

A PERSONAL ODYSSEY

Fred Kantor

Thank you all for coming. The opportunity to go back over one's life and share it with a crowd is unique to this group. When Tom Duffy asked me, I was a little reluctant, but then I warmed to the idea, and I hope that you'll have fun with me as we go through this. I was a young kid of ten or eleven when I got interested in electricity and radio. I built a crystal set. You all are of an age where you may remember a crystal set. I think many of the millennials would not know what I was talking about. Shortly thereafter I got into it seriously and became a radio amateur. I think I was the youngest radio amateur in New York State at the time, and I accompanied air raid wardens during those scheduled blackouts where they practiced how we would behave during air raids. I had a two-meter rig, a little radio that they could communicate with which was very useful. In any event, because of that interest I applied to and went to Brooklyn Technical High School, which I have to say was a wonderful education. First of all, it's four years of shop, which I think was terrific, as well as all the academic subjects. I must say that comparing education at the high school, college, medical school, and graduate school levels and so on, Brooklyn Tech was a highlight, it really was. So I'm a big fan. But I took a biology course and I said, Whoa!! this is a better machine, this is a lot more interesting, so I switched my interest from engineering to medicine. Now my mother's friends all clucked their approval, and I demurred and angrily told them that I wasn't interested in saving lives, helping people. I really wanted to explore the machine. And that's of course what we tell medical students *not* to do, that patients aren't vessels for disease, they're people. It took me a while to learn that, but I finally did and I got interested in biology.

In my second year at Union College, I took a course in comparative anatomy. The subject, or at least the lab, was dissecting the dogfish shark. The book was so bad. It was really awful and I liked to dissect. During the summer I worked at a children's

Fred Kantor, Paul B. Beeson Professor Emeritus of Medicine (Immunology), was born in Brooklyn and educated in the New York public school system. Interested in physics and electronics, he entered Brooklyn Technical High School with a career in electrical engineering in mind, but a biology course introduced him to the wonders of the human body. He graduated from Union College and NYU medical school, finding excitement in both clinical and laboratory work. Upon graduation he interned on the ward medical service of Barnes Hospital, followed by two years as a research associate at the National Institutes of Health. He finished his residency in internal medicine at Yale and became a fellow of Dr. Paul Beeson, who suggested that he start a new section at Yale in allergy and clinical immunology. After additional training with Dr. Baruj Benacerraf, he returned to Yale in 1962 and is convinced there is no better place for a career in academic medicine.

camp not far from where my folks had a summer place. I bought some preserved dogfish sharks, and I would dissect during the week after my waiter activities ended. When my dad came up on the weekend we'd photograph the dissection. I gave the finished film to the college, which used it until a very short time ago. When I applied to medical school I still remember the interviewer looked at that and said, "Are you interested in academic medicine." I said, "What's that?" I had no idea what academic medicine was and I guess he could see more ahead than I.

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I went to NYU College of Medicine, and the chair of microbiology was Colin MacCleod, who with Avery and McCarty discovered that DNA is the genetic material of life. I mean that was not that long ago. It was during our lifetime, 1943, and they had a tough time convincing the scientific world that it wasn't protein, it was DNA. I entered medical school in 1952. When I learned that there was a connection between streptococci and rheumatic fever and asked what the connection was and nobody knew, I decided instead of being a waiter in the summertime, maybe I should start looking into things medical and I thought I'd find the answer in one summer. Well, it's how many years later I'm still working on it? I asked the teachers in microbiology with whom I should work, and it ended up Dr. Gene Stollerman. He was a young director of Irvington House, which was a hospital for children with rheumatic fever. If we had a patient with active rheumatic fever we'd pull in all the students, all the residents. Rheumatic fever still occurs in the world, but in the United States it's just vanishingly absent, and Irvington House doesn't exist anymore. Dr. Stollerman ran this place, and there were two fellows. I was the only student, and the three of them just poured their teaching right at me. It was a wonderful experience and it was a friendship that lasted throughout his life. He advised: "You should go to the NIH, there's a new research associate program designed by Dr. Robert Berliner." Well, you know, he was our dean not long ago and I was so ignorant I said, "Yeah, sure, okay, I'll go to the NIH. It sounds like a good idea." Well, I didn't have any idea what a wonderful opportunity this was. When I was at the NIH, I was still studying the relationship of the streptococcus and its immune reactions. If you immunize an animal or a person with a protein, they develop an antibody, which is a gamma globulin in their serum that reacts with the substance that you immunized them to. I was working with streptococcal M protein, and I had rabbits that I had immunized, and I was a little lazy one day and decided to test the plasma instead of the serum. Usually you look for antibodies in serum. Serum is really the liquid part of blood minus the clotting factors, fibrinogen or fibrin. You let the blood clot and then you take the serum off. Well, that required time and effort and I thought, well if the antibodies are in the serum, it's got to be in the plasma. So I set up the test of streptococcal M protein with plasma and what do you know, it precipitated and I found out later that the M protein was precipitating fibrinogen. When you inject M protein into an animal, fibrinogen goes to the glomerulus. Subsequently, it became clear that M protein and fibrinogen co-localized at the site of streptococcus infection; and biopsies of a patient with necrotizing fasciitis, a very serious and unpleasant illness,

