Denisa Wagner

The Road to Success May Not Be Direct

Susan Ince

Denisa Wagner arrived in the United States in 1975 as a stateless person, a refugee from the former Czechoslovakia who spoke no more than a few pleasantries in English.

"The Massachusetts Institute of Technology (MIT) accepted me and I felt very welcomed in the United States."

In many ways, attending graduate school in the United States was a startling contrast from the University of Geneva, where Wagner received her undergraduate degree in biochemistry. But Wagner made the most of her welcome into the country and created a groundbreaking career at the intersection of vascular biology, inflammation, and thrombosis.

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While studying for her PhD in biology at MIT, Wagner worked with Richard Hynes, PhD, in the Center for Cancer Research. It was the early days of research on the cell biology of adhesion molecules, and Wagner began studying the structure and function of fibronectin, spurring an interest in cell adhesion that she has carried throughout her career.[1] After graduating and establishing an independent laboratory at the University of Rochester School of Medicine and Dentistry, she began work on another adhesion molecule, VWF (von Willebrand factor), on the advice of her junior faculty mentor Victor Marder, MD. In her first articles, she described the discovery of the storage of VWF in Weibel-Palade bodies in endothelial cells,[2] as well as the processing steps in VWF biosynthesis and the rapid secretion of the protein induced by vascular injury.

After becoming an associate professor at Tufts University School of Medicine in Boston, Wagner's lab found that P-selectin was stored along with VWF in Weibel-Palade bodies in human endothelial cells.[3] When they created P-selectin knockout mice along with her former PhD advisor, they were delighted to have the first surviving knockout mice for an adhesion molecule. Here, leukocyte rolling was virtually absent, and there was delayed recruitment of neutrophils in response to inflammation.[4] Double-knockouts for both P- and E-selectin exhibited severe leukocytosis and a great susceptibility to bacterial infection, demonstrating the importance of the selectins in leukocyte recruitment to sites of inflammation and also to bone marrow.[5]

In 1994, Wagner was recruited to Harvard Medical School, where she is currently the Edwin Cohn Professor of Pediatrics in the Program in Cellular and Molecular Medicine and the Division of Hematology/Oncology at Boston Children's Hospital.

At Harvard, Wagner advanced the understanding of platelet biology, observing that platelets are actively involved in inflammatory responses and have similar behaviors to leukocytes, rolling on a stimulated vessel wall in a process dependent on P-selectin.[6] Wagner's laboratory at Harvard was the first to study the blood vessels of knockout mice using intravital microscopy. Visualizing thrombosis in vivo made it possible for the group to challenge several existing dogmas (such as fibrinogen is necessary for thrombus formation) and to recognize the importance of a metalloproteinase that cleaves VWF in reducing both thrombosis and inflammation.[7]

In recent years, Wagner's lab has focused on neutrophil extracellular traps (NETs). Although previously linked to infection and autoimmune disease, the Wagner lab found that platelets bind to NETs and that the enzyme PAD4 (protein arginine deiminase 4), which decondenses chromatin to make NETs, plays a critical role in the development of deep vein thrombosis.[8] They also discovered that NET formation is enhanced by cancer, contributing to cancer-associated thrombosis,[9] by diabetes mellitus, contributing to impaired wound healing,[10] and by aging, contributing to age-related organ fibrosis.[11] The main topic of the lab's investigations is now to determine whether the inhibition of NETosis can improve the outcome of various pathologies involving inflammation and thrombosis, including brain or heart injury following stroke or myocardial infarction.

With the help of a Whitman Center Research Award, during the past 2 summers, Wagner has spent several weeks examining the formation of NETs using the powerful microscopes at the Marine Biological Institute in Woods Hole, Massachusetts. Among numerous other awards, Wagner has received the Robert

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P. Grant Medal, the highest honor presented by the International Society on Thrombosis and Haemostasis (2015); a 7-year Outstanding Investigator Award grant from the National Heart, Lung, and Blood Institute (2016–2023); and the American Heart Association's Distinguished Scientist Award (2017).

Where Were You Born?

