This is an oral history with Dr. Warren Strober on August 13 and 14, 2019 at the National Institutes of Health about his career in clinical research. The interviewer is Dr. Victoria A. Harden, Founding Director, Emerita, of the Office of NIH History and Stetten Museum.

Harden: Dr. Strober, would you please state your full name, that you know that this interview is being recorded, and that you give permission for it to be recorded?

Strober: Yes, I'm Warren Strober, and I give permission for this recording.

Harden: You were born on October 17, 1937, in Brooklyn, New York, the middle child of Samuel and Betty Strober. Would you tell me about your home life, your childhood, and your education through high school, especially about anyone who encouraged you towards a career in medicine and science?

Strober: I was born just prior to World War II, and my father was a roofing contractor in Brooklyn. He worked in the Brooklyn Navy Yard during the war years and then started his own roofing contracting business in the post-war period. I lived in various parts of Brooklyn during my childhood and ultimately went to Brooklyn College in Brooklyn.

I had a very good time, very stimulating time in high school. It was called Tilden High School, which was in the middle of Brooklyn, the

Flatbush section of Brooklyn. We had a rigorous science program at that high school. There I met a man named William Berman. He ran an experimental biology program, and he oriented me toward science. At that time, my junior year in high school, I did a project on the red mold *Neurospora*, and I won honorable mention in the Westinghouse Science Talent Search as a result of that. Mr. Berman gave very liberally of his time to a group of students who were interested in science and inspired me to go into science.

Then at Brooklyn College, I became a chemistry major. Obviously I excelled at Brooklyn College.

Harden: I was going to say that you must have raced through because you graduated at age 20.

Strober: I skipped a year in Junior High School, so I was ahead in college. Ordinarily I would have graduated at 21.

Harden: You were elected to Phi Beta Kappa and Sigma Xi in college. Tell me about your college experience. Were there particular professors who influenced you?

Strober: Brooklyn College also provided a rigorous experience in chemistry and biology. It had a very good faculty during that period. No one person

stood out, but the standards were very high. I was the president of the Chemistry Society in college. I had to decide whether I would become a PhD in chemistry or go into medical science.

Harden: So why did you decide to go to medical school?

Strober: I think the feeling in the family and the culture was that it would be more prestigious to be in medicine than to be a PhD in science, though I had in my mind that I would pursue a research career.

Harden: That early you did? Why is that?

Strober: Because of the earlier experience in high school, competing in the Westinghouse Science Talent Search.

Harden: Did you do any particular projects in college that... ?

Strober: No, no particular project in college. I just went through the four years with a straight A average, and there was enough work just to get through it.

At the end of my college years, as a matter of fact, some of the chemistry teachers said, “If you want to get a PhD in chemistry, we'll get you into a graduate program at Harvard or some other prestigious school.”

That offer was based on my decision not to go into medicine. But I refused that offer and chose medical school.

Harden: You were admitted to the University of Rochester School of Medicine in 1958. Tell me more about your medical school experience.

Strober: The story there is that one of my advisors had a special connection with the University of Rochester. He thought very highly of Rochester. I was the top student at Brooklyn College as far as pre-med was concerned. I won the Salk Scholarship [Jonas E. Salk Award]. I don't know if you've ever heard of that, but that was a four year scholarship for medical school tuition.

Harden: Right and it was only given to one graduate from each New York City college, right?

Strober: That's right. But I never received the Salk Scholarship because I got another scholarship from the University of Rochester.

Harden: That was the Whipple?

Strober: The Whipple, the Katherine Hoyt Whipple Scholarship. Whipple was the founder of the school. He was still there during my period at Rochester

and continued to teach pathology. Unfortunately, Rochester decided that after one year, despite the fact that I was doing quite well, that they would discontinue the scholarship. So I had give up a four year scholarship to get a one year scholarship, but it was okay because at the end of one year I was married, and we were able to handle the tuition. At that time medical school cost $900 a year tuition, $900. The scholarship was for $900.

Harden: I see.

Strober: Now it's closer to $50,000.

Harden: $50,000 a year, right. Tell me about your training in medical school.

Strober: Again it was quite rigorous so I had to work very, very hard. I did very well. I eventually graduated Alpha Omega Alpha. I was one of the top students there. I wanted to go into internal medicine, meaning general medicine. At that time, internal medicine was considered a specialty. Now it's considered general medicine.

Harden: But this is a long way from the chemistry lab in which you liked doing research. Did you get to do any clinical research in medical school?

Strober: No, I did not. At the end of my time there as a medical student, I became an intern and a resident. That was a two-year period of post-graduate education where you actually became a full-fledged doctor. During that period I applied for a program here at the National Institutes of Health.

Harden: Let's drop back a bit. In 1958 you got married and two of your sons [Mark D. Strober and Alan D. Strober] were born while you did your internship and residency.

Strober: Actually, my second son was born on July 4th, three days after I arrived at NIH. When he was born I looked across the street, Wisconsin Avenue, because he was born at what then was called the Naval Medical Center [now known as the Walter Reed National Military Medical Center]. He was born in the tower building. Walter Reed hadn't been reconstructed at that time. I looked across with hope and with anxiety I might say, at the initiation of my research career. I didn't know whether I could be a success in such a career.

That's a difficult transition because in medicine, you're given tasks throughout the day taking care of patients and all that that entails. In research you create conditions for yourself. You create work. That's the main transition. You're much more on your own as a researcher and that's a difficult transition to make.

Harden: I ask all physicians this question. What attracted you to research as a career, as opposed to private practice or public health?

Strober: I thought that research was more creative, basically. It was more challenging. It would stretch my abilities more. I felt that basically that I would become bored with the practice of medicine, that eventually it would be repetitious. And that's what it essentially is for most practicing physicians. Most of the time you're seeing the same kind of problems again and again. You're very rarely seeing new, unusual problems. It is very different in a research career.

Harden: And you have different frustrations in research, I suppose.

Strober: You do because you're always wondering, at least in the early years, whether you can really make it, whether you could be successful. I would say that it took me about 10 years, 10 years from 1964 to '74, to become sufficiently confident in myself, that I felt comfortable being in research, and that I could do it. It was never going to be a problem actually doing it, but it took a decade, I would say, for the confidence to build.

Harden: Why did you join the Public Health Service and come to NIH instead of, let’s say, academia? The Vietnam War hadn't ramped up yet, but the doctor draft was still enforced from the Korean War. Was that an issue?

Strober: No, that was not an issue. Yes, I would have to had some service in the armed services, but I wasn't thinking of that.

Harden: What were you thinking?

Strober: I was thinking of my career as a researcher. I didn't realize that this would be in lieu of a military career, that this would take the place of that.

Harden: And there was nowhere else that you might go to get the same kind of training as you would here?

Strober: In the early years I received a note from somebody at Rochester. This was when I had been two years at Rochester as an intern and resident. They wanted to know whether I would come back after I completed my residency. They asked me if I was coming back, because they had to put me into rotation and tell the people with whom I would be working. They didn't promise me that I would have a certain kind of position there, and I wasn't attracted to go back to Rochester.

Later on, I was also asked whether I wanted to take a position in any university. That was after I had a certain amount of success in research. But, again, nothing appealed to me because the NIH experience is that you don't have to worry about funding. You just have to worry

about doing well and being capable of gaining continued support under the NIH system.

Harden: You don't have to write grant proposals! I have heard this consistently from long-term intramural investigators.

You came into Tom Waldmann's [Dr. Thomas A. Waldmann] lab, and I think you were the only new Clinical Associate in his program to arrive in 1964, at least as far as I can tell. Would you give me a picture of his lab when you arrived? Who was in it? How did people interact? What sort of mentor was he?

Strober: He had come to NIH approximately seven years before I did. By the time I arrived, he was well-established. He had, for instance, described a new disease called intestinal lymphangiectasia. He had done a number of other things. He was interested in erythropoietin, but by the time I came he had dropped that interest.

He sent me a letter before I came. He said that he was interested in immunoglobulin metabolism. That was his main interest at that point and he wanted to study immunoglobulin metabolism in relation to certain immunodeficiency diseases because they had abnormalities of immunoglobulin levels. One of those diseases was called Wiskott-Aldrich syndrome. Another one was called ataxia telangiectasia. This was

described in the letter saying that he wanted to study these from a point of view of metabolism.

He had already done work in metabolism here of several different immunoglobulins [abbreviated IgA, IgE, IgG, IgM]. He had studied the metabolism of IgG and IgM. But he wanted to study IgA and IgE.

Ataxia telangiectasia was a disease where patients had low IgA. So it was interesting to study the metabolism of IgA in ataxia telangiectasia. And we did. When I came, we studied IgA metabolism in normal individuals and the four or five patients with ataxia telangiectasia we were following at that time. It is a very difficult and severe disease affecting the nervous system.

The interesting thing to come out of that was that one of the patients had very rapid metabolism of IgA: the so-called fractional catabolic rate was much faster in this patient without IgA. The reason for that, we discovered, was that the patient had antibodies to IgA which were rapidly eliminating the radiolabeled IgA that we were injecting to study IgA metabolism. That was the first case, the first instance where it was realized that you can get antibodies to IgA and transfusion reactions to IgA if you lacked IgA. It was a very important initial clinical finding.

