2. Review of literature

2.1 Complement system: its activation, regulation and biosynthesis

The complement system is a set of biochemical pathways that removes pathogen components from an organism as part of the innate and acquired immunity defense programs. Activation of the complement system initiates a wide range of cellular responses ranging from apoptosis to opsonization (Holt et al. 2001).

2.1.1 Complement activation

The complement system consists of about 30 soluble and membrane bound proteins, and is activated by 3 distinct pathways either on pathogen surface or in plasma (Figure 1). Activation of these pathways depends on different molecules, present on foreign surface or plasma, for their initiation. The classical pathway is triggered by antigen-bound antibody molecules and is initiated by the binding of the C1 component to the Fc region of the antibody molecule (Morgan 1999). Cleavage of C3 and then C5 activates the alternative pathway which is a humoral component of the immune system’s natural defense against infections. The mannose-binding lectin (MBL) pathway is initiated by the plasma Mannose Binding Lectin (MBL) protein forming a complex with the MBL-associated proteases 1 and 2. MASP1 and MASP2 then bind to arrays of mannose groups on the surface of a bacterial cell (Walport 2001a).

All three activation pathways converge at the level of C3 to form the C5 convertase. The C5 convertase then cleaves C5 to form C5b and C5a. Recently thrombin has also been identified as a C5 convertase in C3 deficient mice, which are capable of cleaving C5 to form C5b and C5a. Thus, thrombin generated from clotting pathway is considered as another complement activation pathway, which provides
another molecular pathway linking clotting pathway to complement activation pathway (Huber-Lang et al. 2006). Terminal complement activation is induced by C5b, and followed by the sequential condensation of C6 to form C5b6, and then C7, C8, and C9. C9 bound to the C5b-8 complex polymerises to forms the membrane attack complex (MAC). The MAC forms a lytic pore in the lipid bilayer membrane that allows the solutes and water passage across the membrane and destroys membrane integrity, followed by destruction of the foreign pathogens and cells (Mayer 1984).

Figure 1: Activation pathways resulting in the formation of MAC (Morgan 1999)

Role of complement in bridging innate and adaptive immunity

Complement activation by-products that bridge innate and adaptive immunity are critical mediators of the host defense against infection. They are also involved in the disposal of immune complexes and products of inflammatory injury (Walport 2001a). C3b and C4b bound to immune complexes potentiate antibody response and
Review of literature

enhance immunologic memory (Walport 2001b). Complement fragments C3a, C4a and C5a act on specific receptors to produce local inflammatory responses. These fragments are termed as anaphylatoxins because they can cause degranulation of mast cell and the release of histamine. Of the three, C5a is the most stable and has the highest specific biological activity, which is being studied in different diseases including liver diseases.

2.1.2 Complement Regulation

Tick over complement activation is a process by which all complement components are activated spontaneously at a low rate in plasma. This tick over activation of complement occurring in vivo is a serious threat to host cells. However, host cells express a plethora of plasma and membrane proteins that can inhibit self complement activation and thereby preventing self damage (Fisicaro et al. 2000) (Figure 2). At least 10 plasma or membrane-bound proteins have been found to keep vigil and put a check on the activation of complement and subsequently prevent the cataclysmic effect of complement activation on host cells. This complement regulatory system generally acts on the point of instability of activation pathway enzymes and reduces the production of activated complement. The soluble plasma complement regulators include C1 inhibitor (C1INH), Complement Factor H (CFH), Complement Factor I (CFI), C4 binding protein (C4bp), S protein, Clusterin. The membrane bound fraction include 3 membrane proteins that are expressed on the surface of almost all cell types have been shown to inhibit autologous complement activation, thereby protecting self cells from consequent complement-mediated injury (Morgan 1999).
2.1.3 Biosynthesis of plasma complements and expression of complement receptors in the liver

The hepatocytes is responsible for biosynthesis of major proportion of plasma complement components and their soluble regulators, including the classical (C1r/s, C2, C4, C4bp), alternative (C3, complement factor B), lectin (MBL, MASP1-3, Map19), terminal (C5, C6, C8, C9) pathways of the complement system, and soluble regulators (factors I, H, and Cl inhibitor) (Morgan and Gasque 1997). A minor contribution to plasma levels of complement system is also attributed to many other types of cells including immune cells and endothelial cells.

Several other plasma components including C1, factor D, properdin, and C7 are mainly produced by cells outside of the liver. C1 is comprised of three subcomponents including C1q, C1r, and C1s. C1q has found to be produced by many cell types including epithelial cells, fibroblasts, and cells of the monocyte/macrophage lineage.
(Tenner and Volkin 1986; Gulati et al. 1993). Major source for plasma C1 is monocytes and they can produce all three C1 subcomponents to form intact C1. Hepatocytes only produce C1r and C1s but not C1q, and therefore intact C1 assembly is not possible in hepatocytes. The biological significance of C1r and C1s synthesized in hepatocytes is elusive. Complement factor D is mainly produced by adipocyte (Choy, Rosen, and Spiegelman 1992; White et al. 1992) while plasma properdin is produced primarily by macrophages and monocytes (Maves and Weiler 1993) and C7 (Würzner, Joysey, and Lachmann 1994). Finally, soluble complement regulatory proteins are produced by hepatocytes (Morgan and Gasque 1997) while membrane bound complement components including CD35, CD59, CD55 and CD46 are expressed omnipresently in all tissues (Mead, Hinchliffe, and Morgan 1999; Harris, Rushmere, and Morgan 1999; X. Qin et al. 2001).

2.2 COMPLEMENT SYSTEM: RELEVANCE TO VIRAL BIOLOGY

This section focuses on the interaction of viruses with complement system, highlighting the protective role of complement against viral infection, the mechanisms used by viruses to evade the effects of complement and the ways in which some viruses can exploit complement to enhance infection.

2.2.1.a. Complement activation during virus infection

It has been found that during viral infection all three pathways of the complement cascade are activated. The binding of the complement component C1q to antibody–antigen complexes activates the classical pathway. In the absence of specific antibodies this activation can also be achieved by C1q binding directly to the glycoproteins of some viruses. These viruses include certain retroviruses (Cooper et al. 1976; Sölder et al. 1989) like human T cell lymphotropic virus (HTLV)(Ikeda et al. 1998) and human
cytomegalovirus (HCMV) (Spiller and Morgan 1998). The antibody independent pathway, lectin pathway, is antibody-independent and is activated by the interaction of viral surface carbohydrates with mannan-binding lectin (MBL). This pathway has been involved in the control of many viral infections, including influenza (Hartshorn et al. 1993; Thielens, Taenett-Delorme, and Arlaud 2002) and hepatitis B virus (HBV) (F.-S. Wang 2003). The alternative pathway was originally defined as the antibody-independent activation pathway, activation of which is triggered by the spontaneous and continual low-level release of the internal thioester bond, called the tick over, of C3 to form C3 convertase. The C3 convertase may either exist attached to the cell surface or remain in the fluid phase. Further activation of the alternative pathway only occurs when there is a foreign surface which lacks factor H bound to its surface.

All the three pathways of complement activation result in several effector functions that contribute to inactivation and elimination of foreign particle. These functions include opsonisation of virions by complement components promoting phagocytosis, virolysis by the membrane attack complex. Immune responses are also enhanced through the production of anaphylatoxins and chemotactic factors.

2.2.1.b. Virus opsonisation

Binding of C1q, C3b, and C4b to the virion surface forms a protein coat and neutralise viral infection in a plethora of ways. Phagocytic cells possess surface receptors that recognise and bind to foreign surface attached to C1q, C3b and C4b, that promote uptake and destruction of virus particles (Krych-Goldberg and Atkinson 2001; McGreal and Gasque 2002; Hannan et al. 2002). The prevention of viral interaction with receptors and viral cell entry is facilitated by covalent attachment of C3b and C4b to the virion surface. In many viruses including human immunodeficiency virus (HIV),
coating of surface with C3 has resulted in functional inactivation of the virus both in vitro and in vivo (Sullivan, Takefman, and Spear 1998). It has been demonstrated that binding of multivalent complement components result in viral particles aggregation resulting in neutralization of virus infectivity and promotion of phagocytosis via Fc receptors.

