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David H. Margulies

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Monoclonal Antibodies: Producing Magic Bullets by Somatic Cell Hybridization

David H. Margulies[1]

"The really profound changes in human life all have their ultimate origin in knowledge pursued for its own sake." Albert N. Whitehead (1)

We purchase them through catalogs and online suppliers; we mail them in polypropylene tubes; we pass them surreptitiously from hand to pocket at scientific meetings; we borrow (with or without permission) a drop from a labmate for a crucial assay; we add microliter amounts to cultures of cells to activate, isolate, kill, block, blot, immunoprecipitate, and stain; we inject them into experimental animals to inhibit or elicit responses or to track specific cell populations; and we introduce them into our patients in an effort to view or destroy their tumors. As scientists, we imagine the one that will define a new molecule, a new cell type, a new signaling pathway. As clinicians, we visualize a better therapy, a complete cure. We hope for the one that will answer *the* central question, make us famous, or make us rich. Each one is different, yet all are the same. No single class of reagents stirs our creativity, or propels our goals, our successes, even our dreams, with as much excitement as do mAbs.

With the single, brief letter to *Nature* in 1975, reproduced here, Köhler and Milstein (2) described the feasibility of producing, from mice, continuous cell lines expressing specific Abs. This represents not only the intersection of many scientists' years of effort in biochemistry, cell culture, immunology, and somatic cell genetics, but has given birth to a new technology deeply tied to diagnostics and therapeutics. In the intervening quarter century, the promise of mAb has been realized and surpassed in the laboratory, particularly when wed to molecular biology and genetic engineering. The clinical applications have been hard-earned, and remain a mixture of art, science, and indeed, luck. For some, the Köhler and Milstein paper seemed to describe the accomplishment of a trivial goal, the somatic cell hybridization of spleen cells from immunized mice to mouse myeloma tumors adapted to tissue culture. For others it was instantly clear that this paper revealed the successful destruction of a technical barrier allowing the production of designer Abs. This had been the goal as well as the source of frustration and failure for many scientists for years. The rapid and widespread applicability of mAb technology as it invaded virtually all aspects of basic research in immunology, cell biology, biochemistry, and medicine, and its clear promise in therapy and diagnosis established the basis for the award of the Nobel Prize for Physiology or Medicine in 1984 to Köhler and Milstein, which they shared with Jerne.

How did this achievement come about? Do we yet know the full implications of the successful production of mAbs? Has the technology fully matured, or are there areas amenable to refinement and improvement?

Our modern comprehension of Abs derives from a series of classical observations, related to early studies of vaccination by Jenner, Pasteur, and Koch, and refined by von Bering and Ehrlich in studies of Abs to diphtheria toxin (3). The appreciation of Abs as identifiable molecules with defined physicochemical properties began with Kabat and Tiselius, was reinforced by the work of Edelman and of Porter, and culminated in the three-dimensional structures of Poljak and of Davies. Central to an understanding of the molecular basis of Ab specificity was the appreciation that clonal myeloma (plasma cell) tumors, both of the human and of the mouse (BALB/c in particular), produced homogeneous Igs, whose antigenic specificity could sometimes be identified (4, 5). However, attempts to screen large numbers of induced myeloma tumors for specific Ab activities proved too laborious for practical application.

Along with the rapidly maturing technology that permitted the growth of mammalian cells in defined medium (6), a keen awareness of the power of bacterial genetics led to the hope of applying similar techniques to the study of mammalian cells (7), and in particular to myeloma cells in culture (8). The keystone of the new field of somatic cell genetics, like any analytical system based on genetic markers, was the ability to perform complementation analysis by somatic cell hybridization. This would establish the dominance or recessiveness of particular traits and shed light on the *cis*- or *trans*-control mechanisms involved. Drawing on a knowledge of the biosynthetic pathways crucial to purine and pyrimidine synthesis, and selection procedures primarily using HAT (hypoxanthine, aminopterin, thymidine)-containing medium, several laboratories demonstrated the feasibility of generating hybrids between myeloma cells and fibroblasts, or between various lymphocytic Ig-producing cell lines (reviewed in Ref. 9). In hybrid cells resulting from fusion of an Ig-producer with a fibroblastic cell, Ig production was consistently found to be extinguished (or profoundly inhibited). Hybrids between various cells of lymphocytic origin continued to express the parental Ig chains. Might fusion of a continuous line to lymphocytes yield a continuous cell line expressing a new Ab specificity? The low frequency ($\sim$1 in 10^6) of hybrid cells produced by such fusion procedures, compounded by the rarity of specific Ab-producing cells (perhaps 1 in 10^4), would make the identification of an Ab-producing hybrid an exceptionally uncommon event.

