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Guest Editorial

IMMUNOLOGY AND NOBEL PRIZE : A LOVE STORY

Several breakthroughs revealing the way in which our bodies protect us against microscopic threats of almost any description have been duly acknowledged by the Nobel Prizes in Physiology or Medicine. Interestingly, Nobel Prizes in Physiology or Medicine including the latest one, for the year 2011, has been awarded for twelve times to the field of Immunology.

The story began in 1901 with the very first Nobel Prize in Physiology or Medicine - it was awarded to ***Emil Von Behring*** for his pioneering work which resulted in the discovery of antitoxins, later termed as antibodies. Working with Shibasaburo Kitasato, Von Behring found that when animals were injected with tiny doses of weakened forms of tetanus or diphtheria bacteria, their blood extracts contained chemicals released in response, which rendered the pathogens' toxins harmless. Naming these chemical agents 'antitoxins', Von Behring and Erich Wernicke showed that transferring antitoxin-containing blood serum into animals infected with the fully virulent versions of diphtheria bacteria cured the recipients of any symptoms, and prevented death. This was found to be true for humans also; and thus Von Behring's method of treatment – passive serum therapy – became an essential remedy for diphtheria, saving many thousands of lives every year.

Shortly after this, the very first explanation about the mechanisms of immune system's functioning was proposed which paved way for extensive research in immunology till today. ***Paul Ehrlich*** had hit upon the key concept of how antibodies seek and neutralize the toxic actions of bacteria, while ***Ilya Mechnikov*** had discovered that certain body cells could destroy pathogens by simply engulfing or "eating" them. Ehrlich imagined the cell to be surrounded by protruding antibody side-chains which could break off and circulate in blood stream and bind in a highly specific manner to the poisons secreted by bacteria. Mechnikov coined the term 'phagocytosis' and the special cells as 'phagocytes' which were deployed by the host to capture and destroy harmful bacteria. We now know that the incredibly complex immune system mounts attacks in both of these ways. It was entirely fitting, therefore, that Ilya Mechnikov and Paul Ehrlich shared the 1908 Nobel Prize in Physiology or Medicine in recognition of their work on immunity.

By that time it was also well recognised that the immune system is *not always* protective and there was evidence that it could damage the body. Understanding how the immune system can be prompted to behave in a self-

destructive manner led to the discovery of anaphylaxis by ***Charles Richet*** for which he received the Nobel Prize in Physiology or Medicine 1913. Charles Richet demonstrated that dogs that had received an injection of sea-anemone poison without any noticeable distress always went into shock and died quickly after receiving a weaker dose a set amount of days later. Instead of raising tolerance towards the toxin, as was generally expected, the initial dose in fact made animals highly sensitive for a set period of time to even miniscule amounts of the poison. This opened up another facet of immune systems' complexity of action and regulation.

The fact that there were other players in the immune systems' orchestra was brought to the focus by ***Jules Bordet*** who revealed that the antibodies recruit a special type of protein to deliver the lethal blow for which he received the 1919 Nobel Prize in Physiology or Medicine. Antibodies created and released into the blood stream to specifically attack bacteria required the presence of a heat-sensitive substance always present in blood, which was initially named 'alexin', and later given the more appropriate name of 'complement'. Taking advantage of the specific way in which complement proteins bind to antibodies, Bordet also developed the Complement-fixation test, which became an invaluable tool for detection of syphilis.

There were many more unresolved questions, probably attributable to immune system and the major one was the uncertainties of blood transfusion; while a few cases of blood transfusion were successful and many more were unsuccessful. This was partially solved by ***Karl Landsteiner*** who discovered that the transfusion reactions occurred when a recipient possessed natural antibodies against a donor's blood cells and proposed the existence of human blood groups. Accordingly people were classified into A, B, O or AB blood group systems based on the presence of antigens and this discovery made blood transfusions not only more safe but also more scientifically based and predictable. In recognition of this major contribution, the 1930 Nobel Prize in Physiology or Medicine was awarded to Karl Landsteiner.

There was still the intriguing question of detection and discrimination of self from foreign antigens by the immune system, and this also coincided with the longest gap between any two successive Nobel prizes in the field of immunology. ***Frank MacFarlane Burnet*** and ***Peter Medawar*** showed how the immune system selectively recognizes and destroys foreign agents but prevents any attack on cells and tissues of the host itself. Finally, the 1960 Nobel Prize in Physiology or Medicine was awarded to them for revealing how self-discrimination is learned at the biological level. Immunological tolerance is a gradual learning process throughout embryonic life, in which the immune system is exposed to some form of self-defining molecules that are present on the host's cells.

A long-standing question in the field was how an almost identical-looking collection of antibody proteins can, at the same time, have the capacity to target specifically any one of an almost infinite range of foreign agents – until their structure was solved by the recipients of 1972 Nobel Prize in Physiology or Medicine. ***Gerald Edelman*** and ***Rodney Porter***'s methods yielded

different perspectives of the general antibody structure, which were later combined to provide a more definitive image of antibody specificity and further refined the understanding of the mechanisms of the immune system.

