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The Germ of an Idea

How Claudio Soto set out on a path that might cure Alzheimer's disease

BY JIM KELLY

Nine years ago, not long after UTMB neurology professor Claudio Soto left his native Chile to do postdoctoral work at New York University (NYU) Medical School, he experienced an epiphany.

It occurred while he was discussing the germ theory of disease with his five-year-old daughter, Claudia. She had contracted chickenpox, and Soto had cautioned her to be careful around her one-year-old brother lest she pass it on to him. The child was puzzled. Why was it, she wanted to know, that sickness could go from a sick person to a healthy person and not healthiness from a healthy person to a sick person? Why did it always have to go the wrong way?

"I started to tell her, you know, there are viruses and bacteria, and if you are infected you can transmit them, and if a person is healthy there's nothing to transmit," he recalls. "But then I started to think about it a little bit more." At NYU, he was investigating an entirely different type of disease process from the one he was describing to his daughter. The lab he had just joined, headed by renowned neuroscientist Blas Frangione, specialized in what are now called protein-misfolding disorders—nightmarish, brain-ravaging degenerative conditions like Alzheimer's disease and the human form of mad cow disease, known as new-variant Creutzfeldt-Jakob Disease (nvCJD).

These disorders weren't caused by viruses or bacteria. Instead, they started when brain cells accidentally made proteins with the wrong shape. The misshapen proteins had the insidious ability to change the shape of other, correctly formed proteins to match their own—to turn "healthy" proteins into "sick" ones. And as the "sick" proteins built up, they stuck together to form steadily growing toxic clumps and slowly but surely killed off the brain cells responsible for memory, reasoning, and the basic bodily functions necessary to sustain life.

Soto was trying to find a way to interfere with or even reverse this deadly process using peptides, small pieces of protein, that were similar in composition to the one that was misshaping but contained certain specific properties that prevented them from adopting the wrong shape. The idea was that these very stable "healthy" peptides would cause "sick" proteins to pop back into their original "healthy" shapes and break up the cell-killing clumps. In fact, he realized, transmitting health instead of sickness was not just possible; it "was actually exactly what we wanted to do."

For Soto, the insight sparked by what he calls his daughter's "illuminating question" marked a critical moment in the development of his ideas about the diagnosis and treatment of diseases like Alzheimer's and nvCJD. It also marked the beginning of a steady flow of scientific productivity that has kept him in the forefront of protein misfolding disorder research ever since.

In 1996, Soto and his colleagues in the Frangione lab succeeded in creating their anti-amyloid plaque peptides. Using them, they demonstrated in test-tube experiments that they could prevent the formation of the abnormal protein plaques blamed for Alzheimer's and also could break up amyloid plaques that had already formed. In 1998, Soto scored again, showing that his peptides could produce similar results in the brains of lab rats. In 2000—having moved to Geneva to become head of neurobiology research for the Swiss-based international pharmaceutical giant, Serono, but still collaborating with his old group at NYU—Soto applied a similar peptide approach to nvCJD, achieving success in both cell cultures and mouse brains.

In 2001, following up on another idea he'd first formulated at NYU, Soto led a Serono team that created the first successful technique for detecting small quantities of the infectious prion protein believed to be responsible for nvCJD. This system, based on the rapid amplification of even the most miniscule amount of "sick" protein in a sample to an easily perceivable level, was hailed as a crucial first step toward developing a much more practical means of determining whether livestock were contaminated with misformed mad cow proteins that could cause nvCJD in humans. It also might lead to an effective early test for Alzheimer's disease, as well as finally settle one of the great arguments of modern biology: the controversy over the "protein-only" explanation for nvCJD and other transmissible brain disorders such as kuru, mad cow, and chronic wasting disease, the partial proof of which won the Nobel Prize for protein chemist Stanley Prusiner of the University of California at San Francisco. Recent work in Soto's UTMB lab—presented at scientific conferences and now being considered for publication in a top scientific journal—includes the first creation of infectious proteins in a test tube, which is widely considered the definitive proof of the controversial prion concept.

In 2003, after five years in industry, Soto left Serono and Geneva for Galveston and UTMB. Tired of helping to run a company and feeling that the corporate imperative to focus on short-term profit and developing products was keeping him from following up on exciting discoveries, he had been looking for a way to return to academic research for some time. And so when new UTMB Neurology Chairman Tetsuo Ashizawa began talking with him about the possibili-

ties of establishing a protein misfolding disorder research effort at UTMB, Soto was immediately interested.

"I wanted to get back to academia, and coming here was a very exciting possibility, because they gave me a very good offer and the challenge to help build a world-class new institute, a center focused on neurological diseases and in particular Alzheimer's disease," Soto says. He arrived in fall 2003, but his office looks as though he moved in last week; a photo-collage of his children is the only visible personal touch.

