Chapter-35

Role of Toll-Like Receptors (Tlrs) in HIV Disease Progression

Authors

Gaurav Kaushik
School of Allied Health Sciences
Sharda University, Greater Noida
Uttar Pradesh 201310, India
Email: gaurav.kaushik@sharda.ac.in

Richa Vashishtha
Department of Medicine
All India Institute of Medical Sciences
New Delhi 110029, India
Email: richavashishtha@yahoo.co.in

Chaitenya Verma
Department of Biotechnology
Sharda School of Engineering and Technology,
Sharda University, Greater Noida,
Uttar Pradesh-201310, India
Email: chaitenya999@gmail.com

Shipra Sharma
Shri Guru Ram Rai Institute of Medical & Health Sciences
Dehradun, Uttarakhand 248001, India.
Email: shiprasharma1405@gmail.com

Raj Narayan Yadav
National Institute of Tuberculosis and Respiratory Disease,
New Delhi, India.
Email: rajnarayan.aiims@gmail.com

E-ISBN: 978-1-68576-521-7

Abstract

Toll-like receptors (TLRs) play an important role in pathogen detection because they selectively attach to different microbial species, triggering both innate and adaptive immune responses to infections. Since their discovery, a growing body of evidence suggests that TLRs play an important role in retroviral infection and disease progression. The investigations show that many Toll-like receptors (TLRs) play a role in the immune response to retroviruses. Furthermore, the interaction of retroviruses and TLRs may have complex and diverse impacts on the infection. Although there has been improvement, many questions remain about the role of TLRs in retroviral infection. This chapter gives a succinct review of current knowledge about the interactions between TLRs and retroviruses, as well as the specific roles these receptors play in immune response and disease development, with a focus on the human immunodeficiency virus (HIV).

Keyword: Toll-Like Receptors, HIV, HIV-TB, Tuberculosis, AIDS

****

1. INTRODUCTION

Study of human immunodeficiency virus (HIV) was initiated in 1981 with identification of rare opportunistic infections (OIs) *Pneumocystis jirovecii* pneumonia and cancers in young homosexual men of United States (1). Since these infections resulted in gradual breakdown of host immune system, Centers for Disease Control (CDC) in 1982 coined the term “acquired immune deficiency syndrome” (AIDS). Researchers believe that this virus was transmitted to humans from different primate species and was present in isolated population groups years before this epidemic began. As the situation changed people moved more frequently, and preferred advanced cities. This also led to change in their lifestyles and sexual behavior pattern. All this provided a favorable environment for the virus to spread through sexual intercourse and contaminated blood. As AIDS affected large numbers of individuals and resulted in several deaths, it becomes imperative to search for its causative agent. In 1983 two independent research groups identified human T-lymphotropic virus-III (HTLV-III) and lymphadenopathy-associated virus (LAV) as the causative agents of disease and belong to the lentivirus family which is a part of larger group known as retrovirus (2, 3). Subsequent research revealed that two viruses (HTLV-III and LAV) discovered as the causative agent of AIDS were similar and thereafter renamed as HIV in 1996 (4).

The AIDS epidemic is characterized by remarkable heterogeneity of HIV infection after exposure and in the clinical course of the disease. Despite natural variability in disease progression, HIV infection proceeds in 3 distinct stages: 1) acute primary infection, 2) asymptomatic phase, 3) symptomatic phase and progression to AIDS. During AIDS development, hosts become susceptible to a variety of OIs caused by diverse range of microorganisms (5). Tuberculosis (TB) is the foremost and commonest OI, complicating the course of HIV-infection (6). It has been observed that, out of the large number of people exposed to *M.tuberculosis*, only 30% acquire TB infection and the remaining 70% remain uninfected. Of these 30% infected individuals, 10% develop active TB disease (i.e. primary disease) while in the remaining 90%, the infection is contained by the host defense system (7). The presence of TB bacilli within the host in dormant or non-replicating state is known as latent tuberculosis infection (LTBI) (8). Individuals with LTBI are asymptomatic, without any radiographic and bacteriological evidence of TB disease, however, resuscitation promoting factors can reactivate the dormant bacteria (9). It is not necessary that TB only occurs during the advanced stage of HIV-infection, compelling evidence has indicated that it may develop at any stage of immunodeficiency, even when host CD4 cell count is high (10). HIV infection not only acts as the most important predisposing factor for LTBI reactivation and progression to clinical TB but also influences the outcome and presentation of TB in the host. Similarly, TB activates the host immune system, enhances HIV replication, and alters the natural history of HIV.

These findings reveal that inter-individual variability exists in susceptibility, transmission, and progression to HIV-infection. It has also been reported that AIDS patients exhibit variability in responsiveness to antiretroviral drugs (11, 12, 13). Therefore, beside precise knowledge of HIV transmission, replication, and disease pathogenesis, it is imperative to define and understand factors which significantly contribute to variability of HIV-infection at clinical, immunological, and virological level. This information will significantly aid in combating HIV/AIDS through development of preventive and therapeutic strategies and will also guide the clinical management of HIV and HIV-TB co-infected patients (14). Studies have demonstrated significant correlation between elements of immune system and susceptibility towards various infections and disease progression. It is well known that immune response of the host contributes towards host-pathogen interaction/host virus- crosstalk. Organisms are frequently exposed to infectious pathogens/agents and in most cases the host escapes the infection and survives. This is possible because organisms can discriminate between self and non self which is an integral function of the human immune system (15).

The human immune system is broadly classified into innate and adaptive immune systems. The innate immune system is evolutionary, more ancient, and acts as the body's first line of defense against foreign antigens (16). Definite work on innate immune system led to remarkable discovery of highly skillful phylogenetically conserved family of proteins known as pattern recognition receptors (PRRs). PRRs had the ability to identify specific pathogen-associated molecular patterns (PAMPs) (17). The most potent of all PRRs which play a central role in innate immune system are toll-like receptors (TLRs). To date, 13 TLRs (TLR1-TLR13) have been identified in mammalian species, these includes 11 TLRs found in humans (18). TLRs are expressed on a wide variety of cells (immune cells like -macrophages, dendritic cells, B cells, T cells and non-immune cells like - fibroblast, epithelial cell) tissues and organs including lymphoid organs. Some TLRs are expressed extracellularly on cell surface (TLRs 1, 2, 4, 5 and 6) while others (TLRs 3, 7, 8 and 9) are intracellularly (19). TLR10 gene is mostly expressed in lymphoid tissues such as spleen, lymph node, thymus, and tonsils. TLR11 is expressed in macrophages and dendritic cells. The exact function of TLR10 and TLR11 gene is still not known in humans. Several studies have demonstrated that innate immune response plays an important role in HIV disease pathogenesis, disease progression and susceptibility to TB (20, 21). The key component of the innate immune system, TLRs are responsible for activating the signaling pathway which regulates HIV replication and disease progression. In other words, the latent and chronic stages of HIV infection are dependent on TLR mediated pathways (22).

