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Arthur Weiss[1]

One could well say that T cell immunology emerged from its "dark ages" of cellular phenomenology in the mid- to late-1970s with the recognition of MHC restriction, which clearly indicated that T cells recognize a fundamentally different form of Ag from B cells and in a different context. However, understanding how T cells could recognize both peptide and MHC ultimately depended on solving the structure of the MHC molecule, which did not come until 1987 (1). In the meantime, many thought the identification of the TCR for Ag would provide the answer, and intense efforts were directed toward discovering the molecular identity of the TCR.

Initial efforts toward finding the TCR were based on the parsimonious view that it should resemble the B cell Ag receptor, which was known to be a membrane-bound form of Ig. During the l970s, a large number of investigators debated whether T cells express a membrane-bound form of Ig and whether TCRs share common idiotypic determinants with Ig. Largely, these misdirected efforts and debates were derived from working with poor cellular and molecular reagents. However, major advances in technology that included the ability to grow monoclonal T cell populations, to produce mAbs, and to clone cDNAs provided the means to finally identify the frustratingly elusive TCR.

The immunological treasure hunt was initiated with the identification of the TCR protein in late 1982 and early 1983 by groups led by Jim Allison, Ellis Reinherz, and John Kappler and Philippa Marrack, who used T cell clone-specific heterosera or mAbs to identify the TCR protein (2–4). All three groups identified a clone-specific disulfide-linked heterodimer, each chain of which was shown by biochemical peptide mapping studies to have variable and constant regions. The Reinherz group also provided the first real evidence for a connection of the heterodimer to the CD3 complex. However, the discovery of the long sought after TCR protein was not the end of the treasure hunt.

The identification of the cDNA encoding these chains was the real Holy Grail yet to be found. With the then-available Ab and cellular reagents in hand, today it would seem to be a simple task given modern mass spectroscopy sequencing techniques, the availability of genomic sequences, and expression cloning techniques. However, in the early 1980s, a true brute force effort involving biochemical purification of large amounts of protein was required for peptide sequencing. Such protein isolation and sequencing of less than abundant proteins represented a near heroic effort.

Working in the Laboratory of Immunology at the National Institutes of Health, Steve Hedrick and Mark Davis, who were postdoctoral fellows at the time, combined their cellular immunology and molecular biology expertise to join and win the race to identify a cDNA clone encoding the TCR. They collaborated to use clever logic and molecular biological strategies to identify the mouse TCR β-chain as described in two articles published back to back in *Nature* in 1984, the first of which is reprinted in this issue of *The Journal of Immunology* (5, 6). In another article in the same issue of *Nature*, Tak Mak joined them at the finish line with identification of the human TCR β-chain (7).

In hindsight, Hedrick's and Davis' strategy seems so logical and elegant. However, their methods really pushed the limits of the existing technology at the time. First, their strategy: They reasoned that the TCR should be 1) expressed in T but not B cells; 2) membrane bound; 3) encoded by genes that rearrange like genes encoding Ig; and 4) composed of constant and variable regions. Here is how they applied it: From a T cell hybridoma, they isolated mRNA from membrane-bound polysomes to enrich for mRNA encoding proteins with signal sequences or transmembrane domains. From this, they made radiolabeled cDNA, eliminated non-T cell-specific cDNAs by hybridizing to an excess of B cell mRNA, and isolated the nonhybridizing, single-stranded, radiolabeled, T cell-specific probe using hydroxyapatite columns (to separate single from double stranded nucleic acids). Because of concerns about sensitivity, they hybridized this labeled T cell-specific cDNA probe to a similarly subtracted T cell-enriched cDNA library. Ultimately, they identified a small number of putative T cell-specific cDNAs and confirmed their T cell-specific expression by Northern blotting. They used these T cell-specific cDNA in Southern blots to look for T cell-specific genomic rearrangements. The results obtained with one clone provided them with that rare "Eureka" moment rarely attained in science, for they had indeed found at least half of the Holy Grail. The sequence of the TCR half they found was presented in the accompanying paper and reveals a predicted membrane bound Ig-L chainlike structure. This was later shown to represent the TCR β-chain of the αβ heterodimer that is responsible for peptide and MHC molecular recognition. Barely able to catch their breath, it was off to the races again to find the TCR α-chain, which Davis, who had moved to Stanford, and colleagues quickly identified using a similar approach (8).

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The seminal work by Hedrick and Davis inspired many to join in the characterization of the TCR genes and accelerated the pace of discovery and understanding in T cell biology. For example, during the course of searching for the TCR α-chain, the TCR γ-chain was unexpectedly identified and ultimately led to the identification of the $\gamma\delta$ TCR and a unique subset of T cells whose existence was previously unappreciated. The identification of the TCR cDNAs enabled the rapid identification of the TCR genes that led to an understanding of how the TCR repertoire is generated. Likewise, having the cDNAs and genes available permitted the development of TCR transgenic mice and transfected cell lines and hybridomas, which helped clarify our understanding of how T cells can recognize both peptide and MHC molecules with a single receptor and to insights into thymic selection. Finally, this work with subtractive hybridization inspired many others to adopt methods to identify genes by differential expression.

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