THE VIRUS HUNTER

Ian Lipkin

When Ian Lipkin chose a career in infectious diseases, he envisioned hunting for pathogens in daring treks around the world. Though disappointed to learn that modern-day virus hunters work largely from the lab, he still wound up a pioneer. At the Scripps Research Institute in La Jolla, then at the University of California, Irvine, and since 2001 as director of the Center for Infection and Immunity at Columbia University's Mailman School of Public Health, Lipkin has developed groundbreaking techniques that have helped a new generation of disease detectives sleuth out the infectious roots of mystery ills, chronic disease, and neuropsychiatric disorders like autism and OCD. Lipkin's signature invention is a technology called Mass Tag PCR, which searches through large numbers of known viral and bacterial genomes to identify a culprit in a few hours. He often complements this test with others, including microbial detection microchips (GreeneChips) and gene sequencers that can complete an exhaustive search for known and unknown pathogens within a tissue sample in less than a day.

When DISCOVER features editor Pamela Weintraub interviewed Lipkin last year, he had to cut his workday short because his dog, Koprowski–a gift from Polish virologist Hilary Koprowski–was desperately sick. Lipkin had a treatment plan: not an antiviral drug or chemotherapy, but red meat. "It has antibiotics, it has growth hormone, it has everything. Koprowski's my best friend in the world," he explained before descending into the subway and heading home.

PHOTOGRAPHY BY GRANT DELIN

Q+A

You were in the first class of men at Sarah Lawrence, where you studied anthropology, even shamanism. Yet you are known for hunting pathogens. How did that come about?

I felt that if I went straight into cultural anthropology after college I'd be a parasite. I'd go someplace, take information about myths and ritual, and have nothing to offer. So I decided to become a medical anthropologist and try to bring back traditional medicines. Suddenly I found myself in medical school.

But you didn't become a medical anthropologist. Instead you studied neurological disease and infection. Why?

By 1977 I had gotten a fellowship at the Institute for Neurology in London, where a professor named John Newsom-Davis was working on myasthenia gravis, a neuromuscular disorder characterized by weakness often so profound that people lose their ability to breathe. Back then, nobody really understood what the disorder was. John was trying something new, treating it with plasmapheresis.

What is plasmapheresis, and why did Davis think it would help his patients?

Plasmapheresis is a method where you take the blood, put it into a centrifuge, and as you spin it the components separate out. You have white cells and platelets in one area, red cells in another. And then you have plasma, which contains antibodies. You introduce what amounts to a straw to suck up the plasma. You replace plasma with albumin to maintain the blood volume, but now the antibodies are gone. With the antibodies gone, the symptoms are relieved.

With antibodies at the root of symptoms, you knew that a pathogen could be the precipitating cause. How did you end up linking infection and diseases of the nervous system and brain?

I took a residency in neuroscience at the University of California, San Francisco (UCSF). It was

1981 when I drove into San Francisco on Gay Pride Day in a Ryder truck right up Castro Street. It was the apex of freedom and joy in the gay community, but we soon started seeing HIV, and I was one of the only people willing to see the patients who were both sick and had neurological disease. My clinic took anybody who had gay-related immunodeficiency, or GRID—an early name for AIDS. One thing I found that was interesting and important was that a lot of people with GRID had idiopathic thrombocytopenic purpura, a bleeding disorder. It's idiopathic, meaning nobody knows what causes it. Thrombocytopenia means you don't have platelets, so the blood doesn't clot properly. Purpura refers to the fact that you get these blotches on your skin where blood vessels break. Their platelets were getting chewed up by their antibodies.

HIV was turning the patients' bodies against themselves, the hallmark of an autoimmune disease. But you found that their immune systems were attacking not just their blood but their nervous systems as well. How did that finding come about?