2. HISTORICAL BACKGROUND: DISCOVERY AND ORIGIN OF HIV

Several theories have been put forward to explain the origin of HIV but none of these have found universal acceptance to date. Retrospective analysis of blood suggests that earliest known case of HIV was from the blood sample collected in 1959 from a man in Kenosha, Democratic Republic of Congo. On genetic analysis of this positive blood sample, it was discovered that HIV-1 must have started disseminating in the late 1940s or early 1950s. However, historically, the study of HIV/ AIDS was started much later, about 30 years ago in 1981, when unexplained presence of rare and unusual diseases like Kaposi sarcoma, *Pneumocystis jirovecii* pneumonia and persistent lymphadenopathy were discovered among young and previously healthy, homosexual men of New York and California (1). It was observed by clinicians that these patients slowly become immune-deficient and succumb to other rare OIs caused by various pathogenic micro- organisms such as *Toxoplasma gondii, Mycobacterium avium, Cryptococcus neoformans* and developed flu like symptoms. As the virus spread, disease that was already in

existence albeit with a low and passive form became an epidemic, which was highly lethal. In that first year, over 1600 cases were diagnosed with around 700 deaths.

The first antigenic variant of HIV also known as HIV-I was discovered in 1986 and the other variant known as HIV-2 was discovered later (23, 24). Although the sequence homology between two HIV variants is approximately 45%, but they appear to cause clinically indistinguishable AIDS. Their distribution indicates that HIV-2 is less easily transmitted and therefore, HIV-1 is more predominant worldwide. HIV-2 type is mainly concentrated in West Africa and rarely discovered elsewhere.

3. HIV EPIDEMIOLOGY

Global HIV Burden: Worldwide estimates have projected that at the end of 2022 there were 39 million [33.1 million-45.7 million] individuals living with HIV, where 1.3 million [1 million – 1.7 million] people become newly infected with HIV in 2022. Globally, HIV prevalence among adults (age 15-49) was 0.7%. However, median prevalence was higher among key population, 2.6% was sex workers, 7.7% was gay men, 5% people indulged in injecting drugs, 10.3% among transgenders, and 1.4% among people in prisons. The number of deaths occurring due to AIDS-related causes was reduced by 69% since the peak in 2004 and by 51% by 2010. In 2022, around 630 thousand [480 thousand – 880 thousand] people died from AIDS related issues (25).

HIV burden in India: According to the National AIDS Control Organization (NACO) report 2023 India accounts 2.4 million cases till 2022, where 45.8 % reports in women only and 68000 were children's, age ranging 0 years - 15 years. AIDS related mortality in India accounts 42000 in 2022. Among the entire population of individuals living with HIV/AIDS (PLWHA), 3.4% of them were children, while around 44% of the total PLWHA who 15 years (26).

4. HIV GENOME AND PATHOGENESIS

Structure and Genome of HIV: The structure of HIV differs from other retroviruses. It is spherical with a diameter of about 120 nm (27). HIV genomes consist of two copies of single-stranded ribonucleic acid (RNA) which codes for nine viral genes. The genetic material is enclosed by capsid made up of viral protein p24 (28). The single -stranded RNA of virus is tightly bound to nucleocapsid proteins named p7, and several other enzymes. These enzymes (reverse transcriptase, proteases, ribonuclease and integrate) are required for the formation of new virions. Another layer or matrix composed of viral protein p17 surrounds the capsid and helps in maintenance of virus integrity. Matrix in

turn is surrounded by another covering known as viral envelope, made up of two layers of fatty molecules called phospholipids. A virus acquires phospholipid envelope from the cell membrane of a host cell during budding. Host cell proteins including 70 copies of HIV protein lie embedded in the virus envelope. This complex HIV protein known as Env is made up of three glycoprotein (gp) 120 molecules and a stem made up of three gp41 molecules respectively. This env protein helps the virus in attaching the host cell and initiating the infectious cycle. The env protein (gp120) serve as the target molecules on which future research such as development of new drugs and vaccines against HIV is being focused (29).

HIV Replication and Life Cycle: The HIV replication cycle can be summarized in nine steps: i) binding and fusion to receptors, ii) infection and viral penetration, iii) reverse transcription, iv) provirus integration, v) transcription and viral protein synthesis, vi) assembly vii) budding, viii) cleavage of immature virions from host cell surface, ix) maturation. The viral entry process involves binding of gp120 to CD4+ T-cells, particularly which induce conformational changes to create, expose or stabilize the coreceptor, conformational changes in the Env protein, and activation of gp41. Fusion of the viral membrane with target cell membrane is mediated by exposing and extending the gp41 fusion peptide, allowing it to insert into the plasma membrane of the target cell.

Viral capsid is released into the cell during the process of viral entry and lipid envelope remains left in the lipid bilayer of the host. This process is followed by reverse transcription of the viral genome into complimentary DNA (cDNA) using cellular lysine tRNA molecule as a primer (30). The degradation of RNA template by the nuclease activity of the RTase facilitates the movement of cDNA inside the nucleus. This nuclear entry is mediated with the help of Vpr and Vif accessory proteins. The viral cDNA integrates to the genomic DNA with the activity of integrase.

5. HIV PATHOGENESIS

Modes of Transmission: The principal modes through which HIV is transmitted are unprotected sexual contact, transfusion of infected blood (in hemophiliacs etc.) and blood derived products. However, transmission also occurs through infected syringes (in intravenous drug users, health care workers, nurses, and doctors) and infected mother to child transmission during pregnancy or delivery or breast feeding. Thus, it has been concluded that various body fluids such as blood, semen, vaginal fluid, breast milk, cerebrospinal fluid (surrounding the brain and the spinal cord), synovial fluid (surrounds bone joints) and amniotic fluid (surrounding the fetus) facilitates viral transmission.

HIV is also found to have various other secretions such as saliva, sweat and urine but in very low amounts and to date there are no recorded cases of transmission through these secretions.

6. HIV DISEASE PROGRESSION OR CLINICAL COURSE OF HIV DISEASE

HIV infection is characterized by CD4 T-cells depletion and reduced naïve cell number, inverted CD4:CD8 ratio, decreased thymic output, altered cytokine profile, increased susceptibility to activation induced cell death and development of OIs. Although, inter individual variability exists, in spite of that disease progression mainly proceeds through three different routes:1) acute primary infection, 2) asymptomatic phase, 3) symptomatic phase and progression to AIDS as described earlier. The disease progression among HIV-positive is basically monitored through CD4+ T-cell count estimation.

Acute Primary HIV Infection: It is the earliest detectable stage of disease, which occurs in approximately 87% of HIV-infected subjects, develops its symptom 2-6 weeks following infection. This stage of infection is characterized by mononucleosis like illness (31, 32). The symptoms include fever, fatigue, lymphadenopathy, headache, and rash (33). This stage is associated with high levels of HIV viremia, decreased peripheral CD4 T-cell count and transient immunosuppression. This stage of infection resolves after a few days or weeks and levels of HIV-1 infection dramatically reduce in the blood. This is followed by seroconversion, with antibodies directed towards different HIV-1 protein. This infection is more rapid and severe in rapid progressors.

Asymptomatic Phase: This phase is clinically quiescent and may last for several years with very little evidence of active TB. There is slow and persistent change in CD4+ T-cell count and PVL and a stage of equilibrium is achieved between virus replication and immune response generated by the host. CD8 T-cells remain slightly elevated during this stage of infection. CD8 cytotoxic T- cells suppress viral propagation. Degree of viremia during this stage of infection predicts disease progression.

