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Pillars of Immunology

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The Power of Dilution: Using Adoptive Transfer To Study TCR Transgenic T Cells

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Of the many tools used to study T cell function, few have been as informative as the development of TCR transgenic (Tg) mice. However, utilizing these mice to study normal immune responses in vivo proved surprisingly difficult and hinged on the insightful approach described in the *Pillars* article featured in this issue by Kearney et al. (1).

TCR Tg mice were developed in the late 1980s (2–4) and were of immediate benefit in confirming and extending the principles of positive and negative selection of thymocytes established in prior studies on bone marrow chimeras and thymus-grafted mice (5). Additionally, with their large content of naive monoclonal T cells in spleen and lymph nodes (LNs), TCR Tg mice were of obvious value for studying the functions of mature Ag-specific T cells. Indeed, with the aid of TCR clonotype-specific Abs, it was rapidly established that TCR Tg T cells gave spectacular primary responses to specific peptides in vitro, both for $CD4^+$ and $CD8^+$ T cell subsets; this was very useful because prior information on primary responses of naive T cells rested on studies with polyclonal T cells and was limited to experiments on superantigens and alloantigens (mixed lymphocyte reactions).

For examining T cell responses in vivo, investigators were presented with the obvious question of what happens when TCR Tg mice are injected with specific Ag. A priori, one might expect that such injection, especially with addition of an adjuvant, would lead to an overwhelming primary response, triggering a massive cytokine storm and rapid death. However, no such immunological eruption occurred: the injections proved innocuous with disappointingly few signs of a vigorous immune response. I remember this myself when Steve Hedrick brought his cytochrome *c*–reactive CD4 TCR Tg line AD10 (6) to my laboratory at Scripps in the early 1990s. We injected these mice by various routes with specific peptide or protein with or without adjuvant (actually, Steve did the injections; I provided pipe smoke as an extra adjuvant), however, except for minor signs of T cell activation, we saw frustratingly few signs of an immune response. Despite these negative findings, which were never published, at least one group did have some success with this approach. Thus, for lymphocytic choriomeningitis virus (LCMV) peptide-reactive $CD8^+$ T cells, Kyburz et al. (7) found that injecting the 318 TCR Tg line with either LCMV peptide or virus did cause a transient mild increase in numbers of the Tg $CD8^+$ T cells in the spleen. However, whether this increase in cell numbers reflected proliferation (which was not easy to measure in vivo in those days) or simply recruitment of circulating T cells to sites of Ag localization (8) was unclear.

We now know that the key to following the response of TCR Tg cells to Ag in vivo is to transfer limiting numbers of these cells into nontransgenic hosts. This adoptive transfer approach originated from models on T–B collaboration dating from the 1960s and was first used successfully for the CD8 TCR Tg line reactive to the male Ag H-Y (9). In that study, the authors found that transfer of small numbers of Tg $CD8^+$ T cells from female mice into T-deficient (nude) male mice led to rapid and prominent expansion of the donor cells, followed by a decline in cell numbers and induction of anergy in the surviving cells.

Because the latter study culminated in tolerance rather than immunity, there was still a clear need for a TCR Tg model in which one could study a normal immune response, especially a primary response of $CD4^+$ cells leading to Th cell generation and Ab production, followed later by formation of memory T cells. The elegant model of Kearney et al. (1) featured in this *Pillars of Immunology* met this need.

The main goal of these authors was to establish why the manner of Ag presentation can have such different effects on the immune response of $CD4^+$ T cells, notably a powerful primary response followed by immunity and memory after injection of Ag with CFA versus induction of anergy and tolerance with Ag given alone or in IFA. Tracing the fate of the responding T cells in these two situations was very difficult in unimmunized normal mice because of the very low frequency of Ag-specific T cells. For this reason the authors turned to TCR Tg mice, namely to the D011.10 $CD4^+$ line specific for chicken OVA peptide 323–339 (OVA-p). In initial studies, they confirmed that direct immunization of this TCR Tg line with OVA-p/CFA had little or no effect on the subsequent reactivity of TCR clonotype-positive (CP) $CD4^+$ T cells, relative to cells from unimmunized mice. Very logically, they concluded that the negative results with intact TCR Tg mice were because of competition from "their artificially high frequency of Ag-specific cells." Hence, they decided to dilute out the specific T cells using an adoptive transfer system.

The system they chose was to inject CP $CD4^+$ T cells i.v. in numbers sufficiently small for the donor cells to comprise

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Abbreviations used in this article: CP, TCR clonotype-positive; LCMV, lymphocytic choriomeningitis virus; LN, lymph node; OVA-p, OVA peptide 323–339; Tg, transgenic.

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only ~0.2% of host LN cells. Their key finding was that, after subsequent s.c. immunization with OVA-p/CFA, numbers of donor CP cells increased dramatically in the draining LN and, to a lesser extent, in other sites. The donor cells in the draining LN were in cell cycle, indicating that the increase in numbers of the donor $CD4^+$ T cells was a reflection of marked proliferation rather than cell trapping. The expansion of the donor cells peaked at 3–5 d after immunization; histologically, the cells accumulated first in the LN paracortex and then moved into the follicles between day 3 and day 5, thereby providing important new insights into the origin and migration of T follicular helper cells. Thereafter, total numbers of donor cells declined to ~20% of peak numbers by day 18, at which time the cells showed increased responsiveness to Ag, indicative of memory.

The results were distinctly different when the T cells were injected with OVA-p without CFA, that is, as peptide given i.v. in saline or s.c. in IFA. In these situations there was a brief proliferative response in all secondary lymphoid organs; the response peaked on day 3, was restricted to the paracortex, and then fell abruptly to very low levels by day 18. Notably, the few cells recovered at this stage were anergic and much less sensitive to Ag than naive donor cells. This finding of an initial immune response culminating rapidly in tolerance induction rather than immunity was reminiscent of the above-mentioned studies on the H-Y TCR Tg line for $CD8^+$ T cells (9) and was also in line with earlier studies on the response of polyclonal $CD4^+$ T cell responses to superantigens (10, 11). Tolerance induction in these various situations presumably reflects the lack of inflammatory cytokines and other "second signals" during initial Ag presentation.

For immunogenicity, the authors extended their studies by transferring a mixture of Tg $CD4^+$ T cells and Tg B cells, thereby providing direct histological evidence on the in vivo events involved in T–B collaboration (12); they also used the system to visualize the migration of effector and memory $CD4^+$ T cells into nonlymphoid tissues (13). The approach was rapidly applied by others to $CD8^+$ T cells and led to the important additional discovery that reproducing normal immune responses and memory generation with Tg cells necessitated transferring trace numbers of cells, equivalent to the precursor frequency of Ag-specific cells in normal mice (14–17).

The adoptive transfer model developed by Kearney et al. (1) for studying TCR Tg T cells was enormously influential and is still an integral feature of many experiments on cellular immunity. In retrospect, it seems such a simple idea: with the long history of adoptive transfer experiments and the intense interest in TCR Tg mice, why didn't someone else come up with this idea? Why indeed!

Disclosures

The author has no financial conflicts of interest.

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