Immunology: Role of β_2-Microglobulin

β_2-Microglobulin may be a small protein, but its potential impact on immunological research is large. Of special interest is the possibility that it is a link between two major areas of immunological research, that on antibodies or immunoglobulins and that on histocompatibility antigens. These are the antigens present on cell surfaces that elicit transplant rejections. This possibility is supported by recent demonstrations that the amino acid sequence of human β_2-microglobulin closely resembles that of regions of the immunoglobulin G (IgG) molecule and that β_2-microglobulin is a component of histocompatibility antigens.

β_2-Microglobulin occurs in small quantities in the urines and serums of normal individuals, and, in larger amounts, in the urines of individuals with certain kinds of kidney damage. Ingemar Berggård of the University of Lund, Sweden, and A. G. Bearn of Cornell University Medical College, New York, isolated the protein from human urine and determined that it consists of a single polypeptide chain of about 100 amino acids. The molecule has a molecular weight of 11,600 and one disulfide bond.

The small size of β_2-microglobulin has made it a suitable candidate for determining the sequence of the amino acid residues that make up the peptide. According to M. D. Poulik of Wayne State University, Detroit, Michigan, and Oliver Smithies of the University of Wisconsin, Madison, the sequence of 44 of the first 46 amino acid residues (residues 31 and 45 were not identified) was similar to that of portions of the IgG molecule.

Immunoglobulin G consists of two identical heavy chains (molecular weight of about 50,000) and two identical light chains (molecular weight of about 20,000). Each of these chains contains a variable and a constant region (Fig. 1). The variable regions confer specificity on the antibody molecule and constitute the sites that combine with the corresponding antigen. The constant region of the heavy chains can itself be subdivided into three regions, each of which displays considerable homology (similarity in the sequences of amino acid residues) with the other two. Each of these regions consists of approximately 100 amino acids and has one disulfide bond —just as does β_2-microglobulin.

Bruce Cunningham and Per Peterson, working in Gerald Edelman's laboratory at Rockefeller University, and Berggård determined the complete sequence of β_2-microglobulin, confirmed the partial sequence proposed by Poulik and Smithies, and identified the remaining 56 amino acids. The Rockefeller group compared the sequence of β_2-microglobulin with the various regions of IgG. They found that amino acids identical to those in β_2-microglobulin occupied 21 to 28 of the corresponding positions in the sequences of the constant regions of IgG.

The two groups have proposed different evolutionary mechanisms to account for these homologies. Smithies and Poulik have suggested that the gene for β_2-microglobulin may have evolved from an ancestor of the present-day IgG genes, possibly as a result of a mutation that led to a large deletion of DNA from the ancestor gene. In contrast, Edelman and his colleagues have hypothesized that the gene specifying β_2-microglobulin evolved from a precursor gene before that precursor underwent the duplication that immunologists think gave rise to the complex immunoglobulin molecules. Independent mutations in the linked duplicated genes thus produced the different immunoglobulin regions.

Demonstration of the homology between β_2-microglobulin and IgG stimulated further investigations into the source and function of this protein. Investigators in a number of laboratories have identified a β_2-microglobulin

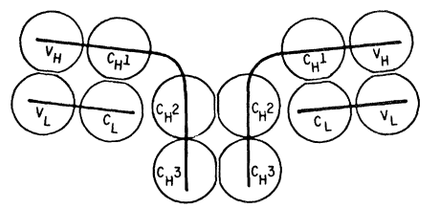

Fig. 1. Schematic diagram of the IgG molecule. The variable and constant regions of the light chain are represented by V_L and C_L, respectively. The variable and constant regions of the heavy chain are represented by V_H and C_H1, C_H2, and C_H3, respectively. [Source: Gerald Edelman, Rockefeller University]

in several additional species, including the dog, mouse, and rat. Moreover, β_2-microglobulin appears to be located on the surfaces of all mammalian cells, including lymphocytes. According to Poulik, both T cells (thymus-derived cells that function in cell-mediated immunity) and B cells (bone marrow–derived cells that secrete antibodies) carry the protein.

β_2-Microglobulin has a widespread distribution as do the histocompatibility antigens. Investigations in at least two laboratories, those of David Pressman at Roswell Park Memorial Institute, Buffalo, New York, and Jack Strominger at Harvard University, Cambridge, Massachusetts, indicate that histocompatibility antigens, which are glycoproteins, consist of two components. The larger component carries the antigenic specificity; its molecular weight is about 45,000. The smaller component appears to be common to histocompatibility antigens of all specificities; it has a molecular weight of about 11,000 to 12,000.

The small component has now been identified as β_2-microglobulin. Pressman and his colleagues compared the properties, including amino acid composition, molecular weight, and migration in an electric field, of the two materials and found that they were the same. Pressman, in collaboration with Poulik, and Ettore Appella, of the National Cancer Institute, Bethesda, Maryland, also showed that the two molecules were indistinguishable immunologically. Moreover, the sequence of the first 24 amino acids of the histocompatibility antigen component was identical with that of β_2-microglobulin.

These analyses were performed on material that had been extracted from cells. Thus, the association between β_2-microglobulin and the larger histocompatibility antigen component could have been an artifact of the isolation procedure and not representative of the situation in the intact membrane. Evidence derived by Poulik, in collaboration with R. Ceppellini of the Basel Institute of Immunology, Switzerland, and by Peterson, now at the University of Uppsala, Sweden, shows that β_2-microglobulin is in fact associated with histocompatibility antigens on the cell surface.