Symptomatic Phase and Progression to AIDS: This stage is associated with active virus replication and rapid decline of CD4+T-cell count (34). Virus replication in lymph node destructs lymphoid tissue architecture. Virus isolates are highly cytopathic and have high replicative potential. Clinical manifestation includes fever, weight loss, diarrhea, enlarged lymph node, fatigue and night sweats. Leukopenia, thrombocytopenia, anemia, increased serum immunoglobulins and absence of delayed type hypersensitivity (DTH)

are also common during this phase. PVL of patients is inversely related to CD4+T- cell count in chronic HIV infection. Profound irreversible immunologic suppression rapidly progresses towards various OIs and development of AIDS.

CDC has stated that AIDS will be diagnosed in an individual by one of the AIDS-defining conditions or if the CD4+T-cell count estimation is <200 cells/µl. Individuals having CD4+T-cell count <200 cells/µl progress to AIDS at least 2-years earlier in comparison to those having clinical symptoms and develop OIs. Survival of any individual infected with HIV after progressing to AIDS depends upon development of AIDS-defining events (35, 36).

7. VARIABILITY IN HIV DISEASE PROGRESSION

HIV infection results in progressive decline of CD4 T-cell numbers, T cell dysfunction, thymic dysfunction and defects in (number and function of) antigen presenting cells such as dendritic cells and monocytes. Large numbers of variations are seen in different HIV- infected patients. It has been observed that without therapeutic interventions, majority of HIV-infected patients (70-80%) progress to AIDS after 8-10 years of latency, however, a small percentage of patients (10%) progress to AIDS within 3 years of infection are known as rapid progressors (37). There are about 5% of individuals who remain asymptomatic for a very long period (more than 10 years), even in absence of treatment (38). These patients maintain low viremia and normal CD4 T-cell count and are termed as long term non progressors (LNTP). This variability in disease progression rate may be accounted for by several factors related to immunological, virological and host genetic aspects. More researchers are warranted to investigate the predictors which lead to inter individual variability in HIV disease progression.

8. GENETIC CROSSTALK: HIV/ AIDS

The Immune System: Immune system is composed of amazingly complex group of organs which collectively resist the host from infections caused by bacteria, parasite, fungus, viruses, environmental toxins, and various other potential threats through specialized defense mechanism (15).

The immune system has evolved in such a manner that it can differentiate between “self” and “non-self”. It is broadly classified into an innate and adaptive immune system (39, 40). The innate immune system acts as the first line of defense against pathogenic microorganisms and is non-specific in nature. The innate immune system generates immediate immune

response against foreign antigen, which is mobilized within seconds to minutes of pathogen invasion.

On the other hand, adaptive immune system mediates a more specialized delayed type of response against those foreign antigens which are not eliminated by innate immune system and is more specific in nature. In contrast to the innate immune system, the adaptive immune system takes time (from days to weeks) to respond against foreign antigen, in other words it generates delayed type response (41). Adaptive immune system on interacting with infectious microorganism activates, proliferates, and creates potent environment to eliminate the pathogen. It is mainly present in vertebrates and has the characteristic of developing immunological memory due to which individual is protected from reinfection by the same pathogen (41). Due to possession of immunological memory adaptive immune system plays an important role in vaccine development (9, 42, 43). Both innate and adaptive immune systems are composed of a variety of cellular and humoral components and generate inflammatory response against foreign antigens (44). The highly evolved adaptive immune system is composed of B and T lymphocytes which identify foreign antigens using the T-cell receptors (TCRs) and immunoglobulins (41). It is basically composed of physical epithelial barrier, macrophages, and dendritic cell, specialized lymphocytes known as natural killer cell and plasma proteins.

It has been realized that immune system activation against these microbes is hierarchical as it has been carried out for centuries. These observations raised the question in the mind of individuals how all these microbes are perceived as foreign antigens or "non-self" by the immune system of the host. However, by this time it was quite evident that in all biological systems recognition of invading pathogens/microorganisms were carried out by the receptors. Therefore, researchers were forced to think that the immune system contains certain receptors which can perceive the molecules of microbial origin. It was discovered that both innate and adaptive immune systems have receptors used for recognizing foreign antigens and initiating signaling pathways in response to eliminate the pathogen. It was discovered that adaptive immune system had clonal B-cell receptors (BCRs or soluble antibody) or TCRs to recognize specific pathogen. This triggered the search for receptors within the innate immune system and led to the discovery of PRRs. It has been well established now that innate immune system besides traditional immune cells contains highly skillful components known as PRRs. These PRRs play a central role in innate immune system and can identify and discriminate different classes of invading pathogen through PAMPs. These PRRs generate a series of immune reactions or signaling pathways in response to invading foreign antigen to eliminate the pathogens. It has been observed

that PRRs get activated on sensing the invading pathogen and activates the cells of innate immune system which quickly accumulate at the site of infection (45) resulting in inflammation. This makes innate immune system respond quickly and immediately against invading pathogen while adaptive immune system does not receive signal from pathogen directly, rather it mediates delayed type response against foreign antigen only when it is directed by the activated innate immune system. Earlier not much importance was given to the innate immune system, however, the discovery of PRRs have renewed interest in further study of this system (42).

9. IMMUNE CORRELATES OF PROTECTION

Toll - Like Receptors: The innate immune systems of vertebrates are endowed with special components known as PRRs. These are prototypical innate sensing receptors which have the capability to recognize and discriminate between different classes of microbes through conserved PAMPs. In response to invading foreign antigen PRRs initiates antipathogen defense mechanism in the form of series of reactions to protect the host from pathogenic microorganism. It has been indicated that TLRs are the most potent family of all PRRs. TLRs belongs to the ancient super family of proteins and this super family also includes proteins found in plants and invertebrates. The first TLR discovered was TLR4 which was found homologous to toll receptor of *Drosophila* and therefore it was named after it. Later, several other proteins, structurally and functionally similar to toll receptor of *Drosophila* and capable of recognizing conserved microbial structures were discovered and helped in creating TLR family.