2.2.1.c. Virolysis

Although opsonisation itself can aptly inactivate and destroy infectious virions, enveloped viruses have also been found to be susceptible to lysis by the membrane attacking complex (MAC). The complement components from C5b to C9 combine and rupture into the viral envelope to form a transmembrane channel. This channel results in a bidirectional flow of ions and macromolecules, which disrupts viral osmotic equilibrium, membrane integrity and eventually to lysis and irreversible loss of viral pathogenicity. Retroviruses, Alphaviruses, herpesviruses and coronaviruses, are some that are susceptible to membrane attack complex based killing (Vasantha et al. 1988; Mochizuki et al. 1990; Spear et al. 1993).

2.2.1.d. Anaphylatoxic and chemotactic role

During complement activation complement components C3, C4, and C5 are enzymatically cleaved resulting in the production of low molecular weight, biologically active peptides. These anaphylatoxins, C3a, C4a, and C5a, are involved in activation of many cell types, co-occurring with the wide range of cells which express receptors on their surface (Ember and Hugli 1997). C5a and C3a functions as a chemo-attractant for leukocytes eosinophils, neutrophils and T-cells and recruits them to the region of complement activation and inflammation. These anaphylatoxins are also involved in plethora of inflammatory responses which includes release of vasoactive amines,
smooth muscle contraction, enhanced vascular permeability and induction of lysosomal enzyme release.

2.2.1. e. Multifaceted effects of complement

Complement system is involved in the induction of humoral immunity against viruses. Enhanced viral antigen presentation to B-cells is achieved by virus particle, opsonised by C3b and iC3b, binding to follicular dendritic cells (FDC) in lymph nodes via the complement receptors CR1 and CR3. C3dg combined viral antigen has been also found to induce cross-linking of the B cell surface attached CR2 and B-cell antigen receptor. Studies have shown that complement component deficient mice fail to initiate normal memory responses to herpes simplex virus (HSV) (Da Costa et al. -1999). Furthermore, augmentation of the antigenicity of the HIV envelope glycoproteins encoded by env (and other viral antigens) was achieved by creating a DNA vaccine for evaluation in mice, whereby env was fused to the human or murine genes encoding C3d (Green et al. 2001).

2.2.2. Viral strategies to evade complement system

Viruses have evolved numerous evasive mechanisms to bypass the destructive effects of complement. They include active strategies in which certain viruses encode their own complement regulatory proteins and passive strategies, where cellular complement regulatory proteins are incorporated into the envelope of viruses as they bud from the cell surface.

2.2.2.a. Incorporation of host complement regulatory proteins into virus envelopes

The mechanism of incorporation of host regulator of complement activation proteins (RCA) and CD59 during emergence from the host cell is utilized by some enveloped viruses to shield the virion from complement-mediated lytic attack. Various isolates of
HIV can be immunocaptured by anti-CD59 antibodies, anti-DAF and anti-MCP antibodies (Saifuddin et al. 1997). Incorporation of all these three proteins has been demonstrated to protect virions from complement-mediated lysis (Saifuddin et al. 1997). Some other viruses like HTLV, HCMV and Vaccinia virus protect themselves against complement mediated attack by incorporating host complement regulatory proteins into their envelope (Spear et al. 1995).

2.2.2.b. Abrogation of complement activation by antibody–antigen complexes and viral Fc receptors

Some viruses, including psuedorabies virus (PRV), a swine a-herpesvirus, have developed the ability to shed or internalise antibody antigen complexes from their surface forbidding complement activation or phagocytosis (Van de Walle et al. 2003). Viruses like Human Simplex Virus, Human Cytomegalo Virus and varicella-zoster virus or virus-infected cells expressing Fc like receptors prevents complement activation or sterically impede access of specific antivirus antibody or by mimicking Fc portion of nonspecific immunoglobulin G (IgG) (Dowler and Veltri 1984a; Litwin and Grose 1992; Antonsson and Johansson 2001). In this regard, binding of nonimmune Immunoglobulin G to Human Simplex Virus-1 infected cells has been shown to protect them against complement-mediated lysis and to protect Human Simplex Virus-1 virions from antibody neutralisation (Dowler and Veltri 1984b). It has been reported that HIV interferes with downstream signaling which impede complement-mediated phagocytosis of infected cells (Kedzierska et al. 2003).
2.2.2.c. Virus-encoded proteins

Homologues of Host complement control protein homologues
Many members of the herpesvirus and poxvirus families incorporate different mechanism, which encode complement control protein homologues that are known to incorporate plethora of mechanism to inhibit the antiviral effects of complement. Some of the poxviruses such as vaccinia virus, variola and cowpox encode functional homologues of complement control proteins. Out of these the vaccinia virus complement control protein has been most extensively characterised. VCP contributes to virulence in animal models, and results in prevention of antibody-complement dependent neutralisation of vaccinia virus (Isaacs, Kotwal, and Moss 1992). VCP acts as a cofactor for CFI-mediated cleavage of C3b and C4b resulting in hastens decay of classical and alternative pathway convertases (McKenzie et al. 1992; Sahu et al. 1998).

Complement control proteins lacking homology to cellular proteins
The only characterized viral complement control proteins lacking any homology to host complement regulatory proteins are the gC glycoproteins of HSV-1 and -2 and was the first viral protein identified as binding complement (Friedman et al. 1984; Fries et al. 1986). Even though there is homology between the gC glycoproteins of HSV-1 and -2, they demonstrate substantial differences in their adroitness to regulate complement. The acceleration of the decay of alternative pathway convertase is carried out by HSV-1 gC protecting it from the complement mediated attack of this pathway (Hung et al. 1994). Mutation in Human Simplex Viruses-1 gC have been reported to incapacitate binding of complement component and abrogates pathogenicity in an animal model of infection (Lubinski et al. 1998). HSV-2 gC does not accelerate the decay of the alternative pathway convertase but provide protective effect against complement-
mediated neutralization which is weaker as compared to HSV-1 gC. Epstein–Barr virus (EBV) ability to regulate complement activity without encoding for any proteins that show homology to host RCA proteins insinuates that much remains to be studied about these viral proteins (Mold et al. 1988a).

2.3 ROLE OF COMPLEMENTS IN DISORDERS PERTAINING TO LIVER

2.3.1 Liver fibrosis

Role of complement component 5 and C5aR have been demonstrate to exhibit important role in pathogenesis of liver fibrosis (Hillebrandt et al. 2005). The proposition that C5 is a strong candidate gene for the liver fibrosis was further substantiated by several experimental evidences (Hillebrandt et al. 2005). Firstly, C5 sufficient strains transformation to C5 deficient strain resulted in the fibrosis-resistant phenotype. Secondly, development of fibrosis-susceptible phenotype by transgenic introduction of C5 gene into C5 deficient inbred strain. Finally, attenuation of liver fibrosis in mice by blocking of C5 receptor 1 (C5aR1). Genetic analyses also suggest that genetic variation in human C5 gene are also involved in liver fibrosis in patients with chronic HCV infection (Hillebrandt et al. 2005). However, the molecular mechanisms responsible for the involvement of C5aR1 in liver fibrosis remain obfuscated. Another pathway contributing to the involvement of C5aR1 in liver fibrosis is the stimulation of C5aR1 by C5a which results in release of prostanoid in hepatic stellate cells and upregulation of fibronectin expression is another pathway by which C5aR1 involves in liver fibrosis (Schieferdecker et al. 1997; Schlaf et al. 2004).

