Profile of Rino Rappuoli

Rino Rappuoli grew up in the shadow of what he describes as a testament to the devastating impact of infectious disease: the unfinished wall of the Siena Cathedral in Siena, Italy. When the plague hit the city in 1348, it slashed the population from 100,000 to 30,000. "It basically shut down one of the most powerful economies of the time, and that momentum was lost forever. I see it as an example of what could happen today with pandemic influenza," says Rappuoli, whose current research focuses on developing a vaccine for avian influenza.

Rappuoli, currently the Global Head of Vaccines Research for Novartis Vaccines & Diagnostics (Siena, Italy), was elected as a foreign associate of the National Academy of Sciences in 2005. He has spent his career developing vaccines for pertussis, meningitis, and *Helicobacter pylori* and is jointly responsible for engineering the carrier protein used in many conjugate vaccines. He is credited with launching the field of reverse vaccinology, the first fruits of which are revealed in his Inaugural Article in this issue of PNAS (1), where he describes a universal vaccine for serogroup B meningococcus.

Rino Rappuoli

Bucolic Beginnings

Rappuoli was born in 1952, in Radicofani, Italy, a village 40 miles south of Siena. When he was 11, his family moved closer to Siena, enabling Rappuoli to attend high school in the city. He spent weekends and summers helping his father produce Chianti, the region's signature red wine. As college approached, Rappuoli was torn between the desires to study poetry or science. "I chose science," says Rappuoli. "The moon landing and the sense of impending scientific revolution probably influenced my thinking."

Rappuoli pursued his undergraduate studies at the University of Siena but yearned to experience science outside of Italy. Wanderlust led him to Washington University in St. Louis, MO, where he studied bacterial mutagenesis during the summer vacation after his third year of college. More important than the research, however, were his observations of the bountiful conditions American scientists enjoyed. "In Italy, the theoretical training was good, but the technology gap was huge," says Rappuoli, "and molecular biology was 5 or 6 years behind the United States."

After graduation, Rappuoli remained at the University of Siena, where he earned a Ph.D. in biological sciences for his NMR studies of proteins and tissue membranes. In 1978, he was offered a fellowship at Siena's Sclavo Research Center, the Italian vaccine institute that had been developing and producing vaccines for almost a century.

Microbiology in America

Almost immediately after joining the institute, Rappuoli left to take on a project in the United States. He knew that to work at the cutting edge of vaccine science he needed to learn new techniques in molecular biology and genetic engineering. In 1979, he spent four pivotal months at The Rockefeller University (New York, NY) as a visiting scientist in the laboratory of Emil Gotschlich, who pioneered meningococcal vaccine studies in the 1960s. "He is probably one of the smartest scientists I've ever met and probably the person who had the greatest impact on me," says Rappuoli. "He would just tell me about science . . . how to approach the problem and spend a lot of time teaching me. Sometimes you meet a person who changes the way you think."

In 1980, Rappuoli spent a year at Harvard Medical School (Boston, MA) in the laboratory of John Murphy, who worked on *Corynebacterium diphtheriae*, which causes diphtheria. Murphy introduced Rappuoli to bacterial genetics and the world of bacterial toxins, and he helped Rappuoli learn new techniques in microbiology. Murphy also introduced Rappuoli to Alwin Pappenheimer, who became a mentor to Rappuoli. "He was one of the fathers of microbiology and immunology. . . . He is another person who really shaped my career," says Rappuoli.

At Harvard Medical School, Rappuoli joined Murphy and Pappenheimer in their search for a new diphtheria vaccine. The project stemmed from work Pappenheimer had done in 1972 (2, 3). Pappenheimer had mutagenized the diphtheria toxin gene and isolated a mutant with a single amino acid change. This minute tweak rendered the molecule, called CRM197, nontoxic and ideal as a diphtheria vaccine. "This was a big change in thinking because instead of using chemicals to detoxify the toxin, you could modify the gene, and the bacteria would produce a molecule you wanted already to go for you. It is perhaps the first example of rational design of natural molecules to yield efficient therapeutics or vaccines," says Rappuoli.