and toxic shock were shown to contain fibrinogen. So the interaction of this bacterial protein with fibrinogen was an important finding.

After the NIH, I wanted to train in infectious disease, and there were two places of interest: one was Yale, and that's of course when Paul Beeson was here; and the other was Johns Hopkins. I applied to both and was lucky enough to be accepted here. After my senior residency Dr. Beeson said, "You know, we have a doctor who is retiring and who runs the allergy clinic. He has never been very much a part of this department anyway, and I think that allergy and clinical immunology is going to burgeon. It's a huge field that's just opening up and if you want to go away and get some extra training, whatever you think you need, and come back and start a one-man section, I think it would be a good idea." My response, "Me! Push pollen? Am I a quack?" I mean, I regarded myself as a scientist and here he was telling me to be an allergist, the worst kind of quack. Anyway, it was a great opportunity, so I went home and talked it over with Linda and we both agreed that it was a good opportunity, and then I had the opportunity to get extra training. Mind you, this was in 1961 or '62. Look at what's happened in the interval. I ended up with Dr. Baruj Benacerraf. He grew up the son of a North African French woman and a South American father. He was educated in France, and he is probably one of the brightest people I ever met. He'd come in in the morning, take his jacket off, put his lab coat on, and stay in the lab all day. The first few weeks he gave me unknowns to weigh and to measure the protein nitrogen. I mean stuff you give to a freshman in college, but he was there at every experiment. He saw every animal. His wife would bring in the mail at about 10:30 or 11 and he'd go through the mail. He was independently wealthy and on the board of a couple of banks, but the laboratory was his main interest. We were immunizing guinea pigs to hapten protein conjugates. A hapten is a small chemical. We did that because we wanted to find out how important the bridge was between the small chemical, hapten, and the protein to which it was conjugated. I immunized these guinea pigs, and not every guinea pig developed reactions. They were tested to this conjugate, which is called DNP-polylysine, DNP being the hapten. For the chemists among you it stands for dinitrophenyl. Okay, so not every guinea pig reacted to these. And I thought I was doing something wrong. There are one, two, three, four, five experiments and every time the same thing would happen. These were outbred guinea pigs, and it was very clear that there were responders and nonresponders. I thought we have an opportunity here to look for immune response genes. Dr. Benacerraf reasoned that there are several steps in immune reaction. The first is when you vaccinate somebody or present an antigen to an animal, it's taken up by antigen-presenting cells and they presented to lymphocytes called T lymphocytes. In subsequent studies, he showed that the responding and nonresponding reactions were dependent not on the T lymphocytes but on the presenting cells and on the histocompatibility antigens on the surface of those cells. You know, histocompatibility antigens are how we recognize ourselves and other peoples, and that's why we don't accept another's transplant without

immunosuppressant drugs because our histocompatibility antigens are incompatible. Well, Benacerraf showed that the response in the guinea pig was dependent upon the histocompatibility antigen, and for that the Nobel assembly gave Benacerraf, Jean Dausset, and George Snell the Nobel Prize in 1980. So, it was thrilling to be in that lab. It was all Benacerraf; he was an incredibly bright guy. In any event, that made a major impression on me, and I thought somewhat later that there must be a genetic basis of the immune response in people. We're all outbred, and so if we took some of these defined antigen chemicals and immunized inbred people with them, maybe we'd find responders and nonresponders.

