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# Peeking Into the Black Box of T Cell Receptor Signaling

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## Abstract

I have spent more than the last 40 years at the University of California, San Francisco (UCSF), studying T cell receptor (TCR) signaling. I was blessed with supportive mentors, an exceptionally talented group of trainees, and wonderful collaborators and colleagues during my journey who have enabled me to make significant contributions to our understanding of how the TCR initiates signaling. TCR signaling events contribute to T cell development as well as to mature T cell activation and differentiation.

## MY PARENTS AND I IMMIGRATED TO THE USA

I am the only child of Jewish Holocaust survivors. My parents grew up in a small Hungarian town, Ermihaiyfalva, located in Transylvania. Neither of my parents had education beyond high school. My parents became sweethearts in their late teens, but their romance was interrupted by the start of World War II. My father was conscripted into Hungarian labor camp service in 1941. In the spring of 1944, my mother and her family, as well as the other Jews in their town, were transported by train to Auschwitz. All of my grandparents, several aunts and uncles, and many other relatives perished in Auschwitz. Over the course of several months, my mother and three of her surviving sisters were moved to several other concentration labor camps. Count Bernadotte, a Swedish nobleman and diplomat, negotiated the release of approximately 21,000 prisoners in the spring of 1945. Among the prisoners rescued were approximately 1,600 Jews who included my mother and one of her younger sisters. They were transported to Sweden. My mother was nursed back to health through the kindness and generosity of a wonderful Swedish woman who had no children and lived in Landskrona, Sweden. After the war, my father returned to the town in which he had grown up, which is now in Romania. Since borders had changed, the town was renamed Valea lui Mihai. There he learned that his family had been taken to Auschwitz. He soon left to start an automobile supply store in Germany with some labor camp friends. In 1947, by chance, a long-lost cousin entered his shop, recognized my father, and informed him that his childhood sweetheart was alive in Sweden. After what must have been a remarkable phone call with his old sweetheart, my father managed to travel rather surreptitiously on a fishing boat to Landskrona to reunite with her. They married in 1948. I was born in Landskrona in 1952. The three of us immigrated to the USA in 1954 as Romanian refugees. We settled in Chicago, where my mother had a brother who had immigrated in 1938. I grew up in Chicago, where my parents ran a dry-cleaning shop.

## AI HAVE HAD INCREDIBLE MENTORS, STARTING IN HIGH SCHOOL

I attended Von Steuben High School, in Chicago. My interest in science was kindled by a fantastic Advanced Placement (AP) Biology teacher, Harold Kiehm. Kiehm knew how to connect with his students. After participating in several science fairs, inspired by my discussions with Kiehm, I was encouraged to apply to National Science Foundation (NSF)-sponsored summer research programs for high school students. I was accepted to one of the rare programs that accepted students between their 10th- and 11th-grade years. The program was in a Public Health Laboratory adjacent to the University of North Dakota campus. There, I compared a catalase test to bacterial plate counts for efficacy in screening dairy milk samples for evidence of mastitis. I was impressed by the hospitality and camaraderie within the lab. While there, I had my first exposure to study of the immune system. I recall being fascinated by the development of antisera raised against the rabies virus by inoculating mice with the virus. The antisera were used for the diagnosis of rabid animals by indirect immunofluorescence of brain specimens. I spent the next summer in another NSF summer research program at the Jackson Laboratory in Bar Harbor, Maine. My project there tested the hypothesis that type A spermatogonia were the testicular stem cells responsible for sperm regeneration following X-irradiation of mice. I was one of 29 students in the program. We gave short talks for each other about our work. One of the students worked on a transplantation immunology project that captured my interest. That summer experience was a fantastic one, not only because of the wonderful setting, the exposure to incredibly diverse fields of science, and the questions being asked, but also because I developed a strong sense that I belonged to a community of students who shared common interests in research. It is perhaps ironic that my UCSF lab has depended on the Jackson Laboratory for mice in our research. Moreover, we have deposited some of the novel strains of mice there that we have developed for other investigators to access.

## MEMBRANE FLUIDITY AT JOHNS HOPKINS UNIVERSITY

On the way to the Jackson Laboratory, I took a diversion and toured the Johns Hopkins University campus in Baltimore and decided to apply. To the great surprise of my high school college counselor, I was accepted and enrolled in Hopkins. Hopkins impressed me with its emphasis on independent study. I wanted to learn more about the immune system and decided to do independent study in a research lab. A contact at the Jackson Laboratory suggested I might find Michael Edidin's research interesting. Edidin was using immunofluorescence technology to study the fluidity of the plasma membrane. He and Larry Frye, his graduate student, had just published an important paper (1) that supported the fluid mosaic model proposed by Singer & Nicolson (2). Frye and Edidin used Sendai virus to fuse mouse and human cells together to form heterokaryons. Using two-color immunofluorescence microscopy, they found the heterokaryons initially exhibited segregation of the plasma membrane proteins from the two cells that had fused. However, they observed a time- and temperature-dependent intermixing of the surface molecules, supporting the fluid mosaic model of the plasma membrane.

Edidin thought that the membranes might be so fluid that there might be intermixing of molecules between cells if they simply touched. He asked me to address this question by coculturing human and mouse adherent cells. To do this, I had to generate some of my own reagents, which was a fantastic experience. I made my own mouse alloantisera, purified and fluorescently conjugated my own anti-IgG reagents, and learned how to culture and passage adherent cell lines. Most challenging were the many hours I spent looking through the two eyepieces of the fluorescence microscope—video imaging was not available in the early 70s.

Although I never convincingly saw exchange of proteins during cell-cell contact, I did see time-dependent aggregation of the fluorescent antibodies on the surfaces of cells. I called these aggregates bundles when I described them to Edidin. Although he initially thought these bundles of stain were artifacts, when I trypsinized the adherent cells off the culture dish these aggregates formed crescentic caps on the rounded cells in suspension. This redistribution of antibody-labeled membrane protein mimicked the immunoglobulin caps that had recently been described on B cells when anti-immunoglobulin was added (3). We published the requirements for cap formation of antigens on fibroblasts in *PNAS*—my first publication (4)!

Although I greatly enjoyed my time in the Edidin lab, I wanted my career in research to relate more to human disease. So, I applied to medical schools and had the good fortune of being accepted to the University of Chicago and entered in 1973.