Soto has had little time in the last year to lavish on interior decoration. His attention has been on other things such as working with Ashizawa to secure additional funding for his research (work that paid off when the Cynthia and George Mitchell Foundation donated \$2.5 million for a UTMB Alzheimer's research center—see "Boosting Alzheimer's Research," p. 27), writing grant proposals for federal support, and jump-starting the experiments under way in his newly outfitted laboratory. There, three researchers Soto brought with him from Serono—assistant professor Joaquin Castilla and researchers Paula Saá and Sylvain Bieler—plus several others recruited from different countries (Karim Abid from Switzerland, Lisbell Estrada and Rodrigo Morales from Chile, Jorge De Castro from Spain, and June Yowtak from the UTMB graduate program) are pushing hard to build on the foundations laid by Soto's research at NYU and Serono. They're also pursuing new avenues of investigation that Soto finds promising but that either didn't match up with Serono's priorities or were deemed too economically risky for the company to support.

"It's like when you play in the stock market, there's always a risk-reward ratio—high risk, high reward; low risk, low reward," Soto says. "I always tell my group that I'm not very interested in doing routine science. We're aiming to do something beyond just adding a little increment of knowledge. We try to concentrate on things that will have a high impact, that change things."

As he speaks, Soto swivels his chair slowly from side to side. Framed by a beard and longish brown hair, his face maintains a curiously calm expression that never appears to change, no matter how excited he may be. Instead, his enthusiasm for his subject seems to channel itself through his body. When he talks about Alzheimer's or nvCJD, his hands act out the parts of the different proteins involved, cupping

the air and twisting to demonstrate the crucial process of “folding” by which the complex molecules take either proper or improper shapes.

One hand placed flat above the other illustrates the way that protein structures misfolded into a flat configuration known as a “beta sheet” align with each other in a very stable—and ultimately dangerous—arrangement. Normally, so-called “chaperone” proteins prevent the formation of too many beta-sheet proteins and keep the ones that do form from stacking up. But when the chaperones don’t do their job, because the aging process or some other factor has disturbed the normal biochemical balance of the cells, beta-sheet proteins appear and begin “aggregating” like dancers forming a conga line.

“We believe this is common for all the protein conformational disorders,” Soto says, shuffling his hands to show the proteins slowly stacking until they form a small “seed.” Once this seed is made, the process moves much faster. The stack of misformed proteins is able to more efficiently add beta-sheet proteins to itself, and in some cases it can actually accelerate beta-sheet formation by encouraging normal proteins to fold incorrectly. In transmissible protein-misfolding disorders like nvCJD, kuru, and mad cow, these seeds (also known as harmful prions) have a chilling property: They are nearly indestructible by high heat, radiation, or other typical means of killing pathogens in the food supply. This enables them to infect animals and humans when the tissue containing them is eaten. The seeds grow into long fibers, which twist around each other to form amyloid plaques.

Soto’s insights into the inner workings of this process led him to the two “high-impact” breakthroughs for which he is best known. The first developed from a notion he says first occurred to him while he was still in Chile. It was to fashion tiny molecules dubbed “beta-breaker peptides” that would attach themselves to beta sheets in just the right way to keep them from stacking together and would encourage their refolding into a healthier shape. “The idea of interrupting the assembly of amyloid beta [the term for the misfolded beta-sheet-rich protein blamed for Alzheimer’s disease] with a ‘breaker peptide’ was a very original contribution in the field,” says Eduardo Castaño, who worked with Soto when he first came to NYU and is now a biochemistry professor at the University of Buenos Aires. Despite initial perceptions of the concept as a “crazy idea” and later concerns that

beta breakers would be unable to get into the brain across the blood-brain barrier, Castaño recalls, Soto persevered. The result, after successful experiments with transgenic mice showed that the peptides could get into the brain, was the creation of a promising new approach to therapy for Alzheimer’s and nvCJD. One of these peptides is currently under evaluation in humans affected by Alzheimer’s disease in Europe.

Soto’s second high-impact innovation came about in response to a problem common to both nvCJD and Alzheimer’s disease. Neither disorder can currently be diagnosed in its earliest stages, when the disease process is moving too slowly to raise concentrations of misfolded proteins to detectable levels. In the case of nvCJD, we have no way of being sure that small quantities of potentially deadly misfolded prion proteins aren’t spreading via beef from apparently healthy cattle slaughtered before they could develop the symptoms of mad cow disease, or from blood donated by humans unaware that they are in the initial phase of nvCJD. In the case of Alzheimer’s, any true therapy that’s developed will depend on early diagnosis; by the time clinical symptoms of the disease appear, far too much damage has already been done.

