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Pillars of Immunology

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Monoclonal Abs opened a new frontier in the late 1970s. In an early example of academic/industrial collaboration, Stuart Schlossman at Harvard Medical School and Gideon Goldstein at Ortho Pharmaceuticals emerged as the fastest gunslingers in the Wild West of making mAbs to define T lymphocytes and their subpopulations. Two papers from this team that stand out as moving this frontier forward are highlighted here as *Pillars of Immunology*. Kung et al. (1) introduced the methodology of using a complex immunogen, purified T lymphocytes, together with the power of screening large numbers of mAbs produced by immortalized B cells from the immunized mice to identify novel lineage-specific reagents. Reinherz et al. (2) went on to demonstrate how one of these mAbs, OKT4, defined a functional subpopulation of Th cells.

We now take for granted the hundreds of surface markers that define WBC subpopulations and developmental lineages. In the 1970s there were a few hard-fought ways to isolate T lymphocytes, the most common of which was to take advantage of serendipitous "rosetting" of human T lymphocytes with sheep erythrocytes (Goldstein's team would later generate an mAb to the sheep erythrocyte receptor, now known as CD2) (3). Some traction on subpopulations had also been gained earlier through the study of alloantisera generated in mice based on polymorphisms in lineage-specific Ags such as Ly2/3 (equivalent to modern-day CD8α and CD8β) that existed between some inbred strains (4). Human T cell subpopulations were initially identified with antisera to T lymphocytes generated in rabbits or horses that were then "adsorbed" to remove all Abs to clonal B and T lineage tumor cells that expressed most of the pan-lineage Ags and some T cell subtype–specific Ags, leaving other subtype-specific Abs in the refined antisera (5). The carefully prepared "heterosera" would then reproducibly identify T cell subtypes. This was a systematic approach, but it still generated a heterogeneous reagent with great batch-to-batch variation. A particularly useful preparation of heterosera defined by adsorption with B and T CLL tumor lines was called T heterosera 2 and defined populations corresponding to cytotoxic (positive) and helper (negative) T lymphocytes. The T heterosera 2 nonreactive population contained the T cells that helped B cell responses and proliferated in response to recall Ags such as tetanus toxoid (6). Both the Ly2/3 Ags in mice and the anti-human heterosera defined populations that cooperated to generate cytotoxic T cells (6, 7).

mAbs to human lymphocytes initially defined major surface Ags that were not subtype specific. For example, Barnstable et al. (8) had immunized mice with human tonsil cells and generated a panel of broadly reactive mAbs, including an Ab specific for a shared determinant of all human MHC class I Ags (HLA-A, -B, and -C) that is still in common use: W6/32. However, there were limitations to the approach used in that most of the other mAbs generated were specific for highly expressed carbohydrate determinants such as blood group Ags.

The method codified by Kung et al. (1) opened a more systematic stage of discovery of T lymphocyte surface Ags through innovations in the immunization and screening process. First, they did the best they could to immunize with a T lymphocyte from human peripheral blood using the best available method, erythrocyte rosetting. Thus, the immunogen was complex, but there was an increased chance of obtaining T cell–specific mAbs using this starting point. Second, following the fusion, they immediately distributed the resulting hybridomas into hundreds of wells that would each contain a single or small number of clones. Although this may have seemed labor-intensive at the time, it was a key innovation because it allowed them to screen for interesting reaction patterns without being swamped by the pan-reactive Abs to abundant Ags that dominated the analysis of Barnstable et al. (8) a year earlier.

These innovations led to the creation of the definitive mAb of T cells: OKT3. To this day, one could say that this mAb, and others made in the same time frame that recognize this complex, truly define human T lymphocytes, as they react with a nonpolymorphic subunit of the TCR that is not expressed elsewhere (9). A second Ab that was defined in this screen was OKT1, which we now know binds the Ag CD5. The flow cytometry analyzer of the time, the Ortho Cytofluorograph, was not sensitive enough to detect the Ag on a small subpopulation of "transitional" B cells that we now know express it (10). CD5 plays an important role as a negative regulator of Ag receptor signaling, and its expression level on T cells is set during development, based on the nature of positively selecting signals in the thymus (11). The third interesting Ab was OKT4, which reacted with roughly half of T lymphocytes. The subpopulation recognized by OKT4 was the focus of the Reinherz et al. (2) study.