2.3.2 Alcohol induced liver injury

The low serum levels of complement components in alcoholic cirrhotic patients than normal healthy individuals has been associated with high risk of bacterial and fungal
infections in these patients (Homann et al. 1997). Recent studies in animal models insinuate that in alcoholic liver disease there is activation of complement system and have contributing role in its pathogenesis. Both endotoxin and oxidative stress have been involved in complement activation and severity in alcoholic liver disease (Collard et al. 1999). Addition of methanol diet in mice and rats resulted in decreased expression of complement regulators Crry and CD59 but increased disposition of C1, C3, C8, and C9 (I. L. Bykov et al. 2004). C6-deficient mice are more predisposed to ethanol-induced hepatic injury and steatosis (I. L. Bykov et al. 2004) while C3-deficient mice are resistant to ethanol-induced hepatic steatosis, serum alanine aminotransferase activity and elevation of liver malondialdehyde level (I. Bykov et al. 2006). These studies suggest that C6 may protect against alcohol-induced liver injury while C3 results in the pathogenesis of alcoholic disease. However, clinical data suggested that there was no difference in the activation of complement in normal healthy control groups and acute alcoholic hepatitis patients and that there was no persisting relationship between clinical or laboratory indicators of complement activation and disease severity in acute alcoholic hepatitis. The above findings are suggestive of the fact that complement activation may have no relation to the clinical and histological features of human alcoholic liver disease (Bird et al. 1995). Further studies are needed to overcome the paucity in defining precise roles of the complement system in alcoholic liver disease.

2.3.3 Liver regeneration

Complement has also been involved in liver regeneration after partial hepatectomy or after toxic injury (Mastellos et al. 2001; Strey et al. 2003; Markiewski et al. 2004). The anaphylatoxins C3a and C5a importance in liver regeneration was demonstrated by
Strey et al., by using a murine model of partial hepatectomy (Strey et al. 2003). C3 or C5 deficiency results in vitiated liver regeneration, often accompanied by fatal or transient liver failure after partial hepatectomy (Strey et al. 2003). Impairment of liver regeneration was observed in C3 and C5 double knockout mice, which was reinstated after administration of C3a and C5a (Strey et al. 2003).

Experiments were also performed to understand the role of C3 and C5 in liver regeneration after CCl₄-induced liver damage (Mastellos et al. 2001; Markiewski et al. 2004). Severely defective liver regeneration was found in C5-deficient mice which also showed persistent parenchymal necrosis post CCl₄ exposure. Their mitotic activity was diminished and the re-entry of hepatocytes into the S-phase of the cell cycle was also delayed (Mastellos et al. 2001). It was observed that C5aR blockage resulted in abrogation of liver cell regeneration during liver injury and the simultaneous reconstitution of murine C5 or C5a restored hepatocyte regeneration in C5 deficient mice (Mastellos et al. 2001). C3a reconstitution resulted in restoration of delayed liver regeneration, post CCl₄ injection, induced by disruption of C3 gene (Markiewski et al. 2004). Lack of C3a receptor also contributed to impaired liver regeneration in mice model (Markiewski et al. 2004). These studies insinuate that after liver injury or loss of tissue both C3 and C5 participate in liver regeneration (Mastellos et al. 2001; Strey et al. 2003; Markiewski et al. 2004). The repertoire involves increasing STAT-3 and NF-κB priming signals, the two most important signaling pathway for the initiation of the regenerating response (Fausto, Campbell, and Riehle 2006).

2.3.4 Liver ischemia/reperfusion injury and transplantation

Liver ischemia/reperfusion (I/R) is a complex inflammatory response occurring during liver surgery and transplantation. The inflammatory response follows temporary
deprivation of blood supply. It comprises of two distinct phases, the initial and later phases of injury. In the initial phase there is increased oxidative stress and Kupffer cell activation whereas massive neutrophil infiltration is the hallmark of the later phase (Hartmut Jaeschke 2006). The involvement of complement activation in liver I/R injury in a rat model was first reported by Jaeschke and colleagues (H. Jaeschke et al. 1993) where they had demonstrated that cobra venom factor induced depletion of serum complement before ischemia attenuated Kupffer cell-induced oxidant stress, hepatic injury caused by ischemia/reperfusion and accumulation of PMNs in the liver (H. Jaeschke et al. 1993). A reduction in total hepatic ischemia/reperfusion induced mortality and accumulation of polymorphonuclear leucocytes in the liver and improvement in partial hepatic ischemia/reperfusion induced liver injury and was observed when treated with C5aR antagonist (Arumugam et al. 2004). sCR1 or C1 inhibitor induced blocking of complement activation also significantly improved inflammation and necrosis during hepatic liver transplantation and I/R injury in rodent models (Chávez-Cartaya et al. 1995; Lehmann et al. 2001; Heijnen et al. 2006). Clinical data substantiate the involvement of complements in liver ischemia/reperfusion injury during human liver transplantation. A positive correlation was found between the number of leukocytes and platelets accumulating within the graft and elevated MAC deposition in the postoperative specimens of patients with liver transplantation. It also associated positively with an increase in postoperative aspartate aminotransferase levels in the serum (Scoazec et al. 1997). I/R by means of the classical pathway likely results in complement activation in the human livers after partial hepatectomy (Heijnen et al. 2006). Schmeding et al., showed that elevation of C4d deposition was detected in 68% of liver allograft biopsies with acute rejection when compared with allografts with
HCV recurrence where it was only 12% and 7% of protocol biopsies from the subjects without rejection or HCV recurrence (Schmeding et al. 2006). This suggests that activation of complement may have a contribution to rejection after liver transplantation (Schmeding et al. 2006) and rejection of hepatic xenotransplantation (Tector et al. 2001). Activation of complement may play a beneficial role in prevention of post liver transplantation infection. Significant decrease in serum mannose binding lectin levels was observed in donor livers carrying MBL variant alleles. It was also found to be associated with an increased incidence of post liver transplant infections. This suggested that activation of complement plays an important role in controlling infection after liver transplantation (Bouwman et al. 2005).

2.3.5 Viral hepatitis

Worldwide, more than half a billion people are chronically infected with the hepatitis C virus (HCV) and hepatitis B virus (HBV), which is a leading cause of liver injury, fibrosis, and cirrhosis. The mechanisms responsible for HBV and HCV persistence and disease pathogenesis remain sketchy, and the interaction of hepatitis viruses and the host immune system is likely involved (Rehermann and Nascimbeni 2005a). Although there has been ample evidence that complement system contribute to the protection of host from infection (Speth, Stoiber, and Dierich 2003; Mehlhop, Fuchs, et al. 2009), the involvement of complement in viral hepatitis has not been fully understood. It was reported that there is cold activation of complement in the patients with chronic HCV infection and that it was useful for monitoring response to interferon therapy in these patients (Nomura et al. 1997; Akahane et al. 1996). However, the reason for high titer of cold complement activation and its role in pathogenesis during HCV infection still remains elusive. A recent study demonstrated that low complement levels were
associated with anti-C1q antibodies an increased prevalence of anti-C1q antibodies was present in HCV-infected patients (Saadoun et al. 2006). The anti-C1q antibodies are associated with immune complex diseases, prominently with systemic lupus erythematosus, hypocomplementaemic urticarial vasculitis syndrome, severe rheumatoid arthritis and diffuse proliferative lupus nephritis (Seelen, Trouw, and Daha 2003). Thus, the high levels of anti-C1q antibodies in HCV patients suggest that immune complex may play a role in the pathogenesis of hepatitis C associated liver injury. Another autoantibody against asialoglycoprotein receptor that is expressed on hepatocytes and mediates clearance of desialylated serum proteins is also involved in the pathogenesis of hepatitis virus-induced liver disease through disrupting clearance of desialylated proteins and activation of the complement-mediated cytolysis (J. Diao, Churchill, and Michalak 1998; Jingyu Diao, Slaney, and Michalak 2003). Membrane attack complex was detected in hepatocytes surrounding necrotic areas in the patients with fulminant and acute hepatitis by Immunohistochemistry analyses (Pham et al. 1995), further supporting the contention that the complement system is activated and is involved in the pathogenesis of Hepatitis C virus-associated liver disease. Impairment of MBL production may result in a decrease of serum MBL levels during Chronic HBV and HCV infection. These findings suggest variant alleles of MBL may dictate plasma levels of MBL and are associated with viral persistence and progression of liver disease after HBV infection.
2.4. HEPATITIS C VIRUS AND CHRONIC HEPATITIS C

Hepatitis C virus (HCV) is a small (55–65 nm in size), single-stranded, enveloped, positive-sense RNA virus. HCV is the cause of hepatitis C in humans (Figure 3).

