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PARADIGM SHIFTS IN PERSPECTIVE

Very Unstable Genetics: How the Confluence of Microsatellite Instability and Immunotherapy Revolutionized the Treatment of Colon Cancer

Jonathan D. Kaunitz[1,3] · Anthony Bejjani[2,4]

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> "…normal science progresses as long as available evidence can be accommodated in the existing paradigm. Once anomalies accumulate from scientific research that can no longer be accommodated into an existing paradigm, the time is ripe for a paradigm shift." [1]

Introduction

In this installment of the "Paradigm Shifts in Perspective" series, Rick Boland and his colleagues describe the extraordinary discovery that they made in their quest to understand the pathobiology of familial colon cancer (CRC), in particular the Lynch syndrome that Rick contributed to naming and to its definitive description. He describes how microsatellite instability (MSI) is related to the pathogenesis of the distinct variety of CRC associated with Lynch syndrome, and more importantly their cardinal findings of why Lynch patients are resistant to traditional cytotoxic chemotherapy but are sensitive in many cases to therapy with immune checkpoint inhibitors—certainly a paradigm shift in the pathophysiological understanding and treatment of CRC.

In this brief introduction, we will provide some context for the accompanying article, weaving the many disparate lines of investigation that coalesced in the described discoveries. Please note that this is not intended to be an exhaustive or comprehensive review but merely a brief introduction intended to provide background and context for the accompanying paper. Accordingly, not every advance or aspect is fully covered and that articles described as first observations may have been preceded by others. Also note that all Nobel laureates are identified by an asterisk (*) when first introduced and are listed in Table 1.

History

Genetics

It would be appropriate to begin this tale with a brief history of the field of genetics, that dates at least to ~ 5000 BC with the monumental feats of genetic engineering by ancient Mesoamericans that lead to the conversion of the wild grass teosinte to domesticated corn (*Zea mays*), arguably the most important cereal crop in the world [2]. The more usual history of genetics begins with Mendel through Darwin, T.H. Morgan, and on through the dawn of the molecular era and beyond, reviewed in multiple publications (cf. [3]) and touched upon to a limited extent in this introduction. The heart of the story, however, is the genetics of colorectal cancer (CRC), more specifically a subtype of familial cancer termed the Lynch syndrome [4].

Colorectal Cancer (CRC)

Although the history of CRC extends to antiquity, with colorectal tumors found in Egyptian mummies and papyri describing the identification and treatment of such tumors [5], the modern history of CRC begins in the late nineteenth century. The first likely verifiable description of adenomatous colonic polyps is attributed to Skilifasowski [6] who in 1881 described (in Russian) a 51-year-old man with several large colonic adenomas. A more recent advance was the discovery that colonic adenomatous polyps were precursors

✉ Jonathan D. Kaunitz
jake@ucla.edu

1 Medical Service, Section of Gastroenterology, Greater Los Angeles VAMC, Los Angeles, CA, USA

2 Medical Service, Section of Hematology Oncology, Greater Los Angeles VAMC, Los Angeles, CA, USA

3 Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

4 Division of Hematology-Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Table 1 Nobel laureates contributing to the development of immune checkpoint inhibitors and the discovery of MSI and MMR (courtesy of [106])

Year	Field	Recipients	Discovery
2018	Physiology or Medicine	James P. Allison and Tasuku Honjo	"For their discovery of cancer therapy by inhibition of negative immune regulation"
2015	Chemistry	Tomas Lindahl, Paul Modrich, and Aziz Sancar	"For mechanistic studies of DNA repair"
2002	Physiology or Medicine	Sydney Brenner, H. Robert Horvitz and John E. Sulston	"For their discoveries concerning genetic regulation of organ development and programmed cell death'"
1996	Physiology or Medicine	Peter C. Doherty and Rolf M. Zinkernagel	"For their discoveries concerning the specificity of the cell mediated immune defence"
1983	Physiology or Medicine	Barbara McClintock	"For her discovery of mobile genetic elements"
1978	Physiology or Medicine	Baruj Benacerraf, Jean Dausset and George D. Snell	"For their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions"
1959	Physiology or Medicine	Severo Ochoa and Arthur Kornberg	"For their discovery of the mechanisms in the biological synthesis of ribonucleic acid and deoxyribonucleic acid"
1908	Physiology or Medicine	Ilya Ilyich Mechnikov and Paul Ehrlich	"In recognition of their work on immunity"