## THE UNIVERSITY OF CHICAGO AND WORKING WITH FRANK FITCH

Within the first six months in medical school, I realized that I sorely missed working in the lab. Using a spring elective, I looked for a lab working on the immune system. Frank Fitch had an ongoing project that related to mechanisms of achieving transplantation tolerance of renal allografts in inbred rats. The connection of the Fitch lab to transplant immunology seemed like a great melding of my interests in the immune system with medicine. During the course of that spring elective, Medical Scientist Training Program (MSTP) positions became available as a result of a successful legal challenge by the Association of American Medical Colleges over funding that had been blocked. Newly available positions were offered to my medical school class. Fitch encouraged me to apply. During several long walks, I thought long and hard about applying to the MSTP. It involved a commitment to several additional years of training. In the end, I decided I wanted a combined career in research and medicine. Obtaining a PhD as well as an MD made great sense given my career aspirations. Moreover, it allowed me to release my parents from the financial pressures of a medical school education. Fortunately, I was one of the eight students that were selected to join the MSTP in 1974.

My initial work in the Fitch lab focused on a topic that was then pervasive in immunology, the notion that a network of anti-idiotypic antibodies regulated immune responses by both B cells and T cells. Previous work by a student in the Fitch lab, Tom McKearn, had reported the detection of anti-receptor idiotypic antibody (ARA) in immunocompetent Lewis × Brown Norway (LBN) F1 rats that were challenged with parental Lewis spleen cells. This suggested such ARA might provide resistance to T cell–mediated graft-versus-host disease (5).

I first focused on the possibility that ARA might be responsible for neonatal self-tolerance in rats. In fact, I found that neonatal rat serum, but not adult rat serum, suppressed proliferative responses by adult rat spleen cells. However, there was no evidence of antigen specificity in this suppressive effect. In retrospect, a high concentration of alpha-fetoprotein in the neonatal serum was likely responsible for the observed inhibition. I mention this work because I published this work as the *sole author* of a paper in the *Journal of Immunology* (6). Yes. . .sole author. Fitch had established a tradition that his students who were mainly responsible for the work in a paper should be sole authors on their first papers. His contribution could simply be noted in the acknowledgments section. Fitch strongly believed that students did not get sufficient recognition for their research work. This undoubtedly also contributed to one of the many Fitch-isms that became well-known. He would begin his lectures by saying, "When I say I, I really mean We. When I say We, I really mean They." Everyone in the lab and in other labs were impressed with Fitch's generosity. Fitch was also inclined to give his trainees considerable independence in the lab—a lesson I carried with me later in my career. That said, he reigned you in when you drifted a bit too far off target, and especially when you overinterpreted your experiments. Fitch continued to work in the lab himself and was the first to sort out how to use a new instrument. He set a great example as a scientist and mentor. He not only was a remarkable scientific mentor for me but also remained my long-term career mentor and friend.

I rapidly switched to a project that was more in line with what drew me to the Fitch lab. Fitch and Frank Stuart, a renal transplant surgeon, had developed an antigen-specific treatment protocol to induce long-term, antigen-specific survival of semi-allogeneic kidneys in inbred rats. Lewis rats were injected with LBN F1 spleen cells intravenously on day 0, followed by 1 mL of Lewis anti–Brown Norway antiserum on day 1. On day 10, the Lewis recipient rats underwent bilateral nephrectomy and received an LBN kidney transplant. Such semi-allogeneic kidneys were typically rejected in 7–10 days in untreated Lewis rats, but in these pretreated rats, the renal transplants were accepted indefinitely. This was referred to as allograft enhancement (7). McKearn and Fitch hypothesized that this might be due to anti-idiotypic ARA induced by the donor spleen cells and antibody. However, after many months of effort, I could not detect anti-idiotypic antibodies directed against the pretreated rat recipients. I felt dejected and wasn't sure where my work was headed.

Many people in the field, including Fitch, were becoming skeptical about the role of the idiotypic network, particularly as it related to T cells. The T cell receptor (TCR) had not yet been identified, and the notion that T and B cells shared idiotypic specificities with antibodies seemed increasingly far-fetched. While in Lausanne, Switzerland, during a sabbatical after I started in his lab, Fitch was unable to detect anti–T cell idiotypic antibodies. He shifted his research to methods for the in vitro generation of cytolytic T cells in the mouse (8). When he returned to Chicago, Fitch suggested that I study the cellular basis for the long-term survival of the LBN renal transplants in the pretreated Lewis rats. I compared the responses of long-term renal transplant survivors to those of normal, previously immunized, and neonatal bone marrow chimeric rats—the latter being truly tolerant to the Brown Norway alloantigen. I first had to solve some problems with rat spleen cell culture systems that were not observed in mouse cultures. I found that activated macrophages in rat spleens suppressed the generation of allogeneic cytotoxic T lymphocytes

(CTLs), but not proliferative responses in vitro (9). Subsequently, I found that the long-term renal allograft survivors rejected LBN skin grafts, made normal primary antibody responses to LBN spleen cells, and mounted normal mixed lymphocyte proliferative reactions and CTL responses to LBN cells. All these responses were absent in truly tolerant allogeneic LBN bone marrow chimeras. However, despite whatever I tried, the enhanced renal allograft survivors could not be induced to mount secondary antibody responses or exhibit memory CTL responses, as were seen with cells from previously immunized normal rats. Thus, I concluded the generation of immunologic memory seemed to be blocked (10). While some investigators were reporting detection of suppressor T cells in various mouse systems at that time, I found no evidence of suppressor T cells in my in vitro or in vivo assays in the long-term renal allograft survivors. To this day, the failure of the immune response to show any evidence of maturation into a memory response still puzzles me.

Despite leaving the Fitch lab after three and a half years with many questions remaining, I returned to medical school clinical rotations feeling quite satisfied that I had learned a lot and had advanced the transplantation field a bit. Fitch, a pathologist, attempted to convince me that I would have more time for research as a pathologist. I considered it, and even did an elective on the autopsy service. However, I greatly enjoyed my clinical clerkships and interacting with patients and decided to focus on internal medicine. There were many obstacles to conquer in clinical organ transplantation. I thought I would likely enjoy a career as a transplant nephrologist.