TLRs are type-I transmembrane proteins and to date 13 TLRs have been discovered in mammals and 11 in humans (46). TLRs basically have extracellular domain made up of LRRs and cytoplasmic tail that consist of another conserved toll/IL-1 receptor (TIR) domain which is homologous to interleukin-1 (IL-1) receptor (47). TLRs are the key component of immune system and by their virtue host is able to sense the invading microorganism (48). TLR on recognizing conserved PAMPS and on binding with specific ligand gets activated. This causes recruitment of adaptor protein near TIR domain and triggers complex signaling cascade leading to series of reactions caused by activation of transcriptional factors and cellular activation. The transcriptional factors or genes activated principally encode proinflammatory cytokines, chemokines, and various co-stimulatory molecules responsible for inflammatory reactions. These reactions play a crucial role in elimination or destruction of invading pathogens. Thus, TLRs not only coordinate multiple innate immune pathways by initiating distinct signaling cascade for efficient elimination of invading pathogen but also empower and direct activation of

highly specialized adaptive immune system against potential pathogenic threats. TLRs expressed extracellularly on cells of innate immune system specifically recognize extracellular microbial products such as lipopolysaccharide (LPS) of Gram-negative bacteria, mannans of fungal pathogens, whereas those expressed intracellularly specifically bind to nucleic acids for detection of intracellular pathogen. It is a common observation that different subsets of immune cells express distinct TLRs and all TLRs do not respond to every invading pathogen, only specific TLR on interacting with pathogen initiates series of reaction. Moreover, the expression of each TLR against different pathogen is different. After the discovery of TLRs they were studied extensively and it was discovered that since they are activated during chronic infections and infectious disease, they play a major role in disease pathogenesis. Results of several studies also suggested that polymorphisms in human TLRs are associated with increased or decreased susceptibility to bacterial or viral infections. Recent studies have indicated that various TLRs, in particular TLR2, TLR4 and TLR9 are capable of recognizing bacterial and viral products and have an important role to play in disease pathogenesis. While TLR2 and TLR4 are expressed on the cell surface TLR9 is located exclusively in the endosomal compartment (49). It has been demonstrated that loss in function of any TLR makes the host vulnerable to pathogenic infections implied that TLRs play a major role in host defense mechanism. Published literature also indicated that if TLRs activity becomes dysregulated during certain infectious or non-infectious diseases they have highly fatal consequences. Therefore, TLRs are being evaluated as an important immune therapeutic target.

10. TLR STRUCTURE

TLRs family is basically type-I transmembrane glycoproteins. Discovery of TLRs generated interest among research fraternity in further study of innate immune system. After TLRs were discovered, it was emphasized that understanding the structure of TLRs is highly important because research have indicated that TLRs can be used in designing novel drugs. TLRs can also be used in pharmacological intervention and serve as targets in immune therapy. It has been elucidated that TLRs structure basically consists of amino terminus extracellular transmembrane domain and carboxy-terminal intracellular region containing highly conserved TIR domain (50).

The extracellular domain of TLRs consists of repeated LRRs and each TLR has 16-28 LRRs. This extracellular domain of TLR having LRR is highly polymorphic and is very essential for TLR dimerization and is mainly involved in ligand binding (51). The LRR family consists of 6000 proteins in the Pfam database. These proteins basically carry out a wide variety of

functions like immune response, signal transduction, enzyme regulation etc. These LRR proteins are made up of multiple LRR modules, short proteins have 2-3 modules while the longer one has more than 40 modules and each module is made up of 20-30 amino acids. Each LRR module has a conserved motif as well as variable region. These modules stabilize the structure of proteins present in TLR and avoid exposure of hydrophobic core to solvents outside It has been observed that conserved motives or sequence of module, formed from parallel β strands are present on the inner concave side of horseshoe shape like structure while the variable part of the module which is generated from helices, β turns and/or loops forms the convex surface in this structure. TLR4 is one of the most extensively studied TLR whose extracellular region in addition to LRR contains highly variable region made up of 82 amino-acids which is essential for carrying out species-specific differences and bacterial LPS recognition (52).

The intracellular region of TLRs contains a highly conserved TIR domain. The intracellular TIR domain is composed of approximately 150 amino acids. This region besides TIR also contains three highly conserved regions. As ligand induces dimerization of TLRs, TIR causes recruitment of specific adaptor molecules including Myeloid differentiation primary response gene 88 (MyD88), MyD88-adaptor-like protein (MAL), TIR-domain-containing adaptor protein inducing IFNβ (TRIF), and TRIF-related adaptor molecule (TRAM). This TIR-TIR interaction occurring between receptor-receptor, receptor-adaptor, and adaptor-adaptor leads to initiation of signaling cascade. Several studies proposed the structure of TIR domain of TLR4 using TLR2 as template. It was suggested that TLR4 uses two different sets of adaptors: TRAM and TRIF, and Mal and MyD88. These adaptor pairs couple two distinct signaling pathways leading to the activation of interferon response factor 3 (IRF-3) and nuclear factor kB (NF-kB) respectively.

11. TLR LOCALIZATION

TLRs play an important role in pathogen recognition and alerting the immune system to the presence of microorganisms. TLRs are mainly located on cells which act as the first line of defense against pathogenic microorganisms. They are mainly located on immune cells like neutrophils, macrophages, dendritic cells, B cells, T-cells; certain non-immune cells like-fibroblast, epithelial cell, tissues and organs like-lymphoid organs. Most TLRs are located extracellular *i.e.* expressed on cell surface while TLR 3, 7, 8 and 9 are located intracellularly. The two of the most important receptors TLR2 and TLR4 which recognize most bacterial lipoproteins and LPS are present on B and T cells (53).

12. TLR SIGNALING

It has been extensively studied and observed that on sensing the invading pathogen TLR initiates signaling pathway. During signaling TIR domain of TLR adaptor molecules interacts with TIR signaling domain (54, 55, 56). Till date 5 adaptor molecules have been identified in TLR. The first TLR adaptor molecule which was identified was MyD88. It contains TIR domain that allows it to interact with IL-1R family members and a death domain (DD) that recruits IL-1R-associated kinases (IRAKs) to the signaling complex (56). MyD88 is the sole adaptor identified for TLR5, TLR7, TLR8, and TLR9, while TLR2 and TLR4 use the MyD88 adaptor molecule along with other adaptor molecules (57, 58). The MyD88-dependent pathway is known to mediate activation of NF- kB and the production of inflammatory cytokines, and the MyD88-independent pathway induces production of interferon (IFN) inducible genes. However, IFN-γ induction by TLR7 and TLR9 ligation is dependent upon MyD88 expression (59, 60, 61).

The TIR-domain-containing adaptor protein (TIRAP), also known as the MyD88-adaptor- like protein (MAL), was the second TLR adaptor discovered. It contains a TIR domain but no DD (62). It was initially thought that TIRAP would mediate the MyD88-independent pathway in TLR signaling. However, studies indicated that TIRAP deficiencies result in inhibition of inflammatory cytokine production (58, 62, 63). It has been determined that TIRAP is utilized in the downstream signaling pathways for TLR4 and TLR2 but no other TLRs.

The TRIF or TIR-domain containing adaptor molecule (TICAM) 1 was simultaneously identified by two groups. TRIF was identified as the adaptor utilized in the MyD88- independent production of IFNβ and induction of IFN-stimulated genes (64, 65). This protein is utilized by both TLR3 and TLR4 (66). The fourth adaptor molecule identified is termed the TRIF-related adaptor molecule (TRAM) or TICAM-2. TRAM associates with TRIF in the TLR4 signaling pathway but not in the TLR3 signaling pathway. In the absence of TRAM, TLR4 signaling results in decreased activation of interferon regulatory factor-3 (IRF-3), suggesting its involvement in the MyD88-independent/TRIF-dependent signaling cascade. The production of inflammatory cytokines was also reduced when TRAM was not expressed. TRAM has only been demonstrated to be involved in the TLR4 signaling pathway (67). The fifth TLR adaptor molecule is the sterile α motif (SAM) and HEAT-Armadillo repeat motif (ARM) - containing protein (SARM) and has only recently been identified as a TIR-containing adapter (68). In TLR4 and TLR2 signaling, TIRAP is utilized in recruitment of the signaling molecules required for NF-kB activation (58). TLR4 requires the TRAM adaptor and the NF-kB subunit p65

to induce IFN-stimulated genes and results in phosphorylation of the N-terminus rather than the C-terminus of IRF3 (69).

