AN IMMUNOLOGIST'S JOURNEY

Nancy H. Ruddle

Introduction and Summary

I am so pleased to be here to be part of this amazing series of stories of how we arrive at the intellectual passions that drive our lives. I have been privileged and so lucky to be part of the field of immunology that just exploded over the course of my career and has become ever more relevant. We see immunology providing more and more direct benefits in infectious diseases, autoimmune diseases, organ transplants, cardiovascular and neurological diseases, and most recently and spectacularly in cancer. My focus throughout my career has been inflammation, and I have studied factors, called cytokines, which mediate this phenomenon. Two of these cytokines are lymphotoxin and tumor necrosis factor, or TNF. I have studied inflammation predominantly in animal models, especially the laboratory mouse, but with constant reference to the human condition. I have been fascinated by how immune cells, called lymphocytes, traffic around the body. They need to be located in specialized lymphoid organs like the lymph nodes, tonsils, and spleen, where they interact with foreign invaders or antigens, such as viruses and bacteria. Then they leave those sites of antigen presentation and go out into the body and attack the invading pathogens. Sometimes the immune system goes terribly wrong, in autoimmune diseases like multiple sclerosis, type 1 diabetes, and rheumatoid arthritis. In those cases, the lymphoid cells attack our own organs, like the brain, pancreas, and joints, thinking they are foreign. Much of what governs these diseases is the ability of lymphoid cells to traffic into those organs and cause tissue damage. In addition to taking you along my intellectual journey in the immune system, I will also provide some insight into the special challenges that I have faced as a woman, wife, and mother as our society and academia have evolved into what I hope is a better place for women and minorities.

Early Background

My parents came from somewhat different backgrounds, but were both highly influenced by the Depression. My mother's family had been very wealthy and fairly prominent

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Nancy H. Ruddle, Professor Emeritus of Epidemiology (Microbial Diseases) and senior research scientist, served on the Yale faculty from 1975 to 2016. She received her Ph.D. from Yale and was an associate research scientist in the Department of Surgery, a lecturer in microbiology, and a postdoc in microbiology. She was appointed assistant professor of microbiology in the Department of Epidemiology and Public Health, now known as the Yale School of Public Health, and rose through the ranks to professor. She maintained a joint appointment in the Department of Immunobiology at Yale School of Medicine from 1991 to 2016 and was awarded the John Rodman Paul Chair in 2002. In the course of her career, she served as head of the Division of Microbial Diseases in the School of Public Health, director of graduate studies, and acting associate dean and chair on two separate occasions. She is a fellow of the American Association for the Advancement of Science and the Connecticut Academy of Scientists and Engineers. Ruddle is known for her discovery of lymphotoxin and its roles in autoimmunity and development and her elucidation of organized lymphoid accumulations, called tertiary lymphoid organs.

in New York State. Her mother's side of the family were involved in shipping on the Hudson River, and her father's family were involved in politics. My great-grandfather was governor of New York State – no, not a Rockefeller or Harriman, but Benjamin B. Odell, a rival of Teddy Roosevelt. On the other hand my father's family were German-Irish living in St. Louis. My grandfather had a dry cleaning business. My dad was a chemistry major at Holy Cross College but had to leave during the Depression, to his great regret. My mother had also planned on going to college but because of the Depression was unable to until an aunt said she would pay for either my mother or her identical twin to go to a four-year college or for both to go to a two-year junior college. They went to Pine Manor and had one dressy dress that they shared. My mother's father managed to get a job in St. Louis, and my parents met there and married. My older sister was born and then I came along in 1940. My father joined the navy in the Second World War and we followed him around the country, finally settling in Elizabeth, New Jersey, because he was stationed in Newark.

