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# The Heart of the Machine: Construction of T Cell Identity, Made Accessible

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**This *Pillars of Immunology* article is a commentary on "Induction of T cell development from hematopoietic progenitor cells by Delta-like-1 in vitro," a pivotal article written by T.M. Schmitt and J.C. Zúñiga-Pflücker, and published in *Immunity*, in 2002. *The Journal of Immunology*, 2022, 209: 1235–1236.**

Twenty years ago, a paper appeared that catalyzed the emergence of a field. Schmitt and Zúñiga-Pflücker (1) described a new monolayer-based tissue culture system in which T cell development from hematopoietic precursors could be supported in vitro outside of the thymus. At a stroke, this work resolved debates about key functions of the thymic epithelium, revealed steps in early T cell differentiation with unprecedented precision, allowed T cell lineage commitment to be studied quantitatively, and created an experimental framework for connecting the T cell lineage to the whole range of other hematolymphoid developmental pathways. As subsequent work showed, the system developed by Schmitt and Zúñiga-Pflücker allowed efficient, routine T lineage development from single progenitor cells, enabling rigorous study of cell fate determination at a clonal level. It also opened up the black box of the thymus, enabling genetic perturbation and titration studies that created a foundation for a molecular understanding of the process through which cells enter into the T cell pathway. Even though this system did not go all the way to generate functional mature T cells, it paved the way for work in numerous other laboratories, ultimately helping to make T cell development one of the best understood developmental systems in mammalian biology.

At the time this work emerged, the thymus was of great interest as the site for T cell development in the body but was technically challenging to study. Since pathbreaking work in the 1980s and 1990s with transgenes coding for TCRs of known specificity (2, 3), it had been recognized that the thymus processed developing T cells differently, depending on the TCR gene rearrangements they happened to have undergone. A major discovery was that the fate of developing T cells, whether they lived, died, or differentiated as a helper or cytotoxic cell, depended not only on TCR expression, but also on details of the interactions between the cells' specific TCRs and the particular MHC–peptide complexes presented in the thymic environment (reviewed in Refs. 4, 5). Thus, the thymus not only permitted T cell development but also shaped the TCR repertoire that emerged. To examine this process more closely, researchers exploited special fetal thymic organ culture systems that combined fetal thymic or fetal liver–derived precursors from one donor with a thymic environment from another donor, either with lymphocyte-depleted fetal thymic lobes or with reaggregates of purified fetal thymic epithelial cells (6); the precursors' development was then studied in the organ culture. The rich knowledge and resources for the genetics of the mouse MHC and Ag presentation machinery that were available at the time were extensively exploited in these studies. Virtuoso work with this fetal thymic organ culture system in the 1990s led to great advances toward understanding the genetic and microenvironmental signaling requirements for TCR-mediated selection (reviewed in Refs. 4, 7).

Insights from these organ culture studies are now textbook bullet points. However, the fetal thymic organ culture system was technically demanding, temperamental about reagents and tissue culture ware quality, and a great logistical challenge for large-scale assays, due to the need to coordinate yields from two cohorts of differentially timed mouse pregnancies. Also, the aspects of T cell development that were readily sampled in these organ cultures were limited to relatively late intrathymic stages. Early events, when the precursors first entered the thymus, were especially hard to study, as the numbers of very immature cells were initially dwarfed by the larger, organized epithelial lobe structures. Exceptionally delicate experiments introducing single precursors into modified fetal thymic organ cultures had given indications that these early events were crucial to determine cell fate (8). However, precursors could not be manipulated or quantitatively tracked once they were embedded within the organ cultures. Attempts to culture hematopoietic precursors on monolayers of thymic epithelial cells, to improve accessibility, failed to promote T lineage development, as the epithelial cells rapidly lost their functional activities when cultured in "two dimensions" rather than in "three dimensions" (9). The black box of an organized thymic lobe appeared essential but impenetrable.

All of these issues were transformed when Schmitt and Zúñiga-Pflücker developed the coculture system that used a Delta-like-1–expressing OP9 cell line (OP9-DL1). This work sprang from evidence that Notch pathway signaling was crucial for T cell development, evidence that had just emerged during the previous 3 y (10, 11). It also built on elegant advances in

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Abbreviation used in this article: OP9-DL1, Delta-like-1–expressing OP9 cell line.



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hematopoietic differentiation in vitro, spearheaded by groups in Japan (12). At one step, it turned out that much of the three-dimensional environmental requirement for the early stages of T cell development had been a requirement to maintain expression of Notch's ligand on thymic epithelium (13). Now, OP9-DL1 monolayer cocultures enabled live developing cells to be tracked clonally, switched from one condition to another quantitatively for lineage commitment assays, and manipulated genetically middifferentiation, and these cultures allowed manipulated cells to be returned to differentiation conditions, picking up where they left off. This system made possible rigorous testing and validation of precursor–product relationships between specific phenotypic stages in the T cell pathway, under conditions that dramatically reduced concerns about selective proliferation and survival as compared with in vivo assays (14–17). It also provided a clear view of the lineage commitment process for T cell development (18–21), which is now better understood than for any other hematopoietic lineages. The simplicity and openness of the system also enabled requirements for T cell developmental and lineage commitment to be related precisely to different Notch–Notch ligand binding strengths (22, 23), among other environmental signaling requirements. Genetic manipulation of cells in OP9-DL1 coculture was also fundamental to the quest to understand the gene regulatory networks that ultimately underlie early T cell developmental changes (reviewed in Ref. 24). This system, therefore, had a transformative impact on the basic science of developmental fate determination at the single-cell level.

The OP9-DL1 coculture system and its sister system using Delta-like 4-expressing OP9 cell line proved to be adaptable to support considerable T lineage differentiation by pluripotent stem cells (25), as well as supporting both human and mouse cells (15, 26). It also has become a springboard for research on visionary translational programs to generate T cells on demand. There have been many refinements and modifications added to the basic OP9-DL1 system since 2002, including culture systems using forced Notch ligand expression in other cell lines (27). Several recent derivatives of the original system are arguably more effective than OP9-DL1 cells, especially for promoting development all the way through TCR-mediated positive selection. Ironically, one of the most effective of these new systems returns to a three-dimensional format, allowing stromal cells with forced DL4 expression to form organoids (28). However, the paper by Schmitt and Zúñiga-Pflücker was a turning point, launching several new fields. For those who were working in T cell development when this paper came out, it was the moment when a barrier fell.

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## Disclosures

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