The hepatitis C virus belongs to the genus Hepacivirus a member of the family Flaviviridae. Initially it was considered to be the only member of Hepacivirus genus. However recently a member of this genus has been discovered in dogs—canine...
hepacivirus (Kapoor et al. 2011). There is also at least one virus in this genus that infects horses (Burbelo et al. 2012). Several additional viruses in the genus have been described in bats and rodents (Quan et al. 2013; Kapoor et al. 2013).

2.4.2. HCV GENOME STRUCTURE AND ORGANIZATION

In this underlying section efforts have been made to explain the HCV genome organization and how its encoded gene products result in a productive life cycle amidst being able to evade the scrutiny of the immune system. HCV belongs to the genus hepacivirus of the Flaviviridae family. The members of the Flaviviridae family are quite similar in their basic structural and virological attributes. They are all lipid bilayer enveloped anchoring to which there are two or more envelope proteins. The envelope contains the RNA genome surrounded the nucleocapsid which is composed of multiple copies of a small basic protein (core or C). The Flaviviridae genome is 9.6 to 12.3 thousand nucleotides molecule, positive-strand RNA, with an open reading frame (ORF) encoding a polyprotein of around 3000 amino acids (aa).

2.4.2.1. 5' UNTRANSLATED REGION

The HCV 5'UTR is the most conserved region of the genome and contains 341 nt located upstream of the ORF translation initiation codon (Choo et al. 1991; Han et al. 1991). The 5'UTR consists of four highly structured domains containing a pseudoknot and numerous stem-loops (E. A. Brown et al. 1992; C. Wang et al. 1995). IRES is comprised of the first 12 to 30 nt of the corecoding region and domains II, III and IV (Honda et al. 1996). The HCV IRES helps in the formation of a stable pre-initiation complex which is achieved by binding the 40S ribosomal subunit without the need of
the orthodox translation initiation factors. This event likely constitutes the first step of HCV polyprotein translation.

### 2.4.2.2. 3' UNTRANSLATED REGION

The 3'UTR is approximately 225 nt long and is organized in three regions. It includes, from 5' to 3', a variable region which is 30-40 nucleotide, a long poly(U)-poly(U/UC) sequence tract, and a highly conserved 3'-terminal, 98 nucleotide, stretch (3'X region). This 3'-terminal includes three stem-loop structures SL1, SL2 and SL3 (Tanaka et al. 1995; Tanaka et al. 1996; Kolykhalov, Feinstone, and Rice 1996). The 3'UTR interacts with the NS5B RdRp and with two of the four stable stem-loop structures located at the 3' end of the NS5B-coding sequence (Cheng, Chang, and Chang 1999; Lee et al. 2004). 52 nucleotide upstream region of the poly (U/C) tract and the 3'X region are associated with RNA replication. The remaining sequence of the 3'UTR appears to enhance viral replication (Ito and Lai 1997; Friebe and Bartenschlager 2002; Minkyung Yi and Lemon 2003).

### 2.4.2.3. STRUCTURAL PROTEINS

#### 2.4.2.3.a CORE PROTEIN

The HCV core protein is a RNA-binding protein, highly basic, which forms the viral capsid. It has also been suggested to interact with cellular proteins and pathways important in the viral lifecycle (McLauchlan 2000). The HCV core protein possess pro- and anti-apoptotic functions and has been implicated in tissue injury and fibrosis progression (Kountouras, Zavos, and Chatzopoulos 2003; Chou et al. 2005; Meyer et al. 2005). The core protein has been found to stimulate growth in Huh-7 cell line by transcriptional upregulation of growth-related genes (Nunez 2004; Fukutomi et al. 2005). The HCV core protein have found to alter transcription of viral promoter and...
also regulate the activity of cellular genes like c-myc and c-fos (Shih et al. 1993; R. B. Ray et al. 1995). It could also induce lipid droplets formation and may directly involved in steatosis (Barba et al. 1997; Moriya et al. 1997; Moriya et al. 1998). Its expression in transgenic mice has also shown to induces hepatocellular carcinoma (Moriya et al. 1997; Moriya et al. 1998; Moriya et al. 1998).

2.4.2.3.b E1 AND E2 ENVELOPE GLYCOPROTEINS

The envelope glycoproteins are essential components of the hepatitis C virion envelope and necessary for viral fusion and entry (Bartosch, Dubuisson, and Cosset 2003; Nielsen et al. 2004) Envelope glycoprotein and E2 plays a crucial role in the early steps of infection. E2 is involved in the initiation of viral attachment via its interaction with components of the receptor complex (Rosa et al. 1996; Flint and McKeating 2000). There is a paucity in knowledge about E1, but is speculated to be involved in intra-cytoplasmic virus-membrane fusion (Rosa et al. 1996; Flint and McKeating 2000).

2.4.2.3.c FRAMESHIFT PROTEIN

The role of frameshift protein in the HCV lifecycle remains obscure but it was proposed to be involved in viral persistence (Baril and Brakier-Gingras 2005).

2.4.2.4 NONSTRUCTURAL PROTEINS

2.4.2.4.a P7

p7 is an integral protein which comprises of two transmembrane α-helices domains, connected by a cytoplasmic loop. In Chimpanzees, Mutation or deletion in p7 appears to suppress infectivity of intra-liver transfection of Hepatitis C virus cDNA (Sakai et al. 2003). In vitro studies have demonstrated that p7 act as a calcium ion channel (Gonzalez and Carrasco 2003). However, these results remain to be substantiated in vivo.
2.4.2.4. b NS2

NS2 is a short-lived protein that loses its protease activity after self-cleavage from NS3. It is degraded by the protein kinase casein kinase 2 in a phosphorylation-dependent manner (Franck et al. 2005). NS2 also interact with host cell proteins and affect reporter genes controlled by liver and non-liver-specific promoters and enhancers such as the liver specific pro-apoptotic cell death-inducing DFF45-like effector (CIDE-B) (Dumoulin et al. 2003; Erdtmann et al. 2003). However, the repercussion of such interactions within the context of the lifecycle of HCV is yet to be deciphered.

2.4.2.4. c NS3-NS4A

HCV NS3 is a multi-functional protein. It contains a serine protease domain in its N terminal and a helicase/NTPase domain in its C-terminal. The NS3/NS4A protease is involved in the life cycle of HCV. HCV NS3-NS4A maybe involved in abrogating interferon induction by antagonizing the double stranded RNA dependent interferon regulatory factor 3 (Foy et al. 2003). During early stage of infection NS3-NS4A protease abrogates innate immune response. It has also been found to be involved in hepatocarcinogenesis (Borowski et al. 1996).

2.4.2.4. d NS4B

This protein is a 261 amino acids integral membrane protein serving as a membrane anchor for replication complex (Hugle et al. 2001; Egger et al. 2002).

