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Pillars of Immunology

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The Reign of Antibodies: A Celebration of and Tribute to Michael Potter and His Homogeneous Immunoglobulin Workshops

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A perfect storm was brewing by the mid-1960s that was destined to pit prevailing assumptions of the new molecular biology against accumulating observations from immunology that would challenge these beliefs. Several intractable enigmas fueled the looming tempest. From the side of molecular biology, the "one gene, one polypeptide" dictum was an accepted extension of the central dogma, as was the notion that the somatic genome was a sacrosanct unchanging entity and that genetic recombination only occurred during germ cell formation. From the side of immunology, the recently appreciated phenomena of self-recognition (1) and acquired tolerance (2) had yielded a set of concepts that comprised the clonal selection theory (3–5). Key to this idea was the notion that B cells had unique AgRs, whose specificity mirrored that of the Ab the cells eventually produced. This, coupled with the well-established specificity of immune responses, implied that the inventory of such receptors, and therefore Abs, must be astronomical. Hence the puzzle: how was a seemingly infinite array of clonally distributed specificities generated by the currently held axioms of molecular biology?

A fundamental roadblock to the answer was that there was no way to interrogate individual Abs at the sequence level, much less their antecedent cellular receptors at the clonal level. Indeed, protein sequencing still required large quantities of homogeneous material, and technologies for single-cell assessment were still years away. It was against this backdrop that inducible mouse plasmacytomas were first described by Michael Potter's laboratory at the National Cancer Institute, National Institutes of Health (NIH) (6). Plasmacytoma induction with mineral oil or tetramethylpentadecane (pristane) had begun to yield a growing library of tumor lines, with monikers reflecting their mode of induction (MOPC or TEPC, respectively). Over the next two decades, Potter's plasmacytomas would become the experimental vehicle to resolve these conundrums and reshape paradigms in immunology and molecular biology. Moreover, they would enable the development of hybridoma technology. These mAb tools not only pervaded the subsequent literature, but nucleated the debate that would propel the field to crack the so-called generation of diversity problem.

Thus, the *Pillars of Immunology* article has a dual purpose. First, it highlights the development of these remarkable tools and the questions answered by studying their Ig products. Equally important, the publication date, 1965, coincides with the last of Mel Cohn's Antibody Workshops and heralded widespread adoption of Potter's plasmacytoma model, spawning the "Homogeneous Immunoglobulin Workshops" convened by Potter on the NIH campus. Accordingly, we have bookended our discussion of the *Pillars of Immunology* article itself with reflections on the genesis and impact of Potter's remarkable workshops.

Mel Cohn's 1965 Antibody Workshop

Mel Cohn (Salk Institute) laid the pioneering groundwork when he organized the first few Antibody Workshops. His vision in 1956 was to entice biochemists and molecular biologists to broaden their horizons and tackle a new biological field: immunology. Cohn assembled his own group of scientists that included Ed Lennox, Herman Eisen, Fred Karush, Jon Singer, Av Mitchison, and Rodney Porter. The fledging group of ∼50 participants met annually, supported by funds from the National Science Foundation. For Cohn's last Antibody Workshop in 1965 at Warner Springs, CA, he wanted to bring together immunology-oriented and canonical molecular biologists. He believed that protein sequencing was critical and carefully chose 100 scientists who were involved in some form of protein sequencing. Norbert Hilschmann (then a postdoctoral researcher at the Rockefeller Institute) dramatically showed that the L chain of human Ig had a V region and a C region and that the variable domain had to be joined to the constant domain somatically. Hilschmann's bombshell abolished forever the dogma of one gene, one polypeptide, and Abs came into their own.

The Article

In that same year, the manuscript selected for this *Pillars of Immunology* commentary was published. The study used several

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Abbreviations used in this article: FWR, framework region; NIH, National Institutes of Health.

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of Potter's mouse plasmacytomas as sources of "L-chain–like proteins" for analysis. With hindsight, these were indeed Ig L chains, but the investigators used cautious wording, reflecting skepticism in the field about whether these cancer-associated proteins were normal. The L chains used for analysis were obtained from the urine of mice transplanted with a plasmacytoma, because about half of the tumors yielded urinary L chains analogous to Bence Jones proteins seen in human multiple myelomas. This was a clever trick, because urine, in contrast to serum, has few, if any, other proteins, obviating the need for laborious purification strategies. This is evident from the electrophoretic analyses of native and reduced proteins shown in plate I in the *Pillars of Immunology* article.