When we returned to clinical clerkships, my MSTP classmates and I learned that the MSTP funds for our final year in medical school had been spent. Funds would not be available for our seventh year of training. The Dean of Students, Joseph Ceithaml, suggested we consider using six months of medical school elective time to work as postdocs with our thesis advisors and be paid as postdocs. That seemed to provide a financial solution to the unanticipated funding shortfall. While discussing this option with Fitch, and without real thoughtful consideration, I asked whether instead of working with him, I might work in the lab in Switzerland where he had done his sabbatical. Fitch thought this was a great idea, as did my wife, Shirley (we had married earlier during my second year in the lab). Neither of them would let me back out of this idea. Fitch was able to arrange a six-month postdoc position for me with Jean-Charles Cerottini and Theodore (Teddy) Brunner at the Swiss Institute for Experimental Cancer Research in Lausanne. I completed most of my interviews for internships in internal medicine before we left and graduated from medical school in December of 1979, the sole MD graduate among students receiving their MBAs at that time of year. When Shirley and I flew off to Switzerland in December of 1979, we had no idea where we would return to after our six months in Lausanne.

## CLONING T CELLS IN LAUSANNE

Shirley and I were lucky to be able move into an apartment in Epalinges, a suburb of Lausanne only a short walk to the lab. I very much enjoyed working with Brunner and Cerottini, who were well-known for their development of the $^{51}$Cr release activity to assess T cell cytolytic activity. I soon became friends with a wonderful group of other foreign scientists including Oreste Acuto, Howard Engers, Osami Kanagawa, Anne Kelso, H. Robson MacDonald, and Marcus Nabholz, who all became successful scientists.

In the lab, I learned how to do limiting dilution analyses for mouse T cells against Moloney leukemia virus (MLV) antigens and studied the frequency of T cell clones with cross-reactivity to alloantigens. I found that approximately 5% of the MLV-reactive clones were cross-reactive with a small sampling of alloantigens (11). I validated this result using technologies for expanding and culturing long-term antigen-specific T cell clones and testing specificity with anti-MHC antibodies. The Swiss scientists in Lausanne had been skeptical about being able to achieve and maintain

long-term growth of antigen-specific cytolytic clones. The key to growing long-term cytolytic T cell clones, I had learned by following ongoing work in the Fitch lab, was to restimulate small numbers of clonal T cells every three to four days with antigen presented by spleen cells and with a source of T cell growth factor (TCGF)-containing medium. IL-2 had not yet been cloned. By isolating T cell clones and using well-characterized alloantisera, I validated such cross-reactivity (12). Moreover, I realized there was a great deal of clonal heterogeneity in the fine specificity in these T cell clones. I thought that the distinct specificities of some of these clones could be used to generate clone-specific monoclonal antibodies (mAbs) to identify the TCR. Unfortunately, just as I began to try to do this with the help of Roberto Acolla, who was making mAbs in Lausanne, the fused hybrids failed to grow. We soon learned that this was due to the outbreak of mouse hepatitis virus. Nearly 75% of the mouse colony at the institute had to be sacrificed.

Despite this setback, Shirley and I had a wonderful six months in Lausanne. I greatly enjoyed the work, my colleagues, and the incredibly supportive environment. Despite working hard in the lab, Shirley and I were able to take weekend trips by train to explore towns in Switzerland as well as in France, Italy, and Germany. In late April, my parents forwarded a letter from the University of Chicago that listed my internal medicine internship match result: the University of California, San Francisco (UCSF). In mid-June, we headed to San Francisco from Lausanne.

## A SWITCH IN CAREER PLANS

UCSF was my top choice because it provided three affiliated hospitals offering a diverse clinical experience and had a very large kidney transplantation program. I started my rotations in the Cancer Research Institute, which was largely a malignant hematology service. This was a clinically challenging service, but I found it very interesting and appreciated the teamwork that went into caring for very ill patients. My second clinical rotation at UCSF was on the kidney transplantation unit, an experience I had very much looked forward to. Unfortunately, I was greatly disappointed. We generally saw patients immediately after renal transplantation, and their clinical problems were very similar. Moreover, the research questions being asked at the time were rather limited.

I then moved on to a general medical service at the Veterans Administration Medical Center (VAMC) affiliated with UCSF. There, I realized that I was most intrigued by patients that presented clinical puzzles. Typically, such patients had unknown infectious diseases or rare autoimmune diseases. During a consult on one of our patients who had a rare autoimmune vasculitis syndrome, I met Bill Seaman, who had a research background in immunology [having trained at the National Institutes of Health (NIH)] and was a rheumatologist. Bill took an interest in me and my research background. We eventually became close friends. When we discussed my career quandary, Bill suggested I consider rheumatology and that I talk about this with the Chief of Medicine at the VAMC, Marvin Sleisenger. Sleisenger supported the idea of a career change and referred me to the rheumatology division chiefs at the San Francisco General Hospital (SFGH) (Ira Goldstein) and the UCSF Moffit Hospital (Jack Stobo). The three UCSF-affiliated hospitals at that time each had their own independent subspecialty training programs.

Basic research at the Parnassus Avenue campus was flourishing. There, the development of recombinant DNA technologies and the cloning of many genes were taking off, the discovery of Src as a proto-oncogene offered exciting new insights into cancer research, and research in prions was emerging. Stobo was very engaging and enthusiastic about my interest in rheumatology and was impressed with my background in immunology. His lab was focused on topics that excited me, namely identifying the TCR and T cell biology related to autoimmunity. So, I jumped at the invitation to do my rheumatology training at Moffitt and eventually joined his lab. At the time, Stobo was also a Howard Hughes Medical Institute (HHMI) investigator, the significance of which I didn't appreciate at the time.

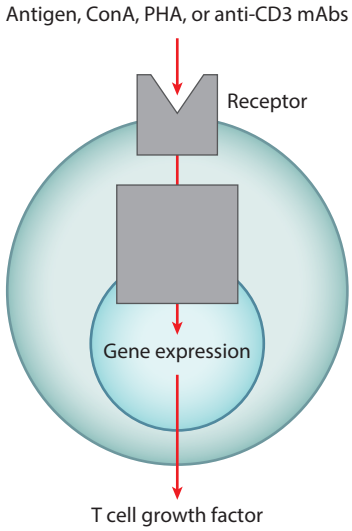


**Figure 1**

How little we knew about the TCR, its signaling mechanisms, and their consequences when we started our studies in 1982! We did not know the identity of the TCR, the molecular events or molecules involved in its signaling mechanism, the genes activated, or the molecular identities of the cytokines the TCR induced. Abbreviations: mAb, monoclonal antibody; PHA, phytohemagglutinin; TCR, T cell receptor.