2.4.2.4. e NS5A

NS5A is a phosphorylated zinc-metalloprotein of 56-58 kDa. It plays important role in regulation of cellular pathways and virus replication.
2.4.3. Prevalence of hepatitis C

2.4.3.1 Prevalence of hepatitis C worldwide

Hepatitis C virus (HCV) continues to be a major disease burden on the world. The worldwide estimated prevalence was about 3% in 1999 with the virus affecting 170 million people worldwide ("Global Surveillance and Control of Hepatitis C. Report of a WHO Consultation Organized in Collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium" 1999). Generally, most prevalence studies use blood donors to report the frequency of HCV. This is usually done by anti-HCV antibodies and follow-up HCV testing is not report. Use of blood donors as a prevalence source may result in the underrating of the real prevalence of the virus because they are a highly selected population (Alter et al. 1999). In a survey by National Health and Nutrition Examination Survey (NHANESIII), an estimated 3.9 million people were infected with HCV in the United States (US) and 2.7 million people with chronic infection. Neither sex nor racial-ethnic group was found to be associated with HCV infection. But it was found that a majority of patients that were HCV positive were below the age of 50 (Alter et al. 1999). Among Central and South America, a study in San Juan, Puerto Rico, showed that the estimated prevalence of HCV in 2001-2002 was 6.3% (Pérez et al. 2005). In Mexico, the prevalence reported was about 1.2% (Uribe and Méndez-Sánchez 2002). Among blood donors in Chile and Brazil, prevalence of HCV Ab was low - 0.3%, 1.14% respectively (Vasconcelos et al. 1994; Muñoz et al. 1998). In Europe, general prevalence of HCV is about 1% but varies among the different countries (Touzet et al. 2000). Prevalence of HCV antibody is 0.87% (1993-1994) in Belgium (Van Damme, Thyssen, and Van Loock 2002). In the United Kingdom, at least 200,000 adults carry HCV. In Northern Italy, prevalence of HCV Ab was 3.2%
(Bellentani and Tiribelli 2001). Three studies in Central and Southern Italy showed a higher rate of HCV (8.4%-22.4%), especially in the older population (Stroffolini et al. 1995; Maio et al. 2000; Raffaele et al. 2001). Among male blood donors in Karachi, Pakistan, the seroprevalence of Hepatitis C Virus was 1.8% with a trend of increasing proportion of positive donors from 1998-2002 (Akhtar et al. 2004).

**2.4.3.2 Prevalence of hepatitis C in India**

Several studies on voluntary or mixed donors have noted a prevalence of hepatitis C below 2% (Arankalle et al. 1995; Irshad, Acharya, and Joshi 1995; Panigrahi et al. 1996; Nanu et al. 1997; Chandrasekaran et al. 2000; Garg, Mathur, and Garg 2001; Das et al. 2002; Sonwane, Birare, and Kulkarni 2003; Singh, Malhotra, and Sarin 2004).

There is a dearth of large population based studies studying the prevalence of hepatitis C in the general population. There are six population studies which give an almost accurate index of the health burden of HCV in India and are depicted in Figure 4. From Andhra Pradesh, two studies are depicted, which were from a group of patients recruited from a gastroenterology camp and from a tribal population which showed a prevalence of 1.4% and 2.02% respectively (Chandra et al. 2003; Khaja et al. 2006). A smaller study from Arunachal Pradesh showed a pretty high prevalence of 7.89% (Phukan et al. 2001). Another survey from Maharashtra which involved more than 1000 villagers showed only 0.09% prevalence rate of HCV infection (Chadha, Tungatkar, and Arankalle 1999). A study from West Bengal, where by a 1:3 sampling method, from 9 villages, 3,579 individuals were selected from a total of 10,737
inhabitants (Chowdhury et al. 2003). Out of HCV 2,973 patients who were finally willing to participate, anti-HCV was detected in 26 (Chowdhury et al. 2003) patients by ELISA. Out of the 26 anti-HCV positive a total of 21 patients were true positive by PCR (0.71%). Older age group >60 years had the maximal prevalence was in the (1.5%) as opposed to prevalence in the age group < 10 years (0.31%).
Review of literature

Figure 4: Prevalence of HCV in India

2.4.4 HCV infection

HCV infection is characterized by its propensity to evolve by a wide clinical spectrum into chronicity. About 85% of patients infected by HCV have found to develop chronic
Review of literature

infection and only 15% resolve acute infection. The severity of the disease varies from asymptomatic chronic infection, with almost normal liver tests, to severe chronic hepatitis, leading rapidly to cirrhosis and hepatocellular carcinoma. The mechanisms responsible for the persistence of HCV infection and for the liver lesions are quite obscure. The lack of an efficient in vitro replication system or an animal model (the chimpanzee model is limited) has greatly hampered the study of these mechanisms.

HCV transmission occurs primarily through exposure to infected blood. This exposure can be through injection, drug use, solid organ transplantation from infected donors, blood transfusion before 1992, unsafe medical practices, occupational exposure to infected blood, vertical transmission (birth to an infected mother), sex with an HCV infected person, high-risk sexual practices, and intranasal cocaine use.

2.4.4.1 Acute Infection

After initial exposure, HCV RNA can be detected in blood within 1 to 3 weeks and is present at the onset of symptoms. Enzyme immunoassay (EIA) detects antibodies to HCV in only 50 to 70 percent of patients at the onset of symptoms, which subsequently increases to more than 90 percent after 3 months. Liver cell injury is manifested by elevation of serum alanine aminotransferase (ALT) levels which is within an average period of 4 to 12 weeks. Acute infection can be severe but rarely is fulminant. Symptoms are uncommon but can include weakness, malaise, jaundice and anorexia. Symptoms usually subside after several weeks as ALT levels decline. Reports have found that HCV is a minor player in the wide spectrum of acute hepatitis. A study from Delhi studied 32 patients with acute hepatitis and found hepatitis C in 12.5% of them (Irshad and Acharya 1994). A similar study from Indore looked at 103 patients and found HCV antibody in only 4.85% of these patients (Jaiswal et al. 1996). A study from
Delhi reported a prevalence of 12% (Kar et al. 1997). The most recent study from the same group of investigators had recruited the maximum number of patients with acute hepatitis, a total of 306 patients of whom 20.6% had evidence of hepatitis C (Kaur et al. 2002, 200). It is possible that these varied estimates may not be entirely accurate in view of the intrinsic referral bias of hospital-based studies.

2.4.4.2 Chronic Infection

Persistence of HCV infection is diagnosed by the detection of HCV RNA in the blood for at least 6 months. Prospective studies have shown that 60 to 85 percent of HCV-infected persons develop chronic infection. Factors that appear to associated with spontaneous clearance of HCV infection include younger age, certain major histocompatibility complex genes and female gender. African American males appear to be least likely to spontaneously clear the virus. Several studies have looked at the prevalence of hepatitis C in chronic liver disease in India. And prevalence has ranged from 10.8% to as high as 48.5% (Amarapurkar et al. 1992; Ramesh, Munshi, and Panda 1992; Issar et al. 1995; Sarin et al. 1996; G. Ray et al. 2000).

2.4.5 Pathogenesis of HCV infection

HCV is a non-cytopathic virus (Irshad and Dhar 2006) that enters the liver cell and undergoes replication simultaneously causing cell necrosis by several mechanisms including immune-mediated cytolysis in addition to various other phenomena such as insulin resistance, hepatic steatosis and oxidative stress. The proteins encoded by different sub-genomic regions of the HCV genome and their quasispecies influence the above mechanism and possibly have a significant role in Hepatitis C pathogenesis and
disease causation. An outline and brief description of HCV pathogenesis in the light of these factors is given in the following section.