Using these as abundant sources of homogeneous L chains that were independently derived from inbred mice, three approaches were used to interrogate their similarities and differences. In toto, the results would show that, although all shared structural and amino acid sequence similarities, each was unique, harboring discrete regions that differed. First, the classic immunochemical technique of two-dimensional immunodiffusion was used (see plate II in the *Pillars of Immunology* article). This Ouchterlony assay (7) used wells punched in an agarose gel; the center well was filled with a rabbit antiserum that had previously been made against one of the L chains (MOPC 63). L chains from this or other plasmacytomas were added to each of the surrounding wells. When the diffusing plasmacytoma L chains met the antiserum, a precipitate would form. Because the resulting precipitin lines were not entirely contiguous, as evidenced by the spurs protruding from the otherwise cleanly hexagonal pattern, one could infer that some of the epitopes on the MOPC 63 protein recognized by the antiserum were not shared by the other L chain proteins.

Second, tryptic peptide maps were performed (Plate III). These involved a standardized trypsin-mediated digestion of each protein, followed by the positional resolution of the peptides using two-dimensional paper chromatography/electrophoresis. Thus, the ending position of a peptide was determined by its size in one dimension and its charge, a surrogate for amino acid composition, in the other. These analyses added further insight, because they allowed assessment of whether the differences between the L chains implied by the immunodiffusion assay were scattered or whether instead there were stretches of similar and stretches of disparate amino acid composition. The latter appeared to be the case, because there were several tryptic peptide spots whose positions were shared across all of the L chains; however, each L chain also had several unique spots.

Third, the amino acid composition was determined (see table I in the *Pillars of Immunology* article). Using a newly developed automated technology, the results revealed that each L chain had a unique amino acid composition, despite their substantial similarity. Considered together, these findings were consistent with the notion that the L chains consisted of regions shared by all, as well as unique, regions that varied from one molecule to the next. These observations led the authors to end their article by stating "The structural nature of this variability and its relation to antibody specificity as well as the mechanisms which produce it, remain for future study." That phrase encapsulated the question that would occupy immunologists for the next 15 y, facilitated in no small part by the workshops that arose from the extensive adoption of this experimental model.

Mike Potter's Homogeneous Immunoglobulin Workshops (1969–1979)

Cohn and Potter met at the 1965 meeting, and they discussed each other's ideas and how to plan experiments. Potter was delighted in Cohn's interest and shipped him breeding pairs of the special strain of mice, BALB/cAnN, that was susceptible to tumor induction, along with detailed protocols of how to induce, transplant, and freeze tumors. Soon, there were two centers of plasmacytoma induction in the United States, with hundreds of tumors being developed in Potter's laboratory at the NIH and in Cohn's laboratory at the Salk Institute. They were on a roll. The big rush was on to see whether the homogeneous Igs could bind to any Ags, reflecting doubt in the community as to whether these were normal Igs or an aberrant tumor-associated version. Sadly, only 5% of the tumors reacted with the limited Ag screens at the time, including phosphorylcholine, dextran, levan, galactan, and interestingly, DNP (8). Indeed, these early screens predated our current belief that naturally occurring Abs are driven by environmental Ags and gut flora. But now there was an available source of proteins to determine structure, genetics, and biology. Human myeloma proteins were passé; plasmacytoma proteins were avant-garde. This brings us to the most striking quality of Potter: his generosity in giving his plasmacytomas to whoever asked, with no expectation of coauthorship. He maintained a catalog of hundreds of cell lines, tumors, and mice and contracted a company to distribute them. Literally thousands of shipments were made to laboratories worldwide, with tumors often shipped directly in mice.

Potter wanted to establish his own meetings in the spirit of the original Antibody Workshops. These annual workshops (Fig. 1) were organized by Potter and Martin Weigert (Institute for Cancer Research, Philadelphia, PA), and were held in Building 1 on the Bethesda NIH campus. Some of us (P.J.G.) remember the long oval table around which the icons in immunology were seated, watching data from two-by-two inch glass slides shown from a carousel projector operated by Potter's graduate student (M.P.C.). Cigarette smoke filled the room, forcing Elvin Kabat (Columbia University) to protest by donning his 1960s-issue gas mask. These were amazing, interactive meetings.

Importantly, there was drama at the workshops. People got excited and argued vigorously, even if it did not end up like the complete meltdown of the 1965 workshop. The major spectacle over the years was the ongoing fight between the germline and somatic mutation opponents to explain the generation of diversity. Basically, there are many Abs, as determined by protein sequencing of variable regions from Potter's plasmacytomas. Is each one encoded by a germline gene from a large inherited gene pool or by a handful of genes that diversify by somatic mutation? The germline camp, spearheaded by Leroy Hood (California Institute of Technology), claimed that each unique framework region (FWR), generally the first 30 aa identified by N-terminal sequencing (FWR1), is coded by a different variable gene (9). Hence, there were many genes, maybe 1000 for H and L chains. The somatic camp, launched by Cohn, claimed that when a sequence was likely known to be germline, such as the rare λ1 L chain, amino acid substitutions in the CDR could only be caused by somatic mutation (10). Thus, a handful of genes formed the initial repertoire, which underwent mutation and selection for CDR residues. These two theories were based on the compilation of sequences by Wu and Kabat, who painstakingly identified