to CRC and that their removal decreased cancer incidence, as comprehensively reviewed previously in this journal in a quasi-installment of this series by Winawer [7], a major force in promoting colonoscopic screening for CRC, which has proven to be a paradigm shift in CRC prevention. One of the most significant achievements in the understanding of CRC genetics was the observation by Vogelstein [8] that the progression of normal mucosa to adenomatous polyp, advanced polyp, and finally cancer involved sequential genetic alterations with gain of oncogenes and loss of genes thought to be involved in tumor suppression.

Familial Colon Cancer

Colon cancer is one of the most heritable human cancers, second only to prostate according to a landmark Swedish twin study by Lichtenstein et al. [9]. Burt and his colleagues [10] from the Huntsman institute in Utah reviewed the major types of human hereditary CRCs, identifying Lynch syndrome, familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP), MUTYH-associated polyposis (MAP), Peutz–Jeghers syndrome, juvenile polyposis syndrome, and hyperplastic polyposis (HPP) as the major syndromes. All of these syndromes confer an elevated lifetime risk, with FAP, MAP, LS, and AFAP conferring lifetimes CRC risks of ~ 60 to 90%. The susceptibility genes for most of these syndromes has been defined with FAP and AFAP linked to the FAP gene termed *APC* and LS linked to the microsatellite instability (MSI) gene cluster such as the *mut S* homolog (*MSH*) and *mut L* homolog (*MLH*) families. Bülow et al. [11] have written a comprehensive review on the history of FAP tracing suspected cases of young individuals who had multiple colonic polyps at autopsy to 1721 to the work of Cuthbert Dukes, Lockhart-Mummery, Eldon Gardner, and many others who identified the familial nature of APC. The *APC* gene was eventually localized to chromosome 5 and unambiguously identified later [12–16]. We will leave the story of the Lynch syndrome to Rick.

Microsatellite Instability (MSI) and DNA Mismatch Repair (MMR)

Although well covered in the accompanying article, we will provide some additional background on the origins of MSI and mismatch repair that are at the heart of this story.

A good place to start would be with the discovery that DNA is the means by which genetic data are stored, originally attributed in 1871 to Friedrich Miescher (see [17] for a detailed historical review) as a non-protein substance extracted from cellular nuclei that he termed "nuclein". The function of DNA is attributed to Avery and co-workers [18] at the Rockefeller Institute in New York, who successfully transformed a strain of pneumococcus from an unencapsulated to encapsulated form with a purified substance that through extensive analysis was concluded to be DNA. Fast forward a decade or so to studies of mammalian or bacterial cells in which DNA synthesis occurred after it was intentionally damaged with UV light or alkylating agents suggesting that repair was occurring [19–22]. Some of the enzymes responsible for this repair, the *phr* gene product and photolyase of *Escherichia coli* were described by Sancar* and Rupert [23] of the University of Texas in 1978. In 1964, Holliday [24] published an important observation regarding "gene conversion" in fungi, in which the many forms of genetic recombination occurred, wondering "…it is rather surprising that recombination by breakage and reunion has often been disregarded on the grounds that it could not be a process precise enough to explain the genetic data…"

implying that a repair mechanism must be present. In 1968, Lindahl* et al. [25] of the Rockefeller University reported the deoxyribonuclease IV due to its propensity for single nucleotide repeats in double stranded DNA was a likely DNA mismatch repair enzyme. The likely first description of the predicted mismatch repair mechanism was published by Brutlag and Kornberg* [26] of Stanford University who described in 1972 an exonuclease specifically directed at excising mispaired nucleotides and other exonucleases directed at "trimming loose ends" and "enlarging nicks and gaps with the helical regions". Many of the pivotal studies on the mechanism of mismatch repair are attributed to the laboratory of Modrich* [27] at Duke University who discovered that an *E. coli* methylase was involved in mismatch repair, later discovering how selective methylation enabled the identification of parent and daughter DNA strands.