Since I had both an MD and a PhD, I was able to fast-track into rheumatology, allowing me to shorten my training in medicine and rheumatology by a year. I began working in the Stobo lab in the fall of 1982. During my PhD training in the Fitch lab, I had developed allergies to many experimental animals. So, with Stobo's encouragement, I decided to grow human alloantigen-specific clones and once again to try to make human T cell clone-specific mAbs. It was remarkable that so little was known about the molecular nature of the TCR and how it signaled, and even about the genes that were induced. Indeed, it was all a mystery at that time, black boxes waiting to be opened (**Figure 1**). Studying the fundamental properties and mechanisms involved in normal T cell responses seemed like the best path forward before I could study the abnormalities of T cell function that might underlie or contribute to the ill-defined autoimmune disease states.

The initial work growing human alloreactive T cell clones was tedious because the T cell clones had to be passaged twice a week and a source of TCGF had to be provided. I initially stimulated human T cells with the plant lectin concanavalin A (ConA) to generate TCGF and used the resultant supernatant, depleted of ConA, to begin to grow alloreactive T cell clones. With the identification around that time of IL-2 as the relevant TCGF, I was attracted to a paper by Gillis & Watson (13) that described a human T cell leukemic line, Jurkat, that could be induced by the plant lectin phytohemagglutinin (PHA) to produce IL-2. The Jurkat line was kindly provided by Kendall Smith, a generous gift that I planned on using only to produce large quantities of IL-2. However, I would leverage the Jurkat line throughout my career to characterize many aspects of the TCR, TCR signaling, and T cell activation (14). Indeed, the Jurkat line has proven to be a valuable resource for many labs through the years.

Within a few short months after I entered the Stobo lab, the TCR was identified. . . but not by us. Three groups led by Jim Allison (15), Ellis Reinherz (16), and Philippa Marrack and John Kappler (17) independently used antibodies to identify clone-specific disulfide-linked αβ heterodimers that turned out to be the antigen recognition subunit of the TCR. The cDNAs for

the β chains were cloned a short time later by Steve Hedrick and Mark Davis (18), as well as by Tak Mak's group (19).

I was particularly intrigued by the paper from the Reinherz group that reported that the αβ heterodimer was cointernalized along with the T3 (now CD3) antigens (16). Since anti-CD3 mAbs were mitogenic (20), there was considerable speculation that the CD3 complex might be the signaling component of the TCR, albeit we knew nothing about TCR signaling mechanisms. However, I knew that Jurkat expressed CD3 and found that these cells produced IL-2 if they were costimulated either by PHA or, as we found, by the combination of anti-CD3 plus phorbol myristate acetate (PMA) (21). It appeared that PMA provided an obligatory second signal that anti-CD3 could not provide. PMA also potentiated the PHA response.

Inspired by the "awesome power of genetics," a mantra frequently used at UCSF, I decided to genetically test the importance of CD3 for TCR signaling. I mutagenized Jurkat and selected for CD3-negative cells. I obtained multiple independent CD3-deficient clones (22). These CD3-deficient clones largely failed to produce IL-2 in response to PHA or PHA + PMA, suggesting the possibility that PHA bound to CD3, among other molecules. But, what if these cells lost the capacity to produce IL-2 due to the chemical or radiation mutagenesis to which they had been subjected? This led me to consider what might substitute for PHA or anti-CD3. Some signaling work from other fields suggested calcium ionophores could synergize with PMA and mimic signaling by various receptors in different cell types. Quite remarkably, I found the calcium ionophore A23187 mimicked the effect of anti-CD3 mAb and synergized with PMA to induce IL-2 by the wild-type Jurkat cells as well as by the CD3-deficient Jurkat mutants (22, 23). This suggested the possibility that the CD3 complex (and possibly the associated TCR αβ heterodimer) induced calcium increases when stimulated by mitogenic anti-CD3 mAb. John Imboden, who was a rheumatology fellow in the Stobo lab, and I discussed this possibility. By coincidence, John's wife, Dolores Shoback, was an endocrinology fellow studying calcium increases induced by the G protein–coupled parathyroid hormone receptor. Dolores was using a calcium-sensitive fluorescent dye called quin2, developed by Roger Tsien (24), as an indicator of cytoplasmic free calcium concentration changes after receptor stimulation. With Dolores's help, we showed that anti-CD3 or PHA induced calcium increases in wild-type, but not CD3-deficient, Jurkat cells (23). Moreover, in a subsequent paper, John found that the TCR was likely to induce the calcium increase via the inositol phospholipid pathway, by hydrolyzing phosphatidylinositol 4,5-bisphosphate ($PIP_2$), linking the response to the possible activation of a phospholipase C activity (25). Interestingly, we very recently emphasized the importance of a PI transfer protein for replenishing $PIP_2$ following in response to pre-TCR or TCR signaling during T cell development and for tonic TCR signaling (26).

Because Jurkat was CD3 surface positive, I suspected it might also express a TCR αβ heterodimer. I immunized mice with Jurkat cells and screened for mAbs against Jurkat and identified an IgM mAb, C305, that was Jurkat-specific and immunoprecipitated a disulfide-linked heterodimer (22). Like the CD3-specific mAbs, C305 could synergize with PMA to induce IL-2 (22). I showed that all mutants that were selected for CD3 deficiency failed to stain with C305 or those selected for deficiency in C305 reactivity lacked cell surface CD3 (22). Ultimately, with the advice of Cox Terhorst, we used biosynthetic labeling to show that all of these mutants, regardless of the mode of selection, contained intracellular CD3 proteins (27). In collaboration with Pam Ohashi, then a graduate student in Tak Mak's lab, we showed that most of the mutants lacked full-length TCRα or TCRβ transcripts. With Pam, we reconstituted the TCR β chain mutant by protoplast fusion (yes, by fusing *Escherichia coli* containing an expression plasmid encoding the cDNA for the β chain to Jurkat cells). This restored expression of a functional TCR αβ heterodimer as well as CD3 on the plasma membrane (27). Our work, together with Michael Brenner's chemical

cross-linking work in Jack Strominger's lab (28), established a close and obligatory physical interaction between the TCR αβ heterodimer and the CD3 chains for plasma membrane expression.