One approach to this problem, which was used in both laboratories, depends on the formation of "caps" when cells are exposed to antibodies against membrane components. The antibodies, usually labeled with a fluorescent molecule, bind to antigens on the membrane. The antibody-antigen complexes coalesce to form caps on the cell surface. When cells were exposed simultaneously to antibodies against β_2-microglobulin, labeled with one fluorescent molecule, and antibodies to histocompatibility antigens, labeled with a different one, the caps that formed contained both antibodies.

The role of β_2-microglobulin and, for that matter, of the histocompatibility antigens is uncertain. Because of the resemblance of β_2-microglobulin to segments of the IgG molecule and its location on lymphocyte surfaces, some investigators have hypothesized that it functions in such aspects of immune responses as recognition of antigens and interactions between T and B lymphocytes.

Poulik, with Marilyn Bach, of the University of Wisconsin, Madison, found that antibodies against β_2-microglobulin can block some in vitro responses of lymphocytes (probably T lymphocytes). The responses are thought to depend on recognition of antigens by receptors on the cell surface. Poulik warns that such data must be interpreted with caution. In other assays, the antibodies did not interfere with lymphocyte activity. The identity of T cell receptors for antigens is now a subject of considerable controversy.

A current hypothesis about the function of histocompatibility antigens involves the possibility that they may be involved in genetic control of immune responses. Most of the immune response genes—those that control an animal's capacity to respond to a given antigen—are located in the same chromosomal segments as the genes that specify histocompatibility antigens. Some immunologists think that the immune response genes and the histocompatibility genes may be identical. They have also speculated that the products of the immune response genes are T cell receptors that function in antigen recognition and cooperation between T and B cells.

Thus, β_2-microglobulin is associated with the histocompatibility antigens. Its structure implies both a common evolutionary origin for immunoglobulins and histocompatibility antigens and also an immunological function for the molecule. Since β_2-microglobulin and the histocompatibility antigens are not restricted to cells of the immune system, they may have additional, although still unknown, functions.

Edelman and J. A. Gally, who is at Meharry Medical School, Nashville, Tennessee, have suggested that the immune system, with its specialized function, has evolved from the more primitive, generalized system of the histocompatibility antigens. If the larger histocompatibility component has an amino acid sequence homologous to that of the immunoglobulins, this will lend additional support to their hypothesis.

The chromosomal location of the gene for β_2-microglobulin is not yet known, but this critical experiment is undoubtedly being vigorously pursued. If the gene is found in the same chromosomal region as the immune response and histocompatibility genes, β_2-microglobulin may be an important clue to the solution of a number of major problems in immunology.

—JEAN L. MARX

Riemann Hypotheses: Elusive Zeros of the Zeta Functions

For more than a century, mathematicians have tried to resolve the classical Riemann "hypothesis"—a conjecture that, if true, would provide information about the distribution of the prime numbers. Attempts to solve this problem have led to generalizations of it and to the advancement of conjectures that resemble, but are distinct from, the original one. Now, although the classical Riemann hypothesis remains unresolved, a class of similar conjectures has been proved true by P. Deligne of Institut des Hautes Etudes Scientifiques in Paris in work that draws on 50 years of research by others in several fields of mathematics. This proof and recent progress toward solutions to problems that are related to the classical Riemann hypothesis have led many to believe that a resolution of the classical conjecture may be forthcoming during this century.

Since all natural numbers which are not primes can be broken down into unique products of primes, describing the distribution of primes is a fundamental problem in number theory. Describing how prime numbers occur in the number system is related to describing the behavior of a function [the Riemann zeta function, $\zeta(s)$*] which is defined for complex numbers. The function is zero when it is evaluated at certain points that lie in the critical strip, an infinite vertical strip in the complex plane (Fig. 1). Answers to many of the outstanding problems in number theory hinge on the location of those points in the critical strip.

Riemann conjectured that all zeros of the zeta function [solutions to the equation $\zeta(s) = 0$] which are in the critical strip lie on the vertical line that divides that strip in half (the critical line). This conjecture is known as the classical Riemann hypothesis. If it is true, knowledge of how the primes occur will be greatly extended. It has long been known that a function, Li(x),† provides a good approximation to the number of primes in the interval from 0 to x. If the classical Riemann hypothesis is correct, then the error term associated with that approximation would be small. In addition, information on the size of the gaps between the primes could be obtained.

The classical Riemann hypothesis resembles other kinds of conjectures known as the Riemann "hypotheses" for algebraic varieties. Like the classical hypothesis, these concern the location of zeros of zeta functions. The zeta functions associated with these analogous proposals have properties similar to those of the zeta function associated with classical hypothesis. However, they are more easily analyzed than the classical function. Nonetheless, a new field of mathematics had to be developed before Deligne could finally resolve the most general of these hypotheses.

Algebraic varieties are defined by polynomial equations (such as $x^3 + y^2 + 1 = 0$). They are associated with zeta functions which can be described

* $\zeta(s) = \sum_{n=1}^{\infty} n^{-s}$ for the real part of s greater than 1 and is analytically continued for all complex $s \neq 1$.

† $\mathrm{Li}(x) = \int_2^x \frac{dy}{\ln y}$