I discovered this because we then had the only MRI scanner in the world at UCSF. As a result, a colleague asked me to see a patient of his, a ski instructor from Vail, Colorado. The man was thought to have MS, multiple sclerosis. So this fellow arrived at my clinic early one morning, and I did a very detailed neurological exam. As I left him and came back to him over the course of an hour, his symptoms changed. He was becoming quite fatigued, because the testing is arduous. So I gave him a break of 15 minutes. And he came back in to see me and now both sides of his face were so weak he couldn't smile. He had numbness and tingling and then, right there in my office, his face became so weak he couldn't close one eye. Then he couldn't close the other eye.

It was clear this wasn't MS, and I thought of a couple of causes for what I saw. One was exposure to some kind of toxin. So I checked the man's spinal fluid for the presence of protein indicating inflammation. His protein was orders of magnitude higher than anything I'd ever seen, and the spinal fluid came out like glue. My colleagues asked what I wanted to do, and I said, I want to do plasmapheresis.

Why did you go back to that technique? Because you wanted to remove the antibodies that you felt were driving his symptoms?

That way I wouldn't be adding a drug that I couldn't remove. I'm just reducing the number of antibodies that might be causing this disease. So I talked with the renal dialysis people who had the machinery required to do this, and they said, this guy's gay, so he has this GRID, right? And he has elevated liver enzymes, so he possibly has non-A, non-B hepatitis. And you want us to contaminate our machines with this guy's blood? I don't think so.

What did you do then? I know you couldn't abandon him.

I'd heard about a Russian guy with a centrifuge at Pacific Medical Center on the other side of town. He was willing to help me, provided two things: Number one, I would take responsibility for inserting the needles in my patient's arm. And two, he would be paid in cash—$600 up front for every treatment. So I put my guy in my Ford Fiesta, and I drove him over to this medical center. He could barely walk, so I walked him in. He lay down on this hospital bed, and I put in two large-bore needles, one in each arm.

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After the first treatment he modestly improved—and he was better yet after the second treatment. I treated him on and off for two years. He started skiing again but eventually progressed to AIDS and died. While he was getting this treatment, though, it helped him function. After that I decided to study infectious disease.

San Francisco in the early 1980s—that would have been a logical time to continue your study of AIDS. But that's not what happened.

I flew down to the Scripps Research Institute in La Jolla to meet with neuroimmunologist Michael Oldstone. I told him I had all these nerves from AIDS patients and all this brain material. But he says to me, AIDS is a flash in the pan. There's a vaccine around the corner. You're going to work on the Rosetta stone of immunobiology: lymphocytic choriomeningitis virus, or LCMV.

So you ended up going to work at Scripps. Why did Oldstone think that virus was so important, more so even than HIV?

Oldstone was interested in infections that can persist and cause damage to the greater organism but not the individual cell. LCMV, a cause of meningitis in humans, shuts down the ability of the pituitary gland to make growth hormone. The virus doesn't kill the cell, but it suppresses transcription and translation of genes, so the organism as a whole suffers but the individual cell looks OK. My contribution was showing that there were specific effects on neurotransmitters linked to the behavioral manifestations of the disease. This became a model for understanding how persistent viral infections affect the central nervous system. Oldstone gave me an opportunity with LCMV, but he was a tough guy.

In your next project you tracked down a virus that caused both physical and behavioral changes in animals. What happened there?

I was at a meeting in 1986 when Kathy Carbone and Bill Narayan, both from the Johns Hopkins School of Medicine, were presenting their work on Borna disease, a neurological syndrome in some mammals and some birds. They'd been working with rats and had two models for what they said was an infectious disease. One model included an adult infected rat and a neonatally infected rat. The adults had profound inflammation in the brain and movement disorders. Some were massively obese, and

Lipkin was a consultant on the 2011 film *Contagion*, which depicts a global pandemic.

many of them died. The neonatally infected rats were smaller than normal rats and more hyperactive, but they didn't die and they didn't have movement disorders. And the researchers were like, really? How could the presentation of the disease be so different in neonates and adults?

Were you able to solve the mystery of Borna disease?