## JOINING THE FACULTY AND THE HOWARD HUGHES MEDICAL INSTITUTE AT UCSF: ON LEAVE FOR 37 YEARS

Jack Stobo had been a great research mentor: always very supportive, positive, and encouraging. Shirley and I were very happy in San Francisco. We now had a son, born during my fellowship, and thought of San Francisco as a great place to call home. So, it was not hard to convince me to stay at UCSF and join the rheumatology faculty in 1985, especially since it included an appointment as an HHMI assistant investigator, the significance of which I then understood.

At that time, investigators could appoint assistant investigators, with HHMI leadership approval. Assistant investigators had an independent budget that included full salary and programmatic support (staff, supplies, etc.) and independent space. The space was carved out of the senior investigator's (Stobo's) space. Through this appointment as an HHMI assistant investigator, I became an employee of HHMI and was formally on leave of absence from UCSF, and I remained so until 2022, despite never leaving UCSF. During this "leave of absence," I taught medical and graduate students, did UCSF service work on various committees, and cared for patients, while devoting 80% of my time to research. I am convinced that becoming an HHMI investigator was transformative for my career—it freed me from the constant cycle of writing and renewing proposals for competitive grants that offered much less support, a process that produces enormous stress. Importantly, HHMI funding also allowed me to take more risks in our research.

I admit that I was a bit wary about staying at the same institution at which I had done my postdoctoral training. Jack and I agreed to continue to collaborate on signaling and that I would work independently on the oligomeric nature of the TCR. My concerns about perceptions regarding remaining at UCSF turned out to be completely irrelevant. On July 1, 1985, the day I joined the UCSF faculty, Jack Stobo left to become Chair of Medicine at Johns Hopkins. I often say this was the worst and the best thing that could have happened to me. No issues of independence persisted, but I lost my main research and career mentor at UCSF.

I inherited half of Jack's space, as did Matija Peterlin who had previously joined the rheumatology faculty as an assistant professor. Matija also received an HHMI appointment. We shared Jack's common space and equipment. Matija was a highly skilled molecular biologist who studied MHC gene regulation and taught me many molecular techniques those first few years on the faculty. Jack's administrative people stayed on and reported to me. Most importantly, I benefitted enormously from having Marianne Newton (later Mollenauer), Jack's lab manager, join my group. Marianne was an excellent scientist and remained my lab manager for 32 years. She was a stabilizing force during this transition and became a close friend. I owe a great deal to her for the success of my lab. The students and postdocs who trained with me regarded Marianne as the "Lab Mom." I was also incredibly fortunate to hire one other long-time technician/specialist, Terri Kadlecek, who was a remarkable experimentalist and taught many of the students and postdocs various techniques, but perhaps most importantly she taught them to become independent. Terri worked with me for 30 years. Marianne and Terri made remarkable contributions to the success of the lab. I can't imagine what we would have accomplished without them. My HHMI appointment made it possible for me to invest in the long-term appointments of Marianne and Terri.

In my very abrupt transition to independence and the departure of Jack, several faculty members played important mentoring roles. In particular the two rheumatology division chiefs, Bill Seaman (VAMC) and Ira Goldstein (SFGH), helped me negotiate the transition to independence. Holly Smith, chair of the Department of Medicine and chair of the HHMI Medical Advisory

Board, was also incredibly supportive. In addition, Henry Bourne, then chair of the Department of Pharmacology, who was interested in signal transduction by G protein–coupled receptors, became a friend and provided me with scientific and career advice.

There was a real sense of a void in leadership in rheumatology at the Parnassus campus after Jack Stobo left. Two efforts, over the course of three years, to recruit a new rheumatology division chief failed. Ultimately, I was invited to take on the position. I initially declined in order to protect my research-focused time. However, in the end I accepted the position with the provision that I could hire a full-time academic clinic chief. I was fortunate to be able to rehire Ken Sack as the clinic chief. With the support of Seaman and Goldstein, as well as the new chair of the Department of Medicine, Richard Root, my transition to division chief went fairly smoothly. We enormously benefitted by an endowed center created by Ephraim Engleman, one of the first rheumatology specialists in the San Francisco Bay Area. The center was named the Rosalind Russell Arthritis Research Center and later renamed the Russell/Engleman Rheumatology Research Center. It has provided important support to the rheumatology research programs at UCSF. Eventually, the three divisions in rheumatology merged their fellowship training programs, which strengthened our rheumatology training program. Our clinical research program had been focused on health services research under Wallace Epstein but was broadened under the leadership of David Wofsy, who transitioned to Parnassus from the VAMC. He led a new clinical trials center that gave UCSF a much more diversified research portfolio and improved its national and international reputation.

The departure of Jack Stobo provided me with an unexpected opportunity. Several new rheumatology fellows who had chosen to come to UCSF for rheumatology fellowship training had anticipated working in Stobo's lab. I was lucky to convince them to become my first postdoctoral rheumatology fellows: Linda Bockenstedt, Tim Laing, and Gary Koretzky. Bernard Manger, a German rheumatologist who had started working with Stobo decided to stay and continue his work with me. I began my independent career at UCSF with a remarkable group of trainees.

I will summarize some of the most notable achievements related to signal transduction in T cells from my lab in the next few sections. I do not mean this to be a comprehensive review of the field or my lab's contributions and apologize for not reviewing the many other important contributions that have been made by members of my lab and many other investigators in other laboratories whose work has contributed to this area of research.

## THE SECOND (COSTIMULATORY) SIGNAL REQUIRED FOR IL-2 PRODUCTION

Using the Jurkat T cell line, while I was a postdoc with Stobo, we had shown that two signals were required for IL-2 transcription (21). One signal was provided by the TCR or a calcium ionophore, and the other could be mimicked by the phorbol ester PMA (22, 23), which at that time was known to activate protein kinase C (PKC). We now know PMA can also activate the Ras pathway via RasGrp guanine nucleotide exchange proteins that are normally responsive to diacylglycerol in T cells (29). This led us to search for physiologic stimuli that could provide the second signal required for IL-2 production. I had originally obtained the 9.3 mAb specific for a 44-kD disulfide-linked dimer on T cells because I thought it possible that 9.3 might react with the TCR. However, this proved not to be the case. The reactivity of 9.3 was unrelated to the TCR/CD3 complex (30). Manger, Imboden, and I found that the 9.3 mAb could in fact provide the second signal necessary for Jurkat production of IL-2 and for highly purified normal human T cells to proliferate. This result was published in the *Journal of Immunology* along with a related paper by Jeff Ledbetter's group (30, 31). Tp44 later became known as CD28.

