I WOULD LIKE TO BEGIN by expressing my thanks to the ASTDA and the Thomas Parran Selection Committee for this wonderful honor. None of us walks alone during our careers, and we depend upon mentors, colleagues, students, fellows, and research scientists who work on our projects with us. We also depend upon our families who give us perspective and balance. I have been blessed by wonderful support from all of these, and I am very grateful. The Parran Lecture frequently provides a retrospective view of a career or a field, and I would like to take you on a chronological journey over the past 30-plus years of syphilis research, describing our efforts to unravel the very complex relationship between Treponema pallidum and the host. I was introduced to syphilis in 1973 and was immediately captivated by the fascinating bacterium that causes this infection.

However, my research career began before that time, when I was an undergraduate at University of California, San Diego. My first research mentor was Willie C. Brown (Fig. 1), a Professor of Biology. Willie introduced me to microbiology and agreed to let me conduct an independent study project in his laboratory. I proposed to make L-forms of Bacillus subtilis, the organism under study in his laboratory. L-forms are bacteria that lack cell walls and they are very difficult to maintain in culture because of their extreme fragility (perhaps presaging my future career focus!). Under Willie’s guidance, I learned to design logical experiments, to critically evaluate my results, and to regroup when things did not work out as planned. Most importantly, I learned that I loved the challenge and the freedom of developing my own research plan. As I prepared to leave his laboratory to begin graduate school in microbiology at UCLA, Willie gave me some advice: “Work on a bug that can be grown in liter quantities overnight” and “Be sure you can put it in the freezer when you go on vacation.” Although I learned much from Willie, I am afraid that I totally ignored this advice, as I have spent the rest of my career working on an organism (T. pallidum) that does not even grow in culture!

T. pallidum must be propagated by passage in rabbits and therefore cannot be manipulated genetically for examination of putative virulence factors. Equally problematic is the unusual ultrastructure of T. pallidum in which the cell wall layer is more closely associated with the cytoplasmic membrane than with the outer membrane, resulting in a very fragile, easily damaged surface structure. Because of these impediments, syphilis research moves slowly compared with other fields. When I was a young investigator, a well-respected senior scientist (unnamed here) spoke words of dubious encouragement to me: “Watching the syphilis field is like watching a glacier move!” Nonetheless, spectacular progress has been made in the past 30 years, due to the passion and commitment of the small number of investigators who work on this organism. Critical to that progress is the support of the STD Branch of the National Institute of Allergy & Infectious Diseases at NIH, whose directors (Judy Wasserheit, Penelope Hitchcock, and Carolyn Deal) have uniformly recognized the continued importance of syphilis and have been very supportive of research in this field.

Syphilis is one of the oldest recognized sexually transmitted infections and has a fascinating history, from the continuing speculation about its hypothesized New World origin to the legsions of famous writers, musicians, painters, and politicians who reportedly suffered from the disease. During the early 1900s, syphilis was a very common infection, estimated to affect ~10% of the population of the United States and Western Europe with much higher prevalence in some demographic groups. Thomas Parran, who became Surgeon General of the United States in 1936, took a very broad approach to syphilis control as described in his famous monograph Shadow on the Land,1 including a widespread educational campaign to make the US population aware of the serious problem around them and the “dragnet” approach of broad serological screening, rapid treatment of patients, and vigorous contact tracing. It is indeed humbling to receive this award that is named in his honor.

The natural history of syphilis was well-understood during Parran’s time and, except for the important interactions with HIV infection and the significant reduction in tertiary disease, is largely unchanged today. I would like to use natural history as the frame-
work for talking about the pathogenesis of syphilis and the evolution of our understanding of this process over the years. You will see that this is a story of research teams, not of any individual.

Syphilis is sexually transmitted by direct contact with an infectious lesion, and the primary and secondary stages are infectious. In each of these two stages, the major manifestations are skin lesions (the primary chancre and the secondary skin rash), triggered by the host’s response to a focus of treponemal multiplication. *T. pallidum* bacteria are highly invasive and gain access to the bloodstream and lymphatics very early in infection; through the circulation the bacteria disseminate, potentially reaching every part of the body including the central nervous system. The skin lesions persist for weeks or sometimes months and then heal spontaneously without medical intervention. The patients then enter the latent stage, in which infection persists but there are no clinical manifestations; this stage can last for many decades or the remainder of the person’s life. In the preantibiotic era, approximately one-third of infected persons developed late, often serious, tertiary clinical manifestations involving skin, bone, the aorta, the brain, and spinal cord. How does this fragile bacterium cause the chronic and complex disease that has been termed the “great imitator”?  

