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Discovery of the γδ TCR: Act II

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In 1986, the discovery of the TCR (1–3), the cloning of the α and β genes that encode it (4–9), and various findings regarding TCR function were still recent events. The latter included the role of the "T3 glycoprotein" (CD3 complex) in TCR signal transduction, its physical association with the TCR, and the requirement of the TCR for its cell surface expression (10–13). More peripherally, Tonegawa's group (14) had uncovered an additional TCR-like gene in mice, which they named γ. γ was enigmatic and somewhat troublesome. Although the gene was transcribed by T cells (15), its predicted protein product had not been detected. It was located on a different chromosome than α or β (16); however, the complexity of the γ locus, containing multiple V and C region segments, suggested it was also an AgR locus (17, 18). In contrast, its strong expression in immature thymocytes, but not in mature T cells (19, 20), and the developmental timing of its rearrangements (21, 22) encouraged speculation about a role in T cell maturation (23). An alternative idea that γ represented the evolutionary relic of a primordial TCR gene was also entertained.

The two complementary articles by Brenner, Bank, and colleagues (24, 25), published in the summer of 1986 and featured in this issue, ended such speculation. What they observed was actually far more exciting than the hypothetical scenarios. Brenner et al. (24) described a small population of human PBLs that expressed T3 but not TCRαβ, because they were not detected by βF1 and WT31, two mAbs thought to recognize determinants on all human αβ TCRs. These cells were enriched in PBLs from patients suffering from bare lymphocyte or ectodermal dysplasia immunodeficiency syndromes and could be further expanded in vitro to obtain near homogeneous lines of $T3^+TCR\alpha\beta^-$ and $T3^+TCR\alpha\beta^+$ cells, as confirmed by immunoprecipitation of cell surface proteins and Northern blot analysis of whole-cell RNA with TCR gene-specific probes. However, chemical cross-linking of cell surface proteins followed by immunoprecipitation with anti-T3 mAb revealed that the $TCR\alpha\beta^-$ cells instead carried two new types of CD3-associated polypeptides of 55 and 40 kDa. Because the predicted M_r for the human γ protein was 40 kDa, and 55 kDa when fully glycosylated, Brenner and colleagues raised antisera against small peptides representing portions of the human Cγ and Vγ protein sequences. These antisera precipitated a 55-kDa molecule from lysates of the $T3^+TCR\alpha\beta^-$ cells. When this molecule turned out to be identical to the 55-kDa T3-associated protein, they concluded that it was γ or at least a protein serologically cross-reactive with γ. The 40-kDa protein also associated with T3 appeared to be a novel unknown polypeptide, which they christened δ. Intriguingly, they suggested that γ and δ might form a T3-associated heterodimer similar to the known αβ TCR (i.e., a second putative TCR), but they could not formally rule out the possibility that γ and δ associated in separate complexes with the T3 glycoprotein.

Instead of PBLs, Bank et al. (25) investigated human thymocytes. To enrich immature thymocytes, in which γ gene expression was known to be strong, they removed $T4^+$ ($CD4^+$) and $T8^+$ ($CD8^+$) cells. They then cultured the remaining double-negative thymocytes in the presence of IL-2 and feeders to expand $T3^+$ cells, managed to isolate several $T3^+$ double-negative cell clones, and studied one (CII) in depth. CII cells produced IL-2, proliferated, and became cytotoxic upon stimulation with an anti-T3 Ab plus additional stimuli, all of which confirmed that the CD3 complex on CII cells was functional. Northern blots showed that CII cells expressed high levels of TCR-γ mRNA but not mature (full-length) TCR-β or TCR-α transcripts, and mAb βF1 did not precipitate TCRαβ from these cells. However, immunoprecipitation of CII cell surface proteins with anti-T3 Abs coprecipitated two additional proteins of 44 and 62 kDa, suggesting that, on CII cells, T3 was not expressed with TCRαβ but instead with a novel heterodimer made up of unknown proteins. Experimentation stops there, and the investigators merely suggest, based on the expressed mRNA, that one of these unknown proteins might be γ. However, a note added in proof shows that they were later able to identify the 44-kDa protein as TCR-γ by immunoprecipitation with Brenner's anti-γ serum.

Combined, the papers by Brenner et al. (24) and Bank et al. (25) told a compelling story. Brenner et al. (24) showed surface expression of the TCR-γ protein and the novel δ protein in association with T3. Bank et al. (25) showed that the two proteins were expressed within a single cell and, hence, perhaps more likely as a heterodimer and that the novel complex supported CD3 function, like the αβ TCR. Bank et al. (25) alone could not make a case that the novel putative TCR was expressed by a separate lineage of cells because they had studied it only on thymocytes, but Brenner et al. (24) had found it on PBLs. The difference in the two studies of the apparent molecular masses of the putative γ proteins and the δ proteins was not immediately addressed. In the following year, another paper from Brenner's group (26) showed that human TCRγδ heterodimers are diverse and that some are disulfide linked, whereas others are not.

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The step-wise uncovering of the γδ TCR could be likened to a dramatic three-act play: the opening act, starting with a "bang," created much smoke and confusion, following the accidental discovery of the TCR-like γ genes (14). The second act, represented by the critical Brenner et al. (24) and Bank et al. (25) studies, cleared much of the smoke, by providing strong evidence that γ might be part of a second alternative TCR. But they also raised new tension with the discovery of the δ protein. Everybody felt that δ was important, but its molecular identity and, with it, the nature of the γδ TCR, were only resolved in the third and concluding act.

In the wake of the Brenner et al. (24) and Bank et al. (25) papers, several other studies further established γδ T cells as a separate and diverse cell lineage (26–31). The molecular identity of δ was resolved in the following year, subsequent to the discovery of the genes that encode it (32–37). Together, these studies confirmed the γδ TCRs as kin to the αβ TCRs and BCRs in structure and function: a third adaptive Ag-recognition system expressed by a distinct lymphocyte lineage. This tripartite organization of the adaptive immune system seems to be important because it has been found in all jawed vertebrates examined, and although γδ T cells in rodents and primates are relatively small in number, they are more prevalent in other vertebrate species. Furthermore, recent evidence suggests that the tripartite organization of the adaptive immune system evolutionarily predates higher vertebrates and their Ig-like AgRs (38). Brenner et al. (24) and Bank et al. (25), by having the insight in 1986 to propose a second TCR expressed by a separate lineage of cells, changed the course of investigations in this field.

Disclosures

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