13. TLRS AND THE IMMUNE SYSTEM

The innate immune system serves as the first line of defense against any foreign antigen. The major components of the innate immune system are macrophages, neutrophils, and dendritic cells. However, recently PRRs are recognized as one of the key components of the innate immune system. These PRRs are highly skillful components and play a central role in the innate immune system. They are responsible for recognizing specific conserved structure present on the surface of pathogens known as PAMPs and directing the course of immune response against these pathogens. PRRs induce immune responses against pathogens by activating both innate and adaptive immune system.

Among all PRRs, TLRs are the most potent and extensively studied receptors. TLRs are phylogenetically conserved family of proteins (57). Till date 13 TLRs are recognized in mammals, of these 11 are present in humans. In humans TLRs were first described by Nomura and colleagues in 1994 (70). They are named after a receptor discovered in fruit-fly; *Drosophila* known as toll which provided protection against fungal pathogen (71). The first TLR recognized in mammals in 1997 was TLR4 and later on several other TLRs were recognized. TLR4 had homology with toll receptor present in Drosophila. Human TLRs are divided into five subfamilies (TLR2, TLR3, TLR4, TLR5 and TLR9) depending on their chromosomal location, structure and amino acid sequences. The TLR2 subfamily includes TLR1, TLR6, and TLR10, the TLR9 subfamily includes TLR7 and TLR8. However, TLR3, TLR4 and TLR5 subfamily are represented only by one family member each respectively. TLRs are composed of extracellular LRR domain and a cytoplasmic carboxy - terminal TIR domain. Pathogen recognition is carried out by proteins present in the membrane of TLR.

Interaction of TLRs with various adaptor proteins of the invading microorganisms activates the TLRs inside the host. These activated TLRs initiate a signaling pathway. This leads to release of various pro-inflammatory cytokines, chemokines, and recruitment of various effector molecules at the site of infection and induction (72, 73). TLR and other PRRs cellular response not only activate innate immunity but also give accessory signal for activation of adaptive immune response. Thus, emergence of TLR was not the turning point for innate immunity of the host but was found necessary for shaping adaptive immune response against the infectious microorganism.

14. TLRS AND THE ANTIVIRAL RESPONSE

Innate immune response serves as the first line of defense against all invading microorganisms inside the human body. TLRs have a central role in innate immune response, and they are activated on interacting with invading pathogens. These invading microorganisms can range from fungus to bacteria or viruses to protozoa etc., TLRs are active against all of them. Similarly on invasion of human body with viruses, TLR initiates antiviral response. Although several TLRs (TLR3, TLR4 TLR7, TLR8 and TLR9) recognize molecular patterns on surface of virus and binding with these motifs but only two TLRs, TLR3 and TLR4 are capable of activating signal cascade (74).

It has been demonstrated that both TLR3 and TLR4 after recognition of viral glycoproteins result in initiation of IRF3 activation or nuclear translocation (75). Although signaling of both TLR3 and TLR4 results in IRF3 activation but the quantity and quality of response generated in both the cases differs. It has been demonstrated that to generate immune response, TLR4 requires help from NF-kB subunit and p65 so that after IRF3 phosphorylation interferon - stimulated response elements (ISREs) are activated. It has also been demonstrated that phosphorylation of IRF3 induced by TLR3 takes place at C-terminal while one induced by TLR4 occurs at N-terminal domain (69).

15. TLR POLYMORPHISMS AND INFLUENCE ON HIV/AIDS

SNPs in TLRs are known to be associated with increased or decreased susceptibility to various infectious diseases. Initial studies demonstrated that the TLR system only interacts with bacterial PAMPs, but it is now well established that TLRs can interact with viruses also. Genetic variations in TLRs could influence individual response to TLR ligands, resulting in altered susceptibility to human pathogens, thus affecting the course of infectious disease (76, 77). SNPs occurring in TLRs are particularly important in reference to HIV, as they alter host immune response affecting disease pathogenesis. Regarding HIV, it is established that variations within the host's genome contribute to the risk of acquiring infection as well as the individual rate of disease progression. It has been observed that SNPs in TLRs may play an important role and provide relevant information about HIV pathogenesis because in vitro activation and signaling through TLR2, TLR4, and TLR9 might accelerate HIV replication (78). Since, growing number of studies have demonstrated that TLRs polymorphism influences individual susceptibility towards various infectious pathogens and therefore it has been implicated that TLRs have pivotal role in activation of both innate and adaptive immune responses of the host. It has been indicated that infecting pathogens results in activation of immune system or HIV gene expression and as a consequence of

this host progresses towards AIDS progression. It has been demonstrated that the responsiveness of immune system to several infectious agents which are associated with HIV relies on a family of PRRs, more specifically known as TLRs. Thus, it has been concluded that HIV associated diseases or OIs are indirectly regulated through TLR-pathogen association or interaction (20). The signals generated by the host immune system in response to antigens or infectious agents trigger stimulation of HIV long terminal repeat (LTR) 3 and is referred as immune activation. (79). The progressive HIV disease is characterized by chronic activation of immune system. The first abnormality described among HIV-seropositive individuals was activation of polyclonal B-cell activation (80). This eventually heightens T-cell turnover (81), subsequently increasing T-cell frequencies with activated phenotype (82) and potentially increases proinflammatory cytokines and chemokines serum levels (83).

The association between TLR signaling pathway and HIV induction is evident through study, which for the first time demonstrated that LPS is responsible for stimulating HIV LTR in macrophage-like cell lines occurs through NF-kB associated activation pathway (84). Since TLR4 is normally not expressed on T cells, it was not yet established whether TLR4 serves as receptor for LPS and initiates NF-kB signaling. However, several studies subsequently demonstrated that HIV replication is stimulated in infected primary human monocyte/macrophage cultures through LPS (85).

In 1997, the discovery of TLR signaling within mammals (43), logically establishes that one of the key events which induces or enhances HIV expression through microbial ligands is TLR ligation. Although these findings were formally accepted in 2001, when Equils et al. demonstrated that LPS stimulation is responsible for transactivation of LTR within human dermal endothelial cells secondarily transfected with wild-type TLR4. Also, Equils et al. in 2001 also established that TLR2 transactivates LTR through TLR2 bacterial ligands (78). In 2004, Sundstrom et al. demonstrated that stimulation *via* ligands of TLR2, TLR4, and TLR9 of HIV-infected mast cells enhances HIV replication in humans (86). The induction of HIV LTR is mediated mainly through cytokines released during NF-kB activation pathway. This mechanism is majorly responsible for enhancing transcription of provirus through several concurrent infectious agents and triggering of TLRs. It was indicated that heightened serum TNF levels in HIV-infected patients co-infected with *M. tuberculosis* was associated with increased viral load (87, 88). As a result of the growing number of HIV infections in sub-Saharan Africa, the number of co-infections of HIV and *M. tuberculosis* is also increasing fast, with a significant contribution to morbidity and mortality (89, 90). It is well known that the incidence of infection with *M. tuberculosis* is

much higher than that of active tuberculosis, even in the case of co-infection with HIV. Research revealed that TLR4 mediates cytokine production stimulated by *M. tuberculosis*, and that TLR4 knockout mice are more susceptible to infection with *M. tuberculosis* (91, 92). TLR4 is known to activate the interferon response, thus inhibiting HIV replication. Therefore, individuals bearing the TLR4 Asp299Gly polymorphism may have a more pronounced viral replication, with an enhanced loss of CD4+ T cells and increased susceptibility to tuberculosis.