The other disease was Wiskott-Aldrich syndrome. We admitted patients with Wiskott-Aldrich and studied the metabolism of immunoglobulins in that disease. Another Clinical Associate who came to NIH a year or two after me, Michael Blaese [Dr. R. Michael Blaese]

ultimately took over the care of these patients but I saw the first few patients. Blaese, and I, together with Tom Waldmann, conducted a study of the immunology of Wiskott-Aldrich syndrome, quite aside from the study of IgE metabolism in this disease. Together with studies of the immunology of ataxia telangiectasia, this marked a big change in all of our careers because we now wanted to study immunodeficiency diseases as immune disorders per se, not just examples of abnormal immunoglobulin metabolism. Blaese, for example, studied Wiskott-Aldrich syndrome very intensively of the next decade and a half and became associated with the studies of immunodeficiency in general. He was one of the first persons to perform bone marrow transplantation for the treatment of immunodeficiency. He was on his own by then, maybe it was 15 years later or 20 years later. This was the first time that anybody in the world had treated patients with bone marrow transplantation. He and two others were involved in that.

Harden: I was struck also, in this early work that you have described with how easily you seem to be able to collaborate with other people in other institutes. In other words, you were not limited to NCI.

Strober: That's right.

Harden: I saw that you worked with Eugene Braunwald [Dr. Eugene Braunwald] from the Heart Institute [now the National Heart, Lung, and Blood Institute]on a paper, and with collaborators in the Neurology Institute [now the National Institute of Neurological Diseases and Stroke, NINDS], and the Arthritis Institute [now the National Institute of Arthritis and Muskoskeletal and Skin Diseases, NIAMS]. How does the intramural program make all this possible?

Strober: I think to some extent it was because the Clinical Associates got to know each other. For instance, people in NIDDK [National Institute of Diabetes and Digestive and Kidney Diseases] housed the GI [gastrointestinal research] program. An Associate named Peter Loeb [Dr. Peter Loeb] contacted me. He wanted me to help him do some work. I'll tell you more about that because it did change my career somewhat. Peter was working with a man named Leonard Laster [Dr. Leonard E. Laster], who was head of digestive diseases at NIDDK some 40 years ago. Leonard Laster left NIH temporarily to become a scientific advisor in the Nixon administration, and he never came back. That was significant because some of the Clinical Associates assigned to him actually gravitated to me. He wasn't around, so I was able to inherit these talented men. We'll get to this in a moment.

Another of my connections was with the Heart Institute. [National Heart, Lung, and Blood Institute, NHLBI]. Again, I was studying

immunoglobulin metabolism, and the Heart Institute approached me to help them study lipoprotein metabolism. They were interested in a form of hyperlipoproteinemia associated with hypercholesterolemia. Bob Levy [Dr. Robert I. Levy], who subsequently became head of the Institute and was, in the late 1960s, Chief of the Section on Lipoproteins in the NHLBI Laboratory of Metabolism, had a Clinical Associate named Terry Langer [Dr. Terry Langer], and he assigned Langer to study lipoprotein metabolism, beta lipoprotein metabolism. Basically, Langer and I did this work under Bob Levy's aegis.

We performed this study and interestingly, we found out that the patients who had hyperlipoprotenemia had a high level of circulating beta lipoprotein, not because they were synthesizing more of it but because they have less catabolism, decreased breakdown, of the beta lipoprotein. We reported that at a major meeting, but the meeting participants couldn't accept that because they just assumed an increase in the level of a circulating protein was always due to increased synthesis of this protein. We speculated in the Discussion of the paper we ultimately published that the high level of beta lipoprotein was due to decreased catabolism, which was due to some problem with the way beta lipoprotein was transported into the cell.

Another Clinical Associate who was at NIH, but subsequently left NIH and then did work on lipoproteins and the cause of hypercholesterolemia was Michael Brown [Dr. Michael S. Brown]. He

won a Nobel Prize with Joe Goldstein [Dr. Joseph L. Goldstein] for that discovery. Subsequently, he came back to NIH, and I saw him. I said, "Did you ever read my paper on this subject?" He said, "I did read it." I'm not trying to take anything away from him. He did the actual studies to show this.

Harden: In 1967 you were named the Senior Investigator in the Metabolism Branch, and you were mentoring your own Clinical Associates. The names I know are Paul Weiden [Dr. Paul L. Weiden], David Nelson [Dr. David L. Nelson], Howard Pitchon [Dr. Howard E. Pitchon], and Marvin Goodenberger [Dr. Marvin D. Goodenberger]. Would you tell me about the research you did with them?

Strober: Yes. Weiden worked on intestinal lymphangiectasia, a disease Tom Waldmann had discovered. It was a very interesting disease because the patients lost protein into the gut, they had what Waldmann identified earlier, protein-losing enteropathy. Tom Waldmann had put this on the map, actually showing that this occurred. He had invented a way of measuring it with, chromium-51 labeled albumin. We used that technique with it to study the patients with intestinal lymphangiectasia. My contribution was to show that they not only lost serum into the gut, but they also lost cells, lymphocytes. They were lymphocytopenic, and as a result of being lymphocytopenic, they were immunodeficient.

You can see through Wiskott-Aldrich syndrome, ataxia telangiectasia, and intestinal lymphangiectasia that we were beginning to orient ourselves toward the study of immunodeficiency diseases.

I took a somewhat different direction by the time 1967-1970 rolled around because I was now expanding on my work on IgA. IgA is the mucosal immunoglobulin made mostly in the GI tract. I was becoming oriented toward studying mucosal immunology.

That's when I began working with Loeb from Len Laster's group, which was in a different Institute (I told you we would come back to this.). They were working on a disease called celiac disease or gluten-sensitive enteropathy.

Harden: How many people in the world have intestinal lymphangiectasia? Is that a common disease?

Strober: No, no, no.

Harden: It's a rare disease?

Strober: Very rare disease, very rare disease. Subsequently, I studied it again and again. A number of other papers appeared in the '70s and '80s from me about intestinal lymphangiectasia and I became known as an expert in the understanding of this disease.

Getting back to celiac disease or gluten-sensitive enteropathy, this disease is caused by the ingestion of and exposure to gluten. There's also a reaction in the GI tract, flattening of villi and malabsorption. Patients have diarrhea, they have loss of weight because they don't absorb nutrients from the food. In other words their GI tract doesn't work as a result.

At the time I was becoming interested in celiac disease or gluten sensitive enteropathy, it was not known whether this was an immunological disease or was a disease due to some toxic effect of gluten on the GI epithelial cells. There were two groups studying this. Some people felt one way, some people felt another way. To make a long story short, it was as a result of my work and that of Pete Loeb, plus the other people that I worked with such as Myron Falchuk [Dr. Z. Myron Falchuk], who subsequently replaced Peter Loeb. We did a series of experiments that show that gluten-sensitive enteropathy or celiac disease was in fact an immunological disease.

One of the most important discoveries that we made in that early period was that celiac disease is associated with a certain HLA [human leukocyte antigen] type, HLA-B8; then later, we showed that this disease was linked to HLA-DR3. At that time, this was a major discovery because the association of histocompatibility antigen with a disease showed that somehow the histocompatibility antigens shaped the kind of immune response we can have. And ultimately it was shown that what actually happens is that fragments of gluten protein, peptides of gluten protein,

bind to certain HLA antigens on antigen-presenting cells and then induce immune responses in T-cells and B-cells.

This was a major discovery, that celiac disease could be associated with a histocompatibility antigen. In addition to that, we did what at that time were ground-breaking experiments. We took little pieces of tissue and cultured the pieces of tissue. We called it organ cultures. Just little, tiny pieces of tissue. We could show that when you put gluten into the culture with these tiny pieces, the gluten had certain negative effects on the tissue. But it didn't have an effect on tissue from patients in remission, only on the patients who had active disease. The significance of this is that something had to have happened in the remission patients. There had to be in place what we called an endogenous mechanism for the tissue to respond to the gluten. That endogenous mechanism was the immune system.

So these organ culture studies basically showed that the disease was an immunological disease. It could not be just the toxicity, because if it were toxicity, the addition of the gluten to the tissues of the patients in remission would cause damage to the remission tissue, which it didn't. Something had to develop in the tissue to be able to react to the gluten.

To study the disease in this way, with pieces of tissue in culture, was of course a breakthrough. By that time, in the mid '70s, I was, you might say, the major researcher in celiac disease. At that time, however, I wanted to redirect my research to concentration on another immunologic

disease of the GI, inflammatory bowel disease simply because it was a more important disease from the point of view of the number of individuals affected. I made that decision in the mid '70s, and my entire career since then--now you're talking about 35, 40 years of research--has been centered on inflammatory bowel disease. It is the place where I made my major contributions to science.