Back at Yale I heard from one of my colleagues in public health who had spent time in Brazil with the Amazonian Indians, that they showed no signs of allergy, no hay fever. They were very inbred, and that's necessary if you're looking for an immune response gene. We decided to study human response genes in Amazonian Indians using defined antigen. At the mouth of the Amazon in Brazil is Belém, and that's where our base was. We selected three Indian tribes who were pacified, which is to say that the Brazilian Indian Service, the FUNAI, had made contact with them through gifts and the like, so we weren't in any danger – I'm sure you all read about this poor young man who got killed by Indians on this isolated island – but they were quite primitive and they were highly inbred. So, the question was, Why should we go? Well, there are many highly inbred groups. We wanted to study human immune response genes, and these tribes were thought to be more inbred than the Amish and other inbred human groups that had been studied before. We were told there was a paucity of allergic disease, which peaked my interest; also that they might have unusual diseases in a Brazilian jungle, which interested me as a physician; and lastly, that we might help some of them. That's a dangerous attitude, because aboriginal peoples in the name of being helped have been just terribly hurt.

Dick and I were both pilots, and we flew to Belém, which looked like a modern city with skyscrapers and the like. Unfortunately, the power would go off every afternoon for several hours, and that created a problem for us because we collaborated with a Brazilian investigator named Jose Piniero who was a biologist. He let us use his lab because we wanted to tissue type the Indians that we were studying. It is a rather long process and when the power goes off, the centrifuge doesn't work, so it creates a problem. The three tribes – Parakanã, Shagrin, and Mecranote – were quite separate in three villages with thatched cottages; there were two or three hundred inhabitants. They were quite peaceful, as I said. Occasionally they'd go on a bit of a war party, and they'd raid another village and take some women. I think somewhere they had the notion, correctly, that they needed some hybrid invigoration and that's how they got it. The FUNAI had them clear a runway at each of the three villages, which is how we got in and out. It is a very interesting matriarchal society about which I'll tell you a little more. A typical member of one of the villages is practically naked. He wears thongs around his waist and around his shoulders to carry things such as a little tobacco; and

his penis is covered with a sheath that's made of a leaf. When that leaf is in place he's totally modest and when it's not, he's very embarrassed and naked. So this is a very primitive tribe. Their skin was darkened with the juice of the *Genipa americana* fruit and they had red feet; these are all decorations passed down from generation to generation, but they have a purpose. They actually are insect repellants; they knew about that and they just passed it off as cosmetic, but it's more than cosmetic. They used to shave their eyebrows and the forehead. They were just warm and healthy people. You know, I mentioned these tribes are all inbred and we're taught that inbreeding produces idiots. Well, in an environment that's threatening it's only the strongest that survive and with this inbreeding, they were healthy people. One of the FUNAI taught one of the tribes to play soccer, which is the Brazilian object of fun, and he took the Indian tribe to the city. They were absolutely not allowed to come back because the Indians could run up and down all day because that's how they used to hunt. They would chase a jungle cat by running it down until they got it in a tree and then they would taunt it with a spear until the cat attacked. They would spear the animal, but meanwhile they got a scar or two, which was their badge of adult manhood.

These aboriginal peoples – we called them Indians then – migrated from across the Bering Strait down through Canada and through the United States down through Middle America to South America. The boys followed us everywhere because they had to be sure we had the same equipment that they did, meaning human equipment. There was nothing private or sacred. We slept in hammocks under a thatched roof occasionally. We made three visits to each of the three tribes and we used protocols that had been approved by the Human Investigation Committee at Yale because we felt it was immoral to ask permission because there's no way you could get a reasonable acceptance; they would do what you asked them to do. Therefore, the responsibility was on us, and we put the protocols through the Human Investigation Committee, who restricted us to very small dosages of these antigens. I think that was the right thing to do. It would have been immoral to use them in that way as they had been in other ways. The other thing was, they had a variety of diseases that were interesting to us because we don't see them in New Haven: ascariasis, a roundworm parasite; malaria; leishmaniasis; TB; polio; impetigo. We took blood and collaborated with a histopathologist in Venezuela. We used to fly the blood back to her after we had tissue typed it. It turned out that these were indeed the most inbred people. Twenty-one percent, twenty-three percent were homozygotes, which means they were really very inbred, more so than any other tribes. I have to tell you that studies on the immune response genes totally failed. I believe because the Human Investigation Committee reduced the amount of antigen that we were giving. We made three visits to each tribe. The first one was to immunize them, then to boost them, and lastly to take blood and study their cells.

We brought down a whole bunch of vaccines: measles, all of the childhood vaccines, because the history is that when aboriginal peoples are exposed to what really

are childhood annoyance diseases, they're killed. We vaccinated all these Indians and we held sick call every day. The mothers would bring their sick kids. So after a while they trusted us, and if we asked them to take their blood, stick out their arm, they did it. It was really just trust. I'm sure they had no understanding of what was going on.

