Plasmacytomas and Basic Immunology

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MILESTONES

Merwin RM, Algire GH: **Induction of plasma cell neoplasms and fibrosarcomas in BALB/c mice carrying diffusion chambers.** *Proceedings of the Society for Experimental Biology and Medicine* 1959, 101:437-439

Dunn TB, Potter M, Fahey JL, Merwin R: **Morphology and serum protein changes in plasma cell neoplasms in mice.** *Archivio "de Vecchi" per l'anatomia patologica e la medicina clinica* 1960, 31:67-77

Michael Potter

These investigators reported the fortuitous discovery and initial characterization of plasmacytomas induced in BALB/c mice. Their findings launched an era of intense research and unprecedented discovery during which some of the most perplexing questions in immunology were definitively resolved. The structural basis of antibody specificity and the genetic basis of antibody diversity were established from investigations with murine plasmacytomas. The cornucopia of immunological information and the insights that were generated in research with plasmacytomas rightfully qualify them as immunological Rosetta Stones.

The initial and critical finding was by Merwin and Algire[1] who implanted cell-impermeable diffusion chambers containing C3H mammary tumor tissue into the peritoneal cavity of BALB/c mice. A goal of the study was to determine if a virus that was present in the tumor, and associated with a high incidence of spontaneous breast cancer in C3H mice, would induce mammary tumors in BALB/c mice, a low incidence strain that did not carry the virus. None of the mice with diffusion chambers developed mammary tumors, but some of them developed peritoneal plasmacytomas[2]. This surprising finding raised the possibility that a virus could cause plasmacytomas. In a subsequent publication - a classic for re-emphasizing the need for controls in any experiment- Merwin and Redmon[3] reported the surprising finding that plasmacytomas also developed in BALB/c mice implanted with empty diffusion chambers. It was soon recognized that a variety of substances induced peritoneal plasmacytomas in BALB/c mice. Potter and Boyce[4] showed that simply injecting mineral oil into the peritoneal cavity of BALB/c mice induced plasmacytomas.

These studies appeared in the literature just as evidence from clinical investigations was suggesting that the so-called M-components, present in the sera of patients with certain autoimmune diseases, were actually homogeneous autoimmune antibodies. Until the discovery of murine plasmacytomas, efforts to investigate antibody structure using serum antibodies were limited by the heterogeneity of antibodies induced by immunization. Since almost every BALB/c plasmacytoma produced a serum M-component, these tumors presented a unique opportunity to investigate the structure and function of molecularly homogeneous immunoglobulins. Michael Potter and colleagues at the National Cancer Institute induced and characterized hundreds of different BALB/c plasmacytomas and generously made them available to investigators throughout the world.

Immunochemists were immediately attracted to plasmacytomas because they provided essentially an unlimited source of homogeneous immunoglobulins. The monoclonal proteins produced by most of the BALB/c plasmacytomas were IgA or IgG. Some produced only a kappa or lambda light chain, and rare plasmacytomas produced IgM or IgD. The amino acid sequences of purified heavy and light chains from dozens of the plasmacytomas indicated that most of them produced a unique monoclonal immunoglobulin. The pooled structural information immediately generated insight into the organization of the genes that encoded antibodies. The amino acid sequence data identified constant and variable regions, hypervariable regions, heavy chain domains, classes and subclasses, hinge regions, allotypic markers, disulfide loops, and a wealth of other information that established most of our current understanding of antibody structure.

A longstanding quest of immunologists was to understand the structural basis of antigen recognition by antibody molecules. The availability of hundreds of different plasmacytomas made it feasible to attempt such structure-function studies. A number of investigators began screening Potter's library of monoclonal immunoglobulins hoping to find some with antibody activity. A considerable number were found to bind conventional haptens, such as 2,4-dinitrophenyl (DNP) and

phosphorylcholine, and some bound carbohydrate antigens. Affinity-labeling reagents began to pinpoint the antigen binding sites. Quantitative and equilibrium analyses of hapten binding provided information about valency and binding kinetics of individual antibody molecules. Eisen and colleagues[5] extensively characterized the DNP-binding IgA proteins produced by plasmacytomas. Subsequent research in several laboratories visualized the stereostructure of antibody combining sites.