Only a few recent studies have investigated the role of TLR9 SNP in the susceptibility to HIV infection. Studies reported that TLR 1635A/G polymorphism is associated with rapid progression of HIV-infection in Swiss cohort. Another study in HIV sero-prevalent cohort of Spain demonstrated that TLR9 1635AA genotype was associated with low CD4 cell count, increased viral load, and more rapid clinical progression compared to AG and GG genotypes (93).

16. DISCUSSION

TLRs, as key component of innate immune system, have the capability to sense the invading pathogen through differential recognition of PAMPs. They are responsible for eliciting innate immune effectors through production of inflammatory cytokines (94). The triggering of TLRs leads to stimulation of TLR induced transcription factor, NF-kB and activation of adaptive immunity of the host (20, 58). Recently, occurrence of genetic polymorphism or mutations has been reported in TLRs. Studies have demonstrated that mutations in TLRs influence signal transduction molecules, resulting in increased or decreased susceptibility to various bacterial and viral infections (76). Several studies have also highlighted the association between TLR polymorphisms and increased susceptibility or protection against several infectious diseases (95, 96, 97).

However, it is interesting to note that majority of genetic studies available, focused on patients and cohorts of Western ancestry, which is in striking contrast with the Indian population, characterized primarily by the presence of HIV clade-C virus. The genetic factors significantly varying among and between different ethnic groups highlighted the importance and a strong need for specific population-based case-control studies to be conducted in HIV-infected patients of Indian ethnic origin. Therefore, it was planned to investigate the role of SNPs in TLRs in susceptibility to HIV and TB in ethnically homogenous ART and ATT naïve HIV-positive patients representing the north Indian population. HIV- seropositivity is the most potent of all known risk factors for LTBI reactivation and TB is the foremost and

commonest opportunistic infection occurring among HIV- seropositive individuals (98). The impact of HIV-TB co-infection is bi- directional and they are often referred to as "cursed duet". The consequence of two diseases occurring together in synergy is greater as compared to either of them present alone (99). TB elevates the cellular markers of immune activation on T-lymphocytes and stimulates viral replication. This facilitates faster disease progression, development of AIDS and reduced human life expectancy (100).

However, the AIDS epidemic is characterized by remarkable heterogeneity of HIV- infection and in the clinical course of full-blown disease. While a vast majority (70-80%) of the HIV-infected individuals develop AIDS after 8-10 years of latency, others develop symptoms at a much earlier stage (101). Indeed, inter-individual variability is the hallmark of HIV-infection and its progression to AIDS (11, 12, 13). HIV-infected individuals exhibiting high degree of variability in susceptibility to associated complications and progression to profound immunodeficiency have encouraged researchers to focus attention towards defining immunological correlates of protection and / or disease progression. It is important to understand the host genetic factors and their interaction with the immune and virological factors, as this could yield important information on the immunopathogenesis of HIV-infection. The advancement of technologies in recent years has allowed us to appreciate the host genetics of HIV/AIDS using candidate gene-based approaches. The whole genome analysis is possible through advanced gene discovery approaches and integration through systems biology.

Large cohort and candidate gene-based studies have highlighted the importance of host determinants comprising several immune response genes that contribute towards differential vulnerability of individuals to HIV/AIDS and TB. A large accumulation of data has demonstrated significant contribution of HLA haplotypes to this variability. Several other genes that regulate HIV cell entry (chemokines coreceptors and their ligands) and influence innate and acquired immunity (TLR, MHC, KIRs and cytokines) are known to influence HIV-infection. There is compelling evidence that genetic variants significantly influence HIV viral load set point, rate of CD4 T cell decline, susceptibility to specific AIDS defining illnesses and response to antiviral therapy (101, 102). It is well established that as soon as the human body is invaded by any pathogen, the innate immune system, acting as first line of defense system, provides natural resistance to the intruders. The first few hours or the days of infection are very crucial in determining the fate of pathogen and establishment of infection. Once pathogen is established within the host, progression to clinical disease occurs. During this period, innate immunity coordinates non-specific defense mechanism within the host. However, soon thereafter adaptive immune facilitates more specific immune response against the pathogen. It

was therefore contemplated that mutation within the elements of immune system may provide insight into the underlying mechanism responsible for causing variability in humans towards infectious pathogen.

This chapter determines the importance of TLR polymorphisms as genetic markers for disease susceptibility and progression in the Indian population. Despite appreciably high number of HIV infected people in the Indian subcontinent, this objective had not been addressed comprehensively in previous studies. The central role of TLRs in innate immune response and initiation of appropriate adaptive response have suggested that regulation of their expression might be important in determining the HIV-1 disease course.

REFERENCE

[1] Masur H, Michalis MA, Greene JB. An outbreak of community acquired pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med 1981; 305: 1431.

[2] Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983; 220 (4599): 865-867.

[3] Sinoussi FB, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983; 220 (4599): 868-871.

[4] Aldrich, Robert, Wotherspoon Garry. Who's who in gay and lesbian history. From Antiquity to World War II, Routledge (UK), 2001.

[5] Zolopa AR, Andersen J, Komarow L, Sanne I, Sanchez A. Early Antiretroviral Therapy Reduces AIDS Progression/Death in Individuals with Acute Opportunistic Infections: A Multicenter Randomized Strategy Trial. PLoS ONE 2009; 4(5).

[6] Ferwerda B, Kibiki GS, Netea MG. The toll-like receptor 4 Asp299Gly variant and tuberculosis susceptibility in HIV-infected patients in Tanzania. AIDS 2007; 21:1375-137.

[7] Manabe YC, Bishai WR. Latent Mycobaterium tuberculosis -persistence, patience, and winning by waiting. Nat Med 2000; 6: 1327-9.

[8] Rothel JS, Andersen P. Diagnosis of Latent Mycobacterium Tuberculosis infection: is the demise of Mantaux test imminent, Expert Rev. Anti Infect. 2005; 3(6): 981-993.

[9] Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. Science 1996; 272:54-60.

[10] Sonnenberg P, Glynn J, Fielding K, Murray J, Godfrey-Fausset P, Shearer S *et al.* How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African Gold Miners. *JID 2005;* **191**, 150-158.

[11] Mallal S, Nolan D, Witt C. Association between presence of HLA-B 5701, HLADR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet 2002; 359:727-32.

[12] Rauch A, Nolan D, Thurnheer C. Refining abacavir hypersensitivity diagnosis using a structured clinical assessment and genetic testing in the Swiss HIV cohort study. Antivir Ther 2008; 13: 1019-28.

