milestones

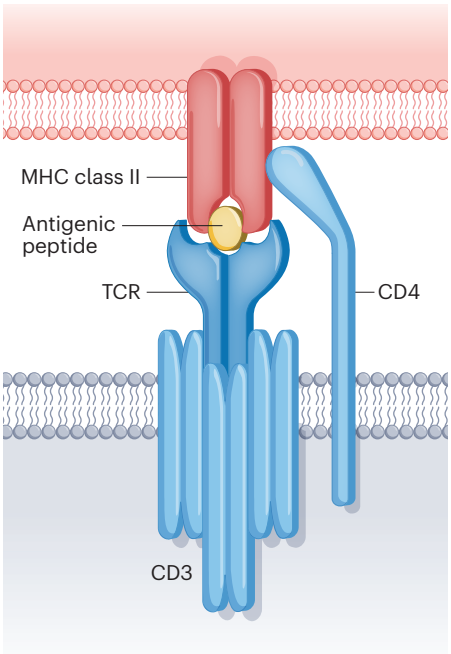
Milestone 8

Identifying and cloning the T cell receptor

T cells are able to detect and respond to specific peptide antigens by virtue of the unique T cell receptor (TCR) complexes they express on their cell surface (see image). The TCR of conventional αβ T cells comprises highly variable α and β chains (which form the antigen-binding αβ heterodimer) and an invariant CD3 signalling complex, which is non-covalently associated with the α and β chains and composed of εγ and εδ heterodimers and a ζ homodimer. The breakthrough experiments that described this multimeric protein complex — thereby identifying the elusive 'antigen-binding structure' of T cells — took place in the early 1980s.

At the time, it was known that T cells, like B cells, can bind to a wide range of antigens via a 'clonally unique recognition structure', which was suspected to bear resemblance to B cell immunoglobulins. However, it was also understood that, unlike B cells, T cells only bind antigen in combination with the correct major histocompatibility complex (MHC) molecules' (Milestone 4), which, in turn, are bound by the 'subset-specific recognition elements' T4 and T8, today known as CD4 and CD8, respectively (Milestone 5). Another lineage-specific molecule that had been described and found to be intimately linked with T cell function was T3, a glycoprotein later identified as CD3ε. Reinherz et al. showed that monoclonal antibodies against T3 inhibited T cell responses, which suggested that T3 was involved in antigen-receptor function. However, as this protein was expressed in a non-clonal fashion, the race was on to identify the antigen-specific part of the TCR.

To this end, antisera and monoclonal antibodies against T cell lymphomas, hybridomas and T cell clones were generated and screened for clonal specificity. The first success was reported in 1982. Allison et al. described the antigen (on a mouse T cell lymphoma) that was reactive with their antibody as a glycoprotein composed of disulfide-bonded subunits of 39 and 41 kDa, and proposed that these corresponded to the antigen-specific subunits of the TCR. In 1983, two papers by Reinherz and colleagues described the generation of monoclonal antibodies against a similar heterodimeric clonal glycoprotein on human



"two papers in *Nature* … provided strong evidence that these encoded TCR subunits"

$T8^+$ and $T4^+$ T cell clones. The targeted protein complex, named T idiotypic (Ti), was found to be similar to surface proteins that were previously shown to co-precipitate with T3. The authors proposed that the heterodimers, non-covalently linked to T3, were, indeed, the antigen-specific α and β subunits of the TCR and demonstrated that antibodies binding to each Ti could replace antigen plus MHC in stimulating the relevant clone. In the same year, similar glycoproteins were described by Pippa Marrack, John Kappler and others on mouse T cells. Moreover, enzymatic digests and proteomic analysis indicated that these glycoproteins, like immunoglobulins, had both constant and variable regions.

The next year (1984), two papers in *Nature* from the laboratories of Tak Mak and Mark Davis described the isolation of T cell-specific cDNA clones and provided strong evidence that these encoded a TCR subunit. Both studies included the construction of extensive T cell cDNA libraries that were screened for T cell-specific clones via subtractive hybridization with B cell-derived mRNA. Working on the assumption that genes encoding antigen-specific TCR proteins should be rearranged as a mechanism to generate diversity, Hedrick et al. used restriction enzyme analysis to compare their clones to genomic DNA from T cell and non-T cell sources, and identified one clone that seemed to be uniquely recombined in T cells. In back-to-back papers, the same authors presented the sequence of this clone and identified variable, constant and joining regions, resembling the genomic organization of immunoglobulin genes. Meanwhile, Mak and colleagues identified, sequenced and translated a T cell-specific cDNA clone that encoded a 30-kDa protein. They found structural similarities between its putative amino acid sequence with mouse and human immunoglobulin light chains. Microsequencing studies subsequently established that the cDNAs identified in these studies encoded the TCRβ chain.

These breakthroughs were followed by the identification of the different CD3 subunits and the γδ TCR (Milestone 9), all the way to our current understanding of the octameric TCR complex, its signalling pathways, and its function as a mechanosensory receptor. They also provided the basis to determine further mysteries of T cell antigen recognition, such as the mechanisms of central tolerance (Milestone 12) and of course have had numerous therapeutic applications, including the generation of CAR T cells (Milestone 20).

Alexandra Flemming Chief Editor, *Nature Reviews Immunology*

Milestone studies

Yanagi, Y. et al. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature* **308**, 145–149 (1984) | Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. Isolation of cDNA clones encoding T cell specific membrane-associated proteins. *Nature* **308**, 149–153 (1984) | Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. *Nature* **308**, 153–158 (1984)

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