DONALD C. SHREFFLER
1933–1994

A Biographical Memoir by
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DONALD SHREFFLER

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DONALD SHREFFLER’s studies on the mouse H2 system played a major role in shaping immunology. Today the major histocompatibility complex (MHC) genes are the focus of study in many areas of immunology. The MHC molecules shape the T cell repertoire in the thymus by positive and negative selection, and in the periphery they generate the CD8 and CD4 T cells restricted by the MHC molecules to play a critical role in immunity. Specific MHC molecules appear to predispose the individual carrying them to develop certain autoimmune diseases. Structure/function studies on the MHC genes, their products, and on their role in human immunity and disease are widespread. In the 1960s the MHC genes were only an obscure curiosity studied by a handful of scientists interested in tumors and transplantation. The pioneers of the mouse H2 system played a critical role in the genetic fine structure studies and in developing inbred, congenic, recombinant strains of mice and reagents that were essential in developing the field. Donald Shreffler was one of those pioneers who paved the way for the explosion that occurred in this field during the 1960s and 1970s. He also provided precious mouse strains and reagents, as well as advice, to many scientists initiating these experimental studies. His work in the mouse also enabled the
rapid progression of studies on the human MHC genes, the HLA system. His place in the “Immunology Hall of Fame” is secure.

Shreffler sprouted from German roots that were transplanted to rural Illinois. His ancestors toiled and cleared the land and established a traditional German farm. Both the family and their farm flourished, and the Shrefflers became leading citizens in that area. They contributed to various community activities such as building churches, schools, and recreational centers. Don was born in the ancestral Shreffler home built in 1846. His father, Cecil, branched out into dairy farming and Don, who was the only boy in the family, became his father’s right hand from the time he could walk. His farm chores included milking the cows in the morning and working in the fields in the afternoons. Sunday was a family day. They would spend the day with church activities, family gatherings, and for Don, playing with his cousins, especially cousin Norman. All of his aunts and uncles remember Don as a shy, serious young man who always did the right things and never got into trouble.

He attended a grade school built by his grandfather and after finishing his elementary education went to high school in Reddick, 15 miles away. That meant getting up even earlier in the morning to finish his chores before taking the bus to school, finishing his evening chores later, and then staying up to finish his homework. Nevertheless, he not only did very well in his schoolwork, but he also participated in extracurricular activities, especially the 4-H club and the Future Farmers of America group. He did not have time for organized sports, but did play clarinet in the school band and the piano accompaniments for the church choir. He graduated at the top of his class of 32 students. His cousin Norman remembers him as a humble person who
preferred to be a face in the crowd at school rather than be recognized as the smartest one in his class.

Don loved farming and planned to join his father as a dairy farmer. In order to be closer to home he enrolled at the University of Illinois College of Agriculture. Besides studying and working part time to defray his college expenses he also found time to participate in intramural sports. Don graduated in 1954 with honors, but before he could rejoin his father on the farm, Uncle Sam called. He spent two years in the service, mostly in Japan, where he learned to appreciate foreign cultures and probably acquired his international outlook on life. After his service he returned to do graduate work in dairy science at the University of Illinois. During that time he met his future lifetime partner, Dorothy, a fellow student at the University of Illinois. It was love at first sight, and they soon married. Dorothy worked as a bookkeeper at the University bookstore while Don continued his graduate work. His master’s thesis project was in hemoglobin variants in dairy cattle, and he published three papers from his research.

