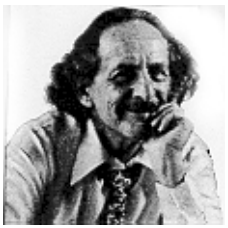
Essays of an Information Scientist, Vol:8, p.416-431, 1985
Current Contents, #45, p.3-18, November 11, 1985



Current Comments®

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The 1984 Nobel Prize in Medicine Is Awarded to Niels K. Jerne, César Milstein, and Georges J.F. Köhler for Their Contributions to Immunology

Number 45 November 11, 1985

The Nobel Assembly of Karolinska Institute awarded the 1984 Nobel Prize in physiology or medicine jointly to Niels K. Jerne, professor emeritus, Basel Institute for Immunology, Switzerland, César Milstein, British Medical Research Council's Laboratory of Molecular Biology, Cambridge, UK, and Georges J.F. Köhler, now at the Max Planck Institute for Immunobiology, Freiburg, Federal Republic of Germany. These scientists were honored for their extraordinary contributions to the field of immunology—Jerne for his theoretical advances that have shaped our concepts of the immune system and Milstein and Köhler for the production of monoclonal antibodies using the hybridoma technique, a methodological breakthrough with broad practical significance.

My discussion on this year's Nobel Prize winners will be in three parts. Next week's essay will focus on the Nobel Prize winners in physics and chemistry. Physicists Carlo Rubbia and Simon van der Meer of CERN, Geneva, Switzerland, shared the physics award for their contributions to the discovery of the heavy field particles W and Z. Biochemist R. Bruce Merrifield, Rockefeller University, New York, was awarded the chemistry prize for his simplification of the method for producing complex proteins. The third essay will discuss the Nobel Prize in literature, awarded to Czechoslovakian poet Jaroslav Seifert, and the Alfred Nobel Memorial Prize in Economic Science, given to Sir Richard Stone, University of Cambridge, UK, for his pioneering national accounts system.

Through citation analysis these essays emphasize the role that scientific literature plays in the discovery process. My previous essays on Nobel Prize winners illustrate how citation analysis can provide a documentary perspective on the field the Nobel award has recognized.[1,2] To establish this perspective, we locate the relevant research fronts on the worldwide map of science. A research front is simply a group of current papers that cite one or more papers in a cluster of core papers.[3]

I am often asked if we can anticipate the winners of the Nobel Prizes. By examining strings of annual research fronts over a long period of time, one can reasonably *forecast* fields that may one day be recognized by a Nobel Prize. Likewise, a long-term analysis of the publication and citation records of notable scientists within these forecasted fields shows who are highly likely to be among future Nobel Prize winners if that field is recognized.

Using this methodology, it is not surprising that our published lists of most-cited contemporary scientists have included many Nobel Prize winners. In Table 1 we have listed those winners who were among the 1,000 most-cited authors for 1965 to 1978. Note that many of these winners were listed before the prizes were awarded. However, without being privy to the rationale involved in the specific annual decisions of the Nobel Assembly, it is impossible to *predict* either the fields or the persons to be recognized.

The 1984 Nobel Prize pays tribute to the field of immunology, the study of the

Table 1: Nobel Prize winners who were among the 1,000 most-cited contemporary scientists, 1965-1978. A = name. B = field. C = year Nobel Prize was won.

A	B	C
Anderson PW	physics	1977
Anfinsen CB	biochemistry	1972
Axelrod J	pharmacology	1970
Baltimore D	virology	1975
Barton DHR	organic chemistry	1969
Benacerraf B	immunology	1980
Berg P	molecular biology	1980
Bloembergen N	physics	1981
Blumberg BS	oncology	1976
Brown HC	organic chemistry	1979
Dausset J	immunology	1980
DeDuve C	cell biology	1974
Edelman GM	immunology	1972
Fischer EO	organometallic chemistry	1973
Flory PJ	physical chemistry	1974
Glashow SL	physics	1979
Guillemin R	endocrinology	1977
Hoffmann R	inorganic chemistry	1981
Hubel DH	physiology	1981
Jacob F	molecular biology	1965
Katz B	biophysics	1970
Khorana HG	organic chemistry	1968
Klug A	molecular biology	1982
Kornberg A	molecular biology	1959
Krebs HA	biochemistry	1953
Lee TD	physics	1952
Lipmann F	biochemistry	1953
Lipscomb WN	physical chemistry	1976
Milstein C	immunology	1984
Mitchell PD	biochemistry	1978
Monod JL	molecular biology	1965
Mott NF	physics	1977
Nathans D	microbiology	1978
Nirenberg M	genetics	1968
Ochoa S	biochemistry	1959
Palade GE	cell biology	1974
Perutz MF	molecular biology	1962
Richter B	physics	1976
Rubbia C	physics	1984
Samuelsson B	biochemistry	1982
Sanger F	molecular biology	1958 & 1980
Schally AV	endocrinology	1977
Sutherland EW	pharmacology	1971
Taube H	inorganic chemistry	1983
Temin HM	oncology	1975
Vane JR	pharmacology	1982
Weinberg S	physics	1979
Wiesel TN	physiology	1981
Wilkinson G	organometallic chemistry	1973
Wilson KG	physics	1982
Woodward RB	organic chemistry	1965

body's ability to resist invasion by foreign organisms or substances. Immunology is a far-ranging field including research from a variety of smaller biological topics such as genetics and virology. As a result, immunology has had a tremendous impact on the research community. Its value is rarely questioned, particularly with the outbreak of new diseases, such as the deadly—and as yet incurable—Acquired Immunodeficiency Syndrome (AIDS). While space cannot permit a complete discussion of immunology, the breadth of the topic compels me to spend more time discussing this topic than usual.

Antibodies and Antigens

Part of the body's power to fight off intruders resides in antibodies. Produced by white blood cells called lymphocytes, each antibody is made to recognize a specific antigenic determinant, a specific site found on a foreign substance. The antibody binds with the antigen in a lock-and-key fashion to inhibit the antigen's biologic activity and mark it for destruction by scavenger cells. After the invader has been neutralized, many antibodies remain in the blood so that the next time the same antigen-bearing molecule invades, the body's defenses are already mobilized.

In his Nobel Prize acceptance speech given in Stockholm, Jerne compared the immune system to language to explain the large variety of foreign invaders that the immune response can handle. "Looking at languages, we find that all of them make do with a vocabulary of roughly a hundred thousand words or less. These vocabulary sizes are a hundred times smaller than the estimates of the size of the antibody repertoire available to our immune system."[4]

Table 2 provides a brief survey of some of the outstanding contributions to the early development of immunology prior to the twentieth century.

From 1900 through 1950, immunological research focused on the underlying mechanisms in the immune response. During this time it was generally supposed that the antigen served as a template on which antibodies were formed. Yet this theory did not explain the presence of antibodies in the serum

Table 2: A selective history of immunology studies.

Scientist	Year	Contribution to Immunology
Edward Jenner	1798	Developed first effective immunization procedure using virulent cowpox against smallpox infection
Louis Pasteur	1881	Demonstrated that vaccination procedure can be applied to many microbial infections. Caused vaccination to gain widespread acceptance
Paul Ehrlich	1897	Proposed side-chain theory of antibody synthesis and promoted his idea that antibody specificity results from antigen recognizing an antibody with complementary molecular structure and that antigen-antibody interaction is a chemical union
Jules Bordet	1899	Found that antibodies can destroy non-threatening cell types in addition to pathogenic bacteria
Karl Landsteiner	1900	Showed that an antibody can distinguish between subtly different antigenic structures

Table 3: Jerne's most-cited papers in 1955-1984, *SCI*®. A = number of citations. B = bibliographic data. *SCI/SSCI*® research front(s) to which the paper is core follows B.

A	B
2400	**Jerne N K & Nordin A A.** Plaque formation in agar by single antibody-producing cells. *Science* 140:405, 1963. 83-7618
1671	**Jerne N K, Nordin A & Henry C.** The agar plaque technique for recognizing antibody-producing cells. (Amos B & Koprowski H, eds.) *Cell-bound antibodies.* Philadelphia: Wistar Institute Press, 1963. p. 109-25.
966	**Jerne N K.** Towards a network theory of the immune system. *Ann. Immunol.—Inst. Pasteur* C125:373-89, 1974. 76-1010, 83-5673, 84-0032
676	**Jerne N K.** The somatic generation of immune recognition. *Eur. J. Immunol.* 1:1-9, 1971. 81-0016, 82-0317, 83-1491
300	**Jerne N K.** The natural-selection theory of antibody formation. *Proc. Nat. Acad. Sci. US* 41:849-57, 1955.
277	**Jerne N K, Henry C, Nordin A A, Fujl H, Koros A M C & Lefkovits I.** Plaque forming cells: methodology and theory. *Transplant. Rev.* 18:130-91, 1974.
272	**Rajewsky K, Schirrmacher V, Nase S & Jerne N K.** The requirement of more than one antigenic determinant for immunogenicity. *J. Exp. Med.* 129:1131-43, 1969. 75-1212
223	**Henry C & Jerne N K.** Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. *J. Exp. Med.* 128:133-52, 1968.
212	**von Boehmer H, Haas W & Jerne N K.** Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice. *Proc. Nat. Acad. Sci. US* 75:2439-42, 1978. 81-0016, 82-0317, 83-1491
139	**Jerne N K.** The immune system: a web of V-domains. *Harvey Lect.* 70:93-110, 1976.

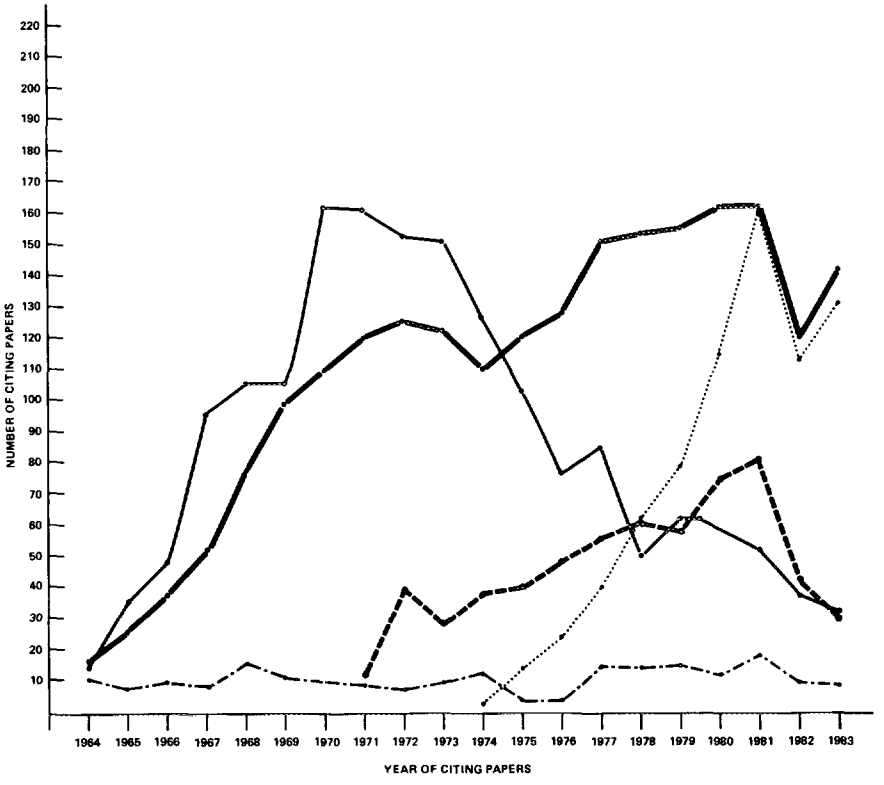
of normal animals. This problem became the focus of studies by Niels K. Jerne.

Niels K. Jerne

Born in London in 1911, Jerne grew up in Holland where he began his studies in physics. After two years, he switched to medicine at the University of Copenhagen, Denmark, where he began studying antibodies. From 1943 to 1956, Jerne continued his studies on antibodies at the Danish State Serum Institute. From there, he spent six years in Geneva, working at the World Health Organization and later at the University of Geneva.

He was chairman of the Department of Microbiology at the Medical School of the University of Pittsburgh between 1962 and 1966, and it was during this period that Jerne and Albert A. Nordin, also of the University of Pittsburgh, described a simple method for counting specific antibody-forming cells among a mixed-cell population. Their paper "Plaque formation in agar by single antibody-producing cells" has been cited over 2,400 times since its publication in 1963, making it Jerne's most highly cited paper. This method has since become a routine technique.[5]

Figure 1: Chronological distribution of citations to Niels Jerne's five most-cited papers. The heavy solid line shows citations to his 1963 *Science* paper; light solid line shows citations to the 1963 paper in *Cell-Bound Antibodies*; dots show citations to the 1974 *Annales d'Immunologie* paper; dashes show citations to 1971 *European Journal of Immunology* paper; dot-dash line shows citations to 1955 *Proceedings of the National Academy of Sciences of the United States of America* paper.



In 1966 Jerne left the US to become director of the Paul Ehrlich Institute in Frankfurt. In 1969 when the Basel Institute of Immunology was founded, Jerne became its first director. During his tenure, he became widely acknowledged as a leader in immunological theory. The Nobel Assembly has recognized Jerne's most influential theories as deserving of the Nobel Prize, noting, "In three main theories he has in a visionary way elucidated essential questions concerning specificity, development and regulation of the immune response."[6]

His first notable theory was proposed in 1955. In his paper "The natural-selection theory of antibody formation," Jerne abandoned the old antigen-template theories and proposed that antibody molecules are already present in the host, having developed during fetal life. An invading foreign antigen selects the antibody molecule that has the best fit for binding. This antigen-antibody binding stimulates production of the chosen antibody.[7]

In addition to providing sharp contrast to the prevailing theories, Jerne's natural-selection theory was the first to forge the idea that a specialized population of cells, now known as lymphocytes, is required for antibody formation. His hypothesis was later proven to be true and today is considered to be the theoretical foundation for immunology.

In his theory on "The somatic generation of immune recognition," published in 1971, Jerne described how antibodies

are developed in the fetus before foreign antigens are encountered.[8] Observing that a single animal can make a very large number of different antibodies, Jerne proposed that antigen-sensitive cells (lymphocytes committed to making antibodies) must have a diversity of antibody genes. The antigen-sensitive cells, stimulated by a self-antigen (an antigen present within the animal), enter cell division. Mutations occur in the rapidly dividing cells, producing a pool of antibodies with new immunological specificities capable of handling any foreign antigen. Meanwhile, the specificities of the animal's antigen-sensitive cells for its own antigens are weakened. This theory explains why individual animals of the same species produce different antibodies when stimulated by the same antigen and why animals usually do not make antibodies against their own antigens. According to Jerne's theory, all animals have different self-antigens, and further diversity results from the random mutation process that generates the final antibody pool.

Jerne's third influential theory, proposed in 1974, attempts to explain how the specific immune response is regulated. "Towards a network theory of the immune system"[9] postulates that idiotypes (antigenic markers found on antibodies) may act as antigens eliciting an antibody response. These new antibodies, called anti-antibodies, also contain idiotypes, which in turn may elicit another antibody response. This cascading effect forms a network between the cells of the immune system. Under normal conditions, Jerne believes the network is balanced, but the equilibrium becomes disturbed when an antigen is introduced. To restore balance, Jerne proposes that the immune system causes a response to the antigen that results in the cascading effect and the production of anti-antibodies.

Hybridoma Technique Development

Jerne's theoretical work laid the foundation for many advances in immunology, including the monoclonal antibody production technique developed by César Milstein and Georges Köhler in 1975. In their announcement of the Nobel Prize, the Karolinska Institute described this discovery as "one of the most important methodological advances in biomedicine during the 1970's."[6]

César Milstein was born in Argentina in 1927. He began working at the National Institute of Microbiology in Buenos Aires in 1957. Between 1958 and 1961, he took a leave of absence from the institute to earn his doctorate at the Department of Biochemistry, University of Cambridge, UK, after which he returned to Buenos Aires. In 1963 he left Argentina to join the staff of the Medical Research Council Laboratory of Molecular Biology in Cambridge, where today he heads the Division of Protein and Nucleic Acid Chemistry. Over the past 20 years, Milstein has pioneered in the study of genetic variation among antibodies[10,11] as well as the intracellular mechanisms underlying antibody synthesis.[12]

Georges J.F. Köhler, born in 1946 in Munich, received his doctoral degree in biology from the University of Freiburg in 1974. His doctoral work focused on his findings that the immune system of the mouse can produce a thousand different kinds of antibody molecules against a single antigenic site on an antigen. To study the mutations in the genes that specify antibodies, he began postgraduate research in Milstein's laboratory in Cambridge. It was during this period that Köhler and Milstein developed the technique for monoclonal antibody formation.

Prior to their studies, antibodies specific for a particular antigen could be made only by injecting an animal with a chosen antigen, removing blood from the animal, and separating the antiserum, that portion of blood containing antibodies. This technique was laborious, since the immune system responds to minute amounts of antigen. Even if the purest antigen is used, extra antibod-

ies are produced as a result of trace amounts of contaminants. In order to obtain the antibodies specific for the injected antigen, unwanted antibodies have to be identified and eliminated, a difficult and time-consuming task.

Another problem resulted from the fact that lymphocytes, the producers of antibodies, are highly specialized cells that lack the ruggedness needed to survive in a tissue-culture medium. Therefore, even if the lymphocyte that made a specific antibody is identified, it is unable to survive the rigors of the laboratory processes that would be required to isolate it and use it as a source of pure antibody.

Milstein and Köhler recognized the need for the long-term growth of specific antibody-forming cells in culture. The work by Michael Potter in 1962 at the National Cancer Institute, Bethesda, Maryland, had already established that myelomas (tumor cells) can be cultivated indefinitely.[13] The fusing of two different cell types had been performed earlier by R.G.H. Cotton, Royal Children's Hospital Research Foundation, Victoria, Australia, and Milstein, who fused rat and mouse myeloma cells.[14] Building upon these earlier studies, Köhler and Milstein produced a hybridoma by fusing myeloma cells with lymphocytes immunized by a selected antigen.[15]

In a hybridoma, the myeloma cell contributes the capacity for survival, while the lymphocyte allows production of antibodies with specificity for certain types of antigens. These hybridomas can be propagated, and the clones that result can provide unlimited production of a highly specific antibody. These specific antibodies are called monoclonal antibodies and have become an essential tool in biomedical research. The paper describing this technique has been cited over 3,000 times since its publication in 1975, making it the most cited of many highly cited papers by both Köhler and Milstein.

It is interesting to note that Köhler and Milstein originally developed this technique to study the rate of mutation of antibody genes to further understand antibody diversity. Milstein, quoted in a *Science News* article, said, "We were working on very esoteric questions. Only later we realized the [general] potential of the procedures."[16] In their paper describing the hybridoma technique, Köhler and Milstein appeared to underestimate the ramifications of their discovery when they modestly concluded that "it is possible to hybridise antibody-producing cells from different origins. Such cells can be grown *in vitro* in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use."[15]

Monoclonal Antibody Applications

Today, monoclonals are having a strong impact on most areas of medicine. A major application for monoclonals is in cancer therapy. One of the limitations of standard anticancer therapies is that cancer drugs and radiation therapy attack both cancerous and healthy cells. However, Robert W. Baldwin and Malcolm V. Pimm, Cancer Research Campaign Laboratories, University of Nottingham, UK, are experimenting with an approach in which monoclonals can be prepared to bind with a specific cancer antigen. By injecting a patient with a cancer drug carried by a monoclonal specific for the cancer type, the monoclonal can lead the drug directly to the cancer cells, sparing the healthy cells from the effects of the drug.[17]

Monoclonals can also make bone-marrow transplants more practical for people suffering from leukemia. Jerome Ritz, Division of Tumor Immunology, Sidney Farber Cancer Institute, Boston, Massachusetts, describes an approach that uses monoclonals to selectively deplete the malignant cells from the bone marrow prior to autologous or self-transplantation of the marrow. In addition, monoclonals can be used to promote marrow transplantation between a donor and recipient who are not normally histocompatible—a state of mutual tol-

Table 4: Milstein's most-cited papers in 1955-1984, *SCI®*. A = number of citations received. B = bibliographic data. *SCI/SSCI®* research front(s) for which the paper is core follows B.

A	B
3040	**Köhler G & Milstein C.** Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495-7, 1975. 78-1440, 79-0363, 81-1664
811	**Köhler G & Milstein C.** Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511-9, 1976. 78-1440, 79-0363, 81-1664
693	**Galfre G, Howe S C, Milstein C, Butcher G W & Howard J C.** Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature* 266:550-2, 1977. 78-1440, 79-0363, 81-1664
490	**Milstein C, Brownlee G G, Harrison T M & Mathews M B.** A possible precursor of immunoglobulin light chains. *Nature—New Biol.* 239:117-20, 1972. 74-0003, 75-0034, 76-0514, 77-0621, 78-0534, 79-0550, 81-0054, 82-0224
346	**Köhler G, Howe S C & Milstein C.** Fusion between immunoglobulin-secreting and nonsecreting myeloma cell lines. *Eur. J. Immunol.* 6:292-5, 1976.
324	**Williams A F, Galfre G & Milstein C.** Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. *Cell* 12:663-73, 1977. 79-0363, 82-1232, 83-0081, 84-3435
313	**Barnstable C J, Bodmer W F, Brown G, Galfre G, Milstein C, Williams A F & Ziegler A.** Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. *Cell* 14:9-20, 1978. 79-0363, 81-0710, 82-0788, 83-1880
257	**McMichael A J, Pilch J R, Galfre G, Mason D Y, Fabre J W & Milstein C.** A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* 9:205-10, 1979. 81-0136, 84-0171
220	**Milstein C.** Linked groups of residues in immunoglobulin k chains. *Nature* 216:330-2, 1967.
178	**Springer T, Galfre G, Secher D S & Milstein C.** Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol.* 9:301-6, 1976. 82-0673

Table 5: Köhler's most-cited papers in 1955-1984, *SCI®*. A = citations. B = bibliographic data. *SCI/SSCI®* research front(s) for which the paper is core follows B.

A	B
3040	**Köhler G & Milstein C.** Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495-7, 1975. 78-1440, 79-0363, 81-1664
811	**Köhler G & Milstein C.** Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511-9, 1976. 78-1440, 79-0363, 81-1664
427	**Shulman M J, Wilde C D & Köhler G.** A better cell line for making hybridomas secreting specific antibodies. *Nature* 276:269-70, 1978.
346	**Köhler G, Howe S C & Milstein C.** Fusion between immunoglobulin-secreting and nonsecreting myeloma lines. *Eur. J. Immunol.* 6:292-5, 1976.
63	**Köhler G, Hengartner H & Shulman M J.** Immunoglobulin production by lymphocyte hybridomas. *Eur. J. Immunol.* 8:82-8, 1978.
47	**Köhler G, Lefkovits I, Elliott B & Coutinho A.** Derivation of hybrids between a thymoma line and spleen cells activated in a mixed leukocyte reaction. *Eur. J. Immunol.* 7:758-61, 1977.
44	**Milstein C, Adetugbo K, Cowan N J, Köhler G, Secher D S & Wilde C D.** Somatic cell genetics of antibody-secreting cells: studies of clonal diversification and analysis by cell fusion. *Cold Spring Harbor Symp.* 41:793-803, 1976.
43	**Forni L, Coutinho A, Köhler G & Jerne N K.** IgM antibodies induce the production of antibodies of the same specificity. *Proc. Nat. Acad. Sci. US* 77:1125-8, 1980.
41	**Köhler G, Pearson T & Milstein C.** Fusion of T and B cells. *Somatic Cell Genet.* 3:303-12, 1977.
35	**Köhler G.** Frequency of precursor cells against the enzyme β-galactosidase. An estimate of the BALB/c strain antibody repertoire. *Eur. J. Immunol.* 6:340-7, 1976.

erance allowing different tissues to be grafted together.[18]

In kidney transplant recipients, the early signs of infection are identical to those of impending organ rejection. Y.C. Smart, Faculty of Medicine, University of Newcastle, Australia, and colleagues have found that monoclonals may accurately diagnose infection versus rejection, which is imperative because these two problems must be treated in different ways.[19]

Major breakthroughs often become the source for new perplexing research questions. While researchers realize the potential benefits that can be derived from monoclonals, Henry Krakauer, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, and Office of Research, Health Care Financing Administration, Baltimore, Maryland, notes that research using monoclonal antibodies has unveiled deterring problems.[20] For example, research is being done to develop methods for obtaining hybridomas that produce human antibodies. However, antibody-producing human lymphocytes fused to mouse myeloma cells have proved unstable; suitable human myeloma cells need to be developed for fusion. In addition, it is not ethical or legal for humans to be injected with viruses or cancer cells to provoke an immune response, making it difficult to obtain human lymphocytes that produce a desired antibody.

Nevertheless, monoclonal research has hinted at significant potential. In 1983 NIH funded 358 research projects in which monoclonal antibodies were either the principal objects of the investigations or the essential tools.[20]

Citation Studies

Although Jerne was honored by the Nobel Committee for his theoretical work, it is interesting, but hardly surprising, to note that his methodological paper "Plaque formation in agar by single antibody-producing cells"[5] is his most-cited paper (Table 3). Of interest to citation analysts is the fact that this article is one of the three most-cited papers published in *Science* from 1961 to 1980.[21] As we reported earlier, it is in fact one of the most-cited papers of all time.[22] Jerne discussed this paper in a *Citation Classic* commentary in 1981, long before being awarded the Nobel Prize.[23] The chronological frequency graph for this paper and his four other most-cited papers is shown in Figure 1. Within five years of publication, this paper had already become a citation classic. In 1966 one might have forecast the growth of its impact.

Jerne's three theories recognized by the Nobel Assembly are discussed in his third, fourth, and fifth most highly cited papers. His natural-selection theory[7] has been cited 300 times, his paper on somatic generation[8] has been cited over 675 times, and his paper on the network theory[9] has been cited over 950 times. This illustrates that theory is not necessarily less cited than methodology.

As Tables 4 and 5 show, the two most-cited articles for Milstein and Köhler are two of their collaborative works. The first article describing the hybridoma technique[15] is one of the most-cited articles between 1961 and 1982.[22] The second most-cited paper for both Milstein and Köhler, "Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion," was cited over 800 times. This article extends the use of fusion techniques for producing specific antibodies.[24]

Milstein's third most-cited paper, "Antibodies to major histocompatibility antigens produced by hybrid cell lines,"[25] was one of the 1977 articles most cited between 1977 and 1979, with 99 citations.[26] Milstein is included in Table 1 as 1 of the 1,000 contemporary scientists most cited between 1965 and 1978.[27] Our annual lists of most-cited current papers have often included works that were accorded early recognition and give forecasters good reason to watch the related fields for future growth potential.

Köhler's third most-cited paper, cited 427 times, describes a method for making a better tumor-cell fusion partner,[28] and his fourth most-cited paper studies the control of synthesis and secretion of antibodies.[29]

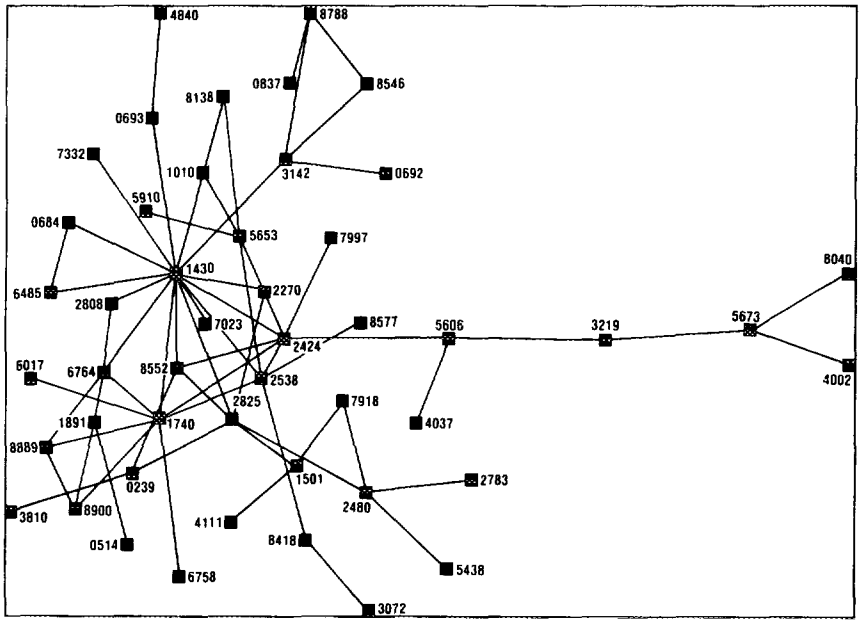
Research-Front Data

Table 6 presents the research fronts in which Jerne's works occur as core documents. Briefly, a research front develops when authors cite a paper to indicate its relevance to their own research. Papers

Table 6: ISI® research fronts in which works by Niels K. Jerne occur as core documents. A = number. B = name. C = number of core papers. D = number of citing papers. Asterisks (*) indicate research fronts that appear on the historiograph in Figure 3.

A	B	C	D
*76-1010	Anti-idiotype antibodies	2	37
*81-0016	Regulation of immune response with emphasis on genetic control	34	423
*82-0317	Growth and regulation of B-cell activation by T cells, helper T cells and antibodies	46	433
83-1491	Major histocompatibility genes of mouse and man; structure, genetics, polymorphism and their role in T-cell immunocompetency	47	931
*83-5673	Expression of T-cell idiotypes and murine antibodies; regulation of T-cell activation	7	229
83-7618	*In vitro* activation of T-cell and B-cell mouse lymphocytes for study of immune responses	3	295
*84-0032	Clinical and experimental studies of immune responses within an idiotype network	10	256

Figure 2: Multidimensional scaled map for #83-0145, "cDNA cloning, gene structure, RNA activity expression and protein structure," showing links between research fronts. A = 1983 research-front number. B = research-front name.



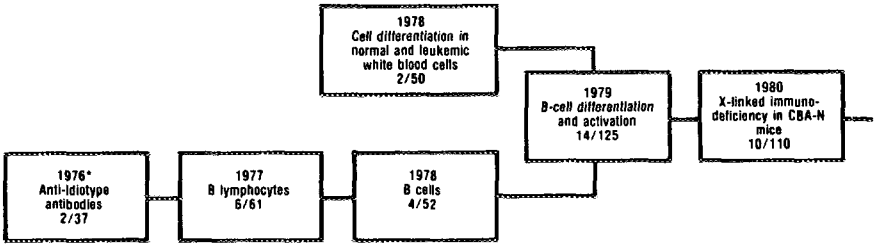
Key

A	B
0239	Mapping and gene structure in *Escherichia coli*, other bacteria and plasmids; *in vitro* transcription and RNA-polymerase isolation
0514	Synthesis, chemical modification and biochemical characterization of histone variants associated with cell differentiation and changes in cell growth

(continued on next page)

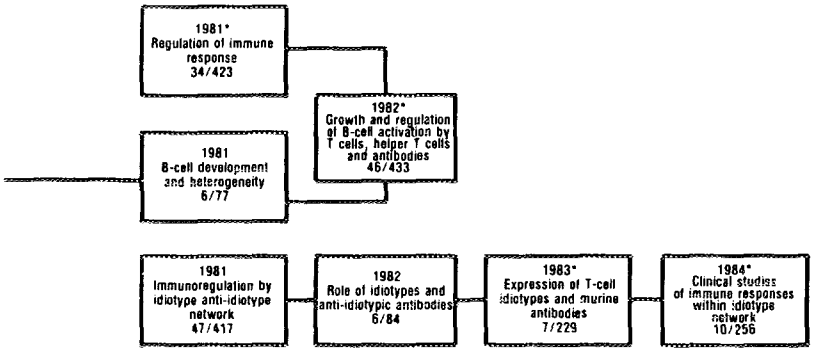
A	B
0684	Methods of biosynthesis of DNA and cDNA cloning into *Escherichia coli* in the production of human insulin
0692	Immunochemical identification of proteins after transfer blotting; characterization of DNA binding proteins
0693	Gene sequences and isolation of cDNA clones; characterization and transcription of messenger RNA
0837	Applications of high-resolution 2-dimensional protein electrophoresis to protein phosphorylation and other chemical modifications
1010	Prenatal diagnosis of beta thalassemia by DNA polymorphism; restriction enzyme mapping and structure of globin gene
1430	cDNA cloning from messenger RNA in *Escherichia coli* as probes of virus and eukaryote gene and protein structure
1501	Gene expression, regulation and cloning in *Escherichia coli* K-12 and its mutants
1740	Oncogenes and the genetics of human cancer; viral transforming genes and their DNA structure
1891	Role and arrangement of nucleosomes, histones and other proteins in the organization of the nuclear matrix and the structure of the chromatin DNA
2270	Isolation, expression, cloning and related studies of *Saccharomyces cerevisiae* and other yeast genes and plasmids
2424	Nucleotide sequence of eukaryotic globin genes; characterization by messenger RNA analysis; use of viral gene expression and other *in vitro* models
2480	Transposable genes and TN5 insertion in *Drosophila melanogaster* and *Escherichia coli*; evolution of transposon DNA sequences in eukaryotes
2538	Gene transcription, expression and sequence including protein structure and RNA activity
2783	Transposable gene elements and hybrid dysgenesis in *Drosophila melanogaster*; role in evolution
2808	DNA methylation; sequence structure and effect on gene activity
2825	Molecular cDNA cloning of genes in *Escherichia coli*; nucleotide sequence and protein structure
3072	Transcription of class-III transfer RNA genes by RNA polymerase-III in *Xenopus laevis* and other eukaryotes
3142	Characterization of proteins via immunochemical and biochemical methods; rapid detection and modifications related to activity
3219	Genetics of immunoglobulin formation in mouse; gene rearrangement and antibody diversity and idiotypes
3810	Characterization of *Escherichia coli* RNA polymerase and its interaction with DNA promoters in the regulation of transcription
4002	T-cell mediated regulation of B-cell activation; suppressor, contrasuppressor and antigen specific immune responses in normal and tumor cells
4037	Characterization of gene polymorphism in yeast, *Saccharomyces cerevisiae*; transcriptional and post-transcriptional regulation during development and differentiation in eukaryotes
4111	Genetic analysis of gene expression in *Escherichia coli* and yeast by gene fusions; characterization of mutations of promoter regions

Figure 3: Historiograph showing development of theories of the immune system. Numbers at the bottom of each box refer to the number of cited/citing papers for each research front. An asterisk(*) indicates research fronts in which Jerne is author of a core paper.



A B

4840 Characterization of factors affecting messenger RNA synthesis, activity and degradation; gene expression measurement by evaluation of *in vitro* translation products
5438 Transposable DNA sequences and satellite DNA in *Drosophila*; role of repetitive elements in evolution
5606 Processing of messenger RNA of murine immunoglobulin and histocompatibility genes; cDNA probes to study B-cell regulation
5653 Nucleotide sequence of human and mouse genes as tools to study evolution; cDNA for beta globin
5673 Expression of T-cell idiotypes and murine antibodies; regulation of T-cell activation
5910 Analysis of mutagenesis and nucleotide sequence of genes of *Escherichia coli*, yeast and other eukaryotes; mutations by frame-shifting, ultraviolet radiation, repeated sequences, hypervariable sites and slipped mispairing
6017 DNA sequences, properties and mutants of simian virus 40 and polyoma virus
6485 Molecular cloning of human genes; enzymes, fibronectin and histocompatible antigen genes
6758 Avian virus oncogene products; characterization of transforming proteins and induction of lymphoma in chickens
6764 Gene expression and relation to transformation in mammary tumor viruses, adenoviruses and mouse-human cell hybrids
7023 Cloning, isolation, and sequence analysis of cDNA and messenger RNA for genes from humans and other animals
7332 Repetitive DNA sequences in the organization of human genomic families
7918 Transposons of *Escherichia coli* and *Salmonella typhimurium*; mutagenesis and construction of new gene elements
7997 Nucleotide sequence and gene structure of tumor and virus antigens; cap structures of messenger RNA
8040 Cross-reactive idiotypes and genetic basis of antibody formation; heterogeneity of idiotypic mouse monoclonal antibodies
8138 Regulation of human globin genes; endonuclease and structural DNA studies in thalassemia
8418 RNA transcription *in vitro*; initiation and expression and characterization of RNA polymerases
8546 Methods of protein purification and characterization using silver staining and 2-dimensional polyacrylamide-gel electrophoresis
8552 Genetic studies of DNA nucleotide sequences, protein activation, messenger RNA structure and related topics
8577 Transcription of ribosomal RNA genes in *Xenopus laevis*, mice and *Escherichia coli*
8788 2-dimensional gel electrophoresis in the characterization of protein synthesis and expression in cells
8889 DNA-mediated gene transfer and expression of herpes simplex virus thymidine kinase gene in mammalian cells
8900 Expression of genes including the thymidine kinase gene and the stability and inhibition of mammalian cell transformation



that are frequently cited together, or co-cited, share common features such as topics, results, methods, or discussion. In this way, the citing authors themselves categorize papers into subject-related clusters of research. These co-citation groups help identify research fronts.

Figure 2 shows the multidimensional scaling map for the C2 research front entitled "cDNA cloning, gene structure, RNA activity expression and protein structure" (#83-0145). The map shows how many related C1 fronts are linked by co-citation. One of the core papers for "Expression of T-cell idiotypes and murine antibodies: regulation of T-cell activation" (#83-5673) is the 1974 paper by Jerne.[9]

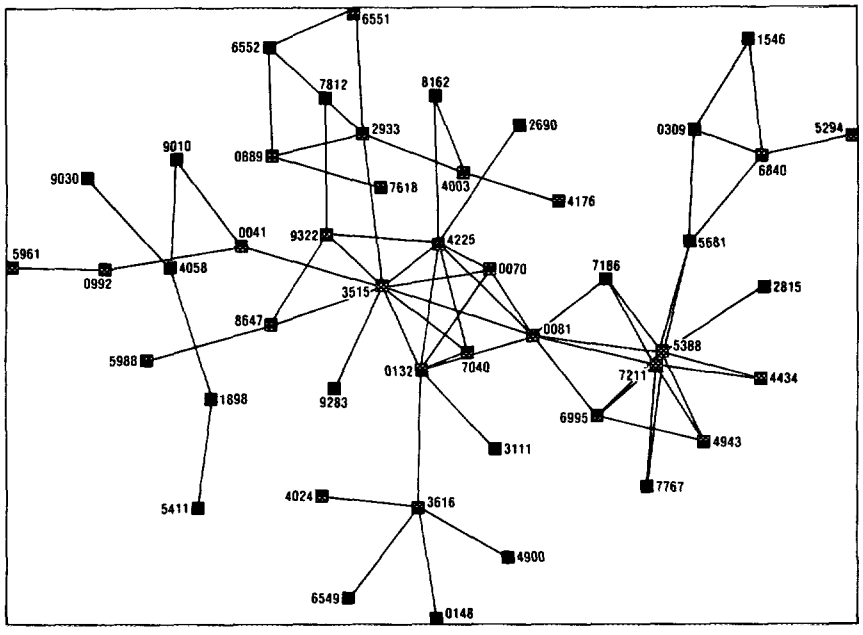
To show how Jerne's field of study has changed and advanced between 1976 and 1984, Figure 3 presents a historiograph (cluster string) of Jerne's contributions to the development and control of the immune system during this period. Each box includes the research-front name, the number of core articles, and the number of citing (published) papers. The fronts that are included are determined by the continuity of the core literature from year to year.[30] If the same core documents are cited at the required thresholds in two adjacent years, then a "string" is established. By continuing this procedure, a historiograph is developed.

Table 7 shows the research fronts that include papers by Milstein and Köhler as core documents. Figure 4 is a higher level map for "Identification of T-cell and B-cell subsets and their role in tumor immunotherapy" (#83-0031). This map shows front #83-0081, "Studies of human natural-killer cells and other T-cell subset activities," which includes a core paper by Milstein.[31]

Table 7: ISI® research fronts in which works by César Milstein and Georges J.F. Köhler occur as core documents. A = number. B = name. C = number of core papers. D = number of citing papers. Asterisks (*) indicate research fronts that appear on the historiograph in Figure 5.

A	B	C	D
74-0003	RNA viruses	490	3573
75-0034	Ribosomes	13	159
*76-0514	Messenger RNA	9	105
*76-0601	Replication of DNA and RNA	21	217
*77-0621	Protein synthesis	12	186
*78-0534	Biosynthetic precursors	11	165
*78-1440	Production of monoclonal antibodies to various antigens	3	99
*79-0363	Hybrid myeloma monoclonal antibody production	6	204
*79-0550	Synthesis of preprohormone precursors	10	174
*79-1716	Sequences of immunoglobulin light-chain genes at recombination sites	4	43
81-0031	Retinoids and cancer	47	519
*81-0054	Protein transfer across membranes	6	270
81-0104	Expression of immunoglobulin genes with emphasis on heavy chain	49	434
*81-0710	Monoclonal antibodies	7	153
81-1664	Production of monoclonal antibody by hybridoma	3	578
*82-0224	Regulation and mechanism of transmembrane translocation of different proteins; role of gene expression and signal peptide sequences in the synthesis, secretion and transport of proteins	41	486
82-1232	Maturation of T-cell subsets and role of T cells in graft vs. host reactions and skin allograft rejection	4	99
83-0081	Studies of human natural-killer cells and other T-cell subset activities	22	546
*83-1880	Monoclonal antibodies in the characterization of HLA antigens, cytotoxic T cells and other cell surface antigens, and their expression	3	149
84-0171	Clinical aspects and characterization of human T-cell subsets	47	1206
84-3435	Monoclonal antibody analysis of rat T cells and their cytotoxic effects in allograft rejection	9	184
84-6424	Biomedical applications of monoclonal-antibody technology	2	68
84-7912	Studies of microtubules using monoclonal antibodies to tubulin	2	40

Figure 4: Multidimensional scaled map for #83-0031, "Identification of T-cell and B-cell subsets and their role in tumor immunotherapy," showing links between research fronts. A = 1983 research-front number. B = research-front name.



Key

A	B
0041	T-cell subset studies of systemic lupus erythematosus and other lymphocyte related diseases
0070	Characterization of epidermal langerhans cells, T cells and others by monoclonal antibodies and other immunological techniques, and studies of histiocytosis-X
0081	Studies of human natural-killer cells and other T-cell subset activities
0132	B-cell and T-cell antigens identified by monoclonal antibody and used in identification of lymphocyte subpopulations and leukemia
0148	Radiolabeled monoclonal antibodies to carcinoembryonic antigen and other antigens to detect and localize tumors via immunoscintigraphy
0309	Human leukocyte interferon gene cloned into *Escherichia coli*; tolerance and antiviral activity of alpha-interferon and gamma-interferon
0889	Regulation of B-cell growth and development by T-cell soluble factors; synergism of immunoglobulin and interleukin
0992	Activity of T-cell subsets in multiple sclerosis and their analysis with monoclonal antibodies
1546	Antiviral and anticellular action and other actions of interferon
1898	Kaposi's sarcoma, cytomegalovirus infection, immunological factors and other aspects of the pathogenesis of acquired immune deficiency syndrome in homosexual men and other populations
2690	Immunofluorescence and other techniques using monoclonal antibodies in the study of T-cell and B-cell lymphocyte, lymphoma leukemia and other human cell antigens
2815	Stimulation of human interferon producing cells by mycoplasma, tumor-cell lines; mediation of natural-killer cytotoxicity
2933	Human T-cell lymphoma virus and adult T-cell leukemia; nucleic acid analysis of virus and expression induced by interleukin-2
3111	Terminal deoxynucleotidyl transferase as immunological markers in human cells with chronic and acute lymphocytic leukemia

(continued on next page)

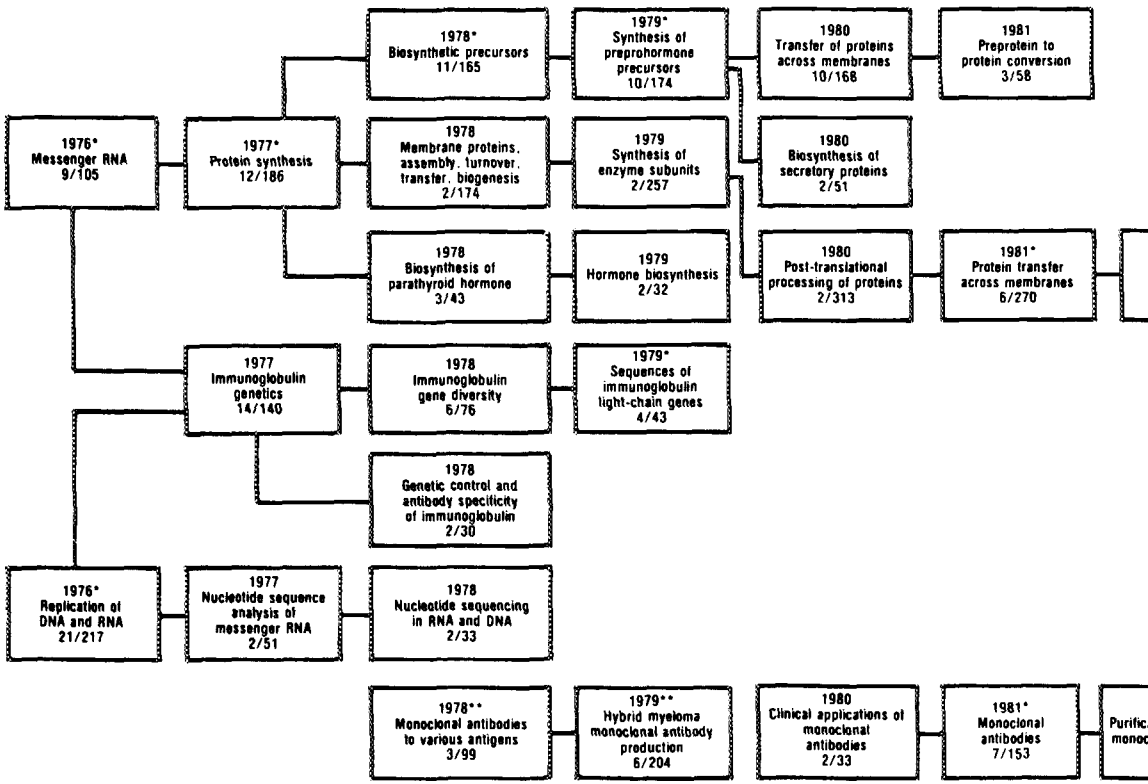
A B

3515 Studies of T-cell subsets in cancer and other diseases by monoclonal antibodies and other techniques
3616 Therapeutic use of monoclonal antibodies in leukemia and other cancers
4003 Cloned cytolytic T cells; functional and immunogenetic analysis of cytolytic lymphocytes T cells and their helper characteristics
4024 Tumor-associated differentiation antigens of human melanoma; analysis of monoclonal antibodies and typing of cell-surface antigens
4058 Circulating T-cell subsets in acquired immune deficiency syndrome, cytomegalovirus mononucleosis and hemophilia; immunoregulatory abnormalities in humans
4176 Mutagens, irradiation antilyt-2 antibodies and other factors in the generation of cytolytic lymphocyte-T precursor clones; applications to tumor immunotherapy
4225 Function of lymphocyte T cells and their antigens including characterization with monoclonal antibodies
4434 Natural-killer cell activity and the presence of sodium-potassium antigens in cytotoxic murine lymphocytes
4900 Generation and characterization of human monoclonal antibody producing hybridomas; applications using lymphocytes from patients with malignant tumors and other disorders
4943 Characteristics and regulation of natural-killer cell activity; role of lymphocytes and other cells in the modulation of cytotoxicity
5294 Production of interferon in lupus erythematosus and its application to acquired immune deficiency syndrome
5388 Natural-killer cytotoxicity of human and mouse lymphocytes affected by interferon and interleukin-2 *in vitro* activity
5411 Acquired immune deficiency syndrome and lymphoadenopathy in homosexuals; morbid anatomy and tumor associations
5681 Effect of recombinant interferon treatment on human natural-killer cell cytotoxicity
5961 Studies of cerebrospinal fluid IGG in multiple sclerosis and encephalomyelitis
5988 Study of human lymphocytes based upon esterase activity and monoclonal antibody reactivity
6549 Monoclonal antibody conjugates in the selective effects of ricin and other toxins
6551 Effect of interleukin-2 on the generation, differentiation and activity of cytotoxic T cells
6552 Characterization of interleukin-2 and its effect on T-cell production in relation to other growth factors
6840 Effect of interferon on human and mouse cells; characterization of antiviral effects and production by human leukocytes
6995 Natural-killer cells and antitumor activity in conjunction with interferon, radiation and chemotherapy; *in vitro* assessment of killer cell suppression
7040 Use of monoclonal antibodies in the characterization of T-cell antigens in the analysis of lymphocyte subsets
7186 Mechanisms of cytotoxicity for human natural-killer cells
7211 Natural-killer cell and cytotoxicity activity and effect on lymphocytes and tumors
7618 *In vitro* activation of T-cell and B-cell mouse lymphocytes for study of immune responses
7767 Studies of natural-killer cell activity, cell-mediated cytotoxicity and resistance to antiasialo GMI antiserum
7812 *In vitro* activity of T cells and B cells including the production of immunoglobulin secreting cells
8162 T-cell surface marker antigens identified with monoclonal antibodies; characterization of expression of cytotoxic clones
8647 Characterization with monoclonal antibodies and other properties of peripheral-blood lymphocyte T cells
9010 Lymphocyte-T suppressor cells and other T-cell subsets after human bone-marrow transplantation
9030 Cytomegalo-virus infection and other opportunistic infections in renal-transplant recipients
9283 Characterization of the receptor for transferrin on T lymphocytes and other cells using monoclonal antibodies and other techniques
9322 Activity, characterization and other studies of human suppressor T-cell subsets

Figure 5 is a historiograph documenting the progress of research and development in monoclonal antibodies between 1976 and 1983. Shifts in research emphasis resulting from new discoveries may cause the flowchart to branch, as in the 1976 messenger RNA front.

In a recent issue of *CC*, I described the *ISI Atlas of Science: Biotechnology and Molecular Genetics, 1981/82*® .[32] Among the 127 chapters in this work, 2 are devoted to topics in which the work of Jerne, Milstein, and Köhler figure prominently.

Figure 5: Historiograph tracing research leading to the monoclonal antibody technique. Numbers at the bottom of each box refer to the nu research front. One asterisk (*) indicates research fronts in which Milstein is an author of a core paper; two asterisks (**) indicate resear Köhler are authors of core papers.



The experienced reader may not need to be told how rapidly the field of immunology has grown in the past dozen years. From the basic research on immunity to infectious diseases, we now realize that the immune system encompasses many other biological topics including genetics, transplantation, cancer, and cell differentiation. But our maps do point to the primordial years when modern immunology advanced as a result of a few unifying principles and techniques. These form the background for an entirely new set of disciplines. I've simply tried to give you another perspective on the contributions of Jerne, Milstein, and Köhler to this remarkable chapter in modern research.

* * * * * *

My thanks to Cecelia Fiscus and Lisa Holland for their help in the preparation of this essay.

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