We were told that there were no allergies in this group of people, none whatever. So we brought with us some ragweed IgE. IgE is the antibody gamma globulin that forms in your blood when you're exposed to an antigen. If you're allergic to ragweed, you have ragweed IgE immunoglobulin antibody. Well, there's no ragweed in the jungle. We wondered whether the Indians would respond, so we brought ragweed extract and we used the four investigators as the controls. I was one, Dick Lee was another, Jose Piniero was a third, and there was a fourth. The response to the extract was negative in the non-allergic controls; one of the controls had a 4+ reaction. He was allergic. Most of us didn't react at all, and the Indians didn't react at all. So, then we injected IgE that we had brought with us. Actually it was IgE ragweed specific; we challenged them again and while some of the Indians reacted with 1+ or 2+, we all, the four controls, reacted at 4+. This was the way it should be. We put known ragweed serum in our skin and then tested it. What was interesting to us was that of the twenty-three Indians tested, some didn't respond at all. That was of major interest. Here we had sensitized them passively and yet they didn't react. Well, did they not make histamine or react to histamine? So we injected histamine in the skin, and they reacted fine. Did they not release histamine when the cells in their skin were stimulated by a chemical releaser called 48/80? Again, they had a perfectly normal response. So, they had cells that released histamine, but when we put the positive serum in they did not react as did the controls. We also tested them to an *Ascaris* extract, and the Indians reacted very strongly to it. We, the controls, didn't have *Ascaris* in our gut as did the Indians. We learned that the Indians could react to a worm, in this case the *Ascaris*, but they couldn't passively react to the ragweed that we gave them. I think we found that out when we got back and measured their IgE units.

The Indians who were unable to accept the ragweed's antigen E had the highest level of total IgE. It was in the thousands, 50,000, some of them 35,000. The usual levels of IgE are in the hundreds or tens. So thousands is very big. We formed a theory that they had so much IgE that it was covering all the cell receptors and when we put on the ragweed IgE there was no place for it to bind to. I think that was one of the early expressions of the hygiene hypothesis of immunization. I want to remind you that Charles Dickens, who was a very keen observer and wrote remarkably about his observations, never described asthma, never described wheal and flare or hives. Neither did any other contemporary authors. In fact, the first paper called "Catarrh estevis," which is hay fever in the spring, was published in the early 1800s. The Broad Street Pump cholera epidemic occurred in the middle of the nineteenth century, at which point the Brits had the housing act and built the Great Northern Sewer; and by 1900 Leonard Noon reported that 10 percent of the people in Britain had allergies. So they went from

none – at least I’m positing none in Dickens’s time. During a period of great advances in hygiene in London, allergies developed and were reported. Hogarth’s pictures of people throwing slop out of the second floor onto the street in the open sewers: that’s the way London was in 1800. In 1900 it had a sewer and it had many, many hygiene improvements, and Noon published the first paper noting that 10 percent of the population were allergic in London. So there was this great change in the population and it made me think that these Amazonian Indians were protected, of course, by their *Ascaris* infection.

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Dr. Beeson was entirely right, of course, about the burgeoning of allergen immunology, and it was not long after coming back from South America that Steve Malawista, a colleague and good friend of mine, and Allen Steere described Lyme disease. A mother brought her child to Yale from Lyme, Connecticut, because the child’s doctor diagnosed rheumatoid arthritis and the mother knew two friends in the neighborhood with the same diagnosis. She looked it up and realized that rheumatoid arthritis does not cluster, it’s an individual thing, so she thought those diagnoses were wrong. She brought her child to Yale and saw Drs. Steere and Malawista. They went up to Lyme and collected serum and studied the cases and really put Lyme disease on the map. For many reasons we know that it has existed for a long time – it exists in Europe in a very different form – but they really put it on the map. It’s caused by a spirochete, and that was discovered by another investigator, and we learned to treat Lyme disease. But the question was, could we prevent it? Steve Barthold was a veterinary pathologist, Richard Favell had just come as head of immunobiology, and Erol Fikrig was a fellow who is now head of infectious diseases at Yale. The four of us got together and decided we could make a Lyme vaccine, because people who got Lyme disease would get a swollen knee or some other manifestation and if they weren’t treated, they got better and usually didn’t get it again. Now, you may know people who have had it several times. Usually they are treated early, so they don’t have a chance for the immune response to develop an immune reaction. But people who are untreated got Lyme disease once. We thought, well, if the bug can make immunity, maybe we can. We decided to immunize rabbits with the organism that caused the disease and then transfer that serum to mice and challenge the mice with cultured spirochetes. We did that experiment. Some of you may know that one of the manifestations of Lyme disease in humans is a heart block because the conduction system runs along here. Happily it’s a transient event; usually they don’t need a pacemaker and they live through it for a week or ten days and it gets better. But the mice had the same exact problem. I’ll go through this with you, it’s probably the only time in my professional life I’ve had results like this. Mice that got anti-*Borrelia burgdorferi* – that’s the Lyme organism serum – when challenged with this organism N40, none of them developed the disease; the controls that got normal mouse serum, eight out of eight developed it. Now, none out of eight and eight out of eight, that’s a result I never had before or since. We really didn’t believe it. We kept doing it, and we showed that normal