In Prague. But in 1968, when I was a teenager, the Soviet Bloc invaded Czechoslovakia. My father was high in the Ministry of Heavy Industry but because he was not a member of the Communist Party we had to leave. Members of the family left separately so the government wouldn't know, and we reunited in Vienna. My parents went on to Switzerland, where my father already had a job promised, and they left me in Vienna to finish high school in a French Lycée that gave me a scholarship. I'm very grateful for that, and my certificate from that school allowed me to transfer to the University in Geneva without an entrance exam.

Have You Been Back?

I couldn't go back for 21 years because it was considered a crime to have left. In 1989, I was able to return and see my cousins and classmates for the first time since I left. I now very much enjoy going back.

When Did You First Spend Time in a Lab?

When I was 12. I always liked nature and insects and flowers. We had neighbors who were scientists and one day I asked if I could go with them and watch what they do. They sent me to the entomological laboratory at Charles University where a woman was treating kitchen flies with toxic chemicals to study the development of ovarian cancer. I'm pretty handy, so I would open up 50 flies and line them up on a plate, removing the ovaries so she could take her measurements. I was good at it, she was very pleased, and she rewarded me with exotic insects to take home. At that point, I knew I wanted to be a biologist.

What Did Your Parents Hope or Expect You Would Do in Your Career?

They thought I might be an architect because I could draw quite well and participated in some sculpture exhibits. My mother was more surprised at my choice because she was a ballet dancer and was hoping I would be inclined towards the theatre. In the end, my biology choice was fine with them.

Why Did You Apply to Grad School in the United States?

In Geneva, I was dating an American fellow who was going to go to graduate school at Harvard. After a one-year separation, while I was finishing in Geneva, we decided to get married, and I was accepted to MIT.

Did You Ever Consider Going to Medical School?

I applied to both Harvard and MIT. Because of my refugee status and lack of residency status, Harvard told me the only way they could pay was if I entered the MD/PhD program. I thought about it, but I am a little bit soft-hearted and I felt I might not do well with all the human suffering. But I knew I wanted to do something medically oriented, so I went to MIT and worked at the Cancer Center.

Did You Speak English When You Came to the United States?

No. I spoke Czech, French, German, and some Russian, but not English. I had never even taken an English class and could only say social things like "hello", "thank you", and "my name is…". When I had notes for a class my husband helped me translate them into French. Because I already knew German (similar to English) and biochemistry, I learned quickly, but I spent many evenings crying because it was so hard.

The teaching methods also made it more difficult. In Geneva, they consider students as adults. You can go to classes or not, and you just have to pass exams scheduled during a specified exam period. Some classmates worked during the day and studied at night, only went to random classes, and passed the exams. MIT was completely different, giving surprise 15-minute quizzes with a paragraph to read that contained the question. It took me 15 minutes just to read a paragraph in English. In that class I got a C because of that. Later at Tufts, I was part of a program project with that professor, Christopher Walsh, but at MIT, he was the most frightening person to me. Life changes things.

I still don't know how to spell, so thank goodness for spellcheckers.

What Drew You to Work on Cell Adhesion at the Cancer Center?

I liked that Richard Hynes worked on cell interactions and how cells responded differently when they were adherent. Those were the early times of this research, before he had identified integrins as a major class of adhesion molecules. We were working on fibronectin—not even called that and not yet cloned—and I studied its structure and function. There was already a little known about fibronectin's function in blood and blood vessels, so that primed my interest in blood.

Are Adhesion Molecules Still as Fascinating and as Full of Open Questions as When You Started?

Yes, I think adhesion is very important. A lot of my career has involved adhesion in blood vessels and interactions among blood cells, including platelets, leukocytes, and endothelium. In 1996, during the boom of research on adhesion molecules, I wrote a review with Paul Frenette for *NEJM* and we depicted in a figure a multitude of interactions relevant to vascular biology. I still work within that depicted field, with more emphasis on neutrophils and NETs.

Did You Do a Post-Doc?