Don’s thesis work gave him an appetite to learn more about biochemical genetics. He realized that state-of-the-art biochemical genetics research was being done by scientists at the California Institute of Technology (Caltech). He planned to stay at Caltech for one or two years to gain sufficient expertise and then to return to Illinois to complete his graduate work. But the longer he stayed, the more excited he became about the science going on at Caltech in the laboratories of professors Owen, Beadle, and Pauling. Finally, Don decided to stay at Caltech to complete his Ph.D. thesis in Ray Owen’s laboratory. He obtained a National Science Foundation predoctoral fellowship for his thesis research on mouse plasma proteins. Ray Owen recalled that
Don speedily settled into the research and teaching in our lab, and revised his intention of a limited stay. Turning mainly to mice as experimental animals, he began to define electrophoretic differences in plasma proteins among inbred lines, using Smithies’ starch-gel techniques. Beginning with antisera that had been prepared by students in the immunology course lab, he defined repeatable genetic patterns in gel diffusion serology, especially in globulins. He also looked at the hemoglobins of monkeys and participated in much that went on in the lab. Our interest in the early days of bone marrow transplantation, immunological tolerance, and the cellular antigens of mice was enriched by extensive working visits from such leaders as Elizabeth Russell of Bar Harbor on the genetics of blood cell formation in mice, David Nanney and his student Eduardo Orías, and Jean-Marie Dubert, Eleanor Brandt, and John Loeffer on immunogenetics and mating in Tetrahymena. Bill Stone, on leave from Wisconsin, looked for blood factors deriving from gene interaction, for human blood typing reagents from immune sera produced in cattle, and for effects of irradiation on the state of immunological suppression. Don worked with Prof. Jerry Vinograd on molecular hybridization of sheep hemoglobins, and studied serum protein types in mouse bone marrow chimeras. Sei Tokuda and Margaret MacGillivray were analyzing “parabiotic intoxication” and “isoimmune anemia” in rats and mice. Much was going on in other labs at Caltech as well, and Don interacted with many of them, to [their] mutual benefit. He was an excellent teaching assistant, devoting time and attention to students and cultivating creativity and substance in wide samples of biological science.

As part of his duties as a teaching assistant for an undergraduate immunology class, Don had to demonstrate immunoprecipitation. He made antisera against mouse serum in rabbits for this demonstration. These antisera gave very strong precipitation lines against most inbred strains of mice, but he noticed a unique reaction with sera from a few mouse strains. He designated these as being high or low in a serum substance (Ss) protein. Mating of Ss high mice with the Ss low mice gave intermediate levels in the F1 and segregated for the high and low phenotype in the F2 population. He realized that all the strains that had the Ss low genotype carried one particular MHC-H2 gene, and eventually showed
that the gene coding for the Ss protein was linked to the H2 locus. This finding was published in *Genetics* in 1963. Don and Dorothy enjoyed their time in Pasadena and also celebrated the birth of their first son, Doug. The group in Ray Owen’s lab became part of their family, and they enjoyed the wonderful hospitality at Ray Owen’s home.

After completing his Ph.D. at Caltech, Don had the option of either going back to the University of Illinois to continue his work on the immunogenetics of cattle or accepting a position at the University of Michigan to continue his work on the mouse Ss protein. His new discoveries in the mouse system convinced him to go to Michigan, where he was appointed research associate in the Department of Human Genetics. His studies on the mouse Ss protein revealed that the Ss gene mapped within the H2 gene complex in the seventeenth chromosome of the mouse. He established a close relationship with George Snell at the Jackson Laboratories in Bar Harbor, Maine. George, the “father of H2,” became his mentor, and he visited with him frequently to exchange ideas. With the help of Snell and Jack Stimpfling, he established a mouse colony at Michigan consisting of inbred, congenic, and recombinant strains of mice. This was before the days of lab animal medicine, and his mice were housed in a small building that was referred to as the “Mouse House.” These mice were bred and maintained by his students and technicians. As a member of the human genetics department he also collaborated with several of the faculty in the department (Neel, Brewer, Gershowitz, Weitkamp, Tashian, Rucknagel, and Sing) on various aspects of human biochemical genetics. During those early years at the University of Michigan he published several papers in these areas. In 1965 he was appointed assistant professor in the department. Shortly after coming to Michigan the Shrefflers welcomed their second son, David. Dorothy became
a full-time mother and a solid home support for Don as he climbed the academic ladder.

