The Journal of Immunology

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*J Immunol* 2016; 197:3429-3430; ;
doi: 10.4049/jimmunol.1601599
http://www.jimmunol.org/content/197/9/3429



*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Pillars of Immunology

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Monoclonal Abs to major components of the immune system are completely taken for granted now; however, at the time of their initial development, they opened broad new vistas on immune biology. The two papers featured in this installment of *Pillars of Immunology*, published in 1980 and 1989, describe Abs to the human and mouse TCR, respectively. They are among the most highly cited papers in the 100-year history of *The Journal of Immunology*. It is interesting to consider the impact that these papers made at the time, as well as how they continue to impact the field.

The article by Van Wauwe et al. (1) from 1980 does not actually report the generation of the OKT3 mAb to human CD3. The creation of OKT3 (and other now-famous anti-human T cell Abs, such as OKT4 that binds to CD4) was reported earlier by Goldstein, Schlossman, and colleagues (2). Rather, the featured article establishes that the OKT3 Ab drives T cell proliferation through TCR cross-linking. In the late 1970s, B cell hybridoma technology was relatively new (3), and Abs of functional interest were often published before their precise specificity was fully understood. This article was a breakthrough because it represented a means to definitively identify human T cells from other blood mononuclear cells and a means to induce human T cell proliferation in vitro.

At the time, the method of choice to enrich human T cells was the rosette assay, wherein PBMCs were mixed with sheep RBCs and centrifuged through Ficoll-Hypaque. The pelleted cells were enriched for T cells because human CD2 binds to an LFA-3 homolog on sheep RBCs, although we did not understand that at the time. Flow cytometry was not yet developed, and the specificity of the OKT3 Ab for human T cells was confirmed by Van Wauwe et al. (1) by the fact that the Ab stimulated proliferation of rosette-positive cells but not rosette-negative cells. They showed that Fab fragments of OKT3 were much less efficient at stimulating proliferation, suggesting that receptor cross-linking was involved. Finally, to prove that the Ab was recognizing a molecule only on T cells (rather than binding a broadly expressed molecule that only stimulated proliferation in T cells), they performed Scatchard binding analysis with radio-labeled Fab fragments. Their results suggested chemical uniformity and estimated 50,000 binding sites per T cell. In the penultimate paragraph of the discussion, the investigators speculated that "it seems plausible to describe the OKT3 lymphocyte receptor as a 'T cell stimulation' receptor"; indeed, their work paved the way for the eventual determination that OKT3 recognizes conserved components of the human TCR.

The OKT3 Ab proved to be a real winner, because it recognizes a nonpolymorphic subunit of the human TCR: CD3ε (4). It and the analogous mouse Ab (2C11) have been used in countless studies to induce proliferation of T cells in vitro, allowing the discovery of costimulatory molecules, inhibitory molecules, and many other cellular features of T cell biology. It is still widely used for the study of human T cells. Indeed, this Ab went on to become the first mAb to be approved for clinical use as muromonab in humans (5).

The second featured article reports the characterization of a mAb to the murine TCRβ-chain (6). By 1989, some Abs to the mouse TCR had been developed, but these were specific to certain Vβ or Vγ subunits or to CD3 (7). There were no Abs that could distinguish the two major types of mouse T lymphocytes: TCRγδ and TCRαβ T cells. The field needed a mAb that would recognize all TCRαβ T cells. Kubo et al. (6) immunized animals with purified TCRs from one T cell hybridoma; when the sera were discovered to cross-react with another hybridoma, they reasoned that the animal had made at least some Abs to a monomorphic part of the TCR.

Several technical advances were made between the 1980 publication of the Van Wauwe et al. (1) article and that of the Kubo et al. (6) article in 1989 to facilitate the generation and study of mAbs. Kubo et al. immunized hamsters instead of mice, an approach that had become popular for making mAbs to mouse proteins. The unique Ig-binding properties of protein A and streptavidin discovered in the 1960s were capitalized upon in the 1980s, and protein A was available conjugated to beads or fluorochromes; in addition, mAbs could be biotinylated. Kubo et al. (6) put these recent advances to good use and succeeded in identifying two mAbs (including H57-597) that stimulated proliferation in both hybridomas. They compared immune precipitates using

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1601599

2C11 and H57-597 by two-dimensional SDS-PAGE to show that H57-597 recognized the αβTCR, but not the γδTCR, thereby ruling out reactivity to the CD3 chains. Finally, because flow cytometry was an emerging technique in the field, the investigators used two-color flow cytometry to elegantly, for the first time, report the ratios of γδ/αβ T cells in lymphoid organs and how they varied with age.

With >1000 citations of this paper, the usefulness of the H57-597 Ab to basic research in immunology cannot be overstated. This unique reagent is widely available commercially and is used for immunoprecipitation, flow cytometry, in vitro and in vivo stimulation, in vivo depletion, and immunofluorescence.

In summary, these two studies represent the best of an era during which application of mAb technology to understand the basic biology of lymphocytes initiated an explosion of new information, commercialization, and new treatment options.

## Disclosures

The author has no financial conflicts of interest.

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