My journey in helping to unravel this mystery began in 1973 when I went to the University of California, Los Angeles, to pursue a graduate degree. New graduate students rotated through different laboratories during their first year to identify a mentor and laboratory for their dissertation research; most students performed three laboratory rotations before making a decision. I knew, and laboratory for their dissertation research; most students pursued a graduate degree. For this achievement and others, Dr. Miller would be selected to receive the Parran Award in 1985. He was, and still is, a wonderful mentor to me.

In addition to the dynamic laboratory atmosphere that Dr. Miller provided, with a number of other graduate students, postdocs, and technologists, he taught me the incredible value of knowing the old literature. “Know what came before you.” I remember many times that we would be talking about a particular topic and he would reach up to his shelf and pull down (from his well-organized files) an old article that had important insights into the topic at hand. With his encouragement, I spent hours in the bowels of the UCLA library searching the old publications on syphilis. The early investigators were not constrained by editors who want to minimize figures, tables, and page length; these early and very lengthy publications were true scholarly works in which exquisite detail was provided. It is often only through the examination of the fine detail of the results that important insights can be gleaned. The discussions in these articles were full of speculation and argument, providing a clear window into the scientific thinking of the author. Through careful reading of these classic papers, we can still today find valuable information that, when put in the context of our modern understanding, can direct our current efforts. When Justin Radolf and I were working on our recent book, *Pathogenic Treponema: Molecular and Cellular Biology*, we spent many hours reading and rereading many of the old classic papers on syphilis, and we found numerous instances in which early scientists speculated on concepts that we considered to be modern.

What did we believe about syphilis immunology in 1973? We believed that antibodies were the key element in the immune response. There was an active body of literature, based upon the study of peripheral blood lymphocytes, purporting that cellular immune function is generally suppressed during early syphilis. *T. pallidum* was believed to be resistant to phagocytosis by macrophages and neutrophils. It was also believed, based upon the vaccine studies of Miller, that protective immunity can be induced only by a labile and scarce surface antigen. There were, however, contradictions between some of these assertions and what we knew about the disease process. For example, antibody titers are very high during the secondary stage—a time at which millions of *T. pallidum* are found throughout the body of the infected person, seemingly oblivious to the antibodies swirling around them. The histopathology of primary and secondary skin lesions was already well recognized as a robust lymphocytic infiltration, which is not consistent with an hypothesis of generalized cellular immunity during early syphilis.

So, I set out to dissect the immune mechanisms in early syphilis, with the goal of figuring out how *T. pallidum* are cleared from the chancre and rash before spontaneous resolution of the lesions. Cellular immunity was coming into its own at this time and I was in a department with a heavy focus on tumor immunology. Consequently, I spent years trying to show that lymphocytes from infected rabbits were directly cytotoxic to *T. pallidum*, just as lymphocytes could be cytotoxic to tumor cells. Many experiments later and being no closer to finishing my PhD degree, I decided to try a new approach. This was a period of very active work, by George Mackaness and others, on the role of macrophages in chronic infections, and I decided to see whether rabbit macrophages could ingest *T. pallidum* in a culture system. My very first experiment was a success and showed evidence of phagocytosis of the bacteria by macrophages—quite a thrill after years of disappointment! Dr. Miller, however, maintained an appropriate level of skepticism, and challenged me to convince him that my conclusions were correct. Eventually, he was persuaded. These cell-focused studies came full circle, back to the role of antibody in
immunity, in that specific antibodies were opsonic for *T. pallidum*, significantly enhancing the phagocytosis of the organism by macrophages.4

Around this time, Dr. Stewart Sell (Fig. 1), a well-respected rabbit immunologist, became interested in working on syphilis using the rabbit model. He called the Miller laboratory looking for someone who was experienced with syphilis, so I moved down the road to UC San Diego to set up a syphilis research program as a postdoctoral fellow in his laboratory. Stew is a pathologist and the most important thing that he taught me was to “Look at the disease that you are studying.” Stew and I spent hours looking through a microscope observing that, during early infection, *T. pallidum* increased in numbers locally in concert with increasing numbers of infiltrating lymphocytes. As treponemes reached peak numbers, macrophages began to infiltrate the lesions and then the bacteria essentially disappeared—from billions to rarely seen within days.5

At this point, the only material that stained with anti-*T. pallidum* antibodies was apparently digested bacteria within the infiltrating macrophages, providing in vivo confirmation of the in vitro phagocytosis that we described earlier. After the bacteria were cleared by the immune response, the lesions resolved, just as in human syphilis. These results were certainly not consistent with a prevailing theory of syphilitic immunosuppression.

Just before my arrival in the laboratory, Stew and his group had developed specific antisera to differentiate rabbit B and T lymphocytes, so we applied these reagents to the study of syphilis. Working with Sharon Baker-Zander, who became a friend and long-time colleague, we showed that splenic and regional lymph node T lymphocytes become sensitized to *T. pallidum* antigens very early during syphilis infection,6 again finding absolutely no evidence for either specific or generalized immunosuppression. In contrast, the cellular immune response is consistently robust and long lasting.