2.4.5.1 Host Immunity

2.4.5.1.a Adaptive immunity

After the virus enters and replicates inside liver cells, they are transported to the endoplasmic reticulum, which then interact with MHC molecules. The MHC viral molecules are then transported to the cell surface which are recognized by T cells. Most of Cytotoxic T Lymphocytes (CTL’s) are CD8+ and basically recognize antigens presented on MHC class I molecules whereas antigens presented on MHC II molecules...
are recognized by CD4+ that comprises of 10% of CTLs. These CTLs eliminate cells infected with virus (Figure 5). There have been evidences that HCV have evolved mechanisms to avoid recognition by CTLs by either reducing the expression of MHC molecules or preventing cell surface presentation of the viral peptide. Thus, CTLs play a major role in immunopathogenesis of HCV infection (Neumann-Haefelin and Thimme 2013) and viral eradication (Zinkernagel et al. 1986).

2.4.5.1.b Innate immunity

Innate immunity is the first line of defense for the control of several viral infections including HCV infection. During HCV infection, Type 1 IFN are produced by cells which induces the cells to resist infection, promote adaptive immunity, check viral replication, and activate natural killer (NK) cells, Dendritic Cells and Kupffer cells etc. Once inside the cell, host recognition of viral macromolecular motifs and glycoproteins, called the pathogen-associated molecular patterns (PAMPs) trigger the innate immunity vs HCV. These receptors includes retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs) and tolllike receptors (TLRs) and (Saito et al. 2008) which activates interferon regulatory factor-3 (IRF-3) for expression of IFN-α/β and antiviral/interferon stimulated genes (ISGs) (H. M. Liu and Gale 2010). The secreted Interferon and cytokines eventually activate Natural Killer cells, Dendritic Cells and Kupffer cells etc. T/B cell-based immunity are also significantly mounted by these cells (Saito and Gale 2008).
2.4.5.1.c Role of genotype in treatment and disease progression

Comparison of the genomes of different HCV isolates showed important variations leading to the classification of HCV into types and subtypes (Simmonds et al. 2005). For nomenclature, HCV was classified into 6 types (1 to 6) each including subtypes (a, b, c,...) according to sequence homology. HCV genotypes are distributed differently depending on geography and etiology (Mondelli and Silini 1999).

The role of HCV genotypes in the progression of liver disease is controversial. Patients with genotype-1b infection are reportedly associated with a more aggressive course and a more severe liver disease than the patients infected with other HCV genotypes. It has been reported that hepatitis C virus genotype-1b was significantly more prevalent among patients with decompensated liver disease requiring liver transplantation and those with liver cirrhosis than among those with chronic active hepatitis C (Silini et al. 1995; Zein et al. 1996; Zein 2000). It was noticed that although steatosis is induced by all HCV-genotypes, steatosis is more prominent and frequent with HCV-genotype 3 infection (Adinolfi et al. 2001; Hui et al. 2002; Abid et al. 2005; Bochud et al. 2009; Probst et al. 2011).

HCV genotype is an important determinant of both treatment strategy and outcome. Patients with HCV genotype 1 and genotype 4 infections need 48 week treatment period whereas patients with genotypes 2 and 3 require 24 weeks. It has been observed that patients with HCV genotypes 1 and 4 are less responsive to PEG-IFN/RBV treatment. Their sustained virological response (SVR) rate is roughly around 50% (manns et al. 2001; Fried et al. 2002; Alfaleh FZ et al. 2004; Sherman KE et al. 2011), while the SVR rate approaches 80% in HCV genotype 2 and genotype 3 infected patients (Manns et al. 2001; Fried et al. 2002)
2.4.6 Immune system in Hepatitis C infection

2.4.6.1 The Role of Humoral Innate Immunity in Hepatitis C Virus Infection:

Special emphasis on the complement cascade

The innate immune system carries out the important roles of recognizing and clearing of viral infections. Its important function is immune surveillance in organ systems and the circulation and directly neutralizing infection (Leikina et al. 2005; Buck et al. 2006; K. S. Brown et al. 2010) It is also involved in triggering inflammation, modulation of adaptive immunity and pathogens opsonization (LeibundGut-Landmann et al. 2008; Avirutnan, Mehlhop, and Diamond 2008; S. Liu et al. 2008; Joffre et al. 2009; Iwasaki and Medzhitov 2010). Cellular components of innate immunity complex, which include dendritic cells, monocytes, Natural Killer cells platelets, and NKT cells, are involved in complex molecular interplay. The pathogens are degraded by these cells which detect pathogens and contribute to pathogen clearance by activating T cells and B cells (Fitzgerald-Bocarsly and Feng 2007; Newman and Riley 2007; Moretta et al. 2008).
The innate immune system and adaptive immunity is highly integrated. Soluble innate molecules are involved in modulation of antigen presentation (Perrin-Cocon et al. 2004; Cretin et al. 2007; Barrionuevo et al. 2007) directing the specificity of antibodies and T cells. In turn, antibodies contributing to enhanced antigen presentation by triggering and modulating innate antiviral effector mechanisms (Bayry et al. 2005). A number of cells including lymphocytes, monocytes and hepatocytes are involved in production of humoral innate immune factors. Importantly, hepatocytes are the primary source of the complement system components, MBL, and the ficolins L-ficolin and H-ficolin. High concentrations of these proteins accumulate in the liver and may have important anti-HCV activity. Infection of these cells with HCV result in increased production of soluble PRRs (J. Liu et al. 2009). Greater understanding of the interplay between components of the innate immune system acting in the liver may reveal novel therapeutic targets.

The importance of complement to Flavivirus infections has been revealed by in vivo studies of West Nile Virus (WNV) infection. Deficiencies in complement receptors CR1 and CR2 or, C3, result in increased pathogenesis of West Nile Virus (Mehlhop et al. 2005). This C5a independent protective effect (Mehlhop, Fuchs, et al. 2009), suggests involvement of opsonisation in limiting pathogenesis. Both classical and lectin pathways play a role in protection (Mehlhop, Fuchs, et al. 2009; Fuchs et al. 2011). Recruitment of complement component C1q increases the potency of specific monoclonal antibodies by modulating the stoichiometry of antibody neutralization (Mehlhop, Nelson, et al. 2009). Despite advances in understanding the pathology of West Nile Virus, there is paucity in the knowledge of the role of complement components in HCV infection. Neutralization of HCV pseudotypes by antibodies is
enhanced by Complement system (Meyer et al. 2002). Although this finding has yet to be substantiated using natural HCV virions, there is a possibility that results similar to those for West Nile Virus particles may be observed. Complement activation is documented in chronic HCV infection, but C4 activity (Dumestre-Perard et al. 2002a) and concentration (Banerjee et al. 2011a) is reduced. C4 may play an important role in HCV infection, as there has been demonstration where both HCV core and NS5A proteins resulted in reduction in C4 production by inhibiting transcription of C4 mRNA (Banerjee et al. 2011a). Patients with greater C4 activity respond to standard HCV treatment better compared to those where C4 activity is lesser (Dumestre-Perard et al. 2002a). This implicates the complement system components in resolving infection.