Education

My love of biology began early on and I received encouragement from a few very different people. From the sixth grade on, I went to a small all-girls private school in Elizabeth, New Jersey. There, I had a rather eccentric science teacher who encouraged me to enter a Westinghouse science contest. My project was to determine how heavy water (deuterium oxide) might influence the growth of peas. Of course, I had no access to heavy water and no training in botany but wrote to Harold Urey, who had won the Nobel Prize in Chemistry for his discovery of deuterium. To my great pleasure, he answered my letter and said that he had studied zoology in college and really loved it. I have no remembrance of what I said in my letter, but I treasured his for many years. I also had no idea what zoology was. I didn't do particularly well in the Westinghouse contest, but my teacher's belief and Harold Urey's encouragement gave me a boost of self-confidence. Another encouraging person was a pediatrician, Bill Rumsey, who was a friend of my parents. He encouraged my desire to go to medical school, something my mother opposed. My father was silent on the subject, which meant that he probably supported it but didn't want to rock the boat. My mother thought it would be okay if I married a doctor. Believe it or not, for awhile I actually considered that a reasonable goal because I would be able to read my husband's textbooks. This was in the 1950s before the feminine mystique and any kind of consciousness raising.

I went to Mount Holyoke, a women's college that was known for being very strong in the sciences, still silently harboring the plan to go to medical school and major in zoology, which I finally understood was biology without the botany! I worked in various hospital labs in the summers and found it fun but somewhat repetitive. One important experience was a research project my senior year on the thymus directed by a professor, Kathryn Stein. I had been introduced to the thymus by a retired professor at Mount Holyoke, Christianna Smith, who was an expert on the histology of that organ. This small, wizened lady had come to our comparative anatomy class with a model as

large as she was and described what a mystery this organ was. At that time the function of that organ that lies right over the heart was unknown. The ancients had considered that the thymus might be the location of the soul. In 1961 Professor Stein went to a meeting at Oak Ridge National Laboratory, where the buzz was all about experiments that had been done involving removal of the thymus. Jacques Miller in the UK, Robert Good in Wisconsin, and Byron Waksman at Harvard had carried out experiments in which they removed the thymus from various animals, and all showed a profound depression in certain immune responses. We now know that this is because the thymus produces cells called T cells or thymus-derived lymphocytes that are crucial for the immune system. T cells are the cells destroyed in HIV/AIDS infections, explaining the profound immunosuppression of individuals infected with that virus. Professor Stein told me about these experiments and we designed some follow-ups in mice. I worked very hard and even won a little prize for the work but did not make any major advances. Nevertheless, I was really hooked on research from then on.

I graduated from Mount Holyoke in 1962. I was still a little unclear about my future plans, and I was somewhat intimidated by the idea of medical school, in part because I was a woman. At that time, the Yale School of Medicine class had only three women out of one hundred. I was debating graduate school and medical school and had been accepted at a few graduate schools, but the courses just didn't seem that interesting. I had avoided taking physics, which was a prerequisite for medical school, and decided to work for a year, begin to pay off my loan (about $1,000 as I remember), decide what I wanted to do, and maybe take a physics course. I came to New Haven to work as a technician with some colleagues of one of my Mount Holyoke professors. Sheila Counce and Bruce Nicklas were in the Zoology department, later Biology when it merged with Botany, now MCDB. On the day of my interview Sheila introduced me to three people. Mr. Roach, who resembled his name, was in charge of glassware and preparing Drosophila food; Sally Wilens had been the technician for Ross Harrison, a luminary in biology for his work on development and tissue culture; Frank Ruddle was a tall assistant professor who had moved into Ross Harrison's lab after Professor Harrison retired. Sheila never told me why she had chosen to introduce me to just those three people. I worked as a technician for Sheila and Bruce for a year and took courses as a special student, still avoiding physics. (I think my avoidance of physics was a reflection of my ambivalence about medical school. If I took it, I would have fulfilled the requirements.) I took a microbial genetics course with Ed Adelberg, who asked me what my plans were. I was pretty well set on going to Washington University, and he invited me to stay at Yale for a Ph.D. in the Microbiology department. I said I was interested in the thymus, and he told me that Byron Waksman, one of the early students of the thymus, was joining the Microbiology department in the fall. That information and the fact that I wanted to stay in New Haven because of the handsome guy I had met on my first day here (Frank Ruddle, not Mr. Roach) sealed the deal and I stayed. One reason that Dr. Adelberg was able to offer me a spot was that the husband

of one of the graduate students felt it inappropriate that she should be paid, although it was okay for her to accept the tuition stipend. The department could give me a half fellowship, which I used to pay tuition and then used the money I had been saving up to pay off my Mount Holyoke debt to live on. One problem was that I would have to take physics, a prerequisite for graduate school in the Micro department. I did that with a bunch of male undergraduates (as the undergrad campus was still not coed) and found that it was interesting and should not have been avoided.