Harden: I want to ask you about one particular thing that interested me. This would have been in the early '70s. I read a paper you co-authored with Sam Broder [Dr. Samuel Broder], Tom Waldmann. And others. It had to do with salivary and intravenous immunoglobulin A.

Strober: Again, at that time I was interested in IgA and it's metabolism. We didn't realize that there were really two different kinds of IgA. One was made in the bone marrow and one was made in the mucosal tissues. They had different sources, and they were different kinds of protein. The mucosal IgA was polymeric and had was associated with so-called secretory component which allowed it to be secreted into the gut. We did some studies to show that the salivary IgA, was different from mucosal IgA.

Harden: Let’s go back to the the mid '70s, when you decided to devote yourself to inflammatory bowel disease. It's also the time, in 1977, when you became Chief of the Immuno-physiological Section in the NCI Metabolism

Branch. So you were a section chief at this point. I noted you were a mentor to two more clinical associates, Robert Yarchoan [Dr. Robert Yarchoan] who played a role in developing AZT as the first drug somewhat effective against AIDS, and Gordon Yenokida [D. Gordon Yenokida], if I'm pronouncing it correctly.

Strober: Gordon Yenokida, yes.

Harden: Do you have more general thoughts about becoming a section chief and about the people that you were now overseeing?

Strober: Tom Waldmann had now assumed the title of Chief of the Metabolism Branch of the Cancer Institute. Prior to that a man named Nathaniel Berlin, Nat Berlin [Dr. Nathaniel Berlin] had been the branch chief.

Harden: Oh yes, I knew Dr. Berlin.

Strober: He was the chief when I first came. John Fahey [Dr. John L. Fahey] was also here. I don't know if you've heard of those names. Sherman Weissmann [Dr. Sherman Weissman].

Harden: Yes, yes. That tells you how long I have been at NIH.

Strober: Sherman Weismann left NIH and became a professor at Yale. Don Tschudy [Dr. Donald P. Tschudy] was interested in porphyria, which is not an immunological disease. He was quite an individual. All these people were not only wonderful scientists, but they were very interesting people as well. Imagine that! John Fahey left NIH and became very prominent in UCLA. I had subsequent interactions with these people from time to time, so the relationships didn't end.

Harden: As you were diving deeply into mucosal immunity, and before we get into more detail of particular things, would you explain just what mucosal immunity means and what happens in the gut? I was very interested in how what happens in the gut is different from the kinds of things that happen in the bloodstream, or the brain, or the bones. It's a completely different.

Strober: Well, you have heard me talk about one thing already, namely that mucosal immunity has a special immunoglobulin IgA. I was interested in why IgA was being made in the mucosal immune system. That was part of the more basic aspects of my studies. I worked with various people on that aspect. Charles Elson [Dr. Charles O. Elson] and Hidenori Kawanishi [Dr. Hidenori Kawanishi] were Clinical Associates, Research Associates of mine. We worked a lot on IgA. Another person was Yoshihiro Wakatsuki [Dr. Yoshihiro Wakatsuki. Ultimately, a person named Markus Neurath

[Dr. Markus F. Neurath] came to the lab. He figures importantly in my story so I'll talk to you more about him later.

Another unique aspect of mucosal immunity was that T-cells in mucosal tissues are involved in a tolerance phenomena. In other words, there was a way that the gut didn't react to antigens in the gut. This had to be true because there were so many antigens present in the gut microflora—now we call it the microbiome—that if the gut reacted to all of the antigens, you would constantly have inflammatory disease. We now believe and some of my work proved that inflammatory bowel disease is due to abnormal response or excessive response to the commensal organisms in the gut microflora. The question is, then, why does this occur?

A third way that mucosal immunity is different is that the antigen presenting cells, the macrophages and dendritic cells, tend to be somewhat different in the gut than they are in the rest of the system. They have somewhat different properties.

So there are many ways that mucosal immunity is different from systemic immunity. One of the things that happened in my career was that when I became more identified with mucosal immunology as a result of my work in celiac disease, or gluten sensitive enteropathy, and also IgA, I became known as a “mucosal immunologist.” At that time this designation was unusual. Most immunologists were systemic immunologists. Most people in immunology didn't think that mucosal immunology was either

separate or important. But there was a person whom you know, Ruth Guyer [Dr. Ruth Guyer], who felt that mucosal immunology was very important. One of the reasons that persuaded her it was important is breast feeding.

Harden: I remember that, yes.

Strober: Ruth Guyer was working at that time with Ken Sell [Dr. Kenneth Sell], who was the Scientific Director of NIAID [National Institute of Allergy and Infectious Diseases]. This was the early '80s (we are now moving into the early '80s in my work). She had Ken Sell's ear and I think she said, "You ought to have somebody in mucosal immunology at NIAID." She told him I was working on this. So Ken Sell became aware of mucosal immunity and acquainted with me as a result of Ruth Guyer. Ken Sell decided that he would give me resources in NIAID, which were considerably greater than the resources I had NCI.

Harden: I was going to ask you what, when you were a rising star at NCI, made you jump to NIAID?

Strober: The fact is that at that point in time, Tom Waldmann wanted to expand on his own. By that time we were quite differentiated, although we were interacting day by day, of course. Tom Waldmann and I shared a little

cubicle for a number of years. So we were, you might say, joined at the hip. But then he became Chief of the Metabolism Branch, and he wanted to expand in his own way. He wanted to study the IL-2 [interleukin-2] receptor. At that time, it was the sexy thing to do. He went off studying that and he hired a number of people who ultimately became very successful, very brilliant people. He had to have room for these people, which limited my expansion, so the offer to leave NCI and join NIAID was very attractive to me at that point. Tom Waldmann and I parted ways amicably.

Harden: NIAID had not started expanding in 1982 when you came. Soon after that, it did expand quickly because of the HIV/AIDS epidemic, but in 1982, it was still a small and relatively poor institute compared to NCI. That fact also made me wonder what it was that made NIAID it so attractive to you.

Strober: My own perception during that period was NIAID was indeed a smaller institute than NCI, but it had some very high-level people. Shelly Wolff [Dr. Sheldon M. Wolff] was the person who ran the Laboratory of Clinical Investigation (LCI). By the time I came, however, LCI was being run by Michael Frank [Dr. Michael M. Frank]. Sadly, he just died a few weeks ago.

Harden: Oh, I didn't know that.

Strober: Yes. He had a group of very, very outstanding people, including Tony Fauci [Dr. Anthony S. Fauci]. Tony Fauci was in the LCI, as well as Steve Strauss [Dr. Stephen E. Straus] who ultimately became the head of the LCI also. NIAID had quite a number of excellent scientists. It was a lot more, I thought, interactive place than NCI. NCI was so large that you didn't know a lot of the people in the NCI. My perception of the NCI during that period is that it was not the premier scientific institution for the study of cancer. I thought it was somewhat behind. That was my perception. I may have been wrong. I think more recently it's come up in quality in terms of scientists.

Harden: Very interesting. Now let’s go back and finish up your work in the late 1970s. This is the period when molecular biology was flowering, when people were learning every week something new about T-cells and B-cells and how they communicated. I noticed a paper you wrote about suppressor T-cells reducing autoimmunity in a certain strain of mice, which developed a disease resembling human systemic lupus. NCB/NCW mice, if this rings a bell.

Strober: Yes.

Harden: You concluded that such mice lose suppressor T-cells as they age. This was a new finding, correct?

Strober: Yes. I don't think that it panned out as a major mechanism in lupus, but I did become very interested in suppressor T-cells.

Harden: Yes, this is what I wanted you to talk more about.

Strober: Especially during the '70s and '80s we were studying suppressor T-cells as they relate to B-cells, how they suppress B-cells. We did studies, for instance, in liver disease, showing that certain patients with liver disease had decreased suppressor T-cells. At that time we didn't have good markers for these suppressor T-cells and we didn't know how they actually functioned.

During that period, we did a lot of studies. Steve James [Dr. Stephen P. James] was one of my associates. I wrote about 20 to 25 papers with him. He studied a disease called primary biliary cirrhosis, PBC. He was a collaborator from the GI department in another institute. He eventually came into my laboratory. He worked closely with me.
He did a lot of studies with PBC and eventually did studies on inflammatory bowel disease. Some of those cases related to suppressor T-cells. Ultimately Steve left NIH, became chief of gastroenterology at the University of Maryland, then came back to NIH and is now high up in the

hierarchy of NIDDK. He's continued to be a friend of mine although not a scientific colleague any longer.

During that period we were studying suppressor T-cells. Then a Japanese scientist, a man named Shimon Sakaguchi [Dr. Shimon Sakaguchi], discovered that suppressor T-cells had IL-2 receptors, (also called) CD25 they're called, on the surface. Ultimately he discovered that they expressed a certain protein called FOXP3. That revolutionized the identification of suppressor T-cells, because you could then identify them as being FOXP3 positive cells. There were other suppressor T-cells, but that was one of the main ones.

