Advances in understanding BACE1, the β-secretase enzyme relevant to Alzheimer disease, stood out as a notable trend at the 10th International Conference on Alzheimer’s Disease and Related Disorders (ICAD), held from July 15 to 20 in Madrid. At ICAD, bits and pieces of news were rustling up a fresh breeze in the air that came as a welcome change after a doldrums of sorts. Hope that this enzyme would serve as a new drug target first rose when researchers led by Martin Citron cloned it in 1999. At first blush, BACE1 looked like a safer target than its big brother γ-secretase, because knockout mice generated by Robert Vassar and several other independent groups all appeared largely normal. When Jordan Tang’s group solved the BACE1 crystal structure a year later—why, it seemed that all that was left to do was for clever drug designers to get busy and, presto, serve up a suitable small molecule drug. But the going got tough when BACE1 proved to be a recalcitrant drug target. What’s more, basic scientists began to whisper that BACE1 might not be as straightforward a target as initially thought. In Madrid, researchers for the first time presented a potent BACE1 inhibitor, fledgling immunotherapy approaches, and new data on its biology and potential as a biomarker. Read on for summaries of a plenary lecture and some of the 48 other presentations on BACE1. As always, Alzforum encourages presenters and attendees to amend our selected notes with their own.

In the plenary reviewing current knowledge on BACE, Citron, of Amgen in Thousand Oaks, California, first recapped that BACE1 and 2 are single transmembrane aspartyl proteases. They are related to the HIV retropepsin, which is a thoroughly studied drug target. One reason why BACE1 is less well understood, besides having been known for only six years, is that it undergoes numerous post-translational modifications that influence its activity in still-mysterious ways, Citron noted. Some things are known, however. BACE2 appears to play little, if any, role in AD pathogenesis. Cell biologists have pieced together that BACE1 traffics through the secretory pathway, moving from the trans-Golgi network to the plasma membrane, where it becomes pinched off into to endosomes and from there is retrieved again for further transport. BACE1 is thought to cleave APP most readily in endosomes and the trans-Golgi network, said Citron. It forms homodimers, and appears to do its work in lipid rafts.

One of the hottest questions in BACE research these days is whether BACE1 is upregulated in AD, and whether this upregulation comes as an epiphenomenon in late-stage AD or plays an early role and contributes to pathogenesis. Numerous reports have found that BACE1 activity increases with age and even more so in AD. Yet no familial AD loci containing BACE1 polymorphisms, much less AD-causing mutations in the BACE1 gene, have been found. This raises the underlying question of what regulates BACE1 expression. Many interactions of BACE1 and other proteins are on the map, including with reticulons, GGA proteins, and sorLa, but which ones participate in AD pathogenesis remains a puzzle. Other research has implicated BACE1 in an inflammatory feed-forward loop, and energy depletion as occurs in an atherosclerotic, underperfused brain is also thought to trigger BACE1.

Tang’s BACE1 crystal structure, and Amgen’s, too, showed that the active site comprises eight subsites, and that it would be difficult for a single small molecule drug to touch them all. Studying which of these sites a drug needs to hit has taken up much of the intervening time since 2000, Citron said. Only clinical trials will show whether BACE1 can be inhibited safely. In the interim, basic research has put
potential concerns to watch for on the drug developers’ radar screen. Potential risks include that interfering with APP metabolism could narrow the therapeutic wiggle room if indeed Aβ turns out to perform an essential biological function, for example, in synaptic activity. Moreover, BACE1 has proven to cleave other substrates more readily than APP, and any physiological consequences of inhibiting these reactions remain unclear at present. The list of published substrates includes ST6Gal I, Psgl-1, LRP, and neuregulin-1 (see ARF related Madrid story).