I had a baby just as I was graduating from MIT and my husband got a job in Rochester. I went with him, set up the house, and looked for a post-doc. When my daughter was 4 months old, I started a post-doc on microtubules with Dr Joanna Olmsted at the University of Rochester. It was pure cell biology and I missed the field of adhesion, so I went to a seminar on fibronectin, the subject of my doctoral thesis. After answering some of the seminar's questions, the host, Victor Marder, asked me who I was and said he heard I was in town and had been looking for me because of my knowledge of adhesion molecules. He offered me a job on the spot, so I was only a post-doc for a few months.

But all experience is good. My first paper as an independent scientist was to localize VWF in a secretory organelle in endothelial cells. It wasn't known at the time that endothelial cells had granules and that they could secrete those on command under stress. To demonstrate that VWF was stored, I needed electron

microscopy. Olmsted was great at that, so we collaborated and published together after all.

In an Obituary of Victor Marder, You Were Described as One of the Young Talents That He Developed. Can You Tell Me About That Mentor Relationship and What You Learned From It?

Victor Marder was a very nice man and showed me in many ways that he was happy to have me in his unit in Rochester. He taught me a lot about writing and how to clearly present my findings. This was very helpful because even after 5 years in America, my written English was not great. He also taught me about politics at a medical school. That was new to me because I had been in a university-type setting at MIT.

Marder also let me work on a schedule that accommodated my children—a 1-year-old girl when he offered me the job and soon after that a baby boy. When we were putting together a program project grant, he tolerated that I brought a 2-week-old baby to the meetings. I also dropped my hours to 60%, so that when the baby was not cooperating, I was able to go home. My husband has always said that I still worked full time, but I appreciated the freedom of not always having to be in the lab—even if it meant making slightly less money.

Why Did You Move to Tufts?

At some point you want to be fully independent, but that is difficult in a clinical department where the chair is on all of your papers, even though Marder deserved to be! I wanted to be independent and was offered a suitable faculty position at Tufts.

How Did You End up at Harvard?

Harvard approached me after several high-profile papers came out of my lab in the adhesion field. After the publication of the P-selectin knockout in *Cell*, I got the call from Dr Fred Rosen who was a legend at Children's Hospital at Harvard. At that time, I had almost signed on the dotted line to join the Department of Cell and Developmental Biology at Tufts Medical School, but Dr Rosen asked me to hold off and meet him the next day. The day after that, he FedExed an offer. Those were different times. I was recruited at a seminar for one job, on the ski slope for another, and in one phone call and a quick meeting for the Harvard job. That kind of thing doesn't exist anymore.

Why Is that? Strictly Money?

Money is most of it. Departments now usually don't have money to recruit possibly risky young talent. If they do, there is a huge search. Nobody can just say, "I really like that paper, let's call her." Now there are major searches where lots of people are interviewed and only one gets the job. What I hear now is that the number of post-docs is declining as people see the level of competition, and our profession no longer looks attractive for the future.

For the Students You're Mentoring, What Advice Do You Give to Help Them Succeed?

First, you have to love what you're doing. Today, science is just too tough to do if you don't love discovery and live for the feeling of a breakthrough. Otherwise you can make better money doing something else.

Second, don't put all your eggs in one basket, because you can be wrong. Follow 2–3 different lines of investigation; one will work and you can stop doing the others that didn't.

Third, networking is more important than when I was young, I didn't quite learn how to network but I now see it's important. So young researchers should make friends among post-docs and their peers. One day it will be important when you need a specific technique or help with a project.

Do You Spend Much Time in Administrative Work?

I try to do only the necessary. That's why I am not a chairman of a department. Several times I was asked to consider the possibility of chairing some department, but I never even went to look because what I like to do is science and discovery and I am not good at politics.

When You Think About Being Helpful to Young Scientists, Are There Things a Mentor or Lab Chief Might Say or Do That You Think Is Never Helpful?

You should encourage people rather than put them down. I try hard not to do that.

One thing I have found horrible in some labs and have never done is to put several people on one project and have them compete with each other. That's cruel and inspires animosity. I always had everybody on a separate project. They could collaborate but everybody knows whose project is whose and knows they will be the first author if they succeed. Competition within a lab is nasty.