We reentered the field of suppressor T-cells, and we made another discovery. I was working with a man named Kazu Nakamura [Dr. Kazuhiko Nakamura] and Atsushi Kitani [Dr. Atsushi Kitani], who still works with me now. Those two are scientists of Japanese origin. We discovered that suppressor T-cells have TGF-beta on the surface. Surface TGF-beta is part of a mechanism by which suppressor T-cells work. That was major discovery.

Subsequent to that we showed that animals with experimental colitis induced by a substance called TNBS, trinitrobenzenesulfonic acid, when you feed them certain compounds, they develop T-cells that have suppressor capability, and these secrete TGF-beta. This tied these suppressor T-cells to the GI tract. One of the features of the mucosal immune system is that it is populated by many more suppressor T-cells

than immune tissue at other sites; that is why the mucosal immune system manifests the phenomenon we mentioned before, immune tolerance.

Harden: That's very interesting.

Strober: The mice have tolerance because they have the tendency to develop suppressor T-cells. A lot of the work even to this day is being done in studying developments in suppressor T-cells in relation to inflammatory bowel disease and other diseases.

Harden: At this point, if you will permit me, I want to make another side diversion. You were talking about coming to immunodeficiency diseases, being interested in them, and in June 1981, you were still in the NCI Metabolism Branch. Dr. Waldmann saw the very first patient with what we now call AIDS when he arrived at the Clinical Center. The person came into his "Omnibus metabolism branch immunodeficiency disease protocol, 77-C-66." Sam Broder told me that everybody in the branch crowded into the room to see this guy because they'd never seen anybody that had such profound immunodeficiency. Did you see this patient or work with him?

Strober: I don't remember seeing that patient. I might have been there, but I don't have a vivid a memory of that particular incident. I certainly remember the impact of AIDS on the NIH and the world, especially on NIAID.

Harden: You participated in a conference later on gastro-intestinal infections in AIDS. Tell me about what all you were doing in relation to that?

Strober: One of the effects of the AIDS virus was to cause malabsorption in the way the gastrointestinal tract works, a malabsorption syndrome and kind of villous atrophy. I wasn't directly involved in most of these studies. Ultimately there was somebody who came into my section and his name was Martin Zeitz [Dr. Martin Zeitz], a German fellow. Martin Zeitz studied inflammatory bowel disease when he was with me. He went back to Germany and concentrated on the effect of HIV on the GI tract. I maintained a relationship with him during that period. Subsequently, he died, unfortunately, at a fairly early age. So I was only peripherally involved with AIDS research. I was not directly involved with the study of AIDS patients.

Harden: In your CV [curriculum vitae], I saw a number of collaborators and co-authors, many people whom I recognized. For example, Jay Hoofnagle [Dr. Jay H. Hoofnagle] , Tom Quinn [Dr. Thomas C. Quinn], Phil Smith [Dr. Phillip D. Smith], Henry Masur [Dr. Henry Masur], and more. Was there a particular line of research that you did with these investigators?

Strober: They were peripheral actors in my universe. Phil Smith: I had a hand in mentoring him. I didn't actually participate in his research, but he used to come every week or every two weeks and have conferences, mentoring conferences with me. He still calls me occasionally. We still have a relationship. He's been at the University of Alabama at Birmingham for many years now. He's made a name for himself in mucosal immunology, but we didn't have any specific relationship. He was studying *Giardia* infection at that time. He was in the NIAID when I was still in the Cancer Institute.

Harden: He might have been working with Ted Nash [Dr. Theodore E. Nash].

Strober: Yes, he was working with Ted Nash.

Hoofnagle, no. I did not have much of a relationship with him, although he worked with Steve James. That was the connection because he was in liver disease. So I knew him and if I see him in the hallway we will talk for a while, catch up on each other, but I didn't really work with him.

Harden: I was fascinated by a 1983 paper you wrote hypothesizing that a genetic mutation for sensitivity to gluten may have made populations more resistant to bubonic plague or other infections. This would be like the sickling of red blood cells as partial protection from malaria in tropical populations. Did this hypothesis go anywhere?

Strober: Yes, because the history of celiac disease, of course, is that gluten was introduced into the human diet. At a certain point in time people, started eating wheat instead of foraging and eating animals, basically. When the domestication of grains was introduced, it was a boon to mankind because they could stay in one place instead of having to move around. There was much easier access to food, but as a result of that certain individuals had problems with eating the grain and namely, they had a kind of celiac disease.

Harden: So gluten sensitivity was cause by a genetic mutation that protected some people from infections, correct?

Strober: Yes. The mutation may have been protective to many people, but it may have been harmful to some others. The same thing is true with the NOD2 [Nucleotide-binding oligomerization domain-containing protein 2] gene. It takes us back to inflammatory bowel disease. We feel that NOD2 is protective in that NOD2 stimulation decreases other kinds of response. It may be that loss of NOD2 suppressor capability is helpful. It's called balanced polymorphism in that if you have a larger response and a lack of NOD2, you get a larger immune response, but you may be more resistant to infection, basically because you have a bigger immune response. Unfortunately, you also have more of a tendency to inflammatory bowel

disease. You may have more resistance to infection of the GI tract, but a greater sensitivity to autoimmune disease.

There's a balance between autoimmunity and infection. With autoimmunity, you have too much response, but that's helpful in terms of dealing with infection. So there's a balance there. I actually did a study showing that under certain circumstances, the lack of NOD2 actually allows you to develop more suppressor cells and you become more resistant to the induction of intestinal inflammation. In most patients a lack of NOD2 makes them susceptible to Crohn's disease because there is a bigger immune response, but at the same time it may make them more resistant to infection because of the bigger cytokine response.

Harden: During the 1980s, you began to receive award after award, including induction into the Association of American Physicians, which is an honor extended to individuals with outstanding credentials in biomedical science and/or translational biomedical research and is limited to only 60 persons per year. In 1991, you took on another administrative responsibility as Deputy Director of the Division of Intramural Research and you held this position until 1995. Now this was the period when NIAID's HIV/AIDS program was expanding and John Gallin [Dr. John I. Gallin] was transitioning to become Director of the Clinical Center. Would you talk about what your job entailed administratively during this time?

Strober: During that period, yes, I was working closely with John Gallin, but I hadn't given up the laboratory. So I was spending about half my time in the lab and half in another office close to John Gallin. We worked pretty closely on more or less the full scope of things involved in the administration of NIAID. At that time NIAID was considerably smaller than it is now. It was a rewarding experience. I had a chance to go to the Rocky Mountain Lab [Rocky Mountain Laboratories, NIAID] a number of times with John and meet all the people there. We reviewed the budgets of everybody at NIAID. This was one of the most important aspects of the job. At times we worked to establish new laboratories or recruit new people.

Harden: This was also the time that Tony Fauci, as NIAID Director, was expanding the institute.

Strober: Yes. I was in the role of Intramural Deputy Director about three years. During that period John Gallin decided that he wanted to become the head of the Clinical Center. He succeeded. He went through a competition, and he won that position. I think that he was excellent in that position, by the way. I never understood why they made a change. He did a very good job.

As a result of that he left his position as the Scientific Director of NIAID and Tony Fauci appointed Frank Neva [Dr. Frank A. Neva], who was a parasitologist, as the Acting Scientific Director. Frank Neva was

one of the grand old men at NIAID, a very distinguished person, very excellent person. Everyone loved him. He was a parasitologist, and he knew a tremendous amount about parasitology, especially the clinical aspects of it. So he was appointed to take an interim role when John left, but he didn't know anything about how to run NIAID as a Scientific Director. He hadn't ever held that position.

This happened during the third year of my tenure. That year I basically ran the institute as co-Scientific Director with Frank Neva. Basically I was steering Frank in that job because he didn't know how to do it. I had essentially been doing the job for two years. So during that period, I had more direct experience being the Scientific Director at NIAID.

Harden: In 1998, you were asked to serve as the acting Scientific Director for the Arthritis Institute [National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIAMS]. This was after Henry Metzger [Dr. Henry P. Metzger] stepped down. You were there for a year until Peter Lipsky [Dr. Peter E. Lipsky] was appointed. Tell me why they looked to you at NIAID instead of somebody within their own institute?

Strober: First of all, Henry Metzger was very distinguished.

Harden: Yes, indeed. I knew Henry well. He sat on my advisory committee for the Office of NIH History and Stetten Museum.

Strober: At that time Steve Katz [Dr. Stephen I. Katz] was the Director of NIAMS. There was criticism of that institute because there was a feeling that there was not enough clinical research or emphasis on clinical studies. Critics said that it emphasized basic research too much over clinical research. I actually was not privy to that criticism. I heard it secondhand. The Board of Scientific Councilors of NIAMS decided that they needed to replace Henry, whom they felt was a very distinguished scientist but was not clinically oriented. My own feeling, after getting the job, was that I thought he was doing all right as far as the clinical aspects were concerned, but they thought that they needed a change.

