Innate Immunity and the 2011 Nobel Prize

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The 2011 Nobel Prize in Medicine or Physiology was awarded to Jules Hoffmann, Bruce Beutler and Ralph Steinman for their contributions in the area of Innate Immunity. This review introduces the readers to innate immunity and links the studies of the awardees to this area of research. The cells and mechanisms involved in the innate immune response are reviewed with a special emphasis on the discovery of the Toll receptors in *Drosophila* and the Toll-like receptors in mammals. The importance of the innate response during inflammatory conditions is discussed and examples of genes related to innate immunity that cause disease are also highlighted. Finally, the roles of innate immunity in modulating adaptive immune response, especially with respect to dendritic cells, are discussed. Further studies in this area are likely to enhance our understanding of the immune response and may lead to drugs to ameliorate excessive inflammatory responses.

The immune system has evolved to protect us against a variety of pathogens that are encountered by our bodies on a daily basis. It consists of a network of interactions between various cells and molecules that keep the body free of disease by coordinating responses against pathogenic or foreign entities in order to bring about their destruction. While the innate (or in-born) non-specific immune response is observed in many less evolved multi-cellular organisms, e.g., plants and insects, the immune system in higher jawed vertebrates consists of both innate and adaptive components (*Table* 1). The innate immune system offers a quick, generic response against many invading microbes by means of the action of different cells such as neutrophils, macrophages, and dendritic cells. These cells are usually the first immune cells to encounter microbes and are capable of engulfing pathogens and processing them so as to present them as antigens to the immune cells of the

Innate Immune System	Adaptive Immune System
Forms the first line of defense.	Forms the second line of defense.
Response is non-specific.	Pathogen and antigen specific response.
Exposure leads to immediate maximal response.	Lag time exists between exposure and maximal response.
No immunological memory.	Exposure leads to immunological memory.
Found in nearly all forms of life.	Found only in jawed vertebrates.

Table 1. Differences between the innate and adaptive immune systems.

adaptive system, namely, B and T lymphocytes. The adaptive immune response, on the other hand, is delayed but displays antigen specificity. In addition, it results in the generation of memory so as to provide greater protection upon subsequent encounters with the antigen. For a detailed review of the adaptive immune response, please refer to our earlier articles [1,2]. Also, see *Box* 1 for a glossary of terms used in this review.

Components of the Innate Immune System

Most pathogens are never encountered by the immune cells because their entry is restricted by a variety of anatomical barriers, comprising of the epithelial surfaces, that constitute the first line of defence of the body. There are several mechanisms involved in this process: sweat, organic acids and microbial flora present on the skin as well as the renewal of the outer skin layer every few days. The surfactant proteins in the mucus, that lubricates the nasopharynx, are an example of the anatomical and physiological barriers that keep a large number of potential pathogens at bay. Other examples are bile salts, acidic pH in the stomach and the gut flora in the gastrointestinal tract. Additionally, the secretions from various glands (e.g., lysozyme in tears) as well as the production of anti-microbial peptides (AMPs) by skin epithelial tissue and other cells are important in innate defence mechanisms. AMPs are a class of conserved small peptide molecules such as defensins and cathelicidins, which are important in innate immunity and have diverse functions: disruption of microbial cell

Box 1. Glossary of Terms

Antigen: A substance that evokes an immune response, either humoral (e.g., antibody) or cell mediated (e.g., T cells).

Anti-microbial peptide: Small peptides secreted by epithelial and immune cells that lead to destruction of microorganisms.

APCs: Antigen presenting cells, e.g., dendritic cells, macrophages, B cells, that display peptides (self and microbe-derived) together with Major Histocompatibility Complex (MHC) encoded molecules on the cell surface.

Complement activation: A cascade-like process by which the complement molecules, upon activation, are enzymatically cleaved, resulting in the killing of the target cell.

Cytotoxic: That which kills or has the capacity to kill a cell.

Inflammation: A biological response characterized by an influx of immune cells and molecules that result in redness, warmth, swelling and pain as a result of infection, irritation, or injury.

LPS: Lipopolysaccharide, also known as endotoxin, is present in the outer membrane of Gram negative bacteria.

NFκB: Nuclear factor kappa B, a transcription factor important for immune and inflammatory responses.

Opsonization: The process of coating of an antigen with antibodies or MBL so as to target it for phagocytosis or complement mediated lysis.

PAMPs: Pathogen associated molecular patterns are molecular motifs or signature patterns conserved in groups of microbes.

Phagocytosis: The engulfment or internalization of particles or microbes by phagocytic cells, e.g. ,neutrophils, macrophages.

PRRs: Pathogen recognition receptors are protein molecules that are capable of recognizing PAMPs, e.g., TLRs.

RNI: Reactive nitrogen intermediates are nitrogen based free radical molecules, e.g., nitric oxide.

ROS: Reactive oxygen species are chemically reactive free radical molecules containing oxygen, e.g., superoxide, hydrogen peroxide.

Sepsis: A condition characterized by a systemic, exacerbated, uncontrolled inflammatory response.

membranes and inhibition of microbial metabolism, eventually resulting in death of microbes. Psoriasin, for example, is an AMP produced by skin epithelial cells that effectively kills *Escherichia coli*, but not *Staphylococcus aureus*. Thus, the first line of protection from pathogens consists of physical, chemical and biological barriers that function together to restrict or block the entry of microbes.