BACE1 knockout mice are fertile, viable, and appear to age normally, but little is known about how they fare when stressed while aging. Some studies have identified subtle memory deficits, though this issue remains controversial, and some BACE1/2 double knockout mice tend to die early. In Madrid, Alex Harper and colleagues from GlaxoSmithKline in Harlow, Great Britain, reported that BACE1 knockouts had trouble gaining weight with age. Removing BACE1 protected the mice against the weight gain usually seen on a high-fat diet. Lack of BACE1 also appeared to increase the mice's insulin sensitivity in the face of a glucose challenge test, pointing to some still-mysterious metabolic role for BACE1. The BACE1 knockout mice also tended to die earlier than did wild-type controls. On the plus side, however, a different safety concern that has been raised about inhibiting APP cleavage by either BACE or its downstream successor γ-secretase appears less worrisome upon further inspection. It concerns a loss of physiological gene expression signaled by the intracellular tail of APP, aka AICD. A few genes, including neprilysin, KAI1, APP itself, or GSK3β, had been implicated as AICD target genes. Yet subsequent studies in different labs have struggled to reproduce these findings, and in Madrid, Sebastian Hébert in Bart de Strooper’s group in Leuven, Belgium, reported that in their hands, too, reducing AICD through secretase inhibition had no major effect on any of those genes (see also Hébert et al., 2006).
Madrid: BACE News Roundup, Part 2

Anti-BACE Drugs Appear on Horizon
On BACE inhibition, Martin Citron of Amgen in Thousands Oaks, California, noted that more than 100 patent applications have been filed, and a growing number of non-peptide inhibitors are being published now that researchers have gained more experience with BACE1. High-throughput screening against BACE was largely unsuccessful as nothing of use stuck to BACE1, so companies switched over to rational design based on the crystal structure. In the patent literature, hydroxyethylamines are a central chemical theme of BACE1 inhibition. In Madrid, scientists for the first time began introducing compounds that appear to work in vivo.

James McCarthy, of Eli Lilly and Co. in Indianapolis, presented the first data on a BACE1 inhibitor that seems to have turned the corner in BACE1 drug development after years of frustration. McCarthy noted that the Eli Lilly team had long worked with a group of sulfone and sulfonamide compounds that are highly potent, but whose physicochemical properties stubbornly kept them outside the blood-brain barrier. The scientists then decided to scrap this class of compounds and instead go after others that started out with a slightly lesser affinity to BACE1 but with more attractive physicochemical properties, such as a lower molecular weight, lower polar surface area, and other parameters.

After describing stereochemical modifications to their initial lead compounds, McCarthy presented an experimental BACE1 inhibitor, LY2434074. This is the first publicly shown BACE1 inhibitor that enters the brain of PDAPP mice and reduces sAPPβ, the product of BACE1 cleavage, in cortex and hippocampus in a dose-dependent manner, McCarthy noted. The product of the alternative α cleavage that processes APP in the absence of BACE1, that is, sAPPα, went up in the brains of the injected mice. Aβ levels decreased in CSF and in plasma, as detailed in a subsequent poster presented by Patrick May of the same group.

Other scientists confirmed that this approach for the first time has demonstrated proof of principle for BACE1 inhibition in brain by a chemical given systemically. They also pointed out that the compound McCarthy presented likely is not the one the company is pursuing for clinical development. It had to be injected in rather large doses, implying problems with its oral availability or possibly its metabolism. Indeed, McCarthy replied in response to a question that Eli Lilly has more suitable compounds in hand. Colleagues from other drug development companies applauded Eli Lilly’s decision to present a potent structure. They added that other firms also have overcome some of the structural challenges posed by BACE1’s unwieldy active site. Indeed, Sethu Sankaranarayanan and colleagues from Merck’s team in West Point, Pennsylvania, presented evidence that intravenous injection of their own inhibitor lowers Aβ in the brain of Bruce Lamb’s human wild-type APP-transgenic mice.

Toward a BACE Vaccine
If anti-Aβ antibodies hold promise, why not hit BACE1 in the same way? Two groups reported progress toward this goal in Madrid. Wan-Pin Chang, in Jordan Tang’s group at the Oklahoma Medical Research Foundation in Oklahoma City, followed in the footsteps of Aβ immunotherapists and injected Tg2576 mice with BACE1.
Chang’s prior experiments had detected reduced Aβ production in cultured cells treated with polyclonal anti-BACE1 antibodies, and he had also noticed that a fraction of injected anti-BACE1 antibodies entered the brain of mice. The underlying rationale for his approach, Chang said, would be that antibodies stick to BACE1 on neuronal cell surfaces and prevent its internalization into endosomes, where BACE1 cleavage of APP finds a conducive pH of 4.5.