Harden: I understood that Henry believed that basic research needed to be emphasized the most within the intramural program. There were very distinct differences at that time in beliefs about what NIH intramural should be doing—whether basic or applied research, laboratory or clinical research, should be emphasized. This went back all the way to the late 1960s, after Jim Shannon [Dr. James A. Shannon] retired as the NIH Director who championed basic research. The health activist and philanthropist Mary Lasker [Mary Woodard Lasker] advocated strongly with Congress for more applied research, especially on cancer. Hans

Stetten [Dr. DeWitt Stetten, Jr.], former Director of Intramural Research for NIH and a friend and colleague of Henry Metzger, believed strongly that basic research should be NIH's priority. He believed that you couldn't be successful in applied clinical research until you understood the basic science underpinning it. Others disagreed with him.

Strober: I think there has to be a balance, frankly. Both are important.

Whether or not NIAMS was correct in removing Henry as Scientific Director was not my role to decide. The reason Steve Katz came to me, somebody outside the institute, was that I had played a role in supporting Steve during his early career here, which we haven't gone into. It was in relation to a particular disease related to celiac disease called dermatitis herpetiformis, a skin disease. We were very, very good friends both scientifically and socially, and so when he had to fill that spot suddenly, he turned to me. For 15 months, it was a rewarding experience for me.

As the acting Scientific Director during that period and having had the experience with John Gallin earlier at NIAID, I was capable of doing that job from every point of view, budgets and everything else. It was a much smaller job than NIAID because NIAMS was a smaller institute.

I worked with Steve Katz quite closely to run that institute for a good period of time. Henry still was in the institute, still was in the lab. Steve got lab space for him very quickly, reestablished him in a lab and he

did science during that period. He was initially upset about being removed, but he came to accept it, and we became quite proper colleagues. It was a good experience and I met a lot of very important people. John O'Shea [Dr. John O'Shea],for instance, who is now the NIAMS Scientific Director, and Dan Kastner [Dr. Daniel Kastner] who is now Director of the Human Genome Institute, worked under me at that time. The institute was incubating some very, very good people at that point.

Harden: Let me ask one more question and then we will stop for today. When you returned to NIAID in 1999, you were named Deputy Chief of the Laboratory of Clinical Investigation. Then in 2003, 2004 you served as Acting Chief of that laboratory, but then the laboratory dissolved. You went back to being a section chief, a position in which you probably had continued to serve anyway, in the Laboratory of Host Defenses (LHD). Can you tell me what was going on in NIAID that caused all this change?

Strober: Yes. At the time that Mike Frank left as Chief of LCI, the NIAID administration appointed Steve Strauss [Dr. Stephen E. Straus] the new head of LCI, the Laboratory of Clinical Investigation. He wanted me to be Deputy Chief during his tenure, so I served as Deputy Chief. Several years later he was offered the directorship of the National Center for Complementary and Alternative Medicine (NCCAM), newly upgraded from an Office in 1998. The person who had headed the search committee

for a director was Steve Katz. In that capacity he asked me, "Do you know anybody who would be suitable to be head of the alternative medicine program?" I said, "I do know somebody, and his name is Steve Strauss." I recommended him because he had done outstanding work in the investigation of chronic fatigue syndrome, so he was already used to dealing with medical therapy that falls under the umbrella of alternative medicine. Straus was ultimately appointed Director of NCCAM but still retained his position as chief of LCI; I continued at Deputy Chief and necessarily assumed the day-to-day responsibilities as Chief. Some time after that Tony Fauci and others in the upper management of the NIAID decided to make a more fundamental change in the laboratory structure of the NIAID as part of a plan to create several clinically oriented laboratories in NIAID, instead of only one, the LCI.

Harden: Was LCI too big?

Strober: Yes. The first reduction in the size of the LCI had occurred earlier with the establishment of the Laboratory of Immunoregulation (about 1989) head by Tony Fauci and largely focused on HIV research. Later, the Laboratory of Host Defenses, headed by John Gallin (1991), was created after Gallin became Scientific Director. It emerged from the part of LCI that was studying neutrophils and chronic granulomatous disease, a neutrophil disorder. Then in 1995, scientists studying allergic diseases was split off

to become the Laboratory of Allergic Diseases (LAD). This occurred at least in part to put more resources into studies of allergy which was after all part of the original orientation of NIAID. Metcalfe [Dr. Dean D. Metcalfe] was appointed head of the new laboratory.

A final re-shaping of the LCI came with the actual elimination of this laboratory and a reshuffling of people in this laboratory. My Laboratory became part of the LHD ultimately headed by Harry Malech and Brian Kelsall [Dr. Brian L. Kelsall]. My section later became part of a new Laboratory called the Laboratory of Molecular Immunology headed by Dr. Philip Murphy.

Harden: Let's stop there for today.

This is Part Two of the oral history with Dr. Warren Strober on August 14, 2019, at the National Institutes of Health about his career in the National Institute of Allergy and Infectious Diseases. The interviewer is Dr. Victoria Harden.

Harden: Yesterday, we leapt ahead, skipping over the transition to molecular techniques in the '70s and '80s. You mentioned to me earlier that you had to teach yourself molecular biology because it had not been available to you when you were training. Will you talk about what you did to learn it?

Strober: Yes. I did two things. First of all, I took a course in molecular biology sponsored by FAES [Foundation for the Advancement and Education in the Sciences]. I also read a book by Benjamin Lewin called *Genes*. There have been a number of different editions of the book. At the time, the most up-to-date edition was called *Genes II.* At the same time, I was overseeing and doing molecular studies in the laboratory, so I was gradually getting used to and understanding both the terminology and the methodology of molecular biology on a daily basis. Eventually, I became mostly oriented to studies using molecular biology to unravel some of the problems we were encountering. In fact, without the use of that technology or the understanding of that technology, I really couldn't proceed after, say, 1985.

Harden: In the 1990s you began clarifying what inflammatory bowel disease is on the molecular level. Would you tell me about this research?

Strober: It started in two ways. One of my former fellows, Charles Elson—I called him Chuck—was one of the first persons to say we had to establish animal models of inflammatory bowel disease in order to really study this disease. He embarked on studying animal models at the University of Alabama in Birmingham and was out of my lab at that point.

About that time, there were three major articles published in a journal called *Cell*, a major journal, about three animal models of

inflammatory bowel disease. Each proposed one of three different molecular defects that resulted in inflammation of the gut. I was asked to write a commentary on these models because I had already done research on inflammatory bowel disease. I recently reread that commentary. It was right in some respects; it was wrong in others in predicting the our present understanding of inflammatory bowel disease. But it did change the direction of my research, together with the previous knowledge of what my former fellow, Chuck Elson, was doing.

We began to study models of inflammatory bowel disease. The first model that we studied was called TNBS colitis, which was induced in mice by injecting them intrarectally with TNBS. This was done in our laboratory by two fellows at that time, Markus Neurath, who was a fellow from Germany. At the moment he's in Erlangen, Germany, but at that time he was in Mainz. My other fellow was Ivan Fuss [Dr. Ivan J. Fuss], who is still with me. These two researchers in my lab established the model of TNBS colitis, which had been established prior to that in rats. But this was the first time this was being performed in mice. The administration of TNBS causes an inflammation of the gut, the colon.

The upshot of that study was the demonstration that TNBS colitis was due to the induction of a cytokine called IL-12. Ultimately, we followed that up by treating the animal with anti-IL-12. Actually, anti-IL-12-p40, which is one of the IL-12 chains. The IL-12 molecule is made up of two chains. One is p40 and one is p19. The treatment of mice with

TNBS-colitis with anti-IL-12-p40 had a dramatic effect on the TNBS colitis. Actually, if you first gave the animal the TNBS to cause the inflammation and waited a while, and then you came back a few days later with the anti-IL-12-p40, it completely cured the animal. At that point we knew that we had a way of curing a type of experimental inflammation of the gut.

Quite a few years later, another person working at that time the laboratory, Peter Mannon [Dr. Peter J. Mannon] conducted clinical studies of patients with Crohn's disease using a recombinant antibody we obtained working with a pharmaceutical company. He worked with the same Ivan Fuss I mentioned earlier—Markus Neurath was no longer in the laboratory at that point. Basically, they set up a clinical study in patients with Crohn's disease in which the patients were given anti-IL-12-p40. To make a long story short, this therapy proved to be very effective in treating the patients. At that point (in the late 1990's) we patented the anti-IL-12-p40 as a method of treating Crohn's disease. NIH provides a technology transfer mechanism to support patent development. Ultimately this therapy was developed by Janssen, the pharmaceutical firm that licensed the patent. They conducted a multi-center clinical trial that showed the treatment of patients with Crohn's disease that did not respond to anti-TNF-alpha [anti-tumor necrosis factor-alpha] treatment still responded to anti-IL-12 p40 treatment. The anti-IL-12 p40 is now called "ustekinumab" and is being sold by Janssen under the trade name Stelara. It is proving to

be one of the most effective biologic treatments of severe Crohn's disease known so the use of animal models to study IBD had come to fruition.

Harden: The technology transfer protocol was instituted so that the federal government was able to recoup some of the taxpayers' investment in your work as a federal employee. If I remember correctly, whoever is named as inventor(s) on the patent gets a certain percentage of the profits from the licensed therapy, and the government gets a larger percentage over the lifetime of the patent.

