No. This Study Does Not Prove What You Think It Does

Part II: Nobel-Worthy Science

Editorial note: This series was originally published in the Parents PACK newsletter, a free monthly e-newsletter for the public that addresses vaccines and related topics. To learn more about the program, visit vaccine.chop.edu/parents.

In part I of this series, we discussed some common misconceptions about science and scientists. In part II of this series, we will look at some studies that led to new understandings. In the first example, Australia antigen, the original authors' ideas about the source of this protein were challenged by further evidence, and as scientists do, they continued to learn about and try to figure out what they had found. In the other two examples —bacteria as a possible cause of ulcers and the existence of infectious proteins, called prions —early observations were questioned because they challenged established norms. As described in part I of this series, the scientists involved, as well as other scientists, further pursued related studies and ultimately, the findings led to new and important understanding for all three examples. Indeed, in each case, the primary scientists received Nobel Prizes.

Australia Antigen

Original study: Blumberg BS, Alter HJ, VisnichS. A "New" Antigen in Leukemia Sera. *JAMA*. 1965 Feb 15;191(7):541-6.

Development of understanding
In 1965, Baruch Blumberg and colleagues published a paper in the Journal of the American Medical Association (JAMA) identifying "Australia antigen." The authors indicated the following:

- The protein was found in high levels in patients who received numerous transfusions.
- In samples from people in the U.S., it was most often found in people with leukemia, but it was otherwise not frequently found in U.S. samples.
- In samples from people in other parts of the world, Aboriginal Australians and a group of individuals from Taiwan had the greatest number of positive samples, but blood samples from individuals in other countries also sometimes contained the antigen.

The authors hypothesized that the antigen could indicate who was more likely to develop leukemia, could develop as a result of leukemia, or could be related to a virus that causes leukemia. They, and other groups of scientists, set out to learn more.

Over the next couple of years, Blumberg and others found that Australia antigen was more likely in patients with Down syndrome who were institutionalized, and in 1968, Alfred Prince identified a critical link with the antigen and hepatitis. At the time, the types of hepatitis were not distinguished by letters (hepatitis A, hepatitis B, etc.), but rather as "serum" or "infectious" in origin. Infectious hepatitis was viewed as being transmitted from one person to another, whereas serum hepatitis was, as suggested, associated with factors in the blood. Ultimately, "infectious" hepatitis became known as hepatitis A, which is transmitted through contaminated food and water, and serum hepatitis became known as hepatitis B. As it turns out, hepatitis B is also infectious, but it spreads through contact with the blood of infected individuals. So-called "Australia antigen" is critical to this bloodborne transmission, but this was not yet understood in the late 1960swhen Blumberg and colleagues were trying to learn more about this protein.

Check the online version of this article to see clips of Dr. Blumberg describing how he and other scientists worked to get answers to questions about Australia antigen, "Tracking a Mystery: Part 1 | Part 2."

By 1970, scientists generally agreed that Australia antigen was related to viral hepatitis, but they still did not understand exactly what it was and how it related. They were working to understand how it spread (e.g., infectious transmission or genetics) and why it was found at different times in different people (e.g., acute or chronic infection). They were also trying to understand why some scientists found different sized antigens in their studies. In 1970, Dane and colleagues proposed an important piece of the puzzle, suggesting that the larger components were intact virus particles and the smaller particles were extra viral proteins.

Part II: Nobel-Worthy Science

Ultimately, Australia antigen was determined to be hepatitis B surface protein, and Dane and colleagues' hypothesis was proven accurate. Intact viral particles as well as large quantities of the surface protein flood the blood of infected individuals. Because some antibodies against hepatitis B bind to the excess surface protein, people can remain infectious and unwittingly spread the virus even through extremely small quantities of blood (not visible to the naked eye).

In 1976, Baruch Blumberg was awarded the Nobel Prize in Medicine for discovery of hepatitis B virus.

Related to vaccines
Even before everything was understood about Australia antigen and its role in hepatitis B infections, scientists started trying to develop a vaccine. By the early to mid-1970s, some scientists were studying how to isolate and inactivate the virus from blood, so that it could be used for immunizations. By the early 1980s, hepatitis B vaccine made from the serum of infected individuals was available, but when AIDS was identified, using blood as a starting material led to questions of whether the virus that caused AIDS could survive the vaccine production process. This led to concerns that the hepatitis B vaccine could potentially be a source of the virus that caused AIDS, called human immunodeficiency virus (HIV). Although the vaccine was not found to contain HIV, a new way of making the vaccine was developed. By the mid-1980s, a new technology for making hepatitis B vaccine became available, removing concerns about hepatitis B vaccine safety and AIDS

Go to the online version of this article to watch a video clip from Watch this video clip from "HILLEMAN: A Perilous Quest to Save the World's Children" to learn more about the two hepatitis B vaccines.

