Introducing the “Inside-Out” Hypothesis

... a logical and unifying explanation ...

There was no doubt in my mind that I was onto something important, something I could not keep to myself. I had organized these findings into a logical hypothesis, all of which I intended to present to the field.

Again, all the findings through my IHC work point to one coherent story of events of the pathology of the AD brain. These novel observations suggest that Aβ42 accumulates in neurons over time, perhaps via the α7 receptor. In the case of AD, these neurons become so engorged with Aβ42 that they die via cell lysis. This leaves the neuronal remnants in place as the Aβ42, dense-core, amyloid plaque; the “neuronal debris field” is the amyloid plaque. I have coined the term “inside-out” hypothesis to capture this suggested series of events, so named for the fact that amyloid plaques come from “inside” the neurons and burst “out” of the neurons, rather than simply existing between the neurons and eventually engulfing them. This is in stark contrast to the amyloid cascade hypothesis suggesting that neurons mysteriously die from the “outside” accumulation of extracellular amyloid. The fundamental approaches of these hypotheses are conflicting and have serious implications on the direction of AD research: the goal of my new hypothesis is to simply prevent the accumulation of Aβ42 in neurons before they die, while the goal of the current amyloid hypothesis was to simply inhibit Aβ42 from depositing outside of the neurons before they die, which failed.

I have found the simplest way to describe this new hypothesis is by way of an analogy. It is useful in this case to think of a neuron as a house. If you happen upon a row of burned out buildings, and have a primitive sense of how fires work, would you look at it and say that the residual black carbon destroyed the buildings? Probably not, because you already know that black carbon residue was the result of the burning of flammable parts in the building such as wood or paper. For decades, AD research has suggested that the “black carbon residue” (amyloid buildup) outside of the cells is the first event leading to more and
more buildup that eventually destroys the houses (the neurons). It would be as if the black carbon residue first “seeded” in the neighborhood, spread and grew by itself, and eventually consumed the houses, leaving nothing but black carbon residue in the neighborhood.

While the notion of “neuronal residue” helps to explain the faulty logic of the amyloid cascade hypothesis, the notion of a fire destroying the “neuron house” is not as accurate. For that, it is more fitting to imagine a house so completely filled with clutter that it is impossible to move around from room to room. While clutter would not lead to the loss of the house, it would make it difficult to easily maneuver. This is like a cell trying to conduct normal business where the parts need to shuffle important substances internally as well as in and out of the cell, and if it was difficult to operate, most of the normal tasks would suffer. I’ve seen neurons (Figure 2.5) that appeared to accumulate so much amyloid that it would be impossible to imagine the cell physically or mechanically operating normally.

Neurons dying by excessive accumulation of amyloid literally burst (lyse), leaving their contents in place. This allows unattended intracellular enzymes (the fire) to consume without control, thereby perishing lytic-sensitive cell parts (flammable items), while leaving lytic-resistant (fire-resistant parts such as metal) cell parts in place, signaling local inflammation (response of the fire engines).

The everyday person would never likely believe that the charred remnants of a home were the catalyst for the fire, and yet this is what the mainstream amyloid cascade hypothesis presumes: the amyloid started the fire between the houses that became increasingly toxic and killed neighboring neurons. Nothing can be further from what my evidence has shown.

**PRESENTATION TIME**

I quickly assembled all of the data into a manuscript and while my first AD paper was in review, I also submitted an abstract to the Annual International Neuroscience meeting in Miami, FL, in 1999. I should note that abstracts (summary of your work) are not peer-reviewed-like manuscripts- but are reviewed for integrity and quality, as well as for
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placement into the proper scientific session. Fortunately, the abstract was accepted as an oral presentation.\(^\text{1}\) About a month before the meeting, I learned the time of my presentation and it was the first one just after lunch at 1:00 p.m. I assembled the slides for the slide projector, and then rehearsed for my first oral presentation in the AD field.

Although presentations of research findings are routine for most, this presentation was anything but usual for me, in spite of giving numerous presentations previously. I was a relative newcomer to the AD field, attempting to sway prevailing scientific consensus toward new findings in a sound and impactful manner. The day of the presentation arrived; lunch had finished and I awaited my introduction by the stage. The chairman of that session opened the meeting, and began to introduce the talk even noting his own interest in the topic and its potential implications, and then he called my name. And with that, my first step into the public domain of Alzheimer’s disease research was taken.

I began by highlighting my background in immunohistochemistry, how my journey with AD research began tangentially through the support of an AD program. And as I learned more and more about the current understanding of AD, I had more and more questions about the currently accepted and highly published amyloid cascade model based on my findings, and then I asked:

*If the amyloid plaques are believed to form from extracellular deposits of \(A\beta_{42}\) that aggregate and grow that are toxic to neurons, then why isn’t there one huge plaque in the AD brain? Why or how do they stop growing? And, why are those plaques of particular sizes and shapes, and located in particular areas of the brain? And why is \(A\beta_{42}\) detected in AD neurons, and more importantly, if \(A\beta_{42}\) is toxic, why do I detect it in normal neurons?*

Then I said, “I’m about to tell you why.”

And then I realized that I had everyone’s attention and then felt a surge of excitement. I essentially reported the litany of compelling experimental data on why it makes more sense to believe that amyloid plaques originate from dying, amyloid-overburdened neurons, than it was to believe that amyloid aggregates and grows between neurons and, for some undefined reason, kills neighboring neurons.\(^\text{2}\) I continued to show slides of intracellular amyloid, specifically \(A\beta_{42}\), in normal, nondemented, age-matched control cortical neurons and when you
compare with those neurons in the AD brains, the Aβ42 in those neurons accumulates to a point that the neuron dies, leaving the dead neuron as the amyloid plaque. I also said that I had not observed any evidence to support the amyloid deposition hypothesis. In sum, I presented a logical and unifying explanation of neuronal death and plaque formation in Alzheimer’s disease. I closed the talk to a nice round of applause and readied myself for questions.

I fielded a few thoughtful inquiries; most were technical questions about methods, antibodies, and whether I had tried other methods to validate those immunohistochemically based findings. However, the inevitable and frankly, exciting, moment came when I received the first criticism. A scientist came forward with a comment, stating that all of my data were suspicious and artifactual based on the cross-reactivity of cellular lipofuscin with the antibodies. Admittedly, at the time I didn’t know that much about lipofuscin and my reply was a bit clumsy. I said that I appreciated his comment and said, “From what I know about lipofuscin, it is a pigment that is typically located in the neuronal perikaryon (cell body), which is around the nuclear area and I had additionally showed intracellular Aβ42 in the dendritic neuronal processes as well where lipofuscin is not typically present.” Nonetheless, I was not convincing and felt a bit uncomfortable that I was not more forthright.

After another round of applause, I walked off the stage, but instead of returning to my seat, I continued to walk out the door to breathe. Just as I left the auditorium, several audience members approached me expressing their fascination. One person actually noted that he also has seen intracellular Aβ42 and his paper was also in review, but, surprisingly, he did not link the intracellular Aβ42 with plaque formation as I clearly presented. After the conference ended, I gave an interview that was published in Science News and summarized the presentation for the AD community at large:

Michael R. D’Andrea offered the controversial idea that plaques observed in patients’ brains arise from clumps of beta-amyloid in living cells. He says that the alpha 7 receptor may draw beta-amyloid into a cell until the cell dies and disintegrates, leaving an extracellular plaque. The distribution, density, and shape of plaques support this idea, he argues.
REFERENCES