Strober: That's right. The Technology Transfer Act was enacted in 1986, during the Reagan administration. Its goal was to encourage federal scientists to develop patents that would be useful in the marketplace.

With respect to our anti-IL-12p40 patent, there was a semi-sweet ending. Every patent has a lifespan of 17 years. By the time ustekinumab was actually cleared for use in patients by the FDA [U.S. Food and Drug Administratoin], 17 years had elapsed. The government, and myself, and other people who were on the patent, which included Ivan Fuss, Markus Neurath, and several other people who were also participating in this study, never received any monetary benefit from the patent. But we did have a psychological benefit in knowing that we had developed an antibody that was useful in the treatment of Crohn's disease.

Harden: Do you remember what year this was?

Strober: The application for the patent was made in 1998. It was introduced as a treatment in 2017. It is possible that the company that developed ustikinumab for clinical use was not proceeding as quickly as possible because it preferred to develop anti-TNF-alpha, which is sold as Humira. They probably didn't want to introduce a treatment that could compete with their other more established product. Whatever the motive, it didn't come into fruition for many years. But we're happy that it is in use now.

Now I'm going to tell you about another use for anti-IL-12p40 that we are currently exploring. We study patients with immunodeficiency, as you know. One of the immunodeficiencies we study is called common variable immunodeficiency, or CVID. A certain percentage of these patients get gastrointestinal disease and malabsorption. We had shown previously before some key studies were done that the malabsorption was associated with and probably caused by excess production of interferon gamma in the gut wall. The person who did that study was the same Peter Mannon who previously had conducted the study of anti-IL-12p40 in patients with Crohn's disease. As a result of this study we reasoned that the secretion of IFN-gamma in the gut of patients with enteropathy was due to the secretion of IL-12, its "parent" cytokine. So under the guidance of Ivan Fuss—we treated patients with CVID who had severe gastrointestinal disease, malabsorption and weight loss due to excess

production of interferon gamma with ustekinumab and they got dramatically better.

Harden: Wow.

Strober: That's a second use for the ustekinumab. Now, interestingly, we had to buy the ustekinumab for the treatment of the patients. It was very, very expensive.

Harden: Had it not already been on the market for something else?

Strober: It was available, but it had not been completely released yet. The Clinical Center under John Gallin had instituted a plan to stimulate joint studies between scientists in the intramural program and those from outside the federal government. This particular study was selected to be funded under that plan and was performed in collaboration with scientists at Oregon State University. Because of this program, we had funding to buy the ustekinumab. It turned out to cost several hundred thousand dollars. The study is still ongoing because we're still following the patients and seeing how they're doing on the ustekinumab therapy over a long period of time.

Harden: When was it started?

Strober: About two years ago. It's ongoing. We have between five and seven patients enrolled in the study. The ustekinumab therapy is life saving. These patients are deathly ill. They have CVID and they have tremendous malabsorption, diarrhea, weight loss. In the past we've had patients die of this condition. This is really a life saving study for those patients.

Harden: It's very interesting to me how this work proceeds brick by brick, discovery by discovery, and then suddenly you're able to figure out how it all comes together to make a useful therapy.

Strober: Yes, because Crohn's disease is also driven by interferon gamma and related cytokines. Interferon gamma is a very important disease-causing cytokine in Crohn's disease. But it is also an important cause of enteropathy in CVID. The CVID immunodeficiency patients we're talking about don't make immunoglobulins. Therefore, they have immunodeficiencies, and they have certain conditions in their gut that are unique. But they do respond to the anti-IL-12-p40.

Harden: By the end of the 1990s, if I understand this, it had become clear that tolerance versus a negative immune response in the gut was cued by the same T-cell subset. Did I get that right and can you explain it?

Strober: Okay, let's talk about that for a bit. Let's talk about Crohn's disease. Over the years, the idea developed that Crohn's disease was due to an excessive, over-exuberant response to normal gut bacteria, that don't ordinarily cause disease. We all have a commensal population of bacteria—and viruses, too—in our guts. It's called the microbiome. The microbiome is now very important and on everybody's mind. It's thought to be involved in quite a number of diseases, but inflammatory bowel disease was particularly involved because the thought was that you're now over-responding to normal gut constituents.

One of the mechanisms that will prevent over responsiveness are certain kind of cells called regulatory T-cells or suppressor cells that tame responses; this process is particularly important in the gut because the gut is being constantly stimulated by antigens and mitogens that are expressed by the bacteria. That's where oral tolerance comes in. It turns out that the gut has a very strong mechanism for generating regulatory T-cells. We were aware of this and we studied regulatory T-cells during the '80s in relation to other diseases, such as autoimmune diseases like primary biliary cirrhosis that I mentioned before. We studied regulatory T-cells, but it wasn't until other investigators had developed markers for the regulatory T-cells that you could really pin down what the regulatory T-cells were doing.

Another investigator, Fiona Powrie [Dr. Fiona Powrie], initially working in the U.S. as a visiting fellow in California but who eventually

moved to Britain, developed another model of inflammatory disease. This model consisted of mice with severe lymphocyte deficiency, such as SCID [severe combined immune deficiency] or Rag2-negative animals who were injected with naïve T-cells, and lo and behold, they would develop severe inflammatory disease of the gut. This was called the transfer model of gut inflammation. This model was very useful for studying regulatory T-cells because if someone injects non-naïve T cells along with the naïve T cells, the mice did not develop colitis. In this way Fiona Powrie showed that the non-naïve T cells contained a sub-population of regulatory T-cells that were very important in preventing immune responses to gut microbial constituents. At that time we conducted studies showing the same thing using the TNBS-model I mentioned above. We showed that if you fed mice proteins that had been modified by TNBS, you could prevent or even cure subsequent induction of TNBS-colitis. Further, we showed that such feeding caused the gut mucosa to produce TGF-beta, a cytokine that was eventually shown to induced regulatory T cells. These studies therefore showed that oral exposure to an antigen, in this case TNBS-modified proteins, could induce regulatory cells (i.e., oral tolerance) that could prevent gut inflammation.

These studies led to the idea that reduced levels or reduced function of regulatory T-cells were very important in causing Crohn's disease. It's a very good concept, but we have not yet proven it; we have not shown that, for instance, you can give patients more regulatory T-cells

and thereby cure their Crohn's disease. Currently, we are conducting studies with a new possible agent that might treat Crohn's disease by increasing their generation of regulatory T cells. This might provide the first evidence that, indeed, Crohn's disease can be ameliorated by increasing the number or function of regulatory T cells that improves their tolerance to intestinal bacteria.

Let me transition into another aspect of this. During the '90s it became apparent that there were certain genetic defects called polymorphisms that were associated with inflammatory bowel disease. What is a polymorphism? It's a change in the DNA composition of a gene, that is similar to a gene mutation which also causes a change in the DNA. However, it differs from a mutation in that it occurs in a fair number of normal individuals in the population who do not have the disease. Its presence puts you at a greater risk for having a particular disease and is therefore only one of several factors responsible for disease occurrence.

Harden: Can you give me an example?

Strober: Well, the best example of a polymorphism associated with an increased risk for developing IBD (Crohn's disease) is the polymorphism in the gene called NOD2. The discovery in the early 2000s of a polymorphism in NOD2 that increased the risk for developing Crohn's was a major advance in our understanding of this disease. However, later on, polymorphisms in

many other genes were discovered to be risk factors in IBD also, although none as strong as NOD2. This means that Crohn's disease could be due to different combinations of genetic defects (polymorphisms). About 10-15% of patients with Crohn's disease have a NOD2 polymorphism.

Harden: And it's just Crohn's, it's not ulcerative colitis as well?

Strober: Ulcerative colitis is associated also with genetic polymorphisms, but only some are the same as those in Crohn's disease and not NOD2. Some polymorphisms affect both ulcerative colitis and Crohn's disease whereas other affect only ulcerative colitis or only Crohn's disease.

Harden: I see.

Strober: When this was discovered, I was approached by a researcher at St. Luke's Hospital in Tennessee. He had made a mouse that lacked NOD2, a NOD2-negative or deficient mouse. He knew that I was interested in Crohn's disease and the study of inflammatory bowel disease. He said, "Why don't I send you these mice so that you may study the role of NOD2 in Crohn's disease." I agreed, he sent the mice, and we bred the mice here to obtain an expanded colony of the NOD2 negative mice.

One of my fellows, very talented fellow, Tomohiro Watanabe [Dr. Tomohiro Watanabe] looked into why NDO2 was associated with Crohn's

disease. He discovered that NOD2, when activated by its ligand, muramyl dipeptide, down-regulates responses to gut bacteria caused by so-called toll-like receptors (TLRs) induced by TLR stimulants associated with the bacteria such as lipopolysaccharides. These TLR responses are “innate” responses that are one of the first reactions of the immune system in the GI tract to components of bacteria and result in the elaboration of cytokines that control immune responses to pathogenic organisms.

