were protected against α-synuclein toxicity. 9E4 reduced the amount of calpain-cleaved α-synuclein. This truncated form of synuclein is believed to serve as a template for aggregation. Much of this work was recently published (see Masliah et al., 2011).

Another antibody seems to encourage glial cells to take up and destroy the protein. Named 274, this antibody completely obliterated α-synuclein accumulation in astroglia, said Masliah, yet had little effect on neurons. Masliah concluded that antibodies can dispatch the protein in at least two ways: by stimulating lysosomal degradation in neurons, and by activating clearance in glia. The latter could be more important for multiple system atrophy (MSA), a disease characterized by glial accumulation of α-synuclein. Masliah said he plans to test 274 in a mouse model of MSA, where the myelin basic protein promoter drives overexpression of α-synuclein in glia.

Martin Ingelsson, Uppsala University, reported a different approach toward synuclein immunotherapy, focusing on antibodies that recognize induced protein oligomers. Because α-synuclein oligomers are fleeting entities, Ingelsson and colleagues developed a screen to find chemicals that coax the protein to form stable oligomers. Two chemicals generated from lipid oxidation, 4-oxo-2-nonenal (ONE) and 4-hydroxy-2-nonenal (HNE), turned out to potently induce α-synuclein oligomerization, though with slightly different outcomes. Atomic force microscopy revealed that ONE-induced oligomers are amorphous and less dense, while oligomers formed in the presence of HNE have a protofibril-like structure, Ingelsson said. Both are toxic to neuroblastoma cells (see Näsström et al., 2011).

Mice immunized against these induced oligomers generated an antibody, 49G, with some promising characteristics. It recognized antigens in brain tissue of PD patients and also brain samples from transgenic α-synuclein mice. In fact, the antibody detected antigens in the brain of A30P α-synuclein mice as young as four months old, some six months before these mice normally begin to display pathology. This finding supports the idea that oligomers form well in advance of visible protein aggregates, and suggests that early treatments with antibodies like 49G could slow or halt the disease process. Ingelsson said that cells take up the antibody, which then seems to attenuate α-synuclein aggregation. While the antibody may help clear such aggregates, it also seems to prevent their formation: When cells express synucleins attached to different domains of green fluorescent protein (GFP), 49G reduces the fluorescence that occurs when synucleins bind together and effectively reconstitute

the full GFP. Ingelsson plans to test the antibody in a preclinical trial using transgenic mice to see if it will limit pathology.

Jean Pierre Julien, from the University Laval in Québec, Canada, outlined two different therapeutic strategies for treating amyotrophic lateral sclerosis (ALS)—traditional antibodies and intrabodies. The latter are antibody genes hidden, Trojan horse-style, inside viral vectors. Julien and colleagues generated traditional monoclonals against the copper-zinc superoxide dismutase 1 (SOD1), which misfolds when mutated to cause rare familial cases of ALS. Three of them, B8H10, D3H5, and A5C3, did not recognize wild-type SOD1, but did recognize a range of different mutants, suggesting that they bind to epitopes that emerge when the protein adopts non-native conformations (see Gros-Luis et al., 2010). In a G93A SOD mouse model of ALS, the antibodies detected aberrant protein aggregates in motor neurons before any onset of symptoms. Slowly pumped into mouse brain ventricles at around the time of disease onset (85 days old) D3H5 increased survival by six days when administered over 28 days. When treated from day 65, mice survived nine days longer than controls. Julien said that the antibody mostly ends up in motor neurons and microglia, which have both been implicated in pathology in ALS (see ARF related news story).

Antibodies lacking the antibody Fc tail also extend lifespan in this mouse model, though less efficiently than the full antibody, said Julien. Nevertheless, this opens up the possibility of using single-chain antibodies, which may penetrate the brain more readily than full-sized immunoglobulins and are less likely to induce unwanted immune reactions. Julien has started treating mice with single-chain antibodies, but said it is too early to know what effect they have. Julien is collaborating with Biogen Idec, Boston, Massachusetts, to develop humanized antibodies for clinical trials. Though SOD mutations cause but a minor fraction of ALS cases, aggregates of the protein form in tissue from sporadic cases, too, (see ARF related news story), suggesting that an anti-SOD therapy might have broad utility.

Furthermore, Julien reported discovering an interaction between TDP-43 and p65/RelA, a member of the NF-κB family of transcription factors. TDP-43 aggregates occur in nearly all cases of ALS and some other neurodegenerative diseases, such as frontotemporal dementia and some Alzheimer's cases, making it an attractive therapeutic target. What role the protein plays in pathology is still unclear. It is an RNA-binding protein, and when it aggregates, it alters expression of many genes (see ARF related news story). Julien found the interaction between TDP-43 and p65 using an immunoprecipitation approach. He said that in ALS spinal cord neurons, p65 shifts into the nucleus, suggesting that it may be playing a role in pathology, which is the opposite of what happens with TDP-43, which mislocates into the cytoplasm. Exactly how the two proteins might conspire to promote disease is unclear, but Julien reported that withaferin A, an NF-κB inhibitor, reduced neuronal toxicity induced by glutamate, and also reduced gliosis and nuclear p65 in TDP-43 transgenic mice, supporting p65 as a potential drug target. Julien said he is working on intrabodies to target the TDP-43/p65 interaction to see if they can reduce pathology in TDP-43 mouse models.