Meanwhile the scene of action was shifting to the molecular and genetic levels and the1980 Nobel Prize in Physiology or Medicine rewarded the achievements of ***Baruj Benacerraf, Jean Dausset*** and ***George D Snell*** for the identification of Major Histocompatibility Complex (MHC). MHC proteins, collectively known as histocompatibility antigens, are unique to each individual. The role played by MHC proteins in immune functions, as well as, in organ transplantations and susceptibility to many diseases was gradually discovered over the years to the present state.

Niels K Jerne described how the immune response is exquisitely controlled, and was built on his premise that antibodies can themselves act as antigens. With the various sets of antibodies stimulating or suppressing the production of each other, he visualized the immune system as a self-regulating network that can switch itself on and off in response to a foreign invasion. ***Georges J F Köhler*** and ***César Milstein*** independently created long living cell lines that could generate large amounts of a particular antibody. Combining their technique, monoclonal antibodies could be synthesised which has led to many medical and biochemical applications. The three scientists were awarded the1984 Nobel Prize in Physiology or Medicine for their seminal contributions.

Susumu Tonegawa, the 1987 award prize winner explained how the gene components can rearrange and shuffle together to form huge number of combinations coding for millions of antibodies. Uncovering this mechanism for antibody diversity revealed that genes are not fixed but can rearrange within the life of an individual and explained how a limited genetic material is used intelligently by the immune system to produce antibodies practically to an unlimited number of different antigens.

All these days the major focus of research and development in immunology was related to humoral immunity. Developments in understanding of the cell mediated immunity was rather slow, but significant. This culminated in the Nobel Prize in Physiology or Medicine 1996 being awarded to ***Peter C. Doherty*** and ***Rolf M. Zinkernagel*** for investigating how the T killer lymphocytes kill selectively the virus infected cells. Simultaneous developments in explaining the role cellular immunity were broadening the scope of this system not only in fighting external threats, but were also explaining the threats by malignant transformations of cells and how these are dealt with by the immune system.

Three scientists won the Nobel Prize in Physiology or Medicine 2011 for their discoveries about the immune system that opened up new avenues for the treatment and prevention of infectious illnesses and cancer. American ***Bruce A Beutler*** and French scientist ***Jules A Hoffmann*** shared the award with Canadian-born ***Ralph M Steinman***. Bruce Beutler and Jules Hoffmann were cited for their discoveries in the 1990s of receptor proteins that can recognise bacteria and other microorganisms

as they enter into the body, and activate the first line of defence in the immune system, known as innate immunity. Ralph Steinman was honoured for the discovery of dendritic cells, which help regulate adaptive immunity, the next stage of the immune system's response, when the invading microorganisms are purged from the body.

Cells of the innate immune system sense host invasion by detecting structural determinants that are broadly conserved among pathogens of a given phylogenetic group. Hoffmann studied in Drosophila (fruit fly) how these structural determinants could be identified to initiate an innate immune response in the host. This insect has no adaptive immune response but is highly resistant to microbial infection. Flies infected with microbes secrete antimicrobial peptides from fat body (equivalent to mammalian liver) that limits the infection.

The receptor mediating the production of antibacterial peptides against fungal and gram positive bacterial infections is Toll protein. Toll protein is a transmembrane receptor with an extracellular domain and an intracytoplasmic region. The microbial components do not bind directly to Toll protein; instead Toll activation is mediated by a distinct proteolytic cascade due to infection resulting in formation of Speatzle. The Toll protein activation by Speatzle leads to a series of signalling cascade resulting in nuclear factor kappa B (NF kB) activation and transcription of several hundreds of genes. Prominent among these genes are those encoding the antifungal peptides, Drosomycin and Metchnikowin. Hoffmann identified that toll deficient Drosophila when infected with fungi failed to produce Drosomycin and succumbed to fungal infection. Though both fungal and gram positive bacteria act upon Toll protein, the extracellular pathways resulting in formation of Spaetzle are different. The Toll-dependent defence against gram positive bacterial infection is mediated through Peptidoglycan receptor protein SA (PGR-SA).

The response to gram negative bacterial infection involves Toll-independent mechanisms and the sensing involves structurally distinct molecules – the peptidoglycan-recognition proteins (PGRPs) and the gram-negative binding proteins (GNBPs/ beta PGRPs). This is linked to Imd pathway which results in activation of I kappa B Kinase (IKK) complex and RELISH, culminating in NF kB and transcription of genes encoding antibacterial peptides, Diptericin, Cecropins, Drosocin and Attacin resulting in defence against these bacteria. Both Toll dependent and Toll independent mechanisms ultimately converge and help in the protection against a wide variety of microbes.

While Hoffmann described innate immunity in Drosophila, Beutler studied innate immunity in mouse models. Bruce Beutler and Anthony Cerami discovered that the substance that induces cachexia and was previously named as 'cachectin' is tumour necrosis factor (TNF). TNF is a cytokine, able to induce fever and inflammation as protective response and sepsis or shock as an exaggerated response.