Another line of investigation dated to 1929 or possibly before in which organisms that had been commonly used for genetic studies were observed to have in some cases frequent mutations that were traced to genetic loci, as Barbara McClintock* from Cold Spring Harbor in the most ancient of genetically modified organisms, the aforementioned *Zea mays,* in which she identified several "mutable loci". The Cold Spring Harbor website [28] that houses a remarkable archive of its symposia remarked about the 1951 symposium chaired by Dr. Miloslav Demerec in which Dr. McClintock presented her data: "Barbara McClintock gave a further example of the dynamic genome in her talk on the Ac-Ds system in maize. It was Richard Goldschmidt who took up these issues in his remarkable opening presentation for the Symposium. Goldschmidt rejected the conclusion that a mutation at a particular point necessarily meant that there was gene there, and he offered a simile: "If the A-string on a violin is stopped an inch from the end the tone C is produced. Something has been done to a locus in the string. But nobody would conclude that there is a C-body at that point." Although Dr. McClintock's research was initially ridiculed by her colleagues, the importance of her groundbreaking work in particular on transposons became accepted by the scientific community. She perhaps had the last laugh when she was conferred a series of prestigious awards, culminating in the Nobel prize in 1983.

Genes associated with increased mutability were reported by Skaar [29] in 1959 in a report published in the *Proceedings of the National Academy of Science* communicated by Demerec in which a genetic locus of *E. coli* termed *mut+* (likely *mut L* due its close linkage with the leucine [L] locus) was identified. This was followed by further studies in *E. coli* in which the *mut S* gene was identified [30] followed by the *uvr D* and *uvr E* genes [31], and later in yeast in genes *MSH1* and *MSH2* [32, 33]. Three years prior to this study, Fujii and Shimada [34] from the NIH isolated a cDNA clone of unknown significance that they later found had substantial homology with the bacterial mutator genes *mut S* and *hex A*. This sequence was later found to correspond to human MSH2. Rapid progress we subsequently made in identifying and characterizing the human mismatch repair system (MMR) [35] with several MSH family members identified in addition to *mut L* homologs (MLH) genes, post-meiotic segregation (*PMS)* genes [32], and their relation to the Lynch syndrome [36], that will be discussed in the accompanying article.

In 1959 Sueoka et al. [37] published an article in *Science*. Using the CsCl gradient equilibrium ultracentrifugation technique developed by Meselson et al. [38] at the California Institute of Technology to fractionate DNA, they identified subfractions of variable buoyant density that varied in GC content. The origin of the term "microsatellite DNA" can be traced to a 1969 publication by Yasmineh and Yunis [39] at the University of Minnesota who labeled these gradient subfractions "satellites" (Fig. 1). These DNA subfractions were studied in a variety of species, with some later identified to contain short repetitive DNA sequences. In 1980, Arnheim et al. [40] at Stony Brook identified the widespread distribution of "spacer sequences" in nontranscribed murine DNA throughout the genome; Weller et al. [41] observed that the human myoglobin gene was flanked by short tandem repeats. The convergence of several lines of investigation identified these spacer sequences as microsatellites that are a subfamily of tandem DNA repeats that affect DNA secondary structure, with profound effects on DNA replication, chromatin assembly, with implications for fragile sites and chromosomal rearrangements in cancer [42].

Another thread dates to 1914 the observation by the genetic pioneer Boveri [43] regarding chromosomal instability in tumors. This observation was subsequently repeated in several tumors such as by Strong et al. [44] identified a deletion on chromosome 13 that was strongly linked to familial retinoblastoma, giving rise to the chromosomal theory of carcinogenesis. Chromosomal instability was linked to the mismatch repair system in yeast [45] and for CRC [36, 46] although the nexus between chromosomal instability and microsatellite instability is controversial [46]. Nevertheless, there is compelling biological plausibility for a link between tandem repeat DNA and chromosome structure [42].

Development of Cancer Immunotherapy

The development of the immune checkpoint inhibitors culminating in the paradigm shift in cancer therapy follows a long path that encompasses fields as diverse as invertebrate ontogeny and biology, classical immunology, and molecular genetics. Since immune checkpoint inhibitors are limited to cellular immunity, we will focus exclusively on that branch of the immune system.