Cells of the Innate Immune Response

Only those microbes that breach epithelial barriers are encountered, recognized by the cells of the innate and adaptive immune system and are eventually destroyed. In *Drosophila*, haemocytes have phagocytic activity while, in mammals, neutrophils (large granulocytic blood cells that contain a multilobed nucleus) are often the first cells of the innate immune system to encounter an antigen. Their primary response is to phagocytose (engulf or eat up) and destroy the pathogen. During infections, the host produces factors that greatly increase the numbers of neutrophils in blood which is often useful for diagnosis of infections. Other innate system cells that are capable of phagocytosis and degradation of the pathogenic agent are macrophages and dendritic cells. Macrophages are present mainly in tissues while monocytes are present in the blood; they are amoeba-like and form phagolysosomes where the invading microbe is destroyed. Dendritic cells are present in the skin and on other body surfaces such as the stomach, gut, nose, and lungs, and function mainly as antigen-presenting cells. This means that, upon encountering a microbe, they phagocytose it and process and present the antigens from the engulfed microbe to other cells of the immune system so as to initiate a subsequent specific adaptive response. Another cell type that is important for innate immune functions is the natural killer cell, a type of large lymphocytic cell that has cytotoxic potential and can kill infected or transformed host cells. In fact, lack of natural killer cells in mice and humans correlates with increase in occurrence of tumours and susceptibility to some viruses.

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Phagocytosis

Once an invading microbe is encountered, a number of processes are activated by the innate immune system: phagocytosis, complement activation, inflammation, etc., (*Figure* 1). These are meant to non-specifically target the invading microbe and eliminate it from the host system. In animals, one of the initial responses to the presence of a foreign agent is engulfment or phagocytosis. The role of phagocytes in engulfing invading microbes was first

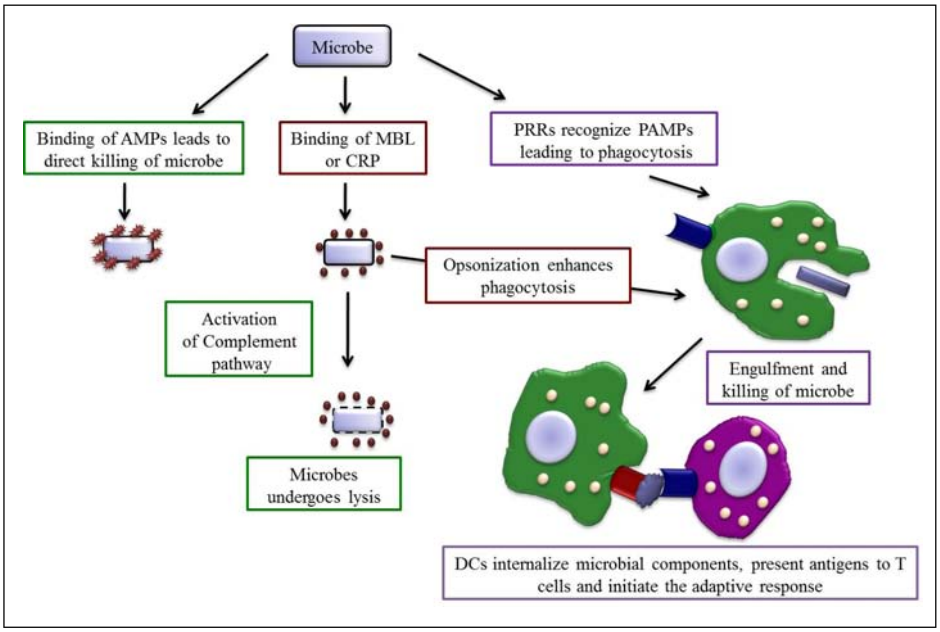
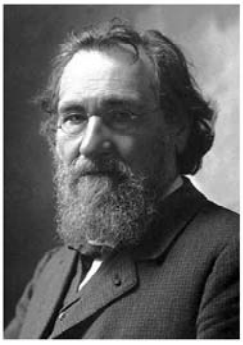


Figure 1. Different consequences upon microbial entry into the host. This involves binding to antimicrobial peptides, mannose binding lectin or C-reactive proteins, followed by opsonization, phagocytosis and complement activation. These processes result in lysis of invading microbes and dendritic cell mediated activation of the adaptive response.

Figure 2. Elie Metchnikoff studied the role of phagocytosis and was awarded the 1908 Nobel Prize in Physiology or Medicine for his studies on cellular immunity.



observed by Elie Metchnikoff, who was awarded the Nobel Prize for his contributions to cellular immunity in 1908 (*Figure* 2). Tissue macrophages, neutrophils and dendritic cells can initiate this process because they bear conserved pattern recognition receptors (PRRs) on their cell surfaces that can recognize pathogen-associated molecular patterns (PAMPs). The term pathogen used in PRRs or PAMPs can be misleading as they recognize microbes (some may be pathogens) or microbial constituents. Upon binding of the appropriate ligands (microbe or microbial component) to PRRs, signals are sent to surrounding cells, indicating the presence of an invader. These signals comprise of chemical factors such as chemokines, prostaglandins, bradykinins, leukotrienes, serotonin, etc., that initiate inflammatory signalling. In addition, these are also accompanied by a state known as acute phase response, wherein, concentrations of specific proteins in plasma are elevated due to enhanced secretions from the liver.