I did my graduate work in the former Yale Microbiology department and had a wonderful time. This was when I really came into my own as a person and scientist. Frank Ruddle and I were married at the end of my first year. (The department suggested that since I was married maybe I didn't need the living stipend. I politely declined.) Frank's brilliant mind, sense of humor, and emotional support made my career and life and its many obligations not only possible, but fun. I did my thesis work with Byron Waksman. His lab was an exciting place with researchers from all over the world; my very best friends to this day include people with whom I trained. Immunology was a small field and Byron seemed to know everyone in it. We would go to the FASEB meetings in Atlantic City, and he would herd us around like a group of ducklings and introduce us to all the people in the field. I am still in contact with some of those people.

My thesis concerned a phenomenon known as delayed-type hypersensitivity. This is the skin test reaction that is the test for tuberculosis exposure. Our interest, and in fact my continuing interest, is in the basis of inflammation not only in the skin test, but also in autoimmune diseases like multiple sclerosis, type 1 diabetes, and rheumatoid arthritis, and graft rejection. The problem at the beginning of my thesis work was that one could only study these issues in a person or a whole animal. Thus, understanding the mechanisms of inflammation—how it happened, and how to inhibit it—was very difficult. Our goal was to develop a tissue culture method to study the phenomenon in vitro or "in glass," that is, outside the body. One group in New York was doing this concentrating on macrophage inhibition. We were more interested in tissue damage and decided to try to figure out a way to evaluate this aspect of inflammation. We were very successful and described an activity mediated by lymphocytes derived from the thymus (my original study subject at Mount Holyoke) that could kill cells, particularly tumor cells. We called it cytotoxic factor, a competitor called it lymphotoxin, and we now know it was a mixture of factors that included lymphotoxin (LT) and tumor necrosis factor, or TNF. In fact, there are more than fifty such factors, which are called cytokines, but since only two were known at the time, our discovery generated a lot of excitement. One of our papers demonstrated that cells in experimental autoimmune encephalomyelitis, or EAE, a murine model of multiple sclerosis, produced this factor. EAE has continued to be a focus of my work.

Since I was finishing my thesis, it seemed to be an ideal time to have a baby. Byron Waksman, my adviser, was going away for the summer, so I decided to tell him that

of one of the graduate students felt it inappropriate that she should be paid, although it was okay for her to accept the tuition stipend. The department could give me a half fellowship, which I used to pay tuition and then used the money I had been saving up to pay off my Mount Holyoke debt to live on. One problem was that I would have to take physics, a prerequisite for graduate school in the Micro department. I did that with a bunch of male undergraduates (as the undergrad campus was still not coed) and found that it was interesting and should not have been avoided.

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I was pregnant before he left. This resulted in the most painful conversation of my career. He swore (something I had never heard him do), slammed his hand on the table, and said: "I told you not to do that." I actually did not remember him telling me "not to do that." But even more devastating was his statement "This is the end of your career." When he returned in September, our interactions were very difficult but we managed to write four papers. I wrote my thesis and had the baby in February 1968, a week after I handed in the thesis. Byron visited me in the hospital and wanted to go over the manuscripts. I had thought I would do a postdoctoral fellowship but decided to take a part-time job so I would have more time at home with the baby.