After my work with LCMV, I knew how to measure neurotransmitter levels. So I did my measurements, and I could see what was different in the adult versus the neonatally infected animals. But I became intrigued by the fact that Bill and several very good people were trying to identify this virus responsible for Borna and couldn't find it. Oldstone didn't have the necessary equipment, so he recommended that I talk to other people at Scripps. There was a guy there, Michael Wilson, whose strategy was eliminating as much irrelevant material as possible from a sample until only the virus was left.

So you just kept eliminating until you found your virus?

It took me more than two years to get the Borna system up and going. First I had to get brain material from an infected rat. Then I had to get permission to infect living rats with this bug because it's potentially dangerous, and the USDA was not excited about testing an agent that can kill horses and sheep that's not native to the United States. By now it's 1987. I injected healthy rats with brain material from infected animals, then I waited until the rats developed disease. I removed the brains of the rats, cut them in half and studied one half microscopically. If a rat brain showed inflammation, I assumed the animal had Borna disease. And I took the opposite half of the brain, pulled out the hippocampus, which is the area described by researchers as the target for disease, and ground it up, saving the RNA. Eventually I succeeded in making a library of genetic material—single-stranded RNA—from the hippocampus of an infected animal. I labeled the RNA with radioactive markers so it was "hot." Then I made another library of RNA from the cerebellum of a normal animal, and this wasn't hot. I put the RNA from both samples in a tube and mechanically shook it for three days behind plastic shields made from discarded Jack in the Box restaurant signs, because they were free and excellent at blocking radiation.

In essence, most of the material from the rat brain bound together, separating out and leaving viral molecules behind. Is that right?

I wound up with a mixture of double-stranded material [because some of the single strands of rat RNA bound together to form a double helix]. The double strands represented material from the rat brain: either the Borna-disease brain, the normal brain, or a combination of the two. I also got single-stranded material—hot material from the Borna brain alone and cold material from the normal brain. All the single-stranded material got separated out on top of a filter, but only the hot Borna brain material appears on film. [Radioactivity exposes photographic film.]

So the hot single-stranded material, which did not bind to the brain tissue in your mixture—was that the Borna virus?

Not yet. I had to eliminate more. Now I take the spleen and I take lymph nodes and I take thymus from normal rats and I grind them all up because, theoretically, these organs contain all the transcripts that might represent inflammatory proteins like chemokines and cytokines. Everything hybridized until I was left with just a 38-kilobase protein and a 24-kilobase protein representing viral DNA. I still didn't have the virus, but I had protein associated with the virus.

You needed many more complex steps, far too elaborate to explain here, to isolate the viral genome and then the virus itself. But when you were done, you had reinvented the field of pathogen discovery with your molecular techniques.

Of all the things I've ever done, I am most proud of that. If the Borna virus had been proved to cause a significant human disease, it would have been an even bigger deal.

Why do you say it's not significant? The Borna virus has been implicated in many different diseases.

A group in Berlin reported Borna in AIDS. A Japanese group reported it in chronic fatigue syndrome at very high levels. People were reporting it in brain tumors, in Alzheimer's disease, in multiple sclerosis. But we could never replicate the findings. Our new blinded, case-controlled study, published in *Molecular Psychiatry*, finds no association between Borna disease virus and psychiatric

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disease. People were contaminating tissue cultures. People were contaminating molecular assays.

So after all that, Borna does not cause any human disease at all?

I’ve seen no evidence of it, and we have not been able to demonstrate Borna virus infection in psychiatric patients or chronic fatigue syndrome patients. Having said that, we have recently shown that Borna virus sequences are integrated into the human genome and have been there for hundreds of thousands of years. Which means that at some point people have been infected with Borna virus. But whether they can currently be infected with it and whether it can be linked in a cause-and-effect relationship with disease are completely separate issues.

Your next big conquest involved a much more famous pathogen. You identified the West Nile virus after the first big U.S. outbreak in New York City in 1999. How did you get involved in that effort?