[13] Mahungu TW, Johnson MA, Owen A, Black DJ. The impact of pharmacogenetics on HIV therapy. Int J STD AIDS 2009; 20:145-51.

[14] Langford SE, Ananworanich J, Cooper DA. Predictors of disease progression in HIV

infection: a review, AIDS Research and Therapy 2007; 4:11.
[15] Medzhitov R, Janeway CA. Decoding the Patterns of Self and Non self by the Innate Immune System. Science 2002; 296 (298-300).
[16] Janeway C.A. The Road Less Traveled: The Role of Innate Immunity in the Adaptive Immune Response - Presidential Address to The American Association of Immunologists. Journal of Immunology 1998; 161(2):539-544.
[17] Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient an MyD88-deficient mice are highly susceptible to Staphylococcus infection. J Immunol 2000 ; 165: 5392-6.
[18] Bowie AG, Haga IR. The role of toll-like receptors in the host response to viruses. Mol Immunol 2005; 42:859-67.
[19] Barton GM. Viral recognition by toll-like receptors. Semin Immunol 2007; 19(1):33-40.
[20] Bafica A, Scanga CA, Schito M, Chaussabel D, Sher A. Influence of coinfecting pathogens on HIV expression: evidence for a role of toll-like receptors. J Immunol 2004; 172:7229-7234.
[21] Heggelund L, Damas JK, Yndestad A. Stimulation of toll-like receptor 2 in mononuclear cells from HIV-infected patients induces chemokine responses: possible pathogenic consequences. Clin Exp Immunol. 2004; 138:116-21.
[22] Mackichan ML. Toll bridge to immunity. The News Letter on International AIDS Vaccine Research. IAVI Report 2005.
[23] Holmes E. On the origin and evolution of the HIV. Biol Rev Camb Philos Soc 2001; 76:239-54.
[24] Lemey P, Pybus OG, Wang B, Saksena NK, Salemi M, Vandamme AM et al. Tracing the origin and history of the HIV-2 epidemic.Proc Natl Acad Sci USA 2003;100:6588-92.
[25] Global report: UNAIDS report on the global AIDS epidemic 2023.
[26] NACO report: National AIDS Control Organization | MoHFW2023.
[27] McGovern SL, Caselli E, Grigorieff N, Shoichet BK. A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. J Med Chem 2002; 45 (8): 1712-22.
[28] Ratner L, Haseltine W, Patarca R. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 1985; 313 (6000): 277-84.
[29] Chan DC, Fass D, Berger JM, Kim PS. Core structure of gp41 from the HIV envelope glycoprotein. Cell 1997; 89 (2):263-73.
[30] Moore RD, Chaisson RE. Natural history of HIV infection in the era of combination antiretroviral therapy. AIDS 1999; 13, 1933-42.
[31] Cooper DA, Gold J, Maclean P, Donovan B, Finlayson R, Barnes TG et al. Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. Lancet. 1985; 1(8428):537-40.
[32] Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. N Engl J Med. 1998; 339(1):33-9.
[33] Vanhems P, Dassa C, Lambert J, Cooper DA, Perrin L, Vizzard J et al. Comprehensive classification of symptoms and signs reported among 218 patients with acute HIV-1 infection. J Acquir Immune Defic Syndr. 1999; 21(2):99-106.
[34] Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995; 373 (6510):123-6.
[35] Osmond D, Charlebois E, Lang W, Shiboski S, Moss A. Changes in AIDS survival time in two San Francisco cohorts of homosexual men, 1983 to 1993. JAMA. 1994; 271(14):1083-7.
[36] Longini IM, Clark WS, Gardner LI, Brundage JF. The dynamics of CD4+ T-

lymphocyte decline in HIV-infected individuals: a Markov modeling approach. J Acquir Immune Defic Syndr. 1991; 4(11):1141-7.
[37] Hay CM, Rahl DJ, Basgoz NO. Lack of viral escape and defective *in vivo* activation of human immunodeficiency virus type-1 specific cytotoxic T lymphocytes in rapidly progressive infection. J of Virol 1999; 73: 5509.
[38] Sheppard HW, Lang W, Ascher MS, Vittinghoff E, Winkelstein W. The characterization of non progressors: Long term HIV-1 infection with stable CD4 T cell levels. AIDS 1993; 7:1159-66.
[39] Janeway CA. How the immune system works to protect the host from infection: a personal view. Proc. Natl. Acad. Sci. U. S. A. 2001; 98:7461-8.
[40] Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. Science 1996; 272:50-3.
[41] Janeway CA, Medzhitov R. Innate immune recognition. Annu. Rev.Immunol 2002; 20:197-216.
[42] Hopkins PA, Sriskandan S. Mammalian toll-like receptors: to immunity and beyond. Clin. Exp. Immunol 2005*;* 140:395-407.
[43] Medzhitov R, Janeway CA. Innate immunity: impact on the adaptive immune response. Curr. Opin. Immunol 1997; 9:4-9.
[44] Luster AD. The role of chemokines in linking innate and adaptive immunity. Curr. Opin. Immunol 2002; 14:129-35.
[45] Wira CR, Grant-Tschudy KS, Crane-Godreau MA. Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. Am. J Reprod. Immunol 2005; 53:65-76.
[46] Tabeta K, Georgel P, Janssen E. Toll-like receptor 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc.Natl.Acad.Sci.U.S.A. 2004; 101(10):3516-21.
[47] Medzhitov R., Preston-Hurlburt P., Janeway CA. A human homologue of the Drosophila toll protein signals activation of adaptive immunity. Nature 1997; 388: 394-397.
[48] Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406:782-787.
[49] Ahmad-Nejad P, Hacker H, Rutz M, Bauer S, Vabulas R. M, Wagner H et al. Bacterial CpG-DNA and lipopolysaccharides activate toll-like receptors at distinct cellular compartments. Eur. J. Immunol 2002; 32:1958.
[50] Bell JK, Mullen GE, Leifer CA, Mazzoni A, Davies DR, Segal DM et al. Trends Immunol 2003; 24(10):528-33.
[51] Smirnova I, Poltorak A, Chan EK, McBride C, Beutler B: Phylogenetic variation and polymorphism at the toll-like receptor 4 locus (TLR4). Genome Biol 2000; 1(1).
[52] Hajjar AM, Ernst RK, Tsai JH, Wilson CB, Miller SI. Human toll-like receptor 4 recognizes host specific LPS modifications. Nature Immunol 2002; 3:354-9.
[53] Imler JL, Hoffmann JA: Toll receptors in innate immunity. Trends Cell Biol 2001, 11:304-311.
[54] Akira S, Takeda K. Toll-like receptor signaling. Nat. Rev. Immunol 2004; 4:499-511.
[55] Takeda K, Akira S. TLR signaling pathways. Semin. Immunol 2004; 16:3-9.
[56] Vogel SN, Fitzgerald KA, Fenton MJ. TLRs: differential adapter utilization by toll-like receptors mediates TLR-specific patterns of gene expression. Mol Interv 2003; 3:466-77.
[57] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu. Rev. Immunol 2003; 21:335-76.
[58] Kawai T, Akira S. Toll-like receptor downstream signaling. Arthritis Res. Ther 2005; 7:12-9.
[59] Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa. Innate antiviralresponses by

