Juvenile diabetes: the antibiotic connection

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A child does not grow up only in a single home. (Omwana takulila nju emoi)

-Bunyoro proverb-

In science, important observations generally do not come from a single person, but rather from an entire context of ideas, people, institutions, and resources. Our linking juvenile diabetes (type 1 diabetes) to antibiotic exposures had many parents.

For a number of years, I had been interested in the possibility that the big increases in the incidence of illnesses like obesity, asthma, and allergies were occurring as a result of antibiotics perturbing our colonizing microbes—the human microbiome – during the early days of childhood. One of the diseases I was considering was juvenile (type 1) diabetes (T1D) because it has been progressively becoming more common, doubling in incidence every 20-25 years since World War II, and with evidence that the age of onset is getting younger. This would fit well within my theory of a lost or perturbed microbiome underlying these diseases (much of the narrative is included in my book *Missing Microbes*, Henry Holt 2014).

At a microbiome research conference in Vancouver in March 2011, I was presenting some of our findings that antibiotics were changing the growth and development of mice, leading to more fat deposition. After my talk, I spoke with Dr. Jessica Dunne, a program officer at the Juvenile Diabetes Research Foundation (JDRF). She indicated that the idea that antibiotic exposures could be playing a role in T1D was of interest to the JDRF, and she asked if I could present our work to her and colleagues. After meeting with them and getting their input, Jessica indicated that we should apply for a Strategic Research Agreement (SRA) with the JDRF. This is another of the starts to the story.

An earlier beginning was the report in 1980 by Makino and colleagues in Japan of a strain of mouse that spontaneously developed an illness strongly resembling T1D. These non-obese diabetic (NOD) mice have since been used by many investigators as a model of the immunological events leading to T1D. In fact, others had tried giving antibiotics to NOD mice and had found that they developed T1D less than untreated mice—this was counter to my hypothesis. However, others had found that under very clean conditions, NOD mice developed T1D at younger ages than mice raised under 'dirty' conditions; that suggested the idea that 'dirty protects.'

Although the literature had many observations about NOD mice that appeared contradictory, it seemed that in total a story could be developed that some aspect related to the microbial compositions of the gut could be important.

Enter Alexandra Livanos. Ali was a medical student at NYU who was interested in the projects on the microbiome that were ongoing in my lab. Ali was interested in the pancreas. In 2010, while a third year student, she told me that she planned to take a year off from medical school to deeply pursue a research project, and asked if I would serve as her mentor. I was thrilled because it was clear to me that she was a brilliant and hard-working student with a sparkling personality. We agreed that she would submit an application to the Howard Hughes Medical Institute (HHMI) to fund her year of research with the focus being the effects of pancreatic disease on the microbiome. In May 2011, she learned that she would be funded by HHMI, and I proposed to her that instead of studying the effects of the pancreatitis on the microbiome, we reverse the question and consider how manipulating the microbiome with antibiotics in NOD mice would affect the pancreas, and affect the development of T1D. Intrigued, Ali agreed.

We planned to use two different antibiotic regimens to perturb the microbiome—a low-dose regimen, which we called STAT (for sub-therapeutic antibiotic treatment), similar to that used on the farm to promote growth of livestock—and a regimen that exposed mice to the levels of antibiotics that children usually receive to treat their ear or throat infections, which we called PAT (for pulsed antibiotic therapy). We applied to the JDRF for an SRA, proposing to study STAT and PAT. They were interested, but could only provide half the support we requested, and thus asked us to only study STAT, since we had strong evidence of effects from our obesity models. We also had very good evidence that PAT substantially affected the microbiome, and we wanted to try both. Fortunately, I had received the long-term philanthropic support for our work on the microbiome from a donor (Diane Belfer), and could use that to supplement the JDRF funds. Ali compared T1D incidence in NOD mice exposed to STAT, PAT, or neither (those were the controls), and it turned out that STAT had essentially no effect, but there were strong effects with PAT. Our intuition to try both models turned out well—for 50% more effort, we doubled our chances for success; it was good that we didn't abandon PAT. And with my encouragement and the JDRF funding in hand, Ali decided to convert her one-year research stint into a Ph. D. with the antibiotic/NOD model of T1D as her dissertation topic.