By August of 1999 I’m doing research in emerging diseases at the University of California, Irvine, and I’m invited to a meeting in Albany, New York, where we learned about an outbreak of what the Centers for Disease Control and Prevention [CDC] said was St. Louis encephalitis virus [SLE]. One investigator noted something unusual in people who had encephalitis that summer: Though otherwise healthy, they became especially weak and even required ventilators. Another noticed that crows were dying, along with a lot of animals in the Bronx Zoo. The CDC said, we don’t want to be bothered by these birds, and these other things are not relevant. We’re trying to address this SLE. But no one could isolate the agent. Then the CDC sent us the samples, and in 48 hours we knew that it was not SLE, and it was growing in the brain. We found material from the brain that matched the flavivirus family, to which West Nile belongs. Then we sequenced the genome. We were the first to clone an entire genome out of degraded human material. We reported West Nile virus in human spinal fluid and blood.

You moved your lab from Irvine to Columbia University in New York City around the time of 9/11. It must have been an extraordinary time for a pathogen hunter. There were the anthrax attacks and the global panic over severe acute respiratory syndrome, or SARS.

SARS was the first plague of the 21st century. In the winter of 2002 we began hearing chatter that there was a new respiratory disease in southern China. Most people thought it was probably some variant of flu, but then we found it was a coronavirus, so called because it’s spherical with a little crown [*corona* in Latin] composed of spikes of protein. It was a surprise because coronaviruses aren’t typically associated with human disease. We found we could grow the virus in certain cell types, and I began trying to develop a rapid molecular test, but it turned out not to be very sensitive. So we obtained some information about the virus’s genetic sequence and developed a real-time PCR assay [using a technique that rapidly replicates a stretch of DNA] that was 50 to 100 times more sensitive.

Chinese public health officials must have been interested in that.

The Chinese consulate invited me to a magnificent banquet at an East Side Chinese restaurant in New York. Halfway through the meal they said to me, we need you to go to Beijing tonight. People were dying there. It was the middle of an outbreak. And there was no treatment. I said, well, I can’t go by myself. So I convinced Thomas Briese, my colleague at Columbia, to come along. The morning we flew out, my kid is crying. Thomas’s wife is crying. I’m traveling with a suitcase filled with booties, gloves, masks, and 10,000 test kits for SARS. Nobody was going into Beijing because people were dying there. There were only three people on the final leg of the flight—Thomas, me, and Elisabeth Rosenthal from *The New York Times*. She was picking up her kid.

What happened when you arrived?

Chen Zhu, now China’s minister of health, was waiting at the airport with a red carpet. The streets were deserted. Tiananmen Square was empty. The Forbidden City was empty. The next morning we went to the Great Hall, and I’m told I am there to design their SARS program. There were 250 people waiting to hear what I wanted them to do.

Eventually they did manage to obliterate SARS in China.

SARS was contained not because of a drug or vaccine but because we identified people who were infected or at risk, and we isolated them. When I went back to see Chen Zhu, he was in a hospital with an unexplained liver problem. At the nursing station they didn’t even have soap. The first thing I did was sit down with him, and I said, you must do two things for me. There can be no spitting on the sidewalks because this spreads all these germs. And doctors and nurses coming to see you must wash their hands. By the time I left his room half an hour later, there was a prohibition against spitting on sidewalks and there was soap and water and paper towels in hospitals.

Your newest research looks at a particularly insidious set of chronic diseases that can result from infection in the womb. These diseases can produce lifelong psychiatric effects. How does that work?

The connection between prenatal infection and damage to the fetus has long been known. Exposure to syphilis, at its most extreme, results in stillbirth. Prenatal exposure to infections could result in microcephaly [a neurodevelopmental disorder in which the circumference of the head is smaller than normal]. But if the damage is more subtle, subtle changes in behavior can result. The child is still breathing, the child is walking, the locomotor function is not so abnormal that it’s incompatible with life in our culture. There was a

time when these children would have died in utero, but now they survive, and you see some of these abnormalities come to the fore.

Have we reached the point where we can link specific infections to specific psychiatric disorders?