These include the positive regulators of the inflammatory response: C reactive protein (CRP), mannose binding lectin (MBL), coagulation factors, complement factors, cytokines such as interleukin 1 (IL 1) (α and β), IL 6, and tumour necrosis factor α (TNFα). Inflammation, characterized by swelling, redness, heat and pain, thus serves as a signal that an invading microbe is present within the host's system.

The surface of the invading microbes is coated with molecules involved in innate immunity, e.g., MBL binding to mannose residues or binding of CRP to phosphorylcholine present on microbe surfaces, which results in molecular tagging for destruction of microbes (*Figure* 1). This process, known as opsonization, increases the ability of host cells to phagocytose the tagged microbes. In fact, deficiency of MBL leads to defects in phagocytosis, which is known as 'common opsonic defect' and results in recurring infections in affected children. Also, this coating may lead to the activation of the complement pathway so as to lyse these microbes. The complement system consists of an array of about twenty globular proteins present in serum and other body fluids, that gets activated upon binding with an antibody-antigen complex (classical pathway) or in the presence of microbial components (alternate pathway) or due to presentation of opsonised entities (lectin pathway). Upon activation of their enzymatic activities in a cascade-like manner, the complement proteins together lead to the formation of membrane attack complexes that drill holes into the cell membrane and cause osmotic lysis of invading microbes.

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Along with the inflammatory and complement activation responses, upon recognition of the presence of an invading microbe, many phagocytic cells rush to the site of inflammation and bind the pathogenic agent. The phagocyte's membrane surrounds the pathogen and engulfs it within a membrane enclosed phagosome. This sub-cellular structure undergoes acidification and fuses with an organelle called the lysosome. Upon fusion, lysozyme, proteases, peptidases, hydrolases and defensins from the lysosome are released into the phagolysosome and the en-

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gulfed microbe is degraded. Some peptides derived from the degraded microbe also bind to major histocompatibility complex (MHC)-encoded class II molecules and are presented by macrophages and dendritic cells to T cells.

Free Radicals

Additionally, phagocytes have another tool in their arsenal, namely a means to generate an oxidative burst that can destroy the engulfed microbe. Upon activation, an enzyme complex, the NADPH oxidase complex, is assembled on the cell surface membrane of the phagocytes and produces superoxide. In fact, people with mutations in the genes of the NADPH oxidase complex are prone to suffer from recurrent infections, resulting in 'chronic granulomatous disease'. These patients are unable to resolve the granulomas formed due to ineffective clearance of engulfed microbes. In addition, macrophages express nitric oxide synthase 2 (Nos2) which leads to the production of nitric oxide (NO). The combination of NO and superoxide (O_2^-) produces peroxynitrite ($ONOO^-$) which is a highly effective anti-microbial agent. Phagocytes produce chemicals such as hydrogen peroxide, hydroxyl radicals, and hypochlorite that can also kill microbes.

Cytokines and Chemokines

Phagocytes also secrete chemo-attractants, such as cytokines and chemokines, which initially attract neutrophils and later monocytes and dendritic cells to the site of infection. For example, IL8 recruits neutrophils. The chemokine receptor, Ccr5 plays an important role in the invasion of cells by the HIV virus that causes AIDS. Some pro-inflammatory molecules, e.g., IL 1 (α and β), IL 6, and TNF α, trigger a rise in body temperature leading to fever, which lowers the division of invading microbes. Other cytokines, such as interferons α, β and γ (Ifnα, Ifnβ and Ifnγ), are released by host cells that are infected with viral, bacterial or fungal pathogens or in the presence of tumours and they have profound anti-viral, anti-tumour and immune-modulatory functions. The combination of interferon α along with an anti-viral drug is used to

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Inflammatory Responses		
Protective		**Harmful**
Microbial constituents	**Endogenous**	**Sepsis/Septic shock**
LPS Poly I:C CpG hypomethylated DNA	Urate crystals Dead cells Cholesterol crystals	Elevated cytokine amounts High fever Low blood pressure Increased heart beat Multi organ failure

Table 2. Consequences of inflammatory responses.

treat infections caused by hepatitis B virus. Ifnβ reduces inflammation and is used to treat patients suffering from multiple sclerosis. Mice or humans lacking Interferon γ or its receptor are very susceptible to intracellular infections, e.g., *Mycobacterium tuberculosis*, *Salmonelle typhi* etc., revealing the importance of this cytokine in host defence.

It may be important to point out that the innate response is useful to the host to initiate responses that protect it from microbes, microbial constituents, dead host cells, endogenous cholesterol, uric acid, etc.; however, an unregulated response may be harmful due to the excessive production of free radicals and cytokines (*Table* 2).

Toll Receptors

Interestingly, the initiation of innate response depends upon the appropriate recognition of microbes and PAMPs by the correct PRR. These receptors are actually a kind of molecular sensor system that conveys the presence of microbes or microbial constitutents to components of the immune system. Such receptors, or sensors, are most often membrane-bound and are expressed on the surface of a cell though a few types are also present in soluble form in body fluids (e.g., complement components, MBL) or within intracellular compartments. These can be broadly classified into either endocytic PRRs (C-lectin type mannose and

The Toll receptor was first discovered in *Drosophila melanogaster* as a transmembrane receptor involved in dorsal–ventral axis formation.

glycoprotein/glucan receptors) or signalling PRRs (e.g., Toll receptors). Of these, the cell surface Toll and Toll-like receptors (TLRs) and the cytoplasmic nucleotide-binding oligomerization domain (NOD) containing receptors are important in innate immunity. The NODs are characterized by N-terminal caspase recruitment domain, a middle NOD domain and a C-terminal leucine rich repeat (LRR) containing domain. NOD1 and NOD2 bind to break down products of peptidoglycan degradation. In fact, mutations in NOD2 result in manifestations of Crohn's disease, an extremely painful inflammatory disease of the bowel.

