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Reviews

Myeloma proteins*

by M. Potter

Department of Health & Human Services, National Institutes of Health, Bethesda (Maryland 20205, USA)

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In 1891, Paul Ehrlich carried out some of the most original and remarkable experiments in immunology. He immunized mice both orally and parenterally to the powerful plant toxins abrin and ricin, and then went on to show that immune mothers could transmit their immunity to their offspring[1]. When one considers the transmission of the immune state by serum (antibody) had only been known for about a year, his daring use of mice, an oral route of immunization and his understanding of maternal transmission were sophistications that were decades ahead of his time. Ehrlich was the first to recognize the utility and value of mice in immunology. Later he was one of the pioneers in tumor transplantation, where again most of his work involved mice. Mice were used in his laboratory as models for other types of diseases such as trypanosomiasis. The influence of Ehrlich was very evident in the early 1950s at the National Cancer Institute where many workers were attempting to understand the pathogenesis of tumor development in inbred mice. The principal systems focused on leukemias and mammary tumors which occurred in high frequency in strains that were intentionally or fortuitously bred for these characteristics. In 1954, Thelma Dunn at the National Cancer Institute published a monograph on reticular neoplasms of the mouse, that remains today as a classic[2]. Many of the common leukemias and lymphomas in man had counterparts in the mouse. A particularly interesting form, the plasmacytoma had been found only in pathological material.

Thelma Dunn had described spontaneous plasmacytomas in old C3H mice, that appeared to arise in the inflammatory tissue that formed under mucosal ulcers in the ileocecum[3]. In Copenhagen, Ragna Rask-Nielsen, influenced by Englebreth-Holms' statement that plasma cell tumors were not available in mice[4], began looking for plasma cell tumors and found lymphomatous tumors with plasmacytoid morphology in old mice[5]. The intriguing aspect of plasmacytomas was of course, the possibility they might resemble human multiple myeloma, and produce large amounts of serum protein (called myeloma proteins) related to γ-globulin (the 1950 version of immunoglobulin), and Bence Jones urinary proteins. Frank Putnam had begun collecting and analyzing by various physical-chemical criteria different Bence Jones proteins and found that they were extraordinarily variable[6]. This made the possibility of finding transplantable plasmacytomas in inbred mice even more exciting because if the mouse tumors produced myeloma and Bence Jones proteins, they would all be products of a single genome. One could now ask whether the biochemical variability in these proteins was due to genetic differences or to some other cause, such as somatic mutation. Using paper electrophoresis the sera of mice carrying the only available transplanted ileocecal tumor of possible plasma cell origin, 70429, was examined and found not to contain a myeloma protein. While this study was underway in 1956, Ira Pilgrim, a graduate student at Berkeley, sent Thelma Dunn tissue sections of two unusual tumors, X5563 and X5647, which he had found and transplanted in old C3H/He CRGL mice. These were clearly plasmacytomas from their morphology and Ira Pilgrim made them available for study. Paper electrophoresis of serum from mice carrying these tumors gave a provocative result. X5563 contained a massive amount of a myeloma protein[7] (later identified as IgG_{2a}). John Fahey showed that X5647 produced a 7,9,11,13S β2A (IgA) myeloma protein[8]. The question of the origin of myeloma proteins could be answered conclusively: were they produced by neoplastic plasma cells or did they represent a host reaction to the tumor? In studies with Daniel Nathans and John Fahey, it was shown that X5563 plasmacytoma cells indeed produced the 7S/IgG myeloma protein[9].

Spontaneous ileocecal plasmacytomas occurred so rarely in C3H mice, that only 4 or 5 have ever been established in transplant. This was not a promising way to study the cause of variability of myeloma proteins. Fortuitously and very unexpectedly, a new rich and unlimited source of mouse plasmacytomas became available in 1958. Ruth Merwin and Glenn Algire at the NCI had been studying the long term survival of C3H mammary tumor tissue that was protected from homograft rejection inside Millipore diffusion chambers[10]. They had chosen to implant these plastic disc-like chambers into BALB/c mice for two reasons, first the histocompatibility type of BALB/c

