The Role of Three-Dimensional Structures in Understanding MHC Restricted Antigen Presentation

Don C. Wiley

The Japan Prize of 1999 cites four scientific accomplishments from my research laboratory: the determination of the three dimensional structures of both Class I and Class II Major Histocompatability Glycoproteins; and, the discovery of how peptide antigens bind to both of those molecules. That research spans a period of about 15 years, from about 1979 to 1994 and involved a number of individuals other than myself. In fact scientific research is usually the combined efforts of a number of individuals, somewhat like the music produced by an orchestra. My role as leader of the laboratory may be thought of as that of an orchestra conductor.

The first and most celebrated scientific result in this series occurred in 1987 when the three-dimensional structure of the human class I MHC molecule was determined by X-ray crystallography at Harvard. This was primarily the work of a graduate student and later postdoctoral fellow in the laboratory, Pamela Bjorkman, over a period of many years. In the final year of the work Mark Saper joined and made a significant contribution as a postdoctoral fellow. The structure was extremely exciting, immediately suggesting answers to long-standing puzzles in the field of cellular immunology. It showed, although only indistinctly, how peptide antigens could be presented by class I MHC molecules on the surface of human cells to be recognized by the receptors on T-killer cells of the human immune system. The structure has been used as the framework for countless experiments in immunology often permitting more precise and informative inquiries. At the clinical level it has been useful in the design of candidate vaccines for infectious agents and for tumors.

The second discovery, showing in atomic detail how class I MHC molecules bound peptide antigens was made by Dean Madden, a graduate student in my laboratory, in collaboration with Joan Gorga, a postdoctoral fellow in Jack Strominger's laboratory, and Ted Jardetzky a postdoctoral fellow in my laboratory. Joan purified and crystallized HLA-B27 a class I molecule associated with susceptibility to autoimmune diseases in humans. Madden and Gorga determined its three dimensional structure, using the earlier results as a starting point. Ted Jardetzky supplied key data by purifying and sequencing a number of the peptides that were bound to the MHC molecule. The combination of a more distinct image of the bound antigens and the knowledge of the amino acid sequence of the bound peptides allowed construction of an atomic model of the interactions between the peptides and MHC molecules. This addressed the mystery of how one MHC molecule could bind very tightly to thousands of different peptide antigens, when the presence of interactions between parts of the peptides and MHC antigens conserved in all peptides (backbone atoms) and MHC molecules (non-polymorphic residues) were described. It also described in atomic detail, how one specific MHC molecule selectively bound specific classes of peptide antigens using polymorphic pockets described earlier by Saper, Tom Garrett, and Bjorkman at Harvard.

The third accomplishment cited was the determination of the three dimensional structure of a class II MHC molecule. These molecules are found on specialized immune cells and present peptides from antigens found outside of cells but brought into cells for presentation to T-helper cells as part of the control and regulation of the cellular immune response. In humans

different alleles of class II molecules are associated with increased susceptibility to a number of autoimmune diseases such as Rheumatoid Arthritis, Multiple Sclerosis, and Insulin Dependent Diabetes. This work was started by Joan Gorga in Jack Strominger's laboratory, who discovered how to proteolytically dissect a human class II molecule, HLA-DR1, off the surface of a human cell and purify it in a form that would crystallize. Gorga and a graduate student, Jerry Brown, in my laboratory began the X-ray structure determination, which was quite difficult until Ted Jardetzky discovered a new crystal form using their HLA-DR1. Larry Stern, another postdoctoral fellow in my laboratory, expressed HLA-DR1 in insect cells and found yet another crystal. Led by Brown and Gorga, these four collaborated to solve the three dimensional structure in 1993.

Larry Stern led the continuing collaboration with Jardetzky, Gorga, and Brown to determine how class II molecules bound peptide antigens by loading a single peptide antigen from the haemagglutinin glycoprotein of influenza virus onto "empty" HLA-DR1 that he had produced in insect cells (that lacked MHC molecules and the peptide presentation system of the vertebrate immune system). The X-ray structure they produced showed in atomic detail what HLA-DR1 looked like when presenting the Flu peptide, just as though we had been looking at the surface of a cell in a person with an influenza infection. The interactions between peptide antigens and class II molecules are different than those seen in class I molecules. The interactions discovered by Stern and colleagues have subsequently been found in all other class II/peptide complexes including those from HLA-DR2 and HLA-DR4, which contribute to increased susceptibility to Multiple Sclerosis and Rheumatoid arthritis.