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Abbreviation used in this article: HLDA Workshop, Human Leukocyte Differentiation Antigen Workshop.

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Reinherz et al. (2) characterized the T cell subsets defined by the newly generated mAb OKT4. They found that OKT4 reacted with just greater than half of T lymphocytes in peripheral blood of healthy individuals. They used the relatively new method of fluorescence-activated cell sorting to isolate the OKT4-reactive and OKT4-negative populations from healthy individuals and tested their responses to lectin mitogens and recall Ags, as well as their cytotoxicity following in vitro MLRs. The most striking results were that the OKT4-reactive population was uniquely able to proliferate in response to recall Ags such as tetanus toxoid and that the OKT4-negative population contained most of the cytotoxic T cells that were generated in an MLR. Therefore, OKT4 seemed to recognize the reciprocal subpopulation of that recognized by T heterosera 2. However, unlike T heterosera 2, which was made in limited batches that varied owing to many factors, one can still purchase OKT4 from many vendors and it will have nearly identical molecular composition to the Ab used in the 1979 study. Reinherz et al. were also able to repeat the observation initially made with T heterosera 2 that the subpopulations reciprocally defined by these reagents collaborated in the MLR to achieve maximal cytotoxic T cell responses.

The approach of immunizing mice with human leukocytes followed by screening for reactivity patterns established by Goldstein and Schlossman led to the generation of hundreds of interesting anti-human mAbs within a year of the Kung et al. report. Many Ags were identified multiple times within a publication year such that priority became increasingly hard to establish and nomenclature systems proliferated. A highly successful approach to organizing this explosion of information came through Human Leukocyte Differentiation Antigen (HLDA) Workshops in which all Abs were distributed to a number of participating laboratories in a "blinded" manner to allow establishment of unbiased reactivity patterns and supporting biochemical analysis through international collaboration (http://www.hcdm.org). This led to the establishment of the now ubiquitous "cluster of differentiation" (CD) system, which was based primarily on patterns of reactivity with normal leukocytes, their developmental progenitors, and large panels of cell lines. The first workshop, held in Paris in 1982, compared results from 139 mAbs reactive to T, B, and myeloid cells and defined CD1–CD15 (12). The ninth HLDA Workshop in 2010 defined up to CD363, the sphingosine-1-phosphate receptor 1. Schlossman was a participant in the first workshop, and the Ag recognized by OKT3 became CD3 and the Ag recognized by OKT4 became CD4, reflecting the priority of these Abs in defining these clusters. This early example of bioinformatics and model for international collaboration was set in motion by the approach of Kung et al. to efficient identification of differentiation Ags with readily shared mAbs. Innovative and highly profitable companies were formed that specialized in delivery of these powerful reagents to scientists around the world.

mAbs to CD4 allowed its cloning in conjunction with subtractive cloning methods (13). Swain's (14) formulation of a functional framework for CD4 and CD8 in MHC recognition was also greatly advanced by function-blocking mAbs. OKT3 was the first mAb to be approved by the U.S. Food and Drug Administration for use in a human in 1986 and has seen service in treatment of acute transplant rejection. In this respect OKT3 paved the way for the wider use of mAbs as therapeutics. Diagnostic use of OKT4 led to the discovery that patients with AIDS lose their $CD4^+$ T cells owing to use of CD4 as the primary receptor for entry of HIV-1 (15). Further innovations, such as screens based on functional assays, led to even greater insights and the birth of new fields, such as the discovery of lymphocyte adhesion receptors by Springer and colleagues (16, 17) and by Butcher and colleagues (18). A more direct method for rapid expression cloning of surface-expressed molecules developed by Aruffo and Seed (19) greatly accelerated cloning of many mAb-defined cDNAs, in part facilitated by the HLDA Workshops. mAb technology based on a principle of immunization with complex, but enriched, immunogens followed by screening for selective binding has also facilitated characterization of intracellular enzymatic complexes (20). In the postgenomic era, mAb production is often farmed out and few academics laboratories still practice this art. However, few reagents remain as useful as a good mAb. These *Pillars of Immunology* articles take us back to a period in the development of immunology where the breakthrough technology of mAbs met one of its greatest challenges in mapping the new territory of the T cell surface. Those quick on the draw reaped great rewards.

Disclosures

The author has no financial conflicts of interest.

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