No, the connection is much more complex. When I worked with LCMV, it became clear that any sort of perturbation could damage the nervous system. Nerves find their way to specific locations through signposts that are part of the immune system. And if you increase immunological molecules of certain types, a nerve may jog this way as opposed to the way it's supposed to go. It may not make a difference what the infectious agent is—bacterial, viral, or parasitic.

If the identity of the infection isn't critical, what is?

The important things are the genetic background of the individual and the timing of the insult. If you look at the original work on the epidemiology of thalidomide [a morning-sickness drug that turned out to cause birth defects], there were specific time points where, if the woman was exposed, the baby had a high probability of having bona fide autism.

One of the most fascinating links between infection and mental disease concerns PANDAS, pediatric autoimmune neuropsychiatric disorders. The bottom line is that strep might cause obsessive-compulsive disorder. How could that happen?

An infection like strep throat provokes an antibody response, but the antibody created to fight the strep also recognizes proteins that are part of your body. Antibodies don't typically traffic much in the central nervous system. But if you have any one of a number of other infections or an insult like head trauma, the blood-brain barrier [which normally protects the brain from pathogens] opens transiently. Depending on how long and where the opening is, the antibodies get access to part of the central nervous system or brain. We are studying this process now in mice, using drugs to open up a portion of the hindbrain or the forebrain or the hippocampus and tracking the effect.

Could autism be another version of a PANDAS-like disease?

It's possible, in some people. There is probably a group of people who have a genetic component to autism, and for them, there may not be much of a trigger or any trigger at all required. Another group is genetically predisposed, and if they encounter some factor or factors, individually or in combination, it could result in either the onset or the aggravation of the neurodevelopmental disorder; by factors, I include everything from heavy metals to infection. And lastly, there is a group that may be relatively or entirely normal but is exposed prenatally to some factor or factors that have an effect on their nervous system and that manifests as autism. This is the hypothesis, at least.

You are trying to put all this research together through a prospective study called the Norwegian Autism Birth Cohort. What's that about?

The idea is that you can get only so far by examining people when they're sick, because the seeds of illness may be laid many years before. In a prospective cohort, you can follow children from before birth in an unbiased way, collecting information and samples and maybe making associations between factors and outcomes after the fact. We're going back as far as we can, which is the first prenatal visit at roughly 17 weeks' gestation. And the study is being done in Norway because there's universal health care; you don't have to be concerned about discrimination for insurance purposes based on disease, and as people get ill, you have long-term follow-up.

"You can get only so far by examining the sick, because the seeds of illness may be laid years before."

What could that study tell you about the nature of autism?

We have thousands of biological samples that will be transferred back here to New York, and we're going to analyze them using all the tools of microbiological analysis we developed to look at acute diseases in the past. Because we have blood samples that are obtained from the mother during the course of pregnancy and at the time of birth, we can examine a whole range of proteins and messenger RNAs that may be reflective of genetic defects or exposures. We may not detect an autism-causing agent specifically, but we will see that there is a marked increase in specific biomarkers. This allows us to define in a very broad sense what proteins, nucleic acids, pathogens, and toxins might be part of the milieu of the fetus.

How would you describe your overall approach to pathogen discovery?

In microbe hunting we start with the result, the disease, and work backward, examining the paths and pathogens that might have led there. Finding footprints of a microbe—whether you're seeing antibodies or the virus itself—is just the beginning of solving the crime. Initially the evidence is circumstantial. Like in criminology: Seeing a suspect on the street corner where somebody was killed is not enough. We still need motive and opportunity. Motive is tropism [seeking nutrients or energy] and virulence [ability to multiply and cause disease]. Some microbes are known to infect the lungs or the intestines or the brain. Finding them in a typical location gives us confidence we are on the right track. Opportunity—could it have done the damage? Some infectious diseases are seasonal. Mosquito-borne diseases, for example, are common in the summer but rare in the winter. Noroviruses are common on cruises. That's where biological plausibility comes in.

You sound a lot like an old Hollywood detective.

If you can find a smoking gun, if you can show that this preceded that and somebody's been shot in the past, then you can get a conviction. That's exactly what it's like.