The substance that activates NOD2, its ligand, is muramyl dipeptide, which is itself a part of the coat of bacteria in the GI tract. Putting this information together, Mannon, Fuss and I developed a general theory of the pathogenesis of Crohn's disease that we published in the *Journal of Clinical Investigation* called “The Fundamental Basis of Crohn's Disease.” Basically, the idea that we put forward was that Crohn's disease develops because of an excessive responsiveness to the gut bacteria which represents a disturbance in oral tolerance that I discussed above. In the case of NOD2 this is the result of decreased NOD2 function caused by the polymorphisms and the inability of NOD2 in patients with the NOD2 polymorphisms to properly down-regulate responses.

Now, one might ask, how does NOD2 work to decrease responses? We puzzled over this question for several years. The insight that Tomo Watanabe, eventually made in further work he did with us was that NOD2 ordinarily induces the generation of a molecule called IRF4 [Interferon Regulatory Factor 4] that has the capacity to down-regulate the function of

other molecules that are necessary for the formation of NF-κB [nuclear factor kappa-light-chain-enhancer of activated B cells], the master regulator of inflammation. However, in patients with Crohn's disease and NOD2 polymorphisms the NOD2 lacks the capacity to generate IRF4 and therefore NF-κB responses are not properly down-regulated and hyper-responsiveness occurs.

Harden: But as a simplified conclusion for public understanding, you had shown that Crohn's disease is a genetically caused disease as opposed to—

Strober: We knew that for a long time that Crohn's disease has a genetic basis because Crohn's disease very frequently occurs within families. Also, Crohn's disease and ulcerative colitis can occur in the same family because there are genes that are shared in Crohn's disease and ulcerative colitis. The question is not whether Crohn's is linked to a genetic effect, the question is: what was the level of the genetic effect and how do environmental factors interact with genetic factors to cause Crohn's disease. For instance, cigarette smoking is a positive environmental factor in some forms of inflammatory bowel disease.

Harden: But it's not enough by itself to trigger it?

Strober: Probably not. You probably have to have the appropriate genetic "background soil" to develop the disease.

The NOD2 story was a very important story and it's still ongoing. Not everybody accepts the idea that NOD2 works through IRF4. Some people have other ideas how NOD2 works, and we can't completely discount those other ideas. We can't completely disprove them; we can only prove that we have shown at least one of the ways that NOD2 works. In any case, it might ultimately possible to perform gene therapy to alter NOD2 function so as to correct NOD2 defects in hematopoietic cells.

Another interesting way in which knowledge of how NOD2 works may lead to new ways of treating Crohn's disease is based on our observation that if you administer excessive amounts of MDP [muramyl dipeptide] to mice you can prevent the mice so treated from developing experimental colitis. What is happening is that the increased amount of MDP causes increased NOD2 function which includes the generation of IRF4. The latter then changes the chemical state of various components that together lead to the downregulation of NF-κB.

Recently, I was called by somebody whose company has developed special compounds related to MDP. Based on our studies, he wants to use MDP to treat patients with gut inflammation. This is a very attractive possibility because MDP already exists in our gut as a component of gut bacteria. Thus, in treating patients with MDP you are

not introducing anything new, you're just giving more of something already there.

Harden: And does the FDA at least require that you demonstrate that giving more of it's not going to have toxic side effects?

Strober: Absolutely. We would have to develop a formulation of MDP that is proven to be safe to administer to patients. In addition, we have to give the MDP in an appropriate fashion, hopefully orally, and show that it can treat Crohn’s disease by stimulating NOD2 regulatory effects.

Harden: Where are you going to get the population to do a proper clinical trial?

Strober: Theoretically, we would focus on patients who had suffered an initial bout of Crohn's disease. Crohn's disease occurs in spurts. You have an episode, it's diagnosed as Crohn's disease, you're treated for it. You'll be treated with steroids for instance, or other substances. Sometimes that clears it up, but usually it reoccurs. That's the problem: it almost always reoccurs. This pattern of disease offers us an opportunity to treat patients with early disease to prove that the treatment prevents further reoccurrences.

Harden: How far along is this therapy in the process of development?

Strober: It's in a very early phase. We're going to have to obtain a CRADA [Cooperative Research And Development Agreement], with the company that will develop the MDP formulation. They will initially provide resources that will allow us to study their compound in mice, who are given TNBS to cause experimental colitis. As part of these studies we will feed the mice the MDP compounds to see whether they are able to prevent or cure experimental colitis. If that is successful we will create a clinical project to test the efficacy of the MDP formulation in patients with Crohn's disease.

Harden: So it's not coming out next year, but down the road sometime.

Strober: Yes. This possibility and related possibilities developed from our work that led to the understanding NOD2 function. To summarize, these ideas are all related to the fact that NOD2 polymorphisms in Crohn's disease cause decreased function, and in the majority of patients who do not have polymorphisms in NOD2 and thus have normal function, it may be desirable to use approaches that increase NOD2 function such as administration of MDP.

Harden: One thing I noted was your discussion of something called toll-like receptors in the epithelial cells of the gut. Is that tied into the NOD2 story?

Strober: Yes, absolutely. These receptors are called toll-like because they were discovered in primitive animals which have similar receptors. These early life forms didn't have immune systems that produced antibodies; instead, they had toll receptors, which were capable of recognizing and mounting primitive immune-like responses to substances in the environment that were components of potential pathogens such as LPS [lipopolysaccharides], like peptidoglycans, and like double stranded DNA (which are part of viruses). These interactions with toll receptors induce so-called innate responses which differ from antibody responses because they are based on recognition of common molecular patterns in the microbial environment rather than specific antigenic components. These early immune responses that developed early are now called innate responses to distinguish them from so-called adaptive immune responses carried out by specific T and B cells. The toll responses in primitive organisms are called toll-like responses in vertebrates and humans, or TLRs.

Harden: And what do they do? It sounds like they are requiring some sort of a toll, such as the kind on a highway you pay in money.

Strober: It's only fortuitous that they're called toll. Toll, the German word for "amazing" is a protein in Drosophila melanogaster that was first shown to be involved in the establishment of its dorsoventral polarity. Later, the toll

protein was found to be structural similar to the cytoplasmic domain of the IL-1 receptor, a receptor whose signaling leads to the activation of NF-κB. This led to the identification of other receptors homologous to toll, that are now called toll-like receptors, that also caused NF-κB signaling and were responsive to various molecular patterns in the microbial environment, such as LPS [lipopolysaccharide]. Today, we recognize at least 11 different TLRs each responding to different microbial component.

Harden: Okay.

Strober: One more story.

Harden: Go ahead.

Strober: It turns out that NOD2 can also cause disease as a result of a mutation rather than a polymorphism. This occurs in patients with Blau Syndrome, so-called because it was first described by Dr. Edward Blau in 1985. He ran across and ultimately published a report of a family in which 11 members had inflammation of the joints, the skin, and the eyes. The eye inflammation was the most important manifestation, because it sometimes caused blindness.

Harden: And this was caused by a mutation of NOD2?

Strober: Yes, it is caused by a DNA abnormality affecting a part of the NOD2 gene that is different from the DNA abnormalities causing polymorphisms in Crohn's disease. Logically, it should cause gut disease similar to that in Crohn's disease, but instead it causes disease in organs where there are no bacterial flora.

Harden: Which tells us that tweaking a gene may have ramifications beyond what you think it's going to have. Correct?

Strober: That's exactly true, yes. We take care of patients with Blau Syndrome occurring in several different families. Their disease is being controlled with anti-inflammatory agents such anti-TNF-alpha. In our studies of these patients we have obtained evidence that they are like Crohn's disease patients in that they also have NOD2 disfunction that leads to inability to control inflammation.

Harden: And yet they don't have Crohn's?

Strober: They don't have Crohn's. The DNA abnormality in Blau syndrome, as I mentioned, is different from that in Crohn's disease. For this reason, there is a subtle difference in the kind of dysfunction that occurs and, correspondingly, a difference in the disease manifestations. In any case, we are going to try to cure these patients with gene therapy. We will

isolate their circulating stem cells and correct their stem cells so that they no longer have the NOD2 mutation. Then we're going to inject the corrected stem cells back into them. They will no longer have a NOD2 mutation and will be cured of Blau Syndrome.

Harden: This procedure would require you to shut down their immune system completely and then reconstitute it?

Strober: Yes. We will have to suppress their immune system temporarily. However, we will re-constitute the suppressed system with their own stem cells and therefore avoid a graft versus host reaction.

Harden: It is, of course, the great hope of genetics that you can do this for many diseases.

Strober: Well, if gene therapy works in Blau syndrome, it will point the way to treating Crohn's disease, wouldn’t it?

Harden: Wow. Yes.

Strober: At least in certain patients.

Harden: You have also published on NOD1. What's the difference between NOD1 and NOD2?