2.4.6.2 Complement system subversion by Hepatitis C virus

Even though complement system forms the first line of defense, there has been ample evidences that many pathogens have developed means by which the complement system component adroitness to protect from infection is subverted. Complement has coevolved with pathogens for millions of years, and is perhaps may be one reason that pathogens have developed mechanisms to inhibit complement activation and effector functions. The subversion of this powerful component of innate immunity have increased their ability to survive within the host cells. Given the disease burden associated with infection with microorganisms and the requirement of novel and effective antibiotics in order to combat them, more insight into the complement system role in defense has significant clinical implications. Deficiency or defect in opsonization pathways, including the production of antibody and phagocytic ability, results in early and recurrent infections with pyrogenic bacteria with the most common organisms being S. pneumoniae and Haemophilus influenza (Walport 2001a).
Activation of the complement system is tightly regulated by regulators of complement activation (RCA), which restrict host self-complement activation thereby preventing self-injury (Ricklin et al. 2010). Several enveloped viruses including human immunodeficiency virus type 1 (HIV-1), cytomegalovirus (CMV), herpes simplex virus 1, Ebola virus, influenza virus and vaccinia virus have been shown to escape antibody-dependent complement-mediated lysis (ADCML) by incorporating and hijacking host RCA proteins into the viral envelopes (Env) (Saifuddin et al. 1995; Spear et al. 1995; Vanderplasschen et al. 1998; Nguyen and Hildreth 2000; Hu et al. 2010). A Study by Amet et al., demonstrates that CD59 is incorporated into both cell line-derived and plasma primary HCV virions at levels that protect against antibody-dependent complement-mediated lysis (ADCML) (Amet et al. 2012). Another study by Ejaz et al., also demonstrated that acquires specifically CD59 to be protected against complement mediatedlysis upon complement activation (Ejaz et al. 2012). Due to the central role of C3 in the complement pathways, C3 deficiency results in defects in opsonization and lysis pathways, which subsequently associated strongly with recurrent infections by many microorganisms (Mold et al. 1988b). Study by Mazumdar et al., demonstrated that sera from patients chronically infected with hepatitis C virus (HCV) displayed significantly lower C3 levels than sera from healthy individuals (B. Mazumdar et al. 2012). They also demonstrated that HCV NS5A protein strongly downregulated C3 promoter activity at the basal level or in the presence of interleukin-1 (IL-1β) as an inducer. It has also been reported that the expression of the transcription factor CAAT/enhancer binding protein beta (C/EBP-β), which binds to the IL-1/IL-6 response element in the C3 promoter region, was suppressed in liver biopsy specimens (B. Mazumdar et al. 2012). A study by Kim et al., demonstrated that in HCV infection
there is attenuation of C9 expression and MAC mediated microbicidal activity. They had also shown C9 promoter activity was significantly inhibited by HCV core protein and, to a lesser extent, by NS2 and NS5A. However NS3/4A protein expression did not inhibit C9 promoter activity (Kim et al. 2013). Another study by Banerjee et al., had demonstrated that in HCV infection C4 expression both at mRNA and protein levels was abrogated by HCV NS5A proteins (Banerjee et al. 2011a). A study by Mawatari et al., had shown that complement C4 was cleaved by HCV NS3/4A and that this cleavage led to attenuation of the activation of classical pathway (Mawatari et al. 2013b). These observations suggest that chronic HCV infection may generate an overall state of immune abrogation and suppression. The role of a specific complement system component(s) in HCV-associated disease progression should be further clarified in the future.

2.4.7. Complement system mutation and manifestations:

As we have seen above that complement system is very crucial for immune response, be it adaptive or innate immunity, we can easily say that any mutation or deficiency in the complement system components and or regulators may help viruses in successfully evading the complement system and to persist in the host tissues and cause serious disorders. The various components of regulators are mentioned below with their deficiency and subsequent manifestation.

C2: Component C2 which is part of the classical pathway of the complement system is cleaved by activated factor C1 into two fragments: C2b and C2a. C2a. It then combines with complement factor 4b to generate the C3 or C5 convertase. Complement component 2 deficiency (C2D) are manifestation of defects in C2. A deficiency of the complement classical pathway associated with the development of autoimmune
disorders, mainly Systemic Lupus Erythematosus (SLE). Renal disease is relatively rare but joint manifestations are common. Patients with complement component 2 deficiency are also reported to have recurrent or invasive infections (Wetsel et al. 1996; Zhu, Atkinson, and Volanakis 1998). C→Y, S→F, G→R at location 131, 209, 464 respectively is involved in C2D.

**C3**: C3 plays a central role in the activation of the complement system. The processing of C3 by C3 convertase is the central reaction in both classical and alternative complement pathways. After activation C3b binds covalently, via its reactive thioester, to immune aggregates or cell surface carbohydrates. Derived from proteolytic degradation of complement C3, C3a anaphylatoxin is a local inflammation mediator. It induces smooth muscle contraction; increases vascular permeability and causes histamine release from mast cells and basophilic leukocytes. Defects in C3 are the cause of complement component 3 deficiency (C3D). It’s a rare defect of the complement classical pathway. Patients develop recurrent, severe pyogenic infections which may be due to ineffective opsonization of pathogens. Some patients also develop autoimmune disorders, such as lupus-like syndrome, arthralgia and vasculitic rashes, and membranoproliferative glomerulonephritis (Nagar et al. 1998; Szakonyi et al. 2001; Singer et al. 1994). Defects in C3 are associated with susceptibility to atypical hemolytic uremic syndrome (AHUS5). It is a complex genetic disease characterized by microangiopathic hemolytic anemia, renal failure, thrombocytopenia, and episodes of enterocolitis and diarrhea (Maga et al. 2010). R→Q, A→V, R→W, D→N, Q→K at location 592, 1094, 592, 1115, 1161 respectively leads to impaired binding to the regulator CD46/MCP and resistance to cleavage by factor I. F→V, R→W, R→L, C→W at location 603, 735, 1042,1158 respectively is observed in AHUS5. The
polymorphism chosen in this study, rs2230201 and rs7951, have been found to be associated with susceptibility to diseases like SLE by Miyagawa et al (Miyagawa et al. 2008).

C4: C4 plays a central role in the activation of the classical pathway of the complement cascade. It is processed by activated C1 which separates from the alpha chain the C4a anaphylatoxin. The remaining product, alpha chain fragment C4b, is the major activation product and is an essential subunit of the C3 convertase (C4b2a) and the C5 convertase (C3bC4b2a) enzymes of the classical complement pathway. Derived from proteolytic cleavage of complement component C4, C4a anaphylatoxin is a local inflammation mediator. It induces smooth muscle contraction, increases vascular permeability and initiates histamine release from mast cells and basophilic leukocytes. Prior to secretion, the single-chain precursor is enzymatically cleaved to yield the non-identical chains (alpha, beta and gamma). During activation, the alpha chain is proteolytically cleaved by C1 into C4a and C4b, and C4b stays linked to the beta and gamma chains. Further proteolytic degradation of C4b by C1 into the inactive fragments C4c and C4d blocks the generation of C3 convertase. Human complement component C4 is polymorphic at two loci, C4A and C4B. A total of 13 alleles of C4A and 22 alleles of C4B have been detected. The C4A alleles carry the Rodgers (Rg) while the C4B alleles carry the Chido (Ch) blood group antigens. The C4A6 allotype totally lacks in haemolytic activity. Defects in C4A are the cause of complement component 4A deficiency (C4AD). A rare defect of the complement classical pathway associated with the development of autoimmune disorders, mainly systemic lupus with or without associated glomerulonephritis. The SNP rs2857009 was selected in this study because
Yang et al., had found that this polymorphism could confer independent effect on affecting the concentration of C4(X. Yang et al. 2012).

C5: Activation of C5 by a C5 convertase initiates the spontaneous assembly of the late complement components into the membrane attack complex. C5b has a binding site for C6 and this complex is the foundation upon which the lytic complex is assembled. C5 anaphylatoxin is a mediator of local inflammatory process and is derived from proteolytic cleavage of C5. It induces smooth muscle contraction of smooth muscle, increased vascular permeability and initiates histamine release from mast cells and basophilic leukocytes. C5a also stimulates and induces the locomotion of polymorphonuclear leukocytes (chemokinesis) and direct their migration toward sites of inflammation (chemotaxis). Complement component 5 deficiency (C5D) is a manifestation of defects in C5. A rare defect of the complement classical pathway associated with susceptibility to severe recurrent infections, predominantly by Neisseria gonorrhoeae or Neisseria meningitides. An association study of C5 haplotypes and genotypes in individuals with chronic hepatitis C virus infection shows that individuals homozygous for the C5_1 haplotype have a significantly higher stage of liver fibrosis than individuals carrying at least 1 other allele (Hillebrandt et al. 2005).