Conjugate Vaccines

In 1981, Rappuoli returned to Sclavo Research Center in Italy and launched his own laboratory. He continued his collaboration with Murphy and Pappenheimer

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This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 10834.

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and was charged with mass-producing CRM197. He succeeded in 1982, using classical genetics, but CRM197 was never used for a diphtheria vaccine. The current diphtheria vaccine was developed in and has been in use since 1924. "That's the vaccine that we still use today," says Rappuoli, "in large part because regulatory agencies and companies did not want to change something that had been working for a century." Thus, CRM197 was not pursued as a diphtheria vaccine.

However, CRM197 found an even broader application than for diphtheria. During the 1980s, other vaccine makers were developing conjugate vaccines for *Hemophilus influenzae*. Research by John B. Robbins had established that linking a polysaccharide to a carrier protein could transform it into a powerful vaccine. Robbins suggested to Rappuoli that CRM197 might make a good carrier. "You vaccinate an infant with a polysaccharide, and there is absolutely no response. You link the polysaccharide to a protein [such as CRM197], and the response is dramatic. It's the basis of many vaccines to date," explains Rappuoli. Today, Rappuoli estimates that every child in the United States and Europe probably receives four or five vaccinations with CRM197. It is a carrier molecule for vaccines such as *H. influenzae*, pneumococcus, and meningococcus.

New Generation of Vaccines

As with diphtheria, the new generation of vaccines against pertussis (whooping cough) was made from a toxin that had been deactivated with formaldehyde. "I didn't waste any time doing that. I cloned and sequenced the gene for pertussis toxin and did what Pappenheimer had done 15 years before with diphtheria," says Rappuoli. But this time he and colleague Mariagrazia Pizza used site-directed mutagenesis to specifically alter amino acids in the active site of the toxin. The result was a nontoxic molecule that made a potent vaccine (4).

The whooping cough vaccine brought attention to Rappuoli and helped establish Sclavo Research Center as a worldwide leader in vaccine development. The center became a part of the California-based biotechnology company Chiron (Emeryville, CA). The pertussis vaccine also established a new generation of so-called acellular vaccines, which, unlike older vaccines, did not contain cells or cell fragments.

A National Institutes of Health (NIH; Bethesda, MD) trial revealed that Rappuoli's acellular vaccine was as effective as traditionally made vaccines, but with an additional advantage—it required 10-fold fewer molecules (5). In 1995, this finding led regulatory agencies in the United States and Europe to shift from use of the old pertussis vaccine to the new acellular one. "That was a milestone in vaccination because it was a switch from traditional whole-cell killed-bacterial vaccines to more sophisticated, modern purified vaccines," Rappuoli says.

The development of the pertussis vaccine was particularly satisfying for Rappuoli. In 1993, Italy adopted the use of the new vaccine, and within 2 years the disease was essentially eliminated. "So that is basically one of the most beautiful things that can happen if you do my job. . . . That's been my motivation ever since," Rappuoli says.

Eliminating Serotype C Meningococcus

"The next [vaccine] I thought I could tackle with my team was meningococcus," says Rappuoli. Five primary serotypes exist for *Neisseria meningitides*, the bacteria responsible for meningitis and septicemia: A, B, C, Y, and W135. Rappuoli knew from Gotschlich that capsule polysaccharides were protective against serotype C. That knowledge had been used to produce the meningococcal C vaccine, but the vaccine was not effective in infants. In 1989, encouraged by promising results of the first conjugate of CRM197 with *H. influenzae*, Rappuoli and colleague Paolo Costantino began making conjugate vaccines for subtypes A and C.

Eradicating whooping cough in Italy was "one of the most beautiful things that can happen if you do my job."