So far I have named 8 scientists whose research was critical to the discoveries cited above. A number of other members of my laboratory worked on these projects and they are listed in the bibliography cited wiht the award (http://www1.mesh.ne.jp/jstf/). A difference in the metaphor of an orchestra with a research group is that the scientists in an academic research group like mine at a University are all in training; some were graduate students receiving Ph. D. degrees for participation in the research outlined and others were recent degree recipients received postdoctoral training. The research results appeared incrementally, as the result of the sequential efforts of small groups of scientists, some of whom had left the laboratory by the time others arrived, in a continuous process of renewal.

MHC proteins and human diseases: A tale of recognition in two immune systems

Jack L. Strominger

MHC proteins (in humans also called HLA proteins) are polymorphic, heterodimeric proteins encoded in the major histocompatibility complex (MHC) of all vertebrate species examined. They were originally called transplantation antigens by Peter Gorer who named them during his studies of graft rejection in the 1930s. The isolation and separation of the two classes of human MHC proteins, description of their primary and secondary structures and their domain organization, and separation of three isotypes in each class led, in collaboration with Don Wiley, to their crystallization and, finally, to elucidation of their three dimensional structures, including that of bound peptides in the "MHC grooves." Thus a detailed description of the molecular interactions involved in immune recognition, i.e., in the initiation of an immune response became available (along with the knowledge that their role in graft rejection was a by-product of their normal role in the immune response). Soon an understanding of the different roles that these MHC proteins play in their interactions with two distinct effector systems, that mediated by the effector T lymphocytes and that mediated by Natural Killer lymphocytes became evident. Not surprisingly for such highly precise systems, each of them is also involved in important human diseases.

The class I HLA-A and -B proteins and class II HLA-DR and -DQ proteins, at least, present foreign peptides for recognition by receptors on the T lymphocytes leading to effector functions such as cytolysis of infected cells or T cell help for antibody formation. Exquisite mechanisms have evolved that result in tolerance to self peptides that are also presented by the MHC proteins while permitting recognition of foreign peptides. Breakdown in the mechanisms of self tolerance leads to autoimmune diseases, i.e., the aberrant recognition of self peptides. The molecular understanding gained from our studies has allowed the precise definition of the self peptides recognized in these aberrant interactions in several autoimmune diseases (as well as of the foreign peptides involved in a normal immune response) and has also permitted rational therapeutic approaches. Examples of the application of these advances to develop approaches to therapy of autoimmune diseases will be described.

Natural Killer cells are the reciprocal of T cells. Peripheral T cells are normally inactive and are *activated* (for proliferation, cytokine release and cytolytic activity) by the recognition of specific MHC/peptide complexes. By contrast, Natural Killer cells are normally active and are *inhibited* (inactivated) by the recognition of class I MHC proteins, particularly HLA-C and HLA-E. One role of Natural Killer cells is to eliminate cells that have lost expression of class I MHC proteins. Class I MHC proteins are absent from cells in at least three circumstances:

1. The fetal extravillous cytotrophoblast forms the fetal- maternal interface. This cell layer of the placenta does not express the normal class I MHC proteins, a physiological regulation which must have evolved to prevent recognition by maternal effector T cells of paternal MHC proteins expressed on nearly all other fetal tissues. A special mechanism that utilizes a novel class I MHC protein (HLA-G) expressed only on the cytotrophoblast evolved to protect the fetus from attack by maternal Natural Killer cells.
2. Some tumor cells, for example, some colon carcinoma and melanoma cells, have also lost expression of class I MHC proteins, and should, therefore, be targets for lysis by Natural Killer cells. However, means of avoidance of this

recognition event have also evolved.
3. Viruses have evolved a variety of mechanisms to down-regulate expression of class I MHC proteins as a means of escape from immunosurveillance. A complex interplay between the virus and the host immune defenses involving both T cells and Natural Killer cells has resulted.
Examples of each of these three phenomena will be described.

The description of MHC molecules and their complexes with self and foreign peptide and their interaction with the two types of effector cells of the immune system has thus revealed many aspects of their normal and abnormal functioning. The molecular knowledge gained may in the future permit many advances in understanding aberrant conditions that result from abnormalities in this exquisitely tuned system.

But the problems before us now and in the future are not so much scientific or medical as they are social and economic. In order to reap the benefits of these advances we must learn to live together and to appreciate and treasure the differences among us and to also treasure and preserve the resources of this beautiful planet for ourselves in the present as well as for future generations. Perhaps we have something to learn from the Japanese people who now live in relative peace and prosperity on these crowded islands, so that in the spirit of the Prize established by the Science and Technology Foundation of Japan with the endorsement of the Japanese government, we may "further world peace and prosperity and thereby make a vital contribution to the positive development of mankind".