C7: Constituent of the membrane attack complex (MAC) that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. C7 serves as a membrane anchor and defects in it are a cause of C7 deficiency (C7D). A rare defect of the complement classical pathway associated with susceptibility to severe recurrent infections, predominantly by Neisseria gonorrhoeae or Neisseria meningitides. R→Q, G→R, R→S, E→Q, R→H at location 220, 379, 521, 682, 687 respectively is involved in C7D.
C8: Called the Complement component C8 beta chain, it is a Constituent of the membrane attack complex (MAC) that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. Defects in C8B are a cause of complement component 8 deficiency type 2 (C8D2). A rare defect of the complement classical pathway associated with susceptibility to severe recurrent infections, predominantly by Neisseria gonorrhoeae or Neisseria meningitidis.

C9: Constituent of the membrane attack complex (MAC) that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells. C9 is the pore-forming subunit of the MAC. Defects in C9 are a cause of complement component 9 deficiency (C9D). A rare defect of the complement classical pathway associated with susceptibility to severe recurrent infections, predominantly by Neisseria gonorrhoeae or Neisseria meningitidis. C→G at location 119 is involved in C9D.

CD55: Also known as the Decay Accelerating Factor. This protein recognizes C4b and C3b fragments that condense with cell-surface hydroxyl or amino groups when nascent C4b and C3b are locally generated during C4 and c3 activation. Interaction of DAF with cell-associated C4b and C3b polypeptides interferes with their ability to catalyze the conversion of C2 and factor B to enzymatically active C2a and Bb and thereby prevents the formation of C4b2a and C3bBb, the amplification convertases of the complement cascade. DAF deficiency is associated with Inab phenotype (absence of all blood group antigens of the Cromer complex). Clinically, PNH (paroxysmal nocturnal hemoglobinuria) usually affects young adults and has a variable course of intravascular haemolysis, pancytopenia, and recurrent (usually venous) thromboses. It often arises in patients with aplastic anaemia, and may transform into acute myeloblastic leukaemia.
Complications include iron deficiency (from chronic haemoglobinuria), progressive renal impairment (from haemoglobinuria), and the Budd Chiari syndrome (hepatic vein thrombosis).

**CD59**: Potent inhibitor of the complement membrane attack complex acts by binding to the C8 and/or C9 components of the assembling MAC, thereby hindering incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore. This inhibitor of MAC formation appears to be species-specific. It is involved in signal transduction for T-cell activation complexed to a protein tyrosine kinase. Defects in CD59 cause CD59 deficiency (CD59D) which causes Paroxysmal nocturnal hemoglobinuria due to hereditary nucleotide deletion in the HRF20 (CD59) gene (Motoyama et al. 1992).

**CFD (Complement Factor D)**: Factor D cleaves factor B when the latter is complexed with factor C3b, activating the C3bbb complex. This complex then becomes the C3 convertase of the alternate pathway. Function of this convertase is homologous to that of C1s in the classical pathway. Defects in CFD are the cause of complement factor D deficiency (CFD deficiency). CFD deficiency predisposes to invasive meningococcal disease. V→G and C→R at sites 213 & 214 respectively are involved in CFD deficiency (Sprong et al. 2006).

**CFH (Complement Factor H)**: Factor H functions as a cofactor in the inactivation of C3b by factor I and also increases the rate of dissociation of the C3bBb complex (C3 convertase) and the (C3b)NBB complex (C5 convertase) in the alternative complement pathway. Defects in CFH are the cause of complement factor H deficiency (CFH deficiency). CFH deficiency determines uncontrolled activation of the alternative complement pathway with consumption of C3 and often other terminal complement
components. It is associated with a number of renal diseases with variable clinical presentation and progression. These include membranoproliferative glomerulonephritis and atypical hemolytic uremic syndrome. Complement factor H deficiency patients may show increased susceptibility to meningococcal infections. Defects in CFH are a cause of susceptibility to hemolytic uremic syndrome atypical type 1 (AHUS1). It's an atypical form of hemolytic uremic syndrome which is a complex genetic disease characterized by renal failure, microangiopathic hemolytic anemia, thrombocytopenia, and episodes of enterococitis and diarrhea. In contrast to typical hemolytic uremic syndrome, atypical hemolytic uremic syndromes have a poorer prognosis, higher death rates and frequent progression to end-stage renal disease. R→G, C→Y, Q→K, V→I, C→W, C→Y, E→K at 78,325,400,609,630,673,850 are involved in AHUS1 (Neumann et al, Pérez-Caballero et al, Richards et al). R→L, C→S, C→S at 127,431,673 respectively are involved in CFH deficiency; with membranoproliferative glomerulonephritis. C→R, C→Y, Q→E, D→G at 536, 959, 1076, 1119 respectively is involved in CFH deficiency (Su et al. 2011).

CFI (Complement Factor I): Responsible for cleaving the alpha-chains of C4b and C3b in the presence of the cofactors C4-binding protein and factor H respectively. Defects in CFI cause susceptibility to hemolytic uremic syndrome atypical type 3 (AHUS3). It's an atypical form of hemolytic uremic syndrome. aHUS is a complex genetic disease characterized by renal failure, microangiopathic hemolytic anemia, thrombocytopenia, and episodes of diarrhea and enterococitis. In contrast to typical hemolytic uremic syndrome, aHUS have a poorer prognosis. They have higher death rates and frequent progression to end-stage renal disease. Defects in CFI are the cause of complement factor I deficiency (CFI deficiency). Complement Factor I deficiency is an autosomal
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CFP (PROPERIDIN): A positive regulator of the alternate pathway of complement system, CFP binds and stabilizes C3- and C5-convertase enzyme complexes. Defects in CFP are the cause of properdin deficiency (PFD). PFD results in higher susceptibility to bacterial infections; especially to meningococcal infections. Three phenotypes have been reported: complete deficiency (type I), incomplete deficiency (type II), and dysfunction of properdin (type III). SLE (systemic lupus erythematosus) and discoid lupus are possible.

CFHR5 (Complement Factor H-related protein 5): Involved in complement regulation. Defects in CFHR5 have been found in patients with atypical hemolytic uremic syndrome and may contribute to the disease. aHUS complex genetic disease characterized by thrombocytopenia, microangiopathic hemolytic anemia, renal failure and episodes of enterocolitis and diarrhea. In contrast to typical hemolytic uremic syndrome, aHUS have a poorer prognosis. Their death rates are higher and frequent progression to end-stage renal disease. N→S at location 216 IS involved in a breast cancer sample.

CD46 (Membrane Cofactor Protein): Acts as a cofactor for complement factor I, which protects autologous cells against complement-mediated destruction by cleaving C4b and C3b deposited on host tissue. It also acts as a co stimulatory factor for T-cells which induce the differentiation of CD4+ into T-regulatory 1 cells. T-regulatory 1 cell suppresses immune responses, basically prevent autoimmunity, by secreting
interleukin-10, and therefore are thought to prevent autoimmunity. A number of bacterial and viral pathogens seem to exploit this property and directly induce an immunosuppressive phenotype in T-cells by binding to CD46. Manifestation of defects in CD46 includes susceptibility to hemolytic uremic syndrome atypical type 2 (AHUS2). An atypical form of hemolytic uremic syndrome and is characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure and absence of episodes of enterocolitis and diarrhea. C→Y, P→S, W→C, P→R, S→P at location 35, 165, 216, 231, 240 respectively is found in AHUS2. C→Y at 228 is seen in a colorectal cancer sample.