was H-2^d while the mammary tumors from C3H which was H-2^k; and secondly, BALB/c was very susceptible to the C3H mammary tumor virus. They were interested in determining if homografts could survive for months in an environment protected from attacking host cells. As a special bonus they could also determine if the MMTV virus present in the C3H tumor tissue could get through the Millipore membrane and induce a mammary tumor in the very susceptible BALB/c host. After the chambers had been in the peritoneum for 6 months some of the mice began developing a complicating hemorrhagic ascites. Ruth Merwin examined these mice and found they had tumors in the peritoneum and gave the tissues to Thelma Dunn for histological diagnosis. To her great surprise Dr Dunn found that most of these mice had peritoneal plasmacytomas. This was a form of plasma cell neoplasm that heretofore had never been seen. The great advantages of the new BALB/c system were first, it provided a method, though somewhat complex, to induce large numbers of plasmacytomas; second, it identified an inbred strain that was susceptible to plasmacytoma induction, and third, it implicated the peritoneal cavity as a tissue site for plasmacytoma development. Since it was recognized that plasma cells were the cellular source of antibodies, it seemed logical to assume that the tissue contained in the Millipore diffusion chambers was acting as a sustained source of antigens and stimulating the continuous formation of antibodies. During this process some of the reactive plasma cells under the intense stimulation had become neoplastic. To prove this hypothesis one would have to find a way to chronically immunize BALB/c mice. The potent heterologous antigens in horse serum were used, as the injection of this antigen was said to induce intense plasma cell reactions in rabbits. BALB/c mice were begun on weekly injections of horse serum but most died after the 4th injection from anaphylaxis, months before plasma cell tumors were expected. A better means for chronic immunization was needed and this lead to the use of mineral oil-water emulsions (e.g., Freunds adjuvants) as these materials were known to release antigens slowly and also to be an excellent means for producing prolonged immune responses without the complication of anaphylaxis. In experiments with Charlotte Robertson Boyce a mineral oil adjuvant containing heat-killed staphylococci prepared by Rose Lieberman was used. Rose Lieberman had shown this mixture was a good adjuvant in mice and the peritoneal tissues of mice injected with the adjuvant contained many plasma cells. Lieberman's adjuvants were injected intraperitoneally into BALB/c mice and the mice were also treated with a series of skin paintings with the carcinogen methylcholanthrene (a procedure that induced lymphomas in mice). After 6 months some of these BALB/c mice began developing peritoneal plasmacytomas[11]. This was an exciting result because it appeared that chronic immunization of BALB/c mice with a xenogeneic antigen was a critical factor. To prove this the components of the adjuvant mixture had to be tested individually. It was soon very clear that the major component of the adjuvants, the mineral oil without antigen or any of the other components was very effective in inducing the plasmacytomas[12]. The chronic immunization concept was now in serious doubt because exogenous antigens were obviously not required. The histogenesis of plasmacytomas was studied with Ross MacCardle and it was found that the intraperitoneal injection of mineral oil induced the formation of chronic granulomatous inflammatory tissue[13] which was characterized by a great excess of macrophages (cells that later have been recognized by others to be important accessories to antibody formation). Not unexpectedly the granulomatous tissue contained focal plasma cell proliferations. The reason why plasma cell tumors developed in this unusual situation was not understood, but it was clear that exogenous antigens were not needed. The important result though, was a relatively easy means to produce large numbers of plasma cell tumors each of which secreted a myeloma protein and could be perpetuated indefinitely by transplantation. The BALB/c system provided an unlimited source of monoclonal immunoglobulins from genetically uniform animals. BALB/c plasma cell tumors also produced Bence Jones proteins[14]. Gally and Edelman showed that Bence Jones proteins were immunoglobulin light chains[15]. The one gene: one chain concept was the prevailing dogma and the BALB/c Bence Jones proteins allowed a direct way to compare light chain gene products. With William Dreyer, we prepared tryptic peptide maps of a large series of kappa chains and found that all of them stared a common sequence, however, each was distinctly different by having many different peptides[16].

When Dreyer, now at Cal Tech, sequenced two of these mouse proteins and compared them with human kappa chains, two things became very clear, the 'common peptides' came from one half of the chain that remained constant for all chains of a species, while the other variable part was associated with a seemingly unlimited variation[17]. This became the basis of the Dreyer-Bennett 'two gene one polypeptide chain' hypothesis in 1965 that broke the 'one gene one polypeptide chain' rule[18].

These and other studies on BALB/c mouse myeloma proteins indicated a part of the variability of these proteins was determined by multiple genes, but the magnitude of the system caused many to propose economical alternatives, by recombination and mutation. Hypotheses abounded and in the end no one could claim victory; multigenes, recombination and somatic mutation all contributed to immunoglobulin variability.