Then the village expanded! Ali's work was supported by a strong analytic team at NYU, with Huilin Li providing statistic approaches, Alexander Alekseyenko leading bioinformatics, and Zhan Gao, with molecular expertise. Dan Knights, a very talented bioinfomatist at the University of Minnesota and his colleague Pajau (PJ) Vangay agreed to perform further analyses. We applied for and were granted support from an NIH research resource for metabolomics studies, which enabled us to work closely with four investigators from RTI International (Delisha Stewart, Wimal Pathmasiri, Susan McRitchie, and Susan Sumner) to understand how the antibiotic exposures were affecting metabolism. When I learned that Fredrik Backhed had a

colony of germ-free NOD mice in Sweden, I asked him if we could send intestinal contents to inoculate their animals. He agreed, and for the next year we worked closely with him and Thomas Greiner on that experiment. When we had unexpected results from that experiment, we teamed up with Ken Cadwell and Xue-Song Zhang back home at NYU to make transfers to C57Bl/6 mice to study their immune responses. A very well-respected pathologist who specializes in the gastrointestinal tract, Arlin Rogers (Tufts University), agreed to blindly examine specimens from the pancreas. Several short-term college (Sandy Ng, Joanne Kim), medical (Sara Kim), and post-baccalaureate (Jiho Sohn, Cecily Barber, Jennifer Chung) students contributed their energy and insights to particular aspects of the project. Hurricane Sandy set us back by almost a year, but with Ali's tenacity, we could recover. All in all, 22 scientists and students, representing a wide array of disciplines and able to measure all kinds of phenomena brought this work to conclusion. This scientific child did not come from a single home.

Now, nearly five and half years since that catalytic conversation with Jessica Dunne got us started, our paper is finally out! Ali successfully defended her dissertation in 2014 and then graduated from medical school. Now a Resident in Internal Medicine at New York-Presbyterian/Columbia University Medical Center, she serves on the front lines of medical care, but with outstanding potential for future contributions to medical science. Using different methods, other groups also have reported antibiotic effects consistent with ours; the principle is holding. We are eager to see how other scientists can use our findings, and in the meantime, we are busy following up on the many leads uncovered by these studies.

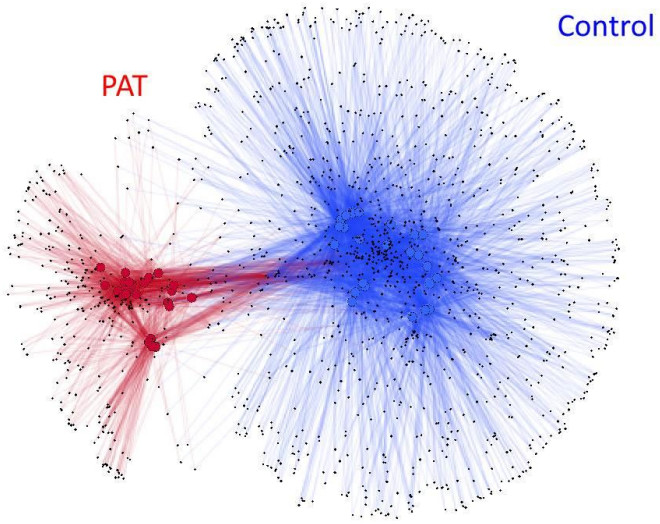

Figure Legend. Co-occurrence network connecting mouse samples (large circles) to the OTUs which they contain (tiny dots around the periphery). This shows that antibiotic-treated mice (red) have a different, and less diverse, set of OTUs than the control mice (blue).
Source: Dan Knights, Ph.D.; University of Minnesota