Jim Fraser, a postdoctoral fellow, identified a sequence motif in the IL-2 and other cytokine upstream regulatory regions that was responsive to the combination of TCR and CD28 signals (32), distinguishing it from typical NFAT sites that were responsive to TCR only signals. Virginia Shapiro, a postdoc in my lab, later showed that that CD28 response element proved to be a composite binding site for c-Rel and AP-1 transcription factors (33). Signaling by CD28 has proven to be rather enigmatic, with multiple pathways seemingly involved, including the PI3K (phosphoinositide 3-kinase) pathway and the NF-κB pathways (34–36). Phospho-proteomic analyses performed collaboratively with the lab of the late Tony Pawson have suggested the involvement of a broader array of pathways (37). It's amazing that our understanding of the molecular mechanism underlying CD28 costimulation is still rather limited nearly three decades later.

## STUDIES OF THE OLIGOMERIC TCR CONTRIBUTED TO CAR-T CELL DEVELOPMENT

As a postdoctoral fellow and junior faculty member, I was fascinated by the TCR oligomeric complexity. Once the sequences of the oligomeric TCR chains became available, the unusual placement of acidic and basic residues within the transmembrane domains of all the CD3 and TCR αβ chains, respectively, became of particular interest. Lee Tan, a postdoctoral fellow, did heroic studies using partial cDNA digests and blunt-end ligation to reconstitute our Jurkat TCR β-chain mutants with CD8 chimeras that included sequences encompassing the transmembrane domains of both TCRα and TCRβ. These CD8 chimeras with TCRαβ transmembrane domains were sufficient to restore CD3 chain expression on the plasma membrane and for linking CD8 extracellular domains to the signaling functions of the TCR (38). This led Bryan Irving, my graduate student, to free the recently discovered ζ chain's long cytoplasmic domain from the rest of the TCR by linking it to the extracellular and transmembrane domains of CD8. Prior to this experiment, it had been thought that the ζ chain did not have an independent signaling function. Instead, different spliced isoforms of the ζ chain were reported to differentially influenced TCR signaling pathways (39, 40). However, Bryan was able to show that the ζ chain cytoplasmic domain alone, linked to the extracellular and transmembrane domains of CD8, was sufficient to create an independently expressed chimera that had the full signaling capability of the TCR (41). The signaling capacity of the ζ chain was later partially mimicked by CD8/CD3$\varepsilon$ chimeras created by the Malissen lab (42). This signaling capability of multiple chains within the TCR was linked to what was eventually named the immunoreceptor tyrosine-based activation motif (ITAM), which was also present in the non-ligand-binding chains of receptors associated with antigen recognition, first noted by Michael Reth (43). Each ITAM in the ζ chain could mediate activation signals, as Bryan showed with sequential truncations or multimers of the same motif (44).

Our finding that the ζ chain cytoplasmic domain could be linked to a heterologous transmembrane domain and extracellular domain inspired the development of chimeric antigen receptors (CARs) based on the ζ chain that are currently used in cell-based tumor immunotherapy. We thought using such therapeutic chimeras and cell therapy was beyond the capabilities of my lab back in 1991. However, we collaborated with scientists at Cell Genesys to patent therapeutic chimeric receptors, which they developed and called universal TCRs (45–47). Cell Genesys's first clinical trials focused on Kaposi sarcomas in HIV-infected patients using T cells that expressed CD4/ζ chimeras (48). However, antiretroviral therapy replaced the need for this cellular therapy just as the methods for such therapy were being optimized. A second clinical trial for colon carcinoma using a single-chain antibody reactive to mucin tumor antigen TAG-72 when fused to the CD4 transmembrane domain and the ζ cytoplasmic domain was not successful (49). Although Cell Genesys abandoned their universal TCR cellular therapy program, Carl June and many of

the former Cell Genesys scientists were pioneers in the subsequent development of successful CAR-T cell therapy for B cell malignancies (50), which has led to explosive efforts in academia and industry to expand this therapy to many solid tumors and other indications.

## TYROSINE KINASES THAT INITIATE TCR SIGNALING

The mechanism by which the TCR signals to increase calcium was unknown. There was speculation that G proteins or tyrosine phosphorylation was involved. My very first graduate student, Mark Goldsmith, took a somatic cell genetic approach to understand how the TCR signaled, and he isolated three distinct complementary TCR signaling mutants from the Jurkat line. Each failed to increase cytoplasmic calcium when their TCRs were stimulated but did increase calcium via stimulation of a heterologously expressed G protein–coupled receptor: the human muscarinic receptor, subtype 1 (51–53). David Straus, a postdoctoral fellow, solved the puzzle of the first isolated mutant once our lab learned how to do Western blots and probe for phosphotyrosine. David showed that J.CaM1 was functionally deficient in the Src family kinase (SFK) Lck (54). At about the same time that we were learning that Lck played a key role in TCR signaling, we attempted to understand how the TCR ζ chain mediated a signal via the ITAM. Terri Kadlecek found that the ζ chain isolated from TCR-stimulated Jurkat cells was rapidly inducibly tyrosine phosphorylated and associated with a 70-kD phosphoprotein. Data from the J.CaM1 mutant suggested Lck was responsible for TCR-induced ζ chain ITAM phosphorylation. I was able to convince Andy Chan, a postdoctoral rheumatology fellow, to identify this 70-kD band. Andy found the CD8/ζ chimeric receptor, when expressed in Jurkat cells, also was inducibly phosphorylated and associated with this 70-kD phosphoprotein when the chimera was stimulated, and he used the CD8/ζ chimera as bait to isolate the 70-kD protein (55). We were able to identify this 70-kDa band with the help of Chris Turk, who did mass spectrometry protein sequencing, and Makio Iwashima, who made degenerate oligo probes and probed a cDNA library. It was a previously unidentified cytoplasmic tyrosine kinase that contained N-terminal tandem SH2 domains (56), most closely related to the Syk kinase previously isolated from porcine spleen (57). We named this kinase ZAP-70, for ζ-associated protein of 70 kDa. In collaboration with John Kuriyan's lab, we later solved the autoinhibited structure of ZAP-70 and studied its mode of activation (58–60). The importance of ZAP-70 was quickly validated by studying patients with a combined immunodeficiency syndrome that had inactivating mutations of ZAP-70. The syndrome was characterized by CD8 T cell deficiency and nonfunctional CD4 T cells in the patients' blood (61–63). Makio Iwashima did important experiments to reveal that Lck and ZAP-70 interact with ITAMs in a sequential hierarchical manner (64). This led me to propose a model that took into account an important role for coreceptors in localizing Lck to the stimulated TCR complex. The model proposed that Lck is localized to the stimulated TCR by the CD4 or CD8 coreceptors in order to phosphorylate ITAMs. ZAP-70, via its tandem SH2 domains, is recruited to doubly phosphorylated ITAMs via a very high-affinity interaction, where it then is phosphorylated and activated by Lck (65). This model has been updated over 30 years as our understanding of the interactions of Lck and ZAP-70 and their substrates has evolved (65–68).