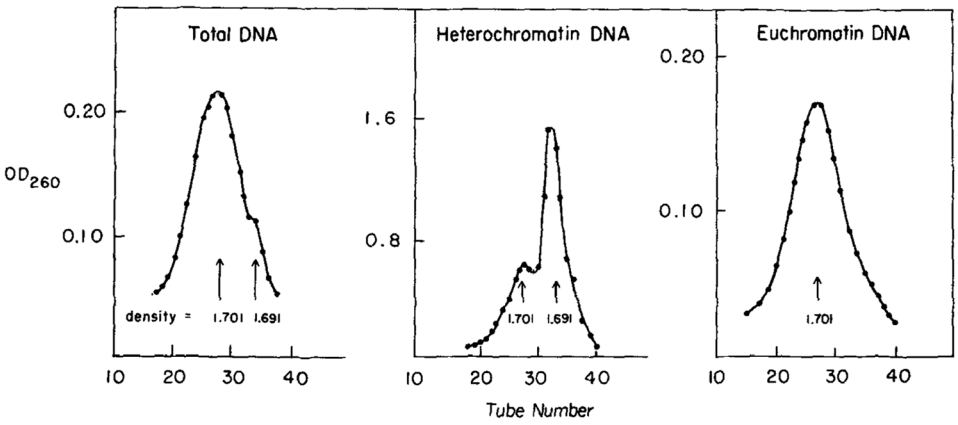

Fig. 1 An early example of a DNA satellite identifiable as a "shoulder" on the usual Gaussian distribution of DNA buoyant densities after gradient centrifugation. Reprinted with permission from [39]

Fig. 1. CsCl sedimentation patterns of total DNA and of DNA from the heterochromatin and euchromatin fractions of mouse chromatin. The amounts of nucleic acid applied were 0.7, 4.0, and 0.6 OD_{260} units, respectively. Following centrifugation as described under methods, the tubes were pierced with a 21 gauge needle and 10 drops aliquots (0.1 ml) were collected, diluted with 0.2 ml of 0.01 M Tris buffer, pH 8.2, and the optical density measured at 260 mμ in a Beckman DU spectrophotometer.

Immunology of Cytotoxic T Lymphocytes

The origins of immunology are described in detail by Kaufman [47, 48] who credits Metchnikoff* with founding the field of cellular immunology with his studies of phagocytosis by a turbellarian flatworm published in 1878 [49], describing his many studies of phagocytosis by immune cells eventually termed macrophages. In 1942, Landsteiner and Chase [50] published a short work in which they reported that cutaneous hypersensitivity of guinea pigs was transferred from the sediment and not the supernatant of peritoneal exudates, suggesting that a cellular element was responsible. The next advance was the discovery that the thymus, previously though the be a vestigial organ, was the source of a specific class of lymphocytes, later termed T lymphocytes, as originally reported by Miller in 1959 [51] and reviewed in 2011 by the same author [52], who recounted his early studies of the prevention of experimental leukemia and lymphoma by thymectomy, followed by recounting the considerable progress made in T cell biology in the ensuing decades. The concept of cellular immunity was further advanced through the study of mice either congenitally or cerebrally infected with the lymphocytic choriomeningitis virus, that became tolerant to the virus in the absence of antibody formation [53] that was attributed to T lymphocytes [54, 55].

A somewhat different take on the subject was provided in a recent review by Golstein and Griffiths [56], the first author credited with the 1986 discovery of the cytotoxic T lymphocyte antigens (CTLA) 1–3 [57], but we am getting ahead of myself. Golstein and Griffiths rather trace the origins of cellular immunity to early studies of transplant rejection such as by Govaerts [58], who recognized in 1960 that lymphocytes obtained from the thoracic duct of the recipient of a kidney transplant were uniquely cytotoxic and by Rosenau and Moon [59], whose experiments in the early 1960s demonstrated that the cytotoxicity of certain sensitized lymphocytes was unrelated to antibodies. The authors expanded on this finding, charting progress in the mechanism of T cell cytotoxicity and the initial cloning of CTLs in 1979 [60]. The initialism "CTLA" was first seen in the medical literature in 1971 to designate the surface antigen termed "chicken T lymphocyte antigen" in a population of thymus-derived lymphocytes [61] although this initialism is now almost exclusively used for "cytotoxic T-lymphocyte antigen" as stated above.

