Indian J Physiol Pharmacol 2001; 45(4) : 389–394

Guest Editorial

EMIL VON BEHRING AND THE LAST HUNDRED YEARS OF IMMUNOLOGY

It was exactly a century ago that the first Nobel Prize for medicine or physiology was awarded to Emil von Behring "for his work on serum therapy, especially its application against diphtheria, by which he opened a new road in the domain of medical science and thereby placing in the hands of the physician a victorious weapon against illness and deaths" (1). After his demonstration that artificial immunity could be passively transferred through serum, we have come a long way in understanding the immunity to a point where we are now able to manipulate the immune responses at cellular, molecular and genetic levels to our advantage.

About a hundred years before von Behring's discovery in 1890, Edward Jenner had already shown the protective effect of vaccinia against small pox. Despite the fact that Jenner's discovery almost led to the eradication of small pox, no significant advances were made regarding the cause and the nature of the infectious diseases. The discovery of microorganisms as the cause of putrefaction by Schwann and works of Louis Pasteur and Robert Koch, giving rise to the germ theory of disease, took us beyond where Jenner had left. It was during this period that von Behring came into the picture. While working first with Koch at the Institute of Hygiene, and later along with Koch and Pasteur at the Institute of Infectious Diseases, both in Germany, von Behring gave the cure for diphtheria.

During the years 1888–1890, E. Roux and A. Yersin, working at the Pasteur Institute in Paris, had shown that bacilli-free filtrates of diphtheria cultures contained a substance (toxin) that produced, when injected into animals, all the symptoms of diphtheria. In 1890, from cultures of diphtheria bacilli, L. Brieger and C. Fraenkel prepared a toxic substance (toxalbumin), which when injected in suitable doses into guinea-pigs, immunized these animals against diphtheria.

Starting from his observations on the action of iodoform (antitoxic but not antimicrobial), von Behring tried to find whether disinfection of the living organisms might be achieved if animals were injected with material that had been treated with various disinfectants. These experiments were performed with diphtheria and with tetanus bacilli. This led to the development of a new kind of therapy for these two diseases. In 1890 von

Behring and S. Kitasato published their discovery that graduated doses of sterilised broth cultures of diphtheria or of tetanus bacilli caused the animals to produce in their blood certain substances, antitoxins, which could neutralize the toxins produced by the bacilli. They also showed that the antitoxins thus produced by one animal could immunize another animal and that it could cure an animal suffering from diphtheria. This great discovery was soon confirmed and successfully used by other workers.

In 1898, von Behring and F. Wernicke found that immunity to diphtheria could be produced by injecting diphtheria toxin, neutralized by diphtheria antitoxin, into animals. This provided a new way to counter diphtheria by preventing it rather than treating it. In 1907 T. Smith suggested that such toxin-antitoxin mixtures could be used to immunize man against this disease.

Emil von Behring's discovery of serum therapy, as it was then known, gave the world a potential weapon to prevent and treat the infections. In the words of Paul Ehrlich, "...this remarkable discovery seemed at one stroke to open up an entirely new and extremely promising prospect of immunising mankind against the majority of the infectious diseases..." (2). It was, however, disappointing to find that serum therapy was not a panacea because it did not work against tuberculosis, leprosy, syphilis and some other important groups of disease produced by gram-positive bacteria, let alone the large number of newly described diseases caused by viruses and parasites. Paul Ehrilich was intrigued by the failure of serum therapy in other diseases. He remarked, "Better success was only to be hoped for when, by an accurate knowledge of the theoretical considerations underlying the question of immunity, explanations of the previous ill-success were forthcoming" (2).

While von Behring and his colleagues were indulging with the serum and blood borne immunologically active principles, 'the humoral factors', Elie Metchnikoff working along with Pasteur at the Pasteur Institute had other notions. He had observed, under the microscope, the phagocytic activity of water *Daphnia* and macrophages. Around the same time, pathologists had observed the presence of multinuclear phagocytic cells in the regions of inflammation. On the basis of these findings, Metchnikoff proposed that it was the cellular components rather than the humoral components, that were responsible for protecting the body against the foreign invaders (3). By the turn of 20th century, there was a sharp divide between those who propounded the cellular basis and those who promulgated the humoral basis of immunity. It is, however, noteworthy that a possibility of both co-existing and working in tandem was generally not considered. Most of the scientific evidence at that time seemed to support the humoral view. Theoretical considerations followed the objective observations. In 1900, in his classical Croonian Lecture, Paul Ehrlich expounded the side-chain theory to explain the mechanisms of humoral immunity. He postulated the presence of antibody (a term then used to encompass anything that could neutralize toxins, the antigens) on the cell surface and proposed that the attachment of antigen to the antibody would lead to increased production of antibodies by that

cell. He postulated that interaction between antibody and antigen is specific, guided by the principle of stereo-chemical nature of both, and he invoked the lock and key analogy to explain his model. This theory rested on the presence of pre-existing endogenous antibodies in an organism. However, at that time it was not clear how such a great diversity of antibodies, even against those antigens to which animal has not been exposed, was produced. As a result, the side-chain theory was rejected.