How Hard Do You Tell Them You Have to Work to Have a Successful Research Career?

If you love it, working all the time happens by itself. For me, most of the time I didn't think of it as work, I would be taking a baby on a walk in the stroller or planting flowers in the garden and thinking about my project. Many scientists around me still work very hard when they could have retired, but they still love it and want to do it. It's not a money-making business but a happiness-making business if it works.

You also need to have other things you love in your life for the times experiments are not working in the lab. If it's not working, go and see your kids or plant your flowers. Tomorrow will be better.

What About Your Own Children? Were They Attracted to Careers in Science?

Our son is an emergency physician at Stanford, and is interested in medical and business innovation. He and his wife are expecting a third child. Our daughter studied law and she was always interested in women and their careers. She now has a start-up company where she finds work-from-home jobs for highly educated moms. Even if they earn less money, women are able to keep their feet in their professions, and later they are in a better position to re-enter work. So both kids share some of my interests. My daughter also has three children, so we have babies everywhere.

What Is the Most Surprising Scientific Result You've Obtained, or the One That Influenced the Direction of Your Career?

The very first paper from my own lab. We localized VWF in the Weibel-Palade bodies and showed that the endothelium on the vessel wall can react to stresses and immediately change its properties by releasing adhesion molecules. Later we found P-selectin in the same organelles. VWF and P-selectin being in the same granule taught us that inflammation and thrombosis are regulated together, and that finding really did direct me through my career.

You've Had a Very Successful Career. Have There Been Failures or Down Times?

Life brings many surprises, but the hardest thing in my life was not in my career but when I was 17 and my parents had to leave me in Vienna. I lived in a children's home with many unhappy children, and could not visit my relatives in Czechoslovakia which was only miles from Vienna. Studying French literature for a French Baccalaureate was extremely hard.

In Recent Years, You've Discovered Many Detrimental Effects of NET Formation. Any Time When This Response Is Useful?

I think NET formation is a vestigial protective response that's no longer needed. It was useful when we were hunters and walked barefoot through mud and cut ourselves. In the mud were invasive bacteria and parasites that got into the wound. It was important to stop bleeding and not get infected. NETs are highly pro-coagulant and generate fibrin. The fibrin and NETs form a barrier which stops us from bleeding and protects from bacterial invasion. NETs may have other roles in activating or accelerating the response of the adaptive immune system, but mice live well without NETs. Inhibiting and destroying NETs may be a good thing because they clog the microcirculation, injure the endothelium, activate platelets, and promote auto-immunity.

What Do You See for Yourself and Your Lab Over the Next 5 Years?

I think we will stick with NETs for a while, but I would very much like to expand that research into new fields. I am starting to be more involved in autoimmune disease. NETs really drive autoimmune disease, and I have a rheumatology fellow from Japan coming in who plans to look at the responses of neutrophils and PAD4 in new models of rheumatoid arthritis.

If I have the possibility, the brain is where I would take the research now. One report shows that neutrophils are implicated in Alzheimer's in animal models and in humans, and NETs have been observed in samples from Alzheimer's patients. I would be very interested to work on that and brain inflammation in general.

I've also started to work as an advisor with a start-up company very recently on something I believe could become a really good drug, so we'll see where that will go. It would be fun at the end of my career to be involved in developing a drug that could really be helpful.

What Do You Like to Do When You Aren't Working?

Flower gardening. I have a green thumb and design and plant everything myself, but I have a helper for maintenance. I like visual art and going to art exhibits, in particular those associated with Art Basel in Miami.

From the Pictures on the Website, It Looks Like You Also Do Fun Things With Your Lab

I like a nice community. This summer I invited the rest of my lab to come to Woods Hole for a day. We had a group meeting and spent time on the water. Every year I have a pool party at my house. Having retreats and doing fun things together is my way of nurturing the fellows. It comes back to me, because when they're successful it's like your children—you're proud of their successes.

Nowadays it is harder to finance research, and sometimes I get a little down. But then I go to an international meeting and former fellows, now having their own labs, make the time to all have lunch together. It is my biggest pleasure and reward.

Disclosures

None.

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