Timeline

- 1965 –Australia antigen was identified.
- 1968 –Australia antigen (referred to as SH antigen) was shown to be related to hepatitis infection.
- 1970 –Although it was generally accepted that Australia antigen was related to serum hepatitis, scientists were still working to understand different sizes of antigen being found in samples. Dane hypothesized that the variation in size was due to viral particles and an excess presence of viral antigen.
- 1971 –Studies were underway to inactivate the virus.
- 1975 –Studies of potential vaccines were completed in animals.
- 1976 –Dr. Blumberg was recognized with the Nobel Prize in Medicine.
- Late 1970s-1980 –Studies of serum-derived hepatitis B vaccine were performed in people.
- Mid-1980s–Serum-derived hepatitis B vaccine was replaced with the recombinant hepatitis B vaccine that is still in use today.

Prions

Original study: Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science*. 1982 Apr9;216(4542):136-44.

Development of understanding
In the summer of 1972, when Stanley Prusiner was a resident at the University of California, San Francisco (UCSF), he saw a patient with increasing memory loss and an inability to complete typical daily tasks. She had Creutzfeldt-Jakob disease (CJD), which Prusiner learned was a condition that slowly caused loss of abilities in individuals and did not appear to be overcome by immune system responses. No one, it seemed, knew what caused this or related conditions. At the time, these conditions were believed to be the result of "slow viruses." Prusiner thought that trying to find and identify these "slow viruses" would be a good research project.

An animal model for studying these conditions came from sheep. Scrapie did to sheep and goats what CJD did to people. In 1974 when Prusiner finished his residency, he accepted a university teaching position at UCSF and opened a laboratory to study scrapie. As it turned out, figuring out what was causing scrapie proved a difficult task. Specifically, Prusiner could isolate a protein that was associated with the disease, but he could not find any genetic material. Any pathogenic agents known at the time (viruses, bacteria, fungi and parasites) included both proteins and genetic material, but Prusiner could not find any genetic component to the agent he was isolating.

After numerous studies proving that what he found was the infectious agent, and several more studies to try to find genetic material, Prusiner started to consider that he had stumbled on a novel type of infectious agent. In 1982, he published his studies identifying prions as "small proteinaceous infectious particles which are resistant to inactivation by most procedures that modify nucleic acids" (p. 141). In the paper, Prusiner indicated that he had not ruled out the presence of nucleic acids, but that he had been unable to find them either. He went on to discuss how these entities might replicate if they did not, in fact, contain genetic material. Other scientists were incredulous; the existence of a novel class of infectious agent that did not contain genetic material seemed impossible. As a result, several other labs started studying this idea. Some worked with Prusiner; others worked to disprove him.

In the year or two that followed, Prusiner's lab identified a protein, called "prion protein" (PrP); however, they still had not resolved the genetic material question.

By the early 1990s, scientists were starting to accept the disease-causing ability of prions, but questions remained about how they did this without genetic material. Ultimately, it was determined that two forms of PrP exist —one that causes disease and one that does not. All people have the non-disease-causing form; however, in some people, this "healthy" form converts to the other form, leading to disease. The form that causes disease can arise in two ways. First, it can be introduced from an outside source, such as by consumption of human (cannibalism) or animal (contaminated bovine meat) products that contain the disease-causing form.

Second, it can arise spontaneously through genetic mutations. In either case, the disease-causing form of the protein serves as a template for conversion of the "healthy" form, leading to a slowly evolving and uniformly fatal condition.

Prusiner was awarded the Nobel Prize in Medicine in 1997 for his discovery of prions.

Check the online version of this article to watch a video interview with Stanley Prusiner talking about his career and experiences discovering prions.

Timeline

- 1972 –Prusiner saw a patient who would set the course of his research.
- 1974 –Prusiner set up a research lab to study scrapie, a disease of sheep similar to the type of disease his patient had.
- 1982 –Paper introducing the concept of prions was published in *Science*.
- 1982-1983 –Prion protein (PrP) was identified.
- 1985 –Use of genetic material from the host's chromosomes was determined
- Early 1990s– General acceptance of the existence of prions as a disease-causing agent.
- 1997 –Prusiner was awarded the Nobel Prize in Medicine in 1997 for his discovery of prions.

Cause of Ulcers

Original study: Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984 Jun 16;1(8390):1311-5.

Development of understanding
In 1940, and again in 1975, researchers reported the presence of bacteria in patients with gastric ulcers, but their results were never confirmed and essentially forgotten.