Subtype C was mostly prevalent in the United States, the United Kingdom, and Europe, whereas subtype A was prevalent in Africa. Vaccines against both subtypes were effective in Phase I and II studies in the United Kingdom. Between 1994 and 2000, Chiron worked with the government to develop and license the meningococcal C vaccine. In 1999, meningococcal C vaccinations began in the United Kingdom, and within a year every person from 2 months to 18 years of age received a vaccination. By 2001, the disease was essentially eradicated.

H. pylori and Cellular Microbiology

In the 1990s, Rappuoli began working on *H. pylori*, which was discovered in the early 1990s and linked to gastric ulcers and cancers. *H. pylori* produced a toxin, but nobody had isolated it. He cloned the *H. pylori* toxin, identified the mechanism of action, and made a vaccine that went into clinical trials. But by the end of Phase I studies, commercial interest in the vaccine was low.

Although the work did not yield a vaccine, Rappuoli found that *H. pylori* first attached to the eukaryotic cell and then injected toxin directly through a hole in the membrane. "It was a totally new mechanism," he says (6). Chiron, which was acquired by the pharmaceutical company Novartis, has continued to probe the basic biology of the microbe. "We are starting to understand how bacteria can cause cancer at the molecular level, but we don't have the full story yet," says Rappuoli. The *H. pylori* toxin triggers molecular changes in the cell that resemble early stages of cancer. The work has led to many productive partnerships, including one with longtime collaborator and fellow National Academy of Sciences member Stanley Falkow, a microbiologist at Stanford University (Stanford, CA) (7, 8).

As Rappuoli studied bacterial pathogenesis, he became increasingly aware that microbiologists were growing bacteria under artificial conditions that barely mimicked the real situations when these microbes encountered humans. "We realized that some very important virulence factors are not expressed when you study the bacteria under those conditions," says Rappuoli. He and his colleagues coined the term "cellular microbiology" to signal to the scientific community, mainly to cell biologists and microbiologists, that these two disciplines should fuse into one. The idea, which was published in a short essay in *Science*, was immediately popular (9). A new journal called *Cellular Microbiology* was launched, two textbooks adopted the name, and several annual meetings are now devoted to this new fusion field.

Reverse Vaccinology

While Rappuoli was working on meningococcal vaccines, serotype B, which causes approximately 50% of meningitis cases worldwide, posed a greater challenge than the other subtypes did. Subtypes A, C, Y, and W135 all have surface polysaccharides that can be coupled to proteins to yield potent conjugate vaccines, but the only polysaccharide on the capsule of subtype B is polysialic acid, which is identical to the sugar on human cells. "So even by the mid-1990s . . . there was no way to make a vaccine," says Rappuoli of serotype B meningitis.

In 1995, Craig Venter, head of The Institute for Genomic Research (TIGR; Rockville, MD), sequenced the *H. influenzae* genome, and Rappuoli saw a new way to tackle subtype B. He visited

Venter in 1997 and asked whether TIGR would sequence *N. meningitides* subtype B. "When I explained the medical need and the opportunity to use a new emerging technology to solve a very old problem, [Venter] was convinced it would be worthwhile," says Rappuoli.

Within 18 months, the entire genome sequence of serotype B was in hand (10). Fifty years of work on subtype B had yielded only about a dozen surface-exposed proteins as potential vaccine targets; the completed genome sequence yielded more than 90. "It became immediately clear that this was the new way for making vaccines, which I call reverse vaccinology, because we start with genes. This was the first time you didn't need the pathogen and could go backwards from the information in the genome," says Rappuoli.

In his PNAS Inaugural Article (1), Rappuoli reveals the spoils of the genome sequence of subtype B meningococcus. He and his colleagues identified five antigens that, when combined with an aluminum hydroxide adjuvant, confer immunity in 78% of mice when challenged with 85 strains of subtype B from around the world. "I hope I'm right that this vaccine will [eliminate] meningococcus B globally," says Rappuoli. Novartis recently completed Phase I clinical trials for this serotype B vaccine.