One of Don’s first two graduate students was Jane Schultz. Jane’s Ph.D. research was on the human serum lipoprotein. Jane Schultz had finished her undergraduate work and had started a family. By the time she started her graduate work, she already had three small children. Don, who was the youngest faculty member in the department, accepted Jane into his laboratory and gave her all the support she needed to complete her research within a reasonable time. Jane remembers Don as magnanimous, allowing her to interact with people outside the laboratory and even outside the department, and providing her with constant advice. Don was a perfectionist, so Jane had to work very hard to come up with an outstanding thesis. Her studies were published in the *Proceedings of the National Academy of Sciences* (PNAS). Jane also remembers Don as being a very social person, joining the graduate students for beer at the local pubs and at impromptu parties at society meetings, and enjoying the hospitality at their home.

The thesis of Don’s other graduate student, Howard Passmore, involved determination of structural differences between the Ss high and the Ss low protein. Howard generated alloantisera by cross-immunizing Ss high and Ss low mouse strains. He identified the presence of an antigen controlled by the Ss locus, expressed only in males, of the proper genotype. He designated this as “sex limited protein” (Slp). Immunological evidence suggested that the Slp antigenic sites resulted from structural variations of the Ss component of the mouse serum, either controlled by the same gene or by closely linked genes.

I met Don for the first time at a genetics meeting in 1968. I was a graduate student and because he looked so young, I assumed he was also one. I was amazed the next
day when I realized he was the keynote speaker, and he gave a masterful presentation. After I finished my Ph.D. and started exploring postdoctoral opportunities I contacted Don through a mutual friend Bill Stone. After visiting him there was no question in my mind about where I wanted to do postdoctoral work. His hospitality, kindness, and humility touched me very much. I was his first postdoctoral fellow, and since I was only three years younger than he and had two children the same age as his boys, he treated me more like a colleague that a trainee. My Ph.D. research had been in the area of chicken immunoglobulins and my knowledge of mouse genetics and MHC was minimal.

Don took hours of his time to painstakingly teach me the intricacies of the H2 system as it was known during the late 1960s. During that first year I had a very hard time understanding the system, and my productivity was nil. Don continued to be patient with me and continued to guide and advise me during that difficult time. At laboratory meetings, even if I made a stupid comment, he never ridiculed or embarrassed me in front of the others. He would talk to me privately in a very nice way, suggesting that maybe what I had said was not correct. Don took me to an H2 meeting in Bar Harbor organized by George Snell. About 20 people involved in H2 research participated. Finally, I was beginning to understand this system. My assigned project was to map conclusively the exact location of the Ss gene within the H2 complex. By undertaking an extensive classical gene mapping study and producing several recombinant strains of mice within the various H2 genes, I mapped the Ss gene to the middle between the major H2 loci, H2K and H2D. During this process several recombinant strains of mice were also identified that were identical at H2K and H2D antigens but differed in the Ss protein.

In 1969 Jan Klein joined our group from Prague. Jan
had been involved in extensive research on the H2 genes in Prague for several years at the Czech Academy of Sciences. He came to our group with a gold mine of knowledge in this area. His studies and his incredibly imaginative analysis of the data with Don resulted in a new hypothesis for the organization of the H2 genes. This was termed the two-gene model for the H2 system, with only two H2 loci, H2K and H2D with the Ss gene mapping in the middle. All the controversies regarding other minor H2 genes in the complex were determined to have resulted from cross-reactions between H2K and H2D antigens. This new model proposed that the H2 gene complex was much simpler than had been previously thought, and made the regulation and function of these genes and their products more comprehensible. This important paper was published in the *Journal of Experimental Medicine* in 1972.