Early Employment

Bernard Lytton had just set up a kidney transplant program at Yale and recruited me to set up a tissue typing lab to immunologically match potential donors and recipients. I accepted that job and was also appointed a lecturer in the Microbiology department. I had the resources to do research as well and could work half-time. After three years, I was pregnant again and I was ready to do postdoctoral work. Frank Richards and Martine Armstrong had come to Yale and had a project studying activation of murine leukemia virus. I was interested in tumor viruses, as I wanted to develop a way to immortalize the cells we had been studying that made lymphotoxin so that I could understand its regulation. I did a postdoctoral fellowship with them for three years, learned a lot of virology, and made some contributions to the field, including mapping the receptor for murine leukemia virus. The Microbiology department was dissolved just when it was time for me to move on to a faculty position. There were many reasons for this — including the establishment of the Human Genetics department and the idea that microbial diseases were not too important, as antibiotics could cure everything (except, of course, viral diseases). Many years later it was realized that this was a mistake, and I served on a committee that encouraged the establishment of the Department of Microbial Pathogenesis at Yale School of Medicine.

Faculty Appointment

In the mid-1970s, Epidemiology and Public Health (EPH) was a department of the medical school with a strong virology emphasis with Frank Black, Robert Shope, Robert McCollum, Greg Tignor, Wilbur Downs, Al Evans, and Dorothy Horstmann, who was also in Pediatrics. With the dissolution of the Microbiology department, EPH was given that teaching function for medical students, and Robert McCollum, the chair of EPH, recruited me to the department. I had already written and obtained an RO1 grant as a postdoctoral fellow, so I was a free agent and a bargain because my salary was completely covered by the grant. Although EPH has not always been a perfect fit, as I don't consider myself an epidemiologist, it has been a supportive home. There was no Immunology department at Yale at that time, although later an outstanding department was established. I have been fortunate to have a joint appointment in the Immunobiology department since its founding, which has provided me with wonderful collaborators

and students. My first appointment in EPH was as an assistant professor at 80 percent effort, as I wanted to be able to spend time with my daughters without feeling guilty. This meant that I probably worked more than full-time and was only paid 80 percent, and I could extend my time in rank. I am not sure that it is the best solution to the sometimes conflicting demands of writing grants and papers, running a lab, teaching, and being there for my family, but it worked for us. This was before the university had parental leave and other benefits that make child-raising a little more doable for young faculty. After five years of this, Dr. McCollum said he was uncomfortable with the fact that my salary was so low, and by that time, I could handle 100 percent effort.

Scientific Contributions

I have spent my entire career focusing on how lymphoid cells move around the body so that they will be in the optimal location for defense against pathogens. It turns out that LT and TNF, the factors we described in my thesis, which are cytotoxic, particularly for tumor cells, are also crucial for the phenomenon of cell trafficking around the body. I have been so lucky along the way, as the development of wonderful technologies has allowed us to probe deep into mechanisms of inflammation. Techniques were developed to clone T cells, the cells that make LT, which allowed us to determine how the cytokine was regulated and which of the various subsets of lymphoid cells could produce these factors. Then my group and others developed monoclonal antibodies that allowed us to precisely determine functions of cytokines. We took the family to Switzerland, where I worked at the Basel Institute for Immunology and learned the basics of molecular biology that allowed us to molecularly clone the gene for lymphotoxin. Frank Ruddle's group developed the technique of transgenesis, which allowed us to make transgenic mice that overexpressed the LT gene in particular organs, and then we were able to study mice in which the LT gene had been selectively eliminated. In addition to the incredible intellectual and emotional support of my husband and my wonderful daughters, over the years I have had many bright and hardworking undergraduates, medical students, doctoral students, postdoctoral fellows, and sabbatical visitors who made major contributions to our work. Science is really a team effort, which is why I describe *our* contributions, rather than *my* contributions. I was also blessed with great research assistants, many of whom stayed on for the duration. My last lab manager was with me for over twenty years and was crucial for the smooth functioning of the group.

Some of our important observations employed the techniques I mentioned above. We used a monoclonal antibody that eliminated the activity of LT and TNF and showed remarkable protection in the EAE model. That is, mice that had been treated to develop paralysis very similar to MS were protected if treated with this antibody. Although this did not work in the human disease, treatment with inhibitors of TNF has become the standard of care in many autoimmune diseases including rheumatoid arthritis, Crohn's disease, and psoriasis. In collaboration with a group at Washington University in St. Louis, we studied a mouse that was selectively deficient in LT. At the time, LT was considered to be a rather poor cousin of TNF because it did pretty much