Lymphocyte Surface Receptors

The first hints that lymphocytes expressed surface receptors was the largely unnoticed (at the time) work of Brondz working in relative isolation at the Gemalya Institute in Moscow in the former Soviet Union, who described in 1968 the specificity of immune lymphocytes [62] later reporting that the absorption of murine lymphocytes to macrophage monolayers correlated with their cytotoxic effects [63] (Fig. 2), supporting his contention that cytotoxicity likely required cell to cell contact and was this suspected to be receptor mediated. His findings were particularly notable and ironic given the hand-wringing regarding the lack of progress in identifying T cell receptors published in the same issue of *Transplantation Proceedings* by Crone et al. [64]. The development of specific antibodies that blocked T cell responses led to rapid advancements in the understanding of the structure and function of T cell receptors (reviewed in [65, 66]) that has

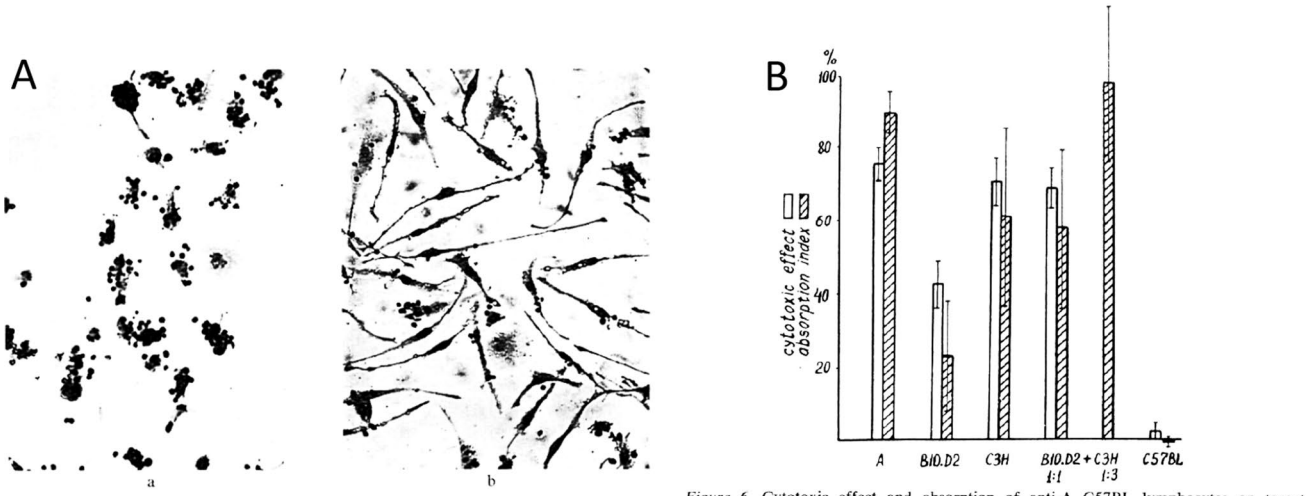

Figure 2. Macrophage cultures of C57BL/10 (a) and B10.D2 (b) mice after incubation with anti-C57BL/10 B10.D2 immune lymphocytes for 6 hours, removing of non-adherent lymphocytes and incubation for one day in the culture medium. Hematoxylin × 200.

Figure 6. Cytotoxic effect and absorption of anti-A C57BL lymphocytes on target cells of different genotypes and on their mixture.

Vertical bars are confidence limits at 95% significance level (mean data of 3–7 experiments).

Fig. 2 Brondz showed that adherence of T lymphocytes to target cells correlated with cytotoxicity, providing preliminary evidence of T cell surface receptors. **A** Adherence of immune lymphocytes to target cells from different mouse strains; **B** Correlation of adherence to target cells and cytotoxicity. Reprinted with permission from [63]

served as the basis for the current understanding of the many surface receptors that control cytotoxic T cell function [48].