After the rejection of side-chain theory, in order to explain the diversities of the antibodies that could be produced in the animal, new theories were put forth. These theories were advanced by biochemists and were quite Lamarckian in nature. The first of these theories was offered by Felix Haurowitz in 1930 when he proposed the template theory (or so-called instruction theory) of antibody production and it was further improved by Linus Pauling in 1940. It was proposed that antibodies were synthesized by complementary folding of a nascent protein over the antigen. Thus antigen contained all the information (or instructions) necessary for production of antibody. It essentially explained how a great diversity of antibodies is produced. The instruction theories could not, however, explain why second exposure led to an increase in antibody production or why antibodies exist even when there has been no exposure to the antigen at all. They also failed to explain newer data showing that antibodies change qualitatively with repeated exposure, sometimes with sharpening of specificity and sometimes with broadening of specificity.

In 1941, in order to overcome the biological shortcomings of the instruction theories, virologist Macfarlane Burnet, proposed an alternative hypothesis. Around this time enzymes were recognized to be important in digestion and synthesis. So he suggested that antigens stimulate an adaptive modification in the enzymes necessary for the synthesis of antibody so that unique molecules could be created. He also suggested that once this happened, the daughter cells of such a cell containing modified enzymes could lead to increased production of antibodies. Here was significant shift back to the role of cell and its role in immunity and antibody production. Equipped with increasing data on nucleic acids, Burnet and Fenner proposed that antigen might influence the genome leading to the production of an indirect template (which may now be considered RNA) for the production of antibody. However, at that time the molecular structure of genes was not known.

Research between 1920's and 1950's produced rich dividends as far as the chemical nature of antigen and antibodies and the precision of their interactions are concerned. However, the instruction theories could not satisfy a flurry of questions raised by the biologists as mentioned earlier. Paradoxically many of these questions were raised by Burnet, who himself had provided modifications of instruction theories.

It is notable that there were reports available in the literature about the role of blood cells (lymphocytes) in immunity from 1920's to 1940's, but they were largely kept

out of the theories of immunity. Karl Landsteiner, based on his experiments regarding passive transfer of immunity against simple chemical compounds and tuberculin, proposed a greater role for cells in immunity. A decade after Landsteiner's observation, N. A. Mitchison, while working on allogenic tumours, concluded that transplantation immunity shares with immunity to simple chemical compounds and tuberculin, the property of being transferred with greater facility by cells than the serum. Cellular immunity thus became an accepted and rapidly developing field of study, complementing the study of the antibodies as mediators of the multifarious responses of the immune system. It also marked the beginning of cellular school of transplantation immunology. New techniques and technologies eventually developed or were in the process of developing. Immunofluoresence staining and hemolytic plaque assay permitted tissue localization and quantitative enumeration of antibody-forming cells. Techniques of passive cell transfer and cell culture were established allowing the analysis of cell-cell interaction and immunocyte dynamics. In 1953, J. D. Watson and F. H. C. Crick proposed the two-chain helical structure of DNA. By 1950's, the stage was set for another conceptual change. The change was brought by Nilse Jerne and Macfarlane Burnet.

The first of purely biological selection theory of antibody formation was outlined by Nilse Jerne working under Delbruke (who favoured the template theory) in 1955, and he called it "natural selection" theory. Jerne proposed, as Paul Ehrlich had proposed six decades before him, that the host possessed an entire repertoire of antibodies in small quantities which were present in the blood as "natural antibody". These antibodies reacted specifically with the appropriate antigens and transported them to specific places in body where the antibody could signal the production of molecules identical to itself. This provided an explanation for the booster antibody response to second exposure to the antigen, which is much more intense than the response to the first exposure. More importantly, it gave for the first time an explanation for the phenomenon of immunological tolerance. It was proposed that antibodies that were against the self-antigens were removed from the repertoire early in the development so that later on the response could not be initiated.

Jerne's theory was followed by clonal selection theory of Burnet, Talmadge and Lederberg in 1959. Central to this concept was the postulate that antibodies are natural products that appear on the cell surface as receptors with which antigens react selectively. Reaction with antigen leads to clonal proliferation of these specific cells with some of these cells differentiating into antibody producing cells and others remaining dormant as memory cells. The memory cells participate in booster response to subsequent antigen challenge. The genetic basis of this diversity was still not given and in effect the theory was similar to the one that Paul Ehrlich had proposed.

In the 1960s Porter and Edelman enzymatically digested antibodies and detailed the physico-chemical structure of antibodies. By 1960, the role of thymus and bursa was recognized. Once it was

established that thymus and bursa had different roles, intense investigation into their roles led to the discovery of T and B lymphocytes.