In Madrid, Chang described a two-pronged study of active immunization with recombinant BACE1. A prevention arm began injecting BACE1 into Tg2576 mice repeatedly at 1 month of age, and a treatment arm began injecting BACE1 at 10 months, when plaques form. The scientists tracked the mice’s behavior and measured Aβ levels at 15 months or 23 months, respectively. In both study arms, but more so in the preventive one, the scientists measured rising antibody titers and waning Aβ40 and Aβ42 levels in serum and brain, as well as a reduced plaque load in brain as the immunization protocol progressed. Immunized mice outdid the untreated mice in negotiating the Morris water maze, Chang added. T cells, microglia, and astrocytes showed no sign of activation.

Michal Arbel, who works with Beka Solomon at Tel-Aviv University in Israel, took a different tack. Because BACE1 cleaves not only APP, Arbel works on devising an immunotherapy that interferes specifically with the BACE1-APP interaction rather than inhibiting or eliminating BACE1 altogether. Arbel develops antibodies directed against the BACE1 cleavage site on APP, which bind to human wild-type APP and human APP carrying the Swedish FAD mutation, but not to Aβ itself (see ARF related news story). (On a broader note: Scientists are realizing, to their surprise, that immunotherapy in mice works quite well across the board. Ajodeji Azuni, working with Einar Sigurdsson at New York University School of Medicine reported initial data of a tau vaccine. A P301L tauopathy mouse model responded to active vaccination with a phospho-tau peptide by mounting a tau-specific antibody response, showing less tau pathology, and performing better on some sensorimotor tasks.)
Madrid: BACE News Roundup, Part 3

BACE Biology: Who Are Its Handlers?
Investigators are probing intensely the question of which other proteins interact with BACE. Perhaps the interacting proteins control BACE activity, and in this way influence Aβ production? Among the list of proteins thought to bind BACE, the nogo family is emerging as an intriguing group. Long studied for its ability to repulse outgrowing neurites in the brain and spinal cord, nogo and its receptor are forming a point of convergence between the formerly separate problems of axonal regeneration and AD pathology.

In Madrid, Rabinder Prinjha of the GlaxoSmithKline research group in Harlow, Great Britain, asked how nogo modulates APP processing via its link to BACE. Prinjha first noted that nogo-A and the nogo receptor are members of a large family of proteins, called reticulons, the physiological function of which is barely known. Four human reticulon genes are known to date with reticulon-4A being better known as nogo-A.

Nogo-A (reticulon4-A) and BACE occur together in the endoplasmic reticulum of cultured cells, Prinjha said. To test if they might interact in vivo, his team looked in APP- and APP/PS1-transgenic mouse lines and saw nogo-A and BACE both being upregulated in cortical areas surrounding plaques. Then the researchers tested the effect of nogo-A and its relatives in the reticulon family on APP processing, and noticed that they all affected Aβ production in distinct ways. In short, both overexpression and RNAi knockdown studies were used to confirm that reticulon3-A1 functions to decrease Aβ production, whereas reticulon4-A (nogo-A) increases it. The precise location of the proteins within the cell drove the effect. Prinjha did not address the mechanism of the interaction, but said that he suspects it to change the endocytosis or subcellular localization of BACE. Notably, besides the large pool in the ER, a second, smaller pool of nogo-A occurs on the cell surface. Similarly, Wataru Araki, of Japan’s National Institute of Neuroscience in Tokyo, reported that reticulon3 and reticulon4-B and -C bind BACE1. (Reticulons4-B/C are smaller isoforms of reticulon4). He suggested that these reticulons appear to inhibit BACE1’s ability to cleave APP by some interaction with BACE1 outside of the enzyme’s active site. For more on this up-and-coming topic, see Park et al., 2006; Gil et al., 2006; Yan et al., 2006).

Different sorting proteins also appear able to control APP processing by directing BACE's journey through intracellular compartments, primarily between endosomes and the trans-Golgi network. They include GGA1 (see ARF 2006 Eibsee conference report; He et al., 2005) and sortilin. On the latter, Gina Finan, working with Tae-Wan Kim at Columbia University in New York, reported that postmortem brain samples of AD patients contain less sortilin than controls. Sortilin forms a complex with BACE1 and appears to reduce Aβ secretion through its role in trafficking BACE1, the researchers suggest.