What’s needed is a test that can pick up infinitesimal amounts of misfolded protein in the blood. Other scientists have tried to solve the problem in what Soto calls “the complicated way,” using sophisticated equipment to drive supposedly ultra-sensitive but ultimately unsuccessful detection systems. Trying to come up with a diagnostic solution for nvCJD, Soto chose a different route. “I always try to make an effort to step outside and look for the easiest and most efficient way to attack a problem, to look for simple things that people usually miss,” he says. “This disease comes from the transformation of the normal protein into the misfolded protein. We start with a minute amount that we can’t see by any means, and then after several years huge amounts of the sick proteins are present in the brain, that anybody can see without any problem. So why don’t we use this process that happens all the time during the disease for diagnostics?”

Soto and his team at Serono devised a test in which a sample containing undetectable amounts of misfolded prion proteins were mixed with normal proteins in a test tube. Ordinarily, it would take months or years for the “bad” prion proteins to convert enough of the “good” proteins to be noticeable,

because of the molecular geometry involved in misfolding and aggregation: the “stacks” of misfolded proteins are linear, and each can add new units only at its two ends. But by bombarding the sample with ultrasonic sound, the Serono group broke each big stack up into many smaller ones, multiplying the number of opportunities for aggregation many times over. By putting the mixture through multiple cycles of “sonication” and incubation, Soto and his colleagues found they could shrink months into hours, boosting the levels of misfolded proteins thirty- to fiftyfold in a short time. In the last year, working at UTMB, the members of Soto’s lab have vastly improved the technique, which they call “protein misfolding through cyclic amplification” (PMCA). They have succeeded in multiplying prion proteins a millionfold—near the level required for a blood test for mad cow and nvCJD, which Soto hopes to have ready soon. A test for Alzheimer’s based on similar principles will take much more work, but Soto is confident one can be developed.

While the development of beta breaker peptides and PMCA demonstrated Soto’s ability to find inspired solutions to the problems posed by protein misfolding disorders, a keen scientific insight is only one of the traits contributing to his success.

Qualities including his easy flow of ideas, productiveness, adaptability to change, and ability to meet changes caught Ashizawa’s attention when he first considered bringing Soto to UTMB. So did one other characteristic that fit perfectly with the direction in which the chairman was steering his new department: Soto’s experience with translating basic science into clinically relevant products. In 1998, Soto and other researchers at NYU had founded a biotechnology company called Axonyx, in part to develop the beta-breaker concept into a marketable product. Thanks to a collaboration between Axonyx and Serono, anti-Alzheimer’s beta-breaker peptides are now in a Phase II clinical study in Europe. Soto’s five years with Serono made him an even more attractive hire.

Ashizawa had decided that emphasizing such “translational research” on Alzheimer’s disease was the most appropriate way for a neurology department that traditionally had focused mainly on education and clinical care to beef up its research muscle. The aim is to complement, not compete with, UTMB’s well-established neuroscience

program. (Neurology is primarily a clinical discipline, while the neurosciences are identified with more basic research; UTMB neuroscience investigators have primarily focused on pain and spinal cord injury and regeneration.) “I recruited basic scientists who were very strong in translational research, who would all be potentially capable of producing new drugs to treat Alzheimer’s disease,” Ashizawa says. “Dr. Soto has already made products. In addition, his people skills are exceptional. He has a real talent for putting investigators from different disciplines together, and he definitely has leadership capabilities.”

That combination—rare in any researcher, but particularly remarkable in one as young as Soto—gives Ashizawa confidence that Soto’s future holds even more impressive accomplishments. “He’s not yet forty, but he’s been quite prolific in his publications, and the quality of his publications has been spectacular,” Ashizawa says. “I have very little doubt that he’s going to be one of the major researchers in the Alzheimer’s disease field in the future, and UTMB will be nationally visible because of his contributions.”

Soto doesn’t deny his high ambitions, and he hints that the near future holds more “high-impact” developments from his lab on nvCJD and Alzheimer’s disease. He expects to soon have enough data to confirm that PMCA can be employed in blood tests for misfolded prion proteins. His team has also been able to refine the PCR-like amplification technique he calls PMCA to study the generation of infectious prion proteins in a test tube—experiments that could lead to the long-sought but never totally accomplished “gold standard” proof of Stanley Prusiner’s controversial Nobel Prize-winning hypothesis that diseases like mad cow, nvCJD, and kuru can be transmitted by misfolded proteins alone. (Despite strong evidence, including a paper published in the August issue of *Science* by Prusiner himself, according to Soto, most experts in the field still don’t regard the protein-only position as completely proven.) And that, Soto says, is only the beginning.

“I’m always working on a few crazy projects,” he says. “I think this is the way big things come. Protein amplification was considered very much a crazy idea, and so were the beta-sheet breaker peptides. Some crazy ideas don’t work out, and that’s okay; this is the risk you take. But if you want to do something different, something important, to create new therapies and diagnostics, I think it’s the only way to go.” ■