The Toll receptor was first discovered in *Drosophila melanogaster* as a transmembrane receptor involved in dorsal–ventral axis formation. The 'scrambled anatomy' of the mutant flies led to its name, Toll, which means 'cool' in German slang. Subsequently, Jules Hoffmann's laboratory in France found these mutants to be highly susceptible to *Aspergillus fumigatus,* whereas wild-type flies were less sensitive to the fungus. Interestingly, the Toll mutants are not sensitive to infection with *Escherichia coli* or other Gram negative bacteria. Further experiments revealed the existence of two pathways in *Drosophila*, which are required for immunity: Toll and Imd (immune deficiency). Toll is involved in resistance to Gram positive bacteria and fungi, whereas Imd is involved in encoding resistance to Gram negative bacteria.

Jules A Hoffmann

Jules Hoffmann, Emeritus Senior Researcher at CNRS and Professor at the Université de Strasbourg, France was born in Luxembourg and derived his enthusiasm for studying insects from his father, an entomologist. During his studies at the University of Strasbourg, France, he joined Prof. Pierre Joly's laboratory that studied hormone regulation in grasshoppers. Although experiments involving transplantation of organs were regularly performed under septic conditions, the grasshoppers did not get infected. This observation triggered Hoffmann's curiosity on the anti-microbial defence system in insects. It was known that AMPs are produced but the molecular details of the receptors or

signalling pathway that triggered the production of AMPs were not known. This was the key question that he set out to understand. An important factor was his decision to move from the study of grasshoppers to *Drosophila* as the genetics and molecular tools in the latter were well known and readily available. Initial work was focussed on setting up infection studies in *Drosophila*, and potent antimicrobial activities that peaked within 24 hr of infection were detected. The majority of the antimicrobial activities were due to the secretion of AMPs by the fat body in *Drosophila*, which is the mammalian equivalent of the liver. Hoffmann's laboratory identified a 44 amino acids long peptide known as Drosomycin which is responsible for anti-fungal immunity. The promoter of the gene encoding drosomycin contains sites for the transcription factor (NFκB) nuclear factor kappa light-chain enhancer of activated B-cells which is activated by the Toll receptor. Subsequently, studies showed that the Imd pathway increases the production of another AMP known as dipterin, which is active against Gram negative bacteria [7].

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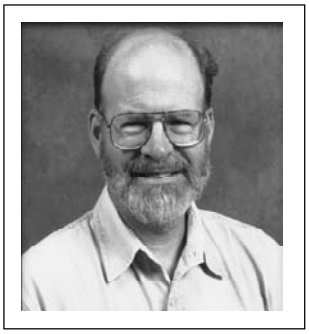
The pathways of activation of Imd and Tolls are distinct: Imd, a death domain containing intracellular adaptor protein, is activated via the binding of peptidoglycan, present in Gram negative bacteria, with a peptidoglycan recognition protein (PGRP)-LC, a transmembrane receptor. Interestingly, Gram negative bacteria contain diaminopimelic acid (DAP) in the third position, whereas most Gram positive bacteria contain Lysine in this position. These differences are recognized by peptidoglycan recognition proteins (PGRPs) and peptidoglycan from Gram positive bacteria are sensed by PGRP-SA and PGRP-SD. Fungal glucans are sensed by glucan-binding proteins (GNBP). Sensing of the presence of Gram positive bacteria or fungi results in the activation of serine proteases which cleave the cytokine spätzle. This ligand of the Toll receptor is a neurotrophin family member. Spätzle is secreted as a precursor polypeptide which needs to be cleaved by a cascade of different serine proteases before activation. Despite the differences in activation of the two main immune receptor pathways in *Drosophila*, the basic intracellular signal transduction

pathway is conserved (although the individual players may be different) between these pathways (*Table* 2).

In response to invading microbes, one of the main defence processes of *Drosophila* involves the production of AMPs, which are secreted in the hemolymph and lyse invading microbes by forming pores in their cell walls. There are nine receptors that belong to the Toll family in *Drosophila* and Toll receptor 9 has the closest resemblance to the mammalian TLR with an extra-cytoplasmic domain of LRRs and an intra-cytoplasmic domain that is homologous to the mammalian IL1 receptor. The remaining eight members are likely to play roles during development. Binding of dimeric and cleaved spätzle activates the Toll receptor and leads to dimerization of Toll and its Toll/Interleukin-1 Receptor (TIR) domain. This in turn leads to the formation of a complex comprising of the adaptor protein MyD88, Tube (a death domain-containing protein) and Pelle (an IL1 receptor kinase family member). This leads to the phosphorylation and degradation of cactus, an ortholog of the inhibitor of κB (IκB) followed by the translocation of NFκB into the nucleus (dorsal in embryo and dorsal-related immunity factor known as DIF in adults). The NFκB protein known as Relish is the main transcription factor involved in the Imd pathway. The phosphorylation of Relish occurs via the activation of the TGFβ-activated kinase 1 (TAK1) and IκB kinase (IKK) complex. TAK1 also activates the c-Jun N-terminal Kinase (JNK) pathway. Following cleavage by the caspase, Dredd, the carboxy terminal fragment remains in the cytosol whereas the amino terminal transcriptional regulatory domain of Relish translocates into the nucleus and induces the expression of several genes, including Dipterin [4].