means of TLR7-mediated recognition of single-stranded RNA. Science 2004*;* 303:1529-31.
[60] Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K et al. Small anti-viral compounds activateimmune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol 2002; 3:196-200.
[61] Heil F, Ahmad-Nejad P, Hemmi H, Hochrein H, Ampenberger F, Gellert T et al. The toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. Eur. J Immunol 2003; 33:2987-97.
[62] Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K et al. Cutting edge: a novel toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the toll-like receptor signaling. J. Immunol 2002; 169:6668-72.
[63] Farina C, Krumbholz M, Giese T, Hartmann G, Aloisi F, Meinl E et al. Preferential expression and function of toll-like receptor 3 in human astrocytes. J Neuroimmunol 2005; 159:12-9.
[64] Imler JL, Hoffmann JA. Toll signaling: the TIR less quest for specificity. Nat. Immunol 2003; 4:105-6.
[65] Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 2003; 301:640-3.
[66] Seya T, Oshiumi H, Sasai M, Akazawa T, Matsumoto M. TICAM-1 and TICAM-2: toll-like receptor adapters that participate in induction of type 1 interferons. Int. J Biochem. Cell Biol 2005; 37:524-9.
[67] Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E et al. LPS-TLR4 signaling to IRF-3/7 and NF-kappaBinvolves the toll adapters TRAM and TRIF. J Exp. Med. 2003; 198:1043-55.
[68] McGettrick AF, O'Neill LA. The expanding family of MyD88-like adaptors in toll-like receptor signal transduction. Mol. Immunol 2004; 41:577-82.
[69] Wietek C, Miggin SM, Jefferies CA, O'Neill LA. Interferon regulatory factor-3-mediated activation of the interferon-sensitive response element by toll-like receptor (TLR) 4 but not TLR3 requires the p65 subunit of NF-kappa. J Biol Chem 2003; 278:50923-31.
[70] Nomura N, Miyajima N, Sazuka T, et al. Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1 [published correction appears in DNA Res 1995 Aug 31;2(4):following 210]. DNA Res. 1994;1(1):27-35. doi:10.1093/dnares/1.1.27
[71] Belvin MP and Anderson K V. A conserved signaling pathway: the Drosophila toll-dorsal pathway. Annu. Rev. Cell Dev. Biol 1996; 12, 393-416.
[72] Kaisho T, Akira S. Pleiotropic function of toll-like receptors. Microbes. Infect. 2004; 6:1388-94.
[73] Datta SK, Redecke V, Prilliman KR, Takabayashi K, Corr M, Tallant T et al. A subset of toll-like receptor ligands induces cross-presentation by bone marrow-derived dendritic cells. J Immunol 2003; 170:4102-10
[74] Vaidya SA, Cheng G. Toll-like receptors and innate antiviral responses. Curr. Opin. Immunol 2003 15:402-7.
[75] Sen GC, Sarkar SN. Transcriptional signaling by double-stranded RNA: role of TLR3. Cytokine Growth Factor Rev 2005; 16:1-14.
[76] Schroder NW, Schumann RR. Single nucleotide polymorphisms of toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis 2005; 5:156-164.
[77] Mockenhaupt FP, Cramer JP, Hamann L. Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. Proc Natl Acad

Sci USA 2005; 103:177-82.
[78] Equils O, Faure E, Thomas L. Bacterial lipopolysaccharide activates HIV long terminal repeat through toll-like receptor 4. J Immunol 2001; 166:2342-2347.
[79] Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science 1993; 12, 262(5136):1011-8.
[80] Lane H.C. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. 1983; 309, 453-458.
[81] Hellerstein, M. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nat. Med 1999; 5, 83-89.
[82] Hazenberg M.D. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). Blood 2000; 95, 249-255.
[83] Valdez H, Lederman MM. Cytokines and cytokine therapies in HIV infection. AIDS Clin. Rev 1997; 187-228.
[84] Pomerantz RJ, Feinberg MB, Trono D, Baltimore D. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. J. Exp. Med 1990; 172:253.
[85] Molina JM, Scadden DT, Byrn R, Dinarello CA, Groopman JE. Production of tumor necrosis factor α and interleukin 1 β by monocytic cells infected with human immunodeficiency virus. J. Clin. Invest 1989; 84:733.
[86] Sundstrom JB, Little DM, Villinger F, Ellis JE, Ansari AA. Signaling through toll-like receptors triggers HIV-1 replication in latently infected mast cells. J. Immunol 2004; 172 (7):4391-401.
[87] Lawn SD. AIDS in Africa: impact of co-infections on the pathogenesis of HIV-1 infection.J.Infect 2004; 48(1):1-12.
[88] Toossi Z. Virological and immunological impact of tuberculosis on human immunodeficiency virus type1disease.J.Infect.Dis 2003; 188:1146.
[89] Nunn P, Williams B, Floyd K, Dye C, Elzinga G, Raviglione M et al. Tuberculosis control in the era of HIV. Nat Rev Immunol 2005; 5:819-826.
[90] Reid A, Scano F, Getahun H, Williams B, Dye C, Nunn P et al. Towards universal access to HIV prevention, treatment, care,and support: the role of tuberculosis/HIV collaboration. Lancet Infect Dis 2006; 6: 483-495.
[91] Abel B, Thieblemont N, Quesniaux VJ, Brown N, Mpagi J, Miyake K et al. Toll-like receptor 4 expression is required to control chronic Mycobacterium tuberculosis infection in mice. J Immunol 2002; 169:3155-3162.
[92] Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ et al. Human toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. J Immunol 1999; 163:3920-3927.
[93] Soriano-Sarabia N, Vallejo A, Ramı´rez-Lorca R, Rodrı´guez MM, Salinas A, Pulido I et al. Influence of the Toll-Like Receptor 9 1635A/G Polymorphism on the CD4 Count, HIV Viral Load, and Clinical Progression J Acquir Immune Defic Syndr 2008;49:128-135.
[94] Medzhitov R, Janeway CA. How does the immune system distinguish self from nonself? Seminars in Immunology 2000; 12:185-188.
[95] Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I et al. The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. Eur. Respir. J, 2004; 23:219-23.
[96] Tal G, Mandelberg A, Dalal I, Cesar K, Somekh E, Tal A et al. Association between common toll-like receptor 4 mutations and severe respiratory syncytial virus disease. J Infect Dis 2004; 189:2057-2063.

[97] Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. J Exp Med 2003; 198:1563-1572.
[98] Sharma SK, Mohan A, Kadhiravan T. HIV-TB co-infection: Epidemiology, diagnosis & management. Indian J Med Res 2005; 121:550-567.
[99] Dye C. Williams B.G. "The population dynamics and control of tuberculosis,"Science 2010; 328, 5980, 856-861.
[100] Sodora DL, Silvestri G. Immune activation and AIDS pathogenesis. AIDS 2008; 22:439-446
[101] O'Brien SJ and nelson GW. Human genes that limit AIDS. Nat Genet 2004; 36(6):565-74.
[102] Kaur G and Mehra. Genetic determinants of HIV-1 infection and progression to AIDS: susceptibility to HIV infection. Tissue Ag 2009; 73 (4):289-301.