In 1979, I traded sun and sand for the drizzly Pacific Northwest when I moved to Seattle to continue my postdoctoral training with Dr. King Holmes. Even at that time, King was recognized as a leader in the growing field of sexually transmitted diseases, and he received the Thomas Parran Award in 1983. King has truly been a great mentor to me. He has pushed me when I needed it, and has, from the beginning, fostered my independence. On my first day in Seattle, he told me that I needed to “write a grant”—essential advice for a young person seeking to establish an independent career! King has also taught me to look outside the laboratory and to study human syphilis to identify important research questions. Over the years, several such questions have been of particular interest, including the invasion of the central nervous system by *T. pallidum* and its persistence there following treatment with benzathine penicillin. After completing her fellowship work in my laboratory, Dr. Christina Marra has continued neurosyphilis studies, focusing on developing evidence-based guidelines for cerebrospinal fluid examination in syphilis and examining the influence of concurrent HIV infection on neurosyphilis progression and response to treatment.7-9

In the laboratory, our group (including Sharon Baker-Zander, Mindy Fohn, Jeanne Shaffer, and Christa Castro) continued our investigations on the mechanisms of immune responses in early syphilis, with a particular focus on opsonic antibody and phagocytosis. Opsonic antibodies were shown to be directed against antigens that are unique to the pathogenic treponemes,10 and not those antigens shared with cultivable treponemes. We also dem-

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**Fig. 2.** Immune mechanisms in early syphilis. Following antigen presentation, CD4⁺ T lymphocytes become sensitized to *T. pallidum* antigens and infiltrate the lesion sites. Locally, T cells produce γ-interferon, which attracts and activates macrophages. CD4⁺ T cells also provide help for maturation of B cells to produce antibodies to *T. pallidum* antigens. These antibodies opsonize *T. pallidum*, facilitating the phagocytosis and killing of the treponemes by activated macrophages, resulting in resolution of primary and secondary lesions.
onstrated that sensitized T lymphocytes produce “macrophage activating factor” (now known to be interferon-γ, IFN-γ) that enhances the ability of macrophages to ingest and kill T. pallidum.\textsuperscript{11}

Although in vitro studies are very interesting and informative, it is critical to demonstrate that phenomena identified in vitro are relevant to the actual disease setting. We thus began a collaboration with Wesley Van Voorhis, Frank Plummer (then at University of Manitoba), and James Nasio (then at University of Nairobi) to examine the nature of the lymphoproliferative activation and the cytokine milieu in early syphilis lesions in humans. Correlative studies were undertaken in the rabbit model with Barbara Molini, Troy Leader, and Charmie Godornes. This work demonstrated that, in both humans\textsuperscript{12,13} and rabbits\textsuperscript{14,15} with early syphilis, the infiltrating lymphocyte populations include both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, and that the cytokine milieu is Th1-type, with IFN-γ as the predominant cytokine.

These studies led to a new paradigm of immune responses in early syphilis (Fig. 2) in which T lymphocytes are specifically sensitized to treponemal antigens and infiltrate the local sites where T. pallidum are multiplying. The T cells produce cytokines (e.g., IFN-γ) that attract and activate macrophages. Some CD4\textsuperscript{+} cells act as helpers for B lymphocytes to produce specific anti-T. pallidum antibodies, including those with opsonic capacity. Clearance of the treponemes occurs as the bacteria are opsonized by antibodies, then ingested and killed by activated macrophages. Importantly, the cellular infiltration and mechanisms of bacterial clearance appear to be the same in both primary and secondary lesions, and the immune response is effective. There is no experimental evidence for immunosuppression or a Th1-to-Th2 shift from the primary to the secondary stage, as had been suggested in the literature.\textsuperscript{16}

I told you at the beginning that, after the early lesions heal, the infected host enters the latent stage in which treponemes persist yet clinical manifestations are absent. Given the argument that we just made about a robust and effective immune response in early syphilis, how do some treponemes escape clearance to cause persistent infection? The question is still under investigation, but we have made some intriguing observations and hypotheses. You remember that early bacterial clearance is mediated by activated macrophages and that only opsonized treponemes are ingested. Is there something different about the treponemes that escape ingestion? The answer is “yes.” When we harvested treponemes from rabbits that had already undergone normal bacterial clearance and subjected these persistent organisms to our normal phagocytosis assay, we found that these treponemes were resistant to phagocytosis even in the presence of immune serum.\textsuperscript{17} These same macrophages were able to ingest “regular” treponemes, so something must have changed in the persistent treponemes themselves. We speculated that the surface antigen(s) of the escapees must have been altered so that opsonic antibody failed to bind.