In a series of elegant studies Coffino, Laskov and Scharff[6,7] used plasmacytoma cell culture and single cell cloning in soft agar to investigate the molecular details of immunoglobulin production. They identified the steps in synthesis, assembly and secretion of antibody molecules, and the role of somatic mutation in the generation of variant cells that only produced a clonal light chain. Non-producer variants would later prove important in the development of hybridomas.

A number of investigators examined pathological and immunobiological aspects of murine plasmacytomas. Zolla-Pazner[8] characterized the mechanisms of the immunodeficiency that occurs in mice bearing plasmacytomas, a condition that mimics the immunodeficiency in patients with multiple myeloma. Those studies showed that plasmacytoma cells elaborate immunological signals that influence the host's immune system. The converse was proven in studies by Lynch and colleagues[9] that showed the growth and differentiation of plasmacytoma cells could be regulated by specific immunological signals programmed into the host by prior immunization.

In the ingenious hybridoma system developed by Kohler and Milstein[10], murine plasmacytoma cells were fused with immune spleen cells to immortalize clones that produced essentially unlimited quantities of a single antibody of defined specificity. Kohler and Milstein shared a Nobel Prize in 1984 for their hybridoma work, the impact of which continues to resonate widely across the biological sciences, human medicine and beyond.

The investigators who initially used plasmacytomas to study antibody structure studied the proteins directly. With the advent of molecular genetic technology, the focus shifted to the analysis of antibody genes. In the mid-1960's observations of the inheritance patterns of human immunoglobulin allotypes suggested that each immunoglobulin chain was encoded by two distinct genes. This idea violated the established dogma that each polypeptide chain was encoded by a single gene. The two gene hypothesis further complicated the central immunologic puzzle of the day: how was the extraordinary diversity of antibodies generated?

The answers came from the research of Susumo Tonegawa, the pioneer in applying recombinant technology in immunology. He was awarded the Nobel Prize in 1987 for his ground-breaking discoveries. Plasmacytomas were a critical element in the design of the research[11]. Tonegawa's research team prepared and fractionated restriction enzyme digests of DNA from a kappa light chain-producing plasmacytoma and from mouse embryo DNA. Using kappa constant and variable region hybridization probes, they found that in the embryonic DNA the kappa constant and variable region gene segments were separated from each other by considerable distances, but in the plasmacytoma DNA they were joined to form a much smaller, contiguous stretch of DNA. This indicated that rearrangement of the DNA took place when a cell produced a light chain. Further studies of kappa light chain DNA from a library of plasmacytomas showed that kappa light chains are encoded by a gene that is formed by combining one constant region gene segment with one of the approximately 250 variable region gene segments present on the same chromosome. Other studies showed that the heavy chain gene was formed by combining a single constant region gene segment with one of the approximately 800 heavy chain variable region gene segments present on the same chromosome.

As many laboratories pursued the study of immunoglobulin genes using murine plasmacytoma cells, it became clear that, in addition to the constant and variable region gene segments, there were other, very small gene segments that were rearranged and incorporated into the functional gene. These additional gene segments, one for light chains and two for heavy chains, were selected from a large number present on the respective chromosome. The process of combining these gene segments into the functional gene was found to be error prone at the splicing sites. This resulted in small changes in the DNA sequences at the joints.

The significance of all this complexity is that it explains how a repertoire of at least 10^9 different antibody molecules can be generated from approximately 10^3 gene segments. The possible gene segment combinations, coupled with the splicing site errors, yields an extraordinary number of functional genes. Even before BALB/c plasmacytomas were discovered, it was estimated that the size of the antibody repertoire was in the range of 10^9. If the dogma of "one polypeptide chain: one gene" was correct, then there was insufficient DNA in the genome for 10^9 antibody genes. Plasmacytomas provided researchers with the experimental system that solved this longstanding immunologic conundrum. It is currently estimated that the mouse genome contains about $3x10^4$ genes.

The tremendous advances that came from research with plasmacytomas showed that tumor cells can provide powerful research tools for understanding the processes of normal cells. This principle has been extended to investigations of other lineages of immune cells using other types of lymphoid tumors.

The straightforward, descriptive research described in the two Milestone articles[1,2] spawned a profusion of basic knowledge that has advanced science and benefited society. The decisions to conduct the initial studies, and to provide resources to support them, can be seen through the "retrospectiscope" as tremendously wise. There might be some lessons here.

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