In 1981, as part of his medical studies, Barry Marshall started working on a research project under the guidance of J. Robin Warren. Warren had identified curved bacteria in a group of patients during gastric biopsies. Warren and Marshall realized that the bacteria resembled *Campylobacter jejuni*, a known cause of food poisoning, but the pair had trouble growing the bacteria from patient samples.

At the time, ulcers were believed to result from stress as well as consumption of spicy foods. These triggers were believed to cause excess production of stomach acid, and as such, patients were treated with acid-suppressing medications. However, as Marshall continued studying patients in the early 1980s, he became more convinced that the bacteria were associated with gastric ulcers. In 1982, after a holiday weekend in which their cultures were kept longer than usual, the scientists and their team were able to grow the bacteria and identify it as something other than *Campylobacter*.

In 1984, Marshall and Warren published a paper sharing their findings and indicating that they were able to grow the newly identified bacteria in the lab using samples collected from patients. The bacteria they identified eventually (1989) came to be known as *Helicobacter pylori*.

The accepted treatment for gastric ulcers at the time was to prescribe acid-suppressing medications, and although patients experienced resolution of symptoms, their symptoms returned after they stopped taking the acid reducers. However, because bacteria can be treated with antibiotics, Marshall and Warren were able to demonstrate that when treated with antibiotics, patients with gastric ulcers recovered completely. Despite their best efforts, Marshall and Warren met with significant resistance to this new model of thought.

Because there was not a useful way to study their theory in animals, Marshall experimented on himself to prove to his colleagues that their model was accurate. In a now widely shared story, Marshall submitted to an endoscopy to prove that he did not have a gastric ulcer, then he drank a mix of the bacteria, which caused him to develop nausea, vomiting and lack of stomach acid. At periods of one and two weeks after drinking the cocktail, Marshall had additional procedures that showed he was infected with the bacteria and had developed gastritis. He recovered from the infection on his own, albeit he did take antibiotics at one point.

It wasn't until 1991 that the U.S. Centers for Disease Control and Prevention (CDC) formally recognized a link between the bacteria and gastric ulcers. Marshall and Warren were recognized with the Nobel Prize in Medicine in 2005.

Find a link to read Dr. Marshall's Nobel Prize speech in the online version of this article.

Timeline

- 1940 –Freedburg and Barron found bacteria they identified as spirochaetes in gastric biopsy samples; their findings could not be confirmed.
- 1975 –Steer and Colin-Jones found bacteria identified as Pseudomonas in biopsy samples from the lower part of the stomach in patients with stomach ulcers. Their findings were considered to be contaminants and forgotten.
- 1981 –Marshall started working with Warren to follow patients whose stomach biopsies revealed "curved bacteria."
- 1982 –Marshall and Warren discovered that the bacteria were not Campylobacter jejuni, but instead a novel type of bacteria.
- 1984 –Marshall and Warren published their findings, including their ability to grow a new type of bacteria isolated from patient samples.
- 1984 –Marshall infected himself with the bacteria.
- 1989 –The type of bacteria was named *Helicobacter pylori (H. pylori)*.
- 1991 –The CDC recognized the link between *H. pylori* and gastric ulcers.
- 2005 –Marshall and Warren were awarded the Nobel Prize in Medicine for their discovery that gastric ulcers are most often caused by *H. pylori*.

- 1989 –The type of bacteria was named *Helicobacter pylori (H. pylori)*.
- 1991 –The CDC recognized the link between *H. pylori* and gastric ulcers.
- 2005 –Marshall and Warren were awarded the Nobel Prize in Medicine for their discovery that gastric ulcers are most often caused by *H. pylori*.

Conclusion

Each of these stories demonstrate that novel scientific findings are often met with resistance, especially if they challenge accepted dogma. However, importantly, they also demonstrate three important takeaways:

1. The scientists who make these discoveries continue to experiment, so they can improve their own and others' understanding of the topic. They do not take the scientific criticism to the media to gain support in the court of public opinion. By continuing to pursue answers in a scientific manner, they demonstrate the way science should be done, often at professional expense and criticism.

2. Science is self-correcting. Even when an idea is supported by evidence, if new ideas come along or unanswered questions remain, by continuing to pursue evidence generated using unbiased, scientific approaches, the pieces of the puzzle eventually make sense.

3. Sometimes, it takes decades of dogged pursuit of answers to get to this point of understanding. As our Director, Dr. Paul Offit is fond of saying, "Nature is slow to give up her secrets." Science allows us a way to uncover those secrets.

FIND MORE INFORMATION

Access the complete 3-part series online: bit.ly/46IkP8M

Find classroom materials: vaccinemakers.org
Get vaccine information: vaccine.chop.edu

the vaccine makers project

Children's Hospital of Philadelphia®
Vaccine Education Center