Of these receptors, the two of primary interest in cancer immunotherapy are programmed death (PD)-1 and CTLA-4 receptors, which serve as the targets for the first approved immune checkpoint inhibitors [67, 68] that will be discussed in later sections. Although Golstein [56] is credited with identifying CTLA-4 in 1987, the original publication is attributed to Allison* then working at the University of Texas, Austin, who described a monoclonal antibody termed 124-40 that recognized a T cell-specific glycoprotein antigen [69] later identified as CTLA-4. Allison, when at UC Berkeley, later published the pivotal study in 1996 [70] that described how CTLA-4 blockade enhanced anti-tumor activity, that served as the basis for development of the current immune checkpoint inhibitors.

Programmed Cell Death

The scientific progress that made these drugs possible began with observations dating to the early twentieth century with the study of cornification, the process by which epithelial cells in the skin and related organs spontaneously convert into a highly keratinized phenotype, as described in detail by Meirowsky and Behr [71]. In 1950, Glucksmann [72] published a detailed review of the many instances of spontaneous cell death that occur in multiple organs during vertebrate ontogeny. The concept that cells can alter their appearance and function without apparent external influence was further described by Lockshin and Williams [73] in 1965, who described the spontaneous degeneration of the intersegmental muscles of the silkworm moth *Antheraæ pernyi.* The first use of the term "apoptosis" to describe the spontaneous death of cells with specific histologic features was reported in 1972 by Kerr et al. [74].

Given this background, Sulston* and Horvitz* [75] then working in the Medical Research Council in Cambridge UK, performed highly detailed studies of the nematode *Cænorhabditis elegans*, that due to its transparency could be studied in detail using Nomarski optics. The investigators recorded the location of all of the 810 cells in the mature adult through developmental stages (Fig. 3), noting that the number of cells decreased during development, that they attributed to a form of programmed death. At the same time, Brenner* [76] working at the same institution published detailed electron microscopic studies of the development of the ventral nerve cord of the same organisms. Sulston and Horvitz continued these studies, fascinated that each cell seem to have a predestined "fate", endeavoring to understand the regulatory mechanism leading to such an elegant orchestration of mitosis, migration, and cell death. In 1980, the same scientists, with Horvitz now in Cambridge, MA published their observation that mutants of *C. elegans* had different cell lineages [77], strongly suggesting genetic control of development. Several subsequent publications [78, 79] culminated in the pivotal observation that the gene egg laying defective (*egl*)-1 was a key component of programmed cell death in nematodes that had many mammalian counterparts that interact with B-cell lymphoma (Bcl)-2-like

