

PILLARS OF IMMUNOLOGY

The TCR of Mice and Men

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 It was the end of a tumultuous year that finally brought clarity to immunology's most famous problem. Abs, and thus B cell Ag receptors, had already been characterized to the angstrom, and their genes were cloned and found to achieve their fantastic diversity by at least two distinctly novel forms of genetic rearrangement. Immunology had moved into the modern age of molecular biology—with one notorious exception. There was no understanding of the basis for Ag recognition by T lymphocytes.² The late Alan F. Williams wrote a perspective for *Nature* in late 1982 (1) concerning the paradigm of T cell Ag receptors as a form of Ig and concluded, "... there is no sound basis for these views." Studies on the biochemistry of Ag recognition by T cells were inconsistent and not interpretable. Jean Marx writing for *Science* opined, "Few issues in science have proved as difficult to resolve and as subject to contention as the nature of the TCR (2)." . . . The story had all the elements of dime-store pulp. There was the mystery of MHC restriction. There were false leads in the form of suppressor networks, anti-idiotypes, and vanishing gene loci. And there was a race, with the winner thought to lay claim to the prize. Few immunologists were untouched by the frenzy.

What a difference 12 months can make. By the end of 1983, several groups described mAbs and antisera that appeared to recognize the TCR. Perhaps because of the many missteps of the previous decade, the evidentiary bar was raised high, and yet beyond a reasonable doubt, studies showed that a cell surface molecule expressed by all human and mouse T cells possessed characteristics that could only be ascribed to the TCR.

The approach was to immunize mice with cloned T cells, either lymphomas, T cell hybridomas, or Ag-dependent T cell clones. For mouse T cells, the theory was that the variable portion of the TCR would be the only structure to which the host was not tolerant. The antisera could be tested directly or the immunized mice used to generate mAbs. Allison et al. (3) led off with a mAb that recognized a tumor-specific Ag not present on normal T cells and showed that it immunoprecipitated a disulfide-linked 80-kDa heterodimer—a structure abundant on normal T cells. They concluded (correctly) that they had generated an anti-idiotypic Ab, but the manuscript lacked proof that the protein in question was directly involved in Ag recognition. Direct evidence would be to show that the heterodimer bound to a specific Ag, but in 1983, the exact epitope (Ag pep-

tide bound to MHC molecules) had itself not been characterized. Rather, over the next year, proof came from several research groups in the form of biochemical characteristics anticipated for a clonally distributed, Ag-specific receptor that, like IgGs, was composed of variable and constant region domains. In November 1983, these studies culminated in a report from a consortium led by Kappler et al. (4), which included an extensive analysis of TCRs derived from mice and men. With this report, there was no remaining doubt that the elusive TCR had at long last been correctly identified.

There were three analyses that could be conducted that would identify mAbs specific for the TCR. First, each Ab should exhibit reciprocal clone-type specificity. In addition, at least some Abs should activate or antagonize Ag-mediated activation. These characteristics were established in various forms by several groups (5–8). Second, putative TCRs immunoprecipitated from different clones should have charge variability that would reflect sequence differences. This, in turn, could be visualized by nonequilibrium pH gradient gel electrophoresis. Again, in each case where it was examined, the acidic α -chain and the more basic β -chain from each clone showed unique characteristics (9–11). The third and final analysis was based on peptide mapping (12). Separating the products of trypsin digestion by electrophoresis and chromatography, a peptide map could be generated for each clone.

In the study by Kappler et al. (4), the panel of five T cell clones used was particularly informative. DO-11.10 was an OVA/H-2A^d-specific hybridoma from BALB/c mice, and it was used by Haskins et al. (6) to produce the KJ1-26.1 mAb. 7DO-286.2 was the one hybridoma screened from ~400 independent OVA-specific clones that reacted with KJ1-26.1, and consistent with this selection, its Ag specificity was indistinguishable from DO-11.10. A third hybridoma, 3DT-52.5, was a self-reactive clone, specific for H-2D^d, and its specific Ab was KJ12-98.15. As a fourth example, the panel included the lymphoma-Ab pair originally published by Allison et al. (3). To compare mouse and human TCRs, they analyzed the immunoprecipitation products of the Ab T20/24 specific for the human T lymphoma, HPB-MLT. This latter Ab-tumor cell pair had been produced several years earlier by Trowbridge and his colleagues (4) but was previously unpublished.

Each mAb was used to purify the receptor from lysates of the appropriate ¹²⁵I surface-labeled T cell hybridoma or lymphoma. Sure enough, the tryptic maps from DO-11.10 and 7DO-286.2 were identical, which were consistent with their Ag specificity and reactivity with KJ1-26.1. Based on this result, the authors argued that their TCR genes might be encoded in the germline and not the result of somatic hypermutation. This

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² The vernacular in the field is to refer to the Ag receptor as the T cell receptor (TCR), when in reality, it is no such thing. It does not bind T cells, it binds Ag/MHC. Pedantry aside, most people refer to it as the TCR with the understanding that it means T cell Ag receptor.

indeed turns out to be true, although junctional diversity plays an important role in Ag specificity (13, 14).

This procedure was repeated for the MHC class I-specific 3DT-52.5, and the result was that there were shared and distinct peptide fragments when compared with the products from DO11.10. This was, of course, consistent with the presence of constant and variable regions. The analysis of C6VL produced a peptide map that was quite different from the first two, although there were two peptides that appeared to be identical in all three peptide maps.

The immunoprecipitation products from HPB-MLT consisted of α - and β -chains that could be separated on the basis of m.w., and this was consistent with the work of Meuer et al. (5). The peptide map analysis revealed a pattern quite different from mouse T cells, with the possible exception of two "constant" peptides from the α -chain and one from the β -chain.

The presence of constant and variable peptides derived from otherwise very similar disulfide-linked heterodimers, combined with compelling functional effects mediated by the mAbs, left little if any doubt that the clonally distributed Ag receptor on T cells had been identified. In a sense, this was the beginning of characterizations that would continue for several years. There were myriad questions concerning the genetic basis for diversity and Ag/MHC recognition that could be answered most readily by cloned genes already in the wind (15).

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