BACE in the Aging Brain: What Goes Wrong?
A number of groups have established that BACE activity tends to go up with age, and more steeply in AD. What could cause this? One hypothesis came to the fore when Robert Vassar’s group at Northwestern University in Chicago picked up findings
from brain imaging, which has shown mild hypometabolism in the aging brain and even more in the AD brain. Wondering if the BACE increase might follow diminished perfusion—that is, insufficient oxygen and glucose supplies to the brain—Rodney Velliquette began to model energy starvation. Last November, the scientists reported that a single injection of chemical inhibitors of ATP generation had a long-lasting effect on the brain in that BACE levels (and Aβ production) shot up, and stayed up for a week (Velliquette et al., 2005). Reflecting Citron’s comment about the importance of post-translational modifications, Vassar noted that this increase occurred at the level of BACE protein, not gene expression.

But one injection does not model a slow disease such as AD, and in Madrid, Vassar followed up with a second, chronic study. It mildly starved ATP production in Tg2576 mice for a 3-month period, beginning prior to amyloid deposition at 9 months of age until 12 months of age when plaques are forming. BACE, Aβ levels, and plaque load all went up in the treated mice, Vassar reported. This suggests that, perhaps, sporadic AD could have an upstream, stress-related beginning that would drive BACE. “It is established that cerebral blood flow decreases in aging and particularly in AD brain. We do not know if this is just a correlation or a pre-existing driving force. It is something to look into because aging is the major risk factor in AD. As we age, cardiovascular disease increases and could put the brain under chronic energy stress,” Vassar speculated.

To sort out the time course of these events, Vassar’s group needed to make a better monoclonal antibody. All antibodies they could get their hands on were “dirty,” showing non-specific binding on Western blots and in brain tissue. With Skip Binder of Northwestern, who is noted for his skill in generating antibodies, the Chicago scientists used BACE knockout mice as immunization hosts, because they have never seen this protein. Out came a cleaner anti-BACE monoclonal antibody that recognizes but a single band on blots, Vassar said. This antibody helped the scientists characterize the BACE increase in various materials, including the Tg2576 and the group’s aggressive 5x-transgenic strain (see ARF SfN meeting story). The BACE antibody co-stains with neuronal markers but not astrocytic ones, particularly in dystrophic neurons. What’s more, BACE staining correlates with plaque development, and visualizes BACE around plaque cores. The BACE increase appears to be associated with Aβ42 deposition. This raises a chicken-and-egg question about which comes first in the course of AD pathogenesis: Does BACE first go up and induce Aβ42 deposition, or do Aβ42 deposits induce a secondary BACE increase? Vassar suspects a stress-induced feedback loop at play here.

The new antibody, Vassar hopes, will help with the analysis of what upstream factors can trigger BACE. Vassar particularly wonders whether any of those upstream factors would make for a good drug target so that, ultimately, a drug would become available that prevents only the age- or AD-related BACE increase, not all BACE altogether. “We may need BACE around for other functions,” Vassar said.

**BACE: The Newest Biomarker?**

Last but not least, one ICAD presentation moved research on BACE into the bustling realm of biomarker research. Yong Shen at Sun Health Research Institute in Sun City, Arizona, reported results of a collaborative study that, tantalizingly, suggested BACE1 might make for a decent biomarker. Shen’s lab was among the first to notice the BACE1 increase in AD brain, (see Li et al., 2004), a finding others have since
confirmed. In Madrid, Shen used the same ELISA assay used for that study to assess BACE1 levels in the CSF of 80 sporadic AD cases, 59 MCI cases, and 69 age-matched controls. His data suggest that BACE levels in these MCI cases were twice as high as in the healthy controls but—surprisingly—returned to control levels once a person has progressed to overt AD. If replicated, this data would suggest that BACE1 might eventually serve to predict AD. Much remains to be sorted out about this prospect. In Shen’s hands, a BACE activity assay tracked with the ELISA for BACE detection and with total Aβ levels in that all three measures correlated in the CSF. These are early days for BACE1 research in CSF, but one study published to date would tend to confirm that enzymatically active BACE1 can be detected in human CSF (Verheijen et al., 2006).

All told, AD researchers still consider BACE the ideal target to test the amyloid hypothesis of Alzheimer disease. To be sure, BACE1 gets more complex the deeper the field digs into its function and regulation. Still, many scientists agree that inhibiting BACE1 may be cleaner than inhibiting γ-secretase, because that enzyme complex has a startling array of functions other than snipping APP once BACE is through with it. If a BACE inhibitor were to get into the brain and remove Aβ as expected, yet failed to improve dementia, then the amyloid hypothesis would be in serious jeopardy. With such inhibitors now coming online, this day of reckoning may be drawing nearer.—Gabrielle Strobel.