The surface of T. pallidum continues to be a mystery, so far evading the best efforts of investigators to define it. In addition to the fragility of the outer membrane, mentioned earlier, 2 groups independently demonstrated by freeze fracture electron microscopy that the outer membrane is relatively devoid of integral outer membrane proteins,\textsuperscript{18,19} having approximately 10% of the density of integral proteins as a typical Gram-negative bacterium. This lack of surface-exposed antigens explains the decades-old observation that antibodies require prolonged incubation times to bind to T. pallidum in neutralization\textsuperscript{20} and immobilization\textsuperscript{21} studies, and it also explains the slow rate of opsonization of T. pallidum.\textsuperscript{22} Despite the rarity of surface proteins, we were convinced of their existence, and several research groups set out to identify them.
To address these questions, we developed a clonal strain of *T. pallidum* that had a single *tprK* sequence. We passed this strain 8 times rapidly in rabbits (passing the organisms before anti-*TprK* antibodies are detectable), then passed it 4 times at longer intervals (during which time the rabbit’s immune response develops and clears the majority of the treponemes from the tissue site). At each of these passes, we sequenced the *tprK* genes contained in the harvested treponemes and found that sequence diversity is generated during infection; importantly, the degree of sequence diversity increases during longer passes, in which the developing immune response may select against treponemes expressing the original *TprK*. If the hypothesis of immune selection is to be viable, though, the immune system must specifically recognize the V regions. Cecilia Morgan, a graduate student, mapped the B and T cell epitopes in *TprK* using synthetic peptides and clearly demonstrated that, while T cells recognize epitopes located in the constant regions of *TprK*, infection-induced antibodies recognize V region epitopes, consistent with the notion that antibodies (perhaps through opsonization) might select against bacteria expressing the recognized *TprK* V regions. New variants, on the other hand, would have a selective advantage. Rebecca LaFond, another graduate student, demonstrated the exquisite specificity of anti-V region antibodies, showing that very small changes in amino acid sequence can obliterate antibody epitopes.

Arturo noted that the sequence variations were caused primarily by apparent insertions or deletions, rather than point mutations, so he looked for the source of the new V region sequences. In searching the *T. pallidum* genome, he identified 2 regions flanking the *tprD* locus that contain over 50 “donor cassettes,” corresponding to portions of the variant V regions that we had identified in the *tprK* locus in our strains. These cassettes appear to provide the new sequences that are recombined into the V regions of the gene in the *TprK* expression site, and it is possible to identify the specific sources of the new V region sequences that appeared during our experiment. Because the donor region sequences do not change, even when the *tprK* ORF sequence does change, the mechanism is thought to be gene conversion, rather than reciprocal recombination.

Our hypothesis, then, is that antibodies develop against the V regions of the infecting strain and that these antibodies opsonize *T. pallidum* for phagocytosis by macrophages, resulting in clearance of the majority of treponemes from the early lesion. Those few organisms that have new variant V region sequences are not recognized by the antibody and are thus able to evade the immune clearance mechanism. This scenario is likely, however, only if the V regions of *TprK* are exposed on the surface of *T. pallidum*. What do we know about the structure of *TprK*? Computer predictions of the structure of *TprK* suggest that it is a porin-like molecule with significant transmembrane β-barrel conformation (unpublished). Interestingly, this structural prediction shows that many of the V regions are extracellular loops that would be exposed on the surface of the bacterium and in potential contact with antibodies.

To bring this journey full circle, how does it fit with what we know of the natural history of syphilis? The treponemes that trigger the development of the lesions of primary and secondary syphilis are eventually cleared by the host’s immune response, via macrophage-mediated phagocytosis and killing of antibody-opsonized bacteria, thus setting the stage for resolution of the early lesions. During the development of that immune response, a small subset of treponemes alters its surface antigens so that it is no longer recognized by antibodies. They may turn off expression (phase variation) of some *Tpr* antigens and may change the surface-exposed antibody epitopes (antigenic variation via gene conversion of the V regions) of *TprK*. The predicted repertoire of possible *TprK* V region sequences is well over 10⁵, allowing for many years of immune evasion. The variant bacteria are selected for survival by the immune system and are thus able to persist in the host for decades, causing the persistent infection of the latent and tertiary stages.

*T. pallidum* is a formidable subject for investigation, and it does not give up its secrets easily. Nonetheless, a cadre of dedicated investigators continues to study this fascinating bacterium and, using new tools and ideas, will continue to make progress in understanding the host-parasite relationship of syphilis. I thank the ASTDA again for this wonderful honor, and I also thank the many colleagues with whom I have had the privilege to work throughout the years. This award belongs to the team.

References