rabbit serum did not protect, but rabbit anti-*B. burgdorferi* totally protected. One of the outer surface proteins of the organism which accounts for a large part of its dry weight is called OSP A, which stands for outer surface protein A. We thought, well, it's abundant, we're going to try it. Lucky Larry, we tried it and it worked. SmithKline made a vaccine in the 1990s. Some of you may wonder why this vaccine is not still available. Well, after 800,000 doses were given, there was a paper suggesting that in a few people, it caused recrudescence of the arthritis that they had when they first had Lyme disease. The CDC, the Centers for Disease Control and Prevention, looked into this and found absolutely no evidence that the vaccine caused arthritis to flare, but that rumor caused sales to drop off. The company decided it was going to take it off the market and now there is no human vaccine. There's a dog vaccine, which is OSP A, but there's no human vaccine, and I doubt that there will be in the near future.

In any event, we then thought Lyme disease is transmitted to humans by ticks and maybe there's such a thing as tick immunity. We looked at the literature and sure enough, way back as far as 1939, tick immunity was described in cattle, sheep, horses, rabbits, and other large mammals induced by repeated tick exposure; the immunity that's produced by this interferes with tick feeding, molting, survival, and egg production. We thought, well, if we could make anti-tick immunity, we could protect people against disease: there are four or five diseases that are transmitted by this tick itself. We made guinea pigs tick-immune by exposing them to three infestations of ticks. We then fed *Borrelia*-infected ticks upon naive or tick-immune animals to see whether or not tick immunity would prevent the transmission of Lyme disease to these animals, and it did. Here's the data in four experiments. In naive guinea pigs, ten out of eighteen developed *Borrelia burgdorferi*; of the immune guinea pigs, only one of eighteen. They were not immune to *Borrelia*, they were immune to ticks. But because the ticks soon found an inimical environment when they sought to attach to these animals, they quickly backed out. They didn't take a big blood meal, they didn't stay long enough to transmit the infection. We thought, well, the notion of anti-tick immunity is a really good idea. So, with collaborators we decided to study the protective role of other tick-borne antigens; and to approach this problem, we produced expression libraries from salivary glands and whole ticks. We probed them with serum from animals made tick-immune. We cloned about forty of the salivary antigens of the tick. When people ask me, "What do you study?" I say, "tick spit." We were able to clone all these genes. Were we able to find one that would produce anti-tick immunity? No! As of now there are twenty or more salivary genes that have been cloned from these libraries. Many have fascinating properties that are useful in other areas. For example, there are anti-coagulants. If ticks didn't have an anti-coagulant in saliva, they would soon have no food because the blood would clot and the tick wouldn't be able to get a blood meal. Since Pasteur, a lot of people have studied the pathogen, but only now it's time to study the vector. A vector is a transporter of something — mosquitos, ticks, a whole variety of other fauna, who carry pathogens and, in their interactions with humans,

transmit them. Vector-borne diseases are very important. I mean if you think about all of the diseases, from malaria on down to Lyme disease, maybe there's a soft spot that we could attack in the vector. In any event, I want to finish by pointing out all of the people who were involved in this study. I think that we're still on a quest for anti-tick immunity; and Erol Fikrig, who, as I mentioned, is head of infectious disease, is studying this very actively. In fact, he brought a fellow to my office just yesterday to talk about it. We went over the negative data that we had so that he wouldn't have to repeat that, and he's going at it tooth and nail, and we're going to help him if we can.

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I invited my wife, Linda, to this talk, but I think not enthusiastically enough, and she decided not to come. We have three children and eight grandchildren and there are some significant others. We're very rich indeed, and that's my trajectory.