While myeloma proteins were regarded by immunochemists as useful structural prototypes of homogenous immunoglobulin molecules they were regarded with great skepticism as prototypes of antibody molecules. First, myeloma proteins had no known antigen binding activities. Second, their origin from tumor cells gave them a dubious background. Third, antibody was defined as a collection of immunoglobulin molecules that bound the same antigen. If myeloma proteins were indeed homogeneous antibodies this could not be demonstrated until one identified the appropriate antigen for each one. Human myeloma proteins with unusual biological activities had been found by Henry Kunkel[19,20]. Jan Waldenstrom had screened a number of human monoclonal components and found several that had an antigen binding activity[21]. In 1967 Melvin Cohn at the Salk Institute created a large library of mouse myeloma proteins and screened them with a battery of antigens and found the

S63 myeloma protein which bound the pneumococcal C polysaccharide[22].

Later that year Herman Eisen began screening our library of myeloma proteins for binding activity to the dinitrophenyl hapten, which surprisingly yielded the IgA producing MOPC 315 and MOPC 460 plasmacytomas, which bound DNP antigens. The careful immunochemical studies by Herman Eisen and Ernest Simms established that MOPC 315 had a high affinity (K_A of 1×10^7 M^{-1}) for DNP haptens and that this myeloma protein of tumor origin resembled what a homogeneous antibody molecule might resemble[23].

The DNP binding myelomas were regarded as fortuitous matches of antibody and antigen since the mice had not been immunized. Gradually attention turned to the question of relevance of environmental antigens to BALB/c myeloma proteins. The first clues began with a chance visit of Myron Leon to the laboratory late one afternoon in the fall of 1967. He was en route from Cleveland to Europe the next day, but had stopped over in Virginia to screen human myeloma proteins with some antigenic cocktails he had brought along, one containing six 'very pure' pneumococcal capsular polysaccharides, the other two dextrans and levans. The day had proven to be frustrating because the myeloma samples could not be located and so, as a second thought, he came across town to Bethesda to discuss some other findings. During the conversation he explained the purpose of his visit to Virginia and the frustrating day. Thinking he had left the antigens in Virginia I told him it was unfortunate he had not come to Bethesda and I pointed to a hundred different myeloma sera in the icebox. With a flourish he reached inside his coat pocket and produced the three antigenic cocktails. It did not take long to cut a micro-Ouchterlony pattern and fill the wells with myeloma protein and antigens. While the proteins were reacting we went out to supper and on our return were delighted to find 3 myelomas that gave strong precipitin bands with pneumococcal polysaccharides (McPC603, a protein that was subsequently crystallized, MOPC 167, and MOPC 299) and one that bound dextrans (MOPC 104E)[24]. Hoping that the myelomas would have pneumococcal type specificities it was disappointing when it turned out that the McPC603, MOPC 167 and MOPC 299 each reacted with all of the individual pneumococcal antigens in the cocktail. It then became clear with the help of Emil Gotschlich that the common denominator was contaminating pneumococcal C polysaccharide (PnC). Melvin Cohn's finding with PnC was confirmed. But what was probably more important was the glimmer of a repetitive pattern. Myron Leon went on to show that the hapten in PnC was phosphorylcholine (PC)[25]. Two other PC binding myeloma proteins, TEPC 15 and HOPC 8 were identified raising the number to 5 among some 300 proteins tested. Groups of myeloma proteins with the same hapten binding specificities could be identified in the collection and these would prove valuable later on in understanding antibody diversity mechanisms.

The first indication that myeloma proteins with the same hapten binding activity were structurally related came from a serological study of idiotypes. With Rose Lieberman idiotypic antisera were prepared to all of the PC binding myeloma proteins and it was found much to our amazement that 5 of the proteins, MOPC 299, TEPC 15, HOPC 8 from the Bethesda collection and S63 and S107 from Melvin Cohn's La Jolla collection all shared the same very unique individual 'T15' idiotope[26]. It was then discovered by Heinz Kohler and Umberto Cosenza[27] that normal PC binding antibodies also had the 'T15 idiotope'. This important finding demonstrated the relevance of PC binding myeloma proteins to induced antibodies. Much later this relationship was first definitively established by amino acid sequences of PC binding myeloma proteins and antibodies which showed the V-regions of both kinds of proteins were controlled by the same V_L and V_H genes. With the advent of recombinant DNA technology nucleotide sequences of genes controlling PC binding myeloma and antibodies established that the same V_H and V_L genes were involved.