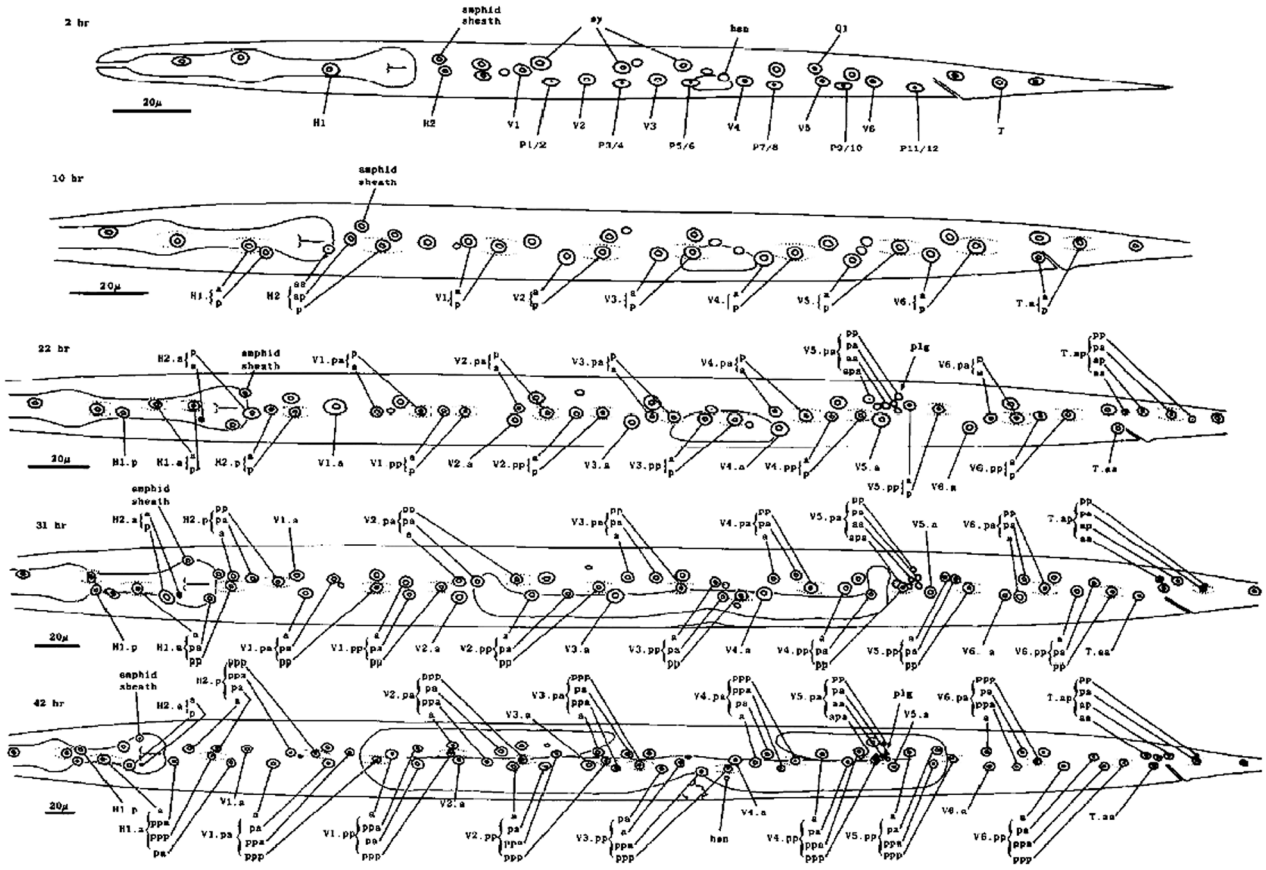

FIG. 9. H, V, and T.a; hermaphrodite, lateral views; development of the left lateral hypodermis. Seam cells are indicated by dotted lines. hsn, hermaphrodite-specific neuron; plg, posterior lateral ganglion.

Fig. 3 Diagram of a section of the nematode *C. elegans* showing the location of every cell, used by Sulston and Horvitz to study programmed cell death. Reprinted with permission from [75]

molecules and caspases, which are involved in cell death pathways [80].

Honjo* and co-workers, working in Kyoto, Japan shared an interest in programmed cell death, in this case working with T cell hybridomas. To better understand their observation that the hybridoma cells when cocultured with a murine hematopoietic died by programmed cell death, they identified in 1992 a gene termed PD-1 by subtractive hybridization [81] which they found was expressed mostly in thymocytes. Honjo's group subsequently reported that PD-1 is expressed on the surface of murine lymphocytes [82]. Another group in the Mayo Clinic identified a protein termed B7-H1 involved in T-cell proliferation that was subsequently termed PD-L1, a ligand for PD-1 [83]. In 1999, Honjo's group reported that disruption of PD-1 produced a lupus-like syndrome [84]. This latter finding was surprising in that the phenotype did not display excess apoptosis as expected but did indicate that PD-1 contributes to systemic immune function to an extent sufficient to justify that PD-1 and one of its cognate ligands PD-L1 [83] should be considered as another checkpoint antigen. CTLs kill cells by a variety of apoptotic and non-apoptotic mechanisms mostly involving the perforin [85, 86], granzyme [87–89], and FAS pathways [90, 91]; reviewed in [92]. Although PD-1 was identified in the context of two cell lines that underwent programed cell death, inconsistencies in the in vitro and in vivo experiments between PD-1 expression and programmed cell death have led Honjo to conclude that evidence linking PD-1 expression and programmed cell death is inconclusive, suggesting that alternative mechanisms may be present [81, 84].