Figure 3. Charles A Janeway predicted the role of bacterial components in stimulating the innate immune response which, in turn, affects adaptive immunity. In addition, work from his laboratory showed the functional roles of mammalian TLRs.



Toll-Like Receptors (TLRs)

As Hoffmann's studies were beginning to be known, C Janeway's laboratory (*Figure* 3) isolated a human ortholog of the Toll receptor, later known as Toll-like receptor (TLR)-4 [8]. Importantly, activation of TLR4 lead to NFκB translocation into the nucleus, similar to the Toll pathway in Drosophila, and led to

increased cytokine production and upregulation of ligands for costimulatory receptors, which are required for optimal T cell activation. This aspect was important as it clearly demonstrated a link between the innate and the adaptive immune response. It also revealed the importance of a proper environment (presence of microbes or microbial constituents) for generating immune responses so as to reduce the risk of autoimmunity. Further studies have shown that basic signal transduction for Toll and TLRs are also conserved between *Drosophila* and humans (*Table* 3), demonstrating conservation of the innate response.

The identification of the natural ligand of TLR4 to be a microbial cell wall constituent known as lipopolysaccharide (LPS), commonly known as endotoxin, came from Bruce Beutler's laboratory who demonstrated that a mutation in *Tlr4* is responsible for the lack of responsiveness of the *Lps* strain of mice to endotoxin [9]. LPS consists of polysaccharide and a lipid part and the latter is important for mediating its biological effects. Richard Pfeiffer, a student of Robert Koch[1] (well known for his studies on *Mycobacterium tuberculosis*; Nobel Laureate in 1905), coined the

[1] See *Resonance*, Vol.11, No.9, 2006.

Table 3. Conservation of innate receptor pathways in *Drosophila* and humans.

Ligand	Spaetzle	PGN (Peptidoglycan in gram negative bacteria)	LPS
Receptor	Toll	PGRP-LC	TLR4
Adaptor	MyD88	Imd	MyD88
Kinases	Pelle Kinase	TAK 1 IKK complex	IRAK, TAK1 IKK complex
Transcription	Cactus/NF-κB NF-κB (Dorsal, Dorsal related immunity factor)	NF-κB (Relish) NF-κB (cleaved Relish)	NF-κB, IκB NF-κB (p65/p65)
Response	Increase in AMP e.g., Drosomycin	Increase in AMP e.g., Diptericin	Increase in cytokines, costimulatory ligands, phagocytosis, etc.

term "endotoxin" (heat stable bacterial toxin) when he found that dead bacteria induced a vigorous immune response in guinea pigs.

Bruce Beutler

Bruce Beutler appears to be a young man in great hurry having completed his undergraduate degree at the University of California, San Diego, USA at age 18 and MD from the University of Chicago, USA at age 23. He has had a remarkable career: As a postdoctoral researcher at Rockefeller University, he was the first to isolate and show that a protein induced upon LPS, which is responsible for lowering lipoprotein lipase amounts in cells and causing tissue wasting, is the cytokine Tnfα [3]. This cytokine is rapidly induced during the invasion of microbes and plays important roles in host immunity and inflammation. A hybrid molecule, known as Enbrel, consisting of the soluble TNFα receptor fused to Fc domain of IgG1, binds to TNFα and is used for the treatment of inflammatory disorders, e.g., rheumatoid arthritis, spondylitis, etc. One must be cautious as this treatment may also increase the susceptibility to infections by microbes. However, it does demonstrate that knowledge gained by studies in innate immunity may have translational outcomes and help to reduce pain and suffering in patients.

A highpoint of Beutler's career was the positional cloning approach and identification of the gene responsible for the *Lps* defect in two strains of mice that are hyporesponsive to LPS. This study involved molecular mapping of the region, and finally, pinpointing of the gene responsible for the *Lps* defect to be *Tlr4*. Interestingly, the mutations in the two strains of mice were distinct: a mutation from Proline to Histidine at position 712 was observed in the C3H/HEJ strain whereas *Tlr4* was not synthesized (null mutant) in the C57BL/10ScCr strain. Further elegant studies involving a variant of LPS that contains 4 acyl chains as compared to the normal LPS molecule with 6 acyl chains revealed that Tlr4 could recognize variants of LPS and is likely to bind directly to LPS. Beutler is now at the Centre for Genetics of Host Defence, University of Texas, Southwestern Medical Centre,

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Receptor	Ligand	Origin of Ligand
TLR1	Triacyl Lipopeptides	Bacteria and Mycobacteria
TLR2	Peptidoglycan	Gram-positive bacteria
	Lipoprotein/lipopeptides	Various pathogens
	Lipoteichoic acid	Gram-positive bacteria
	Lipoarabinomannan	Mycobacteria
	Porins	*Neisseria*
	Zymosan	Fungi
TLR3	Double-Stranded RNA	Viruses, damaged host cells
TLR4	Lipopolysaccharide	Gram-negative bacteria
	Fusion protein	Respiratory syncytial virus
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	*Mycoplasma*
	Lipoteichoic acid	Gram-positive bacteria
	Zymosan	Fungi
TLR7	Single-stranded RNA	Viruses
TLR8	Single-stranded RNA	Viruses
TLR9	CpG-containing hypomethylated DNA	Bacteria and Viruses
TLR10	Not Determined	Not Determined

Table 4. TLRs and their ligands.