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the same things, but was not made by macrophages and did not contribute to sepsis. In fact, David Chaplin was trying to eliminate TNF, and the LT knockout was going to be a rather boring control. He did not knock out TNF, but did knock out LT, which turned out to be a wonderful surprise. The LT knockouts were profoundly deficient in lymphoid organs. They had no lymph nodes, very disorganized spleens, and defects in other lymphoid organs as well. This told us that in addition to its important role in inflammation in response to infection or autoimmunity, LT also plays a crucial role in embryological development. At the same time that we were studying the LT ko mouse, we made mice that selectively overexpressed LT in the pancreas in collaboration with Richard Flavell, thinking we might generate a model of type 1 diabetes, a disease in which there is inflammation in the pancreas and eventual destruction of the cells that produce insulin. The transgenic mice had a tremendous amount of inflammation in the pancreas that looked very much like the early stage of type 1 diabetes, but no destruction of the insulin-producing cells. So then we knew LT was needed for lymph nodes and could cause inflammation. Then we looked more carefully at the inflammation in the pancreas of the transgenic mice and realized that the cells were organized in a way that looked very much like a lymph node. Then we thought a lot more about many autoimmune diseases in people and realized that many of them exhibit the same kind of inflammation, with an organization that resembles lymph nodes. These are now called tertiary or ectopic lymphoid organs. Thus the joints of individuals with rheumatoid arthritis include accumulations of cells that look like little lymph nodes. In multiple sclerosis, cells accumulate in the brain that look like lymph nodes; in Sjögren's syndrome and autoimmune disease affecting tears and saliva, cells accumulate in the salivary gland and lacrimal gland. We also see these ectopic lymph nodes (also called tertiary lymphoid organs, or TLOs) in microbial infections, such as Lyme arthritis, tuberculosis, Hepatitis C, *Helicobacter pylori,* and others. More recently there have been several papers that indicate that the presence of such tertiary lymphoid organs in breast cancer biopsies is a positive prognosticator of outcome. The more TLOs, the better the outcome with regard to survival and freedom from metastases. Thus our data and that of others suggest that these TLOs can serve a function very much like a lymph node. In the case of an autoimmune disease, this is detrimental. However, in the case of cancer or a microbial infection, it may be beneficial.

Much of my work in recent years has been aimed at understanding the vasculature of lymph nodes and ectopic lymphoid tissues. There is a special blood vessel called a high endothelial venule (HEV) that is regulated in part by lymphotoxin. HEVs allow cells that have not experienced antigen to enter into a lymph node and await the entrance of a foreign invader into the lymph node. These vessels are also seen in TLOs. I spent a wonderful sabbatical at the University of California in San Francisco learning more about these vessels from an expert there. More recently, I have become interested in lymphatic vessels that play important roles in fluid balance but are also crucial for moving lymphocytes around the body; I spent two months last year in Zurich with an

expert on these vessels. Some of our most recent exciting work involved developing transgenic mice that have green fluorescent HEVs and red fluorescent lymphatic vessels. These mice allow us to evaluate the events in a living mouse in the course of an immune response.

I have been an emeritus professor for a few years, which has allowed me to concentrate on my research without the distractions of administrative and teaching duties, though I still help out in some courses. Two years ago I decided to close my lab and was in the process of giving away my remaining grants and equipment. But then, on a whim, I applied for and received a small grant from the State of Connecticut to study the role of fat cells in lymphatic vessel development in inflammation. I am doing this in collaboration with a young faculty member with the participation of a student and postdoc. This has been a nice way to keep involved but to gradually slow down and feel less pressure to write papers, get grants, and more time to do other things. I am also having fun consulting one day a week for a small company in New Haven, particularly in its focus on small molecule inhibitors of cytokines.

In summary, my work has taken me from developing methods to study the immune system outside of the organism back to highly sophisticated imaging methods as we study events in real time in the living animal. Along the way we have generated fundamental insights into how the immune system develops normally in ontogeny, and the signals that can cause it to go awry in the adult. Despite the intense pressure to obtain research grants, write papers, and maintain some semblance of a family life, I feel that I have been incredibly lucky and fulfilled having had the opportunity to do such interesting work.