Other Considerations

Major Histocompatibility Complex (MHC) Loci

As was the case with T cell surface receptors, the discovery of the major histocompatibility complex (MHC) loci originated with transplant biology, in particular with the observations of Gorer working at the Lister Institute in London, who in 1937 noted the genetic component to transplant rejection [93], based on studies of the genetics of tissue compatibility dating to 1909 (reviewed by Batchelor [94]). Although

research proceeded with the subsequent identification of the MHC and its importance to transplantation, the next major breakthroughs were the observations by McDevitt [95] at Stanford University and Benacerraf* [96] at Harvard Medical School regarding antibody and cytotoxic responses and by Zinkernagel* and Doherty* [55, 97, 98], reviewed in [38, 99], that MHC Class I (then called the H-2 gene complex) also contributed to T cell cytotoxicity. The authors used methods descussed above that hark to the origins of T cell biology siuch as the mouse lymphocytic choriomeningitis model and several cytotoxicity models, providing convincing data that sensitization with antigens specified by the H-2 complex increased cytotoxicity. The authors and other continued to study how MHC antigens contributed to T cell cytotoxicity, eventuating in the current understanding that MHC class I molecules are involved in antigen presentation by cytotoxic cluster of differentaion (CD)8^+ cytotoxic T cells (reviewed in [100]).

Co-signalling Molecules in T Cell Activation

With the discovery of cytotoxic T cells, their surface antigens and associated molecules, another consideration is the concept of co-activation which invokes the involvement of co-stimulatory and co-inhibitory ligands and receptors on the T-cell surface. In their book, Azuma and Yagita [101] delve into considerable detail about these influences on T-cell activation. In her chapter, Azuma [102] describes how the understanding of negative and positive controls of T-cell activation evolved from the theories of Bretscher and Cohn [103] to the current understanding of the many costimulatory and co-inhibitory T cell surface antigens and ligands. In the book's final chapter, Nakajima and Tamada [68] describe how novel cancer immunotherapies were based on inhibition of the co-inhibitory molecules CTLA-4 and PD-1, finishing with a description of how novel cancer immunotherapeutic drugs could be based on co-stimulatory molecules such as CD137, CD134, and CD357.

Putting It Together: The Advent of Novel Cancer Immunotherapeutic Drugs

The confluence of all of the lines of investigation outlined in this section eventuated in the development a class of drugs termed the "immune checkpoint inhibitors" of which the initially developed drugs inhibit the co-inhibitory CTL surface receptors CTLA-4 and PD-1 and the co-inhibitory ligand PDL-1 described in the previous sections. The preclinical studies described in the previous section provided sufficient data to study these molecules in vivo in experimental tumor models that led to the development of the CTLA-4 blocking monoclonal antibody ipilimumab and the PD-1 blocking antibodies nivolumab and pembrolizumab, and the PD-L1 directed monoclonal antibodies atezolizumab, durvalumab, and avelumab (reviewed in [104]) that are generally used as second-line therapy for advanced cancers, usually resistant to conventional chemotherapy. In the accompanying article, Rick Boland and colleagues [105] provide a remarkable first-hand account of their contributions to the rapid evolution of understanding of the link between defective MMR activity and the triggering of programmed cell death in tumor cells by cytotoxic chemotherapy, paving the way for the paradigm shift in the understanding of cancer therapy with the development of the immune checkpoint inhibitors.

Postscript

One of the themes that we have stressed in this series is that scientific progress is often based on a series of rather simple experiments converging from disparate fields. What most studies have in common includes a carefully-formulated biologically-plausible hypothesis based on existing knowledge, a straightforward, logically-planned experimental design, clear-cut results that do not require advanced statistics to gain meaning, and a willingness by the investigators to think beyond existing paradigms to explain biological phenomena and observations. Most of the pivotal publications cited are relatively short, usually have less than five authors, and do not have complicated figures, in particular computer-generated depictions of multiple complex associations, which of course were not available until recently, but more importantly would not be considered useful even if they were available unless the authors believed that they would support a plausible hypothesis. Though the world has changed since the 1950s when the field of immunology really took off, these fundamental principles still apply.

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Author's contribution JDK: conception, research, writing, editing. AB: editing.

Declarations

Conflict of interest JDK: none AB: none.

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