## TCR SIGNALING MECHANISMS

The consequences of TCR signaling by the proximal cytoplasmic tyrosine kinases required efforts to identify key substrates and the pathways they activated. We were among the first to show that TCR stimulation led to phosphorylation of phospholipase C γ1 (PLCγ1) (69, 70), providing a mechanistic understanding for TCR-induced calcium increases and PKC activation as well as Ras activation via two guanine nucleotide exchange factors, RasGRP and SOS. The latter Ras guanine

nucleotide exchange factors functionally interact to amplify the activation of the Ras pathway responses (71). Subsequently, Tim Finco and Debbie Yablonski, postdocs in the lab, demonstrated that the adaptors LAT (cloned by Larry Samelson's group) and SLP-76 (cloned by Gary Koretzky and collaborators) were critical in TCR downstream signaling and were substrates of ZAP-70 (72–75). In collaboration with the Kuriyan lab, Neel Shah and Terri Kadlecek identified the basis for ZAP-70 substrates' specificity (76). The adaptors were required for optimal TCR signaling leading to PLCγ1 activation and most other downstream pathways, i.e., calcium increases, PKC activation, and Ras/MAPK pathways. LAT contains multiple phosphorylation sites that are phosphorylated by ZAP-70 in order to recruit effector molecules to the plasma membrane (77), and to form condensates that serve to amplify and diversify signaling (78, 79). Recent collaborative studies by Wan-Lin Lo in my lab and Neel Shah in John Kuriyan's lab suggest that the Lck SH3 domain interacts with a conserved proline-rich motif in LAT in order to localize this critical substrate of ZAP-70 to the stimulated TCR-coreceptor complex (68). SLP-76, a cytoplasmic adaptor, is also a substrate of ZAP-70, and via the adaptor GADS localizes to LAT (80). One role of SLP-76 is to coordinate an interaction with the Itk kinase and promote the phosphorylation and activation of PLCγ1 (81).

The critical importance of ZAP-70 in activating downstream pathways and most T cell responses was illustrated by ZAP-70-deficient T cells isolated from patients and cell lines (61–63). However, the role of ZAP-70 was further validated, with the help of Kevan Shokat's lab. Susan Levin, a student, and Byron Au-Yeung, a postdoctoral fellow, took a chemical genetic approach using small-molecule inhibition of a catalytic domain mutation of ZAP-70 (82). This approach proved useful in identifying the functions attributed to ZAP-70 kinase activity in signaling, during thymocyte development and T cell activation (83–87). ZAP-70 does have adaptor function independent of its catalytic function (83). We recently demonstrated that the exceptionally slow phosphorylation by ZAP-70 of the PLCγ1 binding site in LAT is the result of selection to improve ligand discrimination (88). Mutagenesis of a single residue just N-terminal to the PLCγ1 binding site in LAT markedly improved the phosphorylation of this site and led to more rapid and efficient phosphorylation of this site and PLCγ1 in the Jurkat model system and in mice (88, 89). Slow phosphorylation of the PLCγ1 recruitment site in LAT is a key element of kinetic proofreading by which the TCR discriminates ligands, i.e., between agonist-pMHC (peptide–MHC) and self or weak pMHC (90). Improving the phosphorylation of this single site impaired ligand discrimination and led to autoimmunity in mice (88, 89). Thus, ligand discrimination is the result of a complex timing mechanism by which a response to a good agonist is the result of TCR-ligand binding that must be maintained long enough until calcium and diacylglycerol increases result from PLCγ1 activation.

## REGULATION OF SRC KINASES

SFKs, such as Lck and Fyn in TCR signaling, are the most proximal kinases required for signaling by ITAM-coupled receptors in the hematopoietic lineage. Their proper regulation is critical. The work of Gary Koretzky and Joel Picus, postdoctoral fellows, established a positive regulatory function of the very abundant receptor-like tyrosine phosphatase CD45 in TCR proximal signaling events using CD45-deficient T cell lines (91, 92). Monica Sieh, a graduate student, showed that the signaling defects in these mutant lines were a consequence of CD45's requirement to dephosphorylate the negative regulatory tyrosine phosphorylation sites near the C termini of Lck and Fyn (93). Julie Zikherman, a postdoctoral fellow, subsequently used an allelic series of mice that express different levels of CD45 to show that CD45 quantitatively regulates the phosphorylation status of the negative regulatory sites of SFKs, controls the magnitude of TCR signaling ability, and influences T cell development (94). She did similar studies with this CD45 allelic series in