The phosphorylcholine binding myeloma proteins were not the only group of antigen binding myeloma proteins, others were identified[28]. A large group of myeloma proteins bound levans, these were divisible into a group with specificity for inulin, i.e., $\beta 2,1$-fructofuranan containing levans and another with specificity for Aerobacter bacterial levans with $\beta 2,6$ fructofuran linkages. Other groups of proteins that bound $\beta 1,6$ D linked galactan antigens, and $\alpha 1,3$ and $\alpha 1,6$ linked dextrans were also found. In addition, natural antigens were identified from the microbial flora, the diet and even the bedding used in mouse cages that contained antigens to which myeloma proteins reacted[29,30]. The most direct evidence that natural antigens played a role in the natural history of the plasmacytomas was the evidence that the serum of normal BALB/c mice contained natural antibodies to many of these antigens. Thus, some of the myeloma proteins from plasmacytomas induced in BALB/c mice were in fact, monoclonal antibodies, and this idea, once very much in doubt, was accepted by many workers. In 1975 Georges Köhler and Cesar Milstein[31] successfully hybridized plasmacytoma cells with normal antibody forming cells and obtained antibody secreting hybrid cells. This important discovery has revolutionized the world of immunology because it became possible to obtain monoclonal antibodies to virtually any antigen to which mice and rats respond.

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Biological role of major transplantation antigens in T cell self-recognition[1]

by R. M. Zinkernagel

Institut für Pathologie, Universitätsspital Zürich, CH-8091 Zürich (Switzerland)

Summary. The proposal is made, illustrated and supported by experimental evidence that T cell-mediated immunopathology triggered initially by low- or non-cytopathic infectious agents may cause diseases, susceptibility to which is linked to the major histocompatibility gene complex.
Key words. T cell-recognition; major histocompatibility gene complex; HLA-disease association; virus; immunopathology.

Introduction

Major transplantation antigens play a crucial role in lymphocyte interactions amongst themselves as well as with other somatic cells[2-4]. That this is their real biological function and not to make transplantation surgery or tumor research more difficult was first signaled by the finding that susceptibility to certain diseases is linked, albeit weakly, to the major transplantation antigens coded by the major histocompatibility gene complex (MHC, HLA in humans, H-2 in mice)[5-8]. Any list of the more clear cut HLA-disease association immediately suggests that they are all caused by immune mediated pathogenesis. How can these two phenomena, HLA-disease associations and their immune pathogenesis be explained? and can we understand why HLA-diseases associations are often rather weak?

The function of the immune system is to maintain homeostasis in higher vertebrates; its two main arms, cellular and humoral immunity very efficiently defend the host against acute cytopathic infectious agents. The main targets of T cells are intracellular agents, while those of antibodies are primarily extracellular ones. However this immune defence does not cover all viruses and bacteria with the same efficiency and many other causes of disease such as protozoan or metazoan infections, tumors etc. are more or less out of the reach of efficient immune surveillance.

T cell immunity is at its best when dealing with acute intracellular agents, such as cytopathic viruses. This T cell activity is easily measured in a classical ^{51}Cr-release cytotoxicity assay in vitro[4,9]. T cell-mediated lysis of virus-infected target cells is virus specific, since only target cells infected with the proper virus are lyzed. But in addition, target cell lysis of virus-infected cells depends upon T cells and target cells sharing classical transplantation antigens. Many experiments over the past 12 years have clearly documented that the following general rules govern lymphocyte-lymphocyte and lymphocyte-somatic cell interactions[2,4]:

- T cells recognize self-transplantation antigens together with foreign antigenic determinants exclusively on cell surfaces;
- T cell specificity for self-transplantation antigens a) is specific for polymorphic determinants; b) is selected for during differentiation in the thymus; c) determines the effector function of T cells: cytotoxic T cells recognize class I, i.e. the classical transplantation antigens HLA-A, B, C or H-2K, D, L whereas differentiation promoting T cells (helper or DTH T cells) are specific for class II, HLA-D or H-2I; d) regulates T cell responsiveness, i.e. the quality and quantity of cytotoxic T cell response is regulated by HLA-A, B, C or H-2K, D, L, that of differentiation-promoting T cells by HLA-D or H-2I gene products.

Role of cytotoxic T cells

One may question the physiological role of cytolytic T cells: why should T cells mediate cell and tissue destruction to combat intracellular infectious agents[4,10-14]? There is good evidence that cytotoxic T cells destroy virus-infected cells before viral progeny is assembled, thus eliminating virus during the eclipse phase of virus replication. Virus elimination via immunological host cell destruction is, in the case of cytopathic viruses, an efficient way to prevent virus spread and the resulting more extensive virus-mediated cell and tissue damage[4]. In the case of non-cytopathic viruses, this immunological defense mechanism becomes less attractive, because host cells are not destroyed by virus but only by the T cell immune response[4,6,11-13]. Because T cells can apparently not distinguish cytopathic from non-cytopathic viruses, im-