The great surprise of the Köhler and Milstein paper was multifaceted: 1) fusion of a myeloma cell to the nontransformed heterogeneous population of splenic cells from an immunized mouse

[1] Address correspondence and reprint requests to Dr. David H. Margulies, Molecular Biology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, MSC 1892, Building 10, Room 11N311, Bethesda, MD 20892-1892. E-mail address: dhm@nih.gov

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resulted in HAT-resistant, proliferating cells that could be recovered and analyzed (the activated cells fused at a higher rate than resting cells); 2) a proportion of the resulting cells, confirmed as hybrids by chromosome analysis, continued not only to synthesize the Ig H and L chains of the parent myeloma, but also expressed chains that must have derived from the spleen cells themselves; and 3) Ab activity directed against the immunogen, SRBC, was readily detected among clonal populations of the resulting hybrids. The detailed clonal and biochemical analysis of the Ig produced by the hybrid cells confirmed the presence not only of novel H and L chains, but also of unique HL chain pairs that only resulted from intracellular assembly. Thus, in this elegant three-page report, Köhler and Milstein demonstrated the principle that spleen cells producing Ab directed against a specific immunogen could be immortalized by somatic cell hybridization to a myeloma cell. Their final sentence, "Such cultures could be valuable for medical and industrial use," rivals the famous Watson and Crick statement, "It has not escaped our notice . . ." (10) for prescient understatement.

Despite the initial success reported in the *Nature* paper, there were a variety of additional technical barriers to conquer. As related in a Milstein memoir (11), after identifying the three anti-SRBC mAb first reported, and two more lines producing anti-trinitrophenyl Abs, for some 6 mo neither Köhler nor Milstein could derive any new hybrids, related to incorrectly prepared or toxic reagents (As a graduate student in Matty Scharff's laboratory at that time, following up on earlier experiments analyzing the control of Ig production in myeloma-myeloma hybrids and on the recently published Köhler and Milstein methodology, I was readily producing similar myeloma-spleen hybrids. I suspect that this knowledge, communicated through visitors to our lab, may have bolstered Milstein's efforts in those difficult times). Modifications of the fusion protocol, in particular with the use of polyethylene glycol as a fusogenic agent (12, 13), of H or H plus L chain non-producing myeloma cell lines as the fusing partners, and refinement of screening procedures (particularly by enzyme linked immunoassay and flow cytometry), all contributed to a more reliable technology for generating hybridomas and specific mAb at will. (The nomenclature "hybridoma" to describe the somatic cell hybrids producing mAb has been attributed to Len Herzenberg who spent a sabbatical year in the Milstein laboratory). Recent advances including the application of bacteriophage display methods, humanization of the rodent mAbs, exploitation of the uniquely structured camellid Abs, and mutation of particular effector functions have further refined our design and use of mAb.

As research reagents, mAbs are the sine qua non that form the basis of cell and molecular identification. We appreciate the wealth of mAb available from the American Type Culture Collection, from generous investigators who (usually) share their published reagents, or from commercial suppliers that offer exceptionally high quality goods at modest cost. The necessary complexities of clinical trials and FDA approval have limited the number of mAb that are presently available for human use to some 23 preparations. These fall generally into classes of drugs effective against graft rejection, various malignancies, or autoimmune conditions such as rheumatoid arthritis, Crohn's disease, or severe asthma. In addition, several are used for imaging various cancers or inflammatory conditions. With additional experience in addressing many of the scientific questions fundamental to the production of mAb with high specificity for their target Ags, with molecular design directed at minimizing the potential for adverse reactions when used as drugs, and with limits dictated only by our imagination and creativity, we can feel confident that the next quarter century will bring thousands more mAbs, including hundreds with therapeutic and diagnostic value.

The "continuous cultures of fused cells" of Köhler and Milstein are now everyone's reagent, and they do, indeed, promise to continue to be indispensable for both "medical and industrial use."

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