Strober: Well, they are similar. That's why they're called NOD1 and NOD2. But they're also different. Thus, while they both respond to peptides present in the bacteria coat, the peptides they respond to are different; in addition, they signal the cell in different ways. For instance, NOD1, but not NOD2 can induce type one-interferon when stimulated by its specific peptide. As a result, NOD1 (but not NOD2) responses to *Helicobacter pylori* infections lead to protective interferon responses that help control this potentially dangerous infection

One of the most interesting facets of our NOD1 research involves its relation to the development of pancreatitis. The same Tomo Watanabe, I mentioned before in relation to NOD2 function, working at Kyoto University in Japan, has been collaborating with me in Bethesda on studies of pancreatitis. Pancreatitis is a very serious form of inflammation that occurs because of genetic and/or environmental factors. Environmental factors include alcoholism and smoking.

To make a long story short, we discovered that the same commensal bacteria in the gut micro-organisms that I talked about before, can invade the bloodstream as a result of the activity of cytokines released during the initial phases of pancreatitis. Some of these bacteria then encounter NOD1 in the pancreatic acinar cells and this induces an immune

response that is responsible for the pancreatitis. Interestingly, this immune response involves NOD1 induction of type one interferon. The importance of NOD1 in the pancreatitis is proven by the fact that you cannot induce a particularly type of experimental pancreatitis in animals that lack NOD1.

Harden: It's interesting how you managed to trace it from the gut through the blood to the pancreas.

Strober: I would be remiss not to mention our studies in ulcerative colitis.

Harden: Please go ahead.

Strober: Sometime after studying TNBS colitis we decided to create other kinds of experimental colitis. The idea that came to mind was that TNBS is a chemical which, if you inject it into the skin, causes a delayed hypersensitivity immune response. So it occurred to us to determine if other substances that cause delayed hypersensitivity responses also cause experimental colitis. In particular, we wanted to see whether another substance frequently used by investigators to induce a delayed hypersensitivity response, oxazolone, would cause a Crohn's disease-type colitis in mice.

So we said, "We'll administer oxazolone into the rectum of the mouse the same way we administer TNBS, and we'll probably get the

same disease." But, it turned out that oxazolone induces a very different kind of colitis, one that is more reminiscent of ulcerative colitis than Crohn's disease.

Harden: Is it located in the large bowel as opposed to the—

Strober: Both substances induce colonic disease, but while TNBS induces a T-cell mediated disease, oxazolone induces an NK T-cell [Natural Killer T-cell] mediated disease.

Harden: Natural killer.

Strober: Yes, natural killer T cells that in this case produce IL-13 (not interferon-gamma as in TNBS-colitis). Furthermore, we found that the mice with oxazolone colitis can be cured by administration of anti-IL-13.

We went on to study patients with ulcerative colitis. This was done by mostly Ivan Fuss and another German fellow named Frank Heller [Dr. Frank Heller]. Frank Heller was a very good researcher. Now he's in private practice in Germany. Ivan Fuss and Frank Heller looked at patients with ulcerative colitis. Turns out that those patients have a lot of NK T-cells in the wall of their gut that produce IL-13. Ulcerative colitis seems to be a very different disease from Crohn's. There are no NK T-cells in Crohn's disease. This opens up the possibility that you can cure

ulcerative colitis with anti-IL-13. Some people have actually tried to do this. There have been several clinical trials. So far, the results have been very marginal.

Harden: Interesting. Why?

Strober: Don't know. We don't know for sure. Maybe the antibody is not quite the right antibody. Perhaps another antibody would target the receptor for IL-13, not the IL-13 itself. The receptor, which causes the effect of the IL-13, is on the epithelial cells. IL-13 is addressing the epithelial cells and causing epithelial cell death. Therefore, you're develop the ulcerations that are so characteristic of ulcerative colitis. We think an antibody directed to the IL-13 receptor would be more effective than the anti-IL-13 itself. There are actually two receptors for IL-13, alpha1 and alpha2. The antibody would have be directed to the alpha2 receptor. You have to use the alpha2, because that's what's on the NK T-cells.

Harden: So this is ongoing?

Strober: This is ongoing. If we really succeed in that, or other people succeed in this then we'll have a cure for Crohn's disease.

Harden: Very recently you published something about neonatal colonic inflammation that can trigger an inflammatory response that lasts into adulthood.

Strober: Yes.

Harden: Tell me about that.

Strober: Well, we didn't do that study. It was just commentary on somebody else's study, really. But we have a colleague in Boston, at Harvard, whose name is Rick Blumberg [Dr. Richard S. Blumberg]. I've had a longstanding relationship with him. Initially, it was more a mentoring type relationship, and then we became colleagues and he's mentoring me to some extent. He developed the idea that the initial flora, which is inducing NK T-cells, is protective and prevents early enteritis. If you disturb this initial responsiveness to the flora, however, you become more receptive later on to ulcerative colitis. It's an interesting idea that is still being developed. I was just commenting on that.

Harden: What else do you need to tell me about your recent work that we should get on the record?

Strober: One of our major research approaches is to study the genes that are involved in the polymorphisms of inflammatory bowel disease. One of the other polymorphisms we are studying involves a molecule called LRRK2. LRRK2 stands for leucine rich repeat kinase 2. It is a “kinase” that phosphorylates other protein. It turns out that there's a polymorphism in LRRK2 that is associated with increased susceptibility to Crohn's disease. We recently published a paper showing that the reason for this increased susceptibility is that LRRK2 is part of certain kinds of innate immune responses and if you have an LRRK2 polymorphism you have increased responses.

In future studies we are planning to collaborate with scientists at Mount Sinai Hospital in New York who have access to large number of Crohn’s disease patients with this polymorphism. We're going to study how the polymorphism actually causes Crohn's disease. We think it may have something to do with the fact that LRRK2 enhances the responses to another protein molecule called NLRC4 [NOD-like receptor caspase recruitment domain 4] that is an important way the body produces IL-1beta.

Harden: One of the things I have noticed throughout our talk and in preparing for it that you have been very generous in training young scientists and in sharing credit. In the mid 2000s you were heaped with honors. They're all listed in your CV and I won't recite them, but in 2009 there was an article

in the *NIH Record* about you when you got the Beaumont Award. Dr. William Beaumont is well known in medical history as the first person to study a gastroenterological process directly. When you received the Beaumont Award, you stated that the award was really a testament to and recognition of the achievements of the entire lab. That was generous of you. You also stated that your most significant accomplishment was the training of young investigators. Would you tell me more about the people that you've trained?

Strober: As I've said to you during this long interview, quite a number of people have passed through my lab who have gone on to significant positions at various universities in the US and abroad. I mentioned Charles Elson; He became chief of gastroenterology at the University of Alabama. Another person is Steve James, who became chief of gastroenterology at University of Maryland. Now he is a major administrator of NIH. Markus Neurath went back to Germany and now is chief of gastroenterology at University of Erlangen, which is near Nuremberg. I visited him several years ago. He's continuing to do wonderful work, as are the others. Tomohiro Watanabe is now in Kyoto, and although he doesn't have a Chair yet, he probably will obtain one within the next few years.

I should mention Monica Boirivant [Dr. Monica Boirivant]. Monica Boirivant was a major figure unravelling the NK T-cells story; she's the one who developed oxazolone colitis initially. Now she lives and

works in Rome at the equivalent of our Public Health Service. She continues to do research, and I continue to collaborate with her.

I should mention Brian Kelsall [Dr. Brian Kelsall]. We didn't go into my work with him initially. Now he's become a major figure in the study of dendritic cells and macrophages that are present in the GI tract. He is still in NIAID is Chief of the Mucosal Immunobiology Section. He is also the founder and editor-in-chief of major mucosal immunology journal, *Mucosal Immunology*.

From 2001-2004, I was president of the Mucosal Immunology Society. Rick Blumberg, whom I mentioned before, was the person along with me responsible for conceiving the idea that there should be a journal in mucosal immunology. We asked Brian Kelsall to develop this, and he did a beautiful job in developing this idea.

Stefan Fichtner-Feigl [Dr. Stefan Fichtner-Feigl] was another German investigator in my laboratory. He's now a chief of surgery at a major university in Germany. Yes, there are quite a number of outstanding people who passed through the lab. They had positive experiences and continued doing research afterward or became very prominent in clinical practice.

Harden: You spent your whole career here at the NIH, and no doubt you've had all kinds of offers to leave and go to academia. Here you are subjected to all sorts of ethics rules, and travel rules, and everything else that comes with

being a federal employee, but you've never been enticed away. Do you want to comment about why? What does NIAID and NIH offer that keeps you here?

Strober: Well, you're totally free to do what you think is important to do in science. And you have resources to do it without being unduly burdened with having to write grant proposals, which inevitably make you want to do things that you can get funded rather than what you're interested in doing. It's an unusual and free kind of career. You're your own master in this career.

Harden: And you would recommend it?

Strober: I would recommend it. Well, I've said many times, if you're good at it, it can be a tremendously rewarding experience. But if you're not good at it, then you're not going to feel good and you're going to get out of it.

Harden: What does the future hold for you?

Strober: I'm going to continue on for a few years, but inevitably I'm going to retire. I'm not saying when.

Harden: Is there anything else you would like to talk about before we stop? I've come to the end of my questions.

Strober: No, I think that we have covered a great deal of ground.

Harden: Thank you so much. This is a wonderful oral history.