In 1966, H. N. Claman, E. A. Chaperon and R. F. Triplett conducted a series of experiments in which they transferred thymus and/or bone marrow cells from normal mice into irradiated syngeneic mice and then stimulated them with sheep red blood cells. They found that antibody generation was higher when cells from both the sources were transferred simultaneously as compared to when cells transferred were from either source alone. They cautiously concluded that the effector cell is bone marrow-derived and that the thymus provides the auxillary cell. In 1968, Miller and Mitchell went further and conducted experiments with thoracic duct lymphocytes, thymus cells and bone marrow cells, and showed that thymus cells were absolutely essential for the production of antibodies by the bone marrow cells. By 1969, there was enough evidence to enable Ivan Roitt to propose a unified hypothesis according to which thymus dependent lymphocytes were labeled as T cells and thymus independent (bursa or bursa equivalent) cells were named as B cells. In 1969, M. C. Raff identified a cell surface marker (theta) on the T lymphocytes, and thereafter it became possible to distinguish between T and B lymphocytes on morphologic as well as functional grounds.

With advances in molecular biology, it became possible to identify other proteins on the T cells surface and allowed H. Canter and E. A. Boyse to distinguish two subpopulations of T cells on the basis of distinct cell surface markers. This was the foundation for the CD4 and CD8 classification. By the end of 1960s, the terms T and B lymphocytes were in common use and soon the different subsets of the immunoglobulins were discovered.

After the general acceptance of clonal selection theory, attention was diverted to how such a large range of antibodies could be produced. By now genetic control of protein structure, mechanisms and control of protein synthesis, and the amino-acid sequence of antibodies were known. Once again there were two groups; one thought that the entire repertoire was individually coded, and the other group favored somatic mutation or recombination of highly restricted number of germ-line genes. The resolution of this debate, provided by Susumu Tonegawa, is one of the triumphs of 20th century cellular and molecular biology. He proved that this vast repertoire is a result of variable combination of a number of minigene segments, assisted by mutations, to form the large pool of antibody light and heavy chains. In a pioneering study published in 1976, Tonegawa showed through a series of ingenious experiments that parts of the genome are distributed in course of its differentiation from an embryonic cell to an antibody producing B lymphocyte.

In the 1970s Rolf Zinkernagel and Peter Doherty elucidated the mechanisms by which the immune system recognizes virus-infected cells. They demonstrated conclusively the requirement for the cellular immune system to recognize simultaneously both 'foreign molecules' and 'self-molecules' (major histocompatibility antigens). What

also became obvious was the important function of the major histocompatibility antigens (in man called HLA-antigens) in the individual's normal immune response. Their discovery laid the foundation for an understanding of general mechanisms used by the cellular immune system to recognize both foreign microorganisms and self molecules and led to the understanding of the mechanisms of antigen presentation by the antigen presenting cells.

In the last three decades molecular biology has revolutionized the knowledge about antigens present on the surface of immune cells, and about various new molecules serving as intracellular messenger. The list of cell surface markers and interleukins has grown rapidly and is still growing. This enlarging list itself is leading to new questions and is demanding new theories to be put forth regarding the way our immune system works in relation to other systems of the body.

The baton of immunological sciences passed from the early biologists (late 19th century and 1st decade of 20th century) to the biochemists (2nd to 4th decades of 20th century), then back to the biologists (5th and 6th decade of 20th century) and finally to the molecular biologists during the last 4 decades of 20th century. With the wealth of knowledge and tools of cellular and molecular biology, it is time for biologists to solve the jigsaw puzzle and give meaning to the facts that are known.

Emil von Behring holds a special place in the growth of immunology as a discipline. First he showed that immunity against diseases could be actively produced, and later that this immunity could be transferred through serum, opening up immense therapeutic possibilities. From 1901 onwards Behring's health prevented him from giving regular lectures and he devoted himself chiefly to the study of tuberculosis. To facilitate his work he built well-equipped laboratories at Marburg. In 1914 he himself founded, also in Marburg, the Behringwerke for the manufacture of and experimentation with sera and vaccines. His association with the production of sera and vaccines made him financially prosperous. Von Behring himself saw in his production of this toxin-antitoxin mixture the possibility of the final eradication of diphtheria and he regarded this part of his efforts as the crowning success of his life's work. He died in 1917 at the age of 64.

ASHOK KUMAR JARYAL

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi – 110 029

REFERENCES

1. Nobel-e-Museum. http://nobel.sdsc.edu/medicine.
2. Ehrlich P. Croonian Lecture: Side-chain theory. *Proceedings of the Royal Society (London)* March 22 1900; 66: 424–448.
3. Editorial. Elie Metchnikoff (1845–1916). *Indian J Physiol Pharmacol* 1995; 39: 175–176.