Dallas where he heads a large group of researchers and is actively involved in identifying different genes that cause immune defects, e.g., mutations that affect susceptibility to mouse mammary virus etc.

There are twelve TLRs in mice and ten in humans that are involved in binding different ligands (*Table* 4). The basic structure of the mammalian TLR consists of a large domain known as the LRR motif which is involved in ligand binding. The C-terminal region contains the TIR domain which is involved in protein-protein interactions, i.e., binding to other TLRs. TLR4 pairs with itself but other TLRs pair with other members and this may affect ligand specificity. For example, the complex of TLR2:TLR1 recognizes bacterial lipoprotein whereas the complex of TLR2:TLR6 recognizes peptidoglycan, zymosans, and

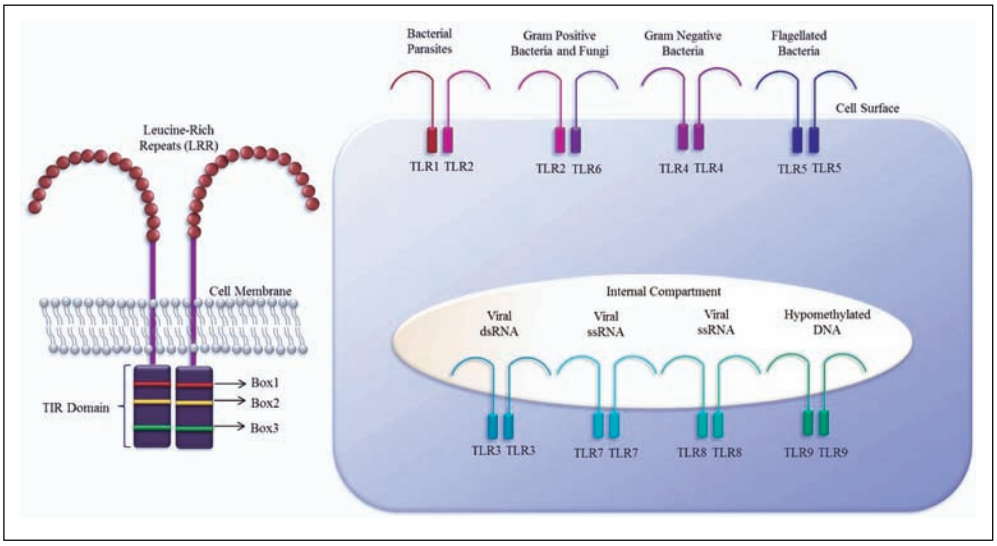

Figure 4. TLR structure, distribution and function.

bacterial lipoproteins. Although TLR4 is the key receptor required for recognition of LPS, other proteins are also important. The LPS binding protein is required to transport LPS in serum and transfers LPS monomers to CD14, a glycosylphosphatidylinositol-linked cell surface protein. A small protein Myeloid Differenciation factor-2 (MD2) is associated with TLR4 and is important for cellular responsiveness to LPS. Most likely, a complex of TLR4/MD2 binds LPS. Activated TLR4 binds to the adaptor MyD88 that mediates interaction with IRAK, a serine threonine kinase. Following the activation of IKK which phosphorylates and targets IκB for degradation, NFκB translocates to the nucleus and mediates its effects (*Figure* 4).

Relationship Between the Innate and Adaptive Responses

As has been discussed extensively, the innate immune response is an essential and important component of the immune system. However, the hallmarks of the vertebrate or mammalian immune response are specificity and memory, which are a result of the adaptive nature of the immune system. These are generated only upon activation of the adaptive response by means of the initial phagocytosis and presentation of antigens by the innate immune cells to the cognate receptor bearing lymphocytes of the adaptive system. Consequently, activation of the adaptive immune cells

signals to the innate system and fine-tunes further responses by means of cytokines, chemokines and cell surface receptors. In order to elicit appropriate adaptive and innate responses, communication between the two arms is essential. Macrophages, dendritic cells, natural killer cells from the innate system and helper T cells, killer T cells from the adaptive system are important for this cross-talk.

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Dendritic Cells

A classic case of cross-talk between the innate and adaptive immune systems is that of the dendritic cells. These professional antigen presenting cells (APCs) function as sentinels that can capture and process antigens and migrate to lymph nodes to present them to naive $CD4^+$ T helper and $CD8^+$ T cytotoxic cells for activation. Additionally, dendritic cells can sense changes in the cytokine milieu in the environment and undergo phenotypic maturation along with secretion of type I interferons and IL12, which are critical to macrophage recruitment and T-cell activation. In addition, the differentiation of the T helper cells into other sub-types, e.g. T_H1, T_H2, etc., is also influenced. In fact, the immense antigen processing, presentation and T-cell activation potential of dendritic cells can be harnessed to develop novel methods of immune therapy. The role of dendritic cells (DC) is tied to the discoveries of Ralph Steinman.