FIGURE 1. The first Homogeneous Immunoglobulin Workshop, 1969. Front row (from left): Kris Hannestad, Michael Potter, Ernest Simms, Paul Anderson, Mason Smith, K. Robert McIntire, William Terry, Frank Richards, Sue Garen, Henry Metzger, Allen Kaplan, Lawrence Levine, Elvin Kabat, Thomas Waldmann, William Konigsberg, Leroy Hood, Henry Azar. Second row: Robert Ashman, J. Frederick Mushinski, Richard Asofsky, Herbert Rapp, Edward Goetzl, Herman Eisen, Philip Periman, Elliott Osserman, Allen Grossberg, Matthew Scharff, Richard Krause, Myron Leon, Oliver Roholt, Tibor Borsos, Michael Mage, Henry Kunkel, Melvin Cohn, Howard Gray. Third row: Norman Talal, John Fahey, Martin Weigert, David Davies. (Photo courtesy of NIH.)

FWR and CDR boundaries based on variability (11). The CDRs were predicted to bind Ag, which was later confirmed by x-ray crystallography (12). Other advances from the Potter workshops were the definition of serologically defined surrogates for heritable variable regions (idiotypes) and constant regions (allotypes). These were used as surrogates for actual genes to track the genetics and biological responses of B cells in different mouse strains (13–17).

Hybridomas: The Holy Grail (1975)

Because the identified Ag specificities of plasmacytoma proteins were so limited, most immunologists dreamed that someday a technique would be discovered that allowed anyone, in their own laboratory, to easily and cheaply generate immortal cell lines that pumped out milligrams of homogeneous Abs that bound to their favorite Ags. This revolution was birthed with Potter's MOPC 21 cell line that had been adapted to grow in tissue culture. In the hands of Georges Kohler and Cesar Milstein (Cambridge University), MOPC 21 variants were fused to normal Ab-secreting plasma cells that came from immunized mice (18). Voila! The world of Abs was changed forever. Potter noted, "The age of hybridomas and monoclonal antibodies rendered the antigen-binding myeloma proteins as prehistoric" (8). He lamented that Kohler and Milstein, who received Nobel Prizes in 1984, would put him out of business. This was certainly true, but of course this discovery was underpinned by the fusing cell line, graciously given to them by Potter. Even the method of growing hybridomas in mice was based on Potter's techniques of pristane priming to induce ascites (19). Nonetheless, the Homogeneous Immunoglobulin Workshops terminated soon after, because everyone was now generating their own hybridomas and no longer needed a specialized meeting to disseminate materials and information.

The Gene Cloning Explosion (1976)

Meanwhile, an undercurrent was fomenting in the nascent gene cloning field and burst to the surface in 1976, with a publication by Susumu Tonegawa (Basel Institute for Immunology, Nobel Prize in 1987) that proved variable and constant genes underwent somatic rearrangement (20). Basically, all of the questions that were raised in the Homogeneous Immunoglobulin Workshops during the protein sequencing years were now decisively answered by molecular biology. Indeed, the most mind-blowing discoveries were centered around Ig genes. Scientists were astounded to learn that the variable gene is actually comprised of three gene segments: variable, diversity, and joining. Combinatorial joining reached new heights of sophistication to provide diversity in the preimmune repertoire and produced the explanation for allelic exclusion, thanks to work by a slew of researchers, including Phil Leder (NIH), Hood, Tonegawa, and Ursula Storb (University of Washington). H chain constant genes, long ignored, took center stage when Tasuku Honjo (University of Kyoto) established that H chain class switching occurs by DNA deletion (21). Hood demonstrated that membrane and secreted forms of Igs are due to novel RNA splicing of the constant gene exons (22). And the long-standing question of germline versus somatic theories of diversity was resolved by gene mapping and sequencing. To everyone's relief, both theories were correct. There are ~100 germline variable gene segments encoding H and κ L chains, certainly a large multigene family by anyone's definition. After Ag stimulation of B cells, mutations are introduced. Somatic mutation became somatic hypermutation, an apt description considering that variable genes accumulate nucleotide substitutions a million times more frequently than spontaneous mutation of other genes. This remarkable feat is due to activation-induced deaminase, a protein that deaminates cytosine to uracil in DNA (23–25).

Summary

Michael Potter encouraged scientists at all levels, eschewing the scramble for patents, business enterprises, and remuneration. Accordingly, a commemorative workshop was held September 15–16, 2016 at the NIH to honor Potter's (1924–2013) foresight in using plasmacytoma models to obtain homogeneous Igs

for analyses of protein structure, generation of diversity, and genome organization. The meeting included some of the major players who participated in the nine workshops that Potter organized, and they illustrated how pivotal his contributions were to their research programs (see https://www.nia.nih.gov/research/labs/commemorative-meeting-michael-potters-homogeneous-immunoglobulin-workshops).

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Disclosures

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