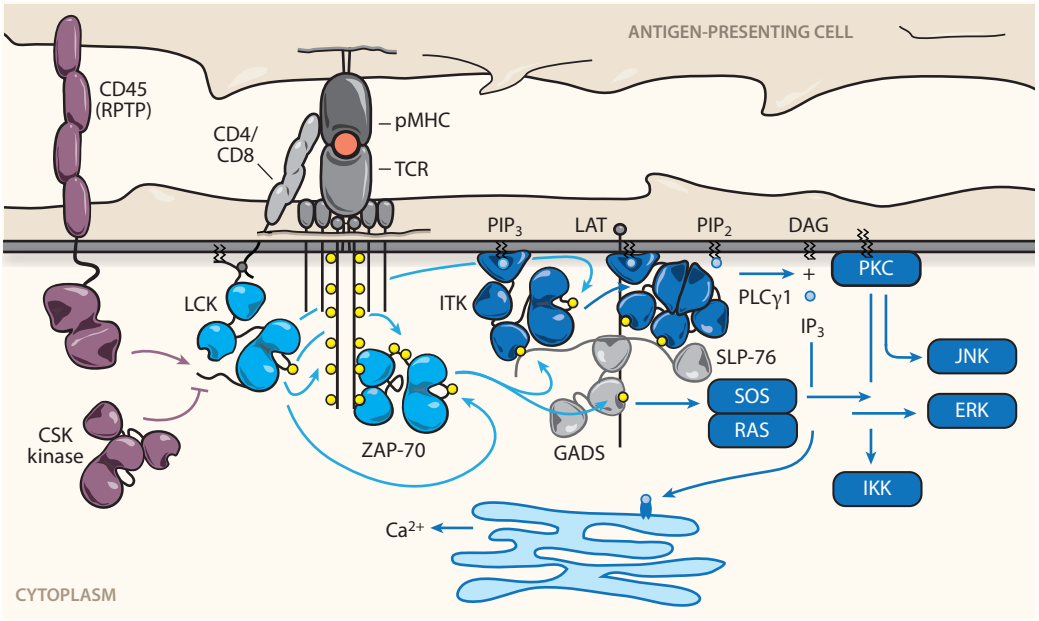


**Figure 2**

A simplified overview of the TCR signaling mechanisms is depicted, focusing mainly on events discussed in the previous three sections of this review. Abbreviations: $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PLCγ1, phospholipase C γ1; pMHC, peptide–MHC; TCR, T cell receptor; ZAP-70, ζ-associated protein of 70 kDa. Figure prepared by Adam Courtney.

B cells (95). However, Jing Zhu, a postdoc, found that in B cells, macrophages, and neutrophils, another transmembrane phosphatase, CD148, can play a partially redundant role with CD45 to control the negative regulatory site of SFKs (96, 97).

In more recent studies, Jamie Schoenborn, Ying Tan, Boryana Manz, and Tanya Freedman, with the help of the Shokat's lab, used a chemical genetic approach to study the Csk cytoplasmic tyrosine kinase. We found that Csk, which phosphorylates the inhibitory tyrosine phosphorylation site near the C termini of SFKs, is a major negative regulator and constrains signaling in the basal state by TCRs, B cell receptors, and macrophage FcRs (98–101). As shown by Simon Davis and P. Anton van der Merwe, by separating the large CD45 receptor phosphatase from proximity with the TCR and substrates during immunologic synapse formation, CD45 also plays an important negative regulatory function via the kinetic segregation model in TCR signaling (102). Work by Adam Courtney, a postdoctoral fellow who collaborated with Arup Chakraborty's group, suggested that the opposing actions of Csk and CD45 control basal signaling in T cells as well as for establishing a threshold for TCR signaling (103). A brief and admittedly incomplete overview of the TCR signaling pathways that we and others have contributed to is graphically depicted in **Figure 2**.

## UCSF AND THE IMMUNOLOGY PROGRAM

I had the good fortune of training and spending my independent career at a remarkable public university where research has flourished. Most importantly, I was able to interact with incredible colleagues with diverse talents and interests. When I began my independent career, six faculty members started the immunology graduate program: Tony DeFranco, Rudi Grosshedl, Dan Littman, Matija Peterlin, Matthias Wabl, and myself. Tony DeFranco deserves special credit for playing a major role in the initial establishment, organization, and leadership needed for the

framework of UCSF's graduate immunology program, which has grown and evolved into a leading international program. Others who have subsequently joined us and/or played important leadership roles in the growth and diversification of the UCSF immunology community include Abul Abbas, Mark Anderson, Mark Ansel, Jeff Bluestone, Francis Brodsky, Jason Cyster, Warner Greene, Nigel Killeen, Lewis Lanier, Richard Locksley, Averil Ma, Mike McCune, Steve Rosen, Bill Seaman, Zena Werb, David Wofsy, and many others. Many of us are being replaced by a wave of new, younger leaders in the newly configured ImmunoX program at UCSF. It has been an honor to work with such a committed, generous, and distinguished group of colleagues at UCSF.

Many of my lab's studies over the last 12 years have benefitted from an NIH-funded program project (PO1) based at UCSF that helped us collaborate with other labs with distinct but complementary abilities and interests. This PO1 involved Arup Chakraborty (Massachusetts Institute of Technology), Jay Groves and John Kuriyan (University of California, Berkeley), Jeroen Roose (UCSF), Art Solomon (Brown University), and my lab. This PO1 greatly extended our abilities, interests, and insights and allowed us to make progress in understanding TCR signaling in ways that would not have otherwise been possible.

## DISCLOSURE STATEMENT

Dr. Weiss previously received royalties for patents that were codeveloped with Cell Genesys and were bundled with patents developed by Cabaret Biotech and licensed to Gilead Sciences for chimeric T cell receptors. Dr. Weiss receives cash compensation and/or stock or stock options for his roles as a cofounder and Scientific Advisory Board (SAB) member of Nurix Therapeutics, an SAB member of BlueSphere Bio, an SAB member of BridGene Biosciences, an SAB member of EpiBiologics, a Scientific Review Board member of Genentech, an Immunology Advisory Board member of IMIDomics, and an SAB member of Jasper Therapeutics.

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I have had the great privilege of having 19 students obtain their PhDs in my lab and 60 postdoctoral trainees train in my lab. Each one has enriched my career, and I am grateful to them for choosing to train with me and for their contributions to our understanding of signaling in the immune system. The lab and its accomplishments have been a group effort—and what a wonderful group it has been!

I apologize to the many trainees and collaborators whose contributions I could not mention here due to space limitations. I am so grateful to the administrative and scientific staff that have worked with me and supported the diverse activities that have been necessary for our lab to function. I especially want to thank my mentors, colleagues, and collaborators. You have had an enormously positive impact on my career and my life.

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