Ralph Steinman

Ralph Steinman completed his undergraduate studies from Montreal, Canada and MD degree from Harvard Medical School, Boston, USA. For his postdoctoral studies, he chose Zanvil Cohn's laboratory at the Rockefeller University, New York. He was intrigued by the initiation of T cell responses. Although he did not have a hypothesis, he initiated work on spleen cells – unlike others in Cohn's laboratory who worked on peritoneal macrophages. He characterized a cell type from lymphoid organs that were dendritic and appeared to be distinct from the previous immune cells described [10]: adherent, present in small numbers with

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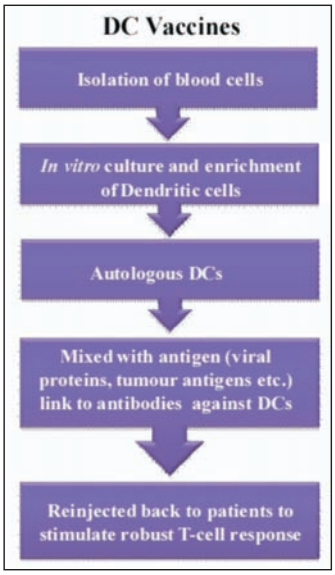

Figure 5. Strategies for the design of dendritic cell-based vaccines.

distinct morphology (large nucleus and many mitochondria). Importantly, these cells did not endocytose particles, unlike macrophages. Subsequent studies showed that the potency of these cells to act as antigen presenting cells and induce the activation of naïve T cells was 100 times more than B cells or macrophages [5]. The ability of a cytokine, GM-CSF, to increase the cell number of DCs proved to be extremely important and led to the possibility of using large number of these cells to process and present antigens to boost T cell responses in case of patients suffering from tumours, etc. Also, targeting of antigens to an endocytic receptor (DEC-205) on DCs enhances the efficacy of antigen presentation. He does mention in his autobiographical write-up that his NIH grant no. 13013 was funded continuously for 36 years [11].

Initial work from the 1990s had demonstrated that dendritic cells loaded with antigens *ex-vivo* could immunize mice as well as human volunteers, thus presenting the idea of a potential dendritic cell based, T cell-immunity inducing vaccine. Subsequent discoveries of uptake receptors (CD205 or DEC-205) on dendritic cells led to further developments of this technology. Briefly, peripheral blood mononuclear cells are harvested from a patient and cultured *in vitro* to enrich dendritic cell populations. The dendritic cells are then mixed with the antigen, which could be a viral protein or of tumour origin, such that they take up the antigen and are 'loaded' with it. These antigen-loaded autologous dendritic cells are then injected back into the patient such that the patient's T cells can be presented with the antigen and a robust antigen specific cell-mediated immune response can be elicited (*Figure* 5).

Although the first evidence for an adaptive immune response is seen only in the chordates, various innate defence mechanisms can be observed in almost every level of the evolutionary tree.

Evolutionary Aspects of the Innate Immune System

Although the first evidence for an adaptive immune response is seen only in the chordates, various innate defence mechanisms can be observed in almost every level of the evolutionary tree. This is due to the constant struggle for survival that has existed amongst the various species that inhabit the earth, and a strong immune defence system being essential for the continued exist-

ence of any given species. When one studies these various innate strategies, a unifying feature observed is the ability to distinguish molecular patterns as self or pathogen associated using germ-line encoded PRRs. Another is the synthesis of soluble molecules capable of neutralizing or destroying the pathogenic agent. Phagocytosis, as an innate immune strategy, is conserved in lower invertebrates (such as protozoans and sponges) and is also an important feature of the more evolved mammalian immune system, e.g., the enteric protozoan *Entamoeba histolytica* utilizes phagocytosis as a food-gathering as well as defence mechanism, while in the lower metazoan *Drosophila*, hemocytes are phagocyte-like cells that are important for both development and innate immune responses. Higher mammals have various phagocytic cells, e.g. neutrophiles and macrophages, with distinct functions. Thus many characteristics of the innate immune system are conserved across the evolutionary scale but there have been constant changes and improvements too as organisms have evolved into multi-cellular and complex structures.

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The study of immunity in plants is an interesting area and the role of LRR domain containing proteins in recognition of microbes is also conserved in plants. These are membrane-bound receptor-like kinases or receptor-like proteins. In fact, Fls2 in *Arabidopsis thaliana* is well studied and recognizes flagellin encoded by *Pseudomonas syringae*. The activation of PRR in plants leads to: changes in ion fluxes, oxidative burst, activation of protein kinases and transcription factors and production of antimicrobials. Some of the plant encoded anti-microbials are: chitinase, β1-3 glucanase (both target microbial cell walls), defensins (AMPs), phytoalexins (phenolics, etc.). Plant hormones also play multiple roles in anti-microbial resistance. The production of salicylic acid by plants is associated with resistance to signalling of biotrophs (pathogen which keeps the plant alive) whereas Jasmonic acid signalling is involved in response to necrotrophs (pathogen which causes necrosis and kills the plant). In addition, plants contain resistance (R) loci, which allows recognition of specific pathogen effectors and there are about 100 R loci in *Arabidopsis*.

Interestingly, the domain organization of R proteins reveals the presence of a C-terminal LRR domain: N-terminal coiled-coil domain–middle nucleotide binding site – C-terminal LRR domain. R loci activation is often associated with increased production of salicylic acid and death of infected and neighbouring cells to block the spread of infection. This form of programmed cell death is known as the 'hypersensitive response' in plants.