One group of neurodegenerative diseases that have been particularly challenging from an immunotherapy perspective are the spongiform encephalopathies, or prion

diseases. As **Claude Carnaud**, INSERM, Paris, France, pointed out, the cellular prion protein (PrPc) is ubiquitously expressed in the body, even in immune cells, so the immune system recognizes PrPc as "self" and will not attack it. The immune system is a poor reservoir for prion antibodies, those that do remain have low affinity, and new epitopes formed upon conversion of PrPc to toxic prions do not seem to trigger immune responses, said Carnaud. In short, researchers must be inventive if they want to evoke the immune system to tackle prion diseases.

Carnaud reported on two possible tacks. Dendritic cell vaccination uses bone marrow-derived dendritic cells (these are cells of the immune system) to spur naive B cells to produce antibodies to PrPc, breaking the immune tolerance for this ubiquitous protein. Adoptive T cell transfer uses T cells from a host that has no immune tolerance, such as a PrPc-negative mouse. Carnaud and colleagues have had some success treating mouse models of prion disease with both approaches.

For the former, the authors first established which prion peptides elicited the best immune response by injecting several into PrPc-negative animals, which lack immune tolerance toward the protein. Next, they used the most antigenic peptides to load up dendritic cells to present the antigens to the immune system of wild-type mice. Dendritic cells harboring a peptide corresponding to PrPc amino acids 98-127 not only induced an immune response, but lengthened the survival of mice injected with a lethal dose of scrapie prion. The animals survived an average of 254 days, versus 212 days for controls (see Bachy et al., 2010). Survival time correlated with the amount of anti-PrPc antibody in the serum.

For adoptive cell transfer, Carnaud and colleagues also used cells from PrPc-negative animals challenged with prion antigens. They transplanted T lymphocytes from those animals to wild-type mice. The transferred cells protected the animals from amassing toxic prion in the spleen. Adoptive T cell transfer also extended the lifespan of mice injected with scrapie by about 30 days, said Carnaud (see also Gourdain et al., 2009). Protection did not correlate with anti-prion antibodies in circulation, suggesting that this method evokes a predominantly cellular immune response. Whether either of these strategies would work in humans remains to be seen. The approach would be to remove a patient's immune cells, expand them to great numbers while challenging them with a suitable antigen, then put them back into the patient. Oncologists are studying this strategy to overcome immune tolerance to cancers (for a review, see Schumacher et al., 2009).

Immunotherapies for AD, PD, and some other neurodegenerative diseases are in their infancy. In contrast, natalizumab (tysabri), produced by Biogen Idec, is approved for the treatment of relapsing multiple sclerosis (MS) in the US., and relapsing-remitting MS in the EU. As Biogen's **Alfred Sandrock** pointed out, many other immunotherapies for MS are in various stages of development. Alemtuzumab, which depletes CD4/CD8 T cells, is currently in Phase 3, and results should be available soon, he predicted. In Phase 2, the therapy reduced disability by 70 percent, and relapses by 75 percent. Ocrelizumab, sponsored by Genentech and Hoffman-La Roche, suppresses B cells and reduces lesions and relapses. Though there was one death reported in Phase 2 and a trial for lupus has been halted, that treatment is entering Phase 3 for MS as well. Daclizumab, being developed jointly by Biogen and Abbot (see related news), is also in Phase 3, and results are expected this summer.

Sandrock said the late progression phase of MS is still difficult to tackle. Losses and gains of the myelin sheath that insulates axons characterize this phase of the disease. Remyelination is a hit-and-miss affair, and it is unclear why, said Sandrock. Blocking the protein leucine-rich repeat and Ig domain-containing 1 (aka LINGO-1), which suppress myelination by limiting maturation of oligodendrocytes, might be one potential avenue for boosting remyelination. Sandrock and colleagues found that knocking down LINGO-1 with interfering RNAs boosts maturation of oligodendrocytes and promotes remyelination. In a mouse model of MS, an anti-LINGO-1 antibody increases myelination. In humans, this can be measured using magnetization transfer ratio, a form of magnetic resonance imaging. Anti-LINGO-1 therapy has entered Phase 1, and Sandrock said Biogen hopes to begin Phase 2 next year using MTR as an outcome measure. Researchers at the meeting took encouragement from the relative success of the MS programs and in the breadth of new potential treatments entering or in clinical trials. The general feeling was that if immunotherapy can work for MS, then similar treatments for AD, PD, and other neurodegenerative diseases may follow suit.—Tom Fagan.