Vaccinating New Zealand

One particularly significant achievement for Rappuoli was making a serotype B meningococcal vaccine specific for the people of New Zealand. Beginning in 1990, New Zealand became mired in a decade-long epidemic of serotype B meningitis. The New Zealand epidemic, unlike those in the United Kingdom and the United States, was caused by a single bacterial strain. Even in the 1990s, the technical solution for a single-strain vaccine was well known. Yet after a decade of disease and consultation with the World Health Organization and the U.S. Centers for Disease Control and Prevention (CDC; Atlanta, GA), no vaccine was available.

In 2000, Rappuoli met with representatives from the New Zealand Ministry of Health. "I told them the problem was economic not technical," he says. New Zealand has a relatively small population of 4 million people and was thus not a lucrative market for vaccine companies. "I told them the New Zealand government had to make the project attractive," Rappuoli says. The government subsequently offered $200 million to encourage vaccine development. Chiron took up the challenge, and from 2004 to 2005, the entire population between 2 months and 18 years of age was vaccinated. "Now the disease is almost gone—it's another powerful example of how effective a vaccine can be if you do it right," says Rappuoli.

Avian Influenza and Adjuvants

In the last decade, another more insidious disease and its microbial source has occupied Rappuoli's attention: avian influenza and the H5N1 virus. In 1997, when avian flu arrived in Hong Kong, Rappuoli's team was the first to make a vaccine against H5N1. In 1999, Chiron launched a clinical trial in the United Kingdom to test the vaccine. Rappuoli's team tested the vaccine with and without a new adjuvant called MF59. Rappuoli says that much of what is known about avian flu vaccines was written in their paper published in *The Lancet* in 2001 (11). "But in 2001, nobody could care less about H5N1, and the paper went unnoticed," he says. The paper contained two key messages. The vaccine was ineffective without the adjuvant, but, with the adjuvant, only half the dose of antigen was required, essentially extending the vaccine's manufacturing capacity. This information is consistent with a recent NIH paper, says Rappuoli (12).

The MF59 adjuvant, which has been used since 1997, also has another advantage. Trials carried out by the CDC showed that only people vaccinated with the vaccine and MF59 adjuvant could recognize both the 1997 and 2003/2004 avian flu virus strains (13). "So the adjuvant probably allows a good immune response with a low antigen dose but also helps to provide protection against a virus that we know changes every year. So this shows two reasons why there is no way to develop a vaccine against avian influenza without a good adjuvant," says Rappuoli. "That [finding] provides the opportunity to really change our strategy by which we tackle influenza pandemic."

Other than aluminum hydroxide and aluminum phosphate, MF59 is the only other adjuvant approved for use in humans in the last century. Hundreds of adjuvants have been tested in animals, but virtually all failed mostly because of toxicity. MF59 was originally developed for use with the annual flu vaccine to enhance immune response in elderly people. "When we were immunizing old mice, this adjuvant allowed the old mice to respond to the vaccination like the young mice. In fact, I call this adjuvant the 'Viagra' of the immune system." (14)

Rappuoli also is supervising the development of another adjuvant, called LTK63. Derived from an *Escherichia coli* toxin, LTK63 improves the immunogenicity of mucosal vaccines. He speculates that engineering "immunogenicity molecules," such as this adjuvant, will be a rapidly evolving field.

"Once I complete the development of vaccines for avian influenza and serogroup B meningococcus, I'll feel pretty satisfied," says Rappuoli, "but I also want to see reverse vaccinology applied to parasites and worms, which pose a huge medical burden." He also suggests that using vaccines to treat chronic diseases and cancer will become increasingly feasible as the understanding of innate immunity improves. Says Rappuoli, "We are about to enter a new era in immunotherapy—what I cannot tell is if it is 2 years away or 10 years away, but it is going to be very exciting."

Bijal Trivedi, *Freelance Science Writer*

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