Perhaps unravelling the complexities of the mammalian immune system might be easier if the simpler immune systems of invertebrates and lower vertebrates are understood from a comparative evolutionary perspective, possibly even providing us with answers regarding the dysfunction of the more complex systems.

Importance of Innate Immunity

Why is the study of innate immunity important? Our body recognizes and responds to microbes and microbial constituents. In fact, the immune response to antigen is greatly enhanced in the presence of microbial constituents. This observation was recognized by C Janeway (1943–2008) who termed it as the "immunologist's dirty secret." Therefore, to generate a good immune response, adjuvants consisting of microbial products are included in antigen preparations and vaccines. Apart from microbial adjuvants, our body also responds to endogenous molecules, e.g., dead cells, cholesterol crystals, uric acid crystals. These may also manifest their effect during disease, e.g., the inflammatory response is important in the clogging up of blood vessels leading to heart failure. Finally, gout is a painful disease that occurs due to accumulation of crystals of uric acid (*Table* 2).

An overt inflammatory response to infections can be harmful and is known as septic shock. This highly inflammatory condition is often observed in patients who develop infections after post-operative surgery.

An overt inflammatory response to infections can be harmful and is known as septic shock. This highly inflammatory condition is often observed in patients who develop infections after post-operative surgery. This response by the body's immune system causes greatly increased cytokine amounts leading to low blood pressure due to vasodilation and increased vascular permeability. Often patients succumb due to multi-organ failure affecting the kidneys, lungs, heart and about 30% of patients who suffer septic

shock die (according to statistics in the US). Needless to add, drug companies are interested in finding cures to septic shock but an effective drug has been elusive against this multi-factorial disease. Overall, it is important to stress that although the innate system is important for our defence, it needs to be regulated and may harm the host under some conditions (*Table* 2). In fact, the amounts of endotoxin or LPS are measured in all fluids that are injected into the body. A common method of testing involves the use of extracts of blood cells from the horse shoe crab, *Limulus polyphemus*, which coagulate in the presence of very small amounts of LPS. This is a good example to illustrate the innate response in lower organisms and one that has a biotechnological application. Further studies on the innate receptors and mechanisms may shed light on the regulation and possibly drug targets that may be effective in regulating innate immunity for the benefit of the host.

The relationship of innate responses to disease has been studied in this review, e.g., the roles of MBL and common opsonic defect, NADPH oxidase in chronic granulomatous disease, NOD2 and Crohn's disease. TLR4 affects our ability to respond to the endotoxin (LPS) and recent studies also show that mutations in TLR4 can affect susceptibility to meningococcal septicaemia. Therefore, further studies are likely to identify more candidates and their ability to modulate immune responses. These, in turn, may help to identify drug targets that may be useful for patients during inflammatory diseases.

Concluding Thoughts

One half of the 2011 Nobel Prize in Medicine or Physiology was jointly awarded to Jules A Hoffmann and Bruce Beutler for the identification of innate immunity receptors in insects and mammals and the other half was awarded to Ralph Steinman for the discovery and roles of dendritic cells (*Figure* 6). Prof. Steinman was suffering from pancreatic cancer and passed away two and a half days before the Nobel Prize was announced. In general, a Nobel Prize is not awarded posthumously, but an exception was made in his case.

Bruce A Beutler | Jules A Hoffmann | Ralph M Steinman

Figure 6. One half of the 2011 Nobel Prize in Physiology or Medicine was awarded jointly to Bruce A Beutler and Jules A Hoffmann while the other half was awarded to Ralph M Steinman.

What lessons do these awardees teach us? It is obvious that a lot of hard work, patience, grant funding and a bit of luck plays roles in success. However, the key is to 'ask a good question' and to 'identify an important area of research'. Often one has to plough a lonely road and Hoffmann's work is illustrative of this aspect. At the time he started working in this area, adaptive immunity was more glamorous and most Nobel Prizes in Immunology were awarded to this area. Only Metchnikoff's studies on the discovery of phagocytosis (Nobel Prize, 1908) can be considered to be a study on innate immune responses. Therefore, it has taken more than a hundred years for the Nobel Prize to be awarded again to scientists studying innate immunity and represent recognition for this area. As there is no guarantee of success, scientists tend to work in areas that they are 'passionate' about. Hoffmann initiated this work because he was intrigued with immune defence systems in insects. This work led to studies on the roles of innate receptors in humans too and C Janeway showed the functional role of TLRs in modulating adaptive immunity. The *in vivo* physiological relevance of TLRs was shown by Beutler's laboratory with the identification of mouse *Tlr4* as the gene responsible for lack of responsiveness to LPS (endotoxin). Also, it may be important not to follow the crowd: Steinman worked on dendritic cells whereas most members in Zalvin Cohn's lab worked on peritoneal macrophages. All three awardees have acknowledged the roles of their mentors and colleagues for 'supporting' their work. After all, 'nurture' is an important aspect in long drawn out scientific studies.

Apart from being a good scientist, it is important to 'manage people' well and keep them motivated especially in large laboratories with myriad personalities. Finally, it may be useful for budding researchers to recall a piece of advice by the late C Janeway [6], "Be inspired by the knowledge that exists at the time you enter research, but be irreverent towards this knowledge – for this is the road to true understanding."

"Be inspired by the knowledge that exists at the time you enter research, but be irreverent towards this knowledge – for this is the road to true understanding."

– C Janeway

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