A prototype inducer of innate immune response is lipopolysaccharide (LPS), an endotoxin released by gram negative bacteria. Sensing LPS is very important to overcome gram negative infections and cytokines (especially TNF) produced by mononuclear phagocytes in response to LPS orchestrate the immune response. An

exaggerated response following LPS detection leads to endotoxic shock.

Interested in the mechanism by which LPS activates mammalian immune cells, Beutler used TNF production as a phenotypic endpoint to identify the LPS receptor. Macrophages were of primary importance in the recognition of LPS. Identification of the receptor hinged on the positional cloning of the mammalian LPS locus, which had been known since the 1960s as a key genetic determinant of all biological responses to LPS. In1998, Beutler's lab identified the mammalian LPS receptor as Toll- like receptor-4 (TLR-4) by genetically mapping and cloning a mutant allele form that was unresponsive to LPS in mice. In contrast to Drosophila where there is no direct contact between the Toll protein and the microbial pathogen, the situation is apparently different in mammals, in which TLR-4 is clearly in direct interface with the microbial world.

Subsequently, with efforts from many other laboratories, 12 mouse TLRs and 10 human TLRs were discovered which can detect signature molecules that herald infection (including LPS, lipopeptides, flagellin, unmethylated DNA, dsDNA, ssRNA). TLRs were thus called as gatekeepers of the most powerful inflammatory responses. Beutler thereafter continued to apply a forward genetic approach to identify the genes that are essential for the innate immune response in mammals. In this process, germ-line mutations that alter immune functions were created through a random process using the alkylating agent N-ethyl-N-nitrosourea (ENU), detected by their phenotypic effects, and then isolated by positional cloning. His work disclosed numerous essential signalling molecules required for the innate immune response and helped to delineate the biochemistry of innate immunity. The ENU mutagenesis effort now underway in the Beutler laboratory is the largest in the world, and presently the only one primarily devoted to the decipherment of innate immunity.

Ralph Steinman's discovery of dendritic cells bridged the adaptive immunity discovered by Ehrlich to innate immunity discovered by Mechnikov. His work contributed immensely to the development of vaccines and the study of autoimmunity. His interest on how the specific immune cell or lymphocyte is selected from the diverse immune system for a particular antigen led him to the discovery of dendritic cell (DC) 35 years ago and he spent the subsequent years elucidating its functions.

Lymphocytes alone were not sufficient and some accessory cells were required to produce specific immune response. This accessory cell was thought to be nothing but a macrophage initially, later on proved to be the dendritic cell by Steinman. 1974 Nobel Prize winning work of George Palade and Christian De Duve helped Steinman in identification and purification of dendritic cell. He put forward that these cells were distinct from macrophages based on the fact that they had multiple projections, had no membrane enzymes, lacked lysosomes, had poor viability, detached easily from cell cultures and lacked key receptors for phagocytosis.

His initial attempt to prove that DC is in fact a different cell with respect to its role in adaptive immune response (Mixed Leucocyte Reaction) was taken with scepticism by immunologists who believed macrophages had the key role as antigen presenting cells. In 1982, Steinman with his colleagues could localise mouse DCs with

monoclonal antibodies.

He proposed that DC captures the antigen and presents it to the T cells, which in turn expand to form Killer cells or Helper cells, also inducing B cell response. There was real breakthrough when DCs could be removed from lymphoid organs or could be generated from progenitors ex vivo and then loaded with appropriate antigen to be re-infused back into the organism for inducing antiviral or antitumor activity. This formed the basis for immunotherapy against cancer. Steinman had tried this technique on himself to fight against cancer.

The next target set by Steinman and his colleagues was to localise the DCs in the human body. These cells were found to be at interface of the body with the environment like skin, airway and other epithelia, also in lymphoid organs where DCs interact with the T cells.

Another important function of DCs discovered was that these cells in steady state capture self or harmless antigens and silence the immune system, either by clonal deletion, anergy or expanding regulatory T cells. Subsequently during infection, DCs channelize the response of T cells only against the pathogen, not on any self antigens. With infection, DCs mature and select the specific T cell from the body's pool of millions of lymphocytes. Thus the DCs were called as the conductors of immune orchestra with its multiple roles.

Inspired by Pasteur, Steinman harnessed the knowledge of DCs to develop vaccines. The vaccine trials were conducted successfully in mice for various diseases like Tuberculosis, Type I Diabetes, HIV, melanoma and allergy. The phase I clinical trials in humans are under way. This definitely has opened up a bright future in research in immunology and its clinical applications for the treatment of not only infections, but also of many non-infectious diseases with an immunological basis. It is indeed a special Nobel Prize for immunology this time but unfortunately Mr. Steinman was not alive to personally receive a richly deserved accolade.

Research in Immunology has had a rich past and present with a long standing love affair with Nobel Prizes and there can be hardly any doubt that the future would be any different, but only better.

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