During this time Hugh McDevitt at Stanford University had discovered immune response genes. He had found that the immune responses against synthetic polypeptide antigens were controlled by genes that he designated as Ir genes, and more importantly he found that these Ir genes were linked to the H2 genes on the seventeenth chromosome. This resulted in a major collaboration between Hugh McDevitt, Donald Shreffler, Jan Klein, Jack Stimpfling, and George Snell to map the location of the Ir gene. Studies using the various recombinant strains showed that the Ir gene mapped inside the H2 gene complex, probably between H2K and the Ss protein. This major breakthrough was also published in the *Journal of Experimental Medicine* in 1972. Until then the H2 genes had been a curiosity worked on by a small number of immunogeneticists and investigators interested in transplantation. The finding that genes controlling immune responses mapped within H2 attracted a much wider group of immunologists. Don and Hugh McDevitt
respected and admired each other, which led to a strong scientific interaction and close friendship. Hugh remembers Don as “a gentle, thoughtful, and rigorous investigator who was extremely generous with his many MHC recombinant mouse strains, as well as with advice.” Hugh remembered that “during our Ir gene mapping studies Don was extraordinarily helpful. In discussions both here at Stanford and [at] the University of Michigan, I was always impressed by Don’s gentle approach to a scientific problem, and his very strict and rigorous application of genetic principles to experiments and experimental results.”

In 1971 Don went to the Basel Institute of Immunology on a sabbatical leave. While there he made two important findings. In collaboration with Bernice Kindred he found an H2 dependence on the cooperation between T and B cells in vivo, a precursor to the MHC restriction studies. In collaboration with Tommy Meo he found that differences in the Ir region were sufficient to stimulate a mixed lymphocyte reaction suggesting an antigen controlled by the Ir genes. During this time Hugh McDevitt and Baruch Benacerraf had speculated that the product of the Ir gene might be the elusive T-cell receptor. This started the race to identify the Ir gene product and possibly the T-cell receptor.

At this time I finished my postdoctoral work and was appointed assistant research scientist in the human genetics department working with Don Shreffler. We were fortunate to have two recombinant strains of mice that were identical for the H2K and the H2D antigens but different in their Ir genes. We decided that cross-immunizations of these two strains of mice (A.TL and A.TH) could potentially generate antisera that could identify the Ir gene product. Similar studies were also initiated in the laboratories of Hugh McDevitt, Jan Klein (who now had his own laboratory at Michigan), and David Sachs at the National Institutes of
Health. The antiserum we produced was unlike any other antisera we had made for the H2 genes. In general it reacted only with 40-50 percent of lymphoid cells, but it had a much higher titer than any other antiserum. It reacted predominantly with B cells, but its reactivity with T cells was controversial. During this time Jeff Frelinger, who like Don had been a graduate student with Ray Owen at Caltech, joined our laboratory and assisted us in this project. Extensive serological, genetic, and immunological studies using this antiserum with the different mouse strains proved that it was indeed detecting a product from the Ir gene itself or from a closely linked gene. The antigen was designated “Ia” for immune-associated antigen. Our first paper was published in the PNAS in 1973. Klein and his associates also made similar observations in their laboratory. The identification of the Ia antigens was a major breakthrough showing a second MHC-linked antigen associated with the genetics of the immune response. Further studies showed that the Ia antigens were expressed predominantly on antigen-presentation cells and not T cells.

Jeff Frelinger fondly remembers many parties at Don’s house, especially the annual Christmas parties. Dorothy collected little mouse figures that the whole family would hide around the house. Hundreds of these little mice (maybe there were thousands!), wooden ones, plastic ones, ceramic, and glass blown mice [were] hidden everywhere: on the book shelves, hanging on the [Christmas] tree, hidden in the intricate carvings of the fine old-world furniture that Don’s father or grandfather had hand-carved, [and] on Don’s desk. [There were] mouse refrigerator magnets, [and some] even sitting in the bottom of the fish aquarium. We’d have a scavenger hunt, and there would be a prize for the person who found the most mice. All of this occurred with a traditional Midwesterner’s openness and friendliness; a sense of honesty and good family values prevailed.
Jeff credits Don for serving as a model for his own career. Don always gave us freedom. Even when he was really involved with a project, such as the beginning of the class II discovery, I never felt that he was trying to manage the project, but rather trying to guide all of us through the process of discovery. Certainly it was an exciting time, every day brought a new result. Don remained calm and supportive throughout the whole process. Don also let us be the young and excitable scientists we were.

Don was a very generous person. He could have kept all of the antisera and the recombinant mouse strains for himself to pursue all the interesting studies. Instead, he freely gave his sera and mouse strains without any strings attached to anyone who wanted them. This was an enormous boon to many laboratories around the world exploring the role and function of the Ir gene products. In a major collaboration between our lab and Stan Nathenson’s laboratory at Albert Einstein University, Susan Cullen showed that Ia antigens consisted of two chains of 33,000 and 31,000 molecular weight whereas the molecular weight of the subunits of the H2K and the D molecules were 45,000. Her findings showed that the Ia antigens were a new class of MHC antigens, and were published in the PNAS in 1974. Jan Klein designated the H2K and the D molecules as class I antigens and the Ir-associated Ia antigens as the class II antigens. The Ss/Slp was designated as class III antigens.

Despite the excitement about the discovery of the class II antigens, Don had not forgotten his first love, the Ss protein. A new graduate student in the laboratory, Ted Hansen, took over the project and made an important observation. He found a significant correlation between the levels of Ss protein and hemolytic complement activity. The results suggesting that the Ss protein might be a component of the complement system were published in the Journal
of Experimental Medicine in 1975. Tommy Meo from the Basel Institute of Immunology joined the lab for a sabbatical and got interested in the possibility that the Ss protein might be a component of the complement system. His studies led to another major finding that a component of human plasma and the Ss protein were cross-reactive. The human plasma component was identified as the fourth component of complement. The conclusion, that the Ss protein was probably the fourth component of complement in the mouse, was published in PNAS in 1975. Until then there had been a question of why there was no homologous gene for Ss in the human, while there was considerable homology in the class I and class II genes in human and mouse. Later the human C4 gene was also found to map within the human MHC region, showing there was complete homology between the human and mouse MHC.

Don had few activities apart from his work and his family, but he did enjoy fishing, where he could completely relax. He was a serious fisherman, allowing him to get his mind away from work in order to focus on what he was doing. Because his sons, David and Doug, were also avid fishermen, it provided a chance to spend time with them. The Shrefflers had a cottage on Little Silver Lake near Ann Arbor, where his family spent most of their summer weekends fishing for bass and bluegill. Don spent several weeks each summer teaching a mammalian genetics course in Bar Harbor, Maine, where he took his whole family. The main activity there was mackerel fishing. He not only loved catching fish but also loved eating them, and my family and I enjoyed many wonderful fish dinners at Don’s house even though I never contributed to catching the meal. As a matter of fact, the one time I went fishing with Don I messed up all his lines so badly it took him an hour to untangle them. He never lost his temper or cool though, and kept smiling
during the whole ordeal. David, his son, feels sad that his children won’t have a chance to get to know their grandfather, but he plans to make sure that they know what a terrific guy he was: his sense of honesty and integrity, his passion for his work and his family, his patience and well-honed listening skills, his wonderful wit, his generosity, his humility, his quiet pride, and his farm-boy roots. The year he spent on sabbatical in Basel, Switzerland, was a special time for his family. They got to travel and see many wonderful things. His family will always remember his mischievous grin.

In 1975 an exciting proposal was offered to Don. Washington University in St. Louis wished to create a new department of genetics composed of several “superstars” in immunogenetics, such as Hugh McDevitt and Stan Nathenson. This was too exciting for Don to turn down. He accepted to move to Washington University, assuming that one of the others would be appointed chairman of the department. Unfortunately, the others decided not to make the move, but since Don was already committed, he agreed to be the acting chairman to start the department. I was recruited as one of the faculty in the department, and Don and I moved to St. Louis in the fall of 1975. The McDonnell Foundation was funding the department, and state-of-the-art laboratories and mouse-breeding facilities with all the amenities were constructed. Don had the opportunity to design one of the best mouse facilities in the country. In 1977 I moved my lab to the Mayo Clinic.

Despite the heavy responsibility of recruiting and building a new department of genetics, setting up the graduate program, and his teaching responsibilities, Don’s lab continued to be very productive on the complement/Ss system. He set up a long-term collaboration with John Atkinson in the Department of Medicine in these studies. The next major breakthrough in the complement system came when Keith
Parker, a graduate student in his lab, showed that the Ss protein and the Slp proteins were discrete components coded by closely linked genes within the S region. Subsequent studies by Parker and another graduate student, David Karp, thoroughly characterized the Ss and Slp proteins biochemically. Keith Parker remembers Don as one of those ideal mentors who was always available for discussion, listened to all sides of a question, had an open mind, and was uncompromising when it came to the integrity of the results and the data.

David Karp remembers Don as

...a very supportive mentor whose scientific thought was both rigorous and creative. He taught us clarity and precision in our writing. He allowed us to pursue independent projects with what I felt was just the right amount of supervision. He was very supportive when it came to students attending and presenting at meetings. On one occasion, he wrote a generic abstract for a meeting that he was asked to present, saying that whoever had the most data could make the actual presentation. Although I am biased because I got to speak at the meeting, I think that was a very generous way to transfer recognition from a lab chief to the people doing the work. The Department of Genetics at Washington University is a lasting legacy to his organization and scientific foresight.

Don worked tirelessly to put together a top-notch group of interactive scientists working in a broad area of genetic model systems. Ted Hansen, a former graduate student in Don’s lab, joined his department to continue his work on immunogenetics of the mouse MHC system. Don didn’t just recruit individuals to his department who were interested in immunology; he engaged geneticists with widely different interests.

In 1980 Don was elected to the Institute of Medicine and in 1982 to the National Academy of Sciences. He also contributed to the American Association of Immunology and in the years 1982-88 served on its council, as vice-
president, and finally as president. In 1984 he stepped down as the chairman of the Department of Genetics to concentrate on his research and other extramural activities. In 1986 the fiftieth anniversary of the discovery of H2 antigens by Peter Gorser was celebrated in Bar Harbor. The pioneers of the H2 field—Peter Grover, George Snell, Bernard Amos, Jack Stimpfling, Don Shreffler, and Jan Klein—were all honored at this happy occasion. In 1989 Don was honored as a distinguished alumni of the California Institute of Technology.

In the late 1980s Don’s health deteriorated, and he was able to spend only limited time in the laboratory. All of his former students, postdocs, and colleagues got together to celebrate his sixtieth birthday in Bar Harbor in June of 1994. He appeared to be doing well both emotionally and physically, and we were all happy that he was on his way to a full recovery. He was looking forward to taking an early retirement to enjoy other things in life. None of us anticipated that he would die within a few months. He was laid to rest in a spot he loved most: his family farm near Kankkee, Illinois, next to four generations of Shrefflers.

Don Shreffler’s death was a big loss to the field of immunogenetics, because we missed the intellectual contributions he had consistently provided. One way to judge the contribution of a person is by the human legacy he leaves behind. The students and the postdocs that Don trained hold important positions in the field and have themselves made major contributions. He gave full credit to his students and postdocs for all his accomplishments. I for one owe my career to Don Shreffler. I worked seven years with him, and credit him with teaching me to become a scientist. Another way to judge an individual is by the contributions he has made to his colleagues. Shreffler selflessly helped many laboratories flourish and succeed.
I recruited the help of many individuals to write this memoir. I thank the members of the Shreffler family who provided me with the information on his childhood. To Don’s wife, Dorothy, and his sons, David and Doug, for their input. I also appreciate contributions from Ray Owen, Jane Schultz, Hugh McDevitt, Jeff Frelinger, Keith Parker, and David Karp. I especially thank Henry Metzger for giving me the opportunity to write this memoir and for reading the manuscript and providing helpful suggestions. The material on Don Shreffler’s early life came from the “In Memoriam” I wrote in 1995 for *Immunogenetics* (vol. 